Restocking of the Western School Prawn (*Metapenaeus dalli*) in the Swan Canning Riverpark



Tweedley, J.R., Loneragan, N.R., Crisp, J.A., Poh, B. Broadley, A.D., Bennett, A.L., Hodson, K.P., Trayler, K.M., Jenkins, G.I., Chaplin, J.A.

October 2017

RSP13MUR01

Murdoch University

Government of Western Australia South Metropolitan TAFE

Australian Government Fisheries Research and Development Corporation

Government of Western Australia

Department of Biodiversity, Conservation and Attractions

Parks and Wildlife Service



Photographs: (Cover) Three generations of recreational prawners enjoying a night on the Swan-Canning Estuary. (This page) Will Smithwick and the former Western Australian Minister for Fisheries the Hon. Ken Baston pulling a prawn net at a Prawn Watch event. Photos taken by Stewart Allen.

Ownership of intellectual property rights annex

Unless otherwise noted, copyright (and any other intellectual property rights, if any) in this publication is owned by the Department of Biodiversity, Conservation and Attractions, Murdoch University, Australian Centre for Applied Aquaculture Research and the Fisheries Research and Development Corporation.

This publication (and any information sourced from it) should be attributed Tweedley, J.R., Loneragan, N.R., Crisp, J.A., Poh, B., Broadley, A.D. Bennett, A.L. Hodson, K.P., Trayler, K.M., Jenkins, G.I., Chaplin, J.A. 2017. Restocking of the Western School Prawn (*Metapenaeus dalli*) in the Swan-Canning Estuary. Report to the Department of Biodiversity, Conservation and Attractions. Murdoch University, October 2017. ISBN: 978-1-921877-22-3.

Disclaimer

The authors do not warrant that the information in this document is free from errors or omissions. The authors do not accept any form of liability, be it contractual, tortious, or otherwise, for the contents of this document or for any consequences arising from its use or any reliance placed upon it. The information, opinions and advice contained in this document may not relate, or be relevant, to a readers particular circumstances. Opinions expressed by the authors are the individual opinions expressed by those persons and are not necessarily those of the publisher, research provider or the Department of Biodiversity, Conservation and Attractions.

Contents

Acknowledgments ..... **vii**

Executive Summary **8**

Awards **11**

Introduction **12**

Objectives **16**

Section 1. Biology and ecology of the Western School Prawn **19**

1.1. Abundance and distribution of the Western School Prawn in the Swan-Canning Estuary 20
- Summary 20
- Rationale and aims 21
- Methods 24
- Results 30
- Discussion 47
- Conclusions 52
- Annexes 54

1.2. Quantitative determination of ovarian development in the Western School Prawn 57
- Summary 57
- Rationale and aims 58
- Methods 60
- Results 64
- Discussion 71
- Conclusions 74

1.3. Environmental factors influencing the reproduction of the Western School Prawn in the Swan-Canning Estuary 76
- Summary 76
- Rationale and aims 76
- Methods 79
- Sampling methodology 80
- Size at maturity 82
- Results 86
- Discussion 98

1.4. Larval development of the Western School Prawn reared in the laboratory 106
- Summary 106
- Rationale and aims 106
- Methods 108
- Results 112
- Discussion 134

1.5. Effects of water temperature and salinity on the survival and development of larval Western School Prawns 138
- Summary 138
- Rationale and aims 138
- Methods 140
- Results 144
- Discussion 147
- Conclusions 149

1.6. Performance of mixed species and mono-specific algal diets for culture of larval Western School Prawns....151
Summary....151
Introduction....151
Methods....154
Results....160
Discussion....165
Conclusions....168

1.7. Quantitative classification of sediments in the Swan-Canning Estuary....170
Summary....170
Rationale and aims....171
Methods....175
Results....180
Discussion....191
Conclusions....195

1.8. Relationship between sediment types and the spatial distribution, size structure and preferences of the Western School Prawn in the Swan-Canning Estuary....198
Summary....198
Rationale and aims....199
Methods....202
Results....213
Discussion....226
Conclusions....231
Annex....232

Section 2. Biological characteristics of the Western School Prawn and bio-economic modelling....234

2.1. Estimation of growth and reproductive parameters of the Western School Prawn in the Swan-Canning Estuary....235
Summary....235
Rationale and aims....236
Methods....237
Results....246
Discussion....257
Conclusions....262

2.2. Bio-economic evaluation of restocking the Western School Prawn in the Swan-Canning Estuary....265
Summary....265
Rationale and aims....266
Methods....267
Results....275
Discussion....280
Conclusions....286

Section 3. Optimise release strategies....289

3.1. Influence of timing-of-release and substrate type on the activity of post-larval Western School Prawns....290
Summary....290
Rationale and aims....290
Methods....292

Results 294
Discussion 295

3.2. Influence of timing-of-release on predation rates of post-larval Western School Prawns 299
Summary 299
Rationale and aims 300
Methods 303
Results 310
Discussion 325
Conclusions 332

3.5. A methodology to develop aquaculture-based release strategies with an application to the Western School Prawn in the Swan-Canning Estuary: the Survival Maximisation-At-Release Tool (SMART) 335
Summary 335
Rationale and aims 336
Methods 338
Results 356
Discussion 377
Conclusions 391
Annexes 393

Section 4. Community engagement and stewardship 411

4.1 Prawn Watch: engaging community in science, restocking and sustainable management of an iconic species that has declined markedly in abundance 412
Summary 412
Rationale and aims 413
Approach 413
Engagement and awareness raising 414
Communications 417
Citizen science monitoring 424
Influencing policy 432
Discussion 432
Annexes 435

4.2. Evaluation of the effectiveness of data collected by recreational prawn fishers to provide low cost monitoring of Western School Prawns 455
Summary 455
Rationale and aims 455
Materials and methods 456
Results 458
Discussion 462

4.3. Recommendations for a sustainable Western School Prawn fishery 473

Conclusion 492

References 495

Appendices 532

Appendix 1. Publications arising from this study 532
Appendix 2. Introduced goby Acentrogobius pflaumii 536

Acknowledgments

Gratitude is expressed to the friends and colleagues for their help with the extensive sampling program and the community members, staff and students of the affiliated organizations who took part in Prawn Watch and gave up substantial amounts of their time to take part in broodstock collection events. Particular thanks to Kevin Reid and Will Smithwick for sharing their intimate knowledge of the prawns and prawning on the Swan-Canning Estuary. We also thank Dr Frances D'Souza (Department of Water and Environmental Regulation) for assisting with the larval studies and Dr Mervi Kangas (Department Primary Industries and Regional Development, Fisheries Division) for sitting on the Project Steering Committee. Robert Michael and Bruno Pais (Australian Centre for Applied Aquaculture) were instrumental in the aquaculture of the post-larval prawns. Jen Eliot, Marnie Giroud, Rachel Hutton, Jason Menzies, Mark Thornley, Dr Steeg Hoeksema and Miranda Jackson (Department of Biodiversity Conservation and Attractions (DBCA), Parks and Wildlife Service) are thanked for their help in the organisation of Prawn Watch and its promotional activities. The development of the Prawn Watch Smartphone App was undertaken with Gaia Resources, who are thanked for their ongoing support in providing technology support at community events. In addition to the funding provided by the Department of Parks and Wildlife (now DBCA), financial contributions were made by the Fisheries Research and Development Corporation, Recreational Fishing Initiatives Fund (which is supported by Recfishwest and the WA Department Primary Industries and Regional Development, Fisheries Division), the Australian Centre for Applied Aquaculture Research (ACAAR) and Murdoch University.

Executive Summary

This report provides the first comprehensive investigation into the biology and ecology of the Western School Prawn (*Metapenaeus dalli*) in the Swan-Canning Estuary in south-western Australia. It provides knowledge to help manage the fishery and evaluate release strategies for the aquaculture-based enhancement of this species. The study involved Murdoch University, the Department of Biodiversity, Conservation and Attractions (DBCA) (formerly Department of Parks and Wildlife and the Swan River Trust) and the Australian Centre for Applied Aquaculture Research (ACAAR). It was designed to complement a concurrent project to develop aquaculture techniques to produce and release *M. dalli* and re-engage the local community with prawning and the estuary (led by ACAAR, DBCA's Parks and Wildlife Service and the West Australian Fish Foundation), funded by the Recreational Fishing Initiatives Fund. The Fisheries Research and Development Corporation provided matching funds for the current study. Biological data on *M. dalli* were collected from 20 sites in nearshore and 16 in the offshore waters of the Swan-Canning Estuary, ranging from the mouth of the system to ~40 and 30 km upstream in the Swan and Canning rivers, respectively, in every lunar month between October 2013 and March 2016. Laboratory studies were also completed to investigate the survival and growth of larval prawns in different salinity, water temperature and algal food conditions. Results were presented as part of the Prawn Watch program to engage the community in the research and encourage stewardship of the fishery and the estuary.

Background

Both *M. dalli* (an estuarine species) and the Western King Prawn *Penaeus (= Melicertus) latisulcatus* (a marine species) were the focus of a small commercial and iconic recreational fishery in the Swan-Canning Estuary before catches declined significantly. The commercial fishery, which had a peak catch of 15 tonnes in 1959, closed in the mid-1970s and recreational fishing, which involved >50,000 people in the 1980s, also declined, with the last significant catches recorded in the late 1990s. The reasons for the decline are unclear and restocking was seen as a possible means of increasing the population size of *M. dalli* in the estuary. The two restocking projects enabled the development of new aquaculture techniques for *M. dalli* and subsequent releases from 2012 to 2016. These were linked to Prawn Watch and community engaged in restocking effort. This project provided the funds to undertake the research and development side of the restocking, enhance our understanding of the biology and ecology of *M. dalli* and the subsequent monitoring of the population post-release of hatchery-reared individuals.

Aims

In light of the above, the aims of the project were to:

1. Evaluate the current stock status and factors affecting the natural recruitment of *M. dalli* in the Swan-Canning Estuary.
2. Evaluate the costs and benefits of releasing *M. dalli* in the Swan-Canning Estuary.
3. Optimise release strategies (*i.e.* stocking densities, size and location at release) for *M. dalli*.
4. Increase stewardship of the recreational *M. dalli* fishery in the Swan-Canning Estuary.

Methodology

The biological characteristics, population size and recruitment of *M. dalli* were studied, over 31 consecutive lunar months, by sampling 20 sites in the nearshore (< 2 m deep) and 16 sites in the offshore (> 2 m deep) waters of the Swan-Canning Estuary using hand and otter trawls. Data from the 1,040 trawls from nearshore and 832 samples in the offshore were used to provide the first quantitative estimates of growth and mortality for *M. dalli*, define the timing of reproduction, size-at-maturity and fecundity and egg production in the estuary and investigate variation in densities of prawns among regions, seasons and years and with salinity, water temperature and dissolved oxygen concentration. The survival and growth of *M. dalli* larvae was investigated under different salinity, water temperature and algal feeding treatments in the laboratory. The sediment preference of juvenile prawns and the significance of fish predation on postlarval prawns (13 days old = PL13) following two releases of ~ 130,000 PL13 were investigated using an innovative new method to inform the best time to release hatchery-reared individuals. The biological parameters estimated from the quantitative models and production practises/costs were used to develop a preliminary bio-economic model

of *M. dalli* releases using the EnhanceFish program. Information from the field and laboratory studies on survival under different conditions were synthesised in a novel tool that facilitates a structured approach to evaluating release strategies – the **S**urvival **M**aximization-**A**t-**R**elease **T**ool (SMART).

Results

The results from this project achieved the following:

- Clearly identified spatial and temporal patterns in the abundance and distribution of *M. dalli* in the Swan-Canning Estuary; including the marked seasonality in their presence in the nearshore waters during the summer breeding season (Oct-Mar) and a migration into offshore waters (> 2 m deep) in autumn/winter. *Metapenaeus dalli* were not found in the entrance channel of the estuary, which, with the information on their reproduction, confirms that they complete their life-cycle in the estuary.

- Described the larval development of *M. dalli* and their response (growth and survival) to different water temperatures, salinities and algal diets. Optimum water temperatures were 26 °C, salinity 35 and the best algal diet was a mixed feed containing *Chaetoceros muelleri* and *Tetraselmis suecica.*

- For the first time, estimated quantitatively the pattern of growth, mortality and size-at-maturity, finding a highly seasonal pattern of growth (virtually no growth in winter) and large differences in growth and survival between male and female prawns. Females grow about 25% longer than males and die faster than males.

- Clearly identified the time of reproduction (mainly November to February) when water temperatures are >18 °C and salinity >25. Described quantitatively changes in reproductive tissue associated with spawning, the number eggs produced by *M. dalli* and identified the factors that influence spawning.

- Estimated rates of predation by a suite of small fish species following the release of ~130,000 post-larval *M. dalli* during the day and night. These studies identified key predators of hatchery-reared prawns (*i.e.* the Gobbleguts *Ostorinchus rueppellii* and the Hardyhead *Atherinomorous vaigiensis*), found that predation is a significant source of mortality and the results provide the basis for recommendations on release-strategies that are likely to increase the immediate post-release survival.

- Produced a quantitative sediment map for the Swan-Canning Estuary and identified areas of preferred habitat based on organic matter content and grain size. *Metapenaeus dalli* was found to have a preference for fine sediments, which facilitate fast and easy burial.

- Developed a structured, quantitative approach for evaluating the potential success of different release sites (SMART) that also provides a mechanism for engaging stakeholders in discussions on release strategies any target species. The SMART synthesized all available information on *M. dalli* and the co-occurring fish/crustacean species collected during this project to identify optimum release sites and times. Releases in the Lower Canning River and Perth Water during early to mid-summer (December to January) were predicted to lead to maximum post-release survival. This tool could readily be applied to other species.

- The preliminary bio-economic model evaluated releases ranging from 650,000 to 5 million *M. dalli* and size-at-release from 1 mm carapace length (CL) to 10 mm CL. The greatest potential returns were obtained when the 5 million prawns were released at a size of 10 mm CL, however, such an aquaculture effort would require substantial capital expenditure to produce the required number of juveniles. At the current low population level, without any restocking, the population biomass was projected to remain virtually unchanged over a five-year period.

- Engaged with the community through the Prawn Watch program to involve them in the collection of mature females for the aquaculture production of *M. dalli*, providing information on the distribution and abundance of prawns, increasing awareness of the biology and ecology *M. dalli,* as well as encouraging stewardship of the recreational fishery and the estuary more broadly. These activities and associated press releases on the project resulted in significant publicity for the project.

- Through data collection, information sharing, training and awareness raising, the project has shown improvements in community understanding of the fishery, sustainable fishing practice and river management issues.

- Discussed the results from the project and their implications for management of the *M. dalli* recreational fishery in the Swan-Canning Estuary with representatives from Recfishwest, the Department of Primary Industries and Regional Development and the Department of Biodiversity, Conservation and Attractions.

- Provided training for four Honours students (all attained first-class Honours) and two PhD students (who are both nearing completion). These studies contributed greatly to the overall extent and scope of studies that were completed as part of this project.

Implications

- As yet, the life-cycle of *M. dalli* has not been closed for aquaculture production, which constrains the production of hatchery-reared prawns for release into the estuary to the months between November and February when prawns in reproductive condition are present in the estuary and can be collected.

- The timing of reproduction and estimates of size-at-maturity and egg production indicate that limited temporal closure may conserve some spawning in the system and complement any aquaculture-based enhancements of *M. dalli.*

- The results from the predation studies suggest that releases should be carried out in shallow waters during the day, when the abundance and feeding activity of key predators is lower than at night.

- The results from the preliminary bio-economic model highlighted the importance of size-at-release and density-at-release to the post-release survival. If larger size-at-releases are required, production methods will need to be developed to release small juvenile *M. dalli*, instead of PL13 prawns.

- The results from the study highlight the importance of multi-disciplinary research for evaluating release programs and the need to adopt a responsible approach to release programs to consider factors such as size-at-release and density-at-release, not just maximising the numbers that can be produced and released in the shortest possible time.

- The data from the studies of larval biology, ecology of *M. dalli*, growth and survival, and from reproduction and predation combined with in the SMART provides an understanding of the optimum areas for spawning and larval survival and, as a consequence, good release sites. The SMART approach could be modified and adapted to consider release strategies for other species/environments.

- The results of this study are valuable for providing a range of managerial, stakeholder and community groups with a greater understanding of release programs, their objectives and the need for responsible programs to maximize their potential success and to complement traditional fisheries management.

Recommendations

- Continue to promote the findings of this research to fishery/environmental managers, Recfishwest and user groups in order to progress a set of recommendations to improve the sustainability of the recreational prawn fishery in the Swan-Canning Estuary.

- Further develop the bio-economic model to incorporate the new knowledge gained from this research and ecological research is initiated to investigate the significance of size-at-release and density-at-release in determining post-release survival. Repeat the predation experiment to determine the role that habitat complexity (the presence of seagrass/algae) may have on predation rates. The results from the above components could be used to further development the SMART.

- Discuss the results from this study in a workshop with researchers, managers, recreational fishers and other interested community members to increase knowledge and understanding of release programs and their potential to contribute to rebuilding fish stocks or enhancing their production.

Keywords: Aquaculture-based enhancement, recreational fishing, restocking, post-release survival, larval ecology, larval taxonomy, fish predation

Awards

This project received a commendation at the 2017 WA Seafood Industry Awards.



Introduction

The global marine fishery catch has not increased since the mid-1990s, despite substantial advances in the effectiveness of fishing effort through technological change (Pauly *et al*., 2002; FAO, 2012). At the same time, declines in the abundance of top-level predators and changes in ecosystem function due to industrial-level fishing have been documented (Myers and Worm, 2003; Baum and Worm, 2009). To reduce the impact of fisheries, Pauly *et al*. (2002) suggested that fishing capacity needs to be greatly reduced and significant spatial closures employed throughout the world's oceans to allow fisheries to recover. However, this places significant economic and social hardship on businesses and communities that previously had open access to these fishing zones (Mascia *et al*., 2010; Bennett and Dearden, 2014).

An alternative potential management intervention for rebuilding stocks and increasing production is the application of release programs to introduce individuals cultured from aquaculture into the natural environment (Bell *et al*., 2005; Bell *et al*., 2008; Leber, 2013). While restocking and stock enhancement both involve the release of aquaculture-raised animals into natural systems, the status of the wild stock and purpose of the release differ significantly between restocking and stock enhancement. Thus, restocking involves the release of cultured juveniles to restore over-exploited or severely depleted wild populations and it attempts to achieve long-term benefits by recovering the spawning biomass (Bell *et al*., 2005; Bell *et al*., 2008; Leber, 2013). In contrast, stock enhancement seeks to improve fishing yield by increasing recruitment when the spawning biomass has not been depleted.

Individuals from several species of prawns or shrimp in the Penaeidae are released on a very large, commercial scale (hundreds of millions to billions) in Japan (Hamasaki and Kitada, 2006) and China (Bell *et al*., 2005; Wang *et al*., 2006; Loneragan *et al*., 2013a). Smaller scale, commercial releases of penaeids have also been practised in Kuwait, Sri Lanka, the United States and Australia (Bell *et al*., 2005; Loneragan *et al*., 2013a). Recent research in Australia has focussed on release programs for penaeids to "enhance" recreational fishing; one for Eastern King Prawns *Penaeus plebejus*, to overcome recruitment limitation caused by a physical barrier

to prawn larval recruitment (Taylor, 2017; Taylor *et al*., 2017b) and the second, to investigate the potential for rebuilding the stocks of the Western School Prawn *Metapenaeus dalli* (this study), *i.e.* evaluating the potential to restock this species.

The genus *Metapenaeus* is one of 25 extant genera belonging to the Penaeidae, and comprises 29 species that occur exclusively throughout the inshore coastal and estuarine waters of the Indo-West Pacific (De Grave, 2014). In this region, species of *Metapenaeus* contribute to important commercial and recreational fisheries and aquaculture production (Dichmont *et al*., 2006; Kompas *et al*., 2010). For example, in subtropical and temperate New South Wales, an average of 1,410 tonnes of prawns, valued at more than AUD $18 million, were caught annually between 2004 to 2009, in inshore and estuarine environments, with *Metapenaeus macleayi* and *Metapenaeus bennettae* comprising 54% and 32% of the total catch by weight and value, respectively (Montgomery, 2010). Prawns found in the estuaries of this region, predominantly *Penaeus plebejus* and *M. macleayi*, are also exploited by recreational fishers who remove ~4,700 tonnes annually (Montgomery, 2010).

Metapenaeus dalli, the Western School Prawn, is the only metapenaeid found in temperate south-western Australia (Racek, 1957). The species typically occurs in shallow, inshore marine waters (< 30 m deep) along the western coast of Australia from Darwin in the north to Cape Naturaliste in the south and also in Java, Indonesia (Grey *et al*., 1983). However, in latitudes below 31º S, it is only found in estuaries and is believed to complete its entire life cycle within these systems (Potter *et al*., 1986b; 1989; Fig. I.1) and is thus classified as a solely estuarine species in this region (Potter *et al*., 2015a; 2015b; Fig. I.1).

Both *M. dalli* and the Western King Prawn *Penaeus latisulcatus* (a species that spawns in the marine environment, but spends significant time in estuaries, *i.e.* a marine estuarine-opportunist; Fig. 1; Penn, 1980) were the focus of a small commercial and iconic recreational fishery in the Swan-Canning Estuary. The commercial fishery catch peaked at 15 tonnes in 1959, but declined afterwards leading to its closure in the mid-1970s (Smith, 2006). At its peak, recreational prawning in this estuary involved over 50,000 people and became an iconic

pastime, particularly during the Christmas period (Smithwick *et al*., 2011). However, recreational catch rates also declined, with the last significant catches recorded in the late 1990s. The reasons for the decline are unclear, however, it is likely due to a combination of overfishing, changing environmental conditions and recruitment failure (Smith, 2006; Smith *et al*., 2007). Smith *et al*. (2007) also concluded that, despite the large reduction in fishing pressure, *M. dalli* populations were still low and had not recovered and, due to their small, discrete breeding stock, were reliant on self-replenishment. Thus, given the long-term recruitment failure, restocking was seen as a possible means of increasing the population size of *M. dalli* in the Swan-Canning Estuary by bypassing the recruitment bottleneck during the high mortality stage of larvae through to juveniles (Smith *et al*., 2007).

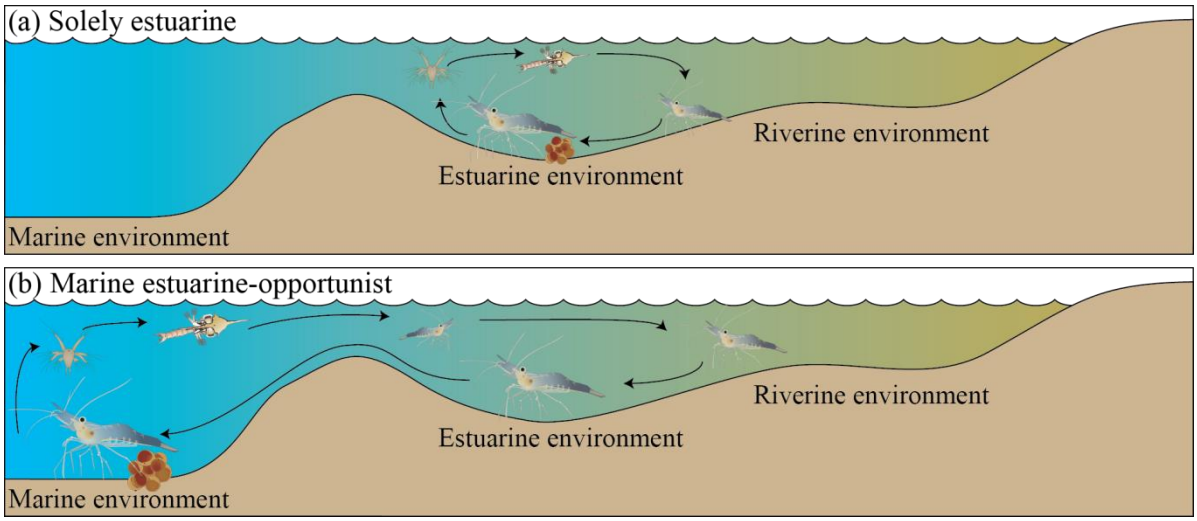


Fig. I.1. Conceptual models of the life cycle of (a) solely estuarine and (b) marine estuarine-opportunist species of prawn.

In late 2011, the Swan River Trust (now within the Department of Biodiversity, Conservation and Attractions) approached Murdoch University and the Australian Centre for Applied Aquaculture Research, to collaborate in a project utilising stock enhancement to investigate the causes of decline of *M. dalli*. Discussions were then held with Department of Fisheries (now Department of Primary Industries and Regional Development) and Recfishwest on the possibility of developing a release program for *M. dalli* in the Swan-Canning Estuary. Both organizations strongly supported a stock enhancement effort and funding of two projects

through the Recreational Fishing Initiatives Fund to pilot the production and release of *M. dalli* from 2012 until 2016 (Jenkins *et al*., 2015; 2017). In addition those two projects (entitled: "Growing community engagement by growing prawns" and "Re-establishing recreational prawning in the Swan-Caning Estuary") also supported a community engagement program, led by the Department of Parks and Wildlife (now Department of Biodiversity, Conservation and Attractions), and known as Prawn Watch.

The projects that were funded through the Recreational Fishing Initiatives Fund only provided for production and release of *M. dalli* and the Prawn Watch program. That is, there was not funding for the necessary research and development side of the restocking or understandings the biology and ecology or *M. dalli* or monitoring of the population following the releases of hatchery-reared individuals. This shortfall was recognised by partner organizations, at that time Department of Parks and Wildlife and the Australian Centre for Applied Aquaculture Research, who provided funding and in-kind support to this project,

This project aims to evaluate the biology and ecology of *M. dalli*, investigate possible reasons as to why the numbers of this iconic species declined in the past and develop methods to help maximise the effectiveness of the release program. The release of cultured *M. dalli* through stock enhancement provides a mechanism for testing whether it is possible to rebuild the stocks and thus the recreational fishery for this species. It also allows us to evaluate whether the current environment of the Swan-Canning Estuary will support released individuals. The stock enhancement and associated community engagement program have the potential to increase the recreational fishing experience in the Swan-Canning Estuary and provide recreational fishers and the broader community with a greater understanding of the biology and ecology of prawns and the environmental conditions of the system.

This project, combined with the Prawn Watch program, also provides opportunities to engage fishers in improved stewardship of the fishery and the Swan-Canning Estuary.

Objectives

The project had seven objectives.

1. Evaluate the current stock status and changes over time of Western School Prawns in the Swan-Canning Estuary (Section 1).

2. Evaluate factors affecting the natural recruitment of Western School Prawns in the Swan-Canning Estuary (Section 1).

3. Establish a bioeconomic model of the Western School Prawn population and the factors influencing it. (Section 2).

4. Evaluate the costs and benefits of releasing Western School Prawns in the Swan-Canning Estuary (Section 2).

5. Optimise release strategies (*i.e.* stocking densities, size and location at release) for Western School Prawns (Section 3).

6. Contribute to the improved understanding of the Western School Prawn, improved stewardship of the fishery and the Swan-Canning Riverpark (Section 4).

7. Contribute to a citizen science program that is complementary to scientific investigation. (Section 4).

Quotation

"This multidisciplinary program has taught us several valuable lessons. First and foremost is the importance of understanding the basic biology and life cycle of the studied model (i.e., the candidate for restocking) within its ecosystem. Specific restoration approaches must be tailored to the species of interest in the context of the targeted system. Second is the importance of integrated approaches to stock restoration. A blue crab restoration program must be based on a holistic approach, which integrates understanding of the released species with the system to be restored, such as the ecology of the habitat, seasonality patterns, and biological cycles. Moreover, blue crab restoration cannot be successful without integrating adequate management strategies to protect the wild and released animals until sexual maturity and spawning. All stakeholders in the resource to be restored must join forces and work in concert, including the fishery and seafood industry, policymakers, environmental activists, and scientists. Finally, a successful stock restoration program must be guided by thorough and solid science that addresses the multiple and complex facets reviewed here."

Zohar *et al*. (2008) on when evaluating a five year project to restock the Blue Crab (*Callinectes sapidus*) in Chesapeake Bay (USA).

Section 1. Biology and ecology of the Western School Prawn

This section details research relating to objective 1, *i.e.* evaluate the current stock status and changes over time of Western School Prawns in the Swan-Canning Estuary and objective 2, *i.e.* evaluate factors affecting the natural recruitment of Western School Prawns in the Swan-Canning Estuary. Six main components of the ecology of this prawn species were evaluated:

1. Abundance and distribution of Western School Prawns in the Swan-Canning Estuary (PhD studies of Brian Poh).

2. Quantitative determination of ovarian development in the Western School Prawn (PhD studies of Jason Crisp).

3. Reproductive biology of the Western School Prawn in the Swan-Canning Estuary (PhD studies of Jason Crisp).

4. Larval development of Western School Prawns reared in the laboratory (PhD studies of Jason Crisp).

5. Effects of water temperature and salinity on the survival and growth of larval Western School Prawns (PhD studies of Jason Crisp).

6. Effects of monspecific and mixed culture algal diets on survival and growth of larval Western School Prawns (PhD studies of Jason Crisp).

7. Quantitative classification of sediments in the Swan-Canning Estuary (Honours studies of Amber Bennett).

8. Relationship between sediment type and the spatial distribution, size structure and preference of the Western School Prawn (Honours studies of Amber Bennett).

1.1. Abundance and distribution of the Western School Prawn in the Swan-Canning Estuary

Summary

The distribution, abundance and size structure of the Western School Prawn *Metapenaeus dalli* Racek were investigated through an extensive, two-year sampling program (832 otter trawls and 1,040 hand trawls) within a microtidal estuary in temperate south-western Australia. Sampling was carried out every lunar month at 20 sites and 9 subregions in shallow waters by hand trawl (< 1.5 m deep) and at 16 sites and eight subregions in deeper waters (> 2 m) by otter trawl. The incidental catch of other species, consisting of other invertebrates and teleosts, was also recorded. Densities of *M. dalli* were highly seasonal, with greatest values recorded in the nearshore waters from October to February, and in the offshore waters from March to July. Prawn densities were also variable across the estuary, with highest densities of *M. dalli* found in the Lower Canning, Middle Swan, and Upper Melville Water regions. Spearman's rank correlation showed a positive relationship between the density of *M. dalli* in nearshore waters and surface water temperature in all nine subregions, whilst densities of *M. dalli* in the offshore waters were negatively correlated with bottom water temperature in most of the estuary, a result of the movement of breeding adults, as well as the subsequent recruitment. Carapace lengths remained relatively unchanged over the late autumn and winter months (May to August), before rapidly increasing in late spring as temperatures increased. The spatial and temporal distribution of *M. dalli* contrasted greatly with the other crustacean taxa in the estuary, particularly that of the Western King Prawn *Penaeus latisulcatus*; which was concentrated mainly in Lower and Upper Melville Water, but not caught further upstream. Non-metric multidimensional scaling revealed that the distribution of *M. dalli* was statistically indistinct from that of the apogonid *Ostorhinchus rueppellii*; which predates heavily on post-larval *M. dalli*, and that of two scyphozoans *Aurelia aurita* and *Phyllorhiza punctata*; which are likely to predate on *M. dalli* larvae. These findings highlight the significance of site selection and potential predation risk for any aquaculture-based enhancement programs aimed at increasing the population size of *M. dalli.*

Rationale and aims

Estuaries are highly productive ecosystems, receiving nutrients from a range of sources including rivers, run-off, tidal water movement, the atmosphere and waste input (Whittaker and Likens, 1975; McLusky and Elliott, 2004; Bianchi, 2006). Their high productivity provides important food sources for many taxa, enabling juveniles to grow rapidly. In addition, these systems lower predation risk due to the reduced presence of large predators (Blaber and Blaber, 1980; Potter *et al*., 2016). For these reasons, estuaries are often used as nursery areas by fish and crustacean species (Haywood *et al*., 1995; Perkins-Visser *et al*., 1996; Beck *et al*., 2001; Tweedley *et al*., 2016b). The ecological value of estuaries for fisheries is reflected in the proportion of commercial and recreational fishery species that utilise these productive, sheltered waters. For example, Lellis-Dibble *et al*. (2008) estimated that estuarine species contributed 46 % by mass and 68 % by value to commercial fish and shellfish landings in the United States between 2000 and 2004, while Creighton *et al*. (2015) estimated that >75 % of commercial fish catch and, in some regions, up to 90 % of all recreational angling catch in Australia comprised species that use estuaries. This significant contribution of estuaries parallels findings on the commercial fisheries of south-western Australia 30 years ago (Lenanton and Potter, 1987).

As estuaries are located at the interface between fresh and marine waters, their physico-chemical conditions change markedly spatially, as well as over a range of temporal scales, *e.g.* tidal cycle, monthly, seasonally and inter-annually (Gallegos *et al*., 2005; Sutherland and O'Neill, 2016; Tweedley *et al*., 2016b). Typically, these are influenced by tidal range and longer-term patterns in weather. For example, salinity in the Fraser Estuary of British Columbia, which has a tidal range of 4 m, varied by almost 30 over a tidal cycle (Geyer and Farmer, 1989). In contrast, in permanently-open microtidal estuaries (tidal range < 2 m), salinity changes little over a tidal cycle, but can change by 30 over the course of a year (Tweedley *et al*., 2016b). Given the dynamic nature of estuaries and their physico-chemical environments, the composition of their faunal communities also changes spatially and temporally (Palma *et al*., 2013; Becker *et al*., 2016). In the temperate estuaries of south-western Australia, the community structure and composition of the fish fauna show major differences associated with the

longitudinal gradient in both the Swan-Canning and Peel-Harvey estuaries (Loneragan *et al*., 1986; 1987; Loneragan and Potter, 1990; Valesini *et al*., 2009; Potter *et al*., 2016).

Temporal differences in the abundance of benthic macroinvertebrate species have been related to the timing of spawning, and recruitment and thus are also influenced by environmental parameters, such as water temperature and salinity, due to their effect on growth, survival and reproductive success (Rainer, 1981; Kalejta and Hockey, 1991; Sardá *et al*., 1995{Platell, 1996 #467). Due to the wide variety of habitats found in estuaries, which typically comprise a different faunal community, it is therefore also expected that the temporal (*i.e.* seasonal) changes in species composition would also vary spatially among the regions or habitats present within the estuary (Young and Potter, 2003).

Many penaeid species are associated with coastal and estuarine systems at some stage of their life cycle, particularly the postlarvae and juveniles, which recruit to nursery areas in estuaries for species that spawn in marine environments (Subramanian, 1990; Rönnbäck *et al*., 2001; Khorshidian, 2002; Macia, 2004). Aspects of the spatial distribution of many species of penaeid has been investigated, including analyzing the distribution of post-larval recruits away from the marine environment (Vance *et al*., 1996; 1998; Galindo-Bect *et al*., 2010) and of adults and/or juveniles between different habitats (Rönnbäck *et al*., 2001; Vance *et al*., 2002) and substrates (De Freitas, 1986; Somers, 1987; Kenyon *et al*., 2004; Gribble *et al*., 2007; Munga *et al*., 2013).

Abiotic variables have been correlated to the distribution of various penaeids species with, for example, water temperature, dissolved oxygen concentration and rainfall influencing the catch of *Metapenaeus macleayi* in the Hawkesbury-Nepean River, New South Wales (Pinto and Maheshwari, 2012). Moreover, water temperature was found to be the major factor influencing the recruitment of *Penaeus esculentus* in Moreton Bay, Queensland (Kienzle and Sterling, 2016) and salinity the main environmental variable influencing the distribution of *Penaeus monodon* and *Peneaus indicus* in the Saadan Estuary, Tanzania (Mosha and Gallardo, 2013). The movement of juveniles of *Penaeus merguiensis* from mangrove areas in the Gulf of Carpentaria, northern Australia and *Metapenaeus macleayi* south-eastern Queensland was also correlated with rainfall and river flow (Loneragan and Bunn, 1999; Vance *et al*., 2002).

Understanding the patterns of abundance and distribution of a species are particularly important for those species that are the target of fisheries and that complete their life cycle in estuaries, as there is unlikely to be recruitment from adjacent marine waters to supplement the population if overexploitation occurs. The Western School Prawn *Metapenaeus dalli*, is found along the western coast of Australia from Darwin in the north to Cape Naturaliste in the south, and also in Java, Indonesia (Racek, 1957; Grey *et al*., 1983). It typically occurs in shallow inshore marine waters (< 30 m deep), however, at the southern limit of its distribution (*i.e.* south of ~31°S), it is found only in estuaries and completes its entire life cycle within these systems (Potter *et al*., 1986b; Broadley *et al*., 2017). This species, together with the Western King Prawn, *Penaeus* (= *Melicertus*) *latisulcatus*, were the focus of a small commercial fishery that closed in the mid-1970s due to sustained low catches (Smith, 2006), and an iconic recreational fishery, which, at its peak, involved over 50,000 people, particularly during the Christmas period (Smithwick *et al*., 2011). However, recreational catch rates also declined, with the last significant catches recorded in the late 1990s (Maher, 2002) and the population has not recovered, despite the large reduction in fishing pressure (Smith *et al*., 2007). A restocking project was initiated between 2012/13 and 2015/16 to evaluate the feasibility of aquaculture-based enhancement to rebuild the population of *M. dalli* in the Swan-Canning Estuary. During this time, about 4.5 million *M. dalli* post-larvae (Jenkins *et al*., 2017) were released into the Swan-Canning estuary.

The general biology of *M. dalli* has been investigated in the Swan-Canning Estuary (Potter *et al*., 1986b) and the Peel-Harvey Estuary (Potter *et al*., 1989), 80 km south. The study in the Swan-Canning Estuary was carried out over 30 years previously at the time when the *M. dalli* recreational fishery was very active. This study and that of Broadley *et al*. (2017) provide valuable information on the patterns of growth and reproduction for the population in the system, but did not investigate the pattern of distribution and abundance within the estuary in detail. There is thus a need to understand the patterns of abundance and distribution of this once iconic fisheries species. The aims of this study were to (i) describe the spatial and temporal patterns of abundance of *M. dalli* and determine whether the patterns are correlated with any abiotic variables, (ii) describe the spatial and temporal patterns of abundance of different size classes of *M. dalli*, (iii) compare these patterns with those of a potential penaeid competitor in

the system, *P. latisculatus*, and (iv) identify the fish and key invertebrate species that exhibit similar spatial and temporal patterns of distribution to those for *M. dalli*, to evaluate potential key predators and how their distribution might affect the *M. dalli* population.

Methods

Study site

The Swan-Canning Estuary is a drowned river valley system located in south-western Australia, which is ~50 km long, covers an area of ~55 km^2 and remains permanently-open to the Indian Ocean {Brearley, 2005 #27}. The estuary comprises a narrow entrance channel that opens into two basins (Melville and Perth Water) and the tidal portions of the Swan and Canning Rivers, which extend ~29 and 13 km upstream from their entry points into Melville Water, respectively. Although the majority of the estuary is shallow, *i.e.* < 2 m in depth, it reaches a maximum depth of ~20 m in the entrance channel. South-western Australia experiences a Mediterranean climate (Gentilli, 1971), with hot, dry summers and cool, wet winters with ~80 % of rainfall occurring between June and September (Hodgkin and Hesp, 1998). This, combined with the microtidal tidal regime (< 1 m variation in tide height), results in marked seasonal variations in water physical-chemical conditions in this salt-wedge estuary. Salinities are typically stable and relatively high throughout much of the estuary during the austral summer (December to February), but during winter, may vary markedly along the estuary following substantial freshwater discharge, leading to marked stratification of the water column and hypoxia (Tweedley *et al*., 2016b).

The estuary flows through the capital city of Perth, which supports ~78 % of the 2.6 million people in the state of Western Australia (Australian Bureau of Statistics, 2015). It is valued highly for its aesthetic, commercial, environmental and cultural importance (Malseed and Sumner, 2001). Recreational fishing is an iconic activity in WA, with an estimated 711,000 participants in 2014/15 (Ryan *et al*., 2015), and the Swan-Canning Estuary is a popular hotspot

for recreational fishers, with an estimated effort of 30,338 fisher days occurring in 1998/99 (Malseed and Sumner, 2001).

Sampling procedure

Metapenaeus dalli were sampled during the night at two locations within 20 sites in the shallow, nearshore waters (< 2 m deep; Fig. 1.1.1) using a hand trawl net, and 16 sites in the deeper, offshore waters (2-17 m deep) using a small otter trawl net, on each new moon phase between October 2013 and 2015 (*i.e.* 26 consecutive lunar cycles). Each site was allocated to both a region and subregion (Fig. 1.1.1), with one trawl sample collected from each of the two locations within each site on each sampling occasion. Sampling in nearshore waters was conducted using a 4 m wide hand trawl constructed from 9 mm mesh. The width of the hand trawl net during trawling was, on average, ~2.85 m, but varied slightly amongst trawls depending on the condition of the substratum, presence of submerged obstacles and localised wind and wave conditions. A trawl of 200 m (swept area of ~570 m^2 each) were carried out at each location in each site on each sampling period, covering a total area of 22,800 m^2 on any single lunar cycle. Sampling in the offshore water employed a 2.6 m wide otter trawl net, with 25 mm mesh in the body, and 9 mm mesh in the cod end. The net was towed at a speed of ~1.6 knots (~3 km h^{-1}) for five minutes, covering a distance of ~250 m. A trawl of ~650 m^2 was completed at each location within each site on each sampling period covering a total area of 20,800 m^2 each lunar cycle.

After each hand or otter trawl, the contents of the net were emptied into a container and each *M. dalli* was counted, sexed, measured and returned alive to the water. The carapace length (CL), *i.e.* orbital indent to the posterior edge of the carapace, of each individual was measured (0.01 mm) using digital vernier callipers. Females were identified by presence of a thelycum and males by the presence of a petasma. Small individuals, without an obvious thelycum or petasma, were recorded as juveniles. Female prawns were also inspected to determine if they were gravid, *i.e.* had large green ovaries, as described by Crisp *et al*. (2017a) and/or possessed

a spermatophore. The abundance of each penaeid, stomatopod, brachyuran, teleost and scyphozoan species collected together with *M. dalli* were also recorded, except in the case of *Craterocephalus mugiloides*, *Atherinosoma elongata* and *Leptatherina presbyteroides*, which were grouped together as 'Atherinidae'. These species have similar morphologies and are very abundant, and it was not possible to identify them to species at night quickly enough whilst still being able to return them live to the water. As with any crustaceans, all teleosts and scyphozoans were returned to the water alive, as per the conditions in Murdoch University Animal Ethics Committee permit #RW2566.

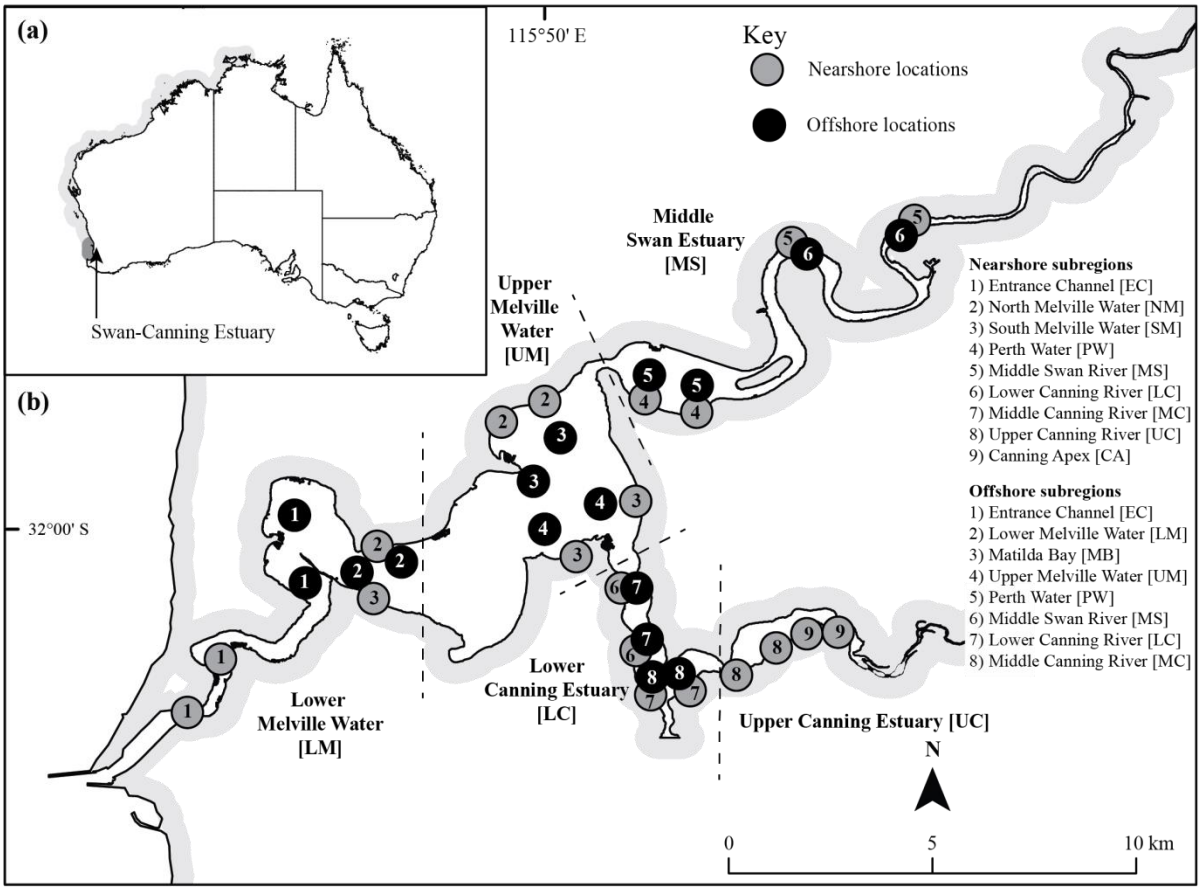


Fig. 1.1.1. Map showing (a) Australia and the distribution of *Metapenaeus dalli* in inshore marine waters (light grey) and solely in estuaries (dark grey) and (b) 20 nearshore and 16 offshore sites in Swan-Canning Estuary sampled over 26 consecutive lunar cycles between October 2013 and October 2015. Dotted lines denote the separation among the five broad regions (bold face) of the estuary. Codes for regions and subregions are given in square brackets.

Salinity, water temperature and dissolved oxygen concentration at the surface and bottom of the water column were recorded at each offshore site on each sampling occasion using a Yellow

Springs International Model 556 water quality meter. Data were also obtained from the Department of Water and Environmental Regulation, Western Australia, who records these variables at sites throughout the Swan-Canning Estuary every week (http://wir.water.wa.gov.au/Pages/Water-Information-Reporting.aspx).

A salinity stratification index was calculated by subtracting the salinity at the surface of the water column from that at the bottom (Jenkins *et al*., 2010). Monthly rainfall and average maximum air temperature data for Perth airport were obtained from the Bureau of Meteorology (http://www.bom.gov.au/climate/data/) between January 2013 and December 2015. The Department of Water and Environmental Regulation provided monthly discharge data from tributaries entering the Swan-Canning Estuary over the same period.

Statistical analyses

Univariate tests

The density of *M. dalli* recorded in each sample from sites in the nearshore and offshore waters, in each of the 13 periods in each year (2013/14 and 2014/15) were subjected to separate two-way Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson *et al*., 2008) tests, to determine whether density differed significantly among Periods (13 levels and fixed), Subregions (8 or 9 levels and fixed; Fig. 1.1.1) or the interaction between these main effects. Tests were conducted separately for data collected from the nearshore and offshore waters in each year. Year was not included as a factor due to the potentially confounding effect of the ongoing restocking program, which released 1,000, 600,000 and 2,000,000 post-larval *M. dalli* into the nearshore waters of the Swan-Canning Estuary during the 2012/13, 2013/14 and 2014/15 periods respectively (Jenkins *et al*., 2017). As the densities of male and female prawns were highly correlated (see results), they were combined to investigate the variation in total *M. dalli* density. When a main effect or interaction term was significant and contributed > 25 % to the mean squares, a pairwise PERMANOVA test was used to identify the pairwise combination of *a priori* groups responsible for that difference.

Spearman's rank correlation tests were employed to elucidate whether the density of *M. dalli* correlated with any of the environmental variables in the water column, (i) within a subregion over time, and (ii) within a period and across the subregions. These variables included water temperature, salinity and dissolved oxygen concentration at the surface of the water column and the density of *M. dalli* in nearshore waters, and water temperature, salinity and dissolved oxygen concentration at the surface and bottom of the water column and the stratification index, and the density of *M. dalli* in offshore waters. The null hypothesis of no significant relationship between two variables was rejected when $p = <0.05$, however, due to the limited number of subregions and thus replicates for correlations within a period, *p* values of 0.05-0.1 were also classed as being influential.

Differences in the mean CL of male and female *M. dalli* found in the nearshore and offshore waters of a region were tested using a non-parametric Kruskal-Wallis test in SPSS v22. Note that a region was only included in the analysis for a given period if a minimum of 30 individuals were caught. To provide a visual indication of the reason(s) for any pairwise differences in mean CL among regions in a period, the number of individuals in each 1 mm CL size class in each region/period combination were calculated. These data were standardized by the percentage contribution each size class made to the total number of *M. dalli* in each region in each period, and used to construct a shade plot, which is a simple visualisation of the data, where a white space for a size class demonstrates that no individuals were collected at that CL in a region/period combination. The depth of shading on the plot, from grey to black, is linearly proportional to the percentage contribution of that size class to the total catch in the region/period combination (Clarke *et al*., 2014b; Valesini *et al*., 2014).

Multivariate tests

The variation in composition of the fauna was investigated using multivariate analyses to determine whether the distribution and abundance of *M. dalli* were similar to that of potential competitors and predators. The densities of all species caught in the nearshore and offshore waters (*i.e. M. dalli* and other penaeid, stomatopod, brachyuran, teleost, elasmobranch and

scyphozoan species) were fourth-root transformed to balance the contributions of common and rare species, by down-weighting the contributions of taxa with high densities (Clarke and Warwick, 2001). The resultant data were used to construct separate Bray-Curtis resemblance matrices for the nearshore and offshore waters, each of which was subjected to a three-way PERMANOVA to determine whether species composition differed among Years (2 levels; fixed), Periods (26 levels; with 13 nested within each Year) and Subregions (8 or 9 levels; fixed) and whether there were any significant interactions between the main effects. As all main effects and the interaction terms differed significantly ($P = 0.001$) in both nearshore and offshore waters, the fourth-root transformed density of each species in each subregion/period combination was averaged.

These data were subjected to coherent species analysis (Somerfield and Clarke, 2013; Tweedley *et al*., 2015) to determine whether the pattern of change in the abundance of *M. dalli* spatially and temporally was statistically indistinguishable to any other species. Species occurring in less than 10 of the 1,040 (< 1%) and 832 (<1.25%) of the total number of samples from the nearshore and offshore waters, respectively, were excluded from this analysis as they add only random noise to the species similarities (Clarke and Warwick, 2001; Veale *et al*., 2014). As *M. dalli* were virtually absent from the nearshore waters between April and September, sampling periods falling within this period were removed from this analysis. The transformed and averaged species density data were used to construct Bray-Curtis resemblance matrices, which were, in turn, subjected to hierarchical agglomerative clustering with group-average linking (CLUSTER) and an associated Similarity Profiles (SIMPROF) test employing the type 3 SIMPROF permutation procedure (Somerfield and Clarke, 2013). Separate analyses were carried out for the nearshore and offshore waters.

Visualization of the 'coherent species groups' was achieved by plotting the transformed densities of each species in each subregion/period combination on a shade plot (Clarke *et al*., 2014b) and placed in optimum serial order using the Bray-Curtis resemblance matrix, constrained by the cluster dendrogram (Clarke *et al*., 2014a). Thus species (*y* axis) are ordered according to their abundance across subregions and periods, with those species with statistically

indistinguishable patterns of abundance grouped together. Subregion/period combinations (*x axis*) were ordered from left to right with increasing distance upstream in the estuary, and within each period in chronological order from October 2013 to October 2015.

Results

Climatic and physico-chemical conditions

Mean maximum air temperatures between January 2013 and December 2015 exhibited a sinusoidal trend, with the lowest values recorded in July of each year (~18°C) increasing sequentially to a peak the following February (~34°C; Fig. 1.1.2a). Total annual rainfall ranged from 704 mm in 2013 to 578 mm in 2015, with the majority of rain (72-86 %) falling between May and September (Fig. 1.1.2a). In contrast, very little rainfall occurred during the austral summer, *i.e.* December, January and February (*i.e.* 3 % over the three years). Annual flows from the Swan and Canning rivers were markedly greater in 2013 and 2014 (218 and 175 GL, respectively) than in 2015 (78 GL; Fig. 1.1.2b). The Swan River was responsible for the between 77 and 86 % of freshwater dischage in to the Swan-Canning Estuary, with the majority of the flow occurring between July and October (82-93 %; Fig. 1.1.2b). Flows were greatly reduced between December and April, typically < 2 GL/month (Fig. 1.1.2b).

As with air temperature, LFDA 5; Kirkwood *et al*., 2001the temperature of the water column in each region underwent a pronounced seasonal pattern. Temperatures of the surface waters typically ranged from ~15 °C in June/July to ~26 °C in Janurary/February (Fig. 1.1.3a). Seasonal differences were greatest in the Upper Canning Estuary and lowest in Upper and Lower Melville Water and the Lower Canning Estuary. Temperatures in surface waters were almost always > 20 °C between October and April and < 20 °C during May-September (Fig. 1.1.3a). Temporal patterns in bottom water temperature mirrored those in the surface waters, but showed less variation than those in the nearshore waters, *i.e.* temperatures were typically greater in the offshore than nearshore waters in the colder months between May and

September, and the converse applied in the warmer months between October and March (*cf* Fig. 1.1.3a,b).

Surface salinity ranged from 1 in the Upper Canning Estuary during October 2014 to 38.4 in that same region in March 2014 (Fig. 1.1.3c). With the exception of October 2013, salinities in Lower Melville Water were > 20, whereas in all other regions they declined to ≤ 10. Ranges in salinity varied markedly among the regions, from 16 in Lower Melville Water to 36 in the Upper Canning Estuary. Within a period, salinities were most similar across regions during summer (January-April), typically differing by < 5, but were as different as ~25 in May and June 2014. The lowest bottom salinity was 9.7 in the Middle Swan Estuary in October 2013, while the highest was 37.7 in the Lower Melville Water during March 2014 (Fig. 1.1.3d). Salinities in the bottom waters ranged far less than the corresponding surface waters, *e.g.* bottom salinities in Lower Melville Water differed by only 7 over the two years. The stratification index (bottom – surface salinity) exceeded 4 in most regions in October/November 2013, between May and October in 2014 and in August to October in 2015 (Fig. 1.1.3e). The water column was most stratified in the Lower Canning and Lower and Upper Melville Water and least stratified in the Middle Swan Estuary.

Dissolved oxygen concentrations in the surface waters in each region/period combination always exceeded 4 mg/L (Fig. 1.1.3f). Although values were lower in the bottom waters, they also typically exceeded 4 mg/L. However, hypoxic conditions (*i.e.* < 2 mg/L; Tweedley *et al*., 2016a) were recorded in the Lower Canning Estuary in October/November 2013 and August/September 2014, and in four periods between August 2014 and February 2015 in the Middle Swan Estuary (Fig. 1.1.3g).

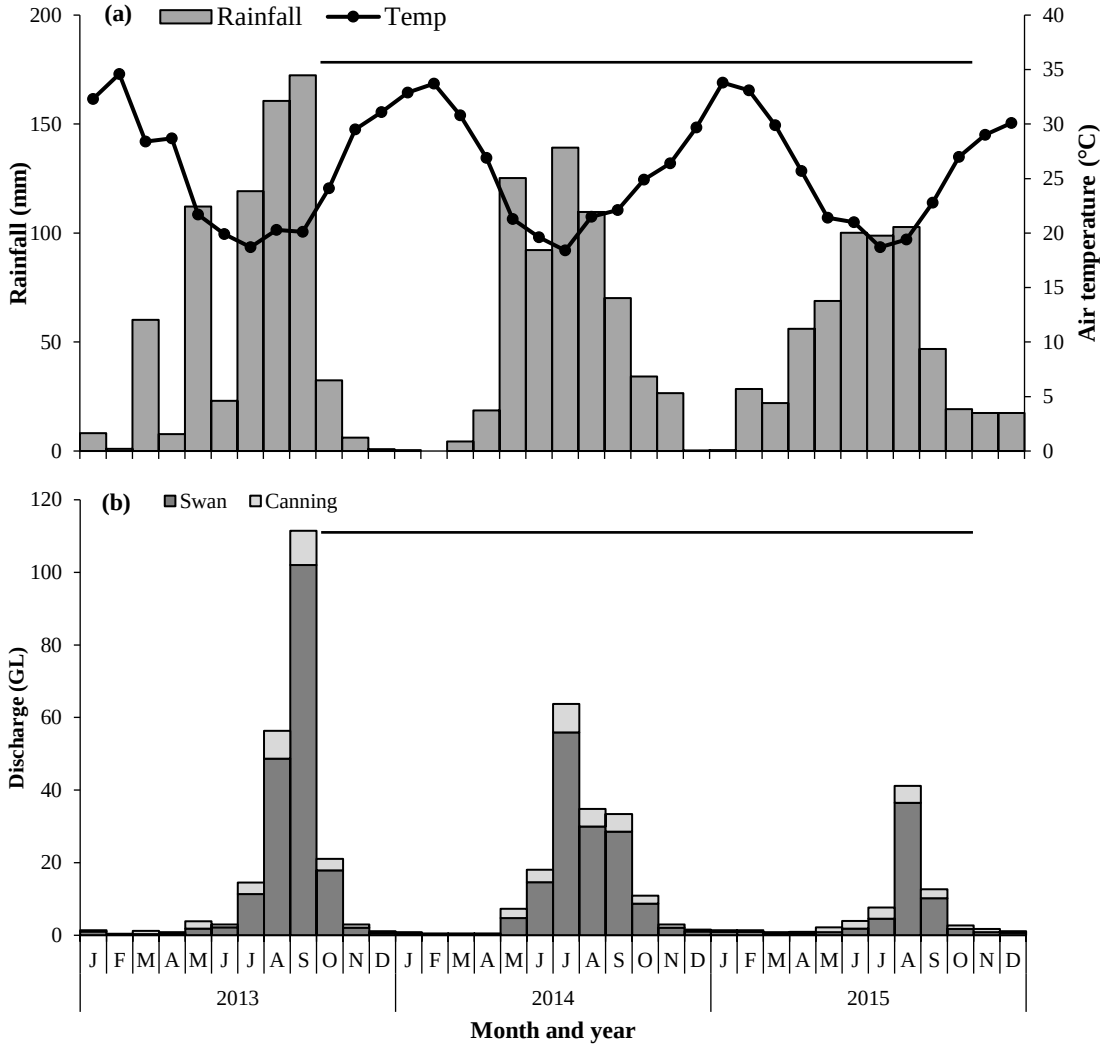


Fig. 1.1.2. Monthly (a) total rainfall (mm, histogram) and average maximum air temperature (°C, line) for Perth and (b) freshwater discharge volumes (GL) into the Swan-Canning Estuary data for the Swan and Canning rivers between January 2013 and December 2015. Climate and flow data obtained from the Bureau of Meteorology (http://www.bom.gov.au/climate/data/) and Department of Water and Environmental Regulation (http://wir.water.wa.gov.au/Pages/Water-Information-Reporting.aspx), respectively. Horizontal line denotes the months in which sampling for *Metapenaeus dalli* occurred (*i.e.* October 2013 to October 2015).

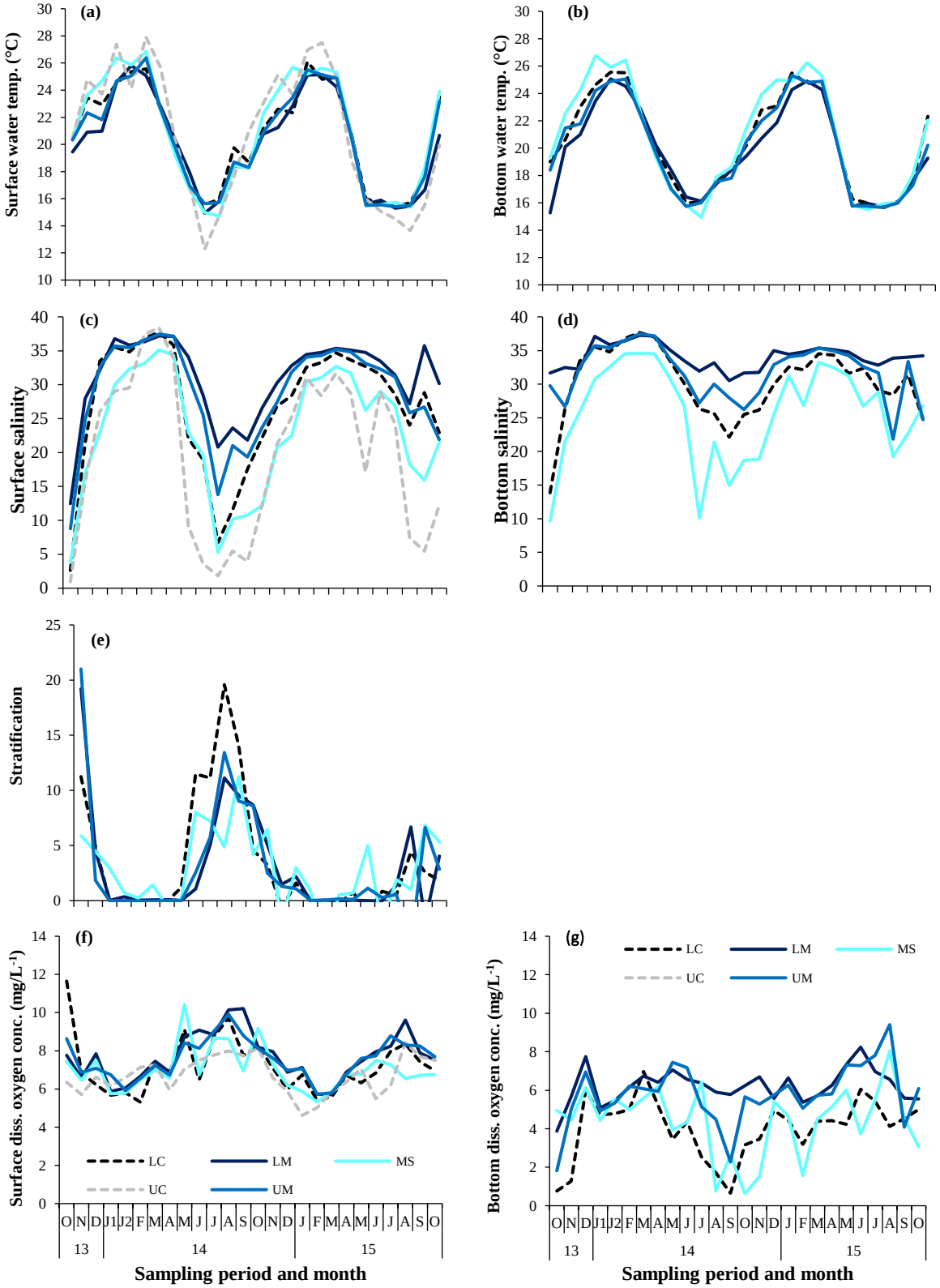


Fig. 1.1.3. Mean values for (a) surface and (b) bottom water temperature, (c) surface and (d) bottom salinity, (e) index of stratification and (f) surface and (g) bottom dissolved oxygen concentration recorded in each of the five regions of the Swan-Canning Estuary in each period between October 2013 and October 2015. Region codes given in Fig. 1.1.1. Note that due to the shallow water depth only surface values for were recorded in the Upper Canning Estuary region.

Density of Metapenaeus dalli

The mean density of male and female *M. dalli* (500 m^{-2}) in the nearshore waters of the Swan-Canning Estuary varied markedly among periods, being substantially greater between October and February (1-5) than other periods (< 1), and few prawns were caught between May and July (Fig. 1.1.4a). This marked seasonal pattern was also present in all four regions of the system. Densities during the October to February portion of the year varied among regions, being greatest in the Lower Canning Estuary, followed by Upper Melville Water and the Middle Swan Estuary (Fig. 1.1.4). Slightly larger densities were recorded during this time of year in 2014/15 than 2013/14. Substantial densities of *M. dalli* were caught in the nearshore waters over a longer period in 2014/15 than in the previous year, except in November/December (Fig. 1.1.4).

A less prominent seasonal pattern in the density of *M. dalli* was present in the offshore waters. Generally, densities (prawns 500 m^{-2}) increased from 0.6 in October 2013 to a peak of 27 in May 2014 before declining until March 2015, reaching a peak in May 2015 (42) and subsequently declining again until October 2015 (Fig. 1.1.4b). Similar seasonal patterns were present in the Middle Swan Estuary and Lower Canning Estuary and, to a lesser extent, in Upper Melville Water. The first two regions recorded by far the largest densities, with far fewer *M. dalli* recorded in Lower Melville Water (Fig. 1.1.4).

The mean densities of male and female *M. dalli* were very similar (Fig. 1.1.4), and were highly correlated in both the nearshore and offshore waters ($r = 0.943$, n = 130 $p = <0.001$; and $r = 0.919$, n = 104, $p = <0.001$, respectively). Thus in all subsequent results, the abundances of males and females were combined.

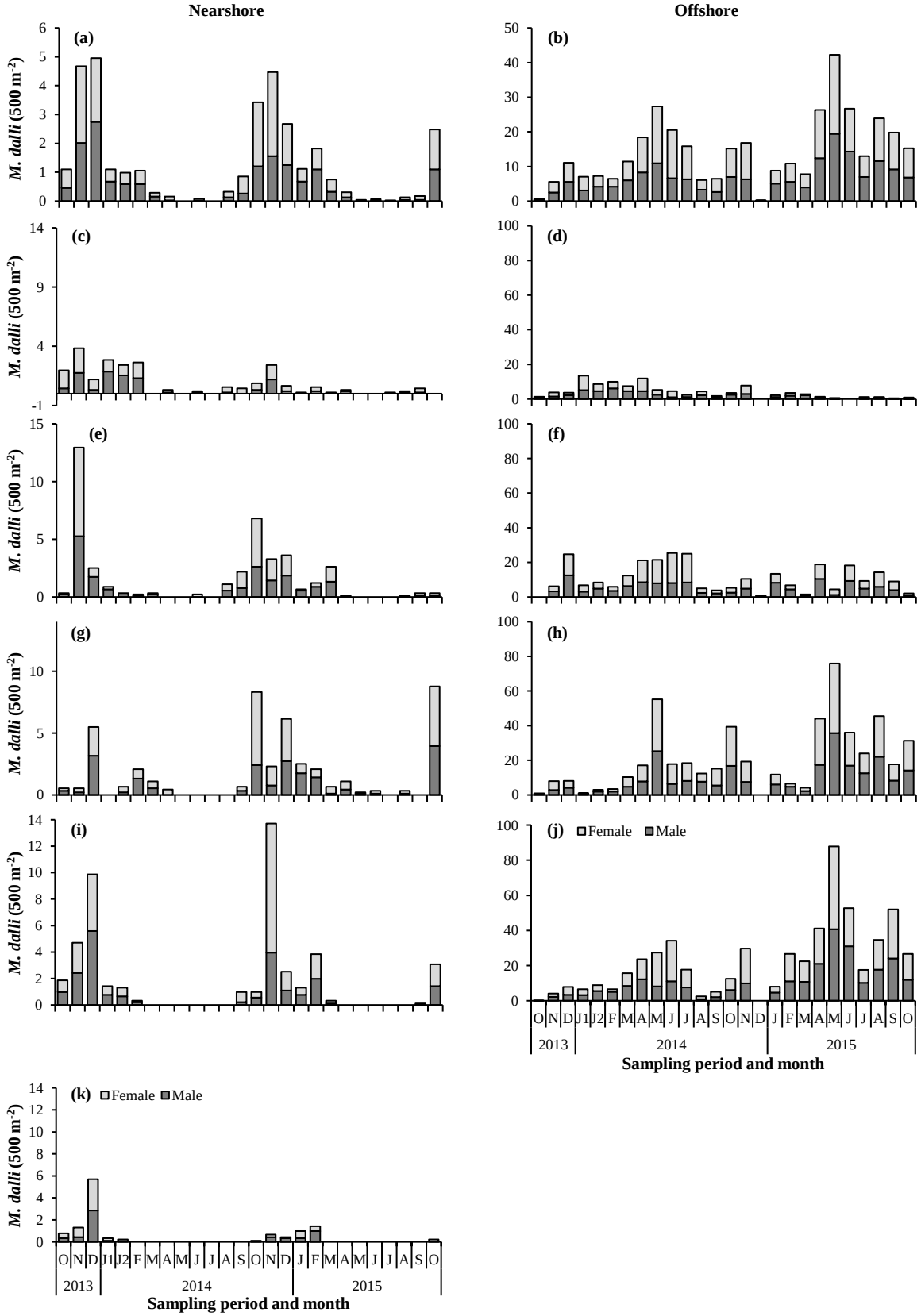


Fig. 1.1.4. Mean density (500 m^{-2}) of male and female *M. dalli* in the (a, c, e, g, i, k) nearshore and (b, d, f, h, j) offshore waters of the Swan-Canning Estuary each period between October 2013 and October 2015 for the system as a whole and for each region separately. (a, b) total estuary, (c, d) Lower and (e, f) Upper Melville Water, (g, h) Middle Swan Estuary and (i, j) Lower and (k) Upper Canning Estuary. Note no offshore data for Upper Canning Estuary, due to shallow depths and a limited data in December 2014 due to engine failure.

Spatial and temporal abundance of* Metapenaeus dalli *and relationship to environmental conditions

Two-way PERMANOVA of the 2013/14 data detected a significant difference in the densities of *Metapenaeus dalli* among Periods and Subregions in the nearshore waters of the Swan-Canning Estuary in 2013/14, but the Period × Subregion interaction was not significant (Table 1.1.1a). The majority of the variation in density was explained by Period (61 %), with densities being significantly greater in December 2014 (~5 *M. dalli* 500 m^{-2}, Fig. 1.1.5a), and, to a lesser extent, October and November of the same year, than in the Periods between March and August 2015 (< 0.25 *M. dalli* 500 m^{-2}; Annex 1.1.1a). Densities in January and February 2015 were also typically greater than those recorded in May-July (Fig. 1.1.5a). Among Subregions (18 % of variation in density), the greatest densities were recorded in North Melville and Perth Water and the Lower and Middle Canning Estuary and least in the Entrance Channel (Fig. 1.1.5b).

Densities of *M. dalli* in 2014/15 differed significantly among Periods, Subregions and the Period × Subregion interaction, with the two main effects explaining the majority of the variation in the mean squares for density (Table 1.1.1b). Significantly greater densities were caught between October 2014 and February 2015 (*i.e.* austral spring and summer) and October 2015 than the periods between March and September 2015 (Annex 1.1.1b; Fig. 1.1.5c). The subregions with the highest mean densities of prawns were those in the middle of the Swan-Canning Estuary, *i.e.* Perth Water and the Lower Canning Estuary and, to a lesser extent, North and South Melville Water, and the Middle Swan and Canning rivers (Fig. 1.1.5d).

In the offshore waters, two-way PERMANOVA detected a significant difference in the densities of *M. dalli* among Periods, Subregions and their interaction term in both 2013/14 and 2014/15 (Table 1.1.1c,d; Annex 1.1.2). In contrast to the nearshore waters, densities in 2013/14 were lowest during the austral spring and summer, *i.e.* September to February (< 10 *M. dalli* 500 m^{-2}) and significantly greater between March and July, with the largest values recorded in May, (~27 *M. dalli* 500 m^{-2}, Fig. 1.1.5e). This seasonal trend was also present in 2014/15, albeit less marked, which is reflected in the reduction of the proportion of the variance explained by

Period (21 %) compared with 2013/2014 (53 %; Table 1.1.1c,d). In both years, mean densities of *M. dalli* typically increased sequentially in an upstream direction, with the lowest values recorded in the Entrance Channel and highest in the Lower Canning Estuary and Middle Swan Estuary (Fig. 1.1.5f, h). Differences among subregions were more pronounced in 2014/15 than 2013/14.

Table 1.1.1. Mean squares (MS), percentage mean squares (%MS), *pseudo-f* (*pf*) and significance value (*P*) from two-way PERMANOVA tests on the density of *Metapenaeus dalli* per 500 m^2 among periods and subregions in the Swan-Canning Estuary between October 2013 and 2015. Significant differences ($P = < 0.05$) highlighted in bold. Grey shading denotes factors that were particularly influential (*i.e.* %MS > 25).

	df	MS	%MS	*pf*	*P*
Nearshore					
(a) 2013/14					
Period	12	**101.21**	**61.11**	**6.652**	**0.001**
Subregion	8	**29.93**	**18.07**	**1.97**	**0.035**
Period × Subregion	96	19.27	11.64	1.27	0.085
Residual	403	15.22			
(b) 2014/15					
Period	12	**88.72**	**36.14**	**3.79**	**0.001**
Subregion	8	**89.22**	**36.35**	**3.81**	**0.001**
Period × Subregion	96	**44.12**	**17.97**	**1.89**	**0.002**
Residual	403	23.40			
Offshore					
(c) 2013/14					
Period	12	**33.56**	**52.80**	**9.58**	**0.001**
Subregion	7	**19.88**	**31.28**	**5.68**	**0.001**
Period × Subregion	84	**6.616**	**10.41**	**1.89**	**0.001**
Residual	312	3.502			
(d) 2014/15					
Period	12	**41.92**	**20.75**	**13.28**	**0.001**
Subregion	7	**145.87**	**72.21**	**46.22**	**0.001**
Period × Subregion	84	**11.06**	**5.47**	**3.50**	**0.001**
Residual	312	3.16			

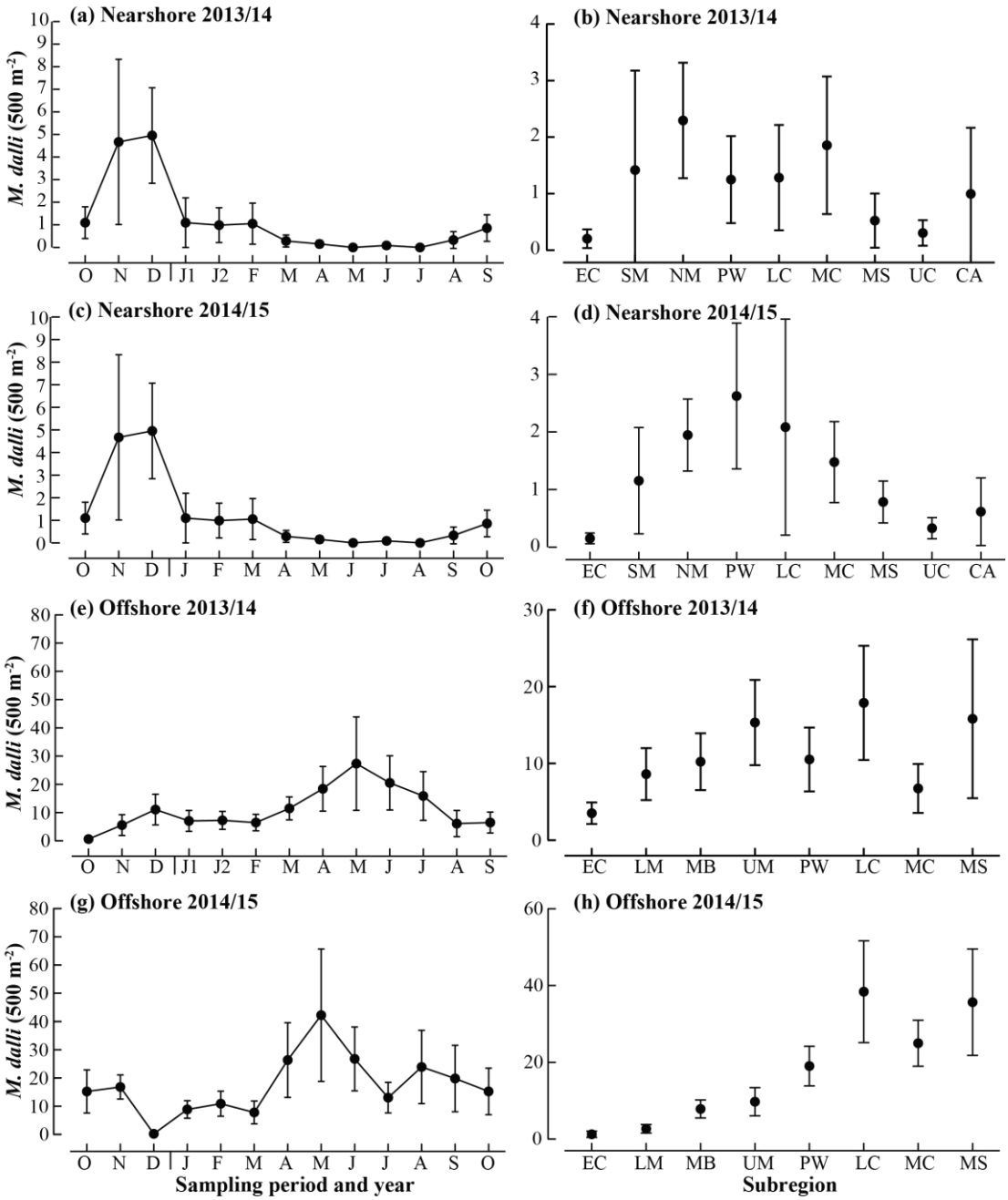


Fig. 1.1.5. Mean and 95% confidence limits of the density of *Metapeanus dalli* among (a, c) periods and (b, d) subregions in the nearshore waters of the Swan-Canning Estuary 2013/14 and 2014/15, respectively and in (e, g) periods and (f, h) subregions in the offshore waters of the Swan-Canning Estuary 2013/14 and 2014/15, respectively. Subregion codes given in Fig. 1.1.1.

Spearman's rank correlations demonstrated that the density of *M. dalli* in seven of the nine subregions in nearshore waters was positively correlated with surface water temperature ($\rho = 0.45 – 0.67$; Table 1.1.2a). Significant and negative correlations were also detected for surface dissolved oxygen concentrations in four regions, while asingle positive and negative correlations were detected in the Middle Swan and North Melville Water subregions respectively (Table 1.1.2a). Trends in the correlation between the density of *M. dalli* and environmental variables were less clear among periods. Density was negatively related to surface water temperature and salinity during February and March 2014, and for salinity, also in February 2015 (Table 1.1.2b). Density was positively correlated to either one or both of these physical-chemical variables in November and December 2014, January 2015, and also some periods between April and September of both years.

Surface and bottom water temperatures were negatively correlated to the density of *M. dalli* in five of the eight offshore subregions ($\rho = -0.28 – -0.61$ and $-0.35 – -0.64$, respectively), and positively correlated in Lower Melville Water ($\rho = 0.50$ and 0.59, respectively; Table 1.1.2c). Surface salinity was positively correlated to density in Lower Melville Water, but both surface and bottom salinity exhibited the reverse trend with density in Perth Water. As with the nearshore waters, the patterns of correlations among periods were less clear than those among subregions. However, surface and bottom salinities, and to a lesser extent, surface and bottom dissolved oxygen concentration, were negatively correlated with density in most periods between October 2014 and October 2015 (Table 1.1.2d).

Table 1.1.2. Rho values from Spearman ranked correlations between the density of *Metapenaeus dalli* (500 m^{-2}) and various water physicochemical variables among (a, c) regions and (b, d) periods in the nearshore and offshore waters of the Swan-Canning Estuary, respectively. Significant differences (p = < 0.05) highlighted in dark grey and those differences where p = < 0.10 in light grey. Subregion codes given in Fig. 1.1.1. S, surface; B, bottom; Temp, water temperature; DO, dissolved oxygen concentration; Sal, salinity.

(a) Nearshore

	EC	NM	SM	PW	MS	LC	MC	UC	CA
S. Temp.	0.03	0.64	0.18	0.66	0.55	0.53	0.67	0.45	0.56
S. Sal.	-0.13	-0.02	-0.33	0.11	0.40	-0.06	0.12	0.04	0.26
S. DO	-0.08	-0.38	0.03	-0.34	-0.21	-0.30	-0.56	-0.51	-0.11

(b) Nearshore

	2013			2014													2015									
	O	**N**	**D**	**J1**	**J2**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**
S. Temp.	0.03	-0.23	0.45	-0.13	0.15	-0.56	-0.67	-0.44	No catch	-0.14	No catch	-0.02	-0.56	-0.10	-0.02	0.55	0.75	-0.08	0.20	0.53	-0.28	0.14	0.55	-0.18	0.02	0.56
S. Sal.	-0.14	0.45	-0.35	0.09	-0.11	-0.70	-0.69	0.06		0.14		0.53	0.65	0.13	0.53	-0.13	-0.35	-0.54	0.26	-0.01	-0.14	-0.41	0.55	0.13	0.51	-0.09
S. DO	0.66	0.39	0.10	-0.51	-0.33	-0.56	0.37	0.09		0.55		0.64	0.45	0.13	0.64	0.08	0.13	-0.54	-0.15	-0.15	0.14	0.00	0.00	-0.28	0.83	0.03

(c) Offshore

	EC	LM	MB	UM	PW	MS	LC	MC
S. Temp.	0.23	0.50	-0.39	-0.28	-0.61	-0.59	-0.52	-0.13
S. Sal.	-0.10	0.47	-0.07	0.23	-0.46	0.10	0.08	0.26
S. DO	0.02	-0.35	0.19	-0.08	0.55	0.38	0.07	-0.15
B. Temp.	0.26	0.59	-0.35	-0.25	-0.64	-0.59	-0.47	-0.13
B. Sal.	-0.13	0.27	-0.18	0.27	-0.45	0.31	0.20	0.22
B. DO	0.00	-0.13	0.35	0.20	-0.19	-0.13	0.31	0.03
Strat.	0.10	-0.28	-0.03	-0.27	0.28	0.28	-0.07	-0.38

(d) Offshore

	2013			2014													2015									
	O	**N**	**D**	**J1**	**J2**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**
S. Temp.	-0.69	-0.05	-0.14	-0.45	-0.21	-0.42	0.12	0.00	-0.64	0.35	-0.31	0.31	0.02	0.74	0.69		0.48	-0.90	0.37	0.38	0.20	-0.35	0.31	0.22	0.30	0.47
S. Sal.	0.34	0.05	0.95	0.65	0.07	0.06	0.14	0.14	-0.81	-0.61	-0.12	0.21	-0.69	-0.93	-0.69		-0.60	-0.71	-0.54	-0.86	-0.60	-0.73	-0.88	-0.71	-0.59	-0.26
S. DO	0.07	0.00	-0.18	0.16	0.07	0.47	-0.29	0.36	0.52	-0.35	0.60	-0.05	-0.38	0.07	-0.69		-0.14	0.00	-0.06	0.19	-0.72	-0.91	-0.59	-0.64	-0.19	-0.78
B. Temp.	-0.49	0.33	-0.14	-0.60	-0.29	-0.47	0.29	-0.02	-0.55	-0.92	-0.32	0.45	-0.08	0.52	0.71		0.76	-0.57	0.24	0.36	0.41	-0.16	0.50	0.60	0.84	0.55
B. Sal.	0.34	-0.10	0.10	0.65	0.24	0.06	0.26	0.19	-0.71	-0.42	-0.12	-0.10	-0.57	-0.76	-0.69		-0.62	-0.52	-0.71	-0.81	-0.69	-0.61	-0.91	-0.45	-0.70	-0.22
B. DO	0.36	0.10	0.19	0.93	-0.57	0.20	-0.21	-0.49	-0.45	-0.06	-0.12	0.07	-0.38	-0.95	-0.48		-0.19	-0.67	-0.73	-0.69	-0.74	-0.76	-0.74	-0.62	-0.05	-0.78
Strat.	0.13	-0.76	-0.07	-0.48	-0.28	-0.11	-0.26	0.17	-0.91	0.28	0.00	-0.60	-0.52	0.10	-0.86		0.19	0.19	-0.41	0.76	0.07	0.71	-0.10	0.02	0.16	0.26

Limited data

Spatial and temporal variation in size of Metapenaeus dalli

The mean CL of individual *M. dalli* in both nearshore and offshore waters combined increased progressively between October 2013 (~13) and February 2014 (~20), before declining markedly the following month to ~12 (Fig. 1.1.6). There was little change in mean CL between March and August 2014, after which CL rapidly rose to ~19 in November 2014 before declining to ~11 in April 2015 and staying relatively consistent until October. The results of Kruskal-Wallis tests indicated that mean CL differed significantly among regions in 19 out of the 24 periods (Table 1.1.3; note this test was not done in October 2013 and December 2014, due to small sample sizes of *M. dalli*). Over the two years, a relatively consistent pattern in mean CL was present, with little difference among regions, due to a similar range of individuals occurring in each region (Figs 1.1.6, 1.1.7) during the period of rapid growth, *i.e.* December-January in 2013/14 and November 2014. Following the decline in mean CL in ~March, however, mean CL was typically lower in the Middle Swan Estuary and Upper Melville Water, due to larger proportions of *M. dalli* ~10 mm CL and fewer ~18 mm CL (Fig. 1.1.6, 1.1.7). In 2013/14, the decline in mean CL in all regions occurred during the same period (March 2014), whereas in 2014/15, mean CL decline was sequential among regions, happening first in the Lower Canning Estuary in January, followed by the Middle Swan Estuary and Upper Melville Water in February, and finally Lower Melville Water in March/April. Although mean CL declined first in the Lower Canning Estuary, it remained fairly consistent (~13 mm) between March and September and was significantly greater than that recorded in both the Middle Swan Estuary and Upper Melville Water during that period (~11 mm; Fig. 1.1.6). During these months, the Lower Canning Estuary contained a greater proportion of *M. dalli* > 17 mm CL and far fewer < 10 mm CL compared to the other regions (Fig. 1.1.7).

Table 1.1.1. Overall and pairwise *p*-values derived from Kruskal-Wallis tests on the carapace length of *Metapenaeus dalli* in four regions of the Swan-Canning Estuary in each period between October 2013 and October 2015. Significant differences are shaded in light grey. NT = no test completed due to < 30 individuals being caught in a region in a period (year/month combination). Region codes given in Fig. 1.1.1.

Period		Overall	Pairwise					
Year	Month		*LC-UM*	*LC-MS*	*LC-LM*	*UM-MS*	*UM-LM*	*MS-LM*
2013	O	NT	NT	NT	NT	NT	NT	NT
	N	0.001	1.000	0.021	0.002	0.172	0.019	1.000
	D	0.270						
2014	J1	0.406						
	J2	0.095						
	F	0.004	1.000	1.000	1.000	0.854	0.002	1.000
	M	0.001	0.124	0.062	1.000	1.000	0.017	0.009
	A	<0.001	<0.001	<0.001	0.008	0.001	1.000	0.001
	M	<0.001	<0.001	<0.001	0.017	1.000	0.064	0.131
	J	0.003	0.504	0.002	0.360	0.130	1.000	1.000
	J	<0.001	<0.001	<0.001	NT	0.028	NT	NT
	A	0.008	NT	NT	NT	0.045	0.011	1.000
	S	0.215						
	O	<0.001	0.297	0.070	0.010	1.000	<0.001	<0.001
	N	0.480	NT	1.000	0.061	NT	NT	0.088
2015	J	<0.001	<0.001	0.004	NT	0.027	NT	NT
	F	<0.001	0.002	0.001	<0.001	1.000	0.002	0.014
	M	<0.001	<0.001	NT	<0.001	NT	<0.001	NT
	A	<0.001	<0.001	<0.001	NT	<0.001	NT	NT
	M	<0.001	0.002	<0.001	NT	0.036	NT	NT
	J	<0.001	<0.001	<0.001	NT	0.032	NT	NT
	J	<0.001	0.018	<0.001	NT	0.117	NT	NT
	A	<0.001	0.006	<0.001	NT	0.001	NT	NT
	S	<0.001	<0.001	<0.001	NT	0.565	NT	NT
	O	<0.001	NT	<0.001	NT	NT	NT	NT

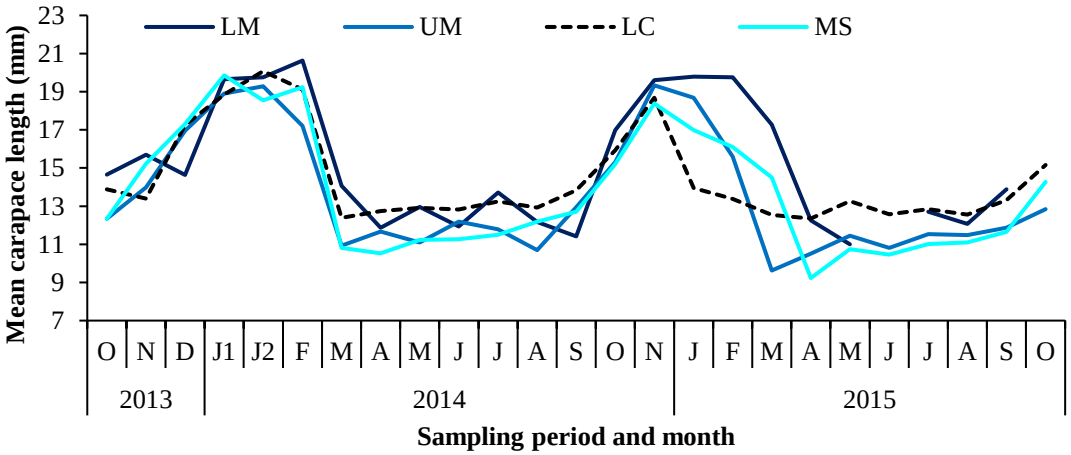


Fig. 1.1.6. Mean carapace length of *M. dalli* caught in the nearshore and offshore waters of each region of the Swan-Canning Estuary in each period between October 2013 and October 2015. Data for December 2014 not shown due to lack of offshore sampling. Region codes given in Fig. 1.1.1.

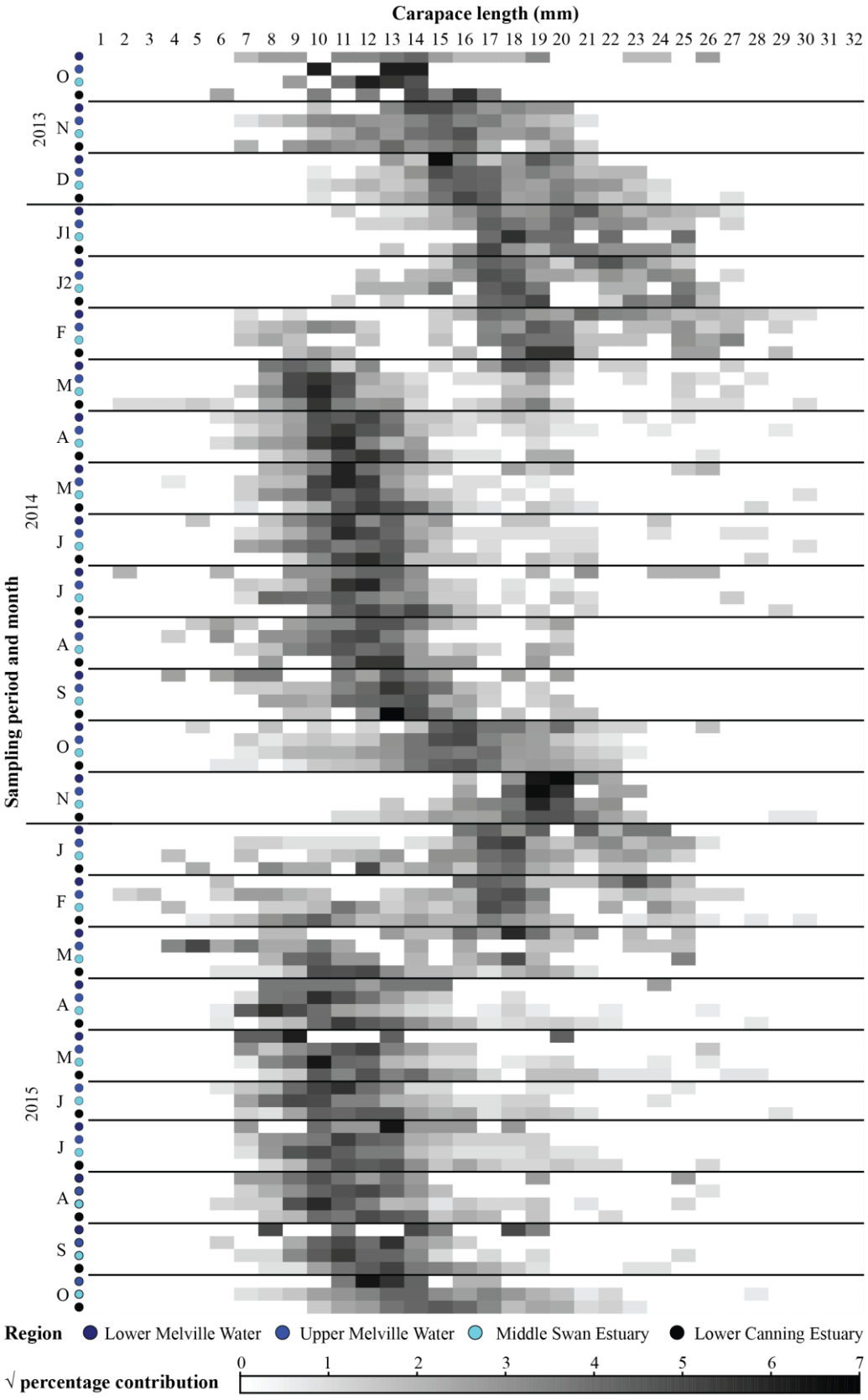


Fig. 1.1.7. Shade plot showing the square-root transformed proportion of *M. dalli* in each 1 mm carapace length size class in each region of the Swan-Canning Estuary in each period between October 2013 and October 2015. White areas denote the absence of a size class from a region/period combination and the shading from grey to black the increasing proportions of that size class. Region codes given in Fig. 1.1.1.

Coherent species groups

Coherent species analysis of the nearshore data found that the 24 species caught in at least 10 samples constituted eight groups and six outliers containing single species (Fig. 1.1.8). A clear serial pattern of progression in species composition was present with species segregating themselves along the linear axis of the estuary during the summer months. *Metapenaeus dalli* had a statistically similar spatial and temporal pattern of distribution and abundance to the apogonid *Ostorhinchus rueppellii*, with these two species consistently occurring in moderate densities in most subregions, except the Entrance Channel and South Melville Water (Fig. 1.1.8). The next most similar species to *M. dalli* and *O. rueppellii* was the atherinid *Atherinomorus vaigiensis, which covered the same spatial extent of the estuary, but was caught less consistently. The scyphozoans, Aurelia aurita and Phyllorhiza punctata, also overlapped in distribution with M. dalli, in the subregions upstream of Melville Water, during some periods. Other larger crustaceans, i.e. the penaeid P. latisulcatus and brachyuran Portunus armatus, were restricted to the most downstream subregions, mainly the Entrance Channel* (Fig. 1.1.8) and showed little overlap with the distribution of *M. dalli.*

In the offshore waters, the 27 species found in 10 or more samples, formed eight groups and four outliers (Fig. 1.1.9). As in the nearshore waters, the distribution of species occurred along a continuum from downstream to upstream, with some species occurring in a limited suite of subregions, and others such as *M. dalli* occurring throughout the entire area sampled. The spatial pattern of distribution and abundance of *M. dalli* was statistically indistinguishable from that of *O. rueppellii* and also similar (albeit not significantly) to *P. punctata* and *A. aurita.* The lack of a significant match in patterns between these two scypohozans and *M. dalli* in offshore waters was likely due to their occurrence in particular periods only (Fig. 1.1.9). Although the distribution of *P. latisulcatus and P. armatus extended further upstream than M. dalli in the offshore waters, they had a far more restricted spatial range.*

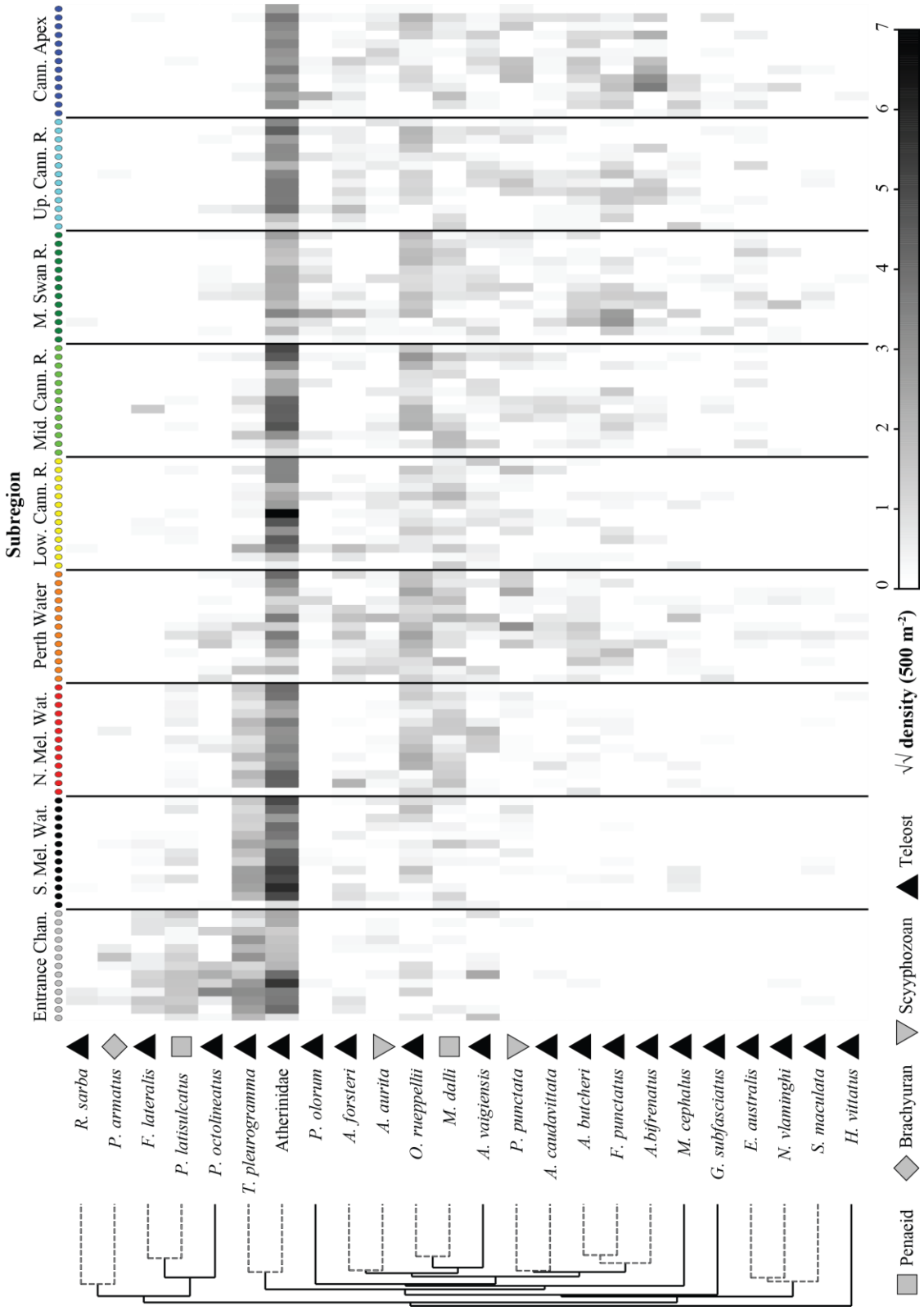


Fig. 1.1.8. Shade plot showing the fourth-root transformed density (500 m^{-2}) of each penaeid, brachyuran, teleost and scyphozoan species found in each subregion and each period between October 2013 and October 2015 in the nearshore waters of the Swan-Canning Estuary. Dendrogram on *y* axis derived by subjecting to CLUSTER-SIMPROF Bray-Curtis resemblance matrix of the fourth-root transformed density of each species. Coherent groups of species are denoted by the dashed grey lines, *i.e.* have statistically indistinguishable patterns of abundance across the subregion/period combinations and are significantly different from those in all other groups.

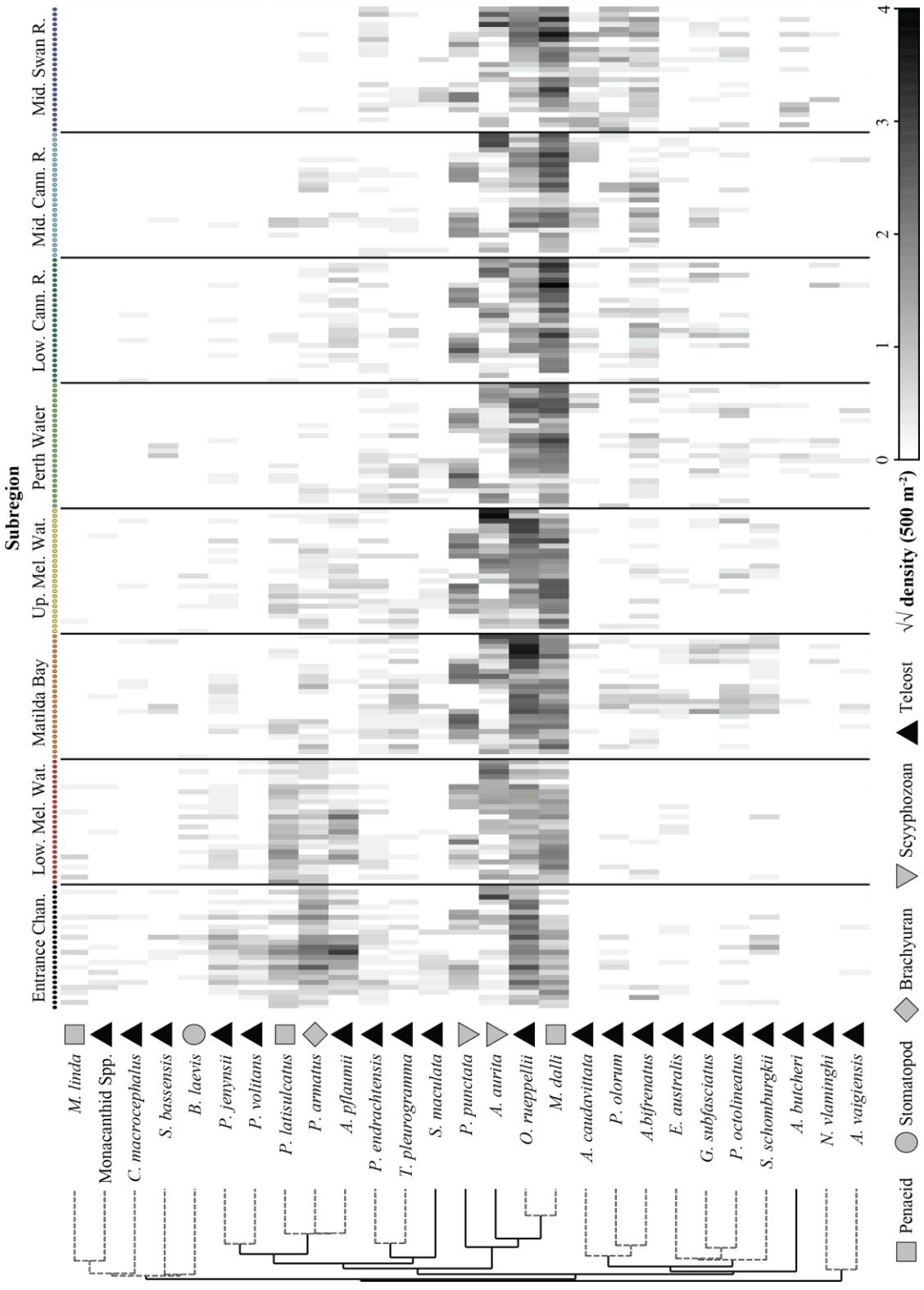


Fig. 1.1.9. Shade plot showing the fourth-root transformed density (500 m^{-2}) of each penaeid, stomatopod, brachyuran, teleost and scyphozoan species found in each subregion and each period between October 2013 and October 2015 in the offshore waters of the Swan-Canning Estuary. Dendrogram on *y* axis derived by subjecting to CLUSTER-SIMPROF Bray-Curtis resemblance matrix of the fourth-root transformed density of each species. Coherent groups of species are denoted by the dashed grey lines, *i.e.* have statistically indistinguishable patterns of abundance across the subregion/period combinations and are significantly different from those in all other groups.

Discussion

Extensive sampling of the population of *Metapenaeus dalli* population in the nearshore and offshore waters of the temperate Swan-Canning Estuary identified the major sources of variation in the population, both seasonally and among regions of the estuary. Contrasting patterns of seasonal abundance were found in the nearshore and offshore waters, and the distribution of *M. dalli* was compared with those of potential competitors and predators to identify suites of co-occurring species.

Spatial and temporal patterns of abundance and distribution of Metapenaeus dalli

Densities of *M. dalli* in the nearshore waters of the Swan-Canning Estuary changed markedly seasonally, with the greatest values recorded in the late austral spring and summer months (October to February) and very few to no individuals recorded between late autumn and winter (May to July). Spearman's rank correlations showed a positive relationship between density and surface water temperature in the nearshore waters of all nine subregions, and were significant in seven of the nine. This reflects the migration of adult prawns into the nearshore waters for breeding during the summer, when water temperatures exceed ~20 °C (Broadley *et al*., 2017). Densities during the October to February period varied between regions, with highest densities recorded in the Lower Canning Estuary, followed by Upper Melville Water and Middle Swan Estuary regions. This pattern of abundance was rarely significantly related to either water temperature or salinity suggesting that the selection of these nearshore regions of the estuary for spawning may be due to factors other than water quality. This may reflect the fact that, in microtidal estuaries in Mediterranean climates, such as the Swan-Canning, environmental conditions in the water column are relatively stable throughout estuaries in the summer and autumn months (Chuwen *et al*., 2009) and thus the estuary provides a conducive environment for the spawning, retention and survival of eggs and larvae (Potter *et al*., 2015b; Tweedley *et al*., 2016b). The lack of correlation between density of *M. dalli* and any of the measured abiotic variables in the Entrance Channel was due to either the absence of *M. dalli* or very low densities of this species being recorded in each lunar month.

In contrast, to the nearshore waters, densities of *M. dalli* in the offshore waters were greatest from autumn to winter (*i.e.* March to July, peaking in May) and generally lower throughout the rest of the year. This is due to (i) the recruitment 0+ individuals that were spawned in Oct-March and which have grown to reach a size where they are able to be caught in the otter trawl (Broadley *et al*., 2017), and (ii) the movement of 1+ individuals from the nearshore waters, back into the offshore areas. This hypothesis is supported by the CL distributions for *M. dalli* (Fig. 1.1.7), which show that over the winter months, the larger individuals (> 18 mm CL), close to the size at maturity (~19 mm CL) are recorded in offshore waters and are virtually absent from the nearshore waters (Fig. 1.1.4a,b). This onshore/offshore migratory pattern was also recorded by Potter *et al*. (1986) in the Swan-Canning Estuary 30 years prior to our study. Moreover, this mirrors the movements of *M. endeavouri* and *M. ensis* in Albatross Bay, Gulf of Carpentaria, Australia, with mature females moving to depths > 40 m in May and July, respectively, and returning to shallower waters (< 35 m) during their spawning season, *i.e.* August to October for *M. endeavouri,* and September to December for *M. ensis (Crocos et al., 2001).* Offshore densities of *M. dalli* were negatively correlated with water temperature in most of the estuary (Table 1.1.2c), which is due to the densities being greatest in the months following recruitment (May-August), when water temperatures are coolest. Among regions, densities of *M. dalli* in the offshore waters were greatest upstream, in the Middle Swan Estuary and Lower Canning Estuary, that were, in general, less saline than the other regions. This could be due to spatial partitioning of the system, with the larger *P. latisulcatus* preferring marine salinities and thus occurring in the regions further downstream (*i.e.* the Entrance Channel).

Although dissolved oxygen concentrations were correlated with the abundance of *M. dalli,* these are not likely to have influenced the distribution of *M. dalli* as conditions in the system were usually normoxic, *i.e.* dissolved oxygen > 4 mgL^{-1}. For example, of the 416 spot measurements at the bottom of the water column, hypoxia (*i.e.* < 2 mgL^{-1}) and anoxia (*i.e.* < 0.5 mgL^{-1}) were detected only 47 and 21 times, respectively. Typically, hypoxic conditions occurred in the Middle Swan Estuary and Lower Canning Estuary regions (Fig. 1.1.3g), and during these times, densities of *M. dalli* in the offshore waters were reduced or zero (B. Poh, Murdoch University, unpublished data). In contrast, larger than 'normal'

densities of *M. dalli* were recorded in the corresponding nearshore waters at these times of hypoxia, where the lowest dissolved oxygen concentration recorded in 520 spot measurements was 4.6 mgL^{-1}, and thus these waters were always normoxic (J. Tweedley, Murdoch University, unpublished data). This suggests that an onshore movement of *M. dalli* occurs as a mechanism to avoid of areas of low dissolved oxygen concentrations. A laboratory study on *Metapenaeus ensis* found that this species was able to detect and avoid areas of hypoxia (Wu *et al*., 2002).

Spatial and temporal patterns of Metapenaeus dalli *size*

Mean carapace lengths differed throughout the year, reaching a maximum between January and February in 2013/14, and between November and February in 2014/15, indicative of the growth of individuals spawned the previous breeding season once water temperatures increase (Broadley *et al*., 2017). The appearance of larger *M. dalli* earlier and for longer during the breeding season in 2014/15 than 2013/14 corresponded with much lower freshwater discharge in this year than the previous year and higher average temperatures in August and September of 2014 than in 2013 (Fig. 1.1.2). This earlier warm weather provides conditions conducive for faster growth (as seen in Fig. 1.1.5) and *M. dalli* reach the size-at-maturity (19 mm CL) sooner in 2014/15 than 2013/14 (Broadley *et al*., 2017).

By April in both years, mean carapace lengths had declined greatly, largely due to the recruitment of the new cohort that were spawned early in the season (~9 mm – 13 mm CL), as well as the loss of the 1+ year males (~17 mm – 20 mm) and females (~24 mm – 30 mm; Fig. 1.1.7) through natural mortality post spawning and, to a far lesser extent, fishing pressure (Broadley *et al*., 2017). In 2013/14, the mean carapace lengths rapidly declined from February to March. In 2014/15, mean CLs declined initially in the Lower Canning, but the overall decline was not as great in the other regions. This reflects the earliest recruitment occurring in this region, and mean CLs remain slightly higher as the early recruits make use of the remaining warm weather to grow in size. In contrast, the Middle Swan Estuary region experienced slower decline in mean CLs, and reach a minimum much later than all other regions. This reflects the delayed breeding occurring in this region, as is shown by the delay in recruitment compared to

the Lower Canning Estuary. Carapace lengths changed little throughout the winter months indicating the minimal growth over this period. Growth resumes in August –September as water temperatures rise (see also Broadley *et al*., 2017). Female *M. dalli* were also shown to grow larger than males; observed in the 1+ cohort. This difference was first recorded by Potter *et al*. (1989) in the Peel Harvey population of *M dalli*, and more recently, by Broadley *et al*. (2017) in the Swan-Canning population, and is a common trait amongst other penaeids (Chu *et al*., 1995; Cha *et al*., 2002; 2004; De Croos *et al*., 2011).

Coherent species analyses

The spatial and temporal pattern of *M. dalli* in the nearshore waters of the Swan-Canning Estuary was statistically indistinguishable from the apogonid *Ostorhinchus rueppellii* and also similar to that of the atherinid *Atherinomorus vaigiensis* and the *scyphozoan Aurelia aurita*. Typically, the individuals of *M. dalli* present in the nearshore waters during this time are sexually mature adults (Potter *et al*., 1986b; Broadley *et al*., 2017) and, due to their relatively large size and tail-flip response (Arnott *et al*., 1998; Guerin and Neil, 2015), are likely to be able to escape predation from *O. rueppellii* and *A. vaigensis* and to avoid the nematocysts of *A. aurita*. However, larvae and postlarvae of *M. dalli* would be extremely susceptible to predation by these species. In particular, *O. rueppellii* are voracious predators of post-larval *M. dalli* (~3 mm total length), with the stomach of one *O. rueppellii* < 50 mm in total length containing 300 post-larval *M. dalli* two hours after the release of 130,000 postlarvae (Poh *et al*., 2018). The total predation by *O. rueppellii* on postlarval *M. dalli* 2 h after this release over a nearshore seagrass meadow at night was estimated to be ≈ 2,000 postlarvae per 100 m^2 (Poh *et al*., 2018).

Although *O. rueppellii* has been identified as the main predator of released *M. dalli* postlarvae, responsible for 68 % of the predation on hatchery-reared post-larval *M. dalli*, the vast majority of the remaining predation (31 %) was attributed to *A. vaigiensis*. Thus, the fact that the distribution and abundance of the two teleost species overlaps with that of *M. dalli*, means that

the postlarvae and small juveniles do not have a spatial or temporal refuge from the two main teleost predators.

The similar pattern of abundance and distribution of *M. dalli* and the *scyphozoans A. aurita* and *P. punctata* in the nearshore waters during the summer period is likely to have a negative effect on the larval stages of the prawn. Jellyfish can be voracious predators, with evidence that these scyphozoans can influence mesozooplankton communities (Schneider and Behrends, 1998; Gueroun *et al*., 2015). Little is known of feeding habits of these two jellyfish in south-western Australia, although there is some information to suggest that their ephyral and small medusa stages predate on rotifers and copepod nauplii, with the rate increasing with size (Jafri, 1997). Moreover, scyphozoans have been implicated in the decline of penaeids in both wild fisheries and aquaculture operations (Purcell *et al*., 2007). Rates of *P. punctata* predation were 18 and 22 prey predator^{-1} hr^{-1} for rotifers and copepods, respectively. Stoecker *et al*. (1987) also found that *A. aurita* selected for large metazoan micro-zooplankton as a key prey item in their diet. This included copepod nauplii, which at ~50 – 800 μm, are similar in size to the nauplii of *M. dalli* at ~300 μm (Crisp *et al*., 2016). The naupli, protozoeal and mysis stages of *M. dalli* are pelagic (Crisp *et al*., 2016) and relatively poor swimmers, and thus would be vulnerable to these scyphozoans (Costello and Collin, 1995; Ruppert and Barnes, 1994).

While larval and post-larval *M. dalli* would be most susceptible to predation by *O. rueppellii*, *A. aurita* and *P. punctata*, those prawns spawned late in the breeding season (*e.g.* February and March) would be most at risk of prolonged predation due to the cooling water temperatures and slowed winter growth of *M. dalli* (Broadley *et al*., 2017). These late recruits would grow little over the winter period and therefore remain vulnerable to predation over an extended period, until water temperatures rise in spring and growth continues.

The second-most abundant penaeid species in the Swan-Canning Estuary, *P. latisulcatus*, is restricted to the lower reaches of the system where salinities remain close to seawater, whilst *M. dalli* occurs throughout the entire sampled range of the estuary. This reflects the superior osmoregulatory capability of *M. dalli*, individuals of which were recorded in the current study in salinities of < 1. In contrast, the abundance of *P. latisulcatus* is correlated with salinity and

inversely correlated with distance from the estuary mouth in the Peel-Harvey Estuary (Potter *et al*., 1991). The minimal overlap in their respective distributions suggests that there is little competition between these two penaeids and is perhaps a mechanism to reduce competition.

Conclusions

The results from this study have clearly identified the times and locations where densities of *M. dalli* are greatest in the nearshore and offshore waters. The data also demonstrate that *M. dalli* move into nearshore waters to spawn during late spring and summer and back into the offshore waters in late summer/autumn. In order to better understand the longitudinal and depth-related movements and home ranges of *M. dalli*, an acoustic tagging program similar to that of Taylor and Ko (2011) for *Penaeus plebejus* in eastern Australia could be valuable. However, the tagged *P. plebejus* (mean = 32 mm CL) were much larger than *M. dalli* in the Swan-Canning Estuary during summer (mean and maximum CLs of 21 and 30 mm, respectively), and therefore a more compact tag would need to be developed in order for tagging to be feasible with *M. dalli*.

Many studies show that recruitment of individuals from marine waters is an important factor in the abundance of prawns in estuaries (Vance *et al*., 1996; 1998). The population of *M. dalli* in the Swan-Canning Estuary is at the southern limit of its geographical distribution and confined within the estuary (Potter *et al*., 1986b), resulting in additional challenges to the survival of this population. Although *M. dalli* is found northward from the Peel-Harvey Estuary to Darwin (and also in Java), there are no published studies on this species outside the Swan-Canning and Peel-Harvey estuaries for comparison. Meager *et al*. (2003) observed that *P. merguiensis* in the Logan River (at its subtropical limit of distribution) had a recruitment period that was restricted in comparison to their tropical counterparts. Moreover, Kienzle and Sterling (2016) found that the population of *P. esculentus* in Moreton Bay (at the southern limit of its distribution) was temperature limited, as recruitment increased with warmer water temperatures. As the growth of *M. dalli* in both the Swan-Canning and Peel-Harvey estuaries essentially stops during winter (Potter *et al*., 1986b; 1989; Tweedley *et al*., 2017), and that the spatial and temporal distribution of *M. dalli* matches those of a number of predators able to consume juveniles, the survival of

juvenile *M. dalli* is compromised as a result as they cannot grow large enough to avoid predation. Studies into the biology of *M. dalli* in warmer marine waters (*e.g.* Shark Bay) would help our understanding of the limitations experienced by these marginalized estuarine populations.

Annexes

Annex 1.1.1. *t*-values derived from pairwise PERMANOVA tests on the density of *Metapenaeus dalli* per 500 m^2 in nearshore waters on the Swan-Canning Estuary among (a) periods in 2013/14 (b) periods in 2014/15 and (c) subregions in 2014/15. Significant differences highlighted in grey. Subregion codes given in Fig. 1.1.1.

(a) Period 2013/14		2013			2014								
		O	**N**	**D**	**J1**	**J2**	**F**	**M**	**A**	**M**	**J**	**J**	**A**
2013	**N**	1.55											
	D	4.55	0.62										
	J1	0.17	1.57	4.26									
	J2	0.31	1.63	4.65	0.08								
	F	0.11	1.56	4.33	0.06	0.15							
	M	2.10	1.98	5.70	1.19	1.54	1.40						
2014	**A**	2.57	2.05	5.89	1.46	1.94	1.70	0.88					
	M	3.12	2.14	6.11	1.78	2.42	2.06	2.31	2.10				
	J	2.92	2.10	6.03	1.65	2.24	1.92	1.75	1.18	2.23			
	J	3.12	2.14	6.11	1.78	2.42	2.06	2.31	2.10	0.00	2.23		
	A	2.10	2.00	5.69	1.25	1.58	1.45	0.22	0.41	1.34	0.97	1.34	
	S	0.58	1.69	4.89	0.25	0.22	0.35	1.52	2.02	2.62	2.39	2.62	1.55

(b) Period 2014/15		2014			2015								
		O	**N**	**D**	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**
2014	**N**	0.55											
	D	0.61	0.79										
	J	2.65	1.45	2.46									
	F	1.54	1.13	1.07	1.37								
	M	3.11	1.68	2.98	1.18	2.07							
	A	3.95	1.84	4.22	3.31	3.38	0.75						
2015	**M**	4.33	1.96	4.75	4.56	4.00	1.40	2.37					
	J	4.29	1.95	4.68	4.38	3.93	1.33	1.96	0.31				
	J	4.37	1.97	4.80	4.71	4.07	1.47	2.76	0.50	0.70			
	A	4.22	1.92	4.59	4.16	3.82	1.21	1.52	0.88	0.50	1.31		
	S	4.18	1.91	4.54	4.06	3.75	1.14	1.29	1.35	0.88	1.90	0.34	
	O	0.38	0.71	0.05	1.36	0.69	1.78	2.18	2.42	2.39	2.44	2.35	2.32

(c) Subregion 2014/15	**EC**	**NM**	**SM**	**PW**	**MS**	**LC**	**MC**	**UC**
NM	4.32							
SM	2.25	1.81						
PW	3.92	2.80	3.62					
MS	3.66	1.38	0.39	2.88				
LC	1.58	0.90	1.39	0.55	1.03			
MC	3.32	1.20	0.50	2.80	0.13	1.00		
UC	1.76	3.44	1.47	3.63	2.40	1.43	3.04	
CA	1.45	3.88	1.84	3.77	2.28	1.50	2.80	0.74

Annex 1.1.2. *t*-values derived from pairwise PERMANOVA tests on the density of *Metapenaeus dalli* per 500 m^2 in offshore waters of the Swan-Canning Estuary among (a) periods in 2013/14 and subregions in (b) 2013/14 and (c) 2014/15. Significant differences highlighted in grey. Subegion codes given in Fig. 1.1.1.

(a) Period 2013/14		2013			2014								
		O	N	D	J1	J2	F	M	A	M	J	J	A
2013	N	3.08											
	D	7.48	2.79										
2014	J1	5.52	1.20	1.84									
	J2	6.24	1.65	1.36	0.51								
	F	5.98	1.21	1.96	0.04	0.57							
	M	7.31	2.95	0.28	2.04	1.59	2.17						
	A	7.09	3.72	1.50	2.99	2.61	3.10	1.22					
	M	6.66	4.04	2.15	3.40	3.09	3.49	1.91	0.80				
	J	8.82	4.55	2.17	3.85	3.44	4.02	1.85	0.43	0.47			
	J	6.62	3.10	0.74	2.29	1.89	2.39	0.47	0.71	1.44	1.22		
	A	4.48	0.59	2.43	0.66	1.16	0.66	2.61	3.45	3.80	4.35	2.79	
	S	5.86	1.07	2.15	0.22	0.76	0.19	2.35	3.25	3.62	4.20	2.55	0.49

(b) Subregion 2013/14	EC	LM	MB	UM	PW	MS	LC
LM	2.70						
MB	4.01	0.96					
UM	6.23	2.91	2.06				
PW	3.47	0.75	0.15	2.05			
MS	2.90	0.69	0.08	1.71	0.04		
LC	4.76	2.29	1.55	0.14	1.59	1.38	
MC	1.60	1.23	2.37	4.49	2.01	1.73	3.47

(c) Subregion 2014/15	EC	LM	MB	UM	PW	MS	LC
LM	3.27						
MB	8.15	5.04					
UM	7.11	4.56	0.22				
PW	13.39	10.38	5.34	4.59			
MS	8.97	7.57	4.95	4.63	1.79		
LC	14.36	12.21	8.34	7.61	3.97	1.12	
MC	13.90	11.37	6.96	6.19	2.11	0.34	1.93

1.2. Quantitative determination of ovarian development in the Western School Prawn

This study has been published in the Journal of Crustacean Biology.

Crisp, J.A., D'Souza, F.M.L., Tweedley, J.R., Partridge, G.J. & Moheimani, N.R. (2017). Quantitative determination of ovarian development in penaeid prawns (Decapoda: Penaeidae). *Journal of Crustacean Biology*. 37(1): 81-89. DOI: 10.1093/jcbiol/ruw001.

Summary

Histological methods were developed to increase precision in measuring oocyte size and to quantify, for the first time, changes in oocyte composition during ovarian development in penaeid prawns. Wild-caught female *Metapenaeus dalli* from the Swan-Canning Estuary were used as a model species to compare the novel method to traditional techniques. Morphological analysis showed that ovarian development in *M. dalli* occurs in five stages *i.e.* immature, early maturing, late maturing, mature and post spawning, which is consistent with other penaeid prawns. Analysis of key morphometric parameters of length and Gonad Somatic Index (GSI) showed that GSI provided the strongest discriminator of ovarian development. Oogenesis was similar to qualitative descriptions of other penaeid prawns and most-closely related to previous descriptions of *Metapenaeus affinis*. Comparisons between the novel perimeter tracing and traditional single linear methods for measuring oocyte dimensions showed greater precision was achieved by tracing. This resulted in a 17 to 40% reduction in the confidence limits of the means for all cell types measured. A novel histological technique of examining oocyte composition was also developed. This technique allowed for the relationship between stages of ovarian development and proportion by volume of oocyte types to be determined. The difference in the proportions of cell types between each stage of ovarian development was found to be statistically significant, except between immature and post spawning females. The novel methods developed in this study provide new opportunities in the study of ovarian development in penaeid prawns and possibly in other species.

Rationale and aims

Understanding the reproductive biology of any penaeid species requires the description and quantification of oocyte development, known as oogenesis. Previous studies of penaeid oogenesis often combined observations of somatic changes in ovarian morphology with histological changes in approximate proportion, size and number of oocyte types. As many penaeid species are the focus of commercial fisheries and aquaculture operations around the world (SEAFDEC, 2012), the oogenesis of a number of species have been described including *Penaeus setiferus* (King, 1948), *Penaeus merguiensis* (Tuma, 1967) and *Penaeus monodon* (Tan-Fermin and Pudadera, 1989). Such is the value of these descriptions that some have been applied to ecological studies of closely-related species *Penaeus* (= *Melicertus*) *latisulcatus* (Penn, 1980), *Metapenaeus endeavouri*, *Metapenaeus ensis* (Courtney *et al*., 1989) and *Metapenaeus dobsoni* (De Croos *et al*., 2011) and in laboratory studies aimed at improving the reproductive potential of domesticated of aquaculture species such as *M. ensis* (Yano, 1985), *Farfantepenaeus paulensis* (Peixoto *et al*., 2005b) and *Penaeus esculentus* (Keys and Crocos, 2006). However, recent histological observations of oogenesis in two species *i.e. Metapenaeopsis dalei* (Sakaji *et al*., 2000) and *Metapenaeus monoceros* (Abraham and Manisseri, 2012) have shown stark differences in development of oocytes compared to the aforementioned studies, particularly in the formation of cortical bodies during the final maturation stage before spawning. This highlights the need for target-specific histological descriptions of oocyte size and composition during development of each species of interest.

Furthermore, histological observations of penaeid prawn broodstock responses, both to environmental parameters (Crocos and Kerr, 1983; Courtney and Masel, 1997; Cha *et al*., 2004), and to laboratory experiments aimed to enhance production in domesticated aquaculture stock (Medina *et al*., 1996; Bindhuja *et al*., 2013), have been limited by their inability to quantify their effects on oogenesis. In the past, a combination of macroscopic and histological descriptions of oogenesis had been conducted by visual approximation only, such as those by Ayub and Ahmed (2002) and Peixoto *et al*. (2003). For oogenesis to be precisely quantified, a microscopic method with replication at the cellular level is the only way to confirm changes in oocyte composition of gonads. For this to occur, two key factors must be addressed. Firstly, more precise methods of measuring dimensions of oocyte cells than are currently available must be found, and secondly, these measures should be applied to a standardized method of

quantifying biovolume contributions of each oocyte cell type in a given area or volume within the gonad.

Cell size has traditionally been determined by taking single linear measures across a pre-determined axis at the equatorial plane with an ocular micrometer, with the equatorial plane defined as the largest possible two-dimensional area of a cell that included complete sections of nucleus in each cell (*e.g.* Peixoto *et al.*, 2005a). However, precise measurements are difficult to obtain using this method, due to the *in-situ* compression of the oocyte cells into non-geometric shapes. For example, inaccuracies have been shown in studies of several penaeids, *i.e. Metapenaeus affinis* (Ayub and Ahmed, 2002), *M. monoceros* (Abraham and Manisseri, 2012) and *P. merguiensis* (Tuma, 1967). New methods would therefore require the use of modern technologies to precisely quantify changes in oocyte size and composition during oogenesis. Solving these problems offers new opportunities to examine whether ovarian development in penaeids is consistent across different regions, fisheries and/or species also in determining whether laboratory treatments to enhance reproductive condition are successful.

While some information about the timing of reproduction and larval development in *M. dalli* is available (*e.g.* Potter *et al.*, 1986; Crisp *et al.*, 2016), detailed reproductive studies are required to ensure that the most mature broodstock are used, which maximizes hatchery production of larvae. With this in mind, the aims of this study were to (i) compare and contrast the ovarian development of *M. dalli* with other penaeid prawns, to verify its use as a model species, by describing the morphological and histological changes the occur during ovarian development, (ii) use preserved histological samples of *M. dalli* ovaries to develop a more precise method for measuring oocyte dimensions and (iii) further use preserved histological samples to develop a quantitative method of assessing the relationship between oogenesis and ovarian development in penaeid prawns. Such novel methods should improve the precision of measures of size, and quantify the number and relative composition of oocyte cells during maturation and subsequent recovery after spawning, allowing for more effective analysis of ovarian maturity in fisheries and aquaculture research.

Methods

Collection of biological material

Female specimens of *M. dalli* were collected at night from the shallow waters of the Swan-Canning Estuary (31°56′50″S 115°54′58″E) between December 2013 and March 2015, using a hand trawl net that was 1.5 m high, 4 m wide and constructed from 9 mm mesh. Upon collection, individuals were categorized using macroscopic observations into one of four stages of ovarian maturity, *i.e.* immature, early maturing, late maturing and mature on the basis of the descriptions in Ayub and Ahmed (2002). All prawns were chilled in an ice slurry until mortality, but not frozen to prevent potential damage to cells. A subset of those individuals whose gonads were classified as mature was first transferred to an aquaculture facility for spawning to demonstrate immediate effects on ovarian tissue of spawning/atresia, *i.e.* the re-absorption of acidophilic oocytes that have failed to be spawned, held under conditions modified from Laubier-Bonichon & Laubier (1976). Briefly, on arrival, the mature females were disinfected with a solution of 1 ppm formaldehyde for 30 minutes and placed in aerated holding tanks overnight (FAO, 1978). They were then stocked into 300 L conical base tanks with a flat mesh-lined base at a density of up to 15 individuals per tank for 48 h. Tanks were filled with water with a salinity of 33 ‰, drawn from a bore accessing a saline aquifer, which was aerated constantly and maintained at a temperature of ~26 °C with 0:24 h light: dark photoperiod. After spawning the females were transferred to an ice slurry as for the other individuals.

Morphology and histology

A subset of 25 individuals from each of the immature, early maturing, late maturing and mature stages together with 25 post-spawned individuals from the aquaculture facility was examined in the laboratory. Each specimen was weighed to the nearest 10 mg (wet weight) using a Sartorius A200S top balance and its carapace length, *i.e.* orbital indent at the anterior end to the posterior edge of the carapace, measured to the nearest 0.1 mm using a Sontax 150 mm digital caliper. Ovarian tissue was then carefully excised and the Gonad Somatic Index (GSI) calculated using the following equation; GSI = (gonad weight / total weight) x 100. Sub samples

of anterior or first abdominal sections of the excised gonads were immediately fixed before histological analysis, for at least 48 h in a solution of tetraborate-buffered 10 % formaldehyde with ~33 ‰ salinity.

The fixed gonad samples were then embedded into paraffin block and 6 µm sections taken as per Bell *et al*. (1988). Sections were then stained using haematoxylin and eosin using the method adapted from Quintero and Gracia (1998). The resultant sections were then imaged using a Tucsen 9 MP camera mounted onto a compound microscope at 100 x magnification. The TSView 7 software package was used to download the images for analysis. Oocytes from these imaged samples were then characterized and compared to published histological descriptions (*i.e.* Tuma, 1967; Ayub and Ahmed, 2002).

From these images, five oocytes of each type per slide were selected *ad-hoc* from 10 haphazardly selected slides and measured via two methods with ImageJ 1.48 64 bit software, producing 50 measures of each cell type overall for each method. The first method was the traditional single linear measure to determine diameter (*i.e.* Ayub and Ahmed, 2002). In this study, diameter was measured parallel to the long axis of each mounted slide. The second method was the measurement of the external circumference by tracing the perimeter of each cell. Note that both measurements were taken as close to the equatorial plane as possible, with this plane being defined by those oocytes that showed the largest possible two-dimensional surface area and included complete sections of nucleus within each cell. In cortical oocytes where the nucleus is not visible, this was defined as the largest possible area of cytoplasm that could be found with cortical bodies of uniform size and shape around the periphery.

Measurements were made at the equatorial plane to ensure that only sections of whole cells were used. Partial sections of cells are often created as oocytes are contorted into non-geometric shapes by being compressed in the ovary. Each measurement was made on a digital image at 100x magnification then re-calibrated to µm using an image of a micrometer at the same magnification. Once perimeter measures were made, equivalent diameter and spherical biovolume were calculated using the following equations, assuming that oocytes released from gonads take on a spherical form.

Diameter (sphere) = circumference / π

Volume (sphere) = (4/3) x π x r^3

Where r = diameter / 2

The diameters measured/calculated using the traditional linear measure and new perimeter method were compared to determine which technique provided the most consistent results, as demonstrated by the size of the 95 % confidence limits.

Oocyte development

The oocyte types present in each developmental stage were described visually from the images taken from the slides, as per traditional methods (*i.e.* Tuma, 1967; Ayub and Ahmed, 2002). The relationship between the relative proportions of each oocyte type present and the stages of development were then examined, to determine trends in oocyte development between stages.

Images of each slide were uploaded to Adobe Photoshop CS6, where 400 x 400 µm gridlines were applied. These dimensions were chosen on the basis that at least six replicate grids could be obtained from each slide for analysis. From this, three grid squares were haphazardly selected to enumerate each cell type over the two-dimensional plane. This involved counting the number of oocyte of each cell type within each square, taking care to only include cells in, or near to the equatorial plane. Cells that overlapped grid boundaries were only considered when crossing the left or top sides of the nominated grid square(s), as adapted from the standard haemocytometer method of counting cells (FAO, 1978).

Once each oocyte type was enumerated, the total biovolume for that type of oocyte in each replicate was calculated by multiplying the number of that oocyte type by its corresponding mean biovolume (see equation below). This is done to standardize the biovolume of each cell type in each replicate. Once calculated, the proportions of each oocyte type at each stage were compared and contrasted to determine the relationship between relative oocyte abundance and macroscopic stage of development.

Total biovolume (oocyte type) = mean biovolume (oocyte type) x n(oocyte type)

Statistical analysis

A one-way Analysis of Variance (ANOVA) was used to determine whether carapace length and GSI differed among various stages of ovarian development. Analysis of the linear relationship between the $\log_e$ mean and $\log_e$ standard deviation of each data set was used to determine which transformations, if any, were required, to meet the test assumption of homogeneity of variance for each of the above two data tests (Clarke *et al*., 2014a). This analysis indicated that a square root transformation of GSI values were required, while carapace length required no transformation. When ANOVA detected a significant difference, *post-hoc* tests were conducted using Tukey's HSD to elucidate the pairs of ovarian stages that were responsible for each of those differences. In this and all tests, a null hypothesis of no significant difference between *a priori* groups was rejected when $p < 0.05$.

To determine which of the two methods for calculating cellular diameter (see above) was the most precise, the data for each measure for each of the five types of oocyte examined was analyzed using multiple pairwise comparisons, via two-tailed *F*-tests. If a significant difference in magnitude of variance was detected, the magnitude of that difference was quantified by calculating the percentage change in variance from the single linear measure to the new trace method. All univariate analyses were performed using SPSS version 22 software.

Multivariate analysis to determine whether the percentage contributions of the biovolumes of each type of oocyte present (*i.e.* oocyte composition) differed among ovarian development stages was conducted using a one-way Analysis of Similarities (ANOSIM; Clarke and Green, 1988) test using the PRIMER v7 software package (Clarke and Gorley, 2015). Prior to analysis, the percentage contributions of the biovolumes of each cell type from each section were visualized using shade plots and an appropriate transformation selected using the criteria in Clarke *et al*. (2014b). In this case, a square-root transformation was used to avoid any tendency for the cell types to be excessively dominant. This pre-treated data was used to produce a Bray-Curtis resemblance matrix, which was, in turn, subjected to the one-way ANOSIM test. To visualize the patterns exhibited by oocyte type among the 125 replicate samples, a non-metric multidimensional scaling (nMDS) ordination plot (Clarke, 1993) was constructed from the above resemblance matrix. To simplify and further illustrate the histological differences between stages, a second (centroid) nMDS plot was produced using a distances among centroids

matrix, which creates averages in the 'Bray-Curtis space' from the 25 replicate samples representing each stage (Lek *et al*., 2011). Stacked bar histograms, representing the untransformed percentage contributions of the various cell types were included to indicate which types of oocytes were responsible for the changes in cellular composition during each stage of oogenesis.

Results

Macroscopic observations

The gonad *in situ*, from the dorsal carapace to the dorso-ventral section of the tail above the anus showed macroscopic changes among the five stages.

Stage 1 (Immature). Gonad not visible through the exoskeleton, requiring dissection to observe. Gonad translucent in appearance and smooth textured, with the diameter in the mid-section smaller than the intestinal tract directly below. Anterior lobes present, although undeveloped.

Stage 2 (Early maturing). Gonad visible through dorsal exoskeleton. Upon dissection gonad appears thin with a white-yellow or green-yellow granular appearance. Anterior lobes enlarged and extend forward into the carapace, while the posterior lobes greater in size than the intestinal tract.

Stage 3 (Late maturing). Gonad now clearly visible through the exoskeleton, taking on a green granular appearance. Texture of the dissected gonad firmer and lobes nearly filled out. Anterior lobes fully-formed, but do not fill the carapace completely. An 'arrow head' shape begins to form posteriorly in the final abdominal segment above the anus.

Stage 4 (Mature). Gonad now clearly visible through the exoskeleton, expanding in size to occupy much of the carapace and a significant portion of the abdominal region. Upon dissection has a dark-green or green-brown granular appearance and a distinct 'arrow head' formed in the posterior end.

Stage 5 (Post spawning). Gonad either slightly visible or no longer visible through the exoskeleton. Upon dissection, gonad appears opaque white or white-yellow in colour and smooth in texture. A red hue present in some samples.

Carapace length and Gonad Somatic Index

Carapace lengths were shown by ANOVA to differ significantly among stages of development ($F = 11.84$, $df = 4, 124$, $p < 0.001$), with Tukey's *post hoc* test determining that immature and early maturing females had a smaller mean carapace length (~ 19 mm) than the late maturing, mature and post-spawned females (~ 21–22 mm; Fig. 1.2.1). Overlap in 95% confidence limits of some of the stages of ovarian development indicates that it is not well defined by carapace length. A one-way ANOVA of the GSI values demonstrated that they differed significantly among the stages of development ($F = 250.8$, $df = 4, 124$, $p < 0.001$), with Tukey's *post-hoc* test demonstrating that significant differences existed between all stages, except between early maturing and post spawning (Fig. 1.2.2). The mean GSI values increased sequentially from 0.58 when immature to a maximum of 6.90 when mature, followed by a sharp decrease at post spawning to 1.97.

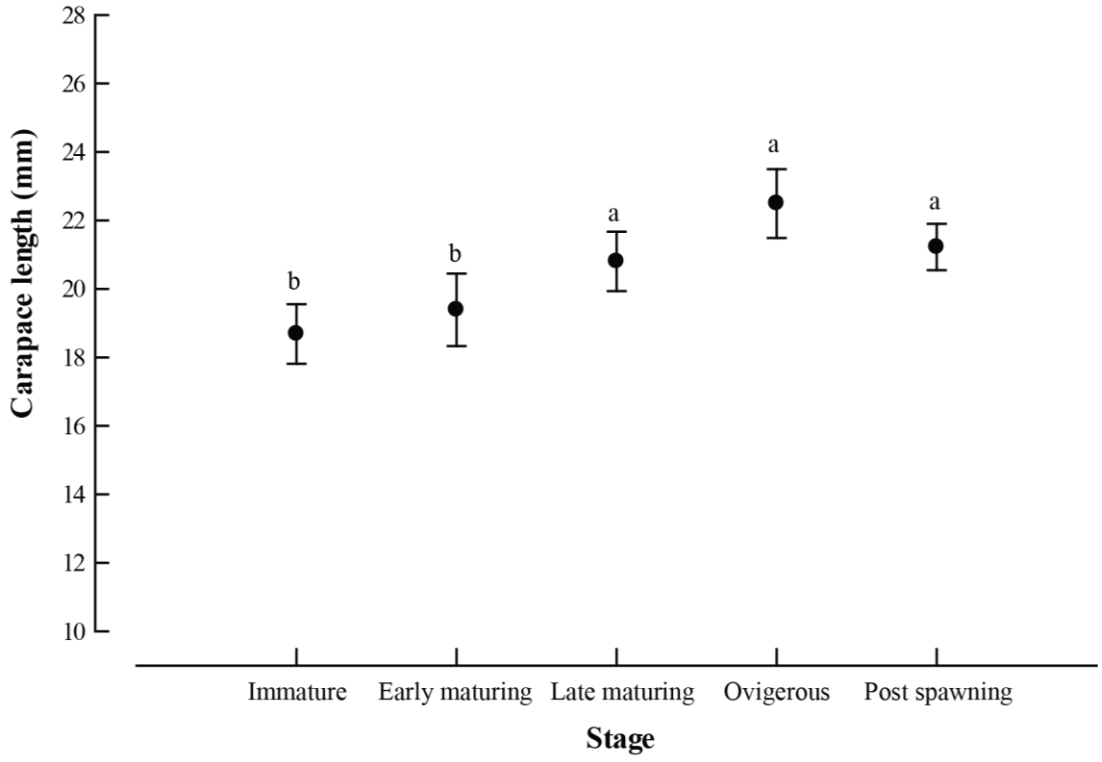


Fig. 1.2.1. Mean carapace length of female *Metapenaeus dalli* at each of the five stages of ovarian development. Error bars represent 95% confidence limits and the letters indicate significant differences among stages as determined by Tukey's HSD test.

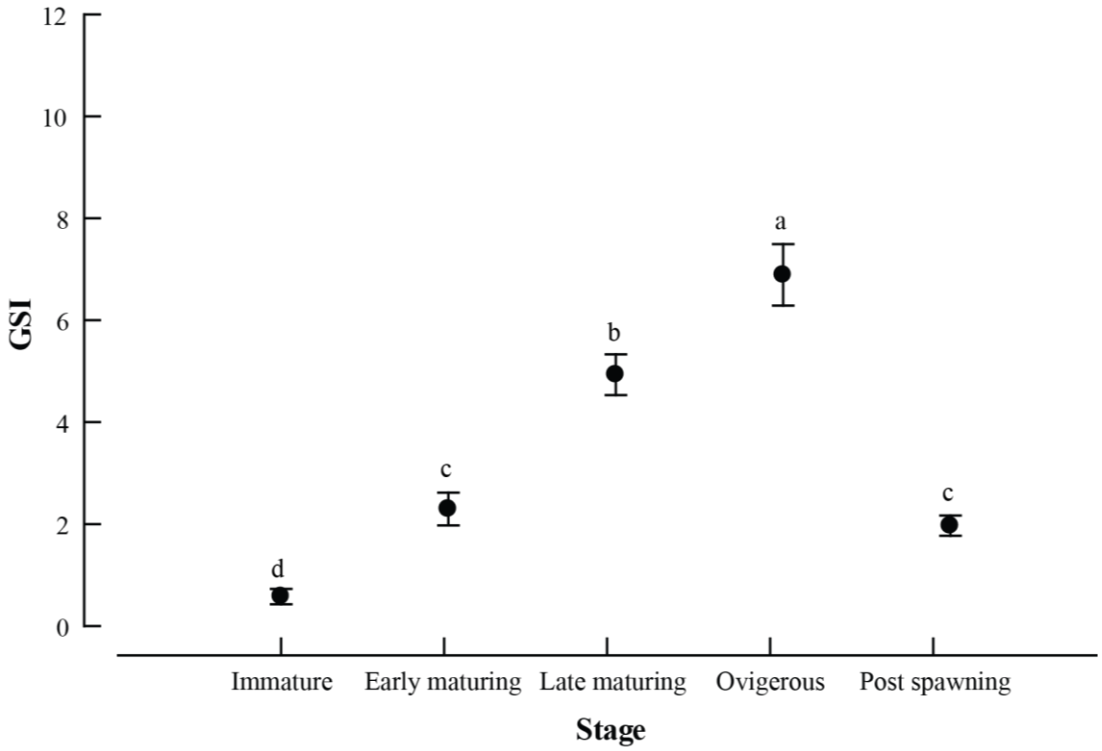


Fig. 1.2.2. Mean Gonad Somatic Index (GSI) values for female *Metapenaeus dalli* at each of the five stages of ovarian development. Error bars represent 95% confidence limits and the letters indicate significant differences among stages as determined by Tukey's HSD test.

Observations of oocyte appearance

Preliminary observations indicated that the arrangement of oocyte cells varied greatly between individuals within each stage of development, with the in situ observations of development indicating that oocyte cells migrate from the germinal zone (Fig. 1.2.3a) to the periphery of the ovary during development. Later, cell types such as yolky and cortical oocytes become bound by follicle cells just prior to spawning. Using the criteria defined by Ayub and Ahmed (2002), five types of oocytes, *i.e.* chromatin nucleolar, perinucleolar, yolkless, yolky and cortical, were identified in addition to follicle cells in haematoxylin and eosin stained ovarian sections. These cells showed a progression in size, with earlier stages comprising primarily basophilic cellular material, staining blue, tending to shift to acidophilic cellular material, staining red, during the later stages of ovarian development. Oocyte cells migrate away from the germinal zone (Fig. 2,2.3a) as they develop from chromatin nucleolar to cortical oocytes, leaving room for new cells to be produced. The appearance of each oocyte type is described below.

Chromatin nucleolar oocyte (Fig. 1.2.3a). Constructed primarily of a densely basophilic nucleus, containing chromatin material in no particular arrangement. Cell has very little cytoplasm that is completely basophilic.

Perinucleolar oocyte (Fig. 1.2.3a). Exhibits a densely basophilic nucleus with a larger basophilic cytoplasm. Basophilic nucleoli are arranged around the periphery of the nuclear membrane. Follicle cells appear to arrange themselves around the outside of some perinucleolar oocytes (Fig. 1.2.3a).

Yolkless oocyte (Fig. 1.2.3b). Cytoplasm now clearly acidophilic, with nuclear membrane clearly defined.

Yolky oocyte (Fig. 1.2.3c). Cytoplasm now exhibits acidophilic yolky 'plates' or granules that include cytoplasmic vesicles and/or cortical crypts. Chromatin material and nucleoli are much greater in number than the yolkless oocyte, resulting in the nuclear membrane becoming denser and less distinguishable. Each yolky oocyte has a layer of follicle cells surrounding each cell.

Cortical oocytes (Fig. 1.2.3d). Nucleus appears absent or near absent. Cortical cells contain small oval-shaped cortical bodies arranged on the internal periphery of the cytoplasm at the cell membrane. These bodies are a defining characteristic of the mature stage of the oocytes.

Atreatic oocytes (Fig. 1.2.3e). Appear as remnants of acidophilic oocytes that failed to spawn, containing no cytoplasmic material or a nucleus.

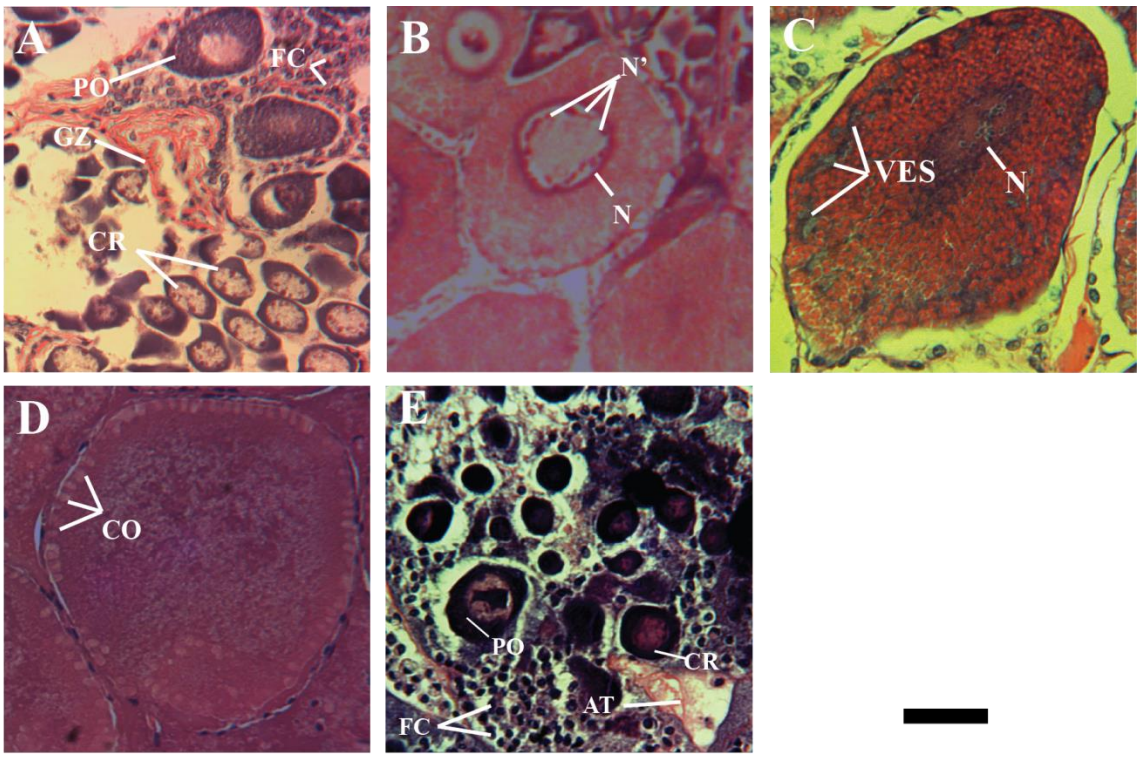


Fig. 1.2.3: Representative photographs of Haematoxylin & Eosin stained oocytes of (A) immature, (B) early maturing, (C) late maturing, (D) ovigerous and (E) post-spawning female *Metapenaeus dalli* at 250x magnification. Images of cells were taken at the ovarian development stage at which they first appear. FC = follicle cells, CR = chromatin nucleolar oocytes, PO = perinucleolar oocytes, GZ = germinal zone, N = nucleus, N' = nucleoli, VES = cytoplasmic vesicles, CO = cortical bodies and AT = atreatic cell. Bar = 50µm.

Oocyte size and composition

Pairwise comparisons of the size of each of the five types of oocyte measured using the single linear or perimeter method, conducted using two-tailed F-tests, demonstrated that in all cases there was a significant difference in the variance (Table 1.2.1). The perimeter method resulted in a 17 to 40 % reduction in the 95% confidence limits of the calculated diameter of the cell, depending on the type of oocyte.

Table 1.2.1. Mean diameters (µm) and ± 95% confidence limits of five types of oocyte present in the ovaries of female *Metapenaeus dalli* during ovarian development, calculated using single linear measure and perimeter methods. The significance values (*p*) of *F*-tests are provided together with the (%) percentage reduction in confidence limits achieved using the perimeter method.

Oocyte type	Linear measure		Perimeter measure		*p*	%
Chromatin	26.59	+1.78	23.32	+1.48	0.001	17
Perinucleolar	55.46	+4.41	60.63	+2.96	0.000	33
Yolkless	94.94	+6.31	102.69	+3.79	0.003	40
Yolky	128.84	+6.95	131.79	+4.48	0.001	35
Cortical	143.05	+7.98	152.55	+5.55	0.000	30

One-way ANOSIM detected a significant difference (Global $R = 0.787$, $P = 0.001$) in the oocyte composition, with pairwise comparisons indicating that the contributions of the various cell types to each ovarian developmental stage were different in all stages, except between immature and post spawning (Table 1.2.2). This is illustrated on the nMDS plot where the points representing the gonads from immature and post spawning stages overlap considerably and are well separated from those points representing the other stages, which all form discrete groups (Fig. 1.2.4a). The centroid nMDS plot illustrates the clockwise progression in oocyte composition (Fig. 1.2.4b); with immature and post spawning ovaries comprised solely of chromatin and perinucleolar oocytes (Fig. 1.2.5). Although early and late maturing ovaries retained chromatin and perinucleolar oocytes, they were characterized by the presence of a substantial proportion of yolkless oocytes, with late maturing gonads also containing yolky oocytes and a low proportion of cortical oocytes. By the mature stage, the ovary is comprised almost exclusively of yolky and cortical oocytes. Following spawning, these yolky and cortical oocytes are expelled or re-absorbed, leaving chromatin and perinucleolar oocytes. Thus, oocyte compositions in immature and post spawning ovaries are almost identical.

Table 1.2.2: Pairwise *R* statistic and significance level (*P*) values derived from a one-way ANOSIM of the square-root transformed percentage biovolumes of each type of oocyte from female *Metapenaeus dalli* in each of the five stages of ovarian development. Insignificant pairwise comparisons ($p > 0.05$) are shaded grey.

	Immature	**Early maturing**	**Late maturing**	**Mature**
Early maturing	0.816			
Late maturing	0.901	0.427		
Mature	1.000	1.000	0.934	
Post spawning	0.018	0.833	0.903	1.000

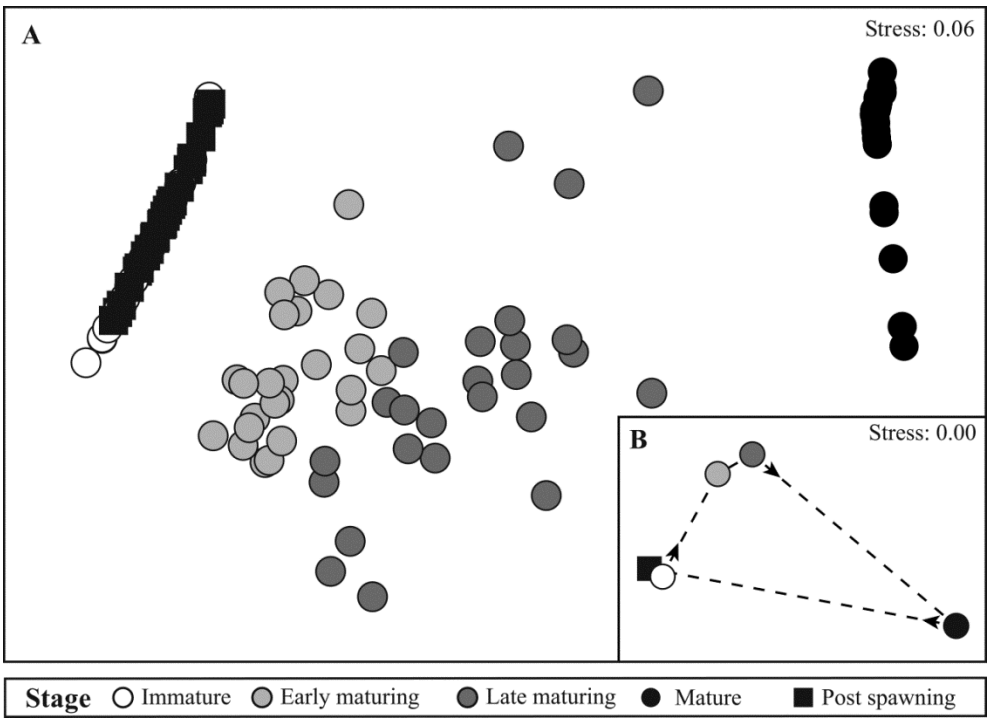


Fig. 1.2.4. (A) Non-metric multidimensional scaling (nMDS) ordination plot derived from a Bray-Curtis resemblance matrix of the square-root transformed percentage bio-volumes of each type of oocyte from 25 female *Metapenaeus dalli* from each of the five stages of ovarian development. (B) Centroid nMDS ordination plot, derived from distance among centroid matrices constructed from the above Bray–Curtis resemblance matrix. Arrows on dotted lines indicate the progression in development of ovaries from immature to post spawning.

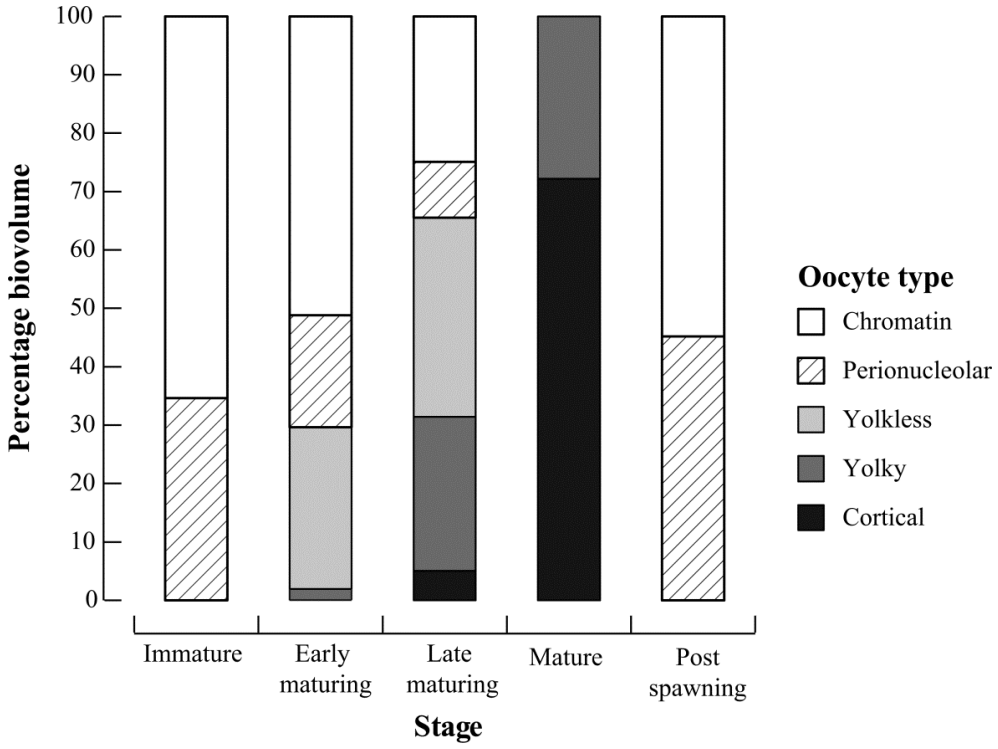


Fig. 1.2.5. Mean percentage contributions of the biovolumes of each type of oocyte in female *Metapenaeus dalli* from each of the five stages of ovarian development.

Discussion

Macroscopic observations

Macroscopic and histological observations of the gonads of wild-caught female *M. dalli* showed that the oogenesis was separated into five distinct developmental stages, progressing from immature, to early maturing, late maturing, mature and finally post spawning. This is similar to previous works on *P. setiferus* (King, 1948), *P. merguiensis* (Tuma, 1967), *Penaeus brasiliensis* (Quintero and Gracia, 1998) and *M. affinis* (Ayub and Ahmed, 2002). Macroscopic differentiation of maturity in *M. dalli* was possible for early maturing, late maturing and mature stages of ovarian development; however, the gonad of immature and post-spawned individuals could not be distinguished from each other using this method. The lack of differences in macroscopic observations for these two stages was due to the fact that the exoskeleton obscured the view of the gonad in situ, and that each gonad was relatively similar in size and colour. Differentiation of these stages was definitive only when gonad was excised and histological analysis was performed; with gonads taken from spawned individuals containing significant amounts of atreatic cells and extraneous material. Late maturing and mature gonads were considerably easier to distinguish externally through the exoskeleton in *M. dalli* than in *P. brasiliensis* (Quintero and Gracia, 1998), but are most consistent with findings from *P. merguiensis* (Tuma, 1967) and *M. affinis* (Ayub and Ahmed, 2002).

Carapace length and Gonad Somatic Index

Strong significant differences were observed in the GSI between stages of ovarian development in this study ($p < 0.001$), with GSI increasing sequentially from a minimum when immature, to a maximum when mature, before declining after spawning. The mean GSI of the mature stage in this study (6.9 + 0.6) was similar to that recorded in the neighbouring Peel-Harvey Estuary (7.0 + 0.4) during the 1987-88 breeding season. The far greater F-value for GSI (250.8) than carapace length (11.84) indicates that GSI provides a more precise measure of ovarian developmental stage. Although it was possible to differentiate overall ovarian development based on carapace length ($p < 0.001$), post hoc analysis indicated that the five ovarian

developmental stages formed only two significantly different groups, *i.e.* immature and early maturing versus late maturing to post spawning. This provides relatively poor discrimination between stages of ovarian development.

The increase in carapace length with ovarian developmental stage is due to the fact that the breeding of *M. dalli* occurs during the warmer summer period (October-March), which coincides with a significant increase in somatic growth rates (Potter *et al*., 1986b; 1989). Carapace lengths of mature *M. dalli* in this study (22.3 mm) closely matched those of female *Metapenaeus bennettae* (22.4 mm) in Moreton Bay, Queensland (Courtney and Masel, 1997). It is interesting that the range of carapace lengths between late maturing to post spawning female *M. dalli* in this study (20.6-22.3 mm) are larger than corresponding values recorded in the neighbouring Peel-Harvey Estuary in 1987-88 (17.9-20.7 mm; Potter *et al*., 1989). This demonstrates that, although the GSI of mature females is similar between these two systems, the size of individual females is greater in the Swan-Canning Estuary.

Oocyte size and development

During ovarian development, distinct changes were observed in oocyte size and composition from the immature stage, through early and late maturation to the mature stage. At post spawning, the ovary was similar in appearance to that of the immature stage, however, the post-spawned ovary could be distinguished by the presence of large deposits of non-oocyte material and atreatic oocytes. While the changes in oocyte composition are consistent with those observed in *M. affinis* (Ayub and Ahmed, 2002), they differ from those of *M. monoceros* (Abraham and Manisseri, 2012) and *M. dalei* (Sakaji *et al*., 2000). This is due to *M. monoceros* and *M. dalei* not observed with oocytes containing cortical bodies at the mature stage.

Measurements of oocyte diameter using the new perimeter method were found to reduce the range of the 95% confidence limits by between 17-40% for each of the five oocyte types when compared to those calculated using the single linear method. This increased level of precision is particularly useful when quantifying the effects of spatial, temporal, nutritional and environmental changes on ovarian conditioning in the field and/or in the laboratory. The method

developed in the current study would have enhanced findings by Rao (1973), who found considerable differences in oocyte size from wild caught *M. dobsoni*, *P. indicus* and *P. stylifera* from Cochin when compared to other parts of India. Similarly, findings by Ayub and Ahmed (2002), which compared oocyte sizes in *M. affinis*, *P. indicus* and *P. stylifera* from coastal waters off Pakistan, with similar environments in India, would have been more precise. In laboratory studies for aquaculture purposes, this quantitative method of assessing ovarian development can be applied to studies exploring the effects of altering food composition, physiology and/or rearing conditions in broodstock domestication. Quantitative analysis of these treatments on *Penaeus kerathurus* (Medina *et al*., 1996), *F. paulensis* (Peixoto *et al*., 2005a), *P. esculentus* (Keys and Crocos, 2006) and *P. monodon* (Marsden *et al*., 2007) would have significantly enhanced the qualitative histological comparisons that were made.

Ovarian oocyte composition

Relationships between oocyte composition and ovarian development in this study indicated that the greatest diversity in oocyte cells existed during proliferation phase of the early and late maturing stages, with cortical oocytes making up the majority of cells present in the mature stage. This phenomenon has been described in several other studies and by Dall *et al*. (1990), but without any statistical analysis to support these claims. Thus, this study is the first to statistically demonstrate changes in oocyte composition with each developmental stage. Given the nature of the pairwise comparisons between stages analyzed histologically, and the absence of chromatin, perinucleolar and yolkless oocytes in mature gonads, it could be assumed that the final maturation process is rapid. Changes in GSI between late maturing and mature stages also support this assertion, indicating that much of the energetic and nutritional reserves during this period are bestowed to the oocytes, particularly lipids (Cahu *et al*., 1994), α-tocopherol and ascorbic acid (Cahu *et al*., 1995). Post-spawning absorption of atreatic oocytes and extraneous material in the gonad may act as a recovery mechanism to stave off mortality after spawning, allowing for recovery and repeated spawning, but additional environmental stressors may increase mortality during this sensitive period.

Conclusions

The novel method of determining the size of oocytes in the ovaries of penaeid prawns developed in this study showed a much greater precision than the traditional techniques previously used. Having using these measures, multivariate statistical analyses were employed to describe the relationship between oocyte composition and ovarian development, resulting in the first quantifiable data to be obtained by any study of oogenesis for a penaeid. New opportunities now arise in the application of these methods in studies of the ovarian development in penaeids, with practical applications in assessing the reproductive performance of wild caught female prawns and those held under the influence of alimentation, relatively different to what would be found in natural environment.

1.3. Environmental factors influencing the reproduction of the Western School Prawn in the Swan-Canning Estuary

This study will be published in Fisheries Management and Ecology.

Crisp, J.A., Tweedley, J.R., D'Souza, F.M.L. Poh, B. & Loneragan, N.R. (In press). Environmental factors influencing the reproduction of an estuarine penaeid population and implications for management. *Fisheries Management and Ecology*.

Summary

This study determined environmental factors influencing and the reproductive dynamics of a recreationally-fished penaeid *Metapenaeus dalli*, in the Swan-Canning Estuary, south-western Australia, during a restocking program. Prawns were collected from nearshore (< 2 m deep) and offshore waters (> 2 m deep) every lunar month from October 2013 to March 2016. Reproduction occurred between November and March, when water temperature > 17 ˚C, salinity > 25 and stratification (bottom – surface salinity) < 3. Densities of gravid *M. dalli* were highest in November each year when 0+ females matured (19 mm; ~ 56 % asymptotic length), and were highest within the Lower Canning Estuary. Individual fecundity ranged from 34,000 (18.1 mm CL) to 132,000 ova (27.1 mm CL). Egg production peaked in December/January differed among years, being greatest in 2015/16. Results suggest that closing fishing between November and December would protect breeding aggregations of *M. dalli*.

Rationale and aims

The managers and users of recreational fisheries see aquaculture-based enhancement (*i.e.* the release of culture-based juveniles into the natural environment) as a mechanism for enhancing the fishing experience and increasing their catch rates when stocks are declining (Garlock and Lorenzen, 2017; Taylor *et al*., 2017a). Such programs can be used to complement more traditional management mechanisms, such as spatial and temporal closures and size and bag limitations. To maximize the potential benefits of aquaculture-based enhancements and ensure a sustainable fishery for the future, however, extensive studies on the biology and ecology of target species are required, particularly the reproductive biology (Lorenzen *et al*., 2010).

Penaeid prawns (= shrimp) are typically short-lived, *i.e.* maximum ~2 to 3 years and reproduce well before their maximum size is attained (Hartnoll, 1985; Dall *et al*., 1990). Environmental factors, such as water temperature and salinity, can influence respiration rates and growth and therefore influence the timing and magnitude of reproduction (Clarke, 1987; Dall *et al*., 1990; Pillai and Diwan, 2002). The reproductive biology of tropical penaeids, including the effects of temperature on the size of maturity, maturation rates and timing of reproduction, are relatively well described for some species (Crocos, 1987; Crocos *et al*., 2001; Cha *et al*., 2004; De Croos *et al*., 2011). However, the reproductive characteristics of penaeids in temperate estuarine environments, where marked seasonal changes in water temperature and salinity occur, are less well known.

In the tropics, prawns grow rapidly and typically reach sexual maturity within six months of eggs being released (Crocos, 1987; Crocos *et al*., 2001). Reproduction continues until senescence at about 15 to 17 months of age. Although these species mature at around six months of age, egg production is far lower than that in larger, older prawns at 10 to 12 months of age, due to smaller percentages of reproductive females (~67 % versus ~75 – 80 %) and egg production increasing with size (Somers and Kirkwood, 1991; Coman and Crocos, 2003). In contrast, penaeids found in temperate marine and environments, such as *Metapenaeus bennettae* and *Metapenaeus macleayi* in eastern Australia, *Penaeus* (=*Melicertus*) *latisulcatus* and *Metapenaeus dalli* in south-western Australia and *Metapenaeus joyneri* in Korea exhibit highly seasonal growth, which declines markedly during the cool, winter months. This results in delayed maturation and reproduction, which only occurs during the warmer summer months (Penn, 1980; Coles and Greenwood, 1983; Cha *et al*., 2004; Broadley *et al*., 2017).

The location and timing of reproduction in prawns is also influenced by the seasonal patterns of freshwater discharge. This may occur in both marine species and in those that utilise estuaries for all or part of their lifecycle. For example, marine species, such as *Metapenaeus endeavouri* and *Metapenaeus ensis* in the Gulf of Carpentaria, exhibit continuous reproductive activity throughout the year in this marine embayment at depth of 20 to 60 m. However, when monsoonal rains occur from November to April, reproduction is reduced and confined to the deeper waters from 40 – 60 m (Crocos *et al*., 2001). In species that inhabit estuaries, rainfall

and freshwater discharge influence the location of reproduction. For example, increased discharge during the summer on the east coast of Australia leads to the movement of *M. macleayi* and *M. bennettae* from estuaries into adjacent coastal waters, where most eggs are produced (Glaister, 1978; Courtney and Masel, 1997; Loneragan and Bunn, 1999).

In Western Australia, *M. dalli* is found only in estuaries below the latitude of 31˚S which are subject to a Mediterranean climate, and in coastal waters at 22˚S in sub-tropical regions such as Shark Bay (Grey *et al*., 1983; Potter *et al*., 1986b). Reproduction of *M. dalli* in temperate estuaries occurs during the summer months (Potter *et al*., 1986b; Potter *et al*., 1989), when rainfall and thus freshwater discharge are low and salinity and temperature increases (Hodgkin and Hesp, 1998; Tweedley *et al*., 2016b). Though previous studies identified the broad timing of reproduction of *M. dalli* in both the Swan-Canning and Peel-Harvey estuaries, the spatial and temporal patterns of abundance and reproduction were not comprehensively examined, nor were the fecundity and reproductive potential estimated (Potter *et al*., 1986b; 1989; Broadley *et al*., 2017). Furthermore the last surveys of the abundance of *M. dalli* in the Swan-Canning Estuary were completed in 1982 when numbers or prawns were much higher, thus the key information relevant to the future management of this fishery is lacking (Broadley *et al*., 2017).

Historically, *M. dalli* and the Western King Prawn (*Penaeus latisulcatus*) formed the basis for a commercial and recreational fishery in the Swan-Canning Estuary. However, commercial catches declined greatly from the peak of ~15 tonnes in 1959 resulting in the closure of the fishery 1970s, while recreational fishing effort also declined markedly since the 1990s, with 1999 having the last substantial catches (Smith, 2006). The causes of the decline in the population of *M. dalli* are not known, but may be related to over-fishing or changes in the environmental conditions in the Swan-Canning Estuary (Smith, 2006). Furthermore, the effect of environmental changes in the system on prawn populations and their lack of recovery since the substantial reduction in fishing pressure, are not known. To address this problem, a study of the reproductive cycle for *M. dalli* was initiated. This study builds on the detailed study of the growth and mortality of *M. dalli* in the Swan-Canning Estuary, which also described the timing of reproduction based on macroscopic examination of gonads (Broadley *et al*., 2017). However, it did not examine the histology of the gonads, estimate the egg production by females

or explore quantitatively the spatial and temporal variation and effects of salinity and water temperature on reproduction in the estuary.

The aims of this study were to: (i) describe the spatial and temporal patterns of abundance of female *M. dalli*, their size at maturity and timing of reproduction within the Swan-Canning Estuary; (ii) determine whether seasonal changes in salinity and water temperature affect maturation, size of breeding females and subsequent fecundity of *M. dalli*; and (iii) estimate the population fecundity of *M. dalli* and investigate how this changes during the breeding period. The knowledge generated from this study provides valuable information for how fishing regulations might be modified to rebuild the *M. dalli* population in the Swan-Canning Estuary.

Methods

Study area

The Swan-Canning Estuary is a wave-dominated microtidal estuary, located in south-western Australia (31°56′50″S 115°54′58″E), is ~50 km long and covers an area of ~55 km^2 (Valesini *et al*., 2014; OzCoasts, 2017). It permanently connected to the Indian Ocean via a narrow entrance channel that opens into a lagoonal basin area and the tidal portions of the Swan and Canning Rivers (Fig. 1.3.1). Although most of the estuary is shallow, *i.e.* < 2 m in depth, it reaches a maximum depth of ~20 m near the entrance channel. The estuary and its catchment experiences a Mediterranean climate, with >70 % of the rainfall occurring during cooler months between May and September (Hodgkin and Hesp, 1998; Broadley *et al*., 2017). As a result of this environmental variation, physico-chemical conditions of the water were monitored at sites throughout the Swan-Canning Estuary to determine the extent of annual, inter-annual and regional environmental effects on densities of gravid *M. dalli* from 2013/14 to 2015/16.

During the course of this study, a restocking program was conducted, with ~4.65 million post-larvae released in the Swan-Canning Estuary during the spring and summer months between December 2012 and March 2016. The number released increased sequentially among years with ~15,000 in 2012/13, ~635,000 in 2013/14 and ~2,000,000 in both 2014/15 and 2015/16 (Jenkins *et al*., 2017).

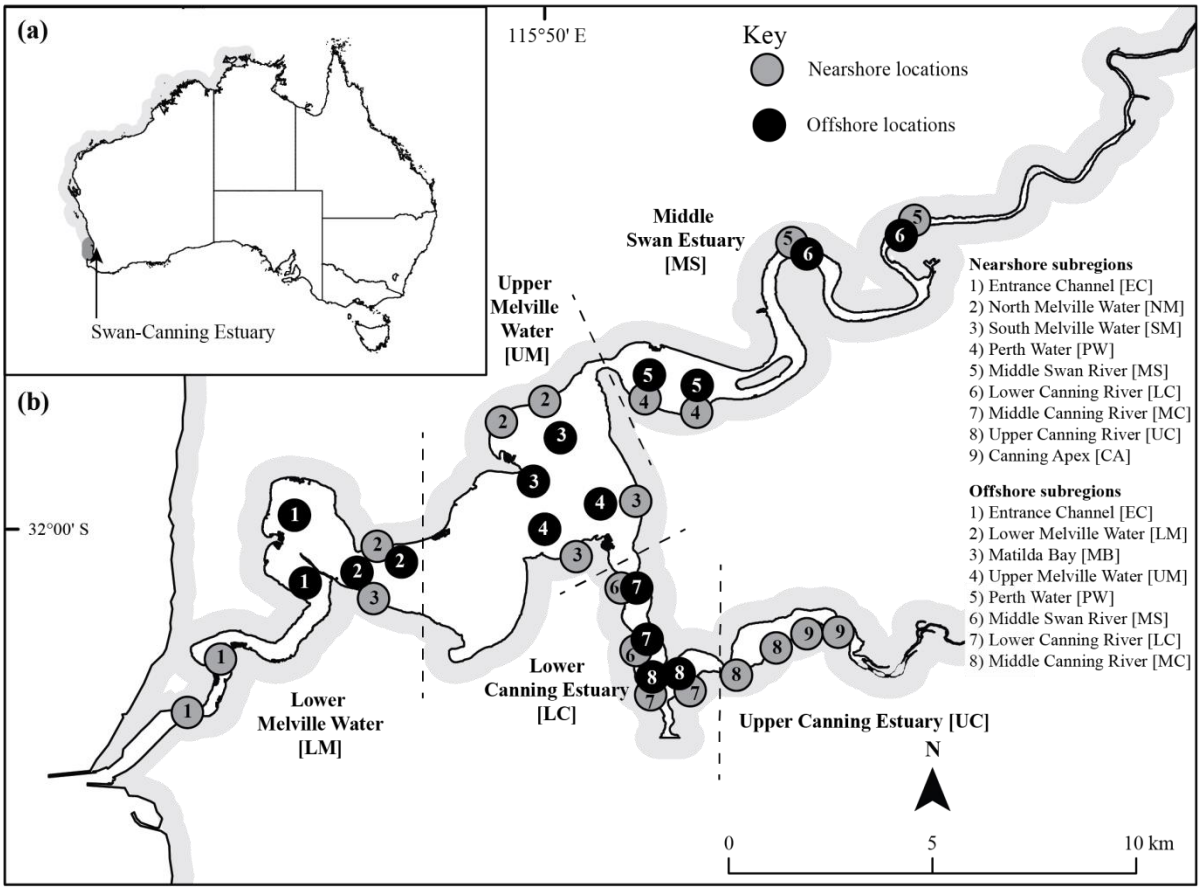


Fig. 1.3.1. (a) Map of Australia showing the distribution of *Metapenaeus dalli* in nearshore marine waters (light grey) and solely in estuaries (dark grey). (b) Location of the 9 nearshore subregions (grey dots with their designated subregion in black numbers), 8 offshore subregions (black dots with their designated subregion in white numbers) and 5 regions (labelled on map in bold) as defined by Broadley *et al*. (2017).

Sampling methodology

Details of the prawn sampling regime are described fully in Broadley *et al*. (2017), but a brief summary is provided here. Prawns were sampled at two locations within 20 nearshore (< 2 m deep) and 16 offshore sites (2-17 m deep) at night between ~18:00 and 01:00, on every new moon phase (moon <10% illumination), between October 2013 and March 2016. The sites were spread from close to the mouth of the estuary to 34 and 27 km upstream in the Swan and Canning rivers, respectively and were categorized into 9 and 8 regions in nearshore and offshore waters, respectively (Fig. 1.3.1). Nearshore waters were sampled using a hand trawl net that was 4 m wide and constructed of 9 mm mesh. A 200 m trawl (swept area of ~570 m^2) was carried out at each location in each site, on each sampling occasion. The offshore waters were

sampled using a small otter trawl that was 2.6 m wide and constructed of 25 mm mesh in the body and 9 mm mesh in the cod-end. Each trawl was towed at an average speed of 1.6 knots (~3 km h^{-1}) for 5 min, covering a distance of ~250 m (swept area ~650 m^2), except at some sites where only a 3 min trawl (swept area ~390 m^2) could be completed. Prawns captured in the first year of study were euthanized in an ice slurry and transported to the laboratory for measurement (see below), except when a large number were caught, when a sub-sample of ~50 prawns were retained and the remainder were counted, sexed and returned to the water alive. In the subsequent years, the carapace length of all prawns was also measured before returning them to the water.

Temperature and salinity were recorded at the top and bottom of the water column at each offshore site, on each sampling occasion, using a YSI 556 Handheld Multiparameter Instrument. Due to a boat engine malfunction in December 2014, data were not obtained from all offshore sites and this month was thus removed from all statistical analyses. Mean monthly temperature and salinity values were calculated for surface and bottom water. Stratification index (*i.e.* the difference between surface and bottom salinities) was calculated as per Jenkins *et al*. (2010). Each mean physico-chemical value was then described according to regions described by Broadley *et al*. (2017). These regions were Lower Melville Water, Upper Melville Water, Middle Swan Estuary, Lower Canning Estuary and Upper Canning Estuary (see Fig. 1.3.1). Mean daily maximum air temperature and mean rainfall for each sample period were calculated from the Perth Metropolitan and Midland sites in Western Australia (Bureau of Meteorology, 2017), while freshwater flow data were obtained for both the Swan and Canning Rivers from the Water Information Reporting system (Department of Water Western Australia Water Information Reporting, 2017).

Prawns retained in the first year were transported to the laboratory where the carapace length (CL), *i.e.* the distance from the post-orbital margin to the dorso-posterior point of the carapace, (± 0.01 mm), the wet weight (± 0.01 g) and their sex were recorded. A subset of individuals was also measured for total length (TL, n = 797), with measurements taken from the anterior tip of the rostrum to the posterior tip of the telson, and wet weight (see below). Females and males were distinguished by the presence of a thelycum and petasma, respectively. The

presence or absence of a spermatophore on females was recorded. Gravid females, with ovaries showing macroscopic signs of being at the late maturing or mature stages, as described by Tuma (1967) and Crisp *et al*. (2017a), were recorded. Carapace length frequency histograms of gravid females in the nearshore and offshore water in each breeding year (*i.e.* October of one year to March of the following year) were constructed.

The CL and wet weight of a subset of 125 female *M. dalli* were recorded and the ovarian tissue of each individual carefully excised and weighed to ± 0.01 g. The histology of each gonad was then assessed and assigned to one of five stages of ovarian maturity, *i.e.* immature, early maturing, late maturing, mature or post-spawned, as described by Crisp *et al*. (2017a). Twenty five individuals were selected from each stage of maturity, except early maturing prawns (stage 2) to describe the relationship between size and stage of maturity. Note that early maturing females were removed from this analysis due to their potential to either develop to maturity, or regress to an immature state of development.

Size at maturity

The relationship between TL and CL was TL = 3.3189 x CL + 20.058 (r^2 = 0.89, n = 797, p = < 0.001). The CL at 50% maturity (CL_{50m}) was estimated from the relationship between CL and maturity, using a three parameter logistic regression. Stages of reproductive development were characterized as a binary response; with individuals with immature gonads assigned a value of 0, while those with late maturing, mature and post spawned gonad were given a value of 1. This method of determining maturity was modified from the method used by Broadley *et al*. (2017), by excluding females with early maturing gonads, as described earlier. Parameters of the logistic regression were as follows:

$$Y = a/(1+(X/c)^{b}),$$

where Y is the proportion that were mature; X is the carapace length in millimeters; a is half the distance between the upper and lower asymptotes of X; b is the slope of the curve at a; and c is the point of inflection of the curve.

Analysis of density data

The numbers of males and females caught in each nearshore and offshore trawl were standardized to numbers per 500 m^{-2}, to allow for uniform comparison between sites. The density of females 500 m^{-2} that were considered reproductive, *i.e.* CL_{50m}, were $\geq CL_{50}$ and had late maturing or mature ovaries macroscopically, was calculated. Preliminary analysis showed that gravid females were found only in the six months between October and March (see also Broadley *et al*., 2017), thus these months were defined as the breeding period.

Initially, four-way Analyses of Variance (ANOVA) tests were used to determine whether the density of gravid females differed significantly among Years (fixed and three levels; 2013/14, 2014/15 and 2015/16), Months (fixed and six levels; Oct, Nov, Dec, Jan, Feb and Mar) and Subregions (fixed; 9 levels for the nearshore and 8 levels for the offshore, see Fig. 1.3.1), with Sites nested within Subregions (random). Before analysis, the distributions of the density data were examined to determine whether any transformations were needed Field (2009). This showed that no transformations were required Separate ANOVAs were undertaken for the densities of gravid females from nearshore and offshore waters. As the Site within Subregion term was not significant (*i.e.* $p > 0.05$) in both the nearshore and offshore analyses, this term was removed and the analyses were re-run as three-way ANOVAs, with Year, Month, and Subregion as the main effects.

Two-way Generalized Linear Models (GLMs) were carried out separately on the densities of gravid females in nearshore and offshore waters to determine whether water temperature, salinity and/or stratification index accounted for a significant component of variation additional to that provided by Month and Subregion. As earlier ANOVA analyses had demonstrated that there was a significant and influential Year effect and the restocking program potentially influenced densities of *M. dalli* (see Results), the data for each year were analysed separately. Data for surface water temperature and salinity from the adjacent offshore sites were used as a surrogate for those in nearshore environments. The stratification index was included as a covariate in the GLMs of densities in offshore waters only. The relative influence of each term in the model was determined by the size of the mean square values and its percentage

contribution to the total mean squares. Significant terms in each GLM with percentage mean squares below 10 % were considered low predictors and not investigated further. Where more than one significant term was found, Akaike's Information Criterion (Akaike, 1973) was used to determine the most parsimonious model to describe the factors that most influence densities of gravid *M. dalli*.

Estimation of fecundity

The fecundities of 35 mature individuals of *M. dalli*, ranging in CL from 18.1 to 27.1 mm and from 5.88 to 12.23 g in wet weight, were estimated by gravimetric analysis of gonadal tissue. Subsamples of gonad, weighing 0.1-0.4 mg, were taken from gonads at an anterior lobe, the first abdominal segment and the last abdominal segment above the anus, following (Crocos and Kerr, 1983). Each subsample was then weighed to the nearest 10 µg and the oocytes counted under a compound microscope at 40 X magnification. Counts of the number of ova per gram were then compared among the three sections using one-way ANOVA to test whether the number of ova varied throughout the gonad, before estimating the mean fecundity per gram of gonad. This test demonstrated that the number of oocytes did not differ significantly among regions of the gonad ($F_{2, 104} = 0.258$; $p = 0.773$) and thus the fecundity per gram of gonad was calculated for each individual from the mean of all three sections.

The fecundity of each gravid *M. dalli* caught in this study was estimated as follows. Firstly, the relationship of carapace length (mm) to wet weight (g) was estimated from 1,366 Stage 4 (*i.e.* mature) females and used to estimate the wet weight of all gravid females caught by each method. The relationship between gonad weight (g) and wet weight (g) from 25 individuals from each of five different ovarian developmental stages (see above) was then used to estimate the gonad weight of the gravid females. Finally, the mean fecundity per gram of gonad weight for 35 mature stage females was calculated. This was then combined with the estimated gonad weight, to estimate the fecundity of maturing females at a given carapace length.

The following relationships were determined:

(1) total wet weight in grams (y) and CL (mm) (x) of females:

$$y = 0.0037x^{2.4445}; r^2 = 0.97, n = 1,366$$

(2) gonad weight in grams (y) and total wet weight in grams (x):

$$y = 0.0005x^{3.089}; r^2 = 0.69, n = 125, \text{ and}$$

(3) mean fecundity per gram of gonad weight (± 95 % confidence limits):

$$80,000 \pm 7,500, n = 35.$$

The CLs ranged from 18.1 to 27.1 mm and wet weights from 5.88-12.23 g, which equated to a fecundity range of 34,000 (18.1 mm CL) to 132,000 ova (27.1 mm CL) per individual.

The fecundity of the population was then estimated for nearshore and offshore waters in two steps. Firstly, mean egg production was calculated for each month in the breeding period. This was done by estimating the total eggs produced for each gravid female caught, based on CL, and converting carapace length to an estimate of wet weight. This was then converted to gonad weight and number of eggs produced. The total number of eggs produced by gravid females in each trawl was standardized to the egg production 500 m^{-2}, with the fecundity over the course of each breeding period (annual egg production) calculated by sequentially adding each monthly estimate of egg production 500 m^{-2}, from October to March for each breeding period. Note that the monthly estimates of egg production assumes that each gravid prawn recorded will spawn before the next new moon ~28 days later. This assumption was made on the basis that *M. dalli* females of similar ovarian condition spawned within ~48 h of transfer to an aquaculture facility, without the need for eyestalk ablation (Jenkins *et al*., 2017).

Results

Environmental conditions

Monthly mean maximum air temperatures ranged from 18.5 to 33.2 ˚C, with lower temperatures (< 20 ˚C) recorded in months between June and August (Fig. 1.3.2a). Monthly mean maximum air temperatures were much higher between October and November of 2015 (27 to 29 ˚C) and 2013 (24 to 30 ˚C), than in the same months of 2014 (25 to 26 ˚C). Most rain (72-86%) fell between May and November (Fig. 1.3.2a), with greatest rainfall occurring from July to August, except in 2013 when September had the second highest monthly rainfall. The total annual rainfall in 2015 (578 mm) was lower than in the previous years (704 mm in 2014 and 621 mm in 2013). The freshwater discharge into the Swan River was much greater than that recorded in the Canning River (Fig. 1.3.2b), accounting for 84 % of the mean fluvial flows into the lower Swan-Canning Estuary from April 2013 to March 2016. The mean daily flows in September 2013 (111 GL) was the highest recorded this study, though the month of peak discharge varied among years; September in 2013, July in 2014 and August in 2015. Freshwater discharges were very low (< 5 GL per month) between November and April each year. In 2015, the year of lowest annual rainfall (609 mm), August was the only month when flows exceeded 10 GL (Fig. 1.3.2b).

Water temperatures were highly seasonal, with lowest means in the austral winter and highest means in summer each year. The greatest variation in mean surface water temperatures was observed in the Upper Canning region, ranging from ~12 (June 2014) to ~28 ˚C (February 2015), while the highest mean (~30 ˚C) occurred in the Middle Swan (February 2016) (Fig. 1.3.3a, b). Mean surface water temperatures for October 2015 in the Middle Swan, Lower Canning and Upper Melville (~23 ˚C) were higher than for the same month in 2014 (~21 – 22 ˚C) and 2013 (20 ˚C; Fig. 1.3.3a). Similar seasonal trends were observed in bottom water temperatures, though winter temperatures were warmer than in the surface waters, with the Middle Swan ranging from ~15 (July 2014) to ~29 ˚C (February 2016; Fig. 1.3.3b).

Mean surface water salinity was also highly seasonal, ranging from ~ 2 (July 2014) to 38.4 (March 2013) in the Upper Canning. Surface salinities < 15 in the Upper Canning persisted for

longer in 2014 (6 months; May to October) than in 2015 (3 months; August to October) (Fig. 1.3.3c). Mean surface salinities started rising in September/October each year until December, when they were ~ 30 in the Upper Canning and ~ 35 in Lower Melville Water. Salinities in the Lower and Upper Melville Water were consistently higher than those in the Canning and Middle Swan (Fig. 1.3.3c). Bottom salinities followed the same pattern as surface salinities but did not fall as low (~ 10; Middle Swan, July 2014), or remain low as long as those on the surface (Fig. 1.3.3d). Bottom salinities in Lower Melville Water were consistently higher than all other regions and were always > 31 (Fig. 1.3.3d).

The mean Stratification Index (= bottom – surface salinity) ranged from ~ 0 (Lower Canning, February 2014) to ~ 21 (Upper Melville Water, October 2013), with the highest stratification index values (> ~5) occurring one month after the maximum recorded freshwater discharge (*cf* Figs 1.3.3e, 2b). In 2015, the mean stratification index did not exceed 8 in any region (Fig. 1.3.3e). The mean stratification index between December and March each year were < 3 in each region, except for the Middle Swan, which recorded 6.5 in January 2016 (Fig. 1.3.3e).

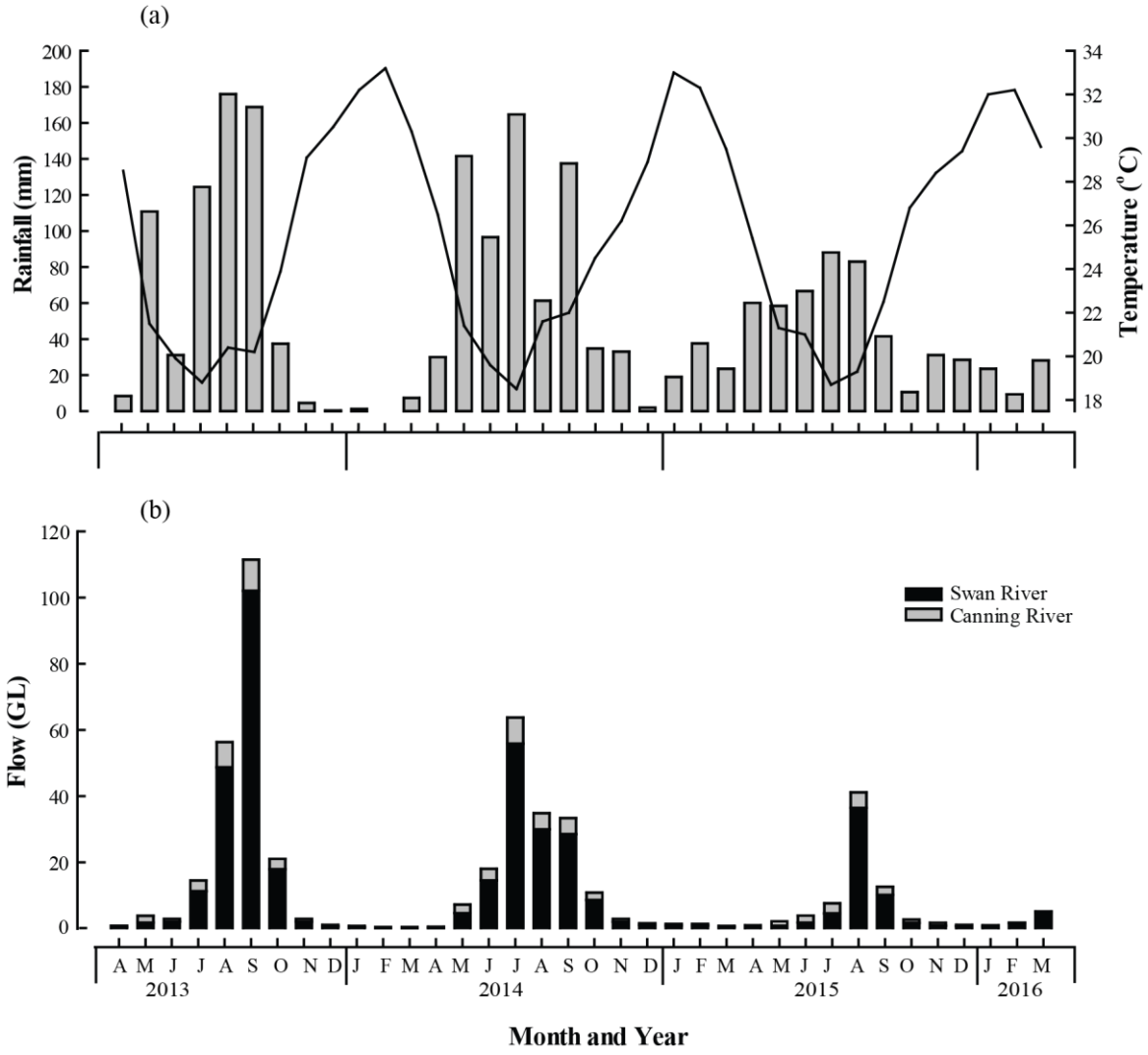


Fig. 1.3.2. Monthly (a) total rainfall (bars) and mean maximum air temperature (line) for Perth, Western Australia. (b) Mean daily fluvial flows from the Swan and Canning rivers into the Swan-Canning Estuary in gigalitres (GL) between April 2013 and March 2016 (Department of Water Western Australia Water Information Reporting, 2017). Rainfall and mean maximum air temperature data sourced from Midland station, Bureau of Meteorology website (Bureau of Meteorology, 2017).

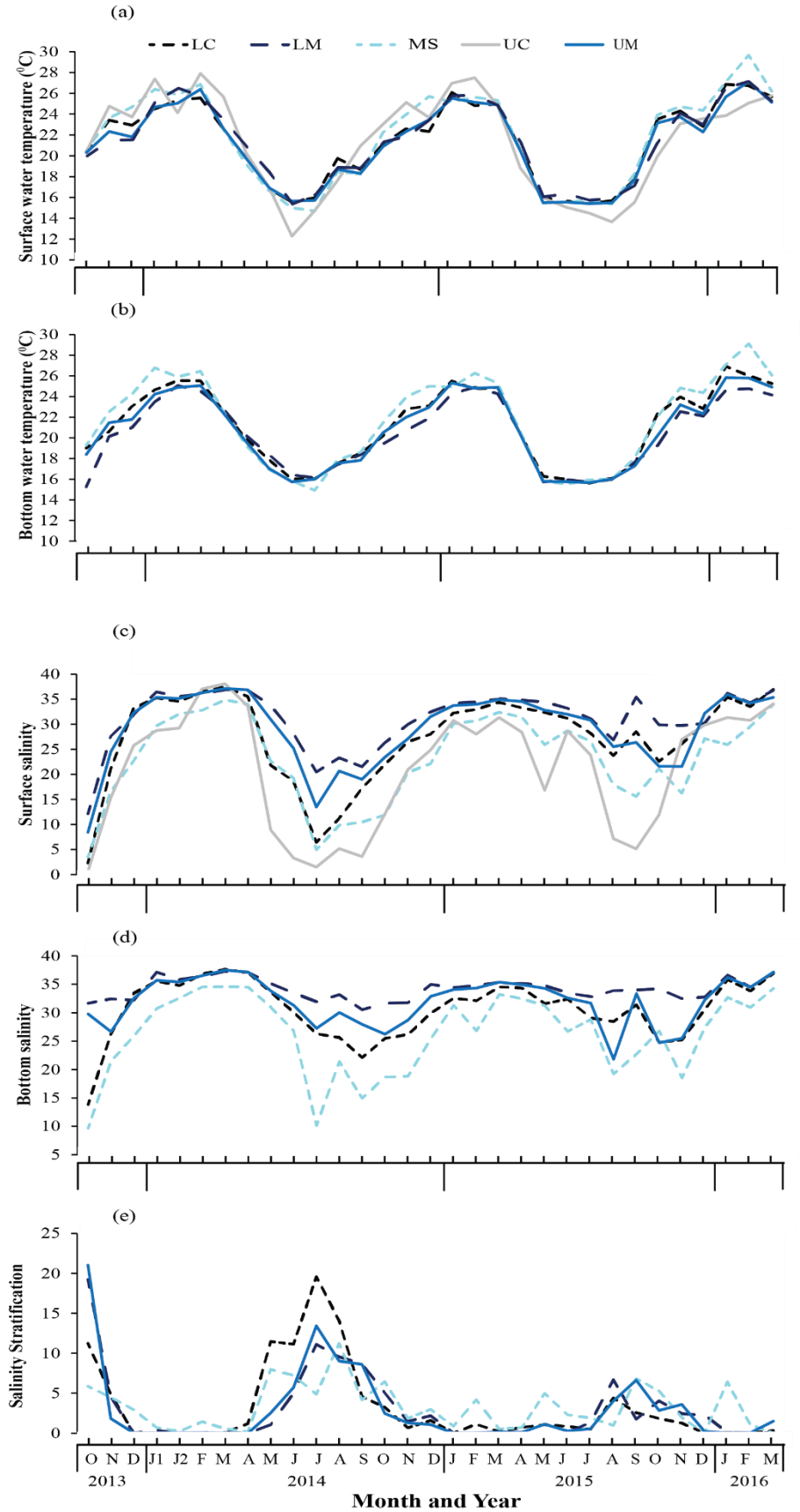


Fig. 1.3.3. Average (a) surface and (b) bottom water temperature; (c) surface and (d) bottom salinity and (e) salinity stratification recorded every 28 days in the Swan-Canning Estuary between October 2013 and March 2016. Mean values of sites are grouped according to five regions defined by Broadley *et al*., (2017), including Lower Canning (LC); Lower Melville Water (LM); Middle Swan (MS); Upper Canning (UC); Upper Melville Water (UM; Fig. 1.3.1). Overall mean and 95% confidence limits all months are provided.

Size of gravid females

The smallest gravid *M. dalli*, *i.e.* females with maturing or mature gonads, had a CL of ~15 mm. The estimated proportion of gravid females increased markedly after 18 mm CL and by 21 mm CL all females were mature, with estimated CL at 50% maturity (CL_{50m} ± 95% confidence limit) of 19.0 ± 1.0 mm (Fig. 1.3.4). The relationship between size at maturity and size in terms of CL was:

$$CL_{50m} = 1.043/(1+(CL/19.03)^{-21.3})$$

The size at maturity of *M. dalli* was similar to that of *M. joyneri* (19.6 mm CL), which were lower than those recorded for other congeners (21 to 31 mm CL) and far lower than those estimated for species in the genus *Penaeus* (32 to 39 mm CL, Table 1.3.1). The smallest mature *M. bennettae* recorded was 14 mm CL, indicating that its CL_{50m} is also likely to be < 20 mm CL.

Carapace length frequencies of gravid *M. dalli*, ranged from 16 to 27 mm, with few individuals > 27 mm. Only 32 gravid *M. dalli* were caught in nearshore waters compared to 199 in offshore waters in 2013/14, however far more were caught in nearshore waters (416) than offshore waters (242) in 2015/16. Greater numbers between 23 – 27 mm were caught in offshore waters than nearshore waters in 2014/15 (Fig. 1.3.5a,b).

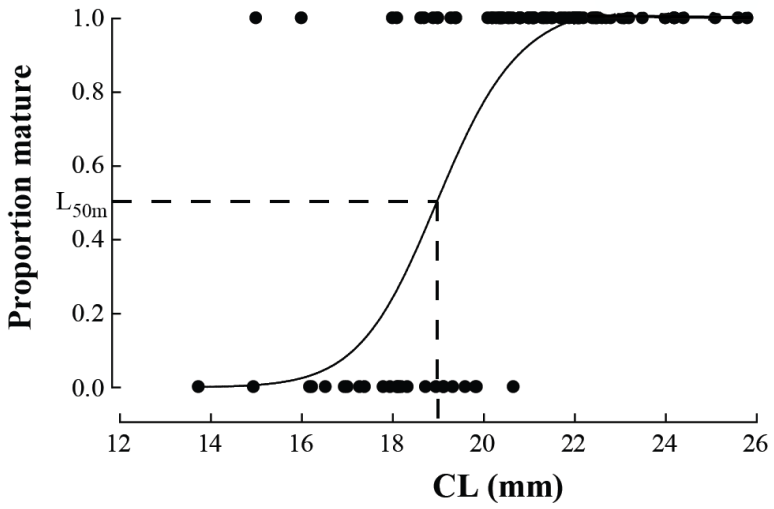


Fig. 1.3.4. Logistic regression curve of the carapace length at maturity of female Metapenaeus dalli collected from the Swan-Canning Estuary between October 2013 and January 2015. Dotted line represents the estimated CL at 50% maturity. N = 100. Females (black dots) with immature ovarian development were assigned a value of 0, while, late maturing, mature and post spawned females were assigned the value 1.

Table 1.3.1. Length at 50% sexual maturity assessed by mature gonads (L50m), insemination rates (L50in) and/or minimum length at maturity (Lmin) of female penaeids in the genera Artemesia, Metapenaeus and Penaeus. Length measures are defined by either total length (TL), carapace length (CL) or body length (BL) in millimetres (mm).

Study	Species	L_{50m}	L_{50in}	L_{min}	Measure
Dumont *et al*., 2011	*A. longinaris*			16.76	CL
Gerami *et al*., 2013	*M. affinis*	27.12			CL
Courtney & Masel, 1997	*M. bennettae*			14	CL
This study	*M. dalli*	18.95		13.74	CL
de Croos *et al*., 2001	*M. dobsoni*	21.3 - 22.3	22.3 - 22.5		CL
Crocos *et al*., 2001	*M. endeavouri*	30			CL
Crocos *et al*., 2001	*M. ensis*	31			CL
Cha *et al*., 2004	*M. joyneri*	19.6	20.4		CL
Chu *et al*., 1995	*M. joyneri*			16.1	CL
Nalini, 1976	*M. monoceros*			118	TL
Nandakumar, 2001	*M. monoceros*			114	TL
Rao, 1989	*M. monoceros*			116	TL
Courtney & Masel, 1997	*P. esculentus*			30	CL
Crocos , 1987	*P. esculentus*	32			CL
Amanat & Qureshi, 2011	*P. Indicus*	133.3			TL
Minagawa *et al*., 2000	*P. japonicus*			130 - 140	BL
Penn, 1980	*P. latisulcatus*			28 / 123	CL / TL
Crocos & Kerr, 1983	*P. merguiensis*			23	CL
Qureshi & Amanat, 2014	*P. merguiensis*	155			TL
Motoh, 1985	*P. monodon*			44	CL
Cha *et al*., 2002	*P. orientalis (chinensis)*	39.19	38.71		CL
Crocos , 1987	*P. semisulcatus*	39			CL

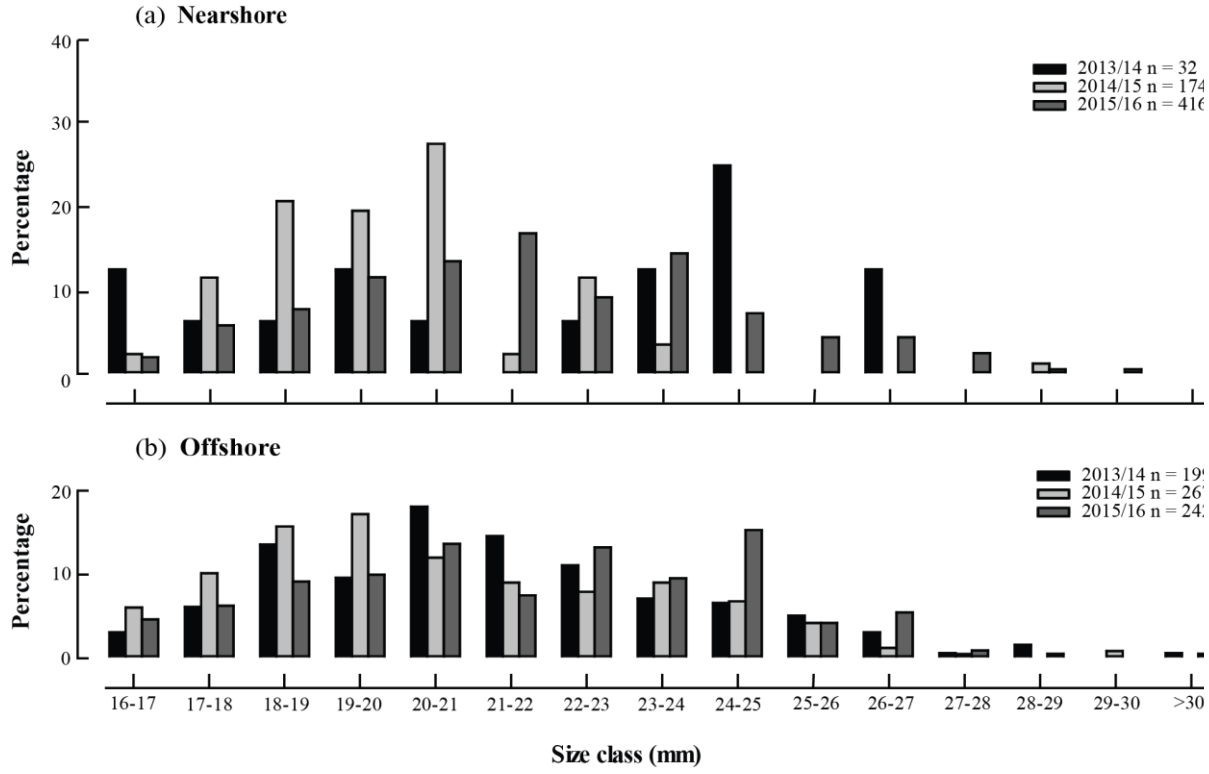


Fig. 1.3.5. Carapace length frequency histogram of gravid *Metapenaeus dalli* recorded in (a) nearshore and (b) offshore waters of the Swan-Canning Estuary in each of 2013/14, 2014/15 and 2015/16.

Abundance and timing of reproduction

Densities 500 m^{-2} of all female *M. dalli* (gravid and non-gravid) were much greater in the offshore than nearshore waters, particularly between February and August. In these nearshore waters, densities were generally greater from October to December than at other times of the year. In contrast, densities were greatest in offshore waters between April and July, with another peak around November (Fig. 1.3.6a). The mean carapace length (CL) of all females during each breeding period was always >14 mm CL. The mean CL was relatively constant and small (~12–13 mm) between April and September each year, increasing to a peak of ~20-24 mm in February/March, before declining rapidly. The mean CL of females in January and February 2014/15 was markedly smaller (~17-20 mm) than in the same months for the other two years (~23-24 mm; Fig. 1.3.6b). Generally, the ratio of female:male *M. dalli* was greater than 1 between April and November and <1 between January and March, particularly in 2016 (Fig. 1.3.6c).

The percentage of females larger than the size at maturity (*i.e.* CL > CL_{50m}) was greater between November and March/April (8.6 to 98.3%) than between April and September (0.7 to 6.9%). The proportions of mature size females were greater during the summers of 2013/14 and 2015/16 (typically > 90 %) than 2014/15 (55-70 %, Fig. 1.3.6d). No females were recorded with either maturing gonads or a spermatophore between April and September of any year, but in the late spring and summer, *i.e.* November to March, the percentage of mature size females that were gravid increased markedly to between 15 and 70 %, while those with a spermatophore increased to between 18 and 48 % (Fig. 1.3.6e,f).

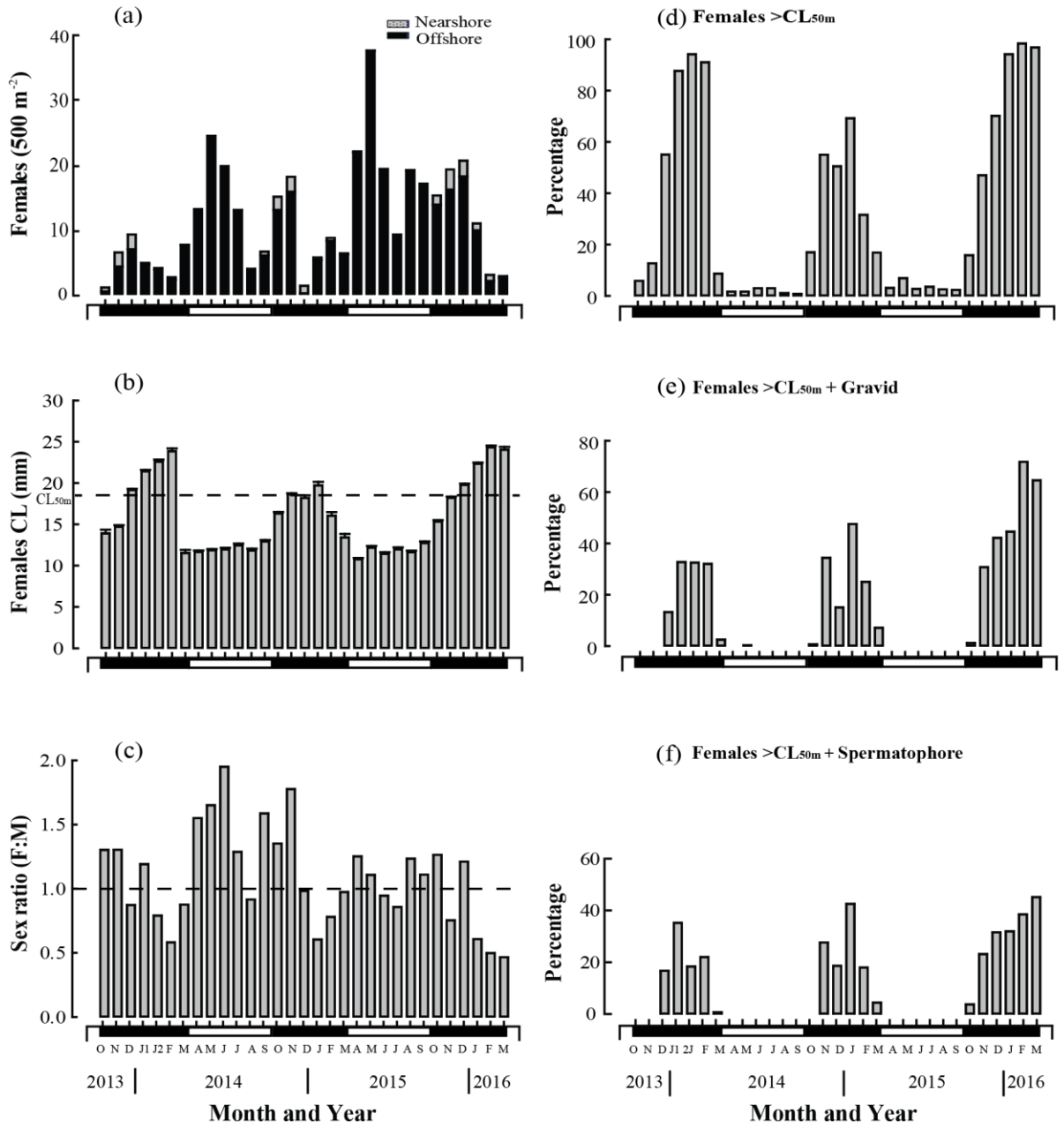


Fig. 1.3.6. (a) Average density of female *Metapenaeus dalli* (500m^{-2}) in nearshore and offshore waters; (b) mean + SE carapace length of females; (c) sex ratio (F:M); percentage of females that are estimated (d) mature (*i.e.* ≥ 18.95 mm CL); (e) mature and gravid and (f) mature and impregnated with a spermatophore recorded in the Swan-Canning Estuary every 28 days between October 2013 and March 2016. Dashed line on (b) represents the carapace length at 50 percent maturity (CL50m; see Fig. 1.3.4) and (c) a 1:1 female:male ratio. Black bars denote month of the year where breeding occurs *i.e.* breeding period.

Densities of gravid females among years, month and regions

A three-way ANOVA demonstrated that densities (500 m^{-2}) of gravid *M. dalli* in the nearshore waters differed significantly among Year, Month and Subregion, with the greatest proportion of variation accounted for by Year (32.4 %), followed by Subregion (20.3 %) and Month (17.9 %; Table 1.3.2a). Although all the interactions terms were also significant, each

interaction accounted for less than 10% of the total variation. The mean densities of gravid *M. dalli* in nearshore waters were greatest in the 2015/16 breeding period (1.5 prawns 500 m^{-2}), followed by 2014/15 (1.1 prawns 500 m^{-2}) and 2013/14 (0.6 prawns 500 m^{-2}; Fig. 1.3.7a). Densities were greatest in November (1.9 prawns 500 m^{-2}), followed by December (1.2 prawns 500 m^{-2}; Fig. 1.3.7b); and highest in the Lower Canning Estuary (3.2 prawns 500 m^{-2}), followed by the Middle Swan Estuary (1.6 prawns 500 m^{-2}). The mean densities in the nearshore of all other regions were less than 0.8 500 m^{-2} (Fig. 1.3.7c). Densities in offshore waters differed significantly among all main effects and all interaction terms. However, Month accounted for by far the greatest proportion of the variation in gravid *M. dalli* in the three-way ANOVA (59.9%), with Year the only other term accounting for 10% of the variation (Table 1.3.2b). The greatest mean densities of gravid *M. dalli* were recorded from the 2014/15 breeding period (1.5 prawns 500 m^{-2}), despite sampling only being conducted in five of the six months (Fig. 1.3.7d). Gravid *M. dalli* were most abundant in offshore waters in November (3.2 prawns 500 m^{-2}; Fig. 1.3.7e), in the Middle Canning Estuary (2.0 prawns 500 m^{-2}; Fig. 1.3.7f).

Table 1.3.2. Mean squares (MS), percentage contribution of mean squares (%MS) and significance levels (p) for three-way crossed ANOVAs on the density 500m^{-2} of gravid female *Metapenaeus dalli* in the (a) nearshore and (b) offshore waters of the Swan-Canning Estuary over three breeding periods from October to March in 2013/14, 2014/15 and 2015/16. *df* = degrees of freedom. Significant differences ($p < 0.05$) are highlighted in bold and those influential factors, *i.e.* a % MS > 10, are shaded in grey.

(a) Nearshore waters	***df***	**MS**	**%MS**	***p***
Main effects				
Year	2	**124.20**	**32.4**	**<0.001**
Month	5	**68.88**	**17.9**	**<0.001**
Region	7	**78.11**	**20.3**	**<0.001**
Interactions				
Year × Month	10	**23.40**	**6.1**	**0.020**
Year × Region	14	**23.88**	**6.2**	**0.005**
Month × Region	35	**32.28**	**8.4**	**<0.001**
Year × Month × Region	70	**22.19**	**5.8**	**<0.001**
Residual	504	10.93	2.8	

(b) Offshore waters	***df***	**MS**	**%MS**	***p***
Main effect				
Year	2	**19.09**	**10.1**	**0.017**
Month	5	**113.48**	**59.9**	**<0.001**
Region	7	**12.31**	**6.5**	**0.010**
Interactions				
Year × Month	9	**9.41**	**5.0**	**0.034**
Year × Region	14	**14.76**	**7.8**	**<0.001**
Month × Region	35	**7.89**	**4.2**	**0.008**
Year × Month × Region	63	**7.81**	**4.1**	**0.001**
Residual	408	4.61	2.4	

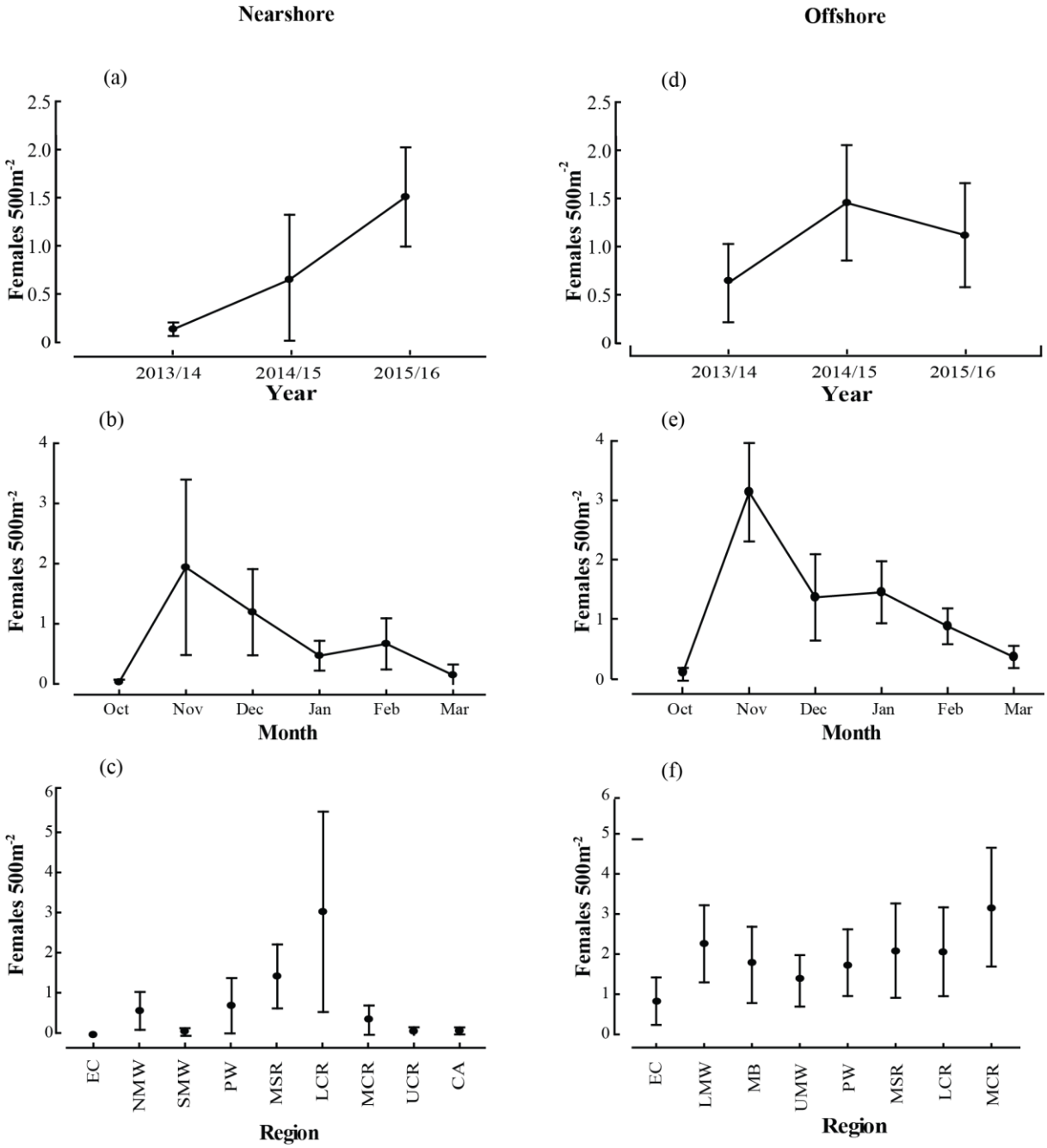


Fig. 1.3.7. Mean density of gravid female *Metapenaeus dalli* 500m^{-2} in (a, d) the breeding period in each year, (b, e) each month of the breeding period (across years) and (c, f) each subregion of the Swan-Canning Estuary (across months and years) in the nearshore (a, b, c) and offshore (d, e, f) waters. Error bars represent ± 95% confidence limits. Subregions in nearshore waters include: Estuary Channel (EC); North Melville Water (NM); South Melville Water (SM); Perth Water (PW); Middle Swan Estuary (MS); Lower Canning Estuary (LC); Middle Canning Estuary (MC); Upper Canning Estuary (UC); Canning Apex (CA). Subregions in offshore waters include: Estuary Channel (EC); Lower Melville Water (NM); Matilda Bay (MB); Upper Melville Water (SM); Perth Water (PW); Middle Swan Estuary (MS); Lower Canning Estuary (LC); Middle Canning Estuary (MC).

In nearshore waters, neither surface temperature, nor surface salinity was significant in two-way GLM models for any year. In 2013/14, no significant factors were found, though it should be noted that the total numbers of gravid *M. dalli* were very low (n = 32) in this year

(Table 1.3.3a). In 2014/15, the Month × Subregion interaction was the only significant term in the two-way GLM (%MS = 26.3, $p = 0.034$; Table 1.3.3b). For the 2015/16 breeding period, Month (%MS = 40.5, $p < 0.001$), Subregion (%MS = 32.7, $p < 0.001$) and the Month × Subregion interaction (%MS = 21.1, $p < 0.001$) terms were all significant (Table 1.3.3c). The Month × Subregion interaction for 2015/16 produced the most parsimonious model (AIC = 1217; AIC full model = 1238).

Table 1.3.3. Mean squares (MS), percentage contribution of mean squares (%MS) and significance levels (*p*) of two-way Generalized Linear Models of density 500 m^{-2} of gravid *Metapenaeus dalli*, caught in nearshore waters of the Swan-Canning Estuary, over three breeding periods from October to March in (a) 2013/14, (b) 2014/15 and (c) 2015/16. Significant differences ($p < 0.05$) are highlighted in bold.

Term	*df*	MS	%MS	*p*
(a) 2013/14				
Water temperature	1	0.20	10.9	0.484
Salinity	1	0.01	0.4	0.890
Month	5	0.41	22.1	0.420
Region	5	0.30	16.1	0.603
Month × Region	25	0.52	28.5	0.180
Residual	130	0.41	22.1	
(b) 2014/15				
Water temperature	1	6.38	3.4	0.675
Salinity	1	2.89	1.5	0.778
Month	5	56.28	29.7	0.177
Region	5	65.42	34.5	0.115
Month × Region	25	**58.51**	**30.9**	**0.044**
Residual	130	36.13		
(c) 2015/16				
Water temperature	1	0.27	0.1	0.876
Salinity	1	3.38	1.5	0.579
Month	5	**97.05**	**42.4**	**<0.001**
Region	5	**73.15**	**32.0**	**<0.001**
Month × Region	25	**54.96**	**24.0**	**<0.001**
Residual	130	10.90		

In offshore waters, bottom water temperature was a significant covariate in two-way GLMs in 2013/14 and 2014/15 (Table 1.3.4a,b), while the stratification index was significant in 2015/16 (Table 1.3.4c). In 2013/14, Month, Subregion, and the Month × Subregion interaction terms were also significant, accounting for 83.8 % of the total variation with bottom water temperature (Table 1.3.4a). The most parsimonious model for the 2013/14 breeding period included water temperature and Month (AIC = 804, AIC full model = 869). In 2014/15, Month and the Month × Subregion interaction terms were also significant, accounting for 78.8% of the total variation with bottom water temperature (Table 1.3.4b). The most parsimonious model included water

temperature and the Month × Subregion interaction (AIC = 723, AIC full model = 781). In 2015/16, Month and Subregion were significant, accounting for 81.0 % of the total variation with stratification index (Table 1.3.4c) and these significant terms were fitted to the most parsimonious model (AIC = 928, AIC full model = 957).

Table 1.3.4. Mean squares (MS), percentage contribution of mean squares (%MS) and significance levels (*p*) of two-way Generalized Linear Models of density 500 m^{-2} gravid *Metapenaeus dalli*, caught in offshore waters of the Swan-Canning Estuary, over three breeding periods from October to March in (a) 2013/14, (b) 2014/15 and (c) 2015/16. Significant differences ($p < 0.05$) are highlighted in bold.

Term	*df*	MS	%MS	*p*
(a) 2013/14				
Water temperature	1	**27.65**	**36.3**	**0.003**
Salinity	1	0.34	0. 5	0.737
Stratification Index	1	8.93	11.7	0.090
Month	5	**24.45**	**32.1**	**<0.001**
Region	7	**6.40**	**8.4**	**0.049**
Month × Region	35	**5.32**	**7.0**	**0.013**
Residual	141	3.07	4.0	
(b) 2014/15				
Water temperature	1	**23.44**	**20.0**	**0.020**
Salinity	1	10.56	9.0	0.116
Stratification Index	1	5.03	4.3	0.276
Month	4	**56.54**	**48.1**	**<0.001**
Region	7	5.07	4.3	0.305
Month × Region	28	**12.59**	**10.7**	**<0.001**
Residual	117	4.20	3.6	
(c) 2015/16				
Water temperature	1	0.85	0.7	0.706
Salinity	1	7.72	6.6	0.258
Stratification Index	1	**41.98**	**36.0**	**0.009**
Month	5	**30.13**	**25.9**	**<0.001**
Region	7	**22.24**	**19.1**	**0.001**
Month × Region	35	7.63	6.5	0.164
Residual	141	5.99	5.1	

Population egg production

The cumulative estimated total number of eggs produced, *i.e.* the population fecundity, for both nearshore (Fig. 1.3.8a) and offshore (Fig. 1.3.8b) waters, increased most from November to February. The estimated number of eggs produced in the nearshore waters was much greater in 2015/16, (163,000 eggs 500m^{-2}), than in either 2014/15 or 2013/14 (34,000, 16,000 eggs 500 m^{-2}, respectively; Fig. 1.3.8a). In offshore waters, the monthly pattern of total egg production showed a similar, pattern to those in the corresponding nearshore waters, with total egg production being greater in 2015/16 than the other years. Most eggs were estimated to be

produced between November and February, with very few eggs produced in October (representing < 3% of total egg production in each breeding year; Fig. 1.3.8b). It should be noted that the total egg production in 2014/15 is an underestimate because of the lack of samples from the offshore waters in December. In 2014/15, the estimated egg production March in the offshore waters was < 10%, but was much greater (22.52 %) in 2015/16.

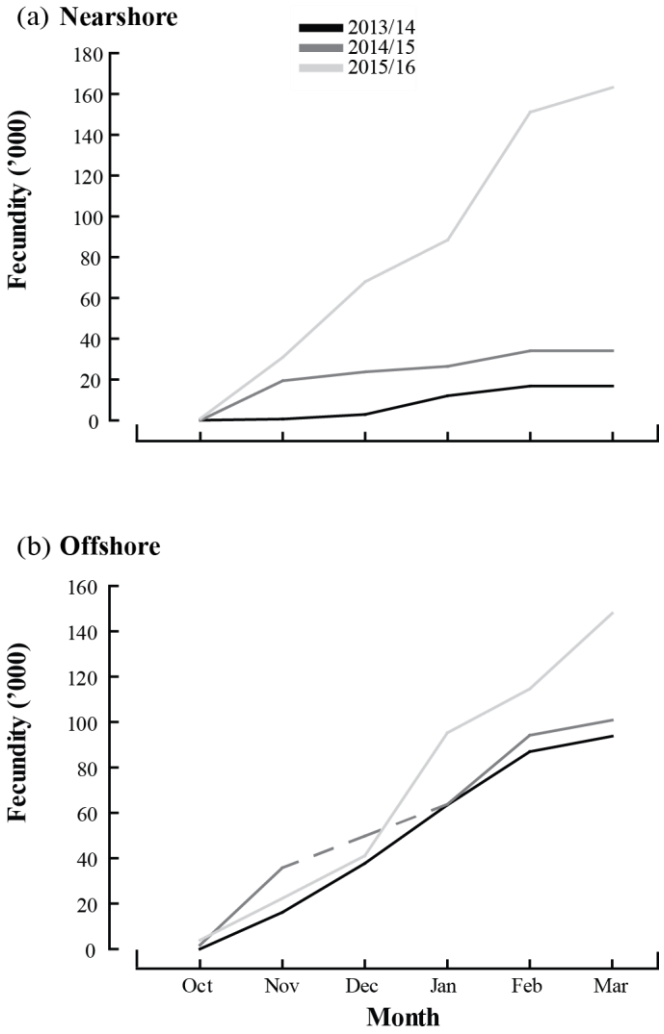


Fig. 1.3.8. Total egg production (cumulative fecundity) by female *Metapenaeus dalli* 500 m^{-2} in (a) nearshore, and (b) offshore waters of the Swan-Canning Estuary in across each month of the breeding period (Oct to March) in 2013/14, 2014/15 and 2015/16. Fecundity is described month-on-month for each breeding period. Dashed line indicates absence of values due to boat breakdown during the month of December 2014.

Discussion

This three year study of the reproductive biology of female *Metapenaeus dalli* in the Swan-Canning Estuary was based on a consistent, spatially comprehensive sampling program in nearshore (< 2 m deep) and offshore (2-17 m deep) waters and supplemented with histological examination of the gonads at different stages of development. Data from this rigorous sampling

regime and studies of gonad histology were used to identify the size at maturity, the timing and location of reproduction and for the first time, estimate the population fecundity for *M. dalli* in this temperate, microtidal estuary. The variation in distribution of gravid females during the breeding season (October to March) and the influence of salinity and temperature on distribution and abundance has also been investigated in each of the three years.

Size at maturity

Female *M. dalli* reach maturity at a size of 19.0 mm carapace length (CL) and an approximate age of 10-12 months in the austral late spring and summer months (November to March). This size at maturity (CL_{50m}) is ~56% of the estimated maximum asymptotic length of 33.6 mm CL (Broadley *et al*., 2017) and ~62% of the size of the largest female caught during the current study (34.7 mm CL). It is slightly larger (~2 mm) than the size CL_{50m} for *M. dalli* estimated by Broadley *et al*. (2017), which estimated the CL_{50m} based on macroscopic examination of the gonads only.

The CL_{50m} estimated for female *M. dalli* in the current study was small compared to many penaeids, although the convention for reporting size at maturity and the size measurements used, *e.g.* carapace length or total length, are not consistent across studies (Table 1.3.1). Metapenaeids such as *M. joyneri* (Cha *et al*., 2004), *M. bennettae* (Courtney and Masel, 1997) and *M. dobsoni* (De Croos *et al*., 2011) had similar CL_{50m} to that of *M. dalli*. Two larger metapenaeids, *M. ensis* (CL_{50m} = 31 mm) and *M. endeavouri* (CL_{50m} = 30 mm), matured at a similar size to *Penaeus* spp. (Crocos *et al*., 2001). Furthermore, *Penaeus latisulcatus*, which co-inhabits the Swan-Canning Estuary with *M. dalli*, has a minimum size at maturity (~ 30 mm CL), similar to the maximum asymptotic length of *M. dalli* (Penn, 1980; Potter *et al*., 1986b; Broadley *et al*., 2017). This indicates that two systematic groups of metapenaeids may exist based on size at maturity.

Seasonal maturation and survival

The absence of gravid *M. dalli* from April to September coincides with periods of almost no growth during the late autumn and winter from April to August (Broadley *et al*., 2017), when water temperatures are < 17 ˚C throughout the estuary. This suggests that 0+ and 1+ individuals *M. dalli* overwinter in the Swan-Canning Estuary (Fig. 1.3.6b,d; Broadley *et al*., 2017). Similarly, overwintering occurred in temperate populations of *M. joyneri* from the west coast of Korea and in *P. latisulcatus* in the marine embayment of Cockburn Sound, adjacent to the Swan-Canning Estuary. In each example, rapid growth occurred when water temperatures continued to increase above 17 ˚C, which lead to a single annual breeding period in these environments (Penn, 1980; Cha *et al*., 2004). This indicates that ~ 17 ˚C acts as a threshold for growth and reproduction in penaeids.

In addition, preference for more stable salinities that occur during spring/summer in the Swan-Canning Estuary may be a significant factor governing the main spawning time for *M. dalli*. During breeding periods, stratification was consistently < 3 in productive regions such as the Lower Canning Estuary (Fig. 1.3.3e). Reproduction in a congener, *M. bennettae* from eastern Australia, has been shown to benefit more from stable osmotic conditions. Metapenaeid prawns in general are euryhaline, thus salinity preference may be less relevant than for species of *Penaeus* (Preston, 1985; Dall *et al*., 1990). Several solely estuarine fish species found in the Swan-Canning Estuary also spawn at similar periods each year to *M. dalli*, including the gobies *Favonigobius lateralis* and *Pseudogobius olorum* and the atherinid *Craterocephalus mugiloides*, which spawn in the spring and summer months, while the sparid *Acanthopagrus butcheri* and the atherinid *Atherinosoma elongata* spawn slightly earlier from September to November (Prince and Potter, 1983; Gill and Potter, 1993; Sarre and Potter, 1999). The spawning time of *M. dalli* and these fish species allows their fertilized eggs to develop under stable osmotic and low flow conditions and remain within the estuary (Potter *et al*., 2015b; Tweedley *et al*., 2016b).

Early in each breeding period a large proportion of reproductive females are 0+ and growth prior to breeding is critical to annual egg production. In the GLM analyses for each reproductive

season, Month was a significant factor in both nearshore and offshore waters, with gravid females being most abundant in November, except for the nearshore in 2013/14, when low catches were recorded. Early in the breeding period (October to November) of each year, growth increases rapidly in response to the increasing water temperatures, and as a consequence, prawns reach the size at maturity. Water temperatures in these months were low in the 2014/15 breeding period (Fig. 1.3.3a), and the mean female CL was only marginally above the CL_{50} from November until March, leading to lower percentages of gravid *M. dalli* (Fig. 1.3.6b). In contrast, water temperatures in October and November of 2015 were warmer than previous years, allowing females to reach the CL_{50} and spawn earlier than in previous years. It also provides them with an opportunity to mature and release eggs on more occasions than when the size at maturity is reached later in the breeding period. Laboratory studies of *P. semisulcatus* collected from the Gulf of Carpentaria on the northern coast of Australia , have shown that small, younger mature females spawned ~50% less often (36.5 mm CL, 6 months; 0.76 spawnings $month^{-1}$) than larger, older prawns (42.6 m CL, 12 months; 1.47 spawnings $month^{-1}$) (Crocos and Coman, 1997). Since the vast majority of gravid *M. dalli* in each breeding period are 0+ recruits (Broadley *et al*., 2017), their ability to spawn repeatedly during breeding periods is already limited. Consequently, the total population fecundity of *M. dalli* in the Swan-Canning Estuary may be further reduced in years when water temperatures are low.

Spatial variation in maturation

The extent of freshwater discharge and time that salinities start to increase, return to higher values and the water column becomes less stratified water may influence the movement of gravid *M. dalli* between nearshore and offshore waters. For example, densities of gravid *M. dalli* in nearshore waters were much greater than in offshore waters in 2015/16 (Fig. 1.3.7a,d), when freshwater discharge was much lower in August and September 2015, than in previous years (Fig. 1.3.2b). Salinities increased earlier in 2015/16 and the stratification declined faster, leading to greater numbers of gravid prawns in nearshore than offshore waters (Fig. 1.3.3c,f). Conversely, densities of gravid prawns were lower in nearshore than offshore

waters in 2013/14 and 2014/15 when freshwater discharge was greater leading up to the November peak in reproduction.

More stable hydrological conditions of higher salinity, low flow and a well-mixed water column may also influence habitat selection for gravid females within nearshore and offshore subregions (Fig. 1.3.2b, Fig. 1.3.3c,d,e). Densities of gravid *M. dalli* nearshore waters were greatest and more variable in the Lower Canning Estuary than other subregions, while in offshore waters the densities were higher in the Middle Canning Estuary but not markedly higher than other subregions (Fig. 1.3.7c,d). In nearshore and offshore waters, few or no *M. dalli* were found in the Estuary Channel, which is exposed to higher tidal energies than upstream environments. During winter and spring, mean fluvial flows into the Canning Estuary were far lower (3.5 GL month^{-1}) than into the Swan Estuary (21.1 GL month^{-1}). In addition, a weir upstream of the areas sampled in this study is closed by local government authorities in July/August each year to prevent saltwater intrusion into sensitive wetlands upstream, leading to the accumulation of freshwater on the upstream side (Department of Water Western Australia Water Information Reporting, 2017). As a result, fluvial flows into the Canning River are further reduced at the beginning of the breeding period, leading to lower hydrological energy input and more suitable conditions for benthic invertebrates (Twomey and John, 2001; OzCoasts, 2017). In contrast, the Swan Estuary continues to experience far higher fluvial flows until December/January, which can affect several other environmental parameters such as salinity, temperature, sedimentation and turbidity (Tweedley *et al*., 2016b).

Fecundity, size distributions and egg production

Fecundity has been well studied across many species in the genus *Penaeus* (Dall *et al*., 1990), particularly those in tropical habitats, where fecundity can range from 50,000 to 1,300,000 ova per individual (Table 1.3.5). However, little information is available on the egg production of smaller prawn species, such as those from the genus *Metapenaeus*, despite their importance to recreational fisheries and commercial fishing. The number of ova produced by female *M. dalli* increased with increasing body size and was estimated to range from ~34,000 to 132,000 ova

per individual, which is relatively low compared to the larger penaeids (Table 1.3.5). From the nine other documented studies of fecundity of metapenaeids, *M. joyneri*, *M. bennettae* and *M. moyebi* in temperate waters had similar sizes at maturity and number of ova per female to those for *M. dalli* in the current study (Table 1.3.1). Other studies of *M. endeavouri* and *M. ensis* in sub-tropical and tropical climates found larger size at maturity and likely their egg production much greater (assuming a relationship between ovarian size and ovarian weight) than those for the smaller *Metapenaeus* spp. (Table 1.3.1; Courtney *et al*., 1989; Courtney and Masel, 1997; Crocos *et al*., 2001).

The data on size, size at maturity and stage of macroscopic development of *M. dalli* in the Swan-Canning Estuary indicated that the estimated population fecundity was far higher in the 2015/16 breeding period than the previous two years, particularly in nearshore waters. The 2015/16 breeding period had the greatest number and largest sizes of gravid females found over the three years (Fig. 1.3.6b,e). Despite the higher densities of gravid *M. dalli* in offshore waters in 2014/15 than 2015/16, their smaller sizes are probably responsible for the much lower estimate of population fecundity in this breeding period, even with an absence of values for December.

Table 1.3.5. Number of ova, given as the total fecundity per individual for the given size range for metapenaeids. Length measures are defined by either total length (TL) or carapace length (CL) in (mm).

Study	Species	length (mm)	Measure	Ova
This study	*M. dalli*	18.1 - 27.1	CL	34,000 – 132,000
This study	*M. dalli*	80.1 - 110.0	TL	34,000 – 132,000
(De Croos *et al*., 2011)	*M. dobsoni*	32 - 48	CL	37,000 – 641,000
(Enomoto, 1971)	*M. dobsoni*	34	CL	240,000
Courtney, unpublished data (See Dall *et al*., 1990)	*M. endeavouri*	45	CL	761,000
(Cha *et al*., 2004)	*M. joyneri*	20.5 - 30.2	CL	73,185 – 206,131
(Deshmukh, 2006)	*M. kutchensis*	132 - 194	TL	380,000 – 570,000
(Nalini, 1976)	*M. monoceros*	146 - 175	TL	157,800 – 348,300
(Rao, 1989)	*M. monoceros*	113 - 181	TL	51,684 – 402,378
(Mane & Deshmukh, 2007)	*M. moyebi*	68 - 112	TL	38,984 – 182,028

Implications for managing for Metapenaeus dalli *in the Swan-Canning Estuary*

Estimates of population fecundity for *M. dalli* in the Swan-Canning Estuary showed an increase in the number of eggs produced during the 2014/15 and 2015/16 breeding periods. This suggests

that hatchery-reared juveniles of the 0+ cohort from each previous breeding period may have contributed to the spawning stock. However, without being able to distinguish wild and hatchery-reared *M. dalli,* it is not possible to attribute this to the restocking program alone. Because of the small size-at-release, and moulting growth strategy of crustaceans, it is not possible to use physical tags to determine recapture rates of stocked prawns. Non-invasive, genetic markers provide a potential "mark" to distinguish stocked from wild individuals (Bravington & Ward, 2004) and have been developed to some extent for *Penaeus* (*Marsupenaeus) japonicus, Penaeus esculentus* and *P. plebejus* (Jerry *et al*., 2004; Liu and Cordes, 2004; Loneragan *et al*., 2004; Chan *et al*., 2014). However, a greater number of markers would be needed for *M. dalli* than the above three *Penaeus* species, as the current practice for collecting gravid females selects those carrying a spermatophore obtained from wild males and thus the genotype of the fathers of the hatchery-reared prawns is not known. Such a marker could be used to determine whether the higher abundance and egg production during the restocking program can be attributed to the release of hatchery-reared individuals (Bravington and Ward, 2004).

Currently, recreational fishing regulations for prawns in the Swan-Canning Estuary permit fishing in the nearshore waters throughout the year by hand netting only, and a combined prawn catch (*i.e. M. dalli* and *P. latisulcatus*) per person of up to 9 L day^{-1} (Department of Fisheries, 2017). Our results indicate that gravid *M. dalli* move into nearshore waters from October to December where they are exposed to recreational fishing. In previous aquaculture-based enhancements of *Fenneropenaeus chinensis* (formerly *Penaeus orientalis*) in the Shanghai and Zhejiang provinces of China (Wang *et al*., 2006), a lack of natural recruitment was attributed to overfishing and capturing released, hatchery-reared prawns before they spawn. As a result, the fishery was almost totally dependent on aquaculture-based enhancement *i.e.* equivalent to sea ranching or a put-grow-take fishery (Taylor *et al*., 2017a). Thus, protection of gravid *M. dalli* in nearshore waters via a closed season from October until early December would allow significant aggregations of females to reproduce and release eggs in nearshore waters before fishing commences.

1.4. Larval development of the Western School Prawn reared in the laboratory

This study has been published in the Journal of Natural History.

Crisp, J.A., Tweedley, J.R., D'Souza, F.M.L., Partridge, G.J., Moheimani, N.R. (2016). Larval development of *Metapenaeus dalli* (Racek) (Crustacea: Decapoda: Penaeidae) reared in the laboratory, including comparisons with other metapenaeids. *Journal of Natural History*. 50(27-28): 1699-1724. DOI: 10.1080/00222933.2016.1155669.

Summary

The six naupliar, three protozoea, three mysis and first post larval forms of the Western School Prawn *Metapenaeus dalli* Racek, 1957 were cultured in the laboratory. These stages were described in detail and compared to those of other metapenaeids. The ontogenetic development occurred in 12 days at 26 °C, with both the growth rate and morphological patterns of development in *M. dalli* broadly following those recorded for other metapenaeids. Differences were found between *M. dalli* and other metapenaeids at corresponding stages of larval development, with these being the number, location and composition of individual setae and other minor spinal development.

Rationale and aims

The morphological development of many of the ~29 metapenaeids species, which occur exclusively throughout Indo-West Pacific (De Grave, 2014) (De Grave and Fransen, 2011), are yet to be fully described. The larval life of such species is short, less than two weeks depending on rearing conditions but is relatively complex. Larvae metamorphose through three stages, *i.e.* nauplius, protozoea and mysis, before reaching the post larval stage (Dall *et al*., 1990). Moreover, each stage can also include multiple sub-stages, which are accompanied with ontogenetic changes in morphology, swimming and feeding behaviour (FAO, 1978; Dall *et al*., 1990; Jones *et al*., 1997).

Studies of larval morphology have traditionally been combined with laboratory rearing techniques, due to difficulties associated with developing reference material from the natural

environment. Although genotypic identification has been developed in many penaeid species (Vaseeharan *et al*., 2013), and in *Metapenaeus affinis* (Lakra *et al*., 2010) and *Metapenaeus dobsoni* (Mishra *et al*., 2009), this method requires taxonomic identification of reference stock prior to development of genetic markers. However, historical literature is often inconsistent in its reporting of the larval metapenaeid taxonomy required for such identifications. Species such as *Metapenaeus ensis* (Leong *et al*., 1992; Ronquillo and Saisho, 1993), *Metapenaeus monoceros* (Mohamed *et al*., 1979), *Metapenaeus moyebi* (Nandakumar *et al*., 1989), *M. affinis* (Thomas *et al*., 1974; Hassan, 1980; Tirmizi *et al*., 1981), *Metapenaeus joyneri* (Lee and Lee, 1968), *Metapenaeus macleayi* and *Metapenaeus bennettae* (Preston, 1985) and *Metapenaeus brevicornis* (Teng, 1971; Rao, 1979) have their larval development described (or partially described), however, many of these lack descriptions of either whole appendages or the detail in the position, number and arrangement of setae on them. Characterising larval development in detail is crucial both for developing and comparing aquaculture rearing techniques and distinguishing between congeners with overlapping geographical distributions (Rothlisberg *et al*., 1983; Jackson and Rothlisberg, 1994).

Larval development is yet to be described for the Western School Prawn *Metapenaeus dalli,* the distribution of which is known to overlap with ten other metapenaeids namely *M. affinis*, *Metapenaeus anchistus*, *M. brevicornis*, *M. dobsoni*, *Metapenaeus elegans*, *M. ensis*, *M. moyebi*, *Metapenaeus papuensis, Metapenaeus endeavouri* and *Metapenaeus suluensis* (De Grave, 2014). Thus, there is a need to (i) provide a comprehensive, well documented and systematic description of the morphological development of three planktonic stages (nauplius, protozoea and mysis) and the first benthic sub-stage (post larva) of *M. dalli* and (ii) compare and contrast discriminatory features with other previously described metapenaeids.

Methods

Broodstock collection

Ovigerous female *M. dalli* were collected at night from the Swan-Canning Estuary (31°56′50″S 115°54′58″E) in Perth, south-western Australia during March 2014, using a hand trawl net that was 1.5 m high, 4 m wide and constructed from 9 mm mesh. Retained individuals were disinfected with 1 ppm formaldehyde for 30 minutes, then immediately transported to the laboratory and placed in aerated holding tanks overnight (FAO, 1978). Females were stocked into 300 L conical base tanks at a density of up to 40 per tank for one to four days. The tanks were filled with seawater at a salinity of ~33 drawn from a bore through a limestone filter, accessing nearshore marine water, aerated constantly and maintained at a temperature of ~26 °C. The base of each tank was fitted with a fine grate that allowed eggs to pass through, separating them from brood stock to prevent any potential cannibalism. After spawning, eggs were collected on 48 µm and 63 µm screens and rinsed, sub-sampled for counting and re-suspended in 300 L of aerated 1 µm filtered seawater. Egg viability was assessed both visually and by hatch rate, with greater than 75% hatch-rate considered suitable for use in the study (FAO, 2007).

Larval culture

After spawning, hatched nauplii were collected and stocked at 250 nauplii L^{-1} (D'Souza and Kelly, 2000) in three 6 L flat bottom cylinder glass culture vessels with 1 µm filtered seawater. Temperature was maintained at 26 + 0.5 °C by housing culture vessels in 115 L temperature-controlled water baths, heated with pre-calibrated Eheim Jager 150 W aquarium water heaters and monitored with Thermocron TCS temperature loggers every 10 minutes. Salinity was monitored daily using an ATAGO PAL-03S digital refractometer. Vessels were exposed to 3.5 µmol photons m^{-2} s^{-1} white incidence light with 12:12 h light:dark photoperiod. The larval cultures were aerated from the base to provide vertical mixing as well as oxygenation.

When the larvae reached protozoea I, culture vessels were fed two microalgae strains obtained from Australian National Algal Culture Collection, CSIRO Marine & Atmospheric Research

Hobart, Tasmania. A chlorophyte *Tetraselmis chuii* (CS-26) and a diatom *Chaetoceros muelleri* (CS-176) were fed in a daily ration at a density of 3 x 10^4 cells mL^{-1} and 9 x 10^4 cells mL^{-1} respectively. Both cultures were maintained under 12:12 h light:dark photoperiod with 180 µmol photons m-2 s-1 white incidence light from fluorescent lights, in 15 L culture vessels with ambient salinity of ~33 and ~25 °C temperature. Each species was cultured in Guillard's F2 medium, with sodium metasilicate added at 30 g L-1 for the diatom *C. muelleri* (Guillard and Ryther, 1962). To avoid carbon limitation, food grade CO_2 was injected and maintained at a pH of 7.4-7.7. Feed concentration was maintained daily by counting cells from sampled culture water with a neubauer haemocytometer until the post larval stage. The volume of water in the culture vessels was topped up to 6 L daily with a combination of microalgae feed and fresh seawater. The gut contents of the larvae were also briefly examined microscopically to confirm that the algal cells were being ingested (D'Souza and Kelly, 2000).

Sampling frequency, fixing, staining and measurements

Routine sampling commenced when the newly hatched nauplii were transferred from spawning tanks to the 6 L culture vessels and continued every 6 h until protozoea I larvae were observed at ~48 h. Subsequently samples were taken every 24 h until post larvae I were observed after ~12 days. At each sampling period, single 200 mL subsamples were taken from each culture vessel, screened over 43 µm nytal mesh and subsequently fixed in 10% tetraborate-buffered formaldehyde, until staining and mounting occurred.

Fixed samples were initially removed from tetraborate-buffered formaldehyde and placed into polyvinyl lactophenol (PVL) medium (Gray and Wess, 1950) with a few drops of 1% Chlorazol Black E stain for 24-48 h before examination, following the methods described by Rothlisberg *et al*. (1983). Samples were then removed from PVL stain medium and placed into fresh PVL medium on a single concave slide for examination under a Leica Dialux 22 compound microscope. Transverse imagery was taken with a top-mounted Tucsen 9 MP camera and downloaded with TSView 7 software. Images were then traced by hand and scanned for digital

labelling and placement in Figures. For each figure, Adobe Illustrator CS6 was used to refine the images so that the morphological details could be clearly presented. In each sub-stage, a minimum of fifteen individuals were examined to describe morphological features, with ten individuals measured for mean + SD total length and carapace length. Measurements were calculated using the ruler tool in Adobe Photoshop CS6, with calibration determined by images of a micrometer. Total length measures were taken from the most anterior point of the carapace, excluding appendages to the most posterior tip of the tail, excluding spines; carapace length was taken from same anterior point to the posterior end of the carapace.

Identification of taxonomic characters

Stages of larval development and taxonomic characters of individual appendages were identified using descriptions by Leong *et al*. (1992) and by Dall *et al*. (1990). Approximate appearance of larval stages nauplius (Fig. 1.4.1a-b), protozoea (Fig. 1.4.1c-d) and mysis and post larva (Fig 1.4.1e-f) and location of individual appendages with labels is shown below (Fig. 1.4.1). Setae arrangement across appendages was generally given from the distal to the proximal end (outer most to inner most), with the most distal point considered as terminal end. One exception was for the first antennae from the protozoea stage onwards, where it was defined from the proximal to the distal end. This was due to the change in the morphology of the coxa and basis affecting continuity of the descriptions. Where setae were arranged across multiple segments or lobes, arrangement was given as the number of setae per segment. For example, arrangement for a four-lobed appendage was given as a+b+c+d setae from distal to proximal. Descriptions of mandibles involved incisor and molar processes with the number of teeth observed. The position of the incisor and molar processes, along with the position of teeth are also shown in Fig. 1.4.1g. Molars were identified as broad oval-shaped grinding plates covering half of the mandible process, incisors as large claw-like peaks in the process at the extremity to the molar and teeth as smaller bristle-like processes between the incisor and molar process.

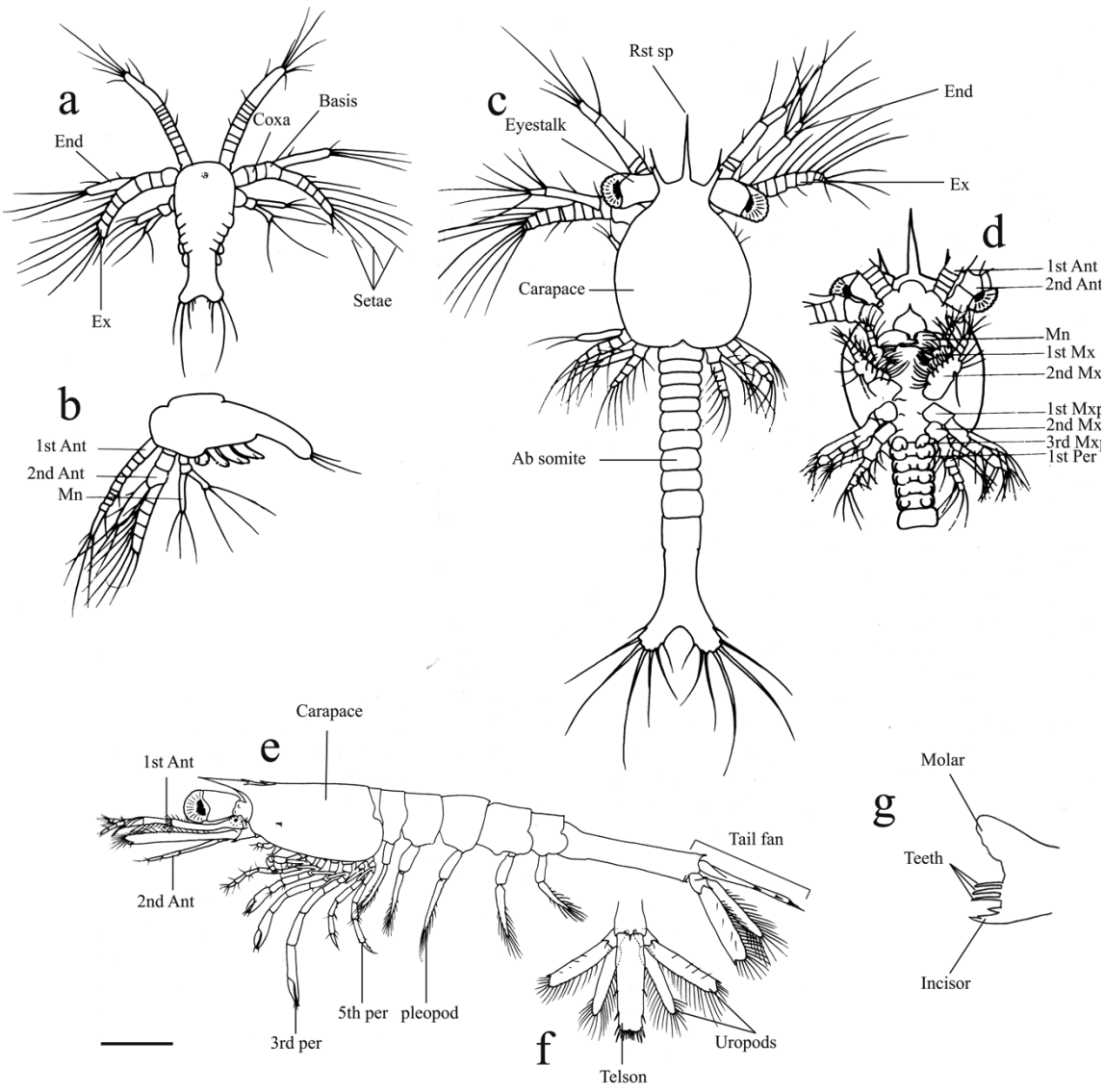


Fig. 1.4.1. Morphological characters of penaeid larval stages adapted from Dall *et al*., (1990); (a) nauplius I dorsal view; (b) nauplius I lateral view; (c) protozoea II dorsal view; (d) protozoea II anterior section ventral view; (e) mysis and post larva I lateral view; (f) mysis and post larval tail fan (g) Right mandible process of protozoea, mysis and post larva sub-stages. Abbreviated labels include: End, endopod; Ex, exopod; 1st Ant, first antenna; 2nd Ant, second antenna; Mn, mandible; Rst sp, rostral spine; Ab somite, abdominal somite; 1st Mx, first maxilla; 2nd Mx, second maxilla; 1st Mxp, first maxilliped; 2nd Mxp, second maxilliped; 3rd Mxp, third maxilliped; 1st per, first pereiopod; 3rd per, third pereiopod; 5th per, fifth pereiopod.

Results

Duration of larval development

The larval development of *M. dalli* comprised six naupliar, three protozoea and three mysis stages before metamorphosing to the post larval stage. The development lasted ~12 days in total under the conditions used in this study, with various time frames for each sub stage and their total and carapace lengths given in Table 1.4.1.

Table 1.4.1. Approximate length and duration of larval sub-stages in the development of *Metapenaeus dalli*, from hatching through to Post larvae I under conditions used in this study (see methods). Nauplius duration is given as time taken to complete all six sub-stages only.

Larval stage	Sub-stage	Approximate duration (days)	Total length (mm ± SD)	Carapace length (mm ± SD)
Nauplius	I		0.300 ± 0.017	
	II		0.302 ± 0.016	
	III		0.310 ± 0.023	
	IV		0.370 ± 0.022	
	V		0.401 ± 0.019	
	VI	2	0.405 ± 0.019	
Protozoea	I	1	0.746 ± 0.034	0.348 ± 0.012
	II	1	1.178 ± 0.091	0.462 ± 0.043
	III	2	1.824 ± 0.069	0.720 ± 0.031
Mysis	I	1	2.422 ± 0.090	0.862 ± 0.027
	II	1	2.557 ± 0.079	0.869 ± 0.028
	III	2	2.810 ± 0.067	0.899 ± 0.027
Post larvae	I	1	2.984 ± 0.060	0.913 ± 0.022

Description of larval development

Nauplius

Morphological development during this stage can be subdivided into six naupliar sub-stages (Figs 1.4.2-7). Both body and appendage forms are relatively consistent, with an eyespot present prior to the development of stalked eyes in protozoea II sub-stage. Identifiable changes are apparent in abdominal shape, mandible protrusion and the number and type of setae. Posterior end morphology is distinct, with a median notch from Nauplius V onwards separating two groups of spines exhibiting bilateral symmetry. Body length also increases through development. 1st antennae consist of a uniramous appendage, 2nd antennae and mandible are both biramous. Each appendage is lightly segmented, often difficult to observe until the protozoea stage.

Nauplius I (N I)

N I (Fig. 1.4.2a) trunk semi-ovoid, anterior half enlarged, posterior end rounded with two spines, all setae are simple.

1st antenna (Fig. 1.4.2b) five segmented, with four terminal setae and seta on 3rd, 4th and 5th segments.

2nd antenna (Fig. 1.4.2c) biramous, coxa two-segmented, basis unsegmented with seta; endopod three-segmented with two terminal setae, seta on 2nd and 3rd segments; exopod four-segmented with three terminal setae, two setae at join to 2nd segment and seta at join to 3rd segment.

Mandibles (Fig. 1.4.2d) biramous, coxa and basis unsegmented; both endopod and exopod two-segmented, each have two terminal setae and seta laterally on 1st segment.

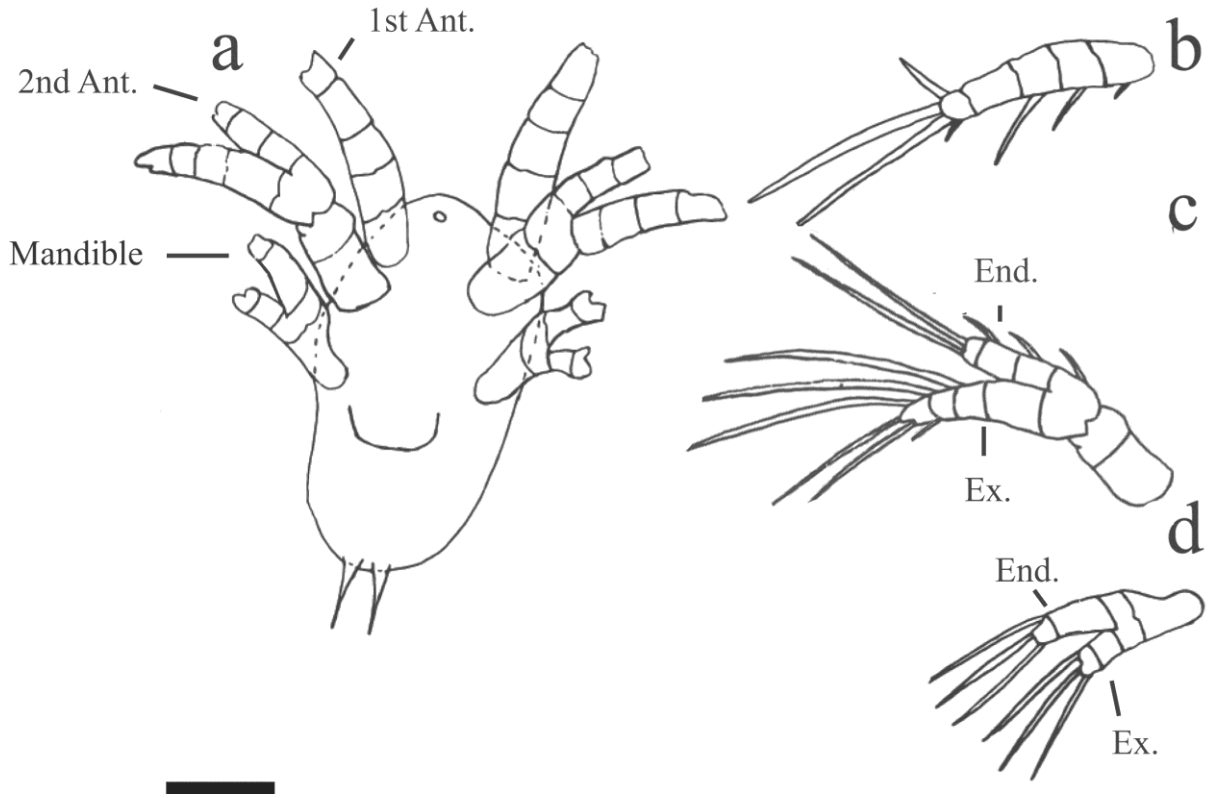


Fig. 1.4.2. *Metapenaeus dalli* Nauplius I ventral view (a); (b) 1st antenna; (c) 2nd antenna; (d) mandible. Endopod (End.) and exopod (Ex.) denoted on 2nd antennae and mandible. Scale bar = 0.1 mm.

N II (Fig. 1.4.3a) trunk similar to N I, with posterior spines outwardly protruding.

1st antenna (Fig. 1.4.3b) has four terminal setae, two setae on 3rd segment, seta on 4th and 5th segment. Longest terminal seta is plumose, all others are simple.

2nd antenna (Fig. 1.4.3c) coxa and basis as per N I; endopod has two terminal plumose setae, simple seta on 2nd and 3rd segment; exopod has three terminal plumose setae and simple terminal seta, plumose seta at joins of 2nd and 3rd segments.

Mandibles (Fig. 1.4.3d) setal position unchanged from N I, setae are plumose.

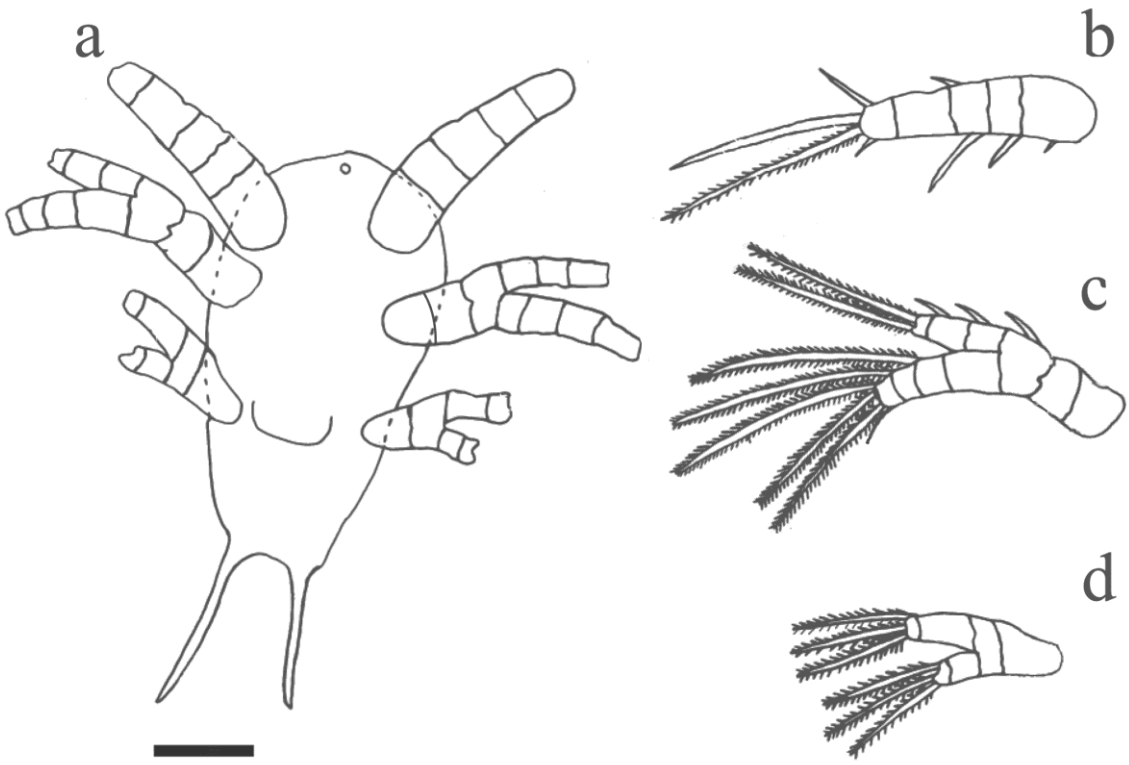


Fig. 1.4.3. *Metapenaeus dalli* Nauplius II ventral view (a); (b) 1st antenna; (c) 2nd antenna; (d) mandible. Scale bar = 0.1 mm.

N III (Fig. 1.4.4a) Posterior has four spines, outer-most exhibiting a claw shape. Inner spines are one third longer than N II.

1st antenna (Fig. 1.4.4b) four terminal setae, longest two terminal setae are plumose, three simple setae on 3rd segment, simple seta on 4th segment.

2nd antenna (Fig. 1.4.4c) coxa, basis and endopod as per N II; exopod has three terminal plumose setae, one is bi-furcated, a simple terminal seta, two plumose setae on 2nd segment and a plumose seta on 3rd segment.

Mandible (Fig. 1.4.4d) Mandibular protrusion forms on basis; endopod and exopod as per N II.

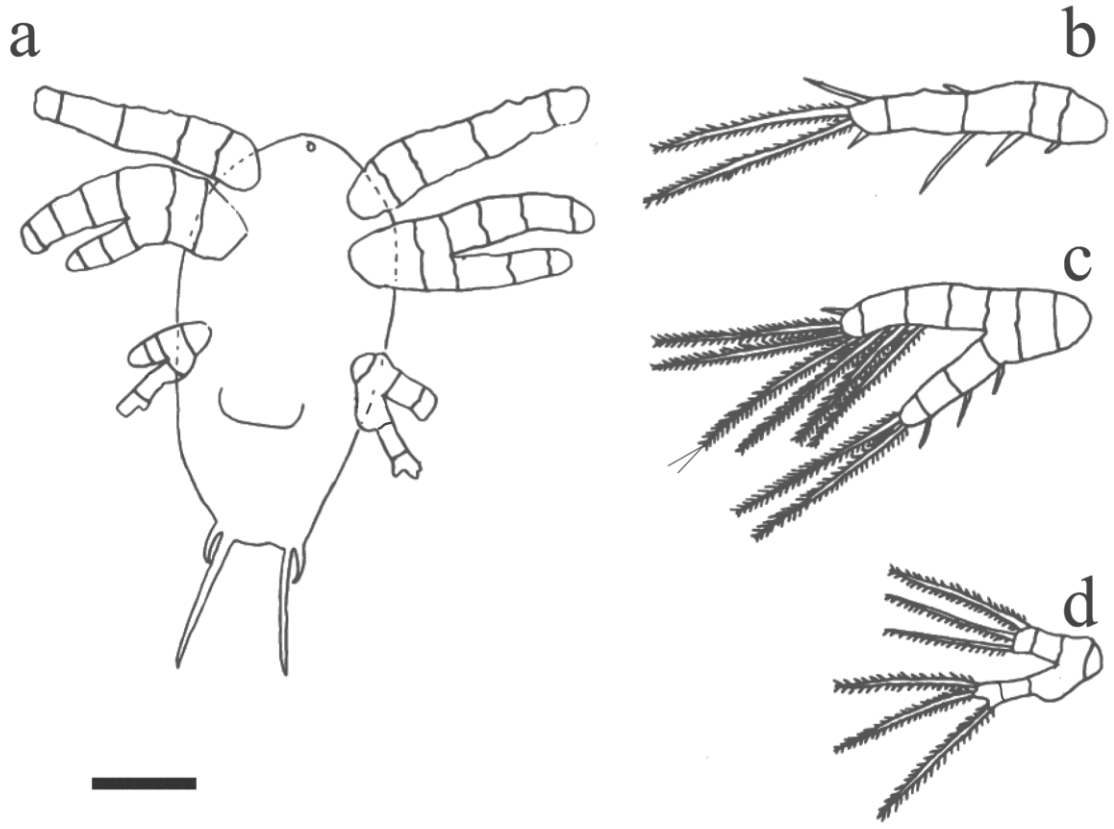


Fig. 1.4.4. *Metapenaeus dalli* Nauplius III ventral view (a); (b) 1st antenna; (c) 2nd antenna; (d) mandible. Scale bar = 0.1 mm.

N IV (Fig. 1.4.5a) trunk is elongated, with two groups of four spines posterior. Two longest spines being twice as long as those in N III.

1st antenna (Fig. 1.4.5b) six segmented, four terminal setae, longest two terminal setae plumose, simple seta at join of 3rd and 4th segment and simple seta on 4th, 5th and 6th segments.

2nd antenna (Fig. 1.4.5c) endopod four segmented, three terminal plumose setae, simple seta at join of 2nd, 3rd and 4th segments; exopod five segmented, three terminal plumose setae, one is bi-furcated, simple terminal seta, plumose seta on 2nd segment, two plumose setae on 3rd segment.

Mandible (Fig. 1.4.5d) Mandibular protrusion now turned inward.

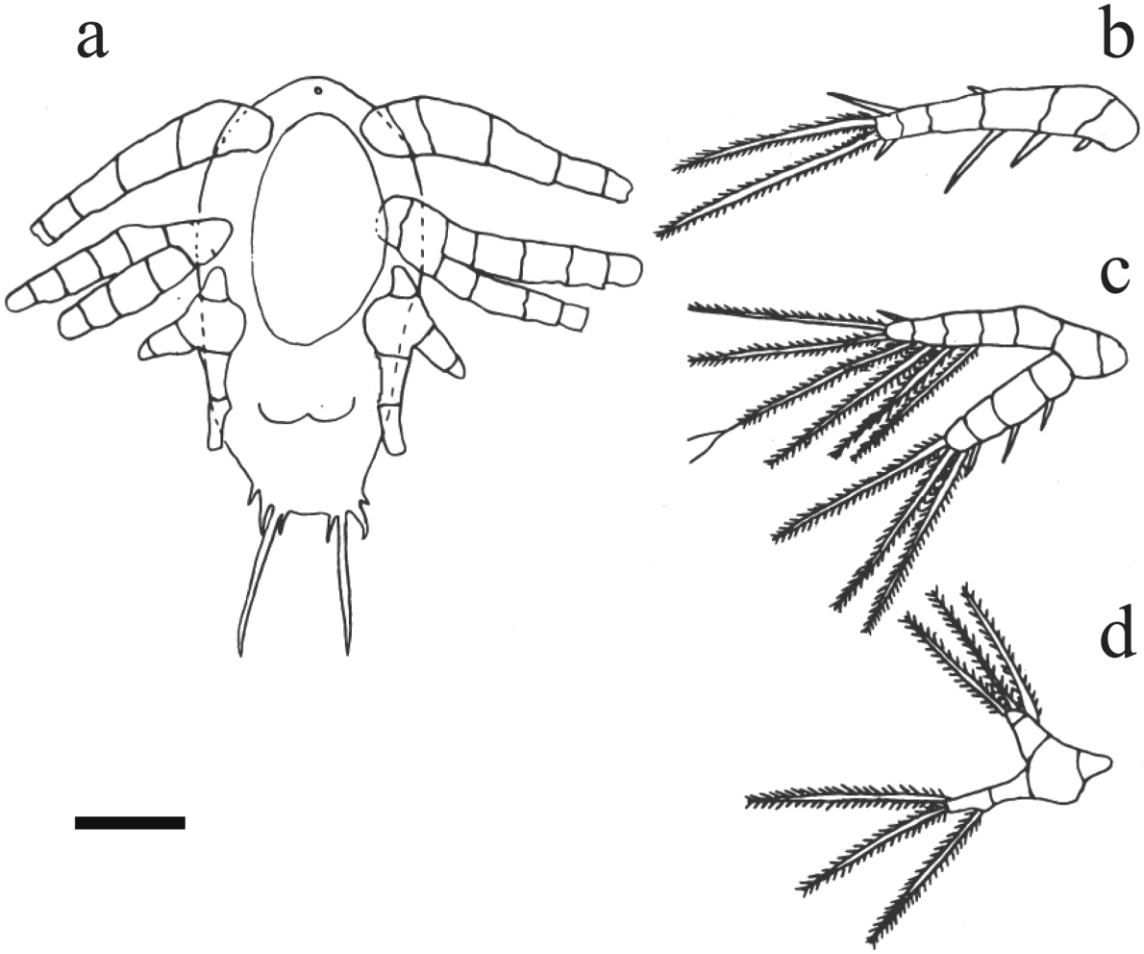


Fig. 1.4.5. *Metapenaeus dalli* Nauplius IV ventral view (a); (b) 1st antenna; (c) 2nd antenna; (d) mandible. Scale bar = 0.1 mm.

Nauplius V (N V)

N V (Fig. 1.4.6a) posterior trunk elongated with median notch forming in the posterior end, separating two groups of spines. Each group has two plumose spines and four that are simple. Abdominal section (*i.e.* below mandible) now exhibits precursory appendages of later stages of development.

1st antenna (Fig. 1.4.6b) has three terminal plumose setae, one simple terminal seta, simple seta at joins of 2nd, 4th, 5th and 6th segments.

2nd antenna (Fig. 1.4.6c) endopod has three terminal plumose setae, one simple terminal seta, simple seta on 2nd and 4th segments; exopod has four terminal plumose setae, one is bi-furcated, plumose seta at joins of 2nd 3rd and 4th segments.

Mandible (Fig. 1.4.6d) unchanged from N IV.

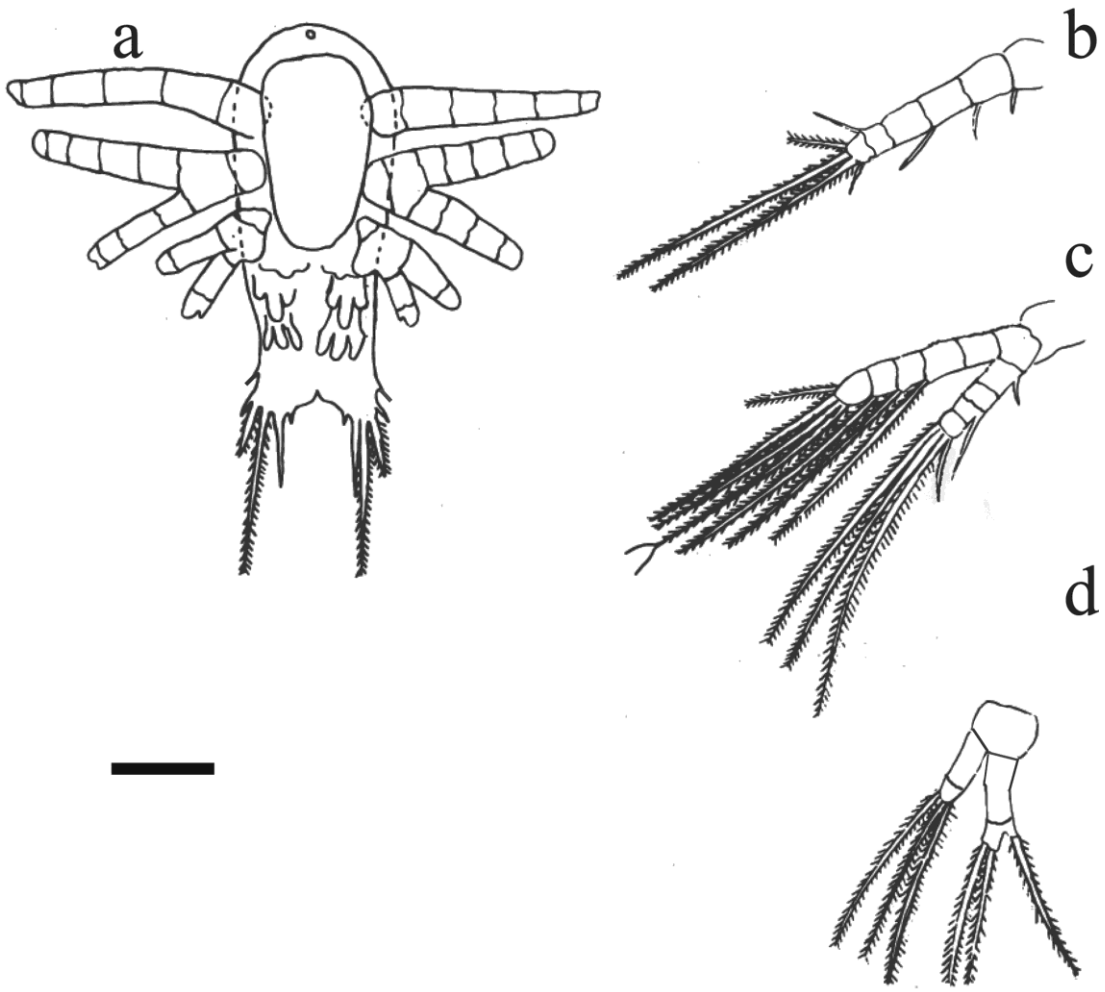


Fig. 1.4.6. *Metapenaeus dalli* Nauplius V ventral view (a); (b) 1st antenna; (c) 2nd antenna; (d) mandible. Scale bar = 0.1 mm.

N VI (Fig. 1.4.7a) trunk as per N V, but with more precursory appendages in the abdominal section. Posterior end now exhibits three terminal plumose spines and three simple spines on each side of the median notch.

1st antenna (Fig. 1.4.7b) has four terminal plumose setae, plumose seta at joins of 2nd, 4th, 5th and 6th segments.

2nd antenna (Fig. 1.4.7c) endopod has three terminal plumose setae, simple seta at joins of 3rd, 4th and basis segments; exopod has two terminal plumose setae, plumose seta at join with 2nd segment, two plumose setae at join to 3rd segment, plumose seta on 3rd and 4th segments, one simple seta on 5th segment. Bi-furcation is now lost on terminal seta.

Mandible (Fig. 1.4.7d) unchanged from N IV.

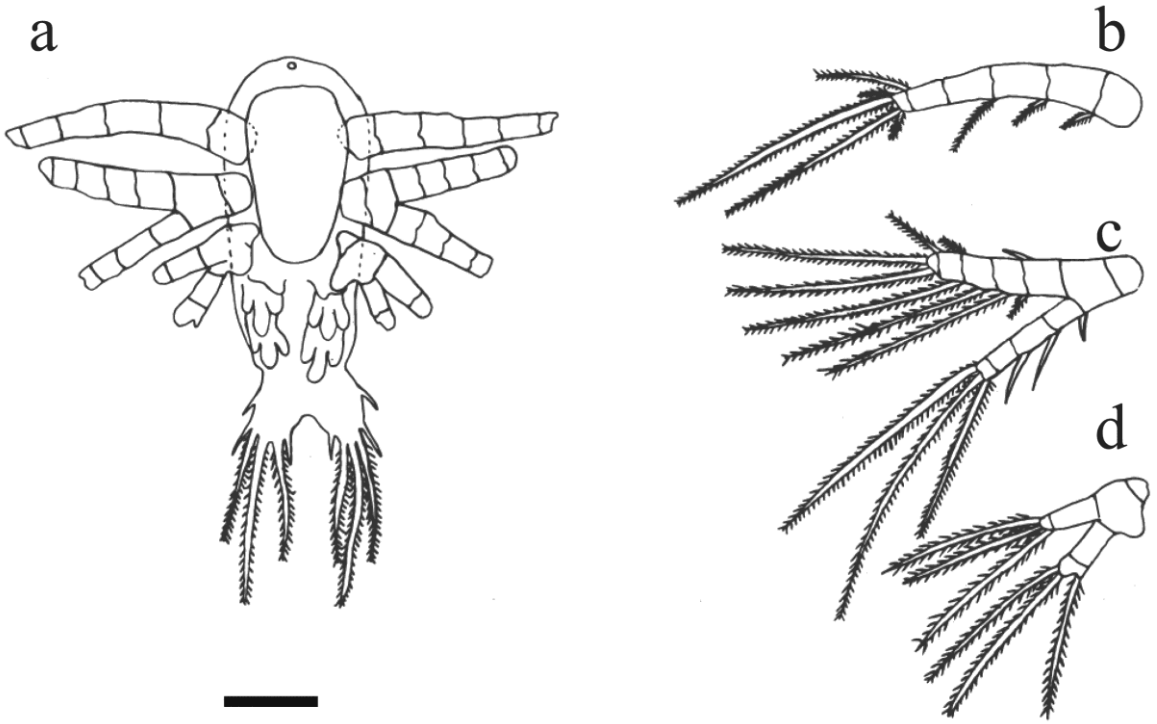


Fig. 1.4.7. *Metapenaeus dalli* Nauplius VI ventral view (a); (b) 1st antenna; (c) 2nd antenna; (d) mandible. Scale bar = 0.1 mm.

Protozoea

The protozoea stage is defined by three distinct sub-stages common to penaeid prawns (Fig. 1.4.8-10). External morphological changes from nauplius are present in the form of a carapace and abdominal somites, with maxillae, maxillipeds and mandibles protected ventrally by the carapace. The nauplius eyespot is replaced by a pair of eyes, sessile in protozoea I, with eyestalks forming in protozoea II. Pereiopods are formed at protozoea III. The telson and number of abdominal somites changes between sub-stages. The 1st and 2nd maxillae and mandibles are partially covered by the carapace and thus are not immediately recognisable in observation. Formation of the mandibular process is important for the switch from endogenous feeding of the nauplius stages to exogenous feeding of marine microalgae from protozoea I. Formation of a rostral spine occurs from protozoea II, and dorso-median spines on the abdominal somites in protozoea III.

Protozoea I (PZ I)

PZ I (Fig. 1.4.8a), rostral spine and eyestalks are yet to form. Seven abdominal somites exist anterior to a telson that appears similar to that of the posterior section of N VI.

1st antenna (Fig. 1.4.8b) is uniramous with coxa, basis and endopod; antenna has three articulations proximal to the coxa, with single seta at the most distal articulation; two setae, one mid-section on the coxa and another at the join to the basis; another one on the basis; endopod has five setae, two of which are terminal; all setae are simple.

2nd antenna (Fig. 1.4.8c) is biramous with protopod, endopod and exopod. Endopod has a single fused segment, with five terminal setae and six setae arranged three in the middle and three near the join to protopod; exopod has ten segments, with four terminal setae, inner seta at each join to the 6th segment and two outer seta at the join to the 4th and 7th segments; setae on the endopod and exopod are plumose.

Mandible (Fig. 1.4.8d) consists of left and right ventral process, located between 2nd antenna and the 1st maxilla. Each process has both incisor and molar processes, with left mandible possessing one tooth and right mandible two teeth.

1st maxilla (Fig. 1.4.8e) has protopod with coxal and basial endites. Coxal endite with seven setae, five are plumodenticulate (leaf-like), flanked by two plumose setae; basial endite with seven cuspidate (pointed) setae, continuing through until mysis III sub-stage; endopod has three segments with five terminal setae and five other in two pairs and a single arrangement from distal to proximal end (hence forth denoted as 2+2+1); scaphognathite is small and knob-like with four setae; exopod and endopod have plumose setae.

2nd maxilla (Fig. 1.4.8f) has coxial and basial endites bi-lobed; coxial endite with 6+2 with plumose setae, basial endite with 2+2 plumose setae; endopod has five segments with three terminal+2+2+2+1 setae. Two terminal setae are simple, the rest are plumose; scaphognathite small and rounded with five plumose setae.

1st maxilliped (Fig. 1.4.8g) has protopod, endopod and exopod; protopod has coxa with five setae and basis with three setae; endopod has five segments with five terminal+2+2+3+3 setae; exopod has four terminal setae, with three setae positioned along the outer margin. All setae are plumose.

2nd maxilliped (Fig. 1.4.8h) has protopod, endopod and exopod; protopod has coxa with three setae and basis two setae; endopod has four segments, with five terminal+2+2+2 setae; exopod has three terminal setae and three positioned along the outer margin. All setae are plumose.

3rd maxilliped (Fig. 1.4.8i) has two segments with two terminal plumose setae.

Telson (Fig. 1.4.8a) has a deep notch with seven spines each side, all of which are plumose.

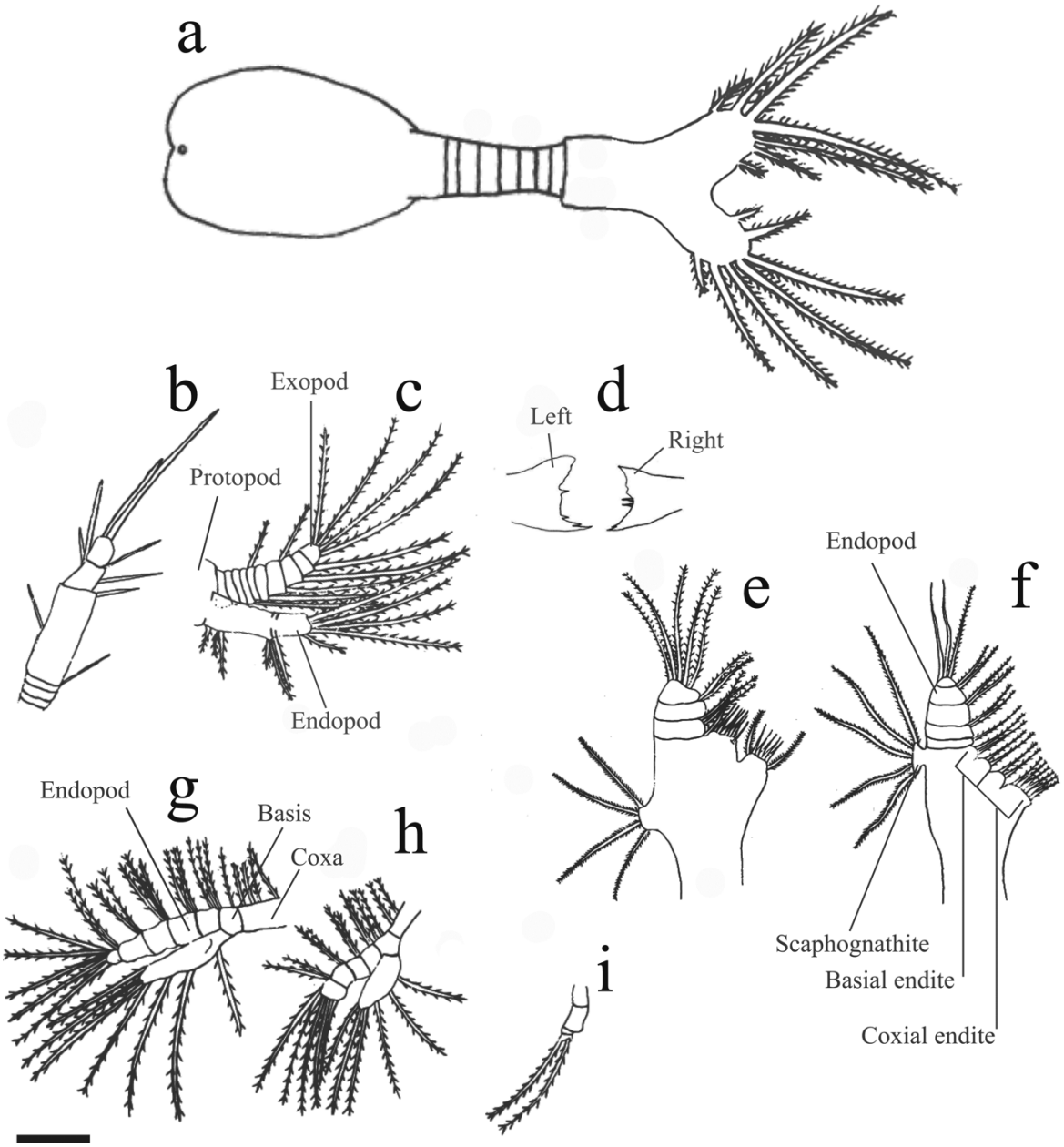


Fig. 1.4.8. *Metapenaeus dalli* Protozoea I dorsal view (a); (b) 1st antenna; (c) 2nd antenna; (d) mandible; (e) 1st maxilla; (f) 2nd maxilla; (g) 1st maxilliped; (h) 2nd maxilliped; (i) 3rd maxilliped. Scale bar (a-c, g-i) = 0.1 mm, (d-f) = 0.05 mm.

Protozoea II (PZ II)

PZ II (Fig. 1.4.9a), rostrum and eyestalks take form; two supraorbital spines are observed; three abdominal somites are added, with one covered by the carapace which begins to extend posterior. 3rd maxilliped now has endopod and exopod (Fig. 9e).

1st antenna (Fig. 1.4.9b) now has four articulations prior to formation of the coxa; a single seta on coxa at join to the most distal articulation; single seta mid-section on the coxa, three at the join to the basis; basis has two setae at join to endopod; endopod has five terminal setae; all setae are simple.

2nd antenna (Fig. 1.4.9c) Endopod now two-segmented, with five terminal +3+2+1 setae; exopod now twelve-segmented, with four terminal setae, inner seta at each join to the 7th segment, outer seta at the 4th and 7th segment; setae on both endopod and exopod plumose.

Mandible (Fig. 1.4.9d) left mandible possesses one tooth and right mandible five teeth.

1st maxilla (Fig. 1.4.9e) is unchanged from PZ I.

2nd maxilla (Fig. 1.4.9f) coxial endite is bi-lobed with 3+7 plumose setae; basial endite tri-lobed with 3+3+5 plumose setae; endopod is unchanged; scaphognathite slightly increased in size.

1st maxilliped (Fig. 1.4.9g) coxa eight setae and basis three setae respectively; endopod and exopod unchanged. All setae are plumose.

2nd maxilliped (Fig. 1.4.9h) coxa two setae and basis three setae respectively; endopod and exopod unchanged. All setae are plumose.

3rd maxilliped (Fig. 1.4.9i) is biramous; both endopod and exopod have three terminal plumose setae each.

Telson (Fig. 1.4.9a) longest spine is now one third longer in comparison to PZ I.

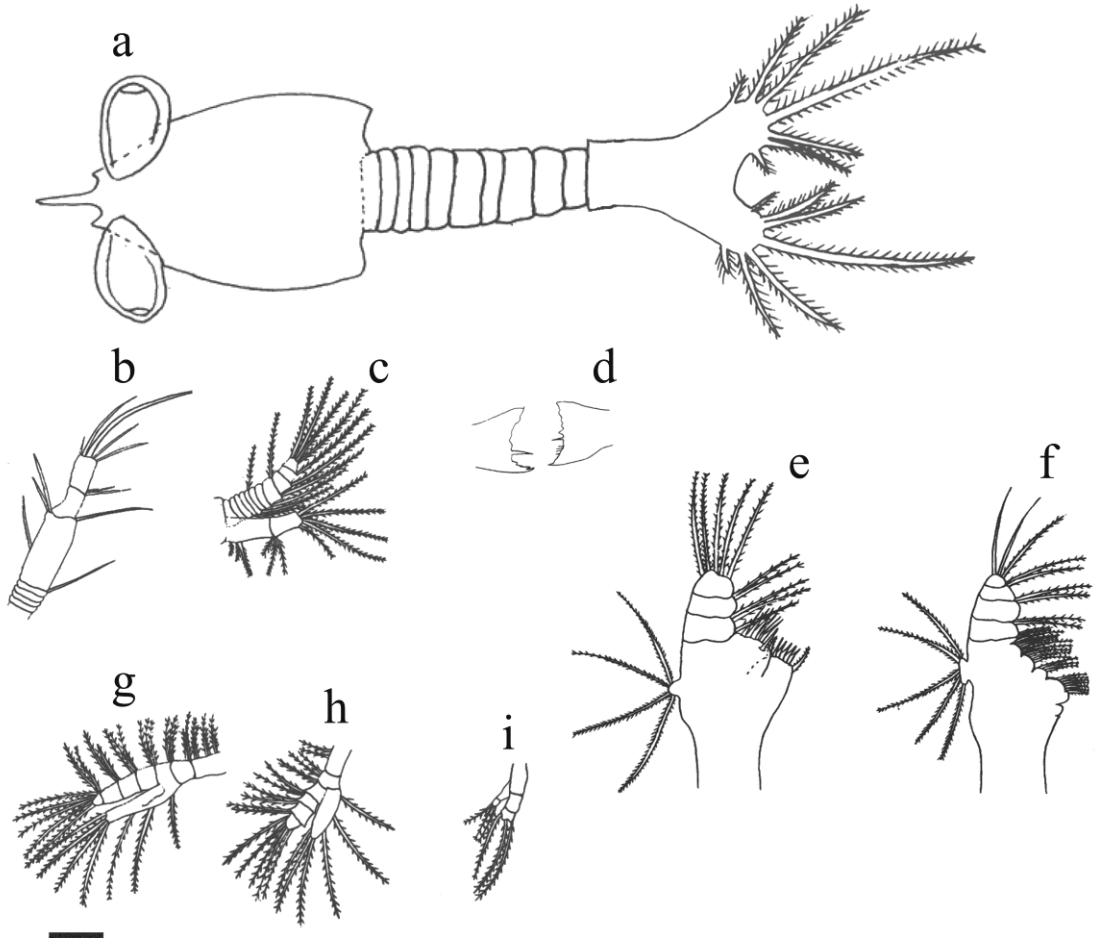


Fig. 1.4.9. *Metapenaeus dalli* Protozoea II dorsal view (a); (b) 1st antenna; (c) 2nd antenna; (d) mandible; (e) 1st maxilla; (f) 2nd maxilla; (g) 1st maxilliped; (h) 2nd maxilliped; (i) 3rd maxilliped. Scale bar (a-c, g-i) = 0.1 mm, (d-f) = 0.05 mm.

Protozoea III (PZ III)

PZ III (Fig. 1.4.10a), rostrum and eyestalks continue to develop; presence of ten abdominal somites ending in a tail fan with a telson and precursory uropods; posterior five abdominal somites have dorso-median spines and the anterior two somites are now covered by the carapace.

1st antenna (Fig. 1.4.10b) coxa has lost its articulations, with two setae at the proximal end of the coxa and one seta half way to basis; basis has five setae at the proximal join and two at the join with the endopod; endopod with five terminal setae. All setae are simple.

2nd antenna (Fig. 1.4.10c) Endopod is unchanged from PZ II; exopod has five terminal setae, with outer seta at joins of the 2nd, 3rd, 4th, 5th, 7th and 9th segments and inner seta at the joins of the 4th and 7th segments; a small tooth-like structure also appears at the proximal end of the exopod.

Mandible (Fig. 1.4.10d) left mandible two teeth and right mandible six teeth.

1st maxilla (Fig. 1.4.10e) is unchanged from protozoea II.

2nd maxilla (Fig. 1.4.10f) coxial endite is bi-lobed with 3+8 plumose setae; basial endite tri-lobed with 3+5+5 plumose setae; endopod is unchanged; scaphognathite is enlarged into a mushroom shape.

1st maxilliped (Fig. 1.4.10g) has coxa with eight setae and basis with six setae; endopod has four segments and five terminal+2+2+3+3 setae; exopod has four terminal setae, four positioned along the outer and one on the inner margin. All setae are plumose.

2nd maxilliped (Fig. 1.4.10h) has coxa with two setae and basis with two outer setae and one inner seta; endopod now has five terminal+2+2+2 setae; exopod has three terminal setae and three setae along the outer margin. All setae are plumose.

3rd maxilliped (Fig. 1.4.10i) endopod has three terminal setae and one seta at the join to the 2nd segment; exopod is two segmented with three terminal setae. All setae are plumose.

Pereiopods (Fig. 1.4.10j) begin to form as five pairs, with each pair positioned ventrally on each of the five anterior thoracic somites; each is rudimentary, biramous and has no setae.

Telson (Fig. 1.4.10a) is now flanked by two pairs of uropods from PZ II; outer pair is major, biramous and located at the dorsal end of the tail section, with six small terminal spines each; inner pair is minor and bare.

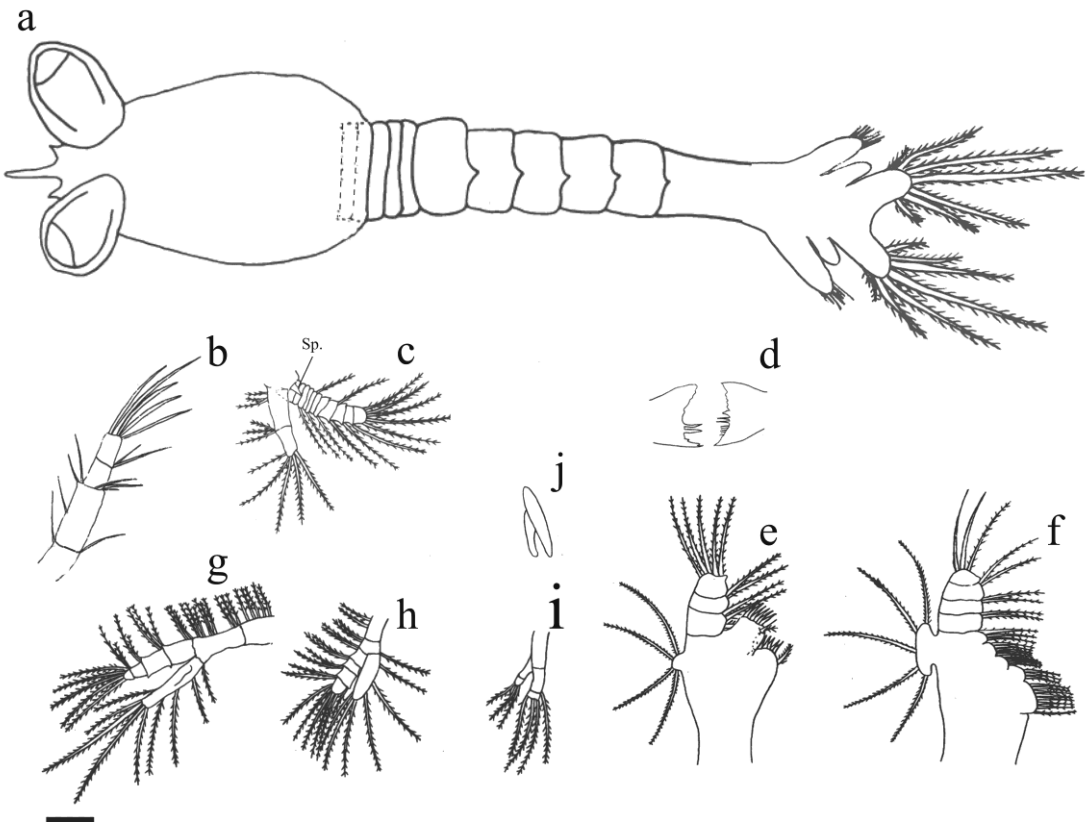


Fig. 1.4.10. *Metapenaeus dalli* Protozoea III dorsal view (a); (b) 1st antenna; (c) 2nd antenna; (d) mandible; (e) 1st maxilla; (f) 2nd maxilla; (g) 1st maxilliped; (h) 2nd maxilliped; (i) 3rd maxilliped; (j) periopod. Scale bar (a-c, g-i) = 0.1 mm, (d-f) = 0.05 mm.

Mysis and post larvae

There are three sub-stages of mysis development (Fig. 1.4.11-1.4.13) prior to the first post larval stage (Fig. 1.4.14). Morphology changes drastically from the protozoea form, beginning to demonstrate precursory features of the adult. Both the carapace and thoracic sections lengthen at each sub-stage, as shown in the measures of carapace and total length. From Mysis I, an antennal spine is formed as a precursor to the rostrum. The posterior section of the carapace extends to cover the first two thoracic somites, including newly-formed pereiopods; exposed thoracic segments have singular dorso-median spines.

From Mysis II, one pterygostomain spine is formed on either side of the anteroventral points of the carapace. All mysis sub-stages have two pairs of antennae, with both distal rami of antennae segmented. In addition, one pair of mandibles, two pairs of maxillae, three pairs of maxillipeds, five pairs of pereiopods and a tail fan with a telson and two pairs of uropods. Of those pereiopods, the 1st three pairs are morphologically similar, with the 3rd pereiopod being the largest. The final two pereiopods are also morphologically similar to each other, thus only the third and fifth pereiopods are described in this section. Five pairs of pleopods appear in the 3rd mysis stage without setae, which develop moderate setation during the first post larval form. A dorso-ventral spine protrudes from the protopod and two more spines over each pair of uropods.

Behaviourally, these forms begin to become increasingly nektonic, eventually becoming capable of capturing prey items such as zooplankton, whilst changing to an omnivorous diet. During these mysis stages they switch from being planktonic and phototactic to increasingly benthic dwellers.

Mysis I (M I)

M I (Fig. 1.4.11a), antennal spine is formed, but without teeth.

1st antenna (Fig. 1.4.11b) uniramous with major and minor rami at terminal end; coxa has three plumose setae and the early formation of an antennal spine on the inner marginal line; basis has three setae at coxa join and one half way to endopod; endopod with three setae at join to basis,

one half way to rami and three at join to rami; minor ramus has two terminal plumose setae, major ramus has seven terminal simple setae.

2nd antenna (Fig. 1.4.11c) has changed markedly from PZ III morphology (Fig. 10b), with a reduction in segmentation and number and composition of setae. Appendage is biramous with protopod and endopod. Exopod flattens from mysis I to become an antennal scale, therefore endopod and exopod results are reversed in text. Exopod has eleven plumose setae positioned marginally; small endopod has three terminal and three simple setae positioned laterally.

Mandible (Fig. 1.4.11d) left mandible three teeth and right mandible seven teeth.

1st maxilla (Fig. 1.4.11e) is unchanged from PZ III (Fig. 1.4.10) apart from the basial endite, which now has ten cuspidate setae, of which one is opposed.

2nd maxilla (Fig. 1.4.11f) coxial endite is bi-lobed with 4+8 plumose setae; basial endite tri-lobed with 2+5+5 plumose setae; endopod four-segmented, with three terminal+2+2+2 setae, two terminal setae are simple; scaphognathite begins to enlarge with eight plumose setae.

1st maxilliped (Fig. 1.4.11g) coxa has five plumose setae and basis seven plumose setae; endopod four-segmented with five terminal + 2+2+3+3 setae, four terminal setae are simple; exopod has four terminal plumose setae and three plumose setae along the outer margin, all are plumose.

2nd maxilliped (Fig. 1.4.11h) coxa six plumose setae and basis three plumose setae; endopod has five terminal setae +2+3+4+4 setae, four terminal setae are simple, arranged as 2+2+3+3 outer setae and 1+1+1 inner setae. This differs from the PZ III unilateral arrangement; exopod has four terminal plumose setae and three setae along the outer margin.

3rd maxilliped (Fig. 1.4.11i) biramous; single simple seta on basis. This appendage changed significantly from PZ III, with additional segmentation and overall size; endopod is five-segmented and has five terminal + 3+1+2+2+2 setae, arranged as 2+0+1+2+2 outer and 1+1+1 inner setae. Plumose seta at the joins of the 4th, 5th and basial segments; exopod two-segmented, with six terminal plumose setae. Plumose seta on the outer and inner margin.

3rd pereiopod (Fig. 1.4.11j) is biramous and single segmented, five terminal plumose setae on endopod and three terminal plumose setae on exopod.

5th pereiopod (Fig. 1.4.11k) is biramous and single segmented, four teriminal plumose setae on the endopod and three terminal plumose setae on exopod.

Telson (Fig. 1.4.11l) now flanked by two sets of uropodsin their adult position dorso-laterally to the telson; outer uropod is two-segmented, with a posterodorsal spine at the join between the two segments on each uropod. Each outer uropod has 11 marginal furcal spines and one posterolateral spine in the out-most position; inner uropod has eight marginal furcal spines; telson with deepening notch in centre and 6+6 furcal spines, with 2+2 spines positioned laterally approximately two thirds posterior.

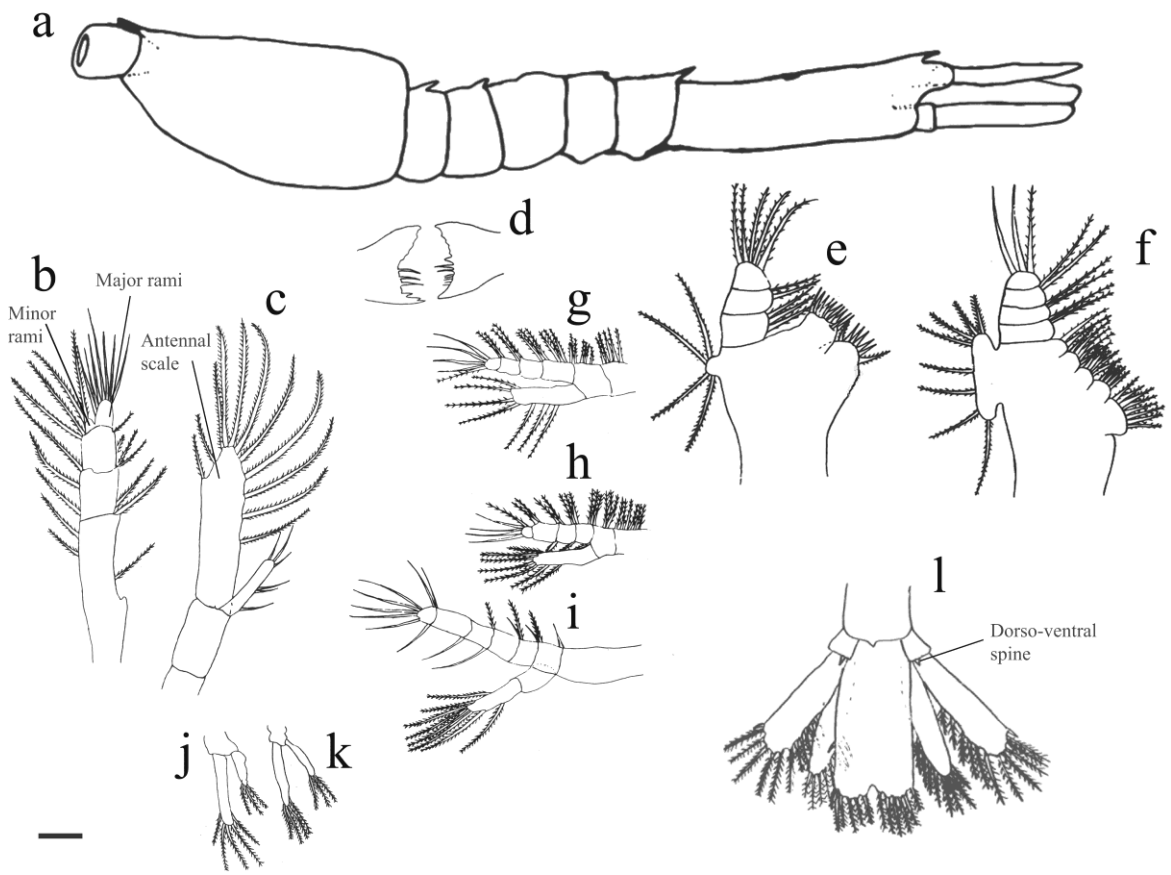


Fig. 1.4.11. *Metapenaeus dalli* Mysis I lateral view (a); (b) 1st antenna; (c) 2nd antenna; (d) mandible; (e) 1st maxilla; (f) 2nd maxilla; (g) 1st maxilliped; (h) 2nd maxilliped; (i) 3rd maxilliped; (j) 3rd pereiopod; (k) 5th pereiopod; (l) telson and uropods. Scale bar (a-c, g-l) = 0.1 mm, (d-f) = 0.05 mm.

Mysis II (M II)

M II, body is more elongated than at M I and pleopods beginning to form, but without structure seen in later mysis sub-stages.

1st antenna (Fig. 1.4.12b) coxa has four marginal plumose setae; basis has six plumose setae at coxa join and two half way to endopod; endopod with five plumose setae at join to basis and five at join to rami; major and minor rami are unchanged from M I.

2nd antenna (Fig. 1.4.12c) protopod develops an antennal spine at the join to the endopod; Exopod has fifteen plumose setae positioned marginally; small endopod has three simple setae along the outer margin.

Mandible (Fig. 1.4.12d) left mandible two teeth and right mandible seven teeth (obscured from view).

1st maxilla (Fig. 1.4.12e) scaphognathite has now lost all setae from M I. Protopod and endopod are unchanged.

2nd maxilla (Fig. 1.4.12f) scaphognathite enlarged with twelve plumose setae.

1st maxilliped (Fig. 1.4.12g) coxa four plumose setae and basis six plumose setae; endopod has five terminal setae+2+2+4+4 setae, with four terminal setae simple. Setae are arranged as 2+2+3+3 outer and 1+1 inner; exopod has six terminal plumose setae and one plumose seta along the outer margin.

2nd maxilliped (Fig. 1.4.12h) coxa has four plumose setae and basis has two; endopod now has five segments with five terminal setae+3+0+3+4+3 setae arranged 2+0+2+3+2 outer and 1+1+1 inner setae. Only the distal inner seta and four terminal setae are simple; exopod has four terminal plumose setae and one plumose seta on the outer margin.

3rd maxilliped (Fig. 1.4.12i) one simple seta on basis; endopod has five terminal+2+1+1+2+2 outer setae and 2+2+1 inner setae, with two outer and two inner setae simple; exopod has six terminal plumose setae and two setae positioned laterally.

3rd pereiopod (Fig. 1.4.12j) endopod has four terminal plumose setae and three plumose setae on the outer margin; exopod is now two-segmented with three terminal plumose setae. Rudimentary chelae begin to form at the distal end of the endopod.

5th pereiopod (Fig. 1.4.12k) endopod has four terminal plumose setae; exopod has three terminal plumose setae; Endopod is now two-segmented.

Telson (Fig. 1.4.12l) unchanged from M I; outer uropods have twelve marginal furcal spines and one posterolateral spine as per M I; inner uropods with nine marginal furcal spines.

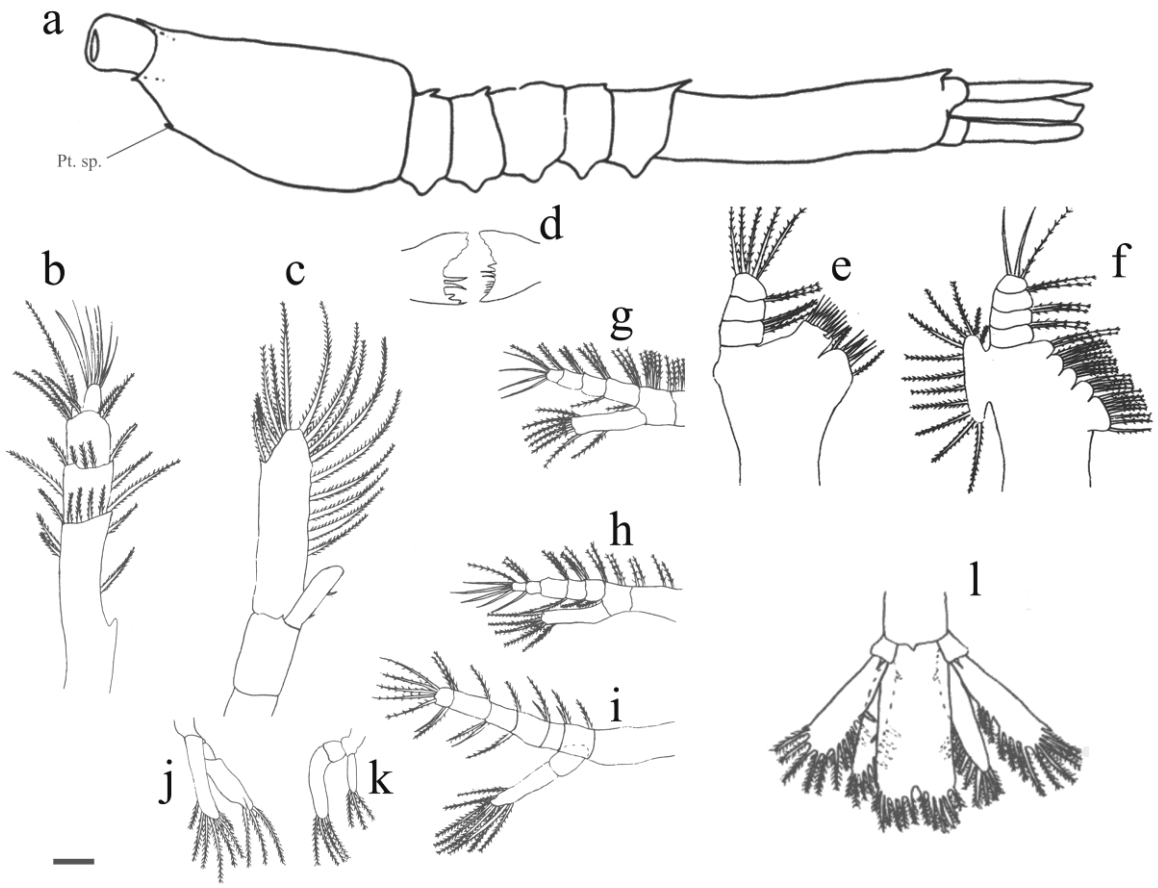


Fig. 1.4.12. *Metapenaeus dalli* Mysis II lateral view (a); (b) 1st antenna; (c) 2nd antenna; (d) mandible; (e) 1st maxilla; (f) 2nd maxilla; (g) 1st maxilliped; (h) 2nd maxilliped; (i) 3rd maxilliped; (j) 3rd pereiopod; (k) 5th pereiopod; (l) telson and uropods. Abbreviated label (Pt. sp.) is pterygostomain spine. Scale bar (a-c, g-l) = 0.1 mm, (d-f) = 0.05 mm.

Mysis III (M III)

M III (Fig. 1.4.13a), body continues to elongate as per mean measures. Pleopods begin to form as five pairs on the five posterior abdominal somites. Rostrum now has an epigastric tooth and a first rostral tooth.

1st antenna (Fig. 1.4.13b) coxa has five plumose setae; basis has seven plumose setae at coxa join and two half way to endopod; endopod with six plumose setae at join to basis and six at

join to rami; minor ramus has three terminal plumose setae, major ramus has seven terminal simple setae.

2nd antenna (Fig. 1.4.13c) exopod has sixteen plumose setae positioned marginally; endopod is now two-segmented with no setae.

Mandible (Fig. 1.4.13d) has a left mandible possessing two teeth and right mandible six teeth.

1st maxilla (Fig. 1.4.13e) coxial endite setae become simple setae.

2nd maxilla (Fig. 1.4.13f) largely unchanged from M I, except for an enlarged scaphognathite with thirteen plumose setae.

1st maxilliped (Fig. 1.4.13g) protopod and endopod are unchanged from M II; exopod has five terminal plumose setae and one plumose seta on the outer margin.

2nd maxilliped (Fig. 1.4.13h) coxa has two plumose setae and basis has four; endopod now has five terminal+3+0+3+4+3 setae arranged as +2+0+2+3+2 outer setae and 1+0+1+1+1 inner setae, four of the terminal setae are simple; exopod has four terminal plumose setae and one plumose seta on the outer margin.

3rd maxilliped (Fig. 1.4.13i) basis has one seta at the endopod join; endopod has 18 setae arranged as five terminal+2+1+1+2+2 outer setae and 2+2+1 inner setae; exopod is two segmented with six terminal plumose setae.

3rd pereiopod (Fig. 1.4.13j) Endopod is two-segmented, with six terminal plumose setae; Exopod is four-segmented with three terminal plumose setae, two outer plumose setae at the 2nd segment join and +one inner (obscured from view) at the join to the 4th segment.

5th pereiopod (Fig. 1.4.13k) protopod has two simple setae; endopod has five segments with five plumose setae arranged one terminal +2+1+1; exopod has five terminal plumose setae.

Pleopods (Fig. 1.4.13l) are two segmented without setae.

Telson (Fig. 1.4.13m) unchanged from M I; outer uropods have fourteen marginal furcal spines and one posterolateral spine; inner uropods with thirteen marginal furcal spines.

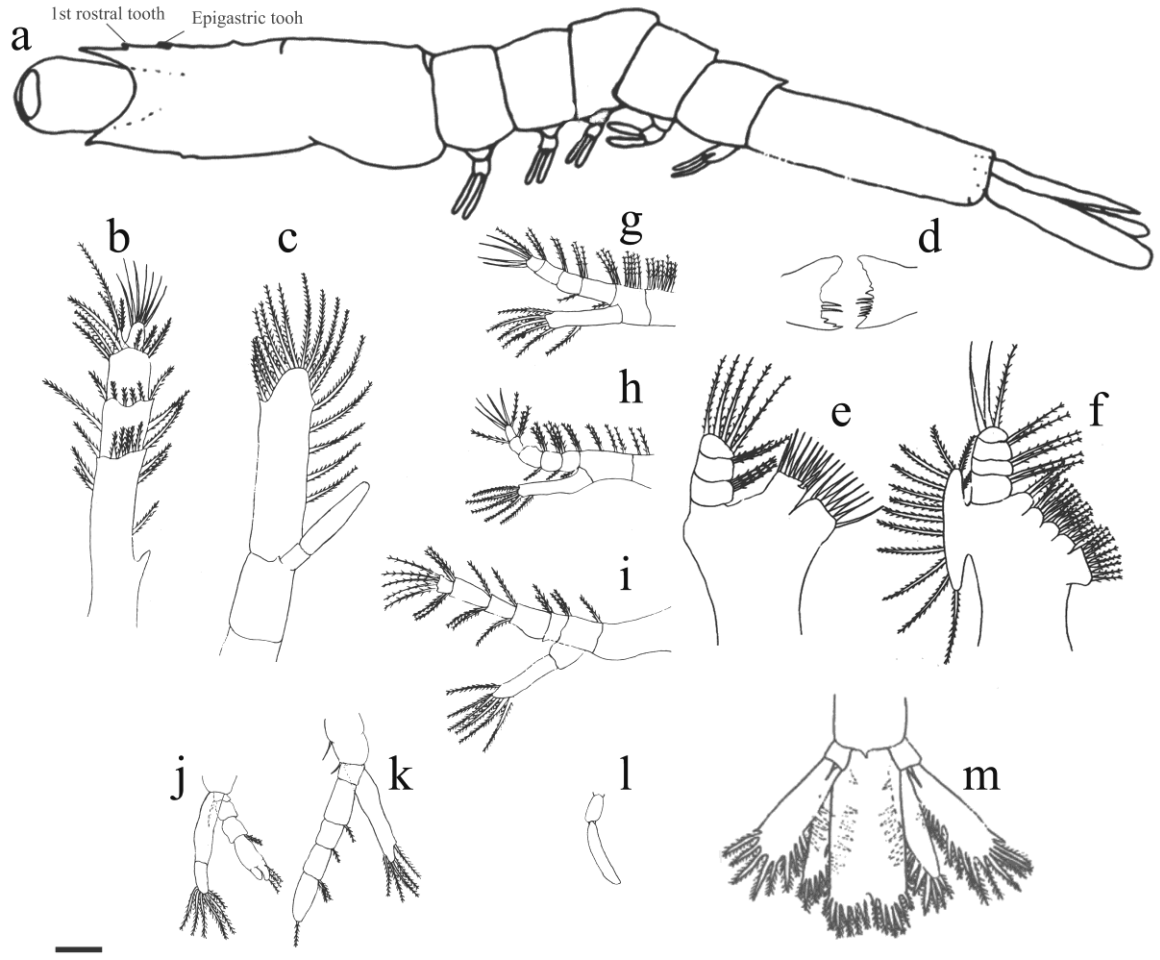


Fig. 1.4.13. *Metapenaeus dalli* Mysis III lateral view (a); (b) 1st antenna; (c) 2nd antenna; (d) mandible; (e) 1st maxilla; (f) 2nd maxilla; (g) 1st maxilliped; (h) 2nd maxilliped; (i) 3rd maxilliped; (j) 3rd pereiopod; (k) 5th pereiopod; (l) pleopods; (m) telson and uropods. Scale bar (a-c, g-m) = 0.1 mm, (d-f) = 0.05 mm.

Post larvae I (PL I)

After the nauplius, protozoea and mysis larval stages, the morphology of the post larval stage begins to resemble the adult form (Fig. 1.4.14a).

1st antenna (Fig. 1.4.14b) coxa has fourteen plumose setae, with antennal spine forming a more defined point; basis has eight plumose setae at coxa join and five half way to endopod; endopod with seven plumose setae at join to basis and six at join to rami; minor ramus has three terminal plumose setae, major ramus is now two-segmented and has four terminal simple setae and four setae positioned laterally, three of which are plumose.

2nd antenna (Fig. 1.4.14c) protopod has two simple setae, one located mid-section and another at the join to the antennal scale; antennal scale has twenty plumose setae positioned marginally, with five additional simple setae positioned adjacent to the marginal line; endopod has five segments with four terminal+2+4+1+3 short and fine simple setae.

Mandible (Fig. 1.4.14d) left mandible three teeth and right mandible nine teeth.

1st maxilla (Fig. 1.4.14e) coxial and basial endites are extended; setae as per M III.

2nd maxilla (Fig. 1.4.14f) coxial endite has 4+9 plumose setae; basial endite has 2+5+5 plumose setae; endopod unchanged from M I; scaphognathite enlarged with twenty-three plumose setae.

1st maxilliped (Fig. 1.4.14g) protopod and endopod are same as M III except for two setae of the inner endopod being lost; exopod is unchanged.

2nd maxilliped (Fig. 1.4.14h) basis now has three setae; endopod has five terminal setae+3+3+4+4 setae, arranged as 2+0+2+3+3 outer setae and 1+0+1+1+1 inner setae, four of the terminal setae are simple; exopod has four terminal plumose setae and one seta on the outer margin. All setae plumose.

3rd maxilliped (Fig. 1.4.14i) endopod is now six segmented and has five terminal+4+3+2+3+4+2 plumose setae, arranged as 2+1+1+2+2+2 outer setae and 2+2+1+1+2 inner setae; exopod has six terminal plumose setae.

3rd pereiopod (Fig. 1.4.14j) has six terminal setae on the endopod; exopod has six setae arranged two terminal +2+2; all setae are plumose; chelae on the first three pereiopods are now functional.

5th pereiopod (Fig. 1.4.14k) coxa and basis have simple seta; endopod and exopod are unchanged.

Pleopods (Fig. 1.4.14l) are elongated from M III with six short terminal simple setae.

Telson (Fig. 1.4.14m) unchanged from M I; outer uropods have fifteen marginal furcal spines and one posterolateral spine; inner uropods with fourteen marginal furcal spines; median notch is faintly-cleft.

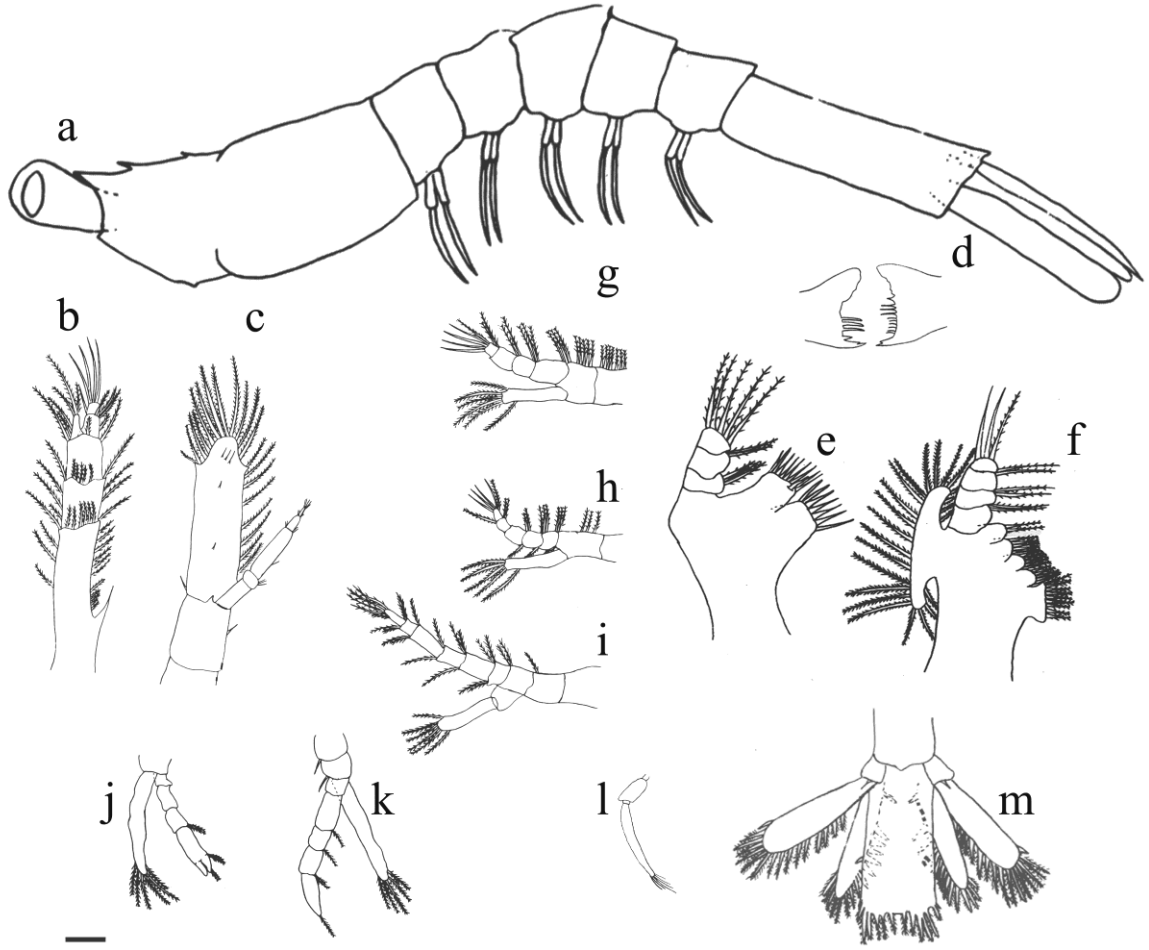


Fig. 1.4.14. *Metapenaeus dalli* Post larvae I lateral view (a); (b) 1st antenna; (c) 2nd antenna; (d) mandible; (e) 1st maxilla; (f) 2nd maxilla; (g) 1st maxilliped; (h) 2nd maxilliped; (i) 3rd maxilliped; (j) 3rd pereiopod; (k) 5th pereiopod; (l) pleopods; (m) telson and uropods. Scale bar (a-c, g-m) = 0.1 mm, (d-f) = 0.05 mm.

Discussion

The larval development of *M dalli* can be subdivided into six naupliar, three protozoea and three mysis stages before individuals metamorphose into the post larval form. This ontogenetic development occurs in ~12 days at 26 °C, with both the growth rate and morphological patterns of development in *M. dalli* described here show, at a broad level, similarity among congeners. However, the number, location and composition of individual setae and other minor spinal developments vary among species at corresponding ontogenetic stages. Differentiation between metapenaeid species was found to be effective when comparing the number and combinations of the 1st and 2nd antennae setal arrangement (Table 1.4.2). Differentiation of each species was possible at each sub-stagevia this method, with subtle differences in the number of setae through the nauplii and protozoea sub-stages, and major differences throughout the mysis and post larval

sub-stages. An example of this is the comparison of *M. dalli* and *M. moyebi*, where at the N I stage, there were seven setae observed on the first antennae of *M. dalli* and five on *M. moyebi*. In addition, there were four setae on the endopod and six setae on the exopod of *M. dalli*, while *M. moyebi* had five setae on each of the endopod and exopod. Unfortunately comparisons of *M. dalli* with other studies and species proved difficult however, as many studies showed incomplete works or lacked appropriate detail. Examples of this are given for the first and second antennae on *M. moyebi* (Nandakumar *et al*., 1989) and *M. monoceros* (Muthu *et al*., 1979) in Table 1.4.2, whereby these works failed to describe these appendages. In addition, comparison of size at each sub-stage between species is prohibitive, due largely to the effects that regional specificity and different culturing techniques can have on growth rates of penaeids (Dall *et al*., 1990).

Differentiation between these species in the wild for distribution and/or abundance studies would prove even more difficult and time consuming without a methodology based on comprehensive frameworks with well-defined criteria. Few examples exist of such approaches except for works based on the second protozoea sub-stage of the penaeid prawns *Penaeus esculentus, Penaeus latisulcatus, Penaeus merguiensis, Penaeus semisulcatus* (Rothlisberg *et al*., 1983) and *M. ensis* with *M. endeavouri* protozoea (Jackson and Rothlisberg, 1994). These studies of distribution and abundance had been successfully achieved with larvae from studies in the Gulf of Carpentaria. This involved the use of between 14 and 17 individual discrimination factors (see Rothlisberg *et al*., 1983; Jackson and Rothlisberg, 1994), focusing on length and width of individual segments of the 1st and 2nd antennae, total and carapace length, using comprehensive larval reference collections within clearly defined research zones. This is yet to be applied to other species or regions, however, largely due to limited availability of detailed larval descriptions, endemic reference material and due to the high degree of overlap among species in both morphometric and meristic characters. Genetic methods of identification may be successful, but markers for *M. dalli* have yet to be determined. Application of morphological techniques for distinguishing *M. dalli* may be possible in areas such as Western Australia, where only three species of metapenaeids occur. However, due to complex overlapping distributions

of many congeneric species, regions such as Indonesia and the Northern Territory would require more comprehensive reference material to distinguish *M. dalli* from other species.

Table 1.4.2. Comparison of the number of setae on the first and second antennae of *Metapenaeus dalli* and six other metapenaeids reared in other laboratory studies. Bi-ramous second antenna is defined by setae on the endopod and exopod, with exopod representing the antennal scale during mysis and post larval stages.

Stage	Character	*M. dalli*	*M. ensis*	*M. affinis*	*M. moyebi*	*M. monoceros*	*M. dobsoni*
		This study	Ronquillo & Saisho, (1993)	Hassan, (1980)	Nandakumar *et al.*, (1989)	Mahomed *et al.*, (1979)	Muthu *et al.*, (1979)
N I	1st ant.	7	6	6	5	-	7
	2nd ant. End/Ex	4/6	4/5	5/5	5/5	5/6	4/5
N II	1st ant.	8	7	7	6	-	7
	2nd ant. End/Ex	4/6	5/6	5/6	5/6	5/6	4/6
N III	1st ant.	8	7	7	6	-	7
	2nd ant. End/Ex	4/7	5/7	5/7	5/7	5/7	5/7
N IV	1st ant.	8	8	7	-	-	7
	2nd ant. End/Ex	6/7	6/8	5/9	-	5/8	5/8
N V	1st ant.	8	9	7	6	-	6
	2nd ant. End/Ex	6/7	6/9	6/10	5/8	5/9	5/9
N VI	1st ant.	8	10	8	7	-	9
	2nd ant. End/Ex	6/9	6/10	8/12	5/9	7/9	6/9
PZ I	1st ant.	9	10	11	9	10	8
	2nd ant. End/Ex	11/11	12/12	10/13	11/12	11/12	11/12
PZ II	1st ant.	12	11	11	-	10	8
	2nd ant. End/Ex	11/12	12/12	10/13	11/12	11/12	11/12
PZ III	1st ant.	15	12	14	-	11	12
	2nd ant. End/Ex	11/13	12/12	10/13	11/12	11/12	11/12
M I	1st ant.	23	34-35	24	-	23	-
	2nd ant. End/Ex	6/11	6/11	3/10	-	5/11	5/11
M II	1st ant.	30	37-38	28	27	-	-
	2nd ant. End/Ex	3/15	6/17	3/15	-/14	0/14	0/13
M III	1st ant.	35	61	30	40-41	32	-
	2nd ant. End/Ex	0/16	6/21	3/17	-/15	0/16	0/15
PL I	Ex. setae	51	51	42	53	50	58-59
	2nd ant. End/Ex	14/25	13/27	14/28	-/26	6/22-25	12/23

Overall, this study will support further works detailing endemic population patterns of penaeid prawns along the Australian coastline and in Indonesia, as well as provide supporting material for the development of commercial aquaculture of this and related species in the future.

1.5. Effects of water temperature and salinity on the survival and development of larval Western School Prawns

This study has been published in International Aquatic Research.

Crisp, J.A., Partridge, G.J., D'Souza, F.M.L., Tweedley, J.R. & Moheimani, N.R. (2017). Effects of temperature and salinity on larval survival and development of the western school prawn *Metapenaeus dalli*. *International Aquatic Research*. 9(1): 1-10. DOI: 10.1007/s40071-016-0151-0.

Summary

The effects of temperature and salinity on the survival and development of larval *Metapenaeus dalli* were investigated in two experiments. Firstly, the effects of four temperatures of 22.6°, 25.8°, 29.4° and 32.6°C on survival and development time were examined from the Nauplius VI to the Mysis I sub-stage. Survival was significantly lower at 32.6° than at 22.6° and 25.8°C. Development times differed significantly across all temperatures, decreasing linearly with temperature from 161.5 h at 22.6°C to 74.8 h at 29.4°C then decreased slightly to 71.4 h at 32.6°C. Secondly, the combined effects of three temperatures (21.4°, 26.5° and 29.7°C) and three salinities (30, 35 and 40‰) on survival and development of larval *M. dalli* were quantified over a 48 h period from the Nauplius VI sub-stage. Only salinity was found to influence survival, with peak survival (77.7%) found to occur at the control salinity (~35‰). Any increase or decrease in salinity from this value resulted in a decrease in survival, with the lower salinity tested (30‰) having a significantly negative effect on survival (58.4%) when compared to the control. Only temperature was found to influence the rate of development, with significant increases in development index values being recorded as temperature increased. The recommended water temperature and salinity conditions for optimal survival and development of *M. dalli* larvae as determined by this study are therefore 25.8 °C and 35‰.

Rationale and aims

Successful rearing of penaeid prawn larvae for stocking requires high survival rates to be attained, however, this must be achieved in conjunction with development rates that limit the time larvae spend in the hatchery to reduce operating costs (FAO, 1978). Two significant

environmental factors that influence the survival and development rates of larval crustaceans are temperature and salinity (Kinne, 1963, 1964). The complex early life stages of penaeid prawns are considered to be particularly vulnerable to temperature and/or salinity changes, with high mortality occurring during the larval stages under adverse conditions (Anger, 2003). Of the three larval stages Nauplius, Protozoea and Mysis in penaeid prawns, Protozoea stage larvae appear to be the most sensitive to changes in temperature and salinity, and thereby provide an effective proxy for assessing suitable culture conditions for all larval and post-larval life stages (Preston, 1985; Zacharia and Kakati, 2004).

Studies of the effects of temperature and salinity on larval penaeid prawns, have demonstrated that salinity has a greater effect on survival than temperature (Kumlu *et al*., 2000; Kumlu *et al*., 2001; Zacharia and Kakati, 2004; Ch and Shailender, 2013), although increases in mortality are known to occur at relatively high temperatures (Jackson and Burford, 2003; Aktas and Cavdar, 2012). In contrast, water temperature has a more pronounced effect on development than salinity (Parado-Estepa, 1998; Jackson and Burford, 2003), with growth rates only shown to be affected by salinity at brackish ranges (Ponce-Palafox *et al*., 1997; Kumlu *et al*., 2000). Temperature and salinity have also been show to interact, further exacerbating mortality rates when both factors are at the extremes of their ranges (Ponce-Palafox *et al*., 1997; Kumlu *et al*., 2000). For the establishment of rearing protocols, however, it is important to determine the temperature and salinity regime that provides the highest possible development rates without compromising larval survival.

Once preferred temperatures and salinities for culture are established, they can be used to predict larval survival and growth rates for future planning. This is particularly important in larval stock management from the Protozoea stage, as this is the point at which penaeid larvae switch to exogenous feeding (Dall *et al*., 1990). It is at this point that hatchery feeding relies on the expensive cultivation of live feeds such as microalgae, which require planning days in advance to produce (D'Souza *et al*., 2000). Live zooplankton is often used from the Mysis and early post-larval stages with similar planning requirements (FAO, 1978; Zacharia and Kakati, 2004). Without predicting survival and development rates, feeding protocols cannot be

calibrated, which may result in increased cultivation costs for live foods and potential negative effects on water quality as a result of over or under feeding.

The purpose of this study was to determine the effects of culture temperature and salinity on *M. dalli* protozoea stage larvae, acting as proxy for all larval developmental stages. This was done in an effort to maximize survival and optimize development rates for the purpose of increasing the number of hatchery-reared *M. dalli* post-larvae that can be restocked into the Swan-Canning Estuary.

Methods

Broodstock collection

Female *M. dalli* were collected at night from the Swan-Canning Estuary in Perth (31°56′50″S 115°54′58″E), south-western Australia from December 2014 to March 2015, using a hand trawl net that was 1.5 m high, 4 m wide and constructed from 9 mm mesh. Maturity was determined as per descriptions by Tuma (1967), with mature prawns immediately transported to the aquaculture facility and placed in aerated holding tanks overnight (FAO, 1978). Females were then stocked into 300 L conical base spawning tanks at a density of up to 40 per tank for two to four days. The tanks were filled with seawater at a salinity of ~35‰ drawn from a bore sunk through limestone rock, accessing near-shore marine water, and aerated constantly and maintained at a temperature of ~26°C. This water temperature and salinity approximated conditions in the Swan-Canning Estuary at the time and place of capture (unpublished data). The base of each broodstock tank was fitted with a fine grate that allowed eggs to pass through, separating them from broodstock to prevent any potential cannibalism. Broodstock spawned naturally within 48 h of capture without eyestalk ablation. After spawning, eggs were collected on 48 µm and 63 µm screens and rinsed, sub-sampled for counting and re-suspended in 300 L of filtered (1 µm) seawater, under constant aeration. Egg quality was assessed by hatch rate, with a value of > 75% survival post-hatch considered suitable for use in the study (FAO, 1978).

Larval rearing system

After spawning, hatched larvae were held until they had metamorphosed into the Nauplius (henceforth denoted as 'N') VI sub-stage (Crisp *et al*., 2016), at which time they were harvested and stocked at a density of 250 larvae L-1 (D'Souza and Kelly, 2000) into 6 L flat bottom cylindrical glass culture vessels containing 1 µm filtered seawater. Water temperature for each experiment was maintained by housing culture vessels in 115 L temperature-controlled water baths, heated with pre-calibrated Eheim Jager 150 W aquarium water heaters and monitored with Thermocron TCS temperature loggers every 10 minutes. A constant salinity was maintained in the culture vessels and monitored daily using an ATAGO PAL-03S digital refractometer. Vessels were exposed to 3.5 µmol photons m-2 s-1 white light with 12:12 h light:dark photoperiod. The larval cultures were aerated from the base to provide vertical mixing as well as oxygenation.

Feed cultivation

Larval *M. dalli* were fed a diet comprising two microalgae strains obtained from the Australian National Algal Culture Collection held by the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Hobart, Tasmania. The chlorophyte *Tetraselmis suecica* (CSIRO strain number CS-187) and the diatom *Chaetoceros muelleri* (CSIRO strain number CS-176) were maintained in batch cultures under 14:10 h light:dark photoperiod with 180 µmol photons m^{-2} s^{-1} white fluorescent light , in 15L culture vessels with ambient salinity (~35‰) and temperature (~25 °C). Each species was cultured in Guillard's F2 medium, with sodium metasilicate added at 30 g L^{-1} to the *C. muelleri* (Ryther and Guillard, 1962). To avoid carbon limitation, food grade CO_2 was injected to maintain a pH of 7.4-7.7.

Feeding larvae

Larval feed in culture vessels was maintained daily at 3 x 10^4 cells mL^{-1} of *T. suecica* and 9 x 10^4 cells mL^{-1} of *C. muelleri*, as per feeding protocols used by the Australian Centre for Applied Aquaculture Research (Jenkins, G. I., Australian Centre for Applied Aquaculture

Research, Fremantle, Western Australia, unpublished data). This was achieved by counting residual cells of each microalgae species in larval culture vessels using a Neubauer haemocytometer and replacing feed that had been consumed. Water in the culture vessels was topped up to 6 L daily with a combination of microalgal feed and fresh seawater. Gut contents of the larvae were also briefly examined under a dissecting microscope to confirm that the algal cells were being ingested (D'Souza and Loneragan, 1999).

Experimental design

Effect of temperature

The influence of four nominal water temperatures, *i.e.* 23°, 26°, 29° and 32°C in the laboratory at a constant salinity of 35‰, were assessed on the development time (h) and percentage survival of larval *M. dalli* from N VI, through the protozoeal stage (PZ I, II and III) to the Mysis I sub stage (M I). Six replicate culture vessels were used for each of the four temperatures. Development time was calculated as the time taken for 50% of the larvae in each vessel to reach the M I stage. Single 200 mL sub-samples were taken every 12 hours from each vessel and each larva staged according to the descriptions in Crisp *et al*. (2016). When M I staged larvae were first observed, each vessel was then subsampled every 0.5 to 1 h. All sampled larvae were carefully returned to the vessels. Once each vessel was found to contain 50% of animals at the M I stage, time was recorded and triplicate 200 mL samples were taken and fixed in 5% tetraborate-buffered formaldehyde solution for later analysis of survival.

Combined effects of temperature and salinity

The combined effects of three nominal temperatures, *i.e.* 23°, 26° and 29°C, and three nominal salinities, *i.e.* 30, 35 and 40‰, on the development and survival of *M. dalli* larvae were quantified in a 3 x 3 fully factorial design. Each combination of salinity and temperature was conducted in two vessels per run, with an average of the vessels used to create a replicate value of each treatment regime. The experiment was run three times, creating three replicates for each treatment. Each run commenced from the N VI larval sub-stage, running for a fixed period of

48 h. This time period was chosen to examine the acute combined effects of temperature and salinity on the Protozoea stage of development.

After 48 h, all culture vessels were sampled via triplicate 200 mL sub-samples, which were fixed with 5% tetraborate-buffered formaldehyde, and from which survival was determined and the metamorphic sub-stage for each larva was recorded. A development index was calculated for each sub-sample based on that described by . Each larvae counted was assigned a value for its developmental sub-stage, where PZ I = 1, PZ II = 2 and PZ III = 3. A development index was then calculated, for each sub-sample, by multiplying the sub-stage value (*i.e.* 1, 2, or 3) by the number of larvae counted at that sub-stage. The product of each sub-stage is then added together then divided by the total number of larvae staged. Development Index = sum (number of larvae at that stage x stage value) / total number larvae staged. The more larvae there are at higher stage numbers the larger the index.

Statistical analysis

Effect of temperature

Analysis of the percentage values for survival indicated that data ranged both > 80% and < 20%. As a result these data were subjected to an arcsine square-root transformation. To determine whether a transformation of the development data was required, the extent of the linear relationship between the loge mean and loge standard deviation of development time was calculated. Examination of the slope of the linear relationship indicated that a square-root transformation was necessary to meet test assumptions of homogeneity of variance (Clarke *et al*., 2014a). Both survival and development time were analyzed separately using a one-way Analysis of Variance (ANOVA). When ANOVA detected a significant difference among temperatures, post-hoc tests were conducted using Tukey's HSD to elucidate the pairs of temperature that were responsible for each of those differences. In this and all tests, a null hypothesis of no significant difference between *a priori* groups was rejected when $p < 0.05$.

Combined effects of temperature and salinity

Analysis of the percentage survival data indicated that there were values > 80%, but not < 20%. As a result, a square-root transformation of the data was employed. Analysis of the relationship between the $\log_e$ mean and $\log_e$ standard deviation of the development index data indicated that no data transformation was required (Clarke *et al*., 2014a). Separate two-way ANOVAs were used to determine whether development index and survival were affected by temperature and/or salinity, or the interaction term. Both factors were considered fixed. If a significant difference in any factor and/or interaction term was detected, post hoc analysis was conducted using Tukey's HSD. All statistical analyses were conducted using SPSS version 22 software (IBM Corp.). Values for all results are given as mean ± 95% CL.

Results

Effect of temperature

Mean water temperatures achieved for the nominal temperatures of 23°, 26°, 29° and 32°C were 22.6 ± 0.2°, 25.8 ± 0.3°, 29.4 ± 0.4° and 32.6 ± 0.4°C, respectively. One-way ANOVA demonstrated that the percentage survival of *M. dalli* larvae differed significantly among temperatures ($p < 0.001$). Survival rates were greatest at 25.8° and 22.6°C, *i.e.* 73.0 ± 17.8% and 66.7 ± 12.4%, respectively and lowest at the highest temperature of 32.6°C, *i.e.* 26.3 ± 12.4% (Fig. 1.5.1). The time taken for *M. dalli* larvae to develop from N VI to M I differed significantly among temperatures ($p < 0.001$). Development time decreased markedly from 161.5 ± 0.9 h at 22.6°C to 74.8 ± 0.3 h at 29.4°C, but only decreased marginally to 71.4 ± 0.1 h at 32.6°C (Fig. 1.5.2).

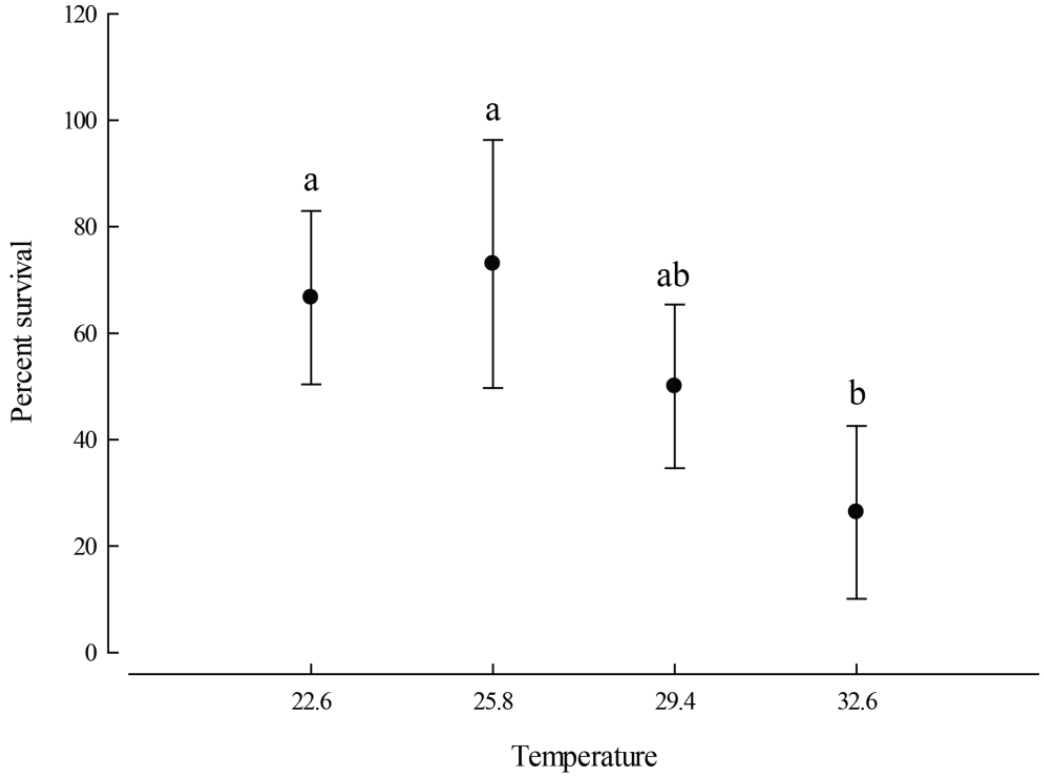


Fig. 1.5.1. Mean percentage survival (±95% CL) of *Metapenaeus dalli* larvae during to development from Nauplius VI to Mysis I at four different water temperatures (in °C). Letters above error bars denote groups of samples identified by Tukey's HSD.

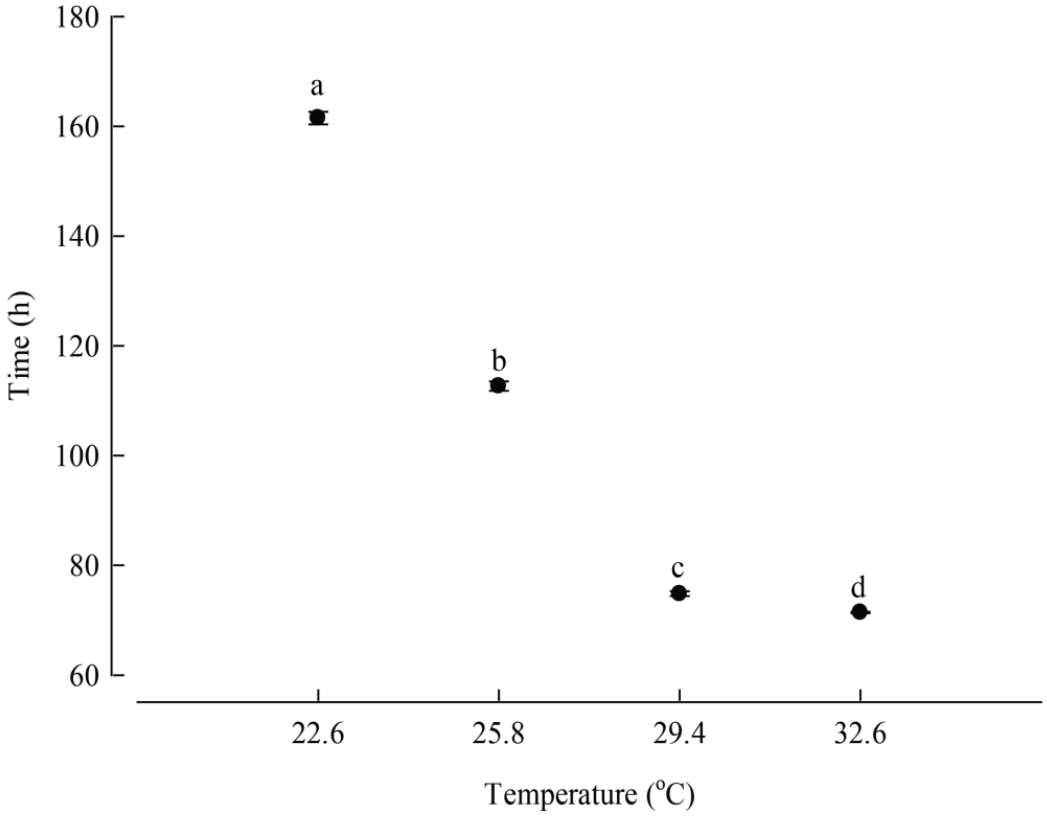


Fig. 1.5.2. Average time (±95% CL) taken for *Metapenaeus dalli* larvae to develop from Nauplius VI to Mysis I at the four different water temperatures tested. Letters above error bars denote groups of samples identified by Tukey's HSD.

Combined effects of temperature and salinity

Mean water temperatures achieved for each of the three nominal temperatures tested (23°, 26° and 29°C) were 21.4 ± 0.3, 26.5 ± 0.2° and 29.7 ± 0.4°C respectively, with the salinities obtained consistent with nominal salinities (30, 35 and 40‰). Two-way ANOVA showed no interacting effect of temperature and salinity on either survival (p = 0.480) or development rate (p = 0.906) of *M. dalli* larvae over a 48 h culture period. There were however two significant main effects, presented below. Firstly, salinity had a significant effect on larval survival (p = 0.010), but not temperature (p = 0.570). Salinity of 35‰ was found to be the best for survival, resulting in a survival of 77.7 ± 5.0%. This survival was significantly higher than that measured at 30‰ (58.4 ± 8.4%). Survival at 40‰ (68.4 ± 7.8%) was not significantly different from either of the other two salinities (Fig. 1.5.3). Secondly, the reverse was found for larval development such that temperature had a significant effect (p <0.001), but not salinity (p = 0.774). Development of larvae was significantly different at all three temperatures. A stepwise increase in development rate occurred from the lowest temperature (1.01 ± 0) to the highest (2.61 ± 0.05), with some larvae observed reaching a peak development of PZ III in the highest two temperatures tested (Fig. 1.5.4).

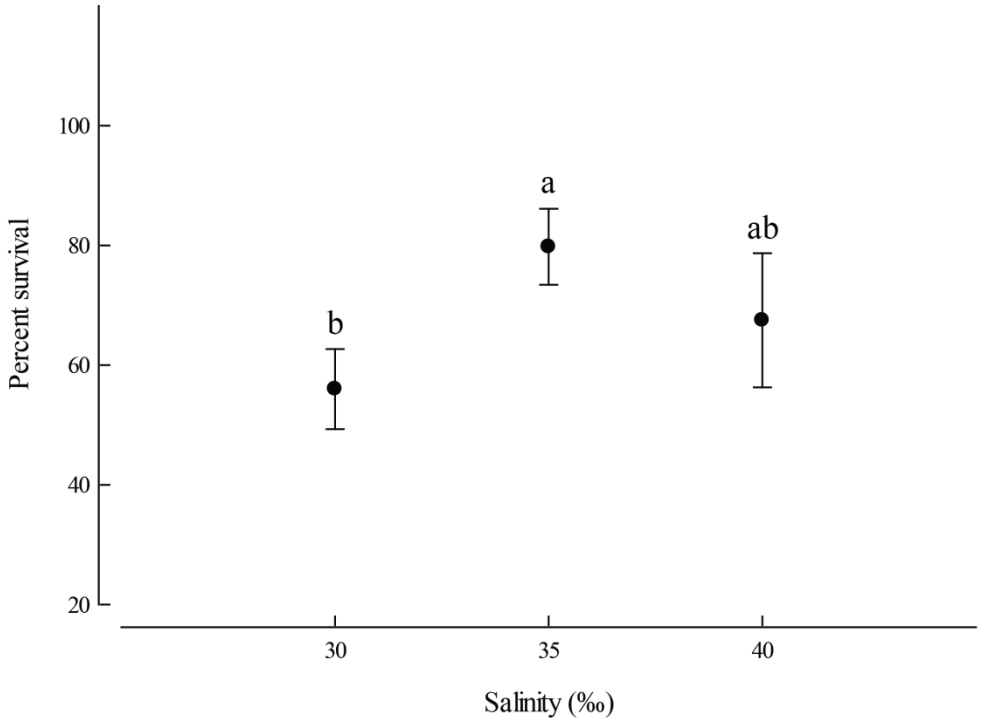


Fig. 1.5.3. Mean percentage survival (±95% CL) of *Metapenaeus dalli* larvae over a 48 h period from N VI sub-stage at three different salinities. Letters above error bars denote groups of samples identified by Tukey's HSD.

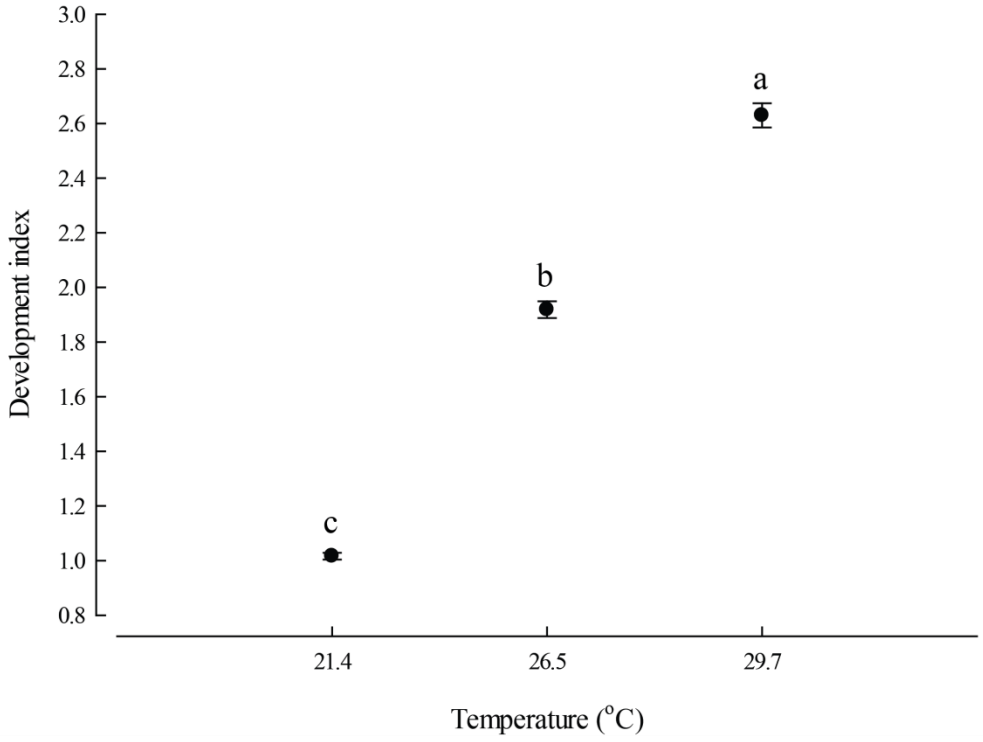


Fig. 1.5.4. Mean development index (±95% CL) of *Metapenaeus dalli* larvae over a 48 h period from N VI sub-stage at three different water temperatures. Letters above error bars denote groups of samples identified by Tukey's HSD.

Discussion

Effect of temperature

In this study, there appeared to be a significant negative impact on survival at the highest temperature tested of 32.6°C, but not at any other temperature tested, though there appeared to be reduced survival at 29.4°C. This result is supported by a study by Kumlu *et al.* (2000), where temperatures above 30°C were found to increase mortality in larval *P. semisulcatus*. Furthermore, preliminary findings by Ponce-Palafox *et al.* (1997) on larval *Litopenaeus vannamei* demonstrated that temperatures above 30°C, with similar salinities to those used in this study, appeared to have a negative effect on survival. The consistency of these results across larval penaeid prawns may indicate there is an upper thermal limit to their biochemical processes.

Results of this study demonstrate that the time taken by *M. dalli* to develop from N VI to M I differ significantly among temperatures. Rapid sequential decreases in development time to M I occurred between temperatures of 22.6° and 29.4°C, with only a marginal decrease in development time at the highest temperature of 32.6°C. These results are consistent with that of larval *Penaeus semisulcatus* (Jackson and Burford, 2003) and *Penaeus monodon* (Parado-Estepa, 1998), where no significant difference was found in development rates between water temperatures of 29° and 32°C during the Protozoea stages.

For commercially cultured crustacean larvae, the effect of water temperatures on larval growth rates are often incorporated into planning tools, such as degree-hours or degree-days calculations (Kittaka *et al*., 2001; 2002; Gendron and Ouellet, 2009), which are used to determine larval duration under various temperatures from spawning to post-larval metamorphosis (Roberts *et al*., 2012). This allows for the determination of potential feed requirements, which is a particularly important factor in larval culture where forward planning is required for the cultivation of live foods (Stevens, 1990). When applied to the temperature ranges tested, it can be assumed that culture temperature should be maintained between 25.8° and 29.4°C provided high survival of larvae can be achieved. Reducing temperature by ~ 3°C would increase development time by > 2 d and thus feed and resource allocation requirements and increasing temperature by ~6°C would increase mortality.

Combined effects of temperature and salinity

When the combined effects of temperature and salinity were examined in this study, salinity significantly affected survival and temperature significantly affected the rate of development of larval *M. dalli*, without any interacting effects in the ranges tested. Unlike in the current study, interacting effects of temperature and salinity on survival have been found in other species such as *P. semisulcatus* (Jackson and Burford, 2003) and *L. vannamei* (Ponce-Palafox *et al*., 1997). However the results of the current study are consistent with these previous studies when similar temperatures and salinities were compared. In addition, any variation in salinity from 35‰ had an apparent negative effect on survival, with a 5‰ decrease having a greater effect than a 5‰

increase; noting that the increase in salinity to 40‰ did not significantly affect survival from that obtained at 35‰. This compares well with the effects of salinity on larval *M. bennettae* (Preston, 1985) and larval *Penaeus merguiensis* (Zacharia and Kakati, 2004). However, these results contrast with those obtained for juvenile *L. vannamei* and larval *P. semisulcatus*, where although temperature was the primary factor in determining growth rates, salinities far lower than those used in the current study were also found to negatively impact on growth as well as survival (Ponce-Palafox *et al*., 1997; Kumlu *et al*., 2000). It may be the case that the energetic demands of osmoregulation under extreme conditions are greater than can be replenished by the larvae. It is only when the extreme ranges are tested that salinity and temperature appear to interact, placing a combined burden on the energetic reserves of the larvae (Anger, 2003).

Finally, the preferred temperatures (25.8° and 29.4°C) and salinity (35‰) determined by this study appear to relate well to the natural spawning environments in the Swan-Canning Estuary, from which broodstock were obtained. Wild *M. dalli* are known to spawn in the middle and lower Swan-Canning Estuary during spring and summer, where the salinity in the river system is 35 to 38‰ and water temperature is 26 to 28°C (Potter *et al*., 1986b), indicating a close relationship between preferred larval hatchery conditions and those expected to influence larvae in the natural population. This relationship appears to be in line with findings on *Metapenaeus bennettae*, where the water temperatures found where broodstock were obtained, appeared to be the most suitable for larval culture in terms of survival (Preston, 1985).

Conclusions

From this study, the most appropriate temperature and salinity to be used in the cultivation of *M. dalli* larvae were 25.8°C and 35‰. It is also clear that any increase in temperature and/or variation in salinity may have a negative impact on survival. However, for short-term gains in growth rate, culture temperatures may be increased, providing they remain below 30°C as this increase has a negligible effect on survival. Using this information, further research into the nutritional requirements of this species may now be conducted under ideal abiotic rearing conditions.

1.6. Performance of mixed species and mono-specific algal diets for culture of larval Western School Prawns

This study is published in the Journal of the World Aquaculture Society.

Crisp, J.A., D'Souza, F.M.L., Tweedley, J.R., Partridge, G.J. & Moheimani, N.R. (2017). Performance of mixed species and mono-specific algal diets for culture of larval western school prawns *Metapenaeus dalli*. *Journal of the World Aquaculture Society*. DOI: 10.1111/jwas.12484.

Summary

The effect of three mono-specific and four combinations of the diatoms *Chaetoceros muelleri*, *Chaetoceros calcitrans* and the chlorophyte *Tetraselmis suecica* on survival, development and dry weight of the prawn *Metapenaeus dalli* was assessed from Protozoea I until Mysis I. The development and dry weight of larvae were significantly greater when fed diets comprising *C. muelleri* and/or *T. suecica*. A fourth diet, consisting of all three microalgal species also performed just as well. Survival alone was a poor measure of the performance of the various diets. Larvae fed with *C. calcitrans*, either alone or in a mixed diet with either *C. muelleri* or *T. suecica*, had significantly slower development and lower dry weight. Overall performance, assessed using the normalized biomass development index, determined that both mono-specific and mixed diets containing *C. muelleri* and *T. suecica* were among the best for *M. dalli* larvae. These results for *M. dalli* are consistent with those found for commercially grown penaeid prawns. This study enhances the limited knowledge on the feeding requirements of metapenaeid prawn larvae. Moreover, the results will help improve hatchery methods for the aquaculture-based enhancement of *M. dalli* in the Swan-Canning Estuary and potentially increase the abundance of this iconic recreational species.

Introduction

Global fisheries production peaked in the 1980s and, as of 2013, 58% of fisheries were classified as almost fully exploited, with a further 31% over exploited (FAO, 2016). Because wild fishery production was not satisfying market demand, aquaculture production rapidly expanded, accounting for approximately 44.1% of global biomass production in 2014,

excluding seaweeds (FAO, 2016). Aquaculture of prawns in particular has increased rapidly and, as of 2014, represented ~56% of total prawn biomass from fisheries and aquaculture (FAO, 2017). While aquaculture production has primarily been used to fill market demand for global food production, the husbandry techniques developed in this sector have also been used in the management and restoration of over exploited fisheries, via aquaculture-based enhancement (*i.e.* stock enhancement, restocking and sea ranching activities; Loneragan *et al*., 2013a; Taylor *et al*., 2017a). For penaeid prawns in particular, emphasis has been placed on hatchery cultivation of individuals prior to release, as this circumvents natural mortality, including predation, that occur during the larval (>70% per week) and the earliest period of post-larval (10-25% per week) stages (Dall *et al*., 1990).

A recent example of a penaeid being restocked is the western school prawn, *Metapenaeus dalli* (Jenkins *et al*., 2017). This species was once the focus of a small commercial and iconic recreational fishery in the Swan-Canning Estuary in south-western Australia, but a decline in stocks led to the eventual closure of commercial fishing activities in the 1970s (Smith, 2006; Smithwick *et al*., 2011). Despite this closure and thus reduced fishing pressure, recreational catches of *M. dalli* continued to decline, particularly from the late 1990s (Maher, 2002). By 2007, the abundance of this species was still very low and had not recovered naturally. Therefore, a restocking program was considered the best option to overcome the long-term recruitment failure, as it would bypass the high mortality rate that often occurs in the early larval stages (Smith *et al*., 2007; Broadley *et al*., 2017).

In addition to preferred abiotic rearing conditions, successful cultivation of larval prawns requires an adequate food supply that provides the diverse nutritional content found in natural feeds. An effective feed would result in larvae exhibiting high survival, while maintaining adequate development and growth (ASEAN, 1978; Piña *et al*., 2006). In commercial marine prawn culture, this management is achieved during the sensitive herbivorous larval stages by feeding live microalgae similar to that found where broodstock naturally reproduce (Brown *et al*., 1997). Although formulated diets have been previously trialled as feed replacement during these stages, their performance has been poor in comparison to live microalgae, often having lower assimilation efficiency. Previous studies have shown that feeding requirements of larval

marine prawns are more likely to be satisfied by mixed microalgae diets than a single microalgal species (Kurmaly *et al*., 1989; Lovett and Felder, 1990; Jones *et al*., 1997; Kesarcodi-Watson *et al*., 2008).

Microalgal species are often used as feeds for marine prawns during the herbivorous stages of development (Brown *et al*., 1997). Species of Bacillariophyceae, such as *Chaetoceros* sp., *Skeletonema* sp. and *Thalassiosira* sp. are considered important for metamorphic development, due, in part, to their complement of long chain polyunsaturated (LC-PUFA) fatty acids (Brown *et al*., 1997; D'Souza and Loneragan, 1999; Conceição *et al*., 2010). Species of Chlorophyceae, such as *Tetraselmis* sp., *Dunaliella* sp. and *Chlorella* sp., are generally fed to provide further nutritional complement (Brown *et al*., 1997).

Although *M. dalli*, reproduces in marine environments in tropical northern Australia and Java (Indonesia), in temperate waters of south-western Australia it is confined to estuaries, where it completes its life cycle (Broadley *et al*., 2017; Crisp *et al*., 2017a). It is unknown whether prawns that reproduce in estuaries, such as *M. dalli* in temperate waters, have related feeding requirements to similar species from tropical marine waters. Studies of phytoplankton communities in the Swan-Canning Estuary have shown that downstream areas with near marine salinities are dominated by diatoms and dinoflagellates (Twomey and John, 2001), whereas phytoplankton in the more upstream brackish and freshwater areas consist mainly of chlorophytes (Chan and Hamilton, 2001).

The aim of this study was to determine the effects of mono-specific and mixed microalgae diets on survival, development and dry weight of larval *M. dalli*, grown under controlled conditions. The result of which will inform future studies devoted to the determination of actual quantitative nutritional requirements of larval *M. dalli* for aquaculture and aid in the aquaculture-based enhancement of this species.

Methods

Brood stock collection and spawning

Gravid *M. dalli*, identified as having a dark green/brown appearance with a distinct 'arrow head' shape in the posterior section (Crisp *et al*., 2017a), were collected at night from the Swan-Canning Estuary (31°56′50″S 115°54′58″E) in Perth, Western Australia in December 2015, using a hand trawl net 1.5 m high, 4 m wide and constructed from 9 mm mesh. Once retained, the prawns were immediately transported to an aquaculture facility and stocked into 300 L conical base tanks, at a density of up to 40 individuals per tank for ~48 h. Spawning tanks were filled with 1 µm filtered seawater at a salinity of ~35 ‰ drawn from a saline aquifer, aerated constantly and maintained at a temperature of ~26 ˚C. This salinity and temperature regime was maintained throughout the entire experiment. Broodstock spawned naturally within 48 h of capture without eyestalk ablation. A 5 mm mesh grate was fitted above the base of each spawning tank to allow eggs to pass through, separating them from broodstock to prevent any potential cannibalism of the eggs (ASEAN, 1978).

After spawning, eggs were siphoned onto a 108 µm screen and rinsed, re-suspended in 10 L of 1 µm filtered sea water and sub-sampled for counting, then stocked into 300 L tanks. Egg viability was assessed both visually and by hatch rate, with greater than 75 % hatch-rate considered suitable for use in the study (FAO, 2007). Hatched nauplii were monitored until reaching sub-stage VI (N VI) at ~48 h, then, just prior to feeding, were collected and stocked at 250 nauplii L^{-1} into twenty four 14 cm diameter x 50 cm height flat bottom glass cylinders, each filled to 6 L with 1 µm filtered seawater resulting in 1,500 larvae per vessel. Larvae were reared in static culture conditions, with aeration delivered with a 4 mm airline and ceramic weight. Remaining larvae were condensed, rinsed with seawater then stored chilled until euthanized, after which samples were prepared for dry weight determination.

Microalgal cultures

Three microalgal species regularly used in the commercial aquaculture of larval penaeids, *i.e.* two diatoms *Chaetoceros muelleri* (CS-176), *Chaetoceros calcitrans* (CS-178) and a

chlorophyte *Tetraselmis suecica* (CS-187), were obtained from the Australian National Algal Culture Collection held at CSIRO Marine & Atmospheric Research in Hobart, Tasmania. Batch cultures of each microalgae were maintained under 14:10 h light:dark photoperiod with 180 µmol photons m^{-2} s^{-1} white incidence light from fluorescent lights, in 15 L culture vessels with ambient salinity of ~35 ‰ and ~25 ˚C water temperature. Each species was cultured in Guillard's F2 medium, with sodium metasilicate added at 30 g L^{-1} for diatoms (Guillard and Ryther, 1962). To avoid carbon limitation, food grade CO_2 was injected and maintained at a pH of 7.4-7.7. Harvesting of microalgae only took place during log phase growth on day five post-inoculation, to avoid possible differences in biochemical composition between batches. Cell densities of stock cultures were calculated via triplicate counts using a neubauer haemocytometer (ASEAN, 1978).

Larval culture

The glass culture vessels were housed in groups of eight vessels per water bath in each of three 115 L clear plastic temperature-controlled water baths, heated with pre-calibrated Eheim Jager 150 W aquarium water heaters and monitored with Thermocron TCS temperature loggers every 10 minutes. Salinity was monitored daily using an ATAGO PAL-03S digital refractometer. Vessels were exposed to 3.5 µmol photons m^{-2} s^{-1} white incidence light from above with 1:23 h light:dark photoperiod (Jenkins *et al*., 2017). Clear plastic was used for the water baths to maximize light scatter and create as close to uniform light intensity throughout entire larval culture vessel. The larval cultures were aerated from the base to provide vertical mixing as well as oxygenation.

The culture vessels were haphazardly allocated one of seven feed regimes (n = 3 for each diet) delivered at known cell densities (Table 1.6.1). These feed regimes consisted of three mono-specific feeds, *i.e. C. muelleri*, *C. calcitrans* and *T. suecica,* each possible combination of two microalgae species, delivered at fifty percent of their mono-specific cell densities, and a seventh experimental diet, used by the Australian Centre for Applied Aquaculture Research,

Fremantle, consisting of all three microalgae species, henceforth known as ACAAR (Jenkins *et al*., 2017). Three remaining replicate vessels were held for a period of 48 h without feed to verify the negative effects of starvation on survival and development, and to ensure sources of nutrition had been removed from the culture water (D'Souza and Loneragan, 1999).

For *C. muelleri* and *C. calcitrans*, mono-specific diets were delivered at a set density of 1×10^5 cells ml^{-1} as recommended by ASEAN (1978), while the feeding density of the mono-specific diet *T. suecica* was determined by delivering the equivalent bio-volume per ml to the *C. muelleri* diet, once the mean ± SE biovolume was determined for each of the three microalgae tested. To determine this relationship for biovolume, the biovolume of each species was calculated by initial selection of fifty cells in log phase on day five of culture of each species. Transverse imagery was then taken of each cell with a top-mounted Tucsen 9 MP camera on a Leica Dialux 22 compound microscope and downloaded with TSView 7 software. Measurements of length, width and depth were calculated using the ruler tool in Adobe Photoshop CS6, with calibration determined by images of a micrometer, as adapted from Crisp *et al*. (2017a). Mean measures of each dimension were then applied to volumetric calculations based on cell geometry described for each species by Hillebrand *et al*. (1999), with the resulting biovolume for each species given in Table 1.6.1.

Feeds were replenished daily for those vessels where microalgae were being tested as diets, initially by conducting residual counts of two culture vessels per feed regime to work out the mean consumption rate of each microalga per regime, followed by replenishment from high density stock cultures of each microalgal species. Feeds were delivered by initially removing and screening 200 ml of culture water from each vessel, then incorporating new algae into a 200 ml daily water exchange. No single microalgae feed source had more than 70 % of cells consumed in a single 24 h period, nor were larvae observed with empty gut contents, indicating that larvae were feeding throughout the experiment.

Table 1.6.1. Contribution of each microalgae stock feed to each diet as cell density (cells ml^{-1}). Included are mean individual cell biovolume ($\mu m^3 \pm$ SE) of each microalgal species tested. Feeds were delivered as mono-specific diets of either *Chaetoceros muelleri* (Cm), *Chaetoceros calcitrans* (Cc) or *Tetraselmis suecica* (Ts), as two-species mixed diets or as a feed mix of all three microalgae, as recommended by the Australian Centre for Applied Aquaculture Research (ACAAR).

Diet	*Density* (cells ml^{-1})		
	C. muelleri	***C. calcitrans***	***T. suecica***
Cm	1×10^5		
Cc		1×10^5	
Ts			1×10^4
Cm+Cc	5×10^4	5×10^4	
Cm+Ts	5×10^4		5×10^3
Cc+Ts		5×10^4	5×10^3
ACAAR	5×10^4	3×10^4	2×10^3
Individual cell biovolume (μm^3)	42.0 ± 1.6	12.8 ± 0.7	407.2 ± 42.2

Sampling of larvae

On day five of the experiment, larval cultures were monitored every ~2 h until larvae in one of the culture vessels metamorphosed to a point where at least 50 % of the individuals were at mysis I (M I) stage, at which time the experiment was terminated. This end point was selected as it is known to provide the greatest discrimination between sub-stages of development in prawn larvae (D'Souza and Loneragan, 1999; D'Souza and Kelly, 2000). Briefly, Nauplii VI (N VI) larvae were identified as having two pairs of antennae, mandibles, an eyespot and a posterior end with two groups of spines with three plumose spines and three naked spines each. Protozoea sub-stages (PZ I, II and III) were defined as having a cephalothorax, abdomen and telson, with eye stalks appearing from PZ II and two pairs of rudimentary uropods from PZIII. M I sub-stage exhibits a dorsal spine as a precursor to the rostrum and a tail fan with a telson and two pairs of uropods almost fully-formed (Fig. 1.6.1; Motoh, 1985; Crisp *et al*., 2016).

At the end of the experiment, triplicate 200 ml samples of water containing larvae were removed from each experimental vessel for determining survival and development index. Samples were preserved in seawater containing tetraborate-buffered 5% formaldehyde (D'Souza and Kelly, 2000). All preserved larvae that were completely intact (*i.e.* had all appendages), were considered live at the point of sampling, thus included in survival and development counts. Samples were then concentrated onto a 43 µm screen and rinsed with distilled water within a fume cupboard to remove formaldehyde. Larvae were then transferred into a petri dish for enumeration and allocation to a developmental stage.

Once all larvae were staged, a development index (DI) was determined by the following Villegas and Kanazawa (1979) equation, modified by D'Souza and Loneragan (1999); where DI = ∑ (Index values) / n (larvae sampled). The index values of individual larvae increased with each developmental sub-stage from the initial N VI to M I, with index values assigned as N VI = 0, PZ I = 1, PZ II = 2, PZ III = 3 and M I = 4, therefore increasing DI values were associated with increased proportions of more developed larvae.

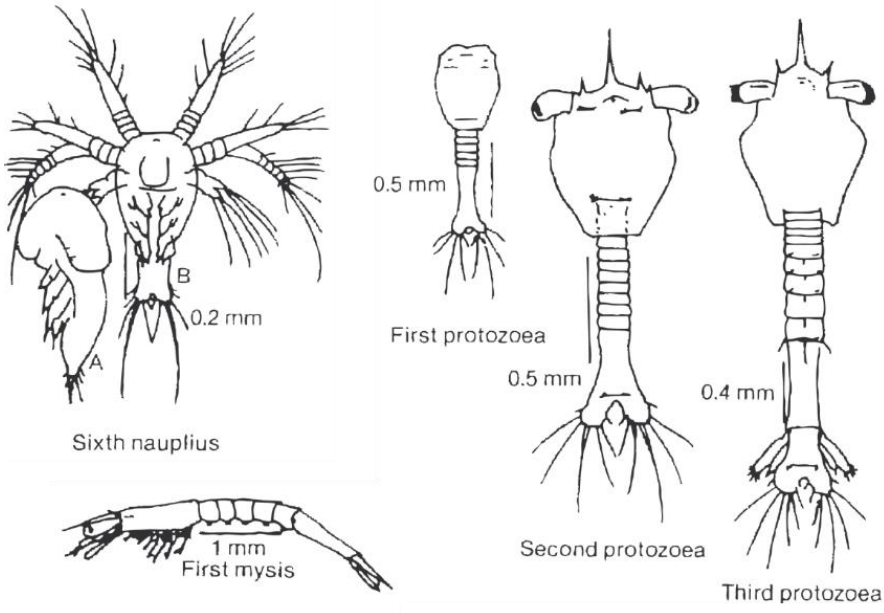


Fig. 1.6.1. Representation of the larval life-cycle of penaeid and metapenaeid prawns from nauplii sub-stage VI, through protozoea sub-stages I to III and on to mysis sub-stages I. Modified from: Motoh (1985).

To prepare the remaining larval biomass for dry weight analysis, all of the remaining larvae from each of the experimental vessels were harvested on a 150 µm screen, rinsed with sea water, re-suspended in their original culture vessels for ~2 h until gut contents were observed to be excreted from larvae, modified from Gamboa-Delgado and Le Vay (2009). Aeration was then removed until suspended solids had settled (~10-15 min), at which time larvae were harvested again, condensed onto a 150 µm screen, briefly rinsed with sea water into 200 ml sample jars and chilled until euthanized. To determine larval body weight as dry weight larva^{-1}, triplicate samples of ~100 pre-fed nauplii larvae and ~10 fed larvae per replicate vessel were first rinsed with 3 x 30 ml aliquots of distilled water to remove external salts, then placed onto tared 25 mm polycarbonate filters (Whatman) with 10 µm pore size (D'Souza and Kelly, 2000). Differences in the number of nauplii and fed larvae used for dry weight analysis were due to the dry weights

of fed larvae being approximately 7-12 times larger than those of nauplii. Filters were then oven dried at 60 ˚C for 24 h, cooled under vacuum for 1 h and weighed.

A normalized biomass development index (NBDI), was calculated for each glass culture vessel for each diet using the percentage survival, development index and dry weight, to determine the overall perform Conklin *et al*. (1975), which was calculated by multiplying the percentage of surviving larvae by the DI, to include a standardized mean dry weight (SMDW). SMDW was calculated for each culture vessel by dividing the mean dry weight (µg larva^{-1}) of larvae from each culture vessel by the maximum mean dry weight (source ACAAR diet; 12.1 µg larva^{-1}) attained for all culture vessels.

SMDW = mean dry weight (µg larva^{-1}) in each vessel / maximum mean dry weight (12.1 µg larva^{-1})

NBDI = (percentage survival) x (DI) x (SMDW)

Statistical analysis

One-way Analyses of Variance (ANOVA) tests were used to determine whether the percentage survival, development and NBDI differed among feeding regimes (7 levels; the three mono and four mixed microalgae feeds) at the end of the experiment. Analysis of dry weight also included samples of N VI larvae in one-way ANOVAs to confirm whether significant changes had occurred from pre-feeding to feeding. This resulted in 8 levels; the three mono and four mixed microalgae feeds, and N VI larvae in this analysis.

Prior to undertaking any analyses, the extent of the linear relationship between the $\log_e$ mean and $\log_e$ standard deviation for each of percentage survival, development, dry weight and NBDI was used to determine which transformations were required to meet the test assumption of homogeneity of variance for each of the above two data tests (Clarke *et al*., 2014a). These analyses indicated that the percentage survival values required a square-root transformation, while development, dry weight and NBDI data did not require transformation. When ANOVA

detected a significant difference, *post-hoc* pairwise comparisons were conducted using Tukey's HSD to elucidate the *a priori* groups that were responsible for each of those differences. The null hypothesis of no significant difference between *a priori* groups was rejected when p was <0.05. All univariate analyses were performed using SPSS version 22 software (IBM, 2013).

To aide in comparing the performance of the diets across survival, development, dry weight and NBDI, all of which are measured in different units, a scaling model was developed. Initially, index values were assigned to diets based upon which groups they were allocated to in the *post hoc* pairwise comparisons. Highest performing groups of diets were assigned the highest values, *i.e.* group a = 3, b = 2, c = 1, etc. Where diet(s) were assigned more than one group, the mean of the index values was used, *i.e.* ab = 2.5, abc = 2, bc = 1.5, etc. To account for the difference in the number of significant groups between each *post hoc* test, index values were standardized as a percentage of the maximum index value for each test. Diets were ranked according to their *post hoc* group for NBDI. In cases where two or more diets were allocated to the same group, they were ordered based upon their mean NBDI score.

Results

One-way ANOVA determined that the percentage survival of *M. dalli* larvae differed significantly among the diets tested ($F_{6, 20} = 5.12$, $p = 0.006$). Pairwise comparisons showed that larvae fed a mixed diet of *C. muelleri* and *C. calcitrans* was the worst performing diet, with a mean (± 95% confidence limits) percentage survival of 53.6 ± 4.2, which was significantly lower than the best performing mixed diets of either *C. calcitrans* and *T. suecica* (94.4 ± 6.3) or *C. muelleri* and *T. suecica* (83.8 ± 18.5; Fig. 1.6.2), and the mono-specific diet of *T. suecica* (83.8 ± 11.5; Fig. 1.6.2). The survival of larvae fed any of the diets aside from the mixed diet of *C. muelleri* and *C. calcitrans* did not differ significantly from each other, with mean percentage survival ranging from 65.3 to 94.4. Survival of starved larvae after 48 h, *i.e.* 23%, was far lower than that of fed larvae.

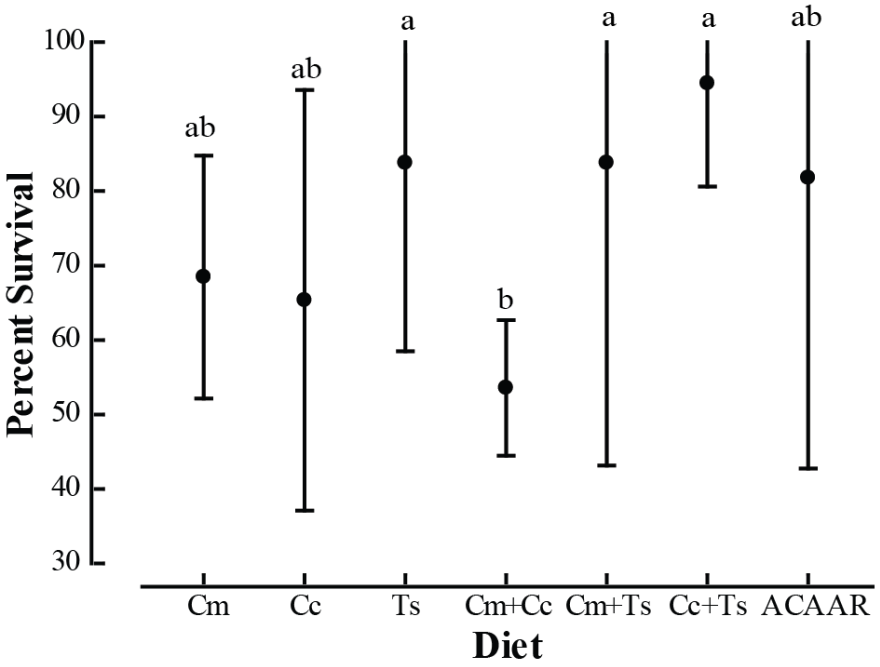


Fig. 1.6.2. Percentage survival of *Metapenaeus dalli* larvae (mean ± 95% confidence limits; *n*=3) fed different algal diets determined at the time when 50 % the control (Cm+Ts) had moulted to M I. *Chaetoceros muelleri* (Cm), *Chaetoceros calcitrans* (Cc), *Tetraselmis suecica* (Ts), Australian Centre for Applied Aquaculture Research (ACAAR) = Cm +Cc+Ts. Bars with the same lower case letters denote groups that are not significantly different.

The development of larvae was shown by ANOVA to differ significantly among the diets tested ($F_{6, 20} = 23.72$, $p < 0.001$). Larvae that were fed the mono-specific feed of *C. calcitrans* had a significantly lower development index than all other diets (2.71 ± 0.07; Fig. 1.6.3). When *C. calcitrans* was fed either *C. muelleri* (3.19 ± 0.10) or *T. suecica* (3.29 ± 0.06), development increased, however, it was still significantly poorer than the mixed diet of *C. muelleri* and *T. suecica* (3.81 ± 0.08). The development of larvae fed the mixed diet of *C. calcitrans* and *C. muelleri* was also found to be significantly lower than larvae fed either the ACAAR diet (3.61 ± 0.12) or *C. muelleri* (3.58 ± 0.21) alone. Starved larvae assessed after 48 h did not develop past PZ I (*i.e.* DI = 1).

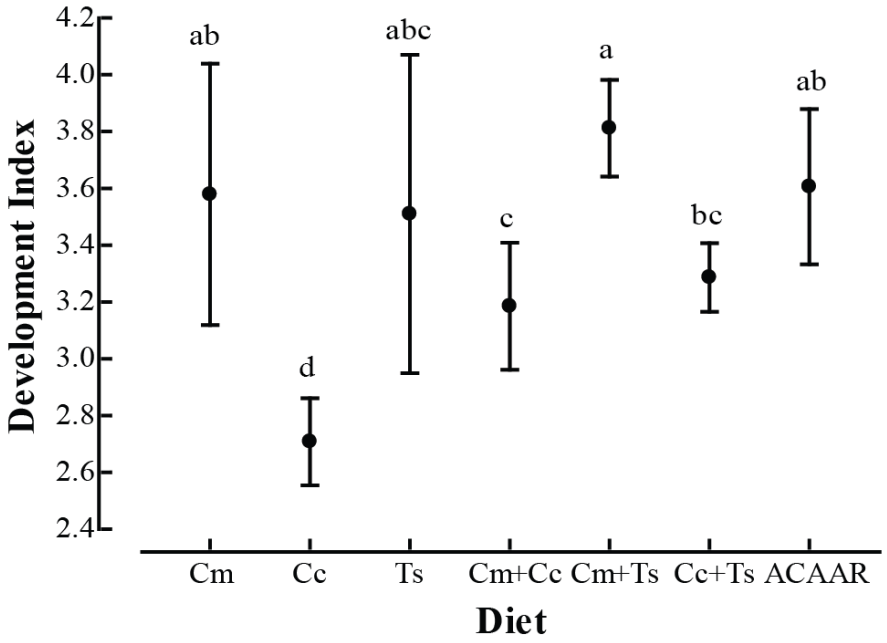


Fig. 1.6.3. Development of *Metapenaeus dalli* larvae (mean ± 95% confidence limits; *n*=3) fed different algal diets determined at the time when 50 % the control (Cm+Ts) had moulted to M I. *Chaetoceros muelleri* (Cm), *Chaetoceros calcitrans* (Cc), *Tetraselmis suecica* (Ts), Australian Centre for Applied Aquaculture Research (ACAAR) = Cm +Cc+Ts. Bars with the same lower case letters denote groups that are not significantly different.

The dry weight of the larvae differed significantly among the various diets tested ($F_{7, 23} = 63.60$, $p < 0.001$). Pairwise comparisons showed that each of the seven diets resulted in a significant increase in dry weight from larvae at the start of the experiment, *i.e.* N VI stage (1.3 ± 0.1 µg larva^{-1}; not shown in Fig. 1.6.4). Of the larvae fed microalgae, a mono-specific diet of *C. calcitrans* (7.0 ± 0.6 µg larva^{-1}; Fig. 1.6.4) was significantly lower than the best performing fed mixed diets including ACAAR diet (11.2 ± 1.2 µg larva^{-1}) and *C. muelleri* and *T. suecica* (10.3 ± 0.6 µg larva^{-1}), or mono-specific diets including either *C. muelleri* (10.0 ± 1.0 µg larva^{-1}) or *T. suecica* (9.7 ± 1.1 µg larva^{-1}). Due to low survival providing inadequate biomass, dry weights of starved larvae were not assessed.

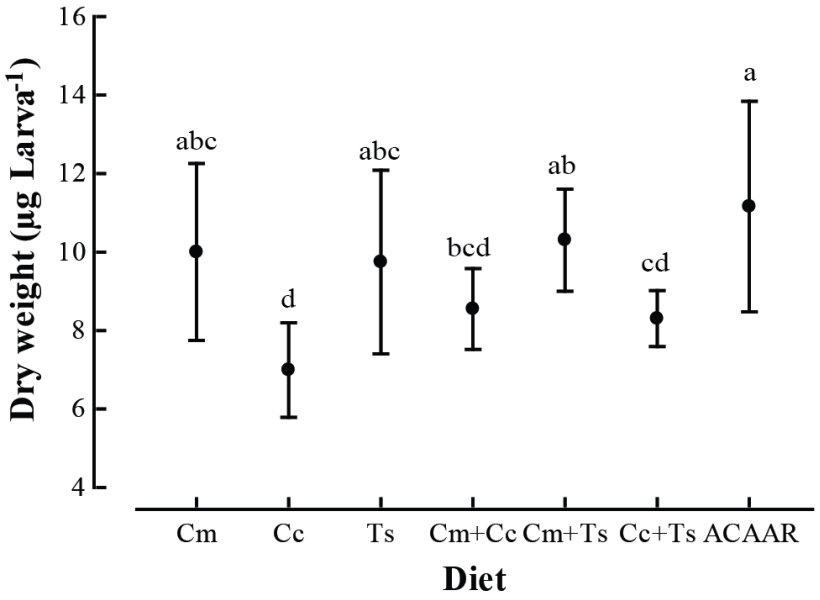


Fig. 1.6.4. Dry weight of Metapenaeus dalli larvae (mean ± 95% confidence limits; n=3) fed different algal diets determined at the time when 50 % the control (Cm+Ts) had moulted to M I. Chaetoceros muelleri (Cm), Chaetoceros calcitrans (Cc), Tetraselmis suecica (Ts), Australian Centre for Applied Aquaculture Research (ACAAR) = Cm +Cc+Ts. Bars with the same lower case letters denote groups that are not significantly different.

The overall performance of diets based on the NBDI was shown by ANOVA to differ significantly among the diets tested ($F_{6, 20} = 9.04$, $p < 0.001$). Larvae fed five of the diets did not differ significantly, performing the best with mean NBDI values ranging from 270.5 to 203.3 (Fig. 1.6.5). Of these five diets, four contained *T. suecica* as either mono-specific or mixed feeds, with the ACAAR diet (270.5 ± 63.0) and a mixed diet containing *C. muelleri* (269.3 ± 46.3) performing the best overall. Two worst performing diets contained *C. calcitrans* as either a mono-specific (102.2 ± 24.1) or mixed diet with *C. muelleri* (119.9 ± 8.9).

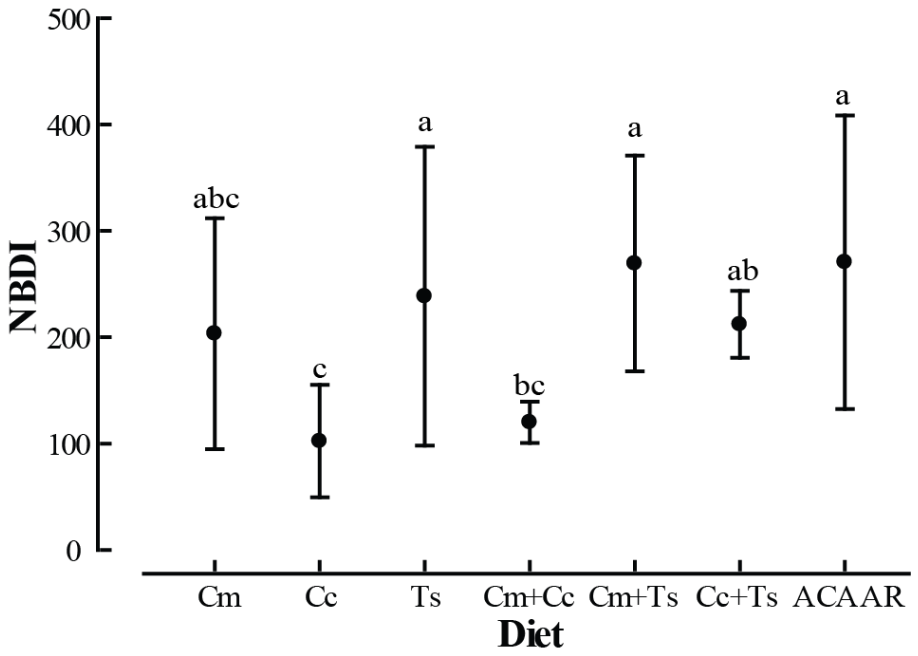


Fig. 1.6.5. Normalized biomass development index (NBDI) of *Metapenaeus dalli* larvae (mean ± 95% confidence limits; *n*=3) fed different algal diets determined at the time when 50 % the control (Cm+Ts) had moulted to M I. *Chaetoceros muelleri* (Cm), *Chaetoceros calcitrans* (Cc), *Tetraselmis suecica* (Ts), Australian Centre for Applied Aquaculture Research (ACAAR) = Cm +Cc+Ts. Bars with the same lower case letters denote groups that are not significantly different.

Comparisons of the ranked performance of each response variable between each diet (Table 1.6.2) indicated that a mixed diet of (i) *T. suecica* and *C. muelleri* and (ii) ACAAR and the mono-specific diet of *T. suecica* performed the best. In each of these diets, a maximum ranking score of 100 was attained in at least one of the response variables, which was also reflected in their high NBDI values (Fig. 1.6.5). A mixed diet of *T. suecica* and *C. calcitrans* also attained a ranking score of 100 for survival, however, larvae fed this diet had poor development and dry weight. The worst performing diets were a mixed diet of *C. calcitrans* and *C. muelleri*, as well as a mono-specific diet of *C. calcitrans*, which had low ranking scores across all response variables.

Table 1.6.2. Performance of larval *Metapenaeus dalli* fed each microalgal diet, scored according to the results of *post hoc* pairwise comparisons for each analysis of survival, development, dry weight and normalized biomass development index (NBDI). Diets were ranked according to their *post hoc* group for NBDI. In cases where two or more diets were allocated to the same group, they were ordered based upon their mean NBDI score. *Chaetoceros muelleri* (Cm), *Chaetoceros calcitrans* (Cc), *Tetraselmis suecica* (Ts), Australian Centre for Applied Aquaculture Research (ACAAR) = Cm +Cc+Ts.

Diet	Survival	Development	Dry weight µg larva^{-1}	NBDI	Mean NBDI
Cm+Ts	100.0	100.0	87.5	100.0	269.3
ACCAR	75.0	87.5	100.0	100.0	270.5
Ts	100.0	75.0	75.0	100.0	238.4
Cc+Ts	100.0	62.5	37.5	83.3	212.1
Cm	75.0	87.5	75.0	66.7	203.3
Cm+Cc	50.0	50.0	50.0	50.0	119.9
Cc	75.0	25.0	25.0	33.3	102.2

Discussion

This study has demonstrated that the performance of various monospecific and mixed microalgae diets affected the survival, development, dry weight and overall performance of larval *M. dalli*.

Survival

Although survival differed significantly among the diets, as a measure of performance this was poor as it failed to distinguish differences between six of seven diets tested as evidenced by the large confidence limits attained with each mean percentage survival value. Mean percentage survival was also found to be highly variable in dietary studies undertaken on larval *Penaeus* spp. and *Litopenaeus vannamei* (D'Souza and Loneragan, 1999; Piña *et al*., 2006). In this experiment survival was considered inadequate at distinguishing between the performance of diets. To overcome this, future trials testing differences in survival of diets should include more replicates to counteract the effect of large variation in survival between replicate culture vessels.

Development and dry weight

Of larvae fed each of the seven diets, mono-specific diets of either *C. muelleri*, *T. suecica* or mixed algal diets containing both species resulted in higher development index and dry weight values at the cell densities tested, while diets containing *C. calcitrans* generally performed

poorly, though one exception to this was the ACAAR diet. When comparing development and dry weight of larval *M. dalli*, there appears to be a positive relationship between these two response variables. This relationship is particularly important, as larval feeding stops during metamorphosis, with larvae switching to limited endogenous reserves until metamorphosis is completed (Barclay *et al*., 1983; Lemos *et al*., 2001). Thus, it is likely lower assimilation of *C. calcitrans* to dry weight by larval *M. dalli* resulted in poor development, leading to poor overall performance of this microalga as feed.

In regards to the performance of *T. suecica*, it is possible that localized environmental adaptability to conditions within estuarine environments has allowed *M. dalli* to survive and grow well on both diatoms and chlorophytes during herbivorous stages. For example, in similar dietary studies of protozoa stage larvae of tropical prawns that reproduce in marine environments, such as *Penaeus semisulcatus* (D'Souza and Loneragan, 1999) and *L. vannamei* (Piña *et al*., 2006), *T. suecica* performed significantly worse than either a mono-specific diet of *C. muelleri* or a mixed feed of *T. suecica* and *C. muelleri*. Given that diatoms are more prevalent in marine environments and chlorophytes more abundant in freshwater and brackish water environments (Twomey and John, 2001; Tweedley *et al*., 2016b), dietary preference for *C. muelleri* by *P. semisulcatus* and *L. vannamei* may occur. It also possible that feed preference by prawn species may be species-specific, as the marine spawning *Penaeus japonicus* larvae, like *M. dalli* in this study, showed no significant difference in development when fed either *T. suecica* or *C. muelleri* as mono-specific feeds (D'Souza and Loneragan, 1999).

When comparing performance between different species of diatoms, *C. muelleri* appeared to be a superior feed to *C. calcitrans* for *M. dalli*. Dietary studies of *Penaeus monodon* protozoea stage larvae also showed that *C. muelleri* was a superior diet in terms of development and growth than *C. calcitrans*, when fed at densities similar to this study (Tobias-Quinitio and Villegas, 1982; D'Souza *et al*., 2002). Biovolume measures of each microalga in this study showed that *C. calcitrans* was in fact the smallest of each microalgal species described, being ~30 % of the biovolume of *C. muelleri*. Although *C. muelleri* was found to be adequate for feeding larval *M. dalli*, another diatom *C. calcitrans* was not. In previous dietary studies of *Penaeus monodon* protozoea stage larvae, *C. muelleri* was found to be a superior diet in terms

of development and dry weight than *C. calcitrans*, when fed at densities similar to this study (Tobias-Quinitio and Villegas, 1982; D'Souza *et al*., 2002). Size preference of microalgae may be a consideration, given that *M. dalli* larvae are ~65 % the length of *P. monodon* during similar metamorphic stages (Robert Michael, pers comm.), however, measures of each microalga used in this study showed that *C. calcitrans* was in fact the smallest of each microalgal species described (Table 1.6.1). Given that dietary feeds of the diatoms were assigned on the basis of cell density alone, it is obvious that *C. calcitrans* provided far less biovolume concentration than *C. muelleri*. This difference may have contributed to the poor performance of diets for larval *M. dalli* and in other penaeid prawns fed *C. calcitrans* at similar densities, due to a shortfall in the gross amount of food consumed.

Previous comparisons of macronutrients for each microalgal species used in this study by Brown (1991) showed substantial differences. *Chaetoceros calcitrans* had constituent weights (pg cell^{-1}) of protein (3.8), carbohydrate (0.68) and lipid (1.8), while *C. muelleri* had protein (9.0), carbohydrate (2.0) and lipid (5.2) and *T. suecica* had protein (52.1), carbohydrate (20.2) and lipid (16.8) respectively, further highlighting the potential for disproportionate delivery of gross macronutrient components. Given that not all the microalgal cells were consumed each day in this study, it is possible that the relative proportions of each macronutrient, particularly in *C. calcitrans*, did not satisfy the feeding requirements of larval *M. dalli*. The lack of nutrients may be due to the greater proportion of indigestible component silica frustule in *C. calcitrans*, which is caused by a surface area to volume ratio smaller than *C. muelleri* (Brown, 1991).

Large-scale production

This research has demonstrated that *M. dalli* larvae can be cultivated in small glass culture vessels through the protozoea stage using a diet comprising a single species of microalgae. However, further trials that examine the role of these microalgae during the mysis stage of development, when prawn larvae are known to become omnivorous are required (Jones *et al*., 1997; Gamboa-Delgado and Le Vay, 2009). Furthermore, larger-scale cultivation of larval *M. dalli* has previously been achieved by the Australian Centre for Applied Aquaculture

Research in Fremantle, Australia, using both the mixed diet of *C. muelleri* and *T. suecica* (~ 2 million post larvae) and ACAAR diets (~ 2 million post larvae) between 2014 and 2016 (Jenkins *et al*., 2017). Whether similar success could be achieved using mono-specific diets of either *C. muelleri* or *T. suecica*, however, has yet to be determined. As part of larger-scale trials, further analysis of tolerance levels of key metabolic by-products by *M. dalli* larvae, such as ammonia, nitrite and nitrite, should be examined to develop baseline requirements for production, particularly if larvae are grown under low/no water exchange. It is also recommended that large-scale production should incorporate monitoring of pH, nitrogen levels and speciation *in situ* to monitor such changes (ASEAN, 1978; Menasveta *et al*., 1989; Kesarcodi-Watson *et al*., 2008).

Conclusions

This study showed that for the herbivorous feeding phase of larval *M. dalli*, both mono-specific and mixed diets containing both *C. muelleri* and *T. suecica* produced the best overall performance. *Chaetoceros calcitrans* performed poorly, both as a mono-specific feed and in mixed culture, except when fed with both *C. muelleri* and *T. suecica*. This study enhances the limited knowledge on the feeding requirements of metapenaeid prawn larvae, however, future studies of assimilation efficiencies of feeds and nutritional requirements of *M. dalli* larvae are required to increase larval performance in commercial production.

1.7. Quantitative classification of sediments in the Swan-Canning Estuary

This study has been published in an Honours thesis by Amber Bennett.

Bennett, A.L. (2014). The influence of sediment compositions on the Western School Prawn *Metapenaeus dalli* in a temperate Australian estuary. Honours Thesis, Murdoch University.

Summary

The composition of the sediment plays an important role in structuring the composition of estuarine biota, as it can provide both habitat and, either directly or indirectly, a food source for invertebrates and fish. While aspects of floral and faunal composition are generally well studied in estuarine environments, less focus is typically placed on elucidating the composition of the sediment. Thus, the aim of this study was to characterise and classify the sediments of the nearshore and offshore waters of the Swan-Canning Estuary. Sediments were collected seasonally from 20 nearshore and 16 offshore sites spread throughout the system. Multivariate statistical analyses indicated that the composition of the sediment differed significantly among water depths, seasons and site, albeit seasonal changes were only detected in offshore waters. Differences between water depths were particularly marked due to higher contributions of particulate organic matter and finer inorganic grain sizes in the offshore waters, due to the reduced levels of water movement creating a depositional environment. Spatial differences in sediment composition were also detected, mainly relating to the distance upstream and thus levels of water movement and extent of tidal transport. A quantitative classification of the sediment composition using the CLUSTER and SIMPROF procedures identified 11 significantly different sediment types that occur throughout the estuary. The sediment compositions were mapped and show that the estuary comprises a mosaic of sediment types. Finally, a decision tree was developed to provide a set of quantitative thresholds to allow the sediment type to which any 'new' sample of sediment from the Swan-Canning Estuary belongs to be calculated.

Rationale and aims

Sediments are comprised of a mixture of organic and inorganic particles, which form the substratum of estuarine and marine environments (Gray and Elliott, 2009). The organic components of the sediment are represented by dead (*e.g.* faecal pellets) and living materials, *e.g.* benthic algae (Araujo *et al.*, 2010), while the inorganic particles are generally comprised of quartz, silicates, calcite and clay minerals that can range in size from clay through silt and sand to gravel (Wentworth, 1922; Dankers, 2002; Araujo *et al.*, 2010). Sedimentary material is transported into estuaries from both terrestrial (via riverine flow or direct runoff) and marine sources (via tidal flow). The main source of sediments differs depending on the estuary, for example, in the Tay Estuary (Scotland), 70% of the sedimentary material is transported into the system via suspended sediment flux from marine waters. This is in contrast to the Loire (France) and Yellow River (China) estuaries, which receive large amounts of clay from the surrounding catchment transported by freshwater discharge (McLusky and Elliott, 2004).

Regardless of the source of sediments, the erosion and deposition of those materials is governed by the speed of water movement and the size of the particles (Postma, 1967) and therefore the extent of tidal influence has been shown to have a major effect on sediment transport within the estuary. For example, while both macro (*i.e.* tidal range >2 m) and microtidal (*i.e.* tidal range <2 m) estuaries typically receive relatively large inputs of terrigenous sediment, the patterns of sediment deposition and erosion are different (Fig. 1.7.1). In macrotidal estuaries, coarse sediment is deposited at the point where freshwater discharge and tidal forces converge (Fig. 1.7.1a). In contrast, in microtidal estuaries these materials are generally deposited at the mouth of the estuary (Pasternack and Brush, 2002; Webster *et al.*, 2002). The strong tidal currents in macrotidal systems continually resuspend fine particles, resulting in high turbidity (*e.g.* Turner *et al.*, 1994; Wells, 1995), whereas in microtidal systems, where no such currents exist, fine particles are transported into the deeper regions of the basin, where current velocity is low and deposition takes place (*e.g.* Hodgkin and Hesp, 1998; Harris *et al.*, 2002; Fig. 3.3.1b). Moreover, this low-energy environment results in the trapping of up to 80% of the fine sediment transported into the

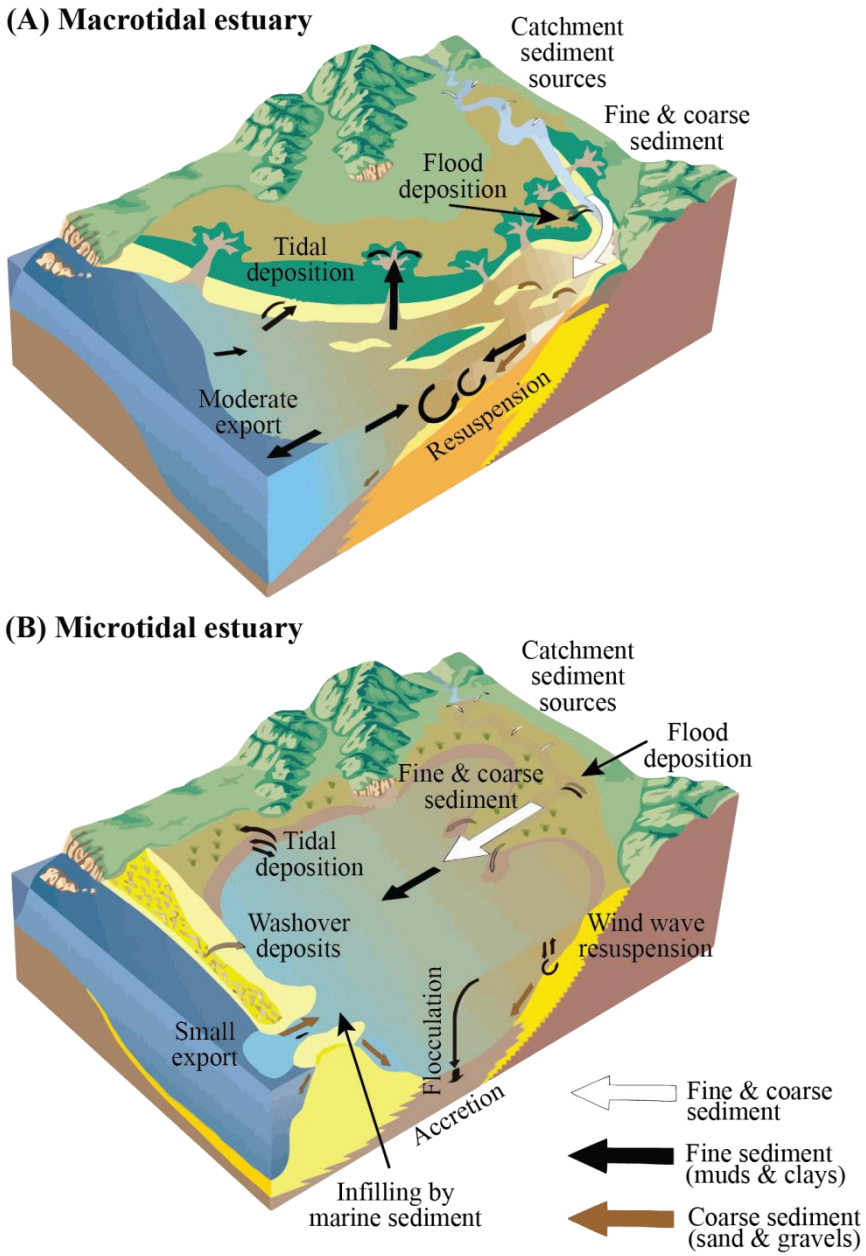


Fig. 1.7.1. Conceptual models of the sediment dynamics of typical (A) macrotidal and (B) microtidal estuaries. Taken from Tweedley *et al*. (2016b).

estuary (Patchineelam *et al*., 1999; Roy *et al*., 2001). It is also important to recognise that, as well as differences between estuaries, the wide range of hydrodynamic environments present within a system can result in a mosaic of sediment types occurring (*e.g.* Gray and Elliott, 2009).

Estuarine sediments can be divided into two broad categories: cohesive and non- cohesive, with the former being fine, muddy, silty sediments that are usually associated with greater quantities of particulate organic matter (POM; Mitchener and Torfs, 1996; Araujo *et al*., 2010). Due to their small size, cohesive particles are highly susceptible to erosion and are only able to settle in areas with low water movement (Postma, 1967). The larger surface area to volume

ratio of these small particles results in them being associated with higher levels of contaminants, such as heavy metals (Ackermann, 1980; Chapman and Wang, 2001). In contrast, non- cohesive sediments are comprised of much coarser grains (*i.e.* > 63 µm), and generally have lower percentages of POM (Araujo *et al*., 2010). Their larger size results in these particles being more commonly found in higher wave action areas (Mitchener and Torfs, 1996).

Many studies have shown that the composition of the substratum strongly influences the floral (*e.g.* Barko *et al*., 1991; Bradley and Stolt, 2005) and faunal assemblages of estuaries (*e.g.* Gray, 1974; Snelgrove and Butman, 1994). While several studies have shown the importance of the sediment composition on benthic, burrowing fish species, such as gobiids (*e.g.* Humphries *et al*., 1992; Gill and Potter, 1993; Gill and Humphries, 1995), most studies have focused on benthic macroinvertebrates. For example, filter feeders have been shown to prefer sandier sediments, as they typically have higher levels of water movement, which erode fine particles and provide an effective food transport mechanism. In contrast, calmer (deeper) waters are often associated with finer sediments, as the reduced rate of water movement allows finer particles to settle and accumulate, resulting in an abundance of food for deposit feeders (Sanders, 1958; Rhoads and Young, 1970; Rhoads, 1974).

The strength of sediment-infauna relationships is supported by work in 15 South African estuaries where differences in sediment compositions were shown to be more influential than the corresponding differences in salinity in structuring the composition of the benthic macroinvertebrate fauna (Teske and Wooldridge, 2003). Furthermore, a number of studies have also shown that epifaunal invertebrate species, such as crabs, prawns and brittlestars, also exhibit a preference for particular types of sediment (*e.g.* Ruello, 1973; Jones and Simons, 1981; Boos *et al*., 2010).

The estuaries of south-western Australia are located in a microtidal region of the world and vary in the temporal duration of their connection to the ocean and are thus classified as either permanently-open, seasonally-open or normally-closed (Potter and Hyndes, 1999; Potter *et al*., 2010). The Swan-Canning Estuary is one of the largest, permanently-open estuaries in this region and, due to its close proximity to the capital city of Perth, is one of the most studied

systems in the state of Western Australia. In particular, extensive knowledge exists on its fish (*e.g.* Loneragan *et al*., 1989; Loneragan and Potter, 1990; Valesini *et al*., 2009; Valesini *et al*., 2014), invertebrate faunas (*e.g.* Kanandjembo *et al*., 2001; Wildsmith *et al*., 2011; Tweedley *et al*., 2016a) and hydrology (*e.g.* Spencer, 1956; Stephens and Imberger, 1996; Hamilton *et al*., 2001). However, with the exception of several studies on heavy metal levels (Gerritse *et al*., 1998; Rate *et al*., 2000), little focus has been placed on the sediment composition of this system. Such is the paucity of information on the sediments, that the most comprehensive studies on sediment composition were conducted by Gill and Potter (1993) and Hourston *et al*. (2009), when determining the factors influencing the spatial distribution of gobiid species and the spatial and seasonal variations of nematode assemblages, respectively. They found that, generally, sediments from sites located in the lower estuary were comprised of a higher proportion of fine sands than those surveyed in the middle and upper estuary, while those in the upper estuary contained larger contributions of clay and silt fractions. However, the study by Gill and Potter (1993) only included 15 shallow water sites located throughout the shallow, nearshore waters of the Swan- Canning Estuary and the study by Hourston *et al*. (2009) only 12 nearshore sites. Moreover, neither study investigated any temporal trends in sediment composition or sediments sampled in the offshore waters.

The main aim of this study is to quantitatively classify the sediment types of the shallow, nearshore and deeper, offshore waters of the Swan-Canning Estuary. These data will be used to test the hypotheses that the composition of the sediment will change between water depths and, within those water depths, will change spatially and temporally. A decision tree will then be developed to predict the sediment type to which any 'new' site within the Swan-Canning belongs to. The resulting categorisation of sediment types will then provide the foundation for investigating the extent to which sediment type influences the distribution of *M. dalli* both in the Swan-Canning Estuary and under controlled laboratory conditions (see later).

Methods

Sediment sampling

Sediment samples were collected from 20 nearshore, shallow sites (*i.e.* <2 m deep) and 16 offshore, deeper sites (*i.e.* 2 to 17m deep) in the Swan-Canning Estuary in both February (austral summer) and August 2014 (austral winter) (Fig 1.7.2). The samples were collected from nearshore sites using a cylindrical corer, and from offshore sites using an Ekman grab. The corer was 3.57 cm in diameter (10 cm^2 in area) and sampled to a depth of 10 cm. Once collected, a tube was placed over the end of the corer and the sample ejected. An Ekman grab was employed to collect substrata from offshore sites. This grab sampled an area of 225 cm^2 and to a depth of 15 cm. The sediment from the grab was emptied into a plastic tray and a subsample of 4 cm in diameter and 10 cm in depth was collected. Two samples were collected from each site on each sampling occasion, stored on ice and subsequently frozen.

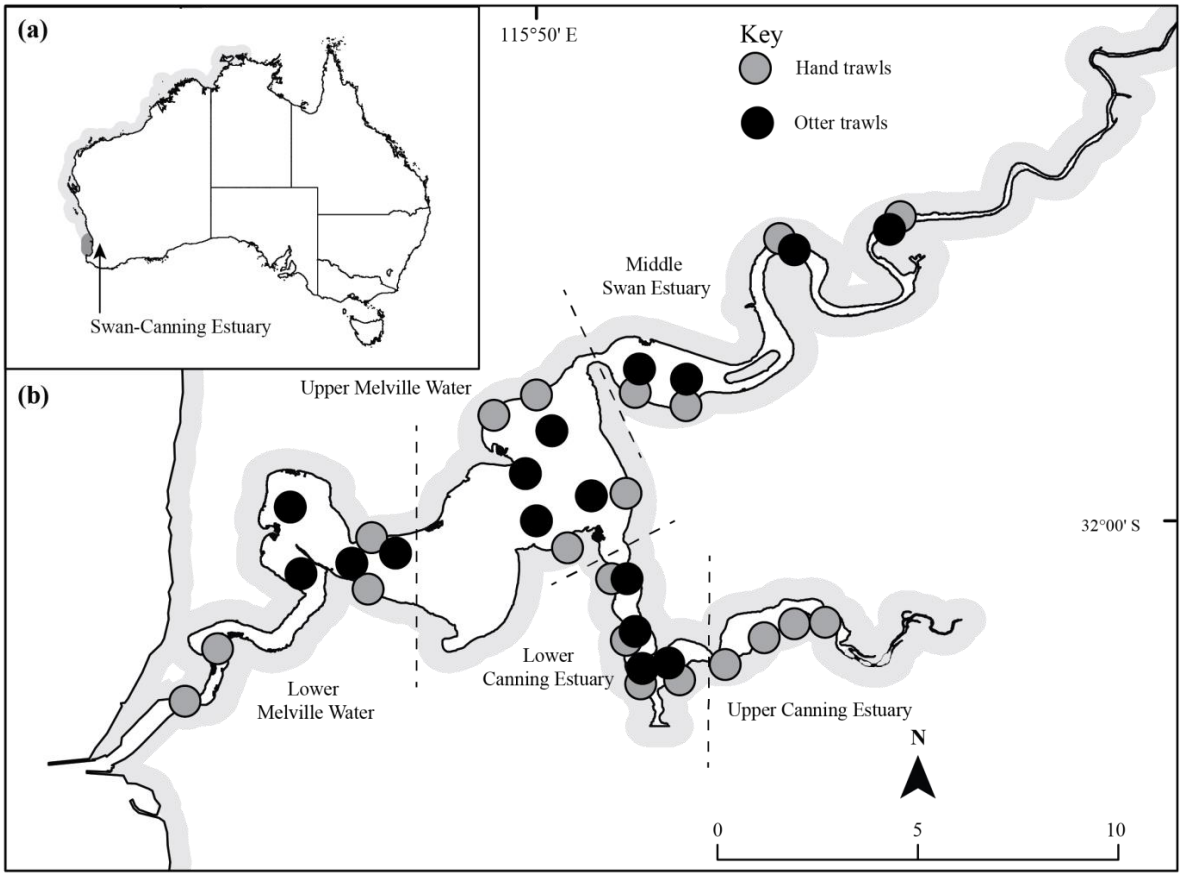


Fig. 1.7.2. A map of the Swan-Canning Estuary, showing the location of the 20 nearshore and 16 offshore sites where sediment was collected in February and August 2014. The arrow on the inset shows the location of the Swan-Canning Estuary in Australia.

For grain size analysis, sediments were defrosted, dried for at least 24 hours at 80 °C and weighed, before being ashed in a furnace at 550 °C for two hours (Heiri *et al*., 2001). The percentage contribution of POM was calculated by subtracting the difference between the dry and ashed weight and converting that number to a percentage (Hourston *et al*., 2009). Fine sediment (*i.e.* sediment particles <63 µm in diameter) were removed from the inorganic portion of the sediment by wet- sieving through a 63µm sieve before drying and re-weighing. Finally, the remaining sediment was then wet-sieved through six mesh sizes corresponding to the Wentworth Scale for grain size (*i.e.* 63 µm, 125 µm, 250 µm, 500 µm, 1000 µm and 2000 µm; Wentworth, 1922). The inorganic fraction for each grain size was then dried, weighed and their percentage contribution by weight determined for each sample.

Statistical analysis

All statistical analyses were performed using the PRIMER v6 multivariate software package (Clarke and Gorley, 2006) and the PERMANOVA+ add-on (Anderson *et al*., 2008), with the exception of the 3-way ANOSIM test, which was conducted in a beta test version of PRIMER v7 (provided by Prof. Bob Clarke, Plymouth Marine Laboratory).

Preliminary investigation of spatial and temporal patterns in sediment composition

The values for a range of sediment characteristics, *i.e.* particulate organic matter content and seven inorganic grain sizes namely (>2,000 µm, 1,000-1,900 µm, 500-

999 µm, 250-499 µm, 125-249 µm, 63-124 µm and <63 µm), were initially examined using pairwise scatterplots between all pairs of variables (*i.e.* a Draftsman plot), with associated Pearson's correlation matrix, which assess whether the distribution of values for each of the variables were skewed and whether any two variables were highly correlated, *i.e.* $r \geq 0.95$; (Clarke and Ainsworth, 1993). This analysis showed that each of the variables required a square-root transformation and that no pair of variables was highly correlated (Fig. 1.7.3). Note

that as all variables were measured using the same units (*i.e.* percentages), no normalization was required (Clarke and Warwick, 2001).

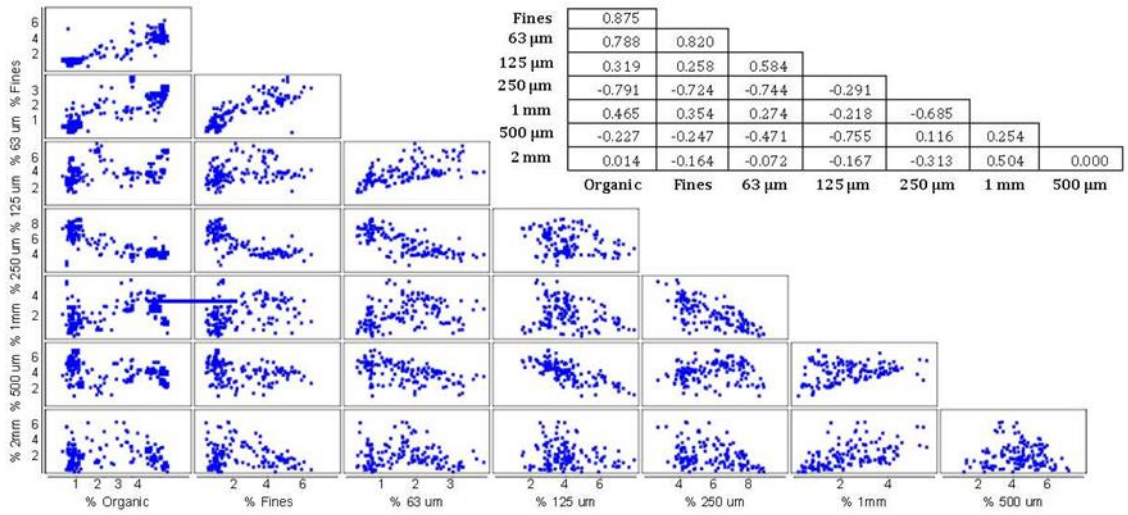


Fig. 1.7.3. Pairwise scatter plots between all variables of the various sediment composition variables and the associated Pearson's correlation matrix. Organic = Particulate Organic Matter (POM), and fines = inorganic particles <63 µm.

To ensure that both the organic (*i.e.* particulate organic matter) and inorganic (*i.e.* the seven grain sizes) variables contributed equally to the multivariate analysis, a weighting procedure was undertaken in which both categories of variables were assigned an arbitrary weight of 100, which was divided equally amongst its component variables (see Valesini *et al*., 2010). Thus, POM was assigned a weight of 100, while each of the seven inorganic grain sizes was given an equal weighting of 14.28. Finally, the pre-treated (*i.e.* transformed and weighted) replicate sediment data for each nearshore and offshore site on each sampling occasion were used to construct a Euclidean distance matrix. This Euclidean distance matrix and was then subjected to 3-way crossed Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson, 2001) using type I sums of squares to determine whether sediment composition varied among sites, water depths and seasons and if these main effects interacted. All main effects were considered fixed and the null hypothesis that there were no significant differences among *a priori* groups would be rejected if the significance level (P) was < 5%. The same matrix was then subjected to 3-way crossed Analysis of Similarities (ANOSIM; Clarke and Green, 1988) test to assess the influence of each of the three main effects. This was achieved by comparing the magnitude of the test statistic (R), which typically ranges ~0, when the average similarities

among and within groups of the target factor do not differ, up to 1, when the compositions of all samples within each group are more similar to each other than to those of any sample from other groups. As the above analyses indicated that there was a significant and large interaction between water depth and season, separate one-way ANOSIM tests were conducted to elucidate whether any seasonal differences in sediment composition were present within each water depth.

Non-metric Multidimensional Scaling (nMDS) ordination plots (Clarke, 1993) were used to display visually any differences and/or similarities in sediment composition among the main effects and the basis for any interactions between those main effects that were identified by PERMANOVA. These ordination plots were constructed by outputting the distances among centroids matrix from the PERMANOVA+ package, which creates averages in the 'Bray–Curtis space' calculated from the replicate samples (Anderson *et al*., 2008). The plots, which show low-dimensional approximations to the pattern of group centroids in the full-dimensional space, are subsequently referred to as centroid nMDS ordination plots (Lek *et al*., 2011).

Quantitative classification of sediment types

To statistically identify those groups of sites that did not differ in their sediment composition, the pre-treated replicate sediment data for each site in each season were averaged, used to construct a Euclidean distance matrix and subjected to hierarchical agglomerative clustering with group-average linking (CLUSTER) and an associated Similarity Profiles test (Clarke *et al*., 2008). As the above test indicated that a significant seasonal effect was present only at sites located in offshore waters, the data for each site in nearshore waters in each season were also averaged. Note that, despite the large differences in sediment composition between the sites in the nearshore and offshore waters, all sites were included in the same analysis as the same variables were measured at all sites and separating the sites in the two water depths would exclude the possibility of a nearshore site being grouped together with an offshore site.

This combination of tests firstly produced a dendrogram (CLUSTER) of the 52 site/season combinations and then, at each node of that dendrogram, a SIMPROF test was performed to determine whether the group of samples being subdivided contained any significant internal structure. This provides an objective basis for determining the point in the cluster procedure at which further subdivision of the sites in unwarranted (*i.e.* are they statistically similar). The null hypothesis of no significant difference in sediment composition between any group of sites was rejected if the significant level (*P*) was < 5%. The raw values for sediment characteristics were averaged for each sediment type and plotted.

Quantitative prediction of sediment types

The Linkage Tree (LINKTREE; Clarke *et al*., 2008) and SIMPROF procedures were employed to produce a binary decision tree whose terminal nodes represented each of the sediment types identified by the CLUSTER and SIMPROF analyses above. A set of quantitative thresholds that best discriminated between two pathways (or sediment types) at each node in the decision tree was also calculated. This enables future workers to classify sediment from new locations within the Swan-Canning Estuary and assign their site to either (i) the most appropriate existing sediment type or (ii) a new sediment type. Note that an identical methodology has been employed to assign sites in five estuaries in south- western Australia to a habitat type based on the measurement of a number of enduring environmental variables (see Valesini *et al*., 2009; 2010; Tweedley, 2011).

In order to construct the decision tree, a fixed 'model' resemblance matrix was produced by averaging the pre-treated (*i.e.* transformed and weighted) data for each sediment variable across the samples employed in the CLUSTER-SIMPROF test (*i.e.* mean data for each site in each season for the offshore sites and the mean data for each site for the nearshore sites over both seasons) representing a sediment type and repeating those average values for each sample belonging to that sediment type. In other words, each sample belonging to a sediment type was given the value equal to the mean for all samples within that sediment type, so that

the data reflect the pattern of differences among sediment types, but without any heterogeneity among samples. These 'model' data were then used to construct a Euclidean distance matrix.

A corresponding data matrix was also constructed, which contained the untreated (raw) measurement for each of the sediment variables in each sample. Both this matrix and the model Euclidean distance matrix were subjected to the LINKTREE and SIMPROF procedures. LINKTREE was employed to construct the decision tree, while the SIMPROF test was used to terminate construction of the tree at those nodes at which the remaining samples contained no significant internal structure (*i.e.* there was no significant difference). Once again the null hypothesis of no significant difference was rejected if the significant level (P) was < 5 %.

Results

Preliminary investigation of spatial and temporal patterns in sediment composition

Three-way crossed PERMANOVA showed that sediment composition (*i.e.* the percentage contribution of POM and seven inorganic grain sizes) differed significantly among water depth, season and sites and that the interaction terms were significant (all P = 0.01%; Table 1.7.1). The pseudo-F values, which provide an indication of the relative strength of each term in the statistical model, demonstrated that among the interaction terms, those containing the depth main effect were more influential, particularly the season x depth interaction (Table 1.7.1). A three-way ANOSIM test demonstrated that the R value for depth (0.968) was considerably greater than those for site (0.697) and season (0.607).

Table 1.7.1. Mean squares (MS), pseudo *F*-ratios and significance levels (*P*) from a three-way PERMANOVA on the sediment characteristics recorded at the 36 sites spread throughout the nearshore and offshore waters of the Swan-Canning Estuary sampled in summer (February) and winter (August) 2014. *df* = degrees of freedom.

Main effects	***df***	**MS**	**Pseudo-*F***	***P***
Season	1	31,547	39	0.01%
Depth	1	2,327,100	2854	0.01%
Site	19	172,440	11	0.01%
Interactions				
Season x Depth	1	26,968	33	0.01%
Season x Site	19	55,280	4	0.01%
Depth x Site	15	177,920	15	0.01%
Season x Depth x Site	14	54,305	5	0.01%
Residual	73	59,519		

The results of these tests were explored visually on centroid nMDS ordination plots. On the plot coded for depth, the points representing the samples collected from offshore waters clearly separated from those representing nearshore waters. Thus, while those samples from offshore waters form a discrete cluster on the left side of the plot, the corresponding samples from nearshore waters formed a relatively tight cluster on the opposite side (Fig. 1.7.4a). Differences between seasons were less marked, however, but the cause of the significant season x depth interaction detected by PERMANOVA (Table 1.7.1) is evident as there is clear separation of the samples from the two seasons in the offshore waters.

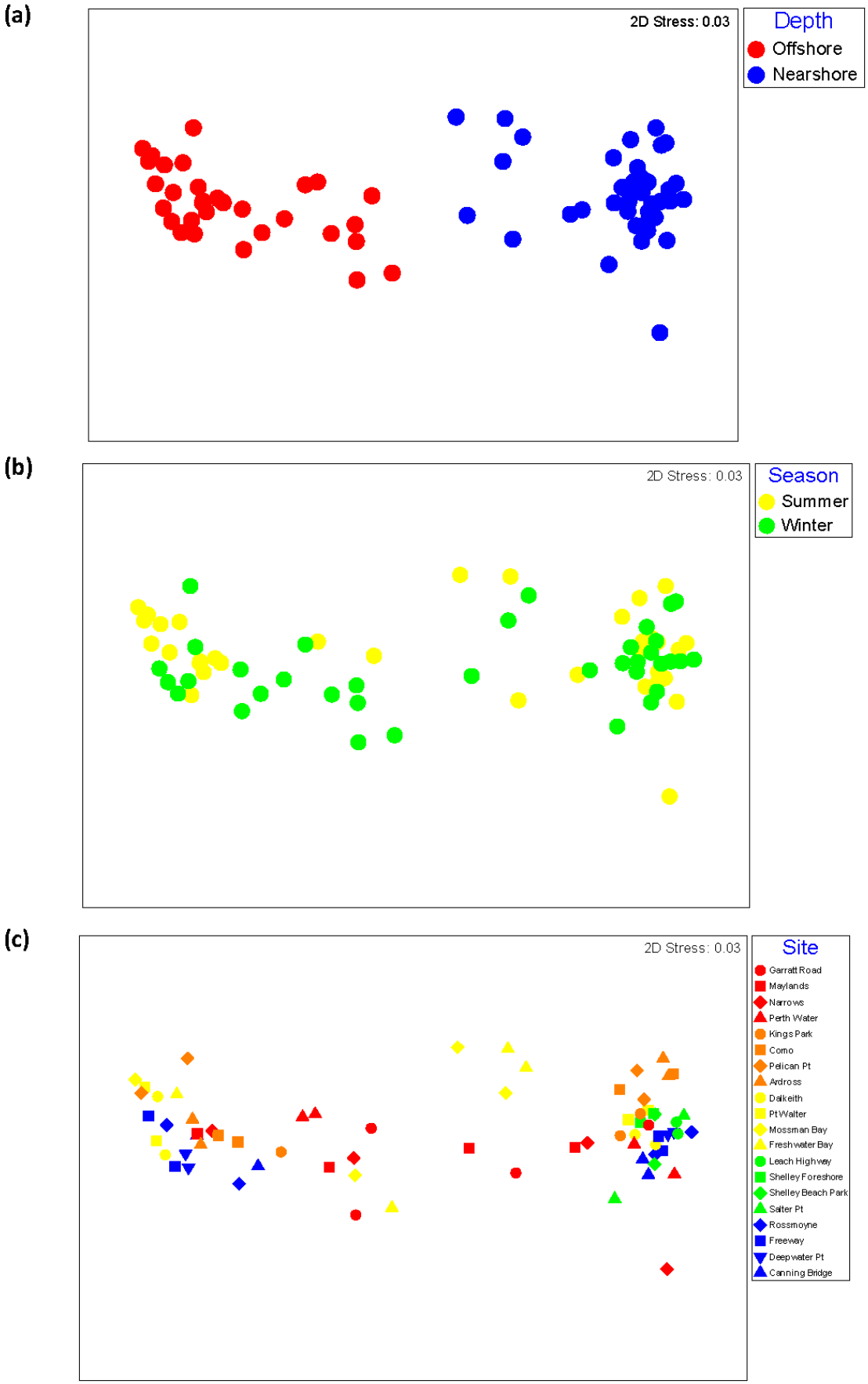


Fig. 1.7.4. Centriod nMDS ordination plots, derived from a distance among centroid matrix constructed from a Bray-Curtis similarity matrix of the sediment composition at the 36 sites in summer and winter 2014. Point coded for (a) water depth, (b) season and (c) site. Sites within the same region are shown in the same colour.

Multiple one-way ANOSIM tests, conducted for offshore and nearshore sites separately showed that sediment composition differed significantly between summer and winter for sites located in offshore waters (R = 0.220; P = 0.01%), but not in nearshore waters (R = 0.00; P = 43.50%). Thus, when classifying the sites into sediment types, the data for nearshore sites in the two seasons can be pooled.

Quantitative classification of sediment types

The CLUSTER and SIMPROF procedures performed on the data for the eight sediment variables recorded at each of the 52 site and season combinations (*i.e.* 20 nearshore sites averaged across seasons and 16 offshore sites in each of two seasons) identified 11 significantly different sediment types (Fig. 1.7.5). This classification contained two sediment types that were represented by a single site and season, (*i.e.* A and B) in the offshore waters at Deep Water Point and Como, respectively (Fig. 1.7.5a).

The analysis split the 11 sediment types into two distinct groups at a relatively high level of dissimilarity, the first group of which contained only sites from offshore waters (*i.e.* A, B, C, D, E, F and G), while the second contained four sediment types (*i.e.* H, I, J and K), all of which were comprised of sites from solely nearshore waters (Fig. 1.7.5). This separation was largely due to far greater percentage contributions of POM being found in the sediment types in offshore waters (*i.e.* 9.5-17%) than those in nearshore waters (*i.e.* 1-3%; Fig. 1.7.6a). In addition, sediment types in the offshore waters contained greater proportions of the smaller inorganic grain sizes, namely those <63 μm and 63-124 μm and consequently less of the grains between 250 and 499 μm than sediment types in nearshore waters (Fig. 1.7.6b).

Note that the linkage tree (see later) provides quantitative thresholds that best discriminated between groups of sediment types and, in this case, the sediment types in nearshore and offshore waters separate at node A.

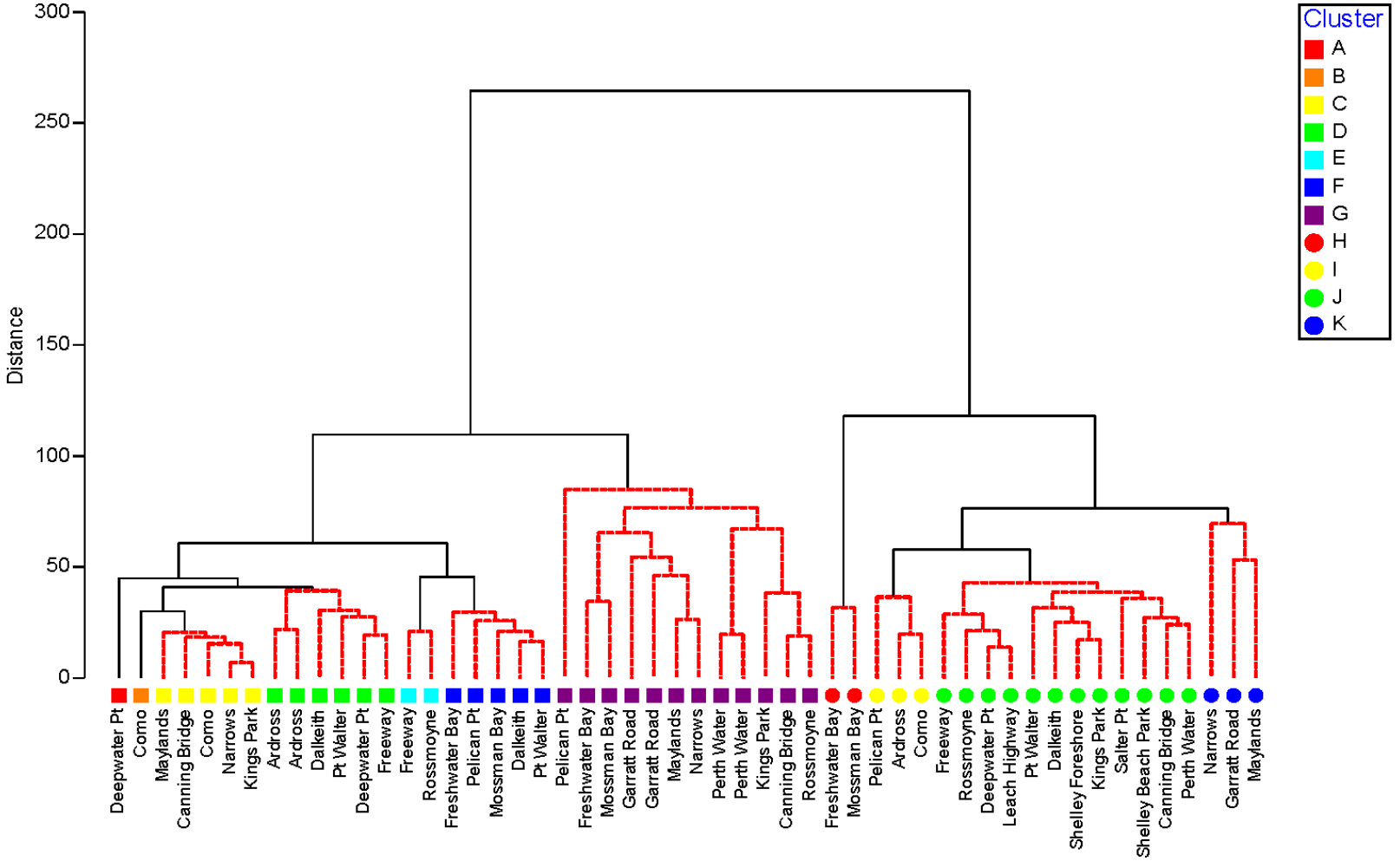


Fig. 1.7.5: Dendrogram derived from CLUSTER analysis using the transformed and weighted percentage contribution of POM and various sediment grain sizes at each of the 20 nearshore sites (averaged for seasons; ●) and 16 offshore sites in each of two seasons (■). The clusters under each solid black line represent sites (and seasons) at which, the sediment composition were shown by SIMPROF not to be significantly different from each other (P = >5%), but to be significantly different from those in all other groups of samples (P = <5%). Circles denote sites (sediment types) in nearshore waters and squares offshore waters.

Among the seven sediment types in offshore waters, six were present in summer (*i.e.* A, C, D, E, F and G) and three in winter (*i.e.* D, F, and G). Of those sediment types found in summer, four were represented by more than a single site. The most numerous of which (*i.e.* C and F) were only recorded in this season and exhibited marked spatial separation within the estuary. Thus, while sites classified as belonging to sediment type F were located in the lower (western) reaches of Melville Water, those classified as C were located further upstream, for example, on the eastern shore of Melville Water, Perth Water and Maylands (Fig. 1.7.7a). These sediment types were distinguished by the far greater contribution of both coarser sediments (*i.e.* inorganic grain sizes > 500 μm) and fine grain sizes (*i.e.* < 63 μm) at sites in sediment type C (~35% and ~26%, respectively) than at corresponding sites in sediment type F (~15% and ~15%, respectively), while the reverse was true for grain sizes between 125 and 249 μm (Fig. 1.7.6b).

Two other offshore sediment types, both represented by two sites, were also present during summer; namely E, located near Rossmoyne and G, situated near Heirisson Island and Bayswater (Fig. 1.7.7a). Sediment type E, was distinguished by its high proportion of inorganic grain sizes < 63 μm (~31%), while the sediment at sites in sediment type G, contained the lowest percentage contribution of POM (*i.e.* 10%) for any offshore sediment type (*i.e.* 12-16%; Fig. 1.7.6a). Moreover, this sediment type also contained relatively low amounts of grains <124 μm, but relatively high amounts of grains >2,000 μm (Fig. 1.7.6b).

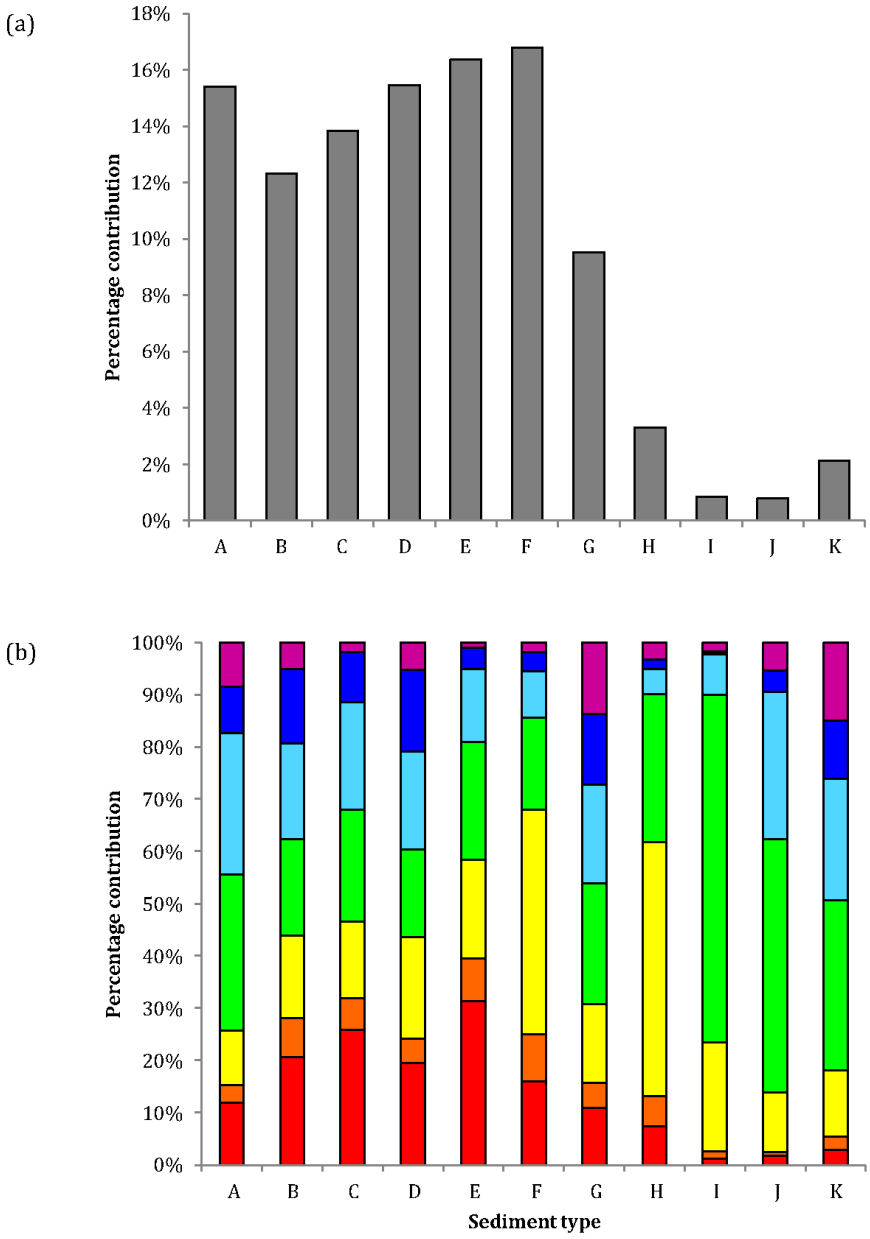


Fig. 1.7.6. Mean percentage (a) particulate organic matter and (b) various inorganic grain size contributions recorded at the 11 different sediment types identified by CLUSTER and SIMPROF from the 36 sites throughout the nearshore and offshore waters of the Swan-Canning Estuary. ■ = > 2,000 µm, ■ = 1,000-1,900 µm, ■ = 500-999 µm, ■ = 250-499 µm, ■ = 125-249 µm, ■ = 63-124 µm and ■ = < 63 µm.

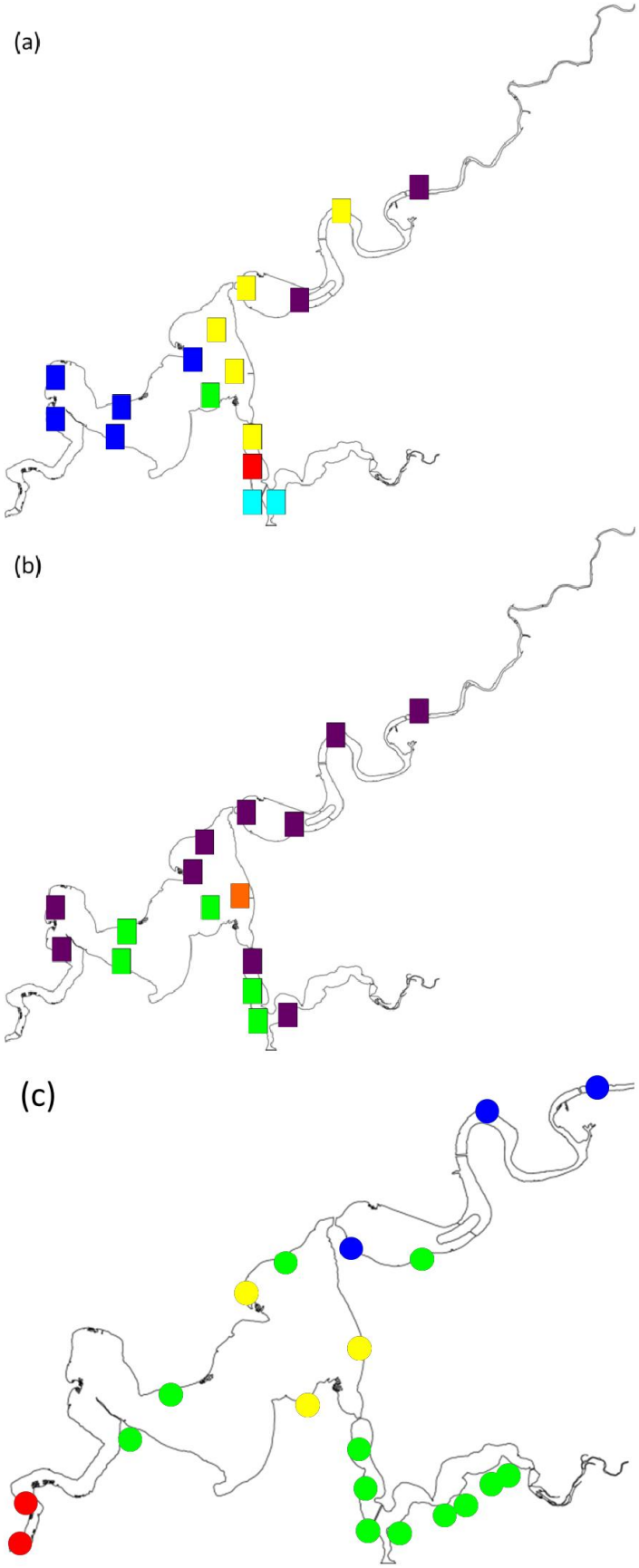


Fig. 1.7.7. A map of the Swan-Canning Estuary showing the spatial distribution of the 11 sediment types identified by the CLUSTER and SIMPROF procedures. Offshore sediment types in (a) summer and (b) winter and (c) nearshore sediment types. Offshore sediment types ■A, ■B, ■C, ■D, ■E, ■F, ■G; nearshore sediment types ●H, ●I, ●J, ●K.

Two main sediment types were identified by the CLUSTER and SIMPROF procedures in offshore waters of the Swan-Canning Estuary in winter, namely sediment type D, which was found at sites located in central Melville Water and around Deepwater Point on the Canning Estuary and sediment type G, found downstream of Point Walter, in upper Melville Water and in the middle Swan Estuary (Fig. 1.7.7b). The main differences between the sediment composition between D and G, was the presence of greater proportions of POM and inorganic grain sizes <63 μm at sediment type D and lower amounts of grains >2,000 μm (Fig. 1.7.6). Note that a third sediment type (B) was also identified during this season, but it was represented by a single site, Como (Fig. 1.7.7b).

The 20 nearshore sites were classified into four sediment types (*i.e.* H-K) and were largely confined to particular regions of the estuary. Hence, sediment type H, was restricted to two sites at the lowermost reaches of the estuary, while sediment type J was located throughout the Canning Estuary and middle reaches of the Swan Estuary (Fig. 1.7.7c). The remaining nearshore sediment types were found in Melville Water, with those sites representing sediment type I being found around Como, Ardross and Pelican Point, while those sites belonging to sediment type K were found upstream at 3 of the 4 sites in the Middle Swan Estuary.

In terms of sediment composition in nearshore waters, sediment types H and K had relatively greater amounts of POM (*i.e.* ~3-4 %) than sediment types I and J (*i.e.* ~1 %; Fig. 1.7.6a). A distinguishing feature between sediment types H and K was that sites comprising of sediment type H had a far higher proportion of inorganic grain sizes between 125 and 249 μm and lower proportions of grain sizes >500 μm in comparison to those in sediment type K (Fig. 1.7.6b). The portions of grain sizes > 500 μm also distinguished between sediment types I and J, with J containing far higher amounts of that grain size and lower amount of grains between 250 and 499 μm.

Quantitative prediction of sediment types

The linkage (decision) tree (Fig. 1.7.8) depicts the separation of 52 site and season combinations into the 11 sediment types by the above CLUSTER and SIMPROF procedures and the associated quantitative thresholds of the proportions of the particulate organic matter and various inorganic grain sizes that best reflect the differences in sediment composition between two 'groups' of samples at each branching node. This tree thus provides a set of quantitative decision rules that enable the sediment type of any 'new' sample of sediment (*i.e.* an additional site and/or new season) from the Swan-Canning Estuary to be classified, providing of course that they follow the methodology for collecting and quantify sediments used in this study. These decision rules also provide an indication of which of the 8 sediment characteristics (*i.e.* the percentage contribution of POM and the seven inorganic grain size categories) are the most important in differentiating between the various sediment types. Whilst all of the eight sediment characteristics were selected at some point on the linkage tree, the percentage organic matter was used infrequently after node A (*i.e.* the separation between nearshore and offshore sites/sediment types) in comparison to the seven inorganic grain size variables.

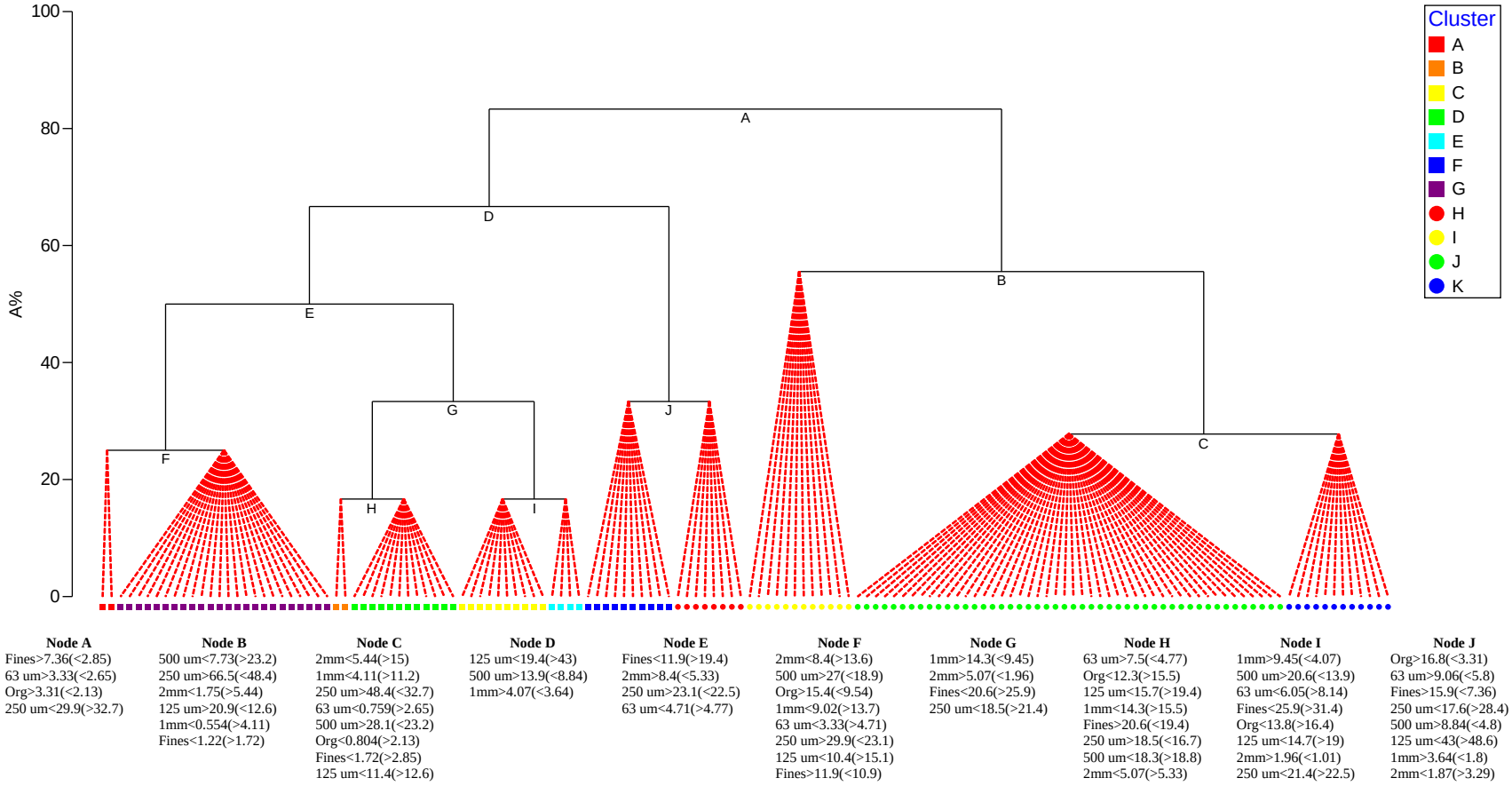


Fig. 1.7.8. Linkage tree and associated quantitative thresholds for assigning new sediments from the Swan-Canning Estuary to their appropriate sediment type. Unbracketed and bracketed thresholds given at each branching node indicate that a left or right path should be followed, respectively. Note that all threshold values have not been subjected to any form of data pretreatment. The terminal node represented by a coloured symbol denotes the sediment type to which any new site (or sample) belongs. A% reflects the extent of inter-sediment type differences as a proportion of that between the most dissimilar sediment types. Org = Particulate Organic Matter (POM), Fines = inorganic particles <63 µm.

Discussion

This study aimed to establish, firstly, whether the composition of the sediment within the Swan-Canning Estuary differed among water depths, seasons and sites, and secondly, to quantitatively group sediment composition into types without employing an *a priori* hypothesis about which sites would be different from which. Sediment composition was shown to differ most notably among water depths, then among seasons and sites, with a significant season effect only present in the offshore waters.

Influence of water depth on sediment composition

The results of a three-way ANOSIM test demonstrated that sediment composition differed among sites ($R = 0.607$), and between seasons ($R = 0.697$), and water depths ($R = 0.968$), with water depth being by far the most influential. This view is supported by the fact that, while nearshore and offshore sites were included in the same CLUSTER-SIMPROF analysis, thus giving sites from both water depths the chance to form composite habitat types, each of the 11 sediment types was comprised of sites from a single depth. Moreover, these nearshore and offshore sediment types were separated at the first node of the CLUSTER dendrogram.

The marked separation in sediment composition between water depths was largely due to high percentage of particulate organic matter (POM) and smaller inorganic grain sizes (*i.e.* those <125 μm) in offshore sediment types and higher proportions of the larger (125-499 μm) grain sizes in nearshore waters. Similar results were also found by Tweedley *et al*. (2012) in Broke Inlet on the south coast of Western Australia. It is therefore relevant that, in both these systems, there is pronounced demarcation between the nearshore (*i.e.* < 2 m deep) and offshore (*i.e.* > 2 m deep) waters, marked by the presence of extensive sand flats. Sediment in these shallow waters in many estuaries throughout the world tend to contain coarser (sandier) sediments due to the higher levels of water movement, which erode and transport the finer particles away from these area (Sanders, 1958; Green and Coco, 2014). Water movement can be generated by a number of sources including freshwater discharge, tidal

currents and wash generated by recreational water craft, which create turbulence in the water column, resuspending and transporting the sediments, particularly those with a smaller grain size (Beachler and Hill, 2003; Green and Coco, 2014). Wave-forced resuspension in microtidal estuaries has been identified as an important component of sediment transport process as these systems do not, by their nature, experience strong tidal flow (*e.g.* Sanford, 1994).

The far greater proportions of POM and smaller grain sizes (*i.e.* those < 125 μm) in offshore waters of the Swan-Canning Estuary can be explained by the fact that water movements in these offshore waters are reduced, as is the effect of any wind. The low energy environments present in the large, deeper central basins of the Swan-Canning Estuary, like in many other microtidal systems, thus act as sediment traps for fine particles. It is estimated that such areas may retain up to 80% of the fine sediment transported into the estuary (Patchineelam *et al*., 1999; Roy *et al*., 2001). This is in clear contrast to the situation in macrotidal estuaries where the substantial tidal water movement results in the continued resuspension of fine particles and thus their transport out of the estuary (Turner *et al*., 1994; Wells, 1995).

Influence of season on sediment composition

Seasonal changes in sediment composition were found to occur offshore waters of the Swan-Canning Estuary, but not in nearshore waters. In the offshore, waters six sediment types were present in summer (*i.e.* A, C, D, E, F and G), but only three in winter (*i.e.* D, F and G). The major variance in the pattern of spatial difference between these seasons was the increase in the number of sites classified as either or G by a factor of five. This noticeable change in sediment type was caused by the reduction in the amount of POM and the percentage contribution of inorganic grain sizes <125 μm and resultant increase in the contribution inorganic grain sizes >500 μm in winter.

The Swan-Canning Estuary experiences a Mediterranean climate, where rainfall is largely confined to the cooler months of the year, *i.e.* May to September (Hodgkin and Hesp, 1998).

Heavy precipitation in those months leads to a great increase in freshwater discharge and thus to scouring of the sediment and a substantial decline in salinity (Spencer, 1956; Stephens and Imberger, 1996). At the same time, the increased freshwater discharge also leads to increased sediment transport into the estuary (Thrush *et al*., 2004). These processes explain the seasonal shift in sediment composition that occurred in the offshore waters. Increased water movement in winter, as a result of the seasonal rainfall, would lead to resuspension and transportation of the fine sediment particles further downstream and out to the ocean. This is shown in this study by the fact that sediment type F, with an average POM of 17%, characterised the five most downstream sites in summer, but in winter these sites belonged to sediment types D and G, which contained lower amounts of POM (*i.e.* ~15.5 and ~9.5% POM, respectively; Figs 1.7.6, 1.7.7). A similar change was also seen in five sites located further upstream, particularly those classified as sediment type C in summer (*i.e.* ~14% POM), which in winter almost all became G (*i.e.* 9.5% POM). As well as scouring the finer inorganic particles, these waters would also be transporting terrestrially derived sediment (Thrush *et al*., 2004), which may explain the increase in the proportion of inorganic grain sizes > 500 µm that was seen at sediment types D and G.

Scouring as a result of the increased water movement was shown to occur in the upper-middle reaches of the Swan Estuary by Kanandjembo *et al*. (2001), who recorded a substantial shift in the benthic macroinvertebrate fauna, which they associated with a change in sediment composition and/or scouring and decreasing salinity. These authors found that the faunal changes were more pronounced in the nearshore than offshore waters, which they claimed experienced greater amounts of scouring due to the reduced depth. However, as, even in summer, when freshwater discharge is minimal, sites in the nearshore waters still contained low contributions of POM and small inorganic grain sizes. The lack of a seasonal change in sediment composition may reflect the fact that the nearshore waters are well mixed and experience a strong and persistent sea breeze during summer (Masselink and Pattiaratchi, 1998). This would resuspend any fine sediment and not allow settlement, thus, during winter, when freshwater discharge and, as a result, scouring increase, their effects are reduced as only the courser grain sizes remain.

Influence of site on sediment composition

ANOSIM detected significant differences in sediment composition among sites, however, the extent of those differences was less than those for water depth and season. When the sediment composition at individual sites (and seasons) was subjected to nMDS ordination, those sites located in the same region were generally located in close proximity, thus indicating that those sites had similar sediment compositions. This conclusion that sediment types of adjacent areas are more similar than those from different regions indicates that the length of the Swan-Canning Estuary is comprised of a variety of sediment types (Fig. 1.7.7), a finding similar to the conclusions of Gray and Elliott (2009), *i.e.*, that estuaries are comprised of a mosaic of sediment types.

Friedman and Sanders (1978) suggested that sediment grain size composition is largely dependent on the parent material, selective and destructive transport processes, and the hydrodynamic characteristics of deposition within the estuary, a view also supported by Postma (1967) and Watson *et al*. (2013). For example, within a water depth, the sediment at locations at the mouth of the Swan-Canning Estuary (*i.e.* sediment types F [offshore] and H [nearshore]) contained by far the largest amounts of the 125-249 µm grain size, one of the most abundant grain size fractions in nearshore waters of the lower west coast of Australia (Wildsmith *et al*., 2005), and represents sediments likely to be derived from marine sands. This result is similar to that recorded in the Knysna Estuary (South Africa), where marine sands make a large contribution to the sediment composition in the lower estuary (Cooper, 2001), probably due to the transport of sediment from the marine environment by tidal currents (Meade, 1969). In general, sediment grain size increased further upstream, a trend that agrees with the work of Gill and Potter (1993). This likely reflects the decrease in the speed of water flow progressively downstream and thus the deposition of the larger terrestrial derived particles first. This trend was also mirrored in the contribution of POM in the offshore waters, which decreased in an upstream direction, presumably as these lighter particles would be transported downstream further.

Utility of the sediment classification

The collection of sediment at 36 sites throughout the nearshore and offshore waters of the Swan-Canning Estuary in two seasons has provided a sound basis for characterising the sediments of this system based on POM and grain size (*i.e.* Fig. 1.7.6). Furthermore, these data represent a substantial extension on those collected by and Hourston *et al*. (2009), as, while those studies covered a similar spatial extent to the current study, they did not include the deeper, offshore waters nor investigate any temporal changes in sediment composition, both of which were shown here to be influential.

In conjunction with this detailed sediment data set, the use of the CLUSTER and SIMPROF provided a quantitative approach to grouping sites with statistically similar sediment compositions. The results of these analyses were the identification of 11 distinct sediment types and the production of the first sediment map for the nearshore and offshore waters of the Swan-Canning Estuary. Moreover, the use of LINKTREE has determined the quantitative thresholds that can be used as part of a decision tree to categorise the sediments of the Swan- Canning Estuary. This provides a mechanism for classifying the sediment type of any new site and thus provides a foundation for future research. With this in mind, one logical extension of this study would be to include sites further upstream in the Swan River axis of the estuary.

Conclusions

In summary, this study has demonstrated that the sediment composition of the Swan-Canning Estuary differs between water depths, season and sites, with the influence of water depth being most important. Differences in water depth were particularly marked due to higher contributions of particulate organic matter and finer inorganic grain sizes in the offshore waters. Interestingly, a significant seasonal effect was only shown only in the offshore waters. Finally CLUSTER- SIMPROF analysis 52 site and season combinations identified 11 different sediment types present in the nearshore and offshore waters of the

Swan-Canning Estuary, thus demonstrating that the substratum of highly dynamic system comprises a mosaic of sediment types.

1.8. Relationship between sediment types and the spatial distribution, size structure and preferences of the Western School Prawn in the Swan-Canning Estuary

This study has been published in an Honours thesis by Amber Bennett.

Bennett, A.L. (2014). The influence of sediment compositions on the Western School Prawn *Metapenaeus dalli* in a temperate Australian estuary. Honours Thesis, Murdoch University.

Summary

The characteristics of sediments are key factors influencing faunal distribution within estuaries. Many species of penaeid display a preference a for particular sediment types, due to their close association with the sediments for burying and feeding. The aim of this study is to establish if there are any relationships between the spatial distribution and size structure of *Metapenaeus dalli*, and the sediment types characterised for the Swan-Canning Estuary in Section 1.7, and to elucidate whether changes in sediment may be responsible for *M. dalli* not being recorded by recreational fishers upstream of Perth Water. The abundance of *M. dalli* differed among sediment types during summer, but not during winter, due likely to the sediments being more homogeneous in winter when heavy freshwater discharge moves finer particles further downstream. While the differences in prawn density in summer were correlated among those sediment types, they were not related to differences in water quality (salinity, water temperature and dissolved oxygen), reflecting the fact that metapenaeids are euryhaline and that stable environmental conditions are present in the Swan-Canning Estuary during summer. Sediment preference experiments in the laboratory demonstrated that prawns had a significant preference for sediment from Dalkeith (sediment type D), rather than further upstream at Garratt Road Bridge (sediment type G). Visual observation also indicated that prawns were able to bury more quickly in the sediments from Dalkeith than those from Garrett Road Bridge. Finally, the laboratory experiments also demonstrated that the emergence and activity patterns of *M. dalli*, like those of many other penaeid species, are strongly linked to photoperiod.

Rationale and aims

The composition of the sediment, *i.e.* the contribution of particulate organic matter and the proportions of various inorganic grain sizes, is a major factor influencing the community structure of benthic macroinvertebrate assemblages (*e.g.* Gray, 1974; Snelgrove and Butman, 1994; Teske and Wooldridge, 2001). For example, work by Teske and Wooldridge (2003) in 15 microtidal estuaries in South Africa showed that differences in sediment composition were more influential than differences in salinity in structuring the composition of the benthic macroinvertebrate fauna. These authors also suggested that the influence of sediment composition may have been underestimated in estuaries, due to the correlation and thus confounding influences between salinity and sediment types. Generally, areas with sandier sediments are exposed to greater levels of water movement, which erode fine particles and provide an effective mechanism for transporting food into the vicinity of filter feeding species, whereas, the deposition of fine sediments occurs in calmer waters, thus clogging the structures used to filter feed, but creating an abundant food source for deposit feeders (*e.g.* Sanders, 1958; Rhoads and Young, 1970).

In addition to the effects of sediment grain size on infaunal species, mobile epifaunal species are influenced by the sediment composition. Penaeid prawns, which are the focus of many important commercial fisheries (Önal *et al*., 1991; Dichmont *et al*., 2006; Kompas *et al*., 2010), not only utilise the benthos as a food source, but also bury in the surface layers as a means to avoid predators and lower their energy expenditure (Williams, 1958; Dall *et al*., 1990). Given their strong association with the benthos, it is not surprising that penaeids have been shown to exhibit species specific preferences for particular types of sediment (Hughes, 1966; Branford, 1980; Somers *et al*., 1987). Both sediment particle size, which influences burrowing ability and the organic content of the sediment, which can be related to food availability (Branford, 1981), have been shown to be important factors affecting the sediment preferences of penaeids. While different species exhibit sediment preferences, these preferences may change during ontogenetic development (Aziz and Greenwood, 1982). For example, a study of the Eastern School Prawn (*Metapenaeus macleayi*) showed that in both field surveys and laboratory experiments, juvenile prawns exhibited a preference for fine

sediments (*i.e.* grains < 180 μm), where they could easily bury by fanning their pleopods. They struggled to bury, however, in sediments with larger grain sizes (*i.e.* 100-500 μm), as the particles were too large for the small prawns to move with their pleopods. Furthermore, as juveniles feed predominantly on small invertebrates, plant material and detritus (Dall, 1967; Ruello, 1973), the fine sediment would presumably contain larger quantities of food (Sanders, 1958). In contrast, *M. macleayi* adults were found buried in coarser sediments, reflecting the fact that they had the strength to move the larger grains and that they feed predominantly on larger crustaceans and polychaetes, which were more abundant in the coarser sediment (Ruello, 1973). This work concluded that the direct influence of sediment grain size on prawns decreased as the prawns grew larger and increased their ability to bury in a range of sediments, whereas the reverse was true for the indirect effects of sediment grain size, *i.e.* resulting in a shift in the abundance of prey taxa.

Burying is a critical component in the life history strategy of penaeids, with many species remaining buried in the benthos during the day and emerging from the sediments during the night to feed and breed on the surface of the substratum (Wickham and Minkler, 1975; Hill, 1985; Dall *et al*., 1990; Wassenberg and Hill, 1994). Typically, emergence peaks shortly after dusk and declines slowly until sunrise, when most individuals bury again in the sediment (Wickham, 1967; Moller and Jones, 1975), however, some species display multiple peaks throughout the duration of the night, *e.g. Penaeus semisculcatus* (Moller and Jones, 1975; Wassenberg and Hill, 1994). Thus, understanding the emergence pattern of a species is a key component of its biology and ecology and crucial to any assessment of its stock structure and how many prawns are likely to be in the path of fishing operations during the diel cycle (Wassenberg and Hill, 1994).

The Western School Prawn (*Metapenaeus dalli*) was once an important target species for commercial and recreational fishers in the Swan-Canning Estuary, in Western Australia. However, the abundance of this penaeid declined markedly, resulting in the cessation of commercial prawning in the 1970s and a dramatic reduction in the numbers of recreational fishers since the 1980s, and particularly the 1990s (Smith *et al*., 2007). There is strong

anecdotal evidence from recreational fishers that, along with the abundance, the spatial distribution of *M. dalli* has changed over the last 30 years, with this penaeid no longer being caught upstream of Perth Water (Smithwick *et al*., 2011). Work on the biology and ecology of *M. dalli* by Potter *et al*. (1986b) demonstrated that individuals of this species moved into the upper reaches of the Swan Estuary once salinity increased to ≥30. However, declining rainfall as a result of climate change has resulted in these waters being more saline now than during the 1980s (Loneragan *et al*., 1986; Cottingham *et al*., 2014) and thus salinity is unlikely to limit the distribution of *M. dalli*. It is therefore hypothesised that the perceived change in spatial distribution of *M. dalli* has been caused by a potential change in the composition of the benthic substrates in the middle and upper Swan Estuary as a result of increased sedimentation since the 1980s.

As little is known on the influence of sediments on the *M. dalli*, the aims of this study were to investigate the influence of sediments on this penaeid species by testing the following four hypotheses:

1. The density and length of *M. dalli* will differ among the 11 sediment types identified in the Swan-Canning Estuary (see Section 1.7).

2. The pattern of relative differences in density and length of *M. dalli* among sediment types will be correlated significantly with differences in the composition of the sediment.

3. Laboratory based sediment preference experiments will demonstrate that *M. dalli* will has a preference for a particular sediment type.

4. *M. dalli* will exhibit a diurnal pattern of emergence and burial.

Methods

Field sampling of M. dalli

Individuals of *M. dalli* were collected from 20 nearshore sites (*i.e.* <2m deep) and 16 offshore sites (*i.e.* 2 - 17 m deep) in the Swan-Canning Estuary every 28 days between October 2013 and August 2014 (Fig. 1 .8.1). Sampling was conducted at least 30 minutes after sunset occurred during the new moon phase, *i.e.* when the percentage of the moon illuminated was <10%. Prawning in nearshore waters was conducted using a hand trawl that was 4 m wide (when fully stretched, but was on average 2.85 m when fishing), 1.5 m high and constricted from 9 mm knotless mesh, The hand trawl was dragged for 200 m, covering an average area of ~570 m^2. Sampling in offshore waters employed an otter trawl that was 2.6 m wide, 0.5 m high and comprised 25 mm mesh. The otter trawl was towed a speed of 3 km/h for five minutes, covering an area of ~650 m^2. Two replicate trawls were conducted at each site (nearshore and offshore) on each sampling occasion.

The number of *M. dalli* in each sample was counted and the carapace length (CL), *i.e.* the distance between the orbital indent and the posterior edge of the carapace, recorded to the nearest 0.1 mm (Fig. 1.8.2), except where a large number of prawns were caught, in which case the lengths of a random subsample of 100 were measured. The number of prawns in each net was then converted to a constant density (*i.e.* 500 m^2) using the formula

$$\frac{\text{Number of prawns}}{\text{Area trawled (either 570 ot 650 m}^2\text{)}}$$

A range of water quality parameters, namely salinity, water temperature (°C) and dissolved oxygen concentration (mg L^{-1}) at the surface and bottom of the water column, were recorded at each site, on each sampling occasion using a Yellow Springs International 556 Handheld Multiparameter Instrument.

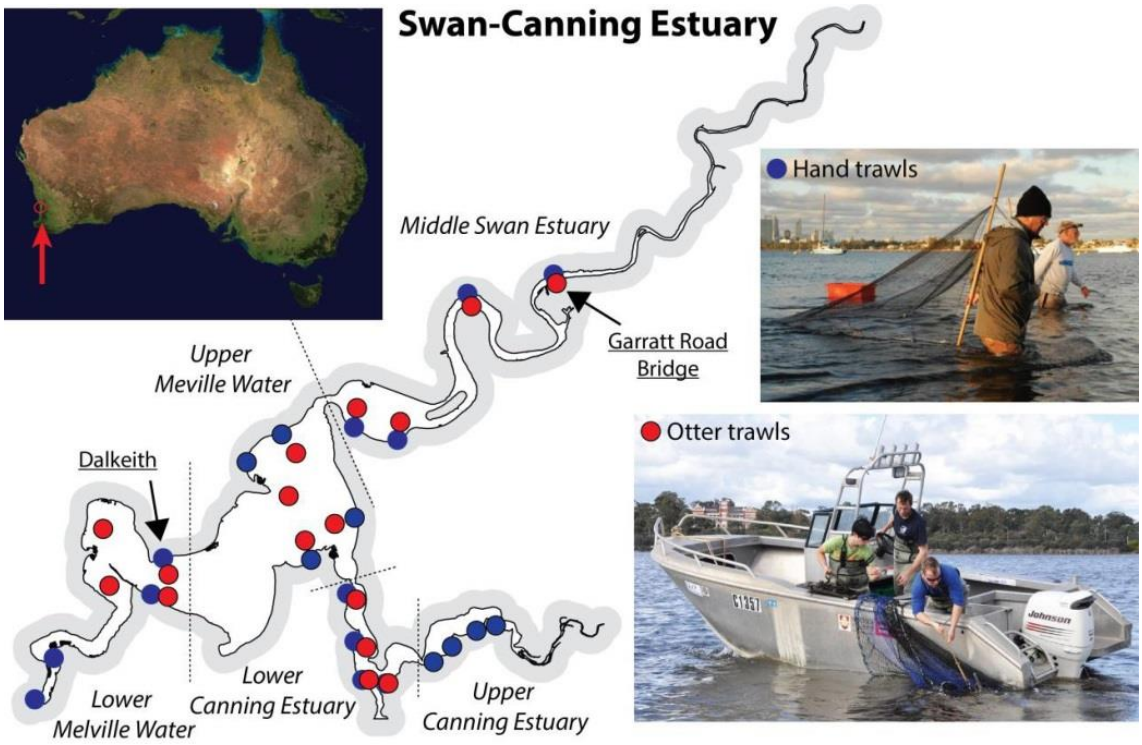


Fig. 1.8.1. Map showing the location of 20 nearshore (●) and 16 offshore (●) sites in the Swan-Canning Estuary sampled every 28 days from October 2013 to August 2014. The red arrow and circle shows the location of the Swan-Canning Estuary in Australia and the photographs the sampling methods employed to collect *Metapenaeus dalli*. The sites with their names underlined are those where sediment was collected for the sediment preference experiments.



Fig. 1.8.2. Photograph of *Metapenaeus dalli* showing the carapace length (CL) measurement.

Differences in the density and length of M. dalli among sediment types

All statistical analyses were performed using the PRIMER v6 multivariate software package (Clarke and Gorley, 2006) and the PERMANOVA+ add-on (Anderson *et al*., 2008), with the exception of the chi-square tests, which were conducted in SPSS Statistics 21.

A subsample of the abundance data for *M. dalli* was extracted from a database containing the data for all 12 sampling occasions from October 2013 to August 2014. These data

corresponded to information collected during sampling occasions that spanned the same season as the one in which the sediment data in Section 1.7 were obtained, namely summer (*i.e.* 30 November 2013 – 4th March 2014) and winter (*i.e.* 24 June 2014 to 28 August 2014). As not all 11 of the sediment types identified above were present in both seasons (Fig. 1.8.7) and because both the density (Potter *et al*., 1986b; Tweedley, unpublished data) and the length of *M. dalli* (Potter *et al*., 1986b; Broadley *et al*., 2017) change seasonally, statistical analyses comparing the density and length of *M. dalli* among the sediment types were conducted separately for each season. Note that as very few *M. dalli* were collected in nearshore sites in winter (only 19 individuals in 120 samples) and nearshore sediment composition did not change significantly between seasons, only the density data for nearshore sites in summer were included in these analyses.

Prior to subjecting the density (individuals per 500 m^2) and CL length of *M. dalli* to Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson *et al*., 2008), the data for each of the two dependent variables were examined separately to determine whether any transformations were required and, if so, the extent to which the data needed to be transformed to meet the test assumptions of homogenous dispersion among *a priori* groups (*i.e.* sediment types). This was achieved by calculating the extent of the linear relationship (slope) between the $\log_e$(mean) and $\log_e$(standard deviation) of each variable at all sediment types and comparing them to the criteria described in Clarke and Warwick (2001) to select the appropriate form of transformation. This analysis demonstrated that density required a fourth-root transformation, while the CL data did not require transformation.

The transformed data for density and CL were used to construct separate Euclidean distance matrices containing all pairs of replicate samples collected in a single season (*i.e.* four matrices, density summer, density winter, CL summer and CL winter). Each of these matrices was, in turn, subjected to a one-way PERMANOVA to test whether each of the dependent variables (*i.e. M. dalli* density and CL) differed significantly among sediment types in a season. Sediment type was considered fixed and the null hypothesis of no significant

differences among *a priori* groups was rejected if the significance level (P) was < 5 %. Where a significant difference was found between a dependent variable and sediment type, a pairwise PERMANOVA test was conducted using the same Euclidean distance matrix, to determine which pairwise comparison(s) where responsible for the difference.

Note that when using a single dependent variable (*i.e.* a univariate test) and employing a Euclidean distance matrix, PERMANOVA's fully-permutational based output (*i.e.* mean squares, [pseudo] *F* and *P* values) is identical to that generated by an ANOVA, where the data also meet the additional assumption of being normally distributed. The use of PERMANOVA in these analyses was to avoid the effects of a highly unbalanced design, due to unequal numbers of sites belonging to the different sediment types. PERMANOVA has been shown to be robust to the effects of an unbalanced design, particularly when using type III sums of squares (Anderson, 2001). However, as the design employed here was particularly unbalanced, for example sediment type A contained a single site (*i.e.* 8 replicate values in summer) compared to sediment types C or F (*i.e.* 40 replicate values in summer), a second version of the analysis was conducted to check the results.

In the second analysis, the first replicate for each dependent variable recorded at each site on each sampling occasion and within a sediment type was averaged, with the same also being done for the second replicate, therefore generating two 'replicates' per sediment type, per sampling occasion. Such an approach produced a balanced design of eight replicates per sediment type in summer and six in winter. These data were then used to calculate the Euclidean distance matrices, which were subject to the same PERMANOVA tests described above. When the outputs of these second PERMANOVA tests were compared to the results obtained using the original PERMANOVA design, they were almost identical, with the only differences being the relative extents of the test statistic *t*, which occur because this value does not provide a universal measure of group separation (Lek *et al.*, 2011). Thus, only the results from the first analyses are presented here.

Relationships between the density and length of M. dalli and sediment types

If a significant difference in density and/or CL was detected among the sediment types in a given season, the RELATE routine was then employed to test the extent to which the relative differences in any dependent variable among sediment types were significantly correlated with those defined by their suite of sediment characteristics. Thus, this procedure determined how similar the pattern of rank orders of resemblance were between the Manhattan distance matrices constructed from fourth-root density data at each sediment type in a particular season and the complementary Manhattan distance matrices constructed from the transformed and weighted sediment data for those sediment types present in that season. The null hypothesis that there was no relationship in the pattern of rank order similarities between the complementary matrices was rejected if the significance level (*P*) was < 5 %. The test statistic, rho (ρ), was used to measure the extent of any significant relationships, with values close to 0 reflecting little correlation in rank order agreement and those close to 1 reflecting a near perfect match.

Non-metric Multidimensional Scaling (nMDS) ordination plots of the sediment characteristics for each sediment type in each season were also constructed to provide a visual indication of any matching between the complementary data sets. Circles ('bubbles') of proportionate sizes that represented the (untransformed) average number of prawns recorded at that sediment type in that season were then overlaid to aid in the visual interpretation of the relationships.

To further explore the relationships between sediment composition and density of *M. dalli* the Biota and Environment matching routine (BIOENV; Clarke and Ainsworth, 1993) was employed to ascertain whether a greater correlation between the complementary sediment and density matrices could be obtained using only a particular subset of the sediment characteristics (*i.e.* any single variable or combination of variables), rather than the full suite as employed in the RELATE analysis (*i.e.* the sediment types determined above). The matrices used in the BIOENV analyses were identical to those employed in the RELATE routine described above, except that to allow all sediment characteristics to have equal

influence all were given an equal weight (*i.e.* 100) prior to constructing the Manhattan distance matrix. The null hypothesis, and test statistic for these BIOENV tests were the same as those for the above RELATE tests.

As the sediment types are located in different areas of the Swan-Canning Estuary (Fig. 1.8.7), they are likely to experience different water quality conditions. Thus, the above RELATE and BIOENV analyses were also conducted on a Manhattan distance matrix constructed from the average values of surface and bottom salinity, water temperature and dissolved oxygen concentration recorded at each sediment type at the same time as prawns were collected, to determine whether differences in density among the sediment types were related to complementary differences in water quality. Prior to construction of the matrix, the relationships between each pair of environmental variable data were displayed in a Draftsman plot to determine whether any of the variables required transformation. No transformations were necessary and the data were then normalised to place all variables on a common scale by subtracting the mean and dividing by the standard deviation for each variable (Clarke and Warwick, 2001). This Manhattan distance matrix was also used to create an nMDS plot and the average values for particular water quality variable(s) (untransformed and normalised) were overlaid on an nMDS plot constructed from the average prawn abundance at each sediment type.

Laboratory sediment preference experiments

Individual *M. dalli* were collected from the offshore waters of Melville Water (Fig. 1.8.1) in the Swan-Canning Estuary using an otter trawl in August 2014. Surface and bottom salinities and temperatures were, on average, 23.9 and 29.1 and 15.7 °C and 16.4°C, respectively, at this time. Once collected, the prawns (all of which were between 10-15 mm CL) were held in an aerated tank and transported to a controlled temperature laboratory and placed in a holding tank. The water temperature in the laboratory was set to 21.5 °C, as below 20 °C prawns become inactive and spend more time buried in the sediments (Wassenberg and Hill, 1994; Park and Loneragan, 1999). The photoperiod was set to 12:12 at 06:00 to 18:00, which

corresponded closely with the time of sunrise and sunset time in Perth in August. All individuals were held in a tank with a salinity of 22 for 48 hours before commencing the experiment, in order to allow them to acclimatise to the temperature regime and photoperiod (following Aziz and Greenwood, 1982). Prawns were fed a diet of small pieces of Southern Calamari (*Sepioteuthis australis*) each day.

To investigate whether a change in the composition of the sediment may have been responsible for the decline in the abundance of *M. dalli* in areas of the Swan Estuary upstream of Perth Water, sediments were collected from two sites (*i.e.* Garrett Road Bridge [upstream of Perth Water] and Dalkeith [downstream of Perth Water]; Fig. 1.8.1). As well as their location, these two sites differed in their prawn density (Table 1.8.1) and sediment composition (Fig. 1.8.7; Section 1.7). Samples of the substrate were collected from nearshore waters using a shovel and from offshore waters with an Ekman grab at both sites. The sediment types in nearshore waters at Garratt Road Bridge were classified as sediment type K, while those in offshore water were sediment type G. The nearshore sediments at Dalkeith belonged to sediment type D and those in the offshore waters to K.

Table 1.8.1. Comparison of the numbers of *Metapenaeus dalli* collected from nearshore and offshore waters of Garratt Road Bridge and Dalkeith in summer (*i.e.* November 2013- March 2014) and winter (*i.e.* June-August 2014).

	Garratt Road Bridge	**Dalkeith**	**% Dalkeith**
Nearshore summer	1	38	97
Nearshore winter	0	2	100
Offshore summer	31	81	72
Offshore winter	13	27	68
Total	**45**	**148**	**77**

Sediment samples were stored in large plastic containers, covered with estuarine water and aerated, so as to maintain their natural flora and fauna. In the laboratory, six 35 L tanks that measured 45 cm long, 25 cm wide and 30.5 cm high, were divided into two equal halves. Each experimental tank was arranged in a 'latin-square' design, to allow each sediment type to occur in every position equally, thus removing any preference prawns may have for a

particular side of the laboratory (Williams, 1958). Sediment from the nearshore waters of Garratt Road Bridge and Dalkeith were transferred into the experimental tanks to a depth of 4 cm (Fig. 1.8.3a,b). A thin plastic petition was placed on the border between the sediment types to prevent mixing of the sediment and/or prawns burying along this divide and thus being in both sediments. A thick plastic mesh was then placed in the tanks to dampen the flow of estuarine water (salinity = 22) as the tanks were being filled and prevent the scouring and resuspension of the different sediments. The tanks were then aerated and left for 24 hours before starting the experiment.

Upon commencing the experiment, a plastic partition was used to divide the tank into two, and five individuals of *M. dalli* were transferred from the holding tank into each side of each experiment tank (*i.e.* ten prawns per tank) during daylight hours. After introduction to the tanks, the prawns immediately buried and the partition was removed, giving prawns access to both sediment types over the duration of the experiment (following Williams, 1958). No biotic data were recorded for the first 24 hours to allow the prawns to interact with both sediment types (following Williams, 1958; Aziz and Greenwood, 1982).

(a)



(b)



(c)



Fig. 1.8.3: Photographs showing (a) an experimental tank prior to filling with water, (b) nearshore sediments from Dalkeith (left) and Garratt Road Bridge (right) and (c) offshore sediments from Dalkeith (left) and Garratt Road Bridge (right).

The prawns were counted, and their stage of activity and position in the tank (*i.e.* location with respect to the sediment) noted at 16:00 the following day after being released into the tank. These observations were repeated every two hours over a 24 hour period. The stages of activity were recorded using the stages described by Pinn and Ansell (1993):

Stage 0: Prawn swimming in water column.

Stage 1: Prawn entirely on sediment surface, *i.e.* not buried.

Stage 2: The whole dorsal surface of the prawn showing above the substratum *i.e.* only the pleopods and pereopods are buried.

Stage 3: The carapace showing above the substratum *i.e.* the abdomen was buried but the carapace and head could be seen, and

Stage 4: The entire body buried beneath the substratum surface. This was the final stage of burial, but the antennae, eyes and occasionally, antennules were left visible on the surface.

Measurements of salinity, water temperature (°C) and dissolved oxygen concentration (mg L^{-1}) were recorded in each of the six tanks every two hours over the course of the experiment. Analysis of these data demonstrated that none of these water quality parameters differed among tanks or changed during the experiment (data not shown).

At the conclusion of each experiment (*i.e.* 16:00 on the following day), a plastic partition was placed between the sediments, the water drained, sediment excavated and the number of prawns buried in each sediment type counted. The experiment was then repeated using the same method, but using sediments from the offshore waters of Garratt Road Bridge and Dalkeith (Fig. 1.8.3c) and a new group of *M. dalli*. However, as the sediment in the offshore waters contained greater proportions of particulate organic matter and fine inorganic grain sizes (Fig. 1.8.6; Section 1.7) prawn activity resuspended the sediment and reduced the accuracy of visual counts. As a result, prawns were counted only at the start and the end of

the experiment and no observations of the stage of activity were recorded during the experiment.

Statistical analyses of sediment preference

The percentage of prawns on each sediment type was calculated. The results of the 24 hours and six tanks were pooled to test the hypothesis that the proportion of prawns would not differ between sediment types. These data were tested using a chi-square test in SPSS, with the expectation that 50% of the prawns would be found on each substrate type.

Description of burrowing behaviours

The burrowing behaviour of *M. dalli* was analysed in a separate, smaller tank that was filled (2.5 cm deep) with nearshore sediment collected from Point Walter. A prawn (CL = 12 mm) was placed just above the sediment and released to bury into the substrate. Burrowing was filmed on a digital camera, and replayed in slow motion to review the burial mechanisms.

Results

Differences in the density of M. dalli among sediment types

Sediment types have separated clearly into those from offshore waters (A to G) and those in the nearshore waters (H to K; see above). The statistical comparisons between densities and CL and sediment type incorporate data from otter trawls only for sediment types A to G and hand trawls only for H to K. Care has been taken not to make comparisons in density and CL between methods below.

One-way PERMANOVA demonstrated that the density of *M. dalli* differed significantly among the 10 sediment types present in summer (P = 0.01%; Table 1.8.2). The pairwise PERMANOVA identified significant differences between 27 of the 45 combinations of sediment type (Table 1.8.3). The *t* statistic, which reflects the magnitude of the differences between pairwise comparisons, was typically greatest for tests involving sediment types H and I in the nearshore waters (hand trawl data). These relatively high values were caused by the far smaller density of *M. dalli* recorded in summer (*i.e.* ~0.25 and ~0.75 500 $m^{-2,}$ respectively; Fig. 1.8.4). Moderately high *t* statistics were generated from comparisons involving sediment types C, D and F (offshore waters and otter trawl data), due to the far greater density of prawns recorded at these sites in summer (*i.e.* ~9.3 - ~11.50 prawns 500 m^{-2}) than the other sediment types (*i.e.* ~0.2 – ~4.2 prawns 500 m^{-2}). In contrast, no significant differences were recorded at sediment types E, G, J and K, where the average density of *M. dalli* was very similar and ranged from ~2.3 – ~2.4 prawns 500 m^{-2} (Table 1.8.3; Fig. 1.8.4).

Table 1.8.2. Mean squares (MS), pseudo F-ratios and significance levels (P) from a one-way PERMANOVA test on the density of Metapenaeus dalli recorded at each of the 10 sediment types present in the Swan-Canning Estuary in summer (*i.e.* November 2013-March 2014). df= degrees of freedom.

Main effect	df	MS	*Pseudo-F*	*P*
Sediment type	9	6.31	13.24	0.01
Residual	278	0.48		

Table 1.8.3. *t*-statistic values derived from a pairwise PERMANOVA test on the density of *Metapenaeus dalli* recorded at each of the ten sediment types present in the Swan-Canning Estuary in summer (*i.e.* November 2013-March 2014). Insignificant pairwise comparisons (*i.e.* *P* >5%) are highlighted in grey. Sediment types A to G were from the offshore waters with matching density data from otter trawls, while those from H to K were in nearshore waters with density data from hand trawls.

	A	C	D	E	F	G	H	I	J
C	0.208								
D	2.267	1.401							
E	1.333	1.834	3.486						
F	0.994	1.163	0.848	3.046					
G	2.044	2.895	3.913	1.069	4.165				
H	6.734	5.889	13.065	4.567	7.897	2.665			
I	3.963	5.140	6.656	2.862	6.949	1.353	1.716		
J	2.529	4.964	4.450	1.557	6.693	0.151	2.890	1.617	
K	2.111	3.698	3.992	1.057	5.242	0.213	3.155	1.907	0.552

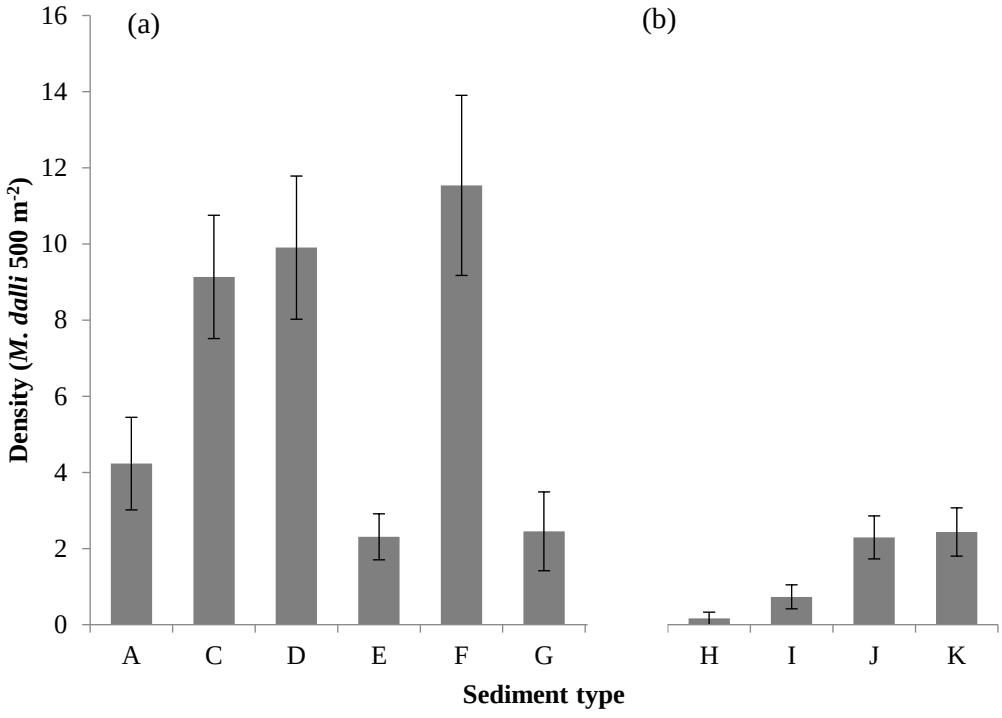


Fig. 1.8.4. The average density of *Metapenaeus dalli* (500m^{-2}) recorded at each of the sediment types present in the (a) offshore and (b) nearshore waters of the Swan-Canning Estuary in summer (*i.e.* November 2013-March 2014). Error bars represent ±1 standard error. Sediment types A to G were from the offshore waters with matching density data from otter trawls, while those from H to K were in nearshore waters with density data from hand trawls.

No significant difference was detected in the density of *M. dalli* among the three offshore sediment types present in winter (P = 70.73%; Table 1.8.4). Although there was a relatively large difference in the average density of *M. dalli* in otter trawls recorded, sediment at types B, D and G in winter (*i.e.* ~27.5, ~16 and ~12 prawns 500 m^{-2}, respectively) varied by more than two fold, there was a large amount of variability in those averages the variation in density was large, particularly that at sediment type B (Fig. 1.8.5), which accounts for the lack of a significant difference among the mean densities.

Table 1.8.4. Mean squares (MS), pseudo F-ratios and significance levels (P) from a one-way PERMANOVA test on the density of *Metapenaeus dalli* recorded at each of the three sediment types present in the Swan-Canning Estuary in winter (*i.e.* June-August 2014). df= degrees of freedom. Sediment types are all from the offshore waters and density data are from otter trawls.

Main effect	df	MS	Pseudo-F	*P*
Sediment type	2	0.26	0.36	70.73
Residual	93	0.74		

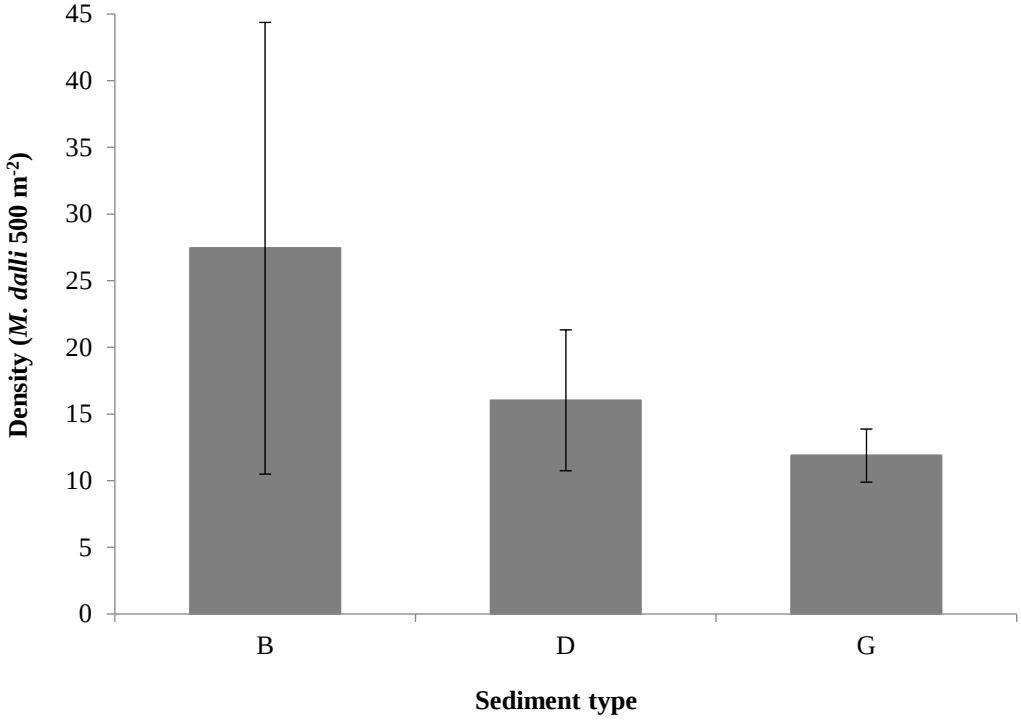


Fig. 1.8.5. The average density of *Metapenaeus dalli* (500m^{-2}) recorded at each of the three sediment types present in the Swan-Canning Estuary in winter (*i.e.* June-August 2014). Error bars represent ±1 standard error. Sediment types are all from the offshore waters and density data are from otter trawls.

Differences in the carapace length of M. dalli among sediment types

One-way PERMANOVA demonstrated that the CL of *M. dalli* found in the 10 sediment types present in summer did not differ significantly (*P* = 99.99%; Table 1.8.5). This lack of a significant difference in CL is reflected in the fact that the average CL was consistent among sediment types and ranged only from ~16.7 to ~20.3 mm (Fig. 1.8.6).

Table 1.8.5. Mean squares (MS), pseudo F-ratios and significance levels (*P)* from a one-way PERMANOVA test on the C1 length of *Metapenaeus dalli* recorded in each of the ten sediment types present in the Swan-Canning Estuary in summer (*i.e.* November 2013- March 2014). df= degrees of freedom.

Main effect	df	MS	Pseudo-F	*P*
Sediment type	2	0.01	0.37	99.99
Residual	152	0.01		

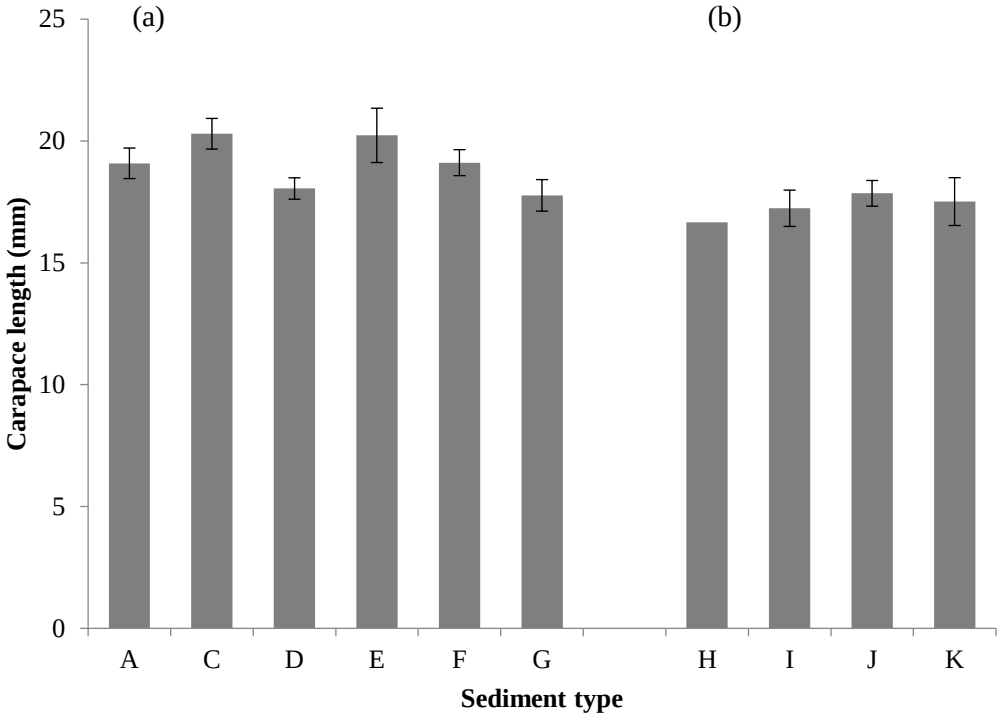


Fig. 1.8.6. The average carapace length (CL) of *Metapenaeus dalli* recorded at each of the sediment types present in the (a) offshore and (b) offshore waters of the Swan-Canning Estuary in summer (*i.e.* November 2013-March 2014). Error bars represent ±1 standard error. Note sediment H has no error bar as only a single individual was recorded. Sediment types A to G were from the offshore waters with matching carapace length data from otter trawls, while those from H to K were in nearshore waters with CL data from hand trawls.

No significant difference was detected in average CL of *M. dalli* recorded at the three sediment types present in offshore waters in winter (*P* = 24.60%; Table 1.8.6). Differences in CL among the sediment types were minimal, the values ranging from ~11.60 mm at sediment type B to ~12.80 mm at sediment type D (Fig. 1.8.7).

Table 1.8.6. Mean squares (MS), pseudo F-ratios and significance levels (P) from a one-way PERMANOVA test on the carapace 1 length of *Metapenaeus dalli* recorded in each of the three sediment types present in the Swan-Canning Estuary in winter (*i.e.* June-August 2014). df= degrees of freedom.

Main effect	df	MS	Pseudo-F	*P*
Sediment type	2	3.98	1.42	24.60
Residual	66	2.80		

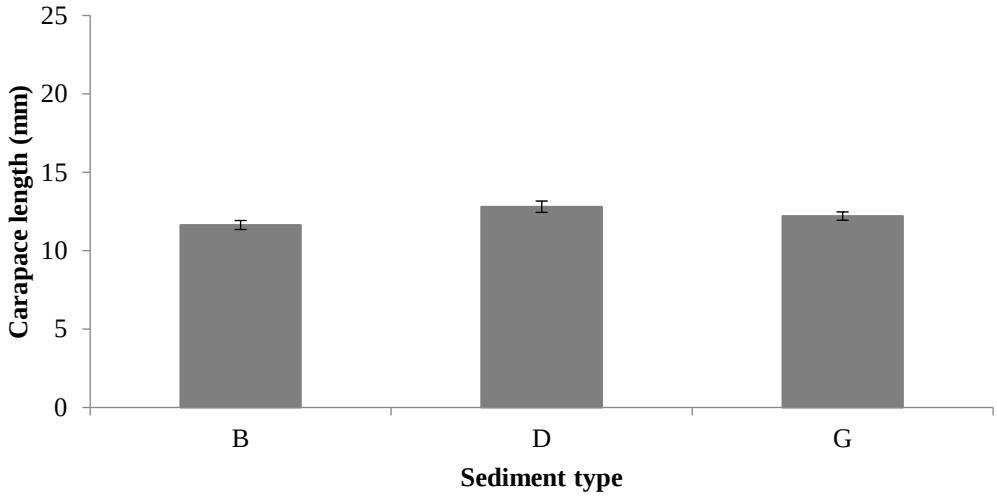


Fig. 1.8.7. The average Carapace length of *Metapenaeus dalli* recorded at each of the three sediment types present in the Swan-Canning Estuary in winter (*i.e.* June-August 2014). Error bars represent ±1 standard error. Sediment types are all from the offshore waters and the matching carapace length data are from otter trawls.

Relationship between the density of M. dalli among sediment types and environmental variables

As the PERMANOVA test above demonstrated that the density of *M. dalli* in summer differed significantly among the ten sediment types present at that time of year, the RELATE test was then employed to ascertain whether the pattern of relative differences among sediment types as defined by their density was significantly correlated with that defined by their sediment composition. This analysis showed that there was no significant correlation between the rank orders of resemblance between the two data matrices (P = 9.38%; ρ = 0.199). This lack of matching patterns can be seen in the nMDS ordination plot (*cf.* Fig. 1.8.8a,b). For example, while the marked separation between the offshore and nearshore sediment types in terms of their sediment characteristics (Fig. 1.8.8a) is generally reflected in those sediment types due to offshore sediment types yielding a greater average density of *M. dalli* (Fig. 1.8.8b), this was not the case for A and G *vs* K and J, which exhibited similar densities of prawns despite markedly different sediment compositions. Furthermore, while sediment types E and F were closely related in terms of their sediment characteristics, F contained far larger densities of prawn (Fig. 1.8.8b).

BIOENV was then used to determine whether a significant and greater correlation could be achieved between sediment composition and density of *M. dalli* by employing a subset of the eight sediment characteristics. This was achieved when the sediment matrix was constructed from only the <63, 125-249 and 1,000-1999 µm inorganic fractions (*P* = 3.20%; ρ = 0.354). Once again, there was clear separation of the nearshore and offshore sediments types, with the former group containing greater proportions of the <63 and 125-249 µm fractions, while the 1,000-1,999 µm fraction helped to account for the differences in sediment types within a water depth.

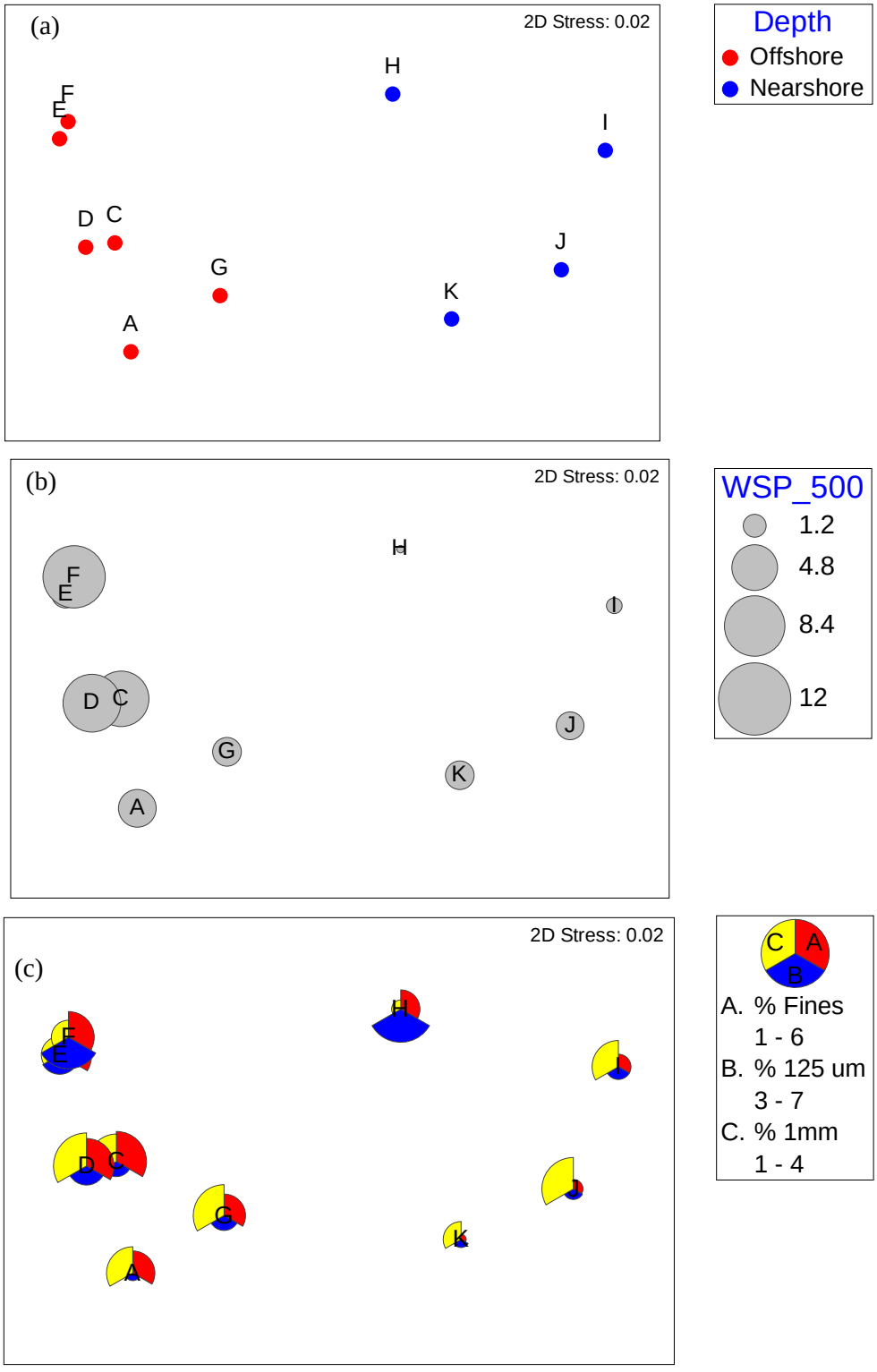


Fig. 1.8.8. (a) nMDS ordination plots constructed from the average of the full suite of eight sediment characteristics at each sediment type. (b)and (c) Bubble plots of mean density of *M. dalli* and the subset of three sediment characters selected by the BIOENV procedure that best match the spatial pattern displayed in the sediment types are superimposed on the nMDS ordinations as circles of proportionate sizes in (a). Sediment types A to G are from offshore waters with prawn density data from otter trawls, while sediments H to K are from nearshore waters with density data from hand trawls.

RELATE was then employed to determine whether the differences in the density of *M. dalli* among sediment types were also correlated to changes in water quality (*i.e.* surface and bottom salinity, water temperature and dissolved oxygen concentration). This analysis demonstrated that there was no correlation between the two matrices (P = 68.80%; ρ = -0.106). Visual comparisons of the spatial pattern of sediment types as defined by their average water quality characteristics and average density of *M. dalli* in summer show that there was no obvious trend (Fig. 1.8.9a,b). For example, while sediment type G, experienced relatively distinct water quality conditions (higher temperatures and lower dissolved oxygen concentrations), the average density of prawns was similar to other sediment types located on the opposite side of the nMDS plot (*i.e.* E, J, and K). Furthermore, sediment types D, F H and I formed a distinct cluster on the right side of the nMDS plot, yet their density of prawns differed markedly (Fig. 1.8.9b).

BIOENV analysis demonstrated that even with a subset of water quality parameters, there was no significant correlation to the density of *M. dalli* (P = 45.70%; ρ = 214). Thus, while the values of bottom temperature increased and those for surface dissolved oxygen concentration decreased from left to right on the nMDS plot these did not match those patterns in prawn density (*cf.* Figs. 1.8.9b,c).

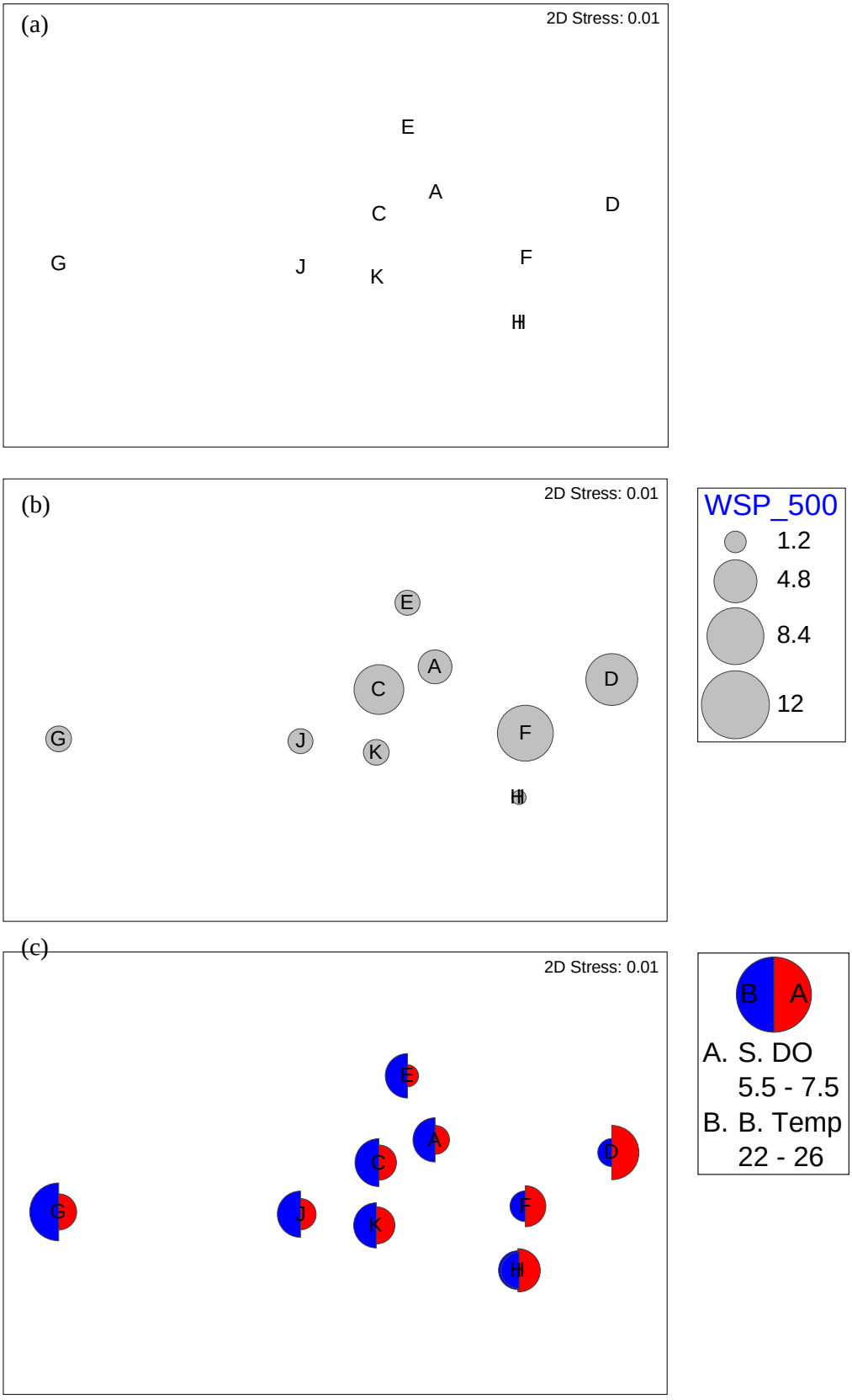


Fig. 1.8.9. (a) nMDS ordination plots constructed from the average of the full suite of six water quality parameters at each sediment type in summer (*i.e.* November 2013-March 2014). (b)and (c) Bubble plots of mean density of *M. dalli* and the subset of two water quality parameters selected by the BIOENV procedure that best match the spatial pattern displayed in the density of *M. dalli* are superimposed on the nMDS ordinations as circles of proportionate sizes in (a). Sediment types A to G are from offshore waters, while sediments H to K are from nearshore waters.

Daily activity patterns of M. dalli

During daylight hours (*i.e.* 06:00-17:59) the vast majority of prawns remained buried in the sediment (stage 4; Fig. 1.8.10). Prawns first emerged at 18:00 when 35% of the prawns had emerged completely (Stage 1), while another 17% had reduced their burial depth and were classified as either stage 2 or 3. During this time the prawns were actively foraging in the sediment. The number of emerged prawns decreased gradually throughout the night from a maximum of 35% at dusk (18:00) to a minimum of 13% at 02:00, before increasing slightly to 22% at 04:00 (Fig. 1.8.10).

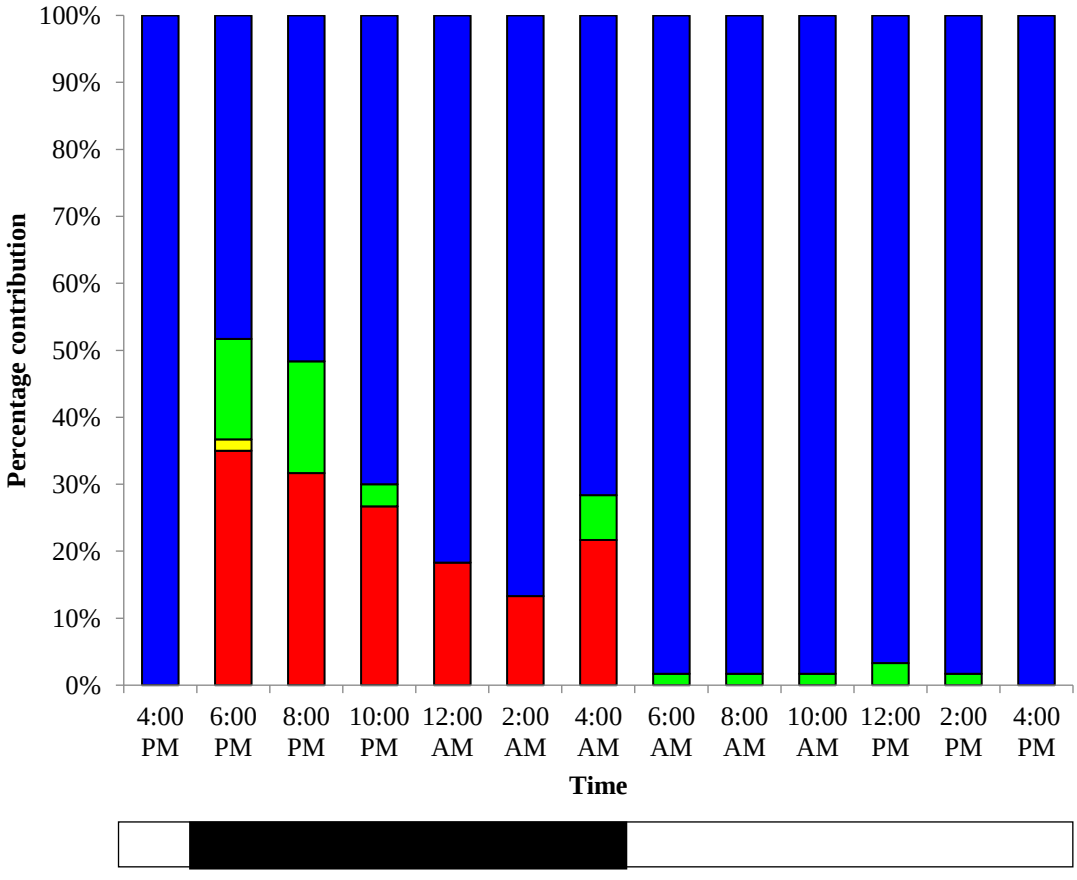


Fig. 1.8.10. Mean percentage of *Metapenaeus dalli* in different stages of emergence at 2h intervals over 24h. Stage 0 ■, Stage 1 ■, Stage 2 ■, Stage 3 ■ and Stage 4 ■. The bar on the *x*-axis shows the photoperiod, with white bars denoting daylight and black bars darkness.

Laboratory sediment preference experiments

Pooling the results from all replicates of the nearshore sediments, the percentage occurrence of *M. dalli* was significantly higher on the sediments from Dalkeith (*i.e.* 39 prawns, 65%) than between the nearshore sediments of Garratt Road Bridge (*i.e.* 21 prawns, 35%; X^2= 4.82; p = 0.028). Variation in sediment preference was found between tanks: in three tanks between 80-90% of individuals were found buried in the Dalkeith sediments, while in the other three tanks no clear pattern was found, *i.e.* 40-50% of prawns were found in the Dalkeith sediments (Fig. 1.8.11a).

For the offshore sediment comparison, a stronger preference was found for the Dalkeith sediments than those from Garratt Road Bridge. Over all tanks, 68% of the prawns were found in Dalkeith sediments (X^2 = 7.36; p = 0.007) and in four tanks, 70-80% of individuals were found in the Dalkeith sediments, with a 50:50 split in the other two tanks (Fig. 1.8.11b).

Description of burrowing behaviours

Metapenaeus dalli buries itself by fanning sediment backwards with pleopods (Fig. 1.8.12a), whilst simultaneously moving forwards and downwards into the sediment using the pereopods. A "flicking" motion using the whole body, ending with a flick of the tail, helps push the prawn further into the sediment (Fig. 1.8.12b). The antennae push together in a swiping motion and push the sediment over the back of the prawn as it continues to bury deeper into the substrate (Fig. 1.8.12c). Within about 17 seconds, the prawn is completely buried within the sediment. Sometimes the whole body is buried while the eyes, antennae and rostrum, remain visible. For the following ~30 seconds, the prawn usually continues to slowly bury itself even further into the sediment using occasional movements of the pleopods and pereopods. After this, the antennae are usually pulled into the sediment, and the prawn is not visible. The time taken to complete the burial process depends on the sediment type; thus the time taken for *M. dalli* to bury in the sediments from Garratt Road Bridge was almost twice that required for Dalkeith sediments. The depth of burial was ~1 cm under the surface of the sediment and individuals buried in very close proximity to one another.

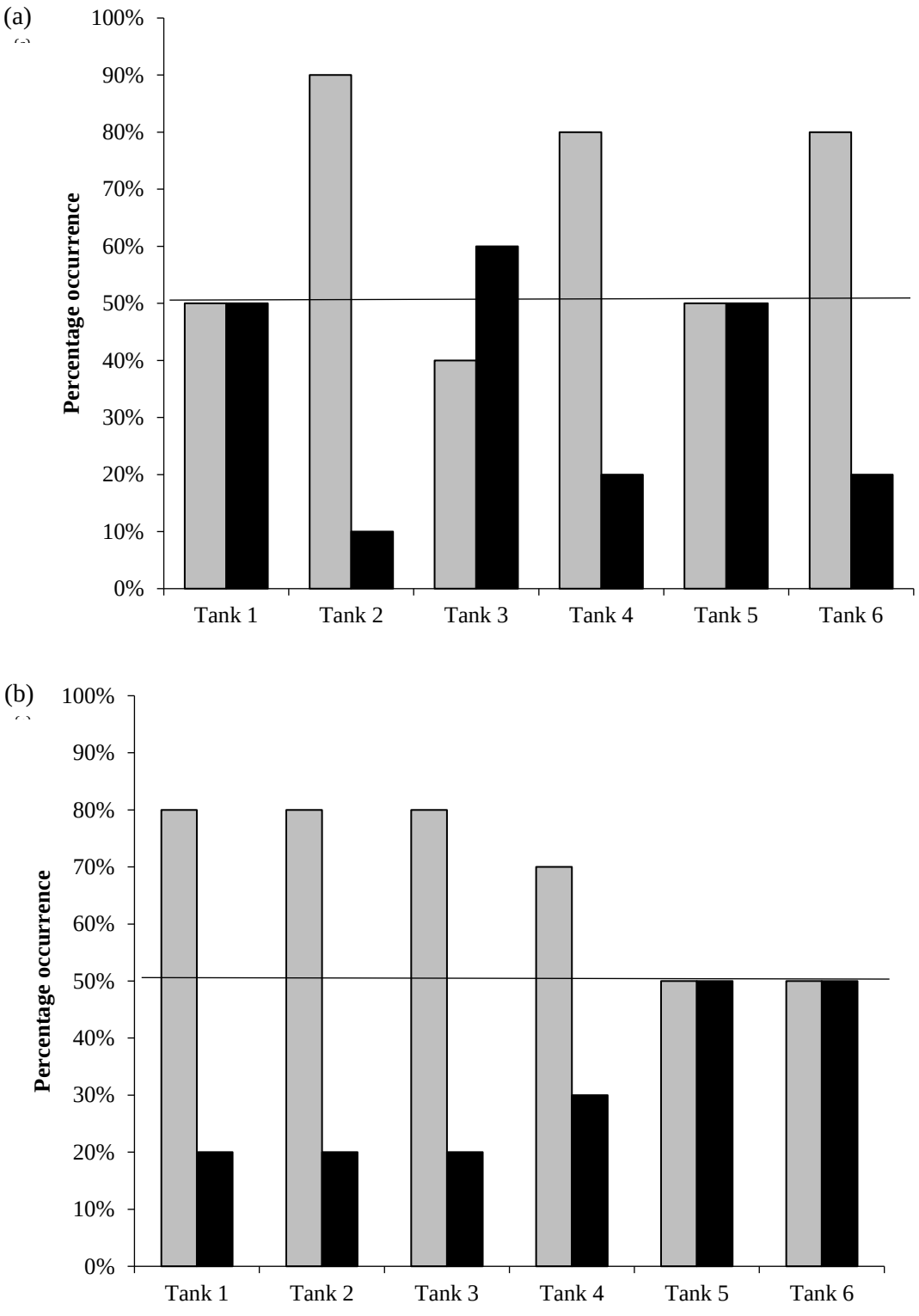


Fig. 1.8.11. The percentage occurrence of *Metapenaeus dalli* found buried in the Garratt Road Bridge (■) and Dalkeith (▒) (a) nearshore and (b) offshore sediments after 48h in the experimental tanks. The line demarks the 50% mark (*i.e.* no preference between sediments).

(a)



(b)



(c)



Fig. 1.8.12. Still frames of the burial process of *Metapenaeus dalli* in the laboratory at (a) 0, (b) 2 and (c) 8 seconds and by 17 seconds the prawn is completely buried.

Discussion

This study aimed to establish firstly, whether the density and length of *M. dalli* differed among the various sediment types identified in Section 1.7 and, if so, whether the pattern of relative differences matched those defined by the sediment and water quality characteristics. Secondly, sediment preference experiments were conducted in the laboratory to assess, in a controlled environment, whether prawns had a preference for one of two contrasting sediment types. This latter experiment also provided an opportunity to describe the diel pattern of emergence and activity of *M. dalli* and its burial behaviour.

Influence of sediment type on M. dalli density in summer

The results of a one-way crossed PERMANOVA demonstrated that prawn density differed significantly among the 10 sediment types during summer (*i.e.* November 2013 - March 2014). This difference was due, in part, to greater densities of *M. dalli* being recorded in the offshore sediment types, particularly C, D and F, and lower densities being recorded in the nearshore sediment types, particularly H and I. Greater densities of *M. dalli* in the offshore compared to the nearshore waters of the Swan-Canning Estuary was also recorded by Potter *et al*. (1986b). The absence of prawns (either *M. dalli* or the Western King Prawn *Penaeus* (= *Melicertus*) *latisulcatus*) in seine net samples collected during daylight hours in this system (Tweedley, unpublished data) suggests that the majority retreat and bury in the sediments in offshore waters during the day. Therefore the lower densities of *M. dalli* recorded in the nearshore waters may reflect both the fact that only a subset of the population move into the nearshore waters at night and/or that these species exhibit a preference for the sediment with a higher proportion of organic matter and finer grain sizes.

This latter theory is supported by the work of by Aziz and Greenwood (1982), which demonstrated that *Metapenaeus bennettae* had distinct preferences for sediments containing finer particles, a common trend with many penaeid prawns (*e.g*. Ruello, 1973; Moller and Jones, 1975). The latter study, in particular, used different grades of natural sediments and a

similar methodology to the one employed here, and results showed that *Metapenaeus macleayi* exhibited a preference for fine sandy substrates. This was attributed to the smaller grain sizes being physically less taxing to move and thus easier for the prawn to bury in (Ruello, 1973; Aziz and Greenwood, 1982). This is likely to also be the case with *M. dalli* as this species is slightly smaller in size than *M. macleayi* (cf. Grey *et al*., 1983; Potter *et al*., 1986b). Furthermore, the high organic content of these sediments, coupled with the calmer conditions associated with these environments, presents a much more desirable environment for the prawns to live on, thus being advantageous to their survival (Sanders, 1958; Ruello, 1973; Aziz and Greenwood, 1982).

The lowest density of *M. dalli* in the offshore waters was recorded in sediment type G, which agrees with the anecdotal evidence from recreational fishers that there are fewer prawns in the areas upstream of Perth Water. It is therefore relevant that sediment of this type (*i.e.* G) contained the lowest percentage contributions of organic matter and finer inorganic particles (Fig. 1.8.6 and see later for discussion of sediment preference involving sediments collected from this site).

Influence of sediment type on M. dalli *density in winter*

In contrast to the situation in summer when the density of *M. dalli* differed among sediment types, no such trend was detected in winter. This could be attributed to a lesser number of sediment types present throughout the estuary during winter (*i.e.* 3) compared to summer (*i.e.* 10). As mentioned in earlier, the Mediterranean climate experienced by Perth (Spencer, 1956; Hodgkin and Hesp, 1998) results in heavy freshwater discharge during the winter months (Kanandjembo *et al*., 2001; Thrush *et al*., 2004; Hoeksema and Potter, 2006). This flow scoured the sediments and reduced the environmental heterogeneity (Fig. 1.8.6, 1.8.7). While in summer, due to the typically very low rainfall and thus freshwater discharge and the small tidal range, the environmental conditions in the Swan-Canning Estuary are relatively stable (Potter *et al*., 2015b). This is not the case in winter and a pronounced longitudinal salinity gradient is

present along which fauna partition themselves (Loneragan *et al*., 1989; Wildsmith, 2007; Hourston *et al*., 2011). However, the two major sediment types present in this season were both spread throughout the full spatial extent of the area sampled (Fig. 1.8.7), removing any confounding effects of changes in salinity.

Influence of water quality on M. dalli density

Significant differences in the density of *M. dalli* among the sediment types were detected in summer, however, RELATE demonstrated that the pattern of relative differences in density did not match those in water quality (*i.e.* salinity, water temperature and dissolved oxygen concentration). Furthermore, even when all possible combinations of variables were employed, no significant correlations were detected. This is a reflection of a number of factors. Firstly, *M. dalli* in south- western Australia exhibits the life history characteristics of an estuarine species *sensu* Potter *et al*. (2015a), *i.e.* completes its life cycle within the estuary (Potter *et al*., 1986b). As such, it must have adapted to the estuarine mode of life and to exposure to the seasonal changes in salinity that occur in this system. It is therefore relevant that individuals of this species have been recorded in salinities of 0.5 in the Swan-Canning Estuary (Tweedley, unpublished data) and in marine water, as well as the hypersaline waters of Shark Bay (Slack-Smith, 1967; Grey *et al*., 1983) and that its congener *M. bennettae*, which occurs in south-eastern Australia (Grey *et al*., 1983), can osmoregulate over a salinity range of 1-62 (Aziz and Greenwood, 1981; Preston, 1985).

Secondly, as mentioned above, environmental conditions within the Swan-Canning Estuary are relatively stable during the warm, dry summer months and thus water quality, particularly salinity and temperature do not differ much among sediment types (Annex 1.8.1). Furthermore, while Tweedley *et al*. (2016a) demonstrated that crustaceans are particularly sensitive to the effects of hypoxia in the Swan-Canning Estuary and that levels of dissolved oxygen have been significantly negatively correlated with commercial catches of *M. macleayi* in the Hawkesbury river in New South Wales (Pinto and Maheshwari, 2012), no major hypoxic events were

detected during the course of this study. On several occasions hypoxia (*i.e.* dissolved oxygen concentrations < 2 mg L-1) was detected at individual sites in the offshore waters and almost always resulted in no prawns being caught. These findings agree with the work of Wu *et al*. (2002), who showed that *Metapenaeus ensis* was able to detect and avoid areas with low oxygen.

Influence of sediment type on M. dalli *length*

No significant difference was detected in the average CL of *M. dalli* and sediment type in either summer or winter. The results of other studies have revealed that many species of prawns do exhibit size differences across different sediment types (Williams, 1958). However, Courtney *et al*. (1995) showed that there were no clear trends in the size distribution of the Eastern King Prawns, *Penaeus plebejus,* in Morton Bay in Queensland (Australia). These authors suggested that this may be a result of the mobility and migratory behavior of the species within the estuary. A similar explanation may be valid for *M. dalli* in the Swan-Canning Estuary, where marked changes in the spatial pattern of distribution and abundance have been reported over relatively short temporal scales (two to four weeks) indicating that these species are highly mobile (see earlier).

Laboratory sediment preference experiments

Sediment preference experiments were conducted under controlled conditions in the laboratory to help validate the above correlations between the density of *M. dalli* and sediment type recorded from the Swan-Canning Estuary. These experiments were controlled for all other environmental factors, and thus removed the confounding effects of differences in the overlying water phyisco-chemistry from the analyses of the field data. The results of the sediment preference experiments demonstrated that *M. dalli* are sensitive to light, with all individuals remaining almost completely buried in the sediment during daylight hours (06:00-17:59), but

emerging and being active as soon as it was dark. A similar and immediate emergence to dark has also been recorded for many species of penaeid, such as *Metapenaeus endeavouri* (Park and Loneragan, 1999), *Penaeus semisulcatus* (Kutty and Murugapoopathy, 1968; Lui and Loneragan, 1997) and *Penaeus aztecus* (Dall *et al*., 1990), and for a number of other species (Wassenberg and Hill, 1994). Such a dramatic response to the presence or absence of light has long been thought of as a response to the risk of predation, which also has the added bonus of reducing an individual's energy demands (Dall *et al*., 1990). Activity reached a peak upon darkness and declined steadily throughout the remaining hours of darkness, a trend also known to occur in *Penaeus duorarum*, although the reasons why are unclear ((Fuss, 1964; Fuss and Ogren, 1966; Wickham, 1967).

A chi-square test demonstrated that in both the nearshore and offshore sediments *M. dalli* exhibited a preference for the sediment at Dalkeith rather than Garratt Road Bridge, with this preference being slightly greater in the offshore sediment.

The offshore sediment at Dalkeith had a higher percentage contribution of organic matter and larger proportions of inorganic grain sizes < 124 µm, indicating that *M. dalli* have a preference for finer grained sediments. These results have been reflected in numerous other studies of sediment preference for penaeids (*e.g.* Ruello, 1973; Moller and Jones, 1975; Aziz and Greenwood, 1982), where species such as *M. macleayi*, *M. bennettae* and *Penaeus monodon* all displayed preferences for finer sediment types. The preference is thought to correspond to the physical ease of moving the particles to bury, enabling the animal to bury in the sediment more rapidly and thus avoid predators (Ruello, 1973; Aziz and Greenwood, 1982). It is also noteworthy that visual observation of the burial behavior (Fig. 1.8.12) indicated that the length of time required to bury completely into the sediment was far longer in the sediment from Garratt Road Bridge than that from Dalkeith, with the prawns sometimes struggling to dislodge and move large particles that had become bound together in the former sediment.

In the nearshore sediments experiment, prawns again exhibited a preference for the Dalkeith sediment. However, as both sediments contained similar levels of fine sediments and the

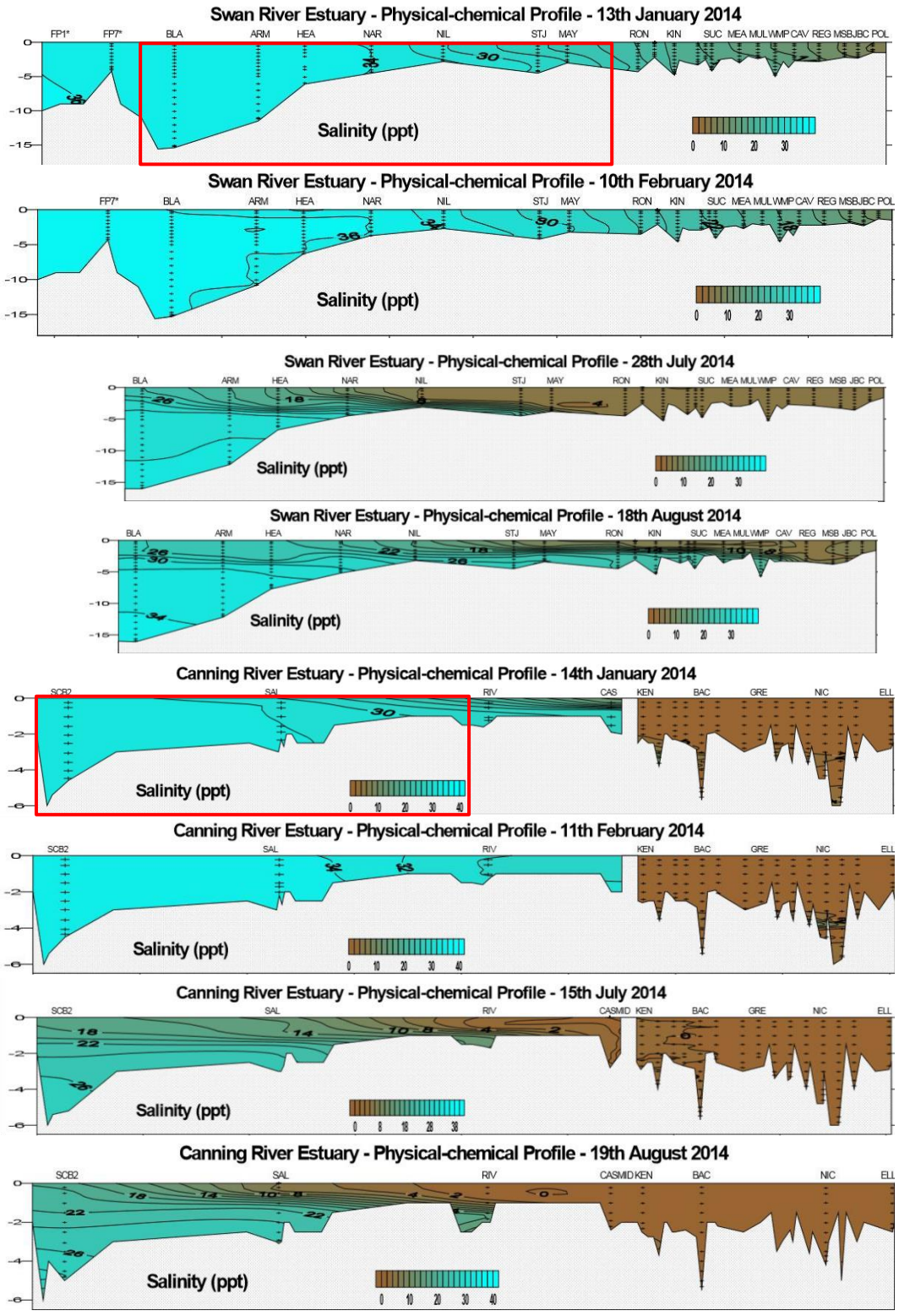
percentage amount of organic matter was greater at Garratt Road Bridge, it is suggested that the preference for Dalkieth sediment is a reflection of the higher proportion of inorganic grains > 1,000 µm present in the Garrett Road Bridge sediments. These larger grains of sediment would be far harder to move and thus make it more difficult for the prawns to bury quickly in (Ruello, 1973).

Conclusions

In summary, this study on sediments and *Metapenaeus dalli* has demonstrated that there was a significant difference between the density of *M. dalli* among sediment types during summer, but not during winter. The lack of a difference in winter reflects the homogeneity among sediment types caused by the effects of heavy freshwater discharge on the presence of fine particles. While the changes in the density of *M. dalli* in summer were correlated to those among sediment types, they were not related to differences in salinity, water temperature or dissolved oxygen concentration. This reflects both the euryhaline ability of metapenaeids and the fact that during summer, water quality is fairly consistent throughout the area of the Swan-Canning Estuary where prawns were caught. The sediment preference experiments conducted, under controlled conditions, in the laboratory demonstrated that *M. dalli* had a significant preference for sediment from Dalkeith, rather than further upstream at Garratt Road Bridge, and that prawns were able to bury more quickly in the sediments from Dalkeith. These findings support the view that a change in sediment composition, if it occurred, may have been partially responsible for the lower recreational catches of *M. dalli* in the waters around Garrett Road Bridge. Finally, the laboratory experiments also demonstrated that the emergence and activity patterns of *M. dalli*, like those of many other penaeid species, are strongly linked to photoperiod.

Annex

Annex 1.8.1. Weekly vertical contour plots of salinity measured at monitoring stations along the length of the (a-d) Swan Estuary and (e-h) Canning Estuary for two occasions in summer (a, b, e, f) and two in winter (c, d, g, h). Red boxes denote the spatial extent of prawn sampling. Data taken from http://www.swanrivertrust.wa.gov.au/swan-river-trust/publications/monitoring-and-evaluation



Section 2. Biological characteristics of the Western School Prawn and bio-economic modelling

This section details research relating to objective 3, *i.e.*establish a bioeconomic model of the Western School Prawn population and the factors influencing it and objective 4, evaluate the costs and benefits of releasing Western School Prawns in the Swan-Canning. Two main components of the biology of this prawn species and the implications for release programs were evaluated:

1. Estimation of growth and reproductive parameters of the Western School Prawn in the Swan-Canning Estuary (Honours studies of Andrew Broadley).
2. Bio-economic evaluation of restocking the Western School Prawn in the Swan-Canning Estuary (Honours studies of Andrew Broadley).

2.1. Estimation of growth and reproductive parameters of the Western School Prawn in the Swan-Canning Estuary

This study has been published in Fisheries Research.

Broadley, A.D., Tweedley, J.R. & Loneragan, N.R. (2017). Estimating biological parameters for potential penaeid restocking in a temperate estuary. *Fisheries Research*. 186(2): 488-501. DOI: 10.1016/j.fishres.2016.09.007.

Summary

Robust estimates of growth, mortality and reproduction provide fundamental information for evaluating release programs. Length frequency data and mixture analysis were used to estimate a suite of biological parameters for the Western School Prawn (*Metapenaeus dalli*). This was an iconic recreational species, which is being evaluated for restocking in the Swan-Canning Estuary in temperate, south-western Australia. Monthly length frequency data, collected from hand and otter trawls over 26 consecutive lunar cycles showed that *M. dalli* exhibits highly seasonal patterns of growth and reproduction. Growth occurred predominantly during the warmer months (October-March), with little to no growth in cooler months (May-August). A von Bertalanffy growth model, incorporating seasonal growth, estimated that female prawns grew significantly larger ($L\infty$ = 33.6 mm CL) than males ($L\infty$ = 22.8 mm CL), but that the rate of reaching the asymptotic size was the same for both sexes (K = 0.98). Gravid females were found only from October to March and spawning activity was greatest from November to February, when surface and bottom water temperatures ranged from 20 to 28 °C. The instantaneous rate of total mortality (Z) was greater for females (0.069 $week^{-1} \cong 3.57\ year^{-1}$) than males (0.043 $week^{-1} \cong 2.28\ year^{-1}$). Since fishing mortality is now very low, these estimates provide a close approximation to natural mortality (M). A similar approach was applied to estimate the growth parameters from the length distributions of *M. dalli* reported in this system 30 years earlier, when the population biomass was likely to be much higher than the current biomass and *M. dalli* was heavily exploited by recreational fishers. The maximum size and $L\infty$ of *M. dalli* are now between 10 and 20% larger than 30 years previously, which may reflect the current lower fishing pressure and lower population biomass. From this study, the optimal release times for *M. dalli* are from December to March, when prawns grow rapidly and can be cultured successfully under current production systems.

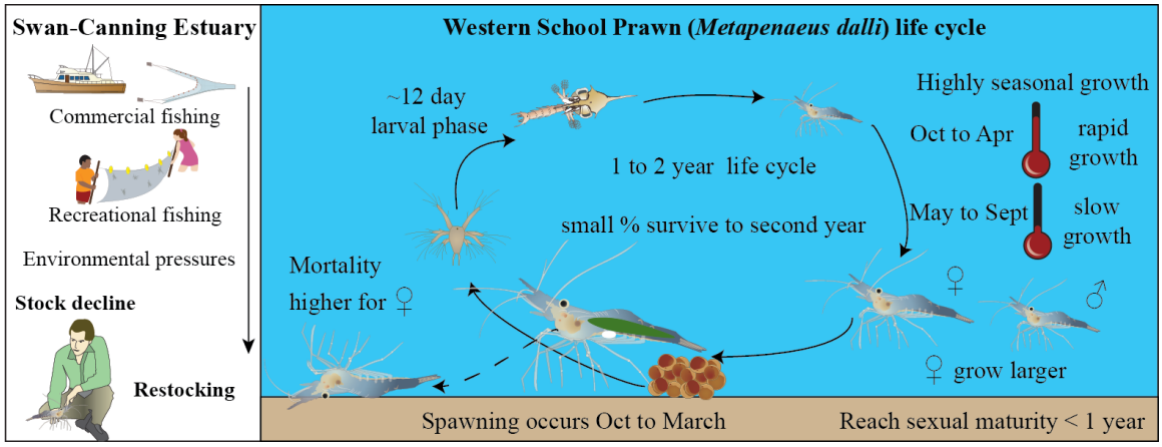


Fig. 2.1.0. Conceptual model of the life cycle of *Metapenaeus dalli* in the Swan-Canning Estuary.

Rationale and aims

Individuals from a several species of prawns or shrimp in the Penaeidae are released on a very large, commercial scale (hundreds of millions to billions) in Japan (Hamasaki and Kitada, 2006) and China (Bell *et al*., 2005; Wang *et al*., 2006; Loneragan *et al*., 2013a). Smaller scale, commercial releases of penaeids have also been practised in Kuwait, Sri Lanka, the United States and Australia (Bell *et al*., 2005; Loneragan *et al*., 2013a). Recent research in Australia has focussed on release programs for penaeids to "enhance" recreational fishing; one for Eastern King Prawns *Penaeus plebejus*, to overcome recruitment limitation caused by a physical barrier to prawn larval recruitment (Taylor, 2017) and the second, to investigate the potential for rebuilding the stocks of the Western School Prawn *Metapenaeus dalli* (this study), *i.e.* evaluating the potential to restock this species.

In response to the depleted status of the *M. dalli* population in the Swan-Canning Estuary, a trial restocking program was initiated in 2012. This focused on estimating the biological parameters of the school prawns to provide the information to better evaluate the costs and benefits of restocking and optimise the potential success of releases. The overall objective of this study is to use the data from the systematic, intensive sampling program of *M. dalli* in the Swan-Canning Estuary in 2013/14 and 2014/15 to determine, for the first time, the biological parameters for growth, mortality and size at maturity to evaluate optimal release times. Growth curves were also fitted to the historical data collected over 30 years ago (1977 to 1982, Potter *et al*., 1986) when the biomass of the population, catch and intensity of recreational fishing effort were much higher than currently.

Methods

Study area

The Swan-Canning Estuary (Fig. 2.1.1) in south-western Australia, is ~50 km long and covers an area of ~55 km^2 (Valesini *et al*., 2014). This drowned river valley system is permanently-open to the Indian Ocean via a narrow entrance channel that opens into two basins and the tidal portions of the Swan and Canning Rivers. Although the majority of the estuary is shallow, *i.e.* < 2 m in depth, it reaches a maximum depth of ~20 m in the entrance channel. The region experiences a Mediterranean climate, with hot, dry summers and cool, wet winters (Gentilli, 1971). Approximately 70% of the rainfall occurs between May and September (Hodgkin and Hesp, 1998), leading to marked seasonal variations in environmental conditions in the estuary: salinities are stable and relatively high throughout much of the estuary during the austral summer (December to February), but during winter, may vary markedly along the estuary following substantial freshwater discharge (Tweedley *et al*., 2016b).

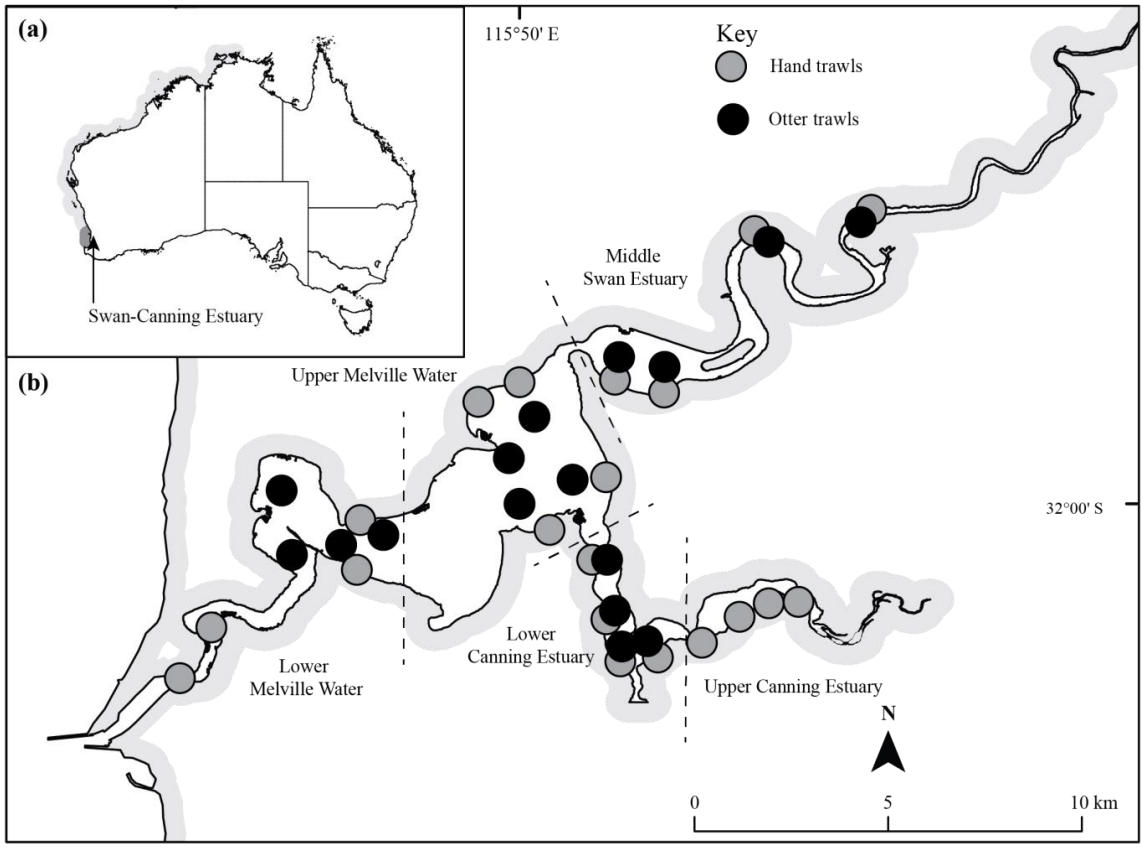


Fig. 2.1.1. Map showing (a) Australia and the distribution of *Metapenaeus dalli* in inshore marine waters (light grey) and solely in estuaries (dark grey) and (b) location of the 20 nearshore sites and 16 offshore sites in Swan-Canning Estuary sampled over 26 lunar cycles in the two years between October 2013 and October 2015. Dotted lines denote the separation among the five regions of the estuary.

The estuary flows through the *capital* city of Perth, which supports ~78% of the 2.6 million people in the state of Western Australia (Australian Bureau of Statistics, 2015). Both the estuary and its catchment have been highly modified by anthropogenic activities (Commonwealth of Australia, 2002), which has led to multiple stressors on the system, such as the increased delivery of sediments and nutrients, in addition to changes to salinity and hydrological regime, including periodic hypoxia (Stephens and Imberger, 1996; Tweedley *et al*., 2016a). Despite these perturbations, the estuary is valued highly by the Western Australian community for its aesthetic, commercial, environmental and cultural importance and recreational fisheries (Malseed and Sumner, 2001).

Rainfall and water quality data for the Swan-Canning Estuary

Rainfall data for Perth airport were obtained from the Bureau of Meteorology (http://www.bom.gov.au/climate/data/) from October 2013 until October 2015. Weekly data for salinity and temperature throughout the water column were obtained for sites in the Swan-Canning Estuary from the Department of Water and Environmental Regulation (http://wir.water.wa.gov.au) for the same period.

Sampling procedure

Prawns were sampled at night at 20 nearshore (< 2 m deep) sites using a hand trawl net and 16 offshore sites (2-17 m deep) using a small otter trawl net, on each new moon phase (*i.e.* every 28 days when the moon < 10% illumination) between October 2013 and October 2015 (*i.e.* 26 lunar cycles over two years). Note that, due to a mechanical failure, no samples were collected from the offshore sites in December 2014 and thus the corresponding data from the nearshore waters was also excluded for this lunar cycle. The sites extended from close to the mouth of the Swan-Canning Estuary to ~34 and ~27 km upstream in the Swan and Canning rivers, respectively (Fig. 2.1.1). The total area within the bounds of the sampling sites was 35 km^2, with 15.5 km^2 in nearshore water and 19.6 km^2 in offshore water.

Nearshore sites were sampled using a 4 m wide hand trawl constructed from 9 mm mesh. The width of the hand trawl net during trawling was, on average, ~2.85 m, but varied slightly amongst trawls depending on the condition of the substratum, presence of submerged obstacles and localised wind and wave conditions. Two replicate trawls of 200 m (swept area of ~570 m^2, were carried out at each site on each sampling period and on any single lunar cycle covered a total area of 22,800 m^2. A 2.6 m wide otter trawl net, with 25 mm mesh in the body, and 9 mm mesh in the cod end was employed to sample prawns in the offshore waters. The net was towed at a speed of ~1.6 knots (~3 km h^{-1}) for 5 min, covering a distance of ~250 m. Two replicate trawls of ~650 m^2 were completed at each site on each sampling period covering a total area of 20,800 m^2 at the 16 sites. After each trawl, individuals of *M. dalli* were euthanised in an ice slurry and returned to the laboratory to be sexed, measured and weighed, expect when > 50 prawns were caught. In such instances, a small sub-sample (~50 individuals was retained) and the majority of prawns were identified, sexed and measured (see below) in the field and returned alive to the water.

The catchabilities of the hand trawl and otter trawl nets used in this study have not been estimated. A catchability of 0.4 has been used in the estimation of the biomass of *M. dalli* in the estuary, based on a range of estimates for catching juvenile *Penaeus esculentus* and *P. semisulcatus* in a small beam trawl (Loneragan *et al*., 1995) and those for *Penaeus* (= *Melicertus*) *latisulcatus* in a large, commercial otter trawl (Joll and Penn, 1990).

In the laboratory, the carapace length (CL), *i.e.* orbital indent to the posterior edge of the carapace, of each individual was measured (to 0.01 mm) using digital vernier callipers, and the wet weight (to 0.01 g) and sex of the prawn were also recorded. Females were identified by presence of a thelycum and males by the presence of a petasma. Individuals without a thelycum or petasma were recorded as juveniles. Female prawns were also inspected to determine if they were gravid, *i.e.* had large green ovaries, as described by Tuma (1967) and Crisp *et al*. (2017a) and/or possessed a spermatophore.

Length-weight relationship

Initially, the relationship between carapace length (CL) and wet weight (W), was evaluated with a non-linear least squares (NLS) model in *R* (R Core Team, 2014). Since the residuals increased with increasing CL, a log relationship ($log(W) = log(a) + blog(CL)$) was calculated. A bias correction factor for back-transforming mean weight values for a given length was calculated using:

$$e^{\frac{s^2_{Y|X}}{2}},$$

where $s^2_{Y|X}$ is the mean square error from the linear model (Ogle, 2014).

The length-weight relationships for female and male *M. dalli* were:

Female: $\log(W) = -6.29 + 2.68 \log(CL)$, ($R^2 = 0.98$, n = 1,721),

Male: $\log(W) = -6.78 + 2.89 \log(CL)$, ($R^2 = 0.98$, n = 1,394).

Alternatively, on the original scale, with bias correction:

Female: $W = (0.0019 CL^{2.68}) \times 1.0067$;

Male: $W = (0.0011 CL^{2.89}) \times 1.0058$;

Estimation of growth

The length frequency data from hand and otter trawls were used to estimate growth and mortality. Growth was estimated from the pooled data from both the hand and otter trawls after adjusting for swept area (see below) by mixture analysis and modal progression. Growth estimates for *M. dalli* in 1977-82 were calculated from modal progression using the data in Potter *et al*. (1986b)

In the absence of data on net efficiency, the catchability of *M. dalli* using the hand and otter trawls were assumed to be equal, and the length frequency data from the otter trawls were scaled

up by a factor of 1.096, *i.e.* the ratio of the total swept area of hand trawls: otter trawls each sampling period (*i.e.* 22,800 m^2: 20,800 m^2). Juveniles were assigned equally to each of the female and male groups. The CL measurements from these data were then allocated to 1 mm size classes.

Identifying cohorts

Monthly histograms of the weighted 1 mm CL data were created in *R* and reviewed visually to gain an understanding of changes in length frequency distribution over time and identify potential modal groups (cohorts). Finite mixture analysis was conducted in R using the Mixtools package (Benaglia *et al*., 2009). Starting values for the mean, standard deviation (SD) and weighting for each potential mixture component (cohort) in a monthly sample were estimated from the histograms visually. A two-step iterative process was employed to generate normal distributions for each of the components using the Expectation-Maximisation (EM) algorithm from the Mixtools package (Benaglia *et al*., 2009). Hypothesis testing (a = 0.05) using 1,000 bootstrap replicates was used to produce a likelihood ratio statistic for the null hypothesis of a k component fit versus an alternative hypothesis of $k+1$ (up to a maximum of 10) components for each monthly sample (Benaglia *et al*., 2009). The resultant outputs were optimised estimates of the mean, SD, weighting and number of prawns in each mixture component for each monthly sample.

Analysis of historical data

Data on the biology of *M. dalli* in the Swan-Canning Estuary collected by Potter *et al*. (1986b) between 1977 and 1982 were analysed to estimate growth rates for comparison with the current study (2013/15). These authors used the same sized mesh in the otter trawl (25 mm), but employed a larger mesh (19 mm) in the hand trawl compared to that used in the current study (9 mm). The means and SD of the female and male CL frequency distributions were estimated

visually from Fig. 3 in Potter *et al*. (1986b). These data were used to reproduce the Potter *et al.* (1986) modal progression graph in *R*.

Parameter estimation from length frequencies

The methods outlined in this section were applied only to the 2013/15 data, because the original length frequency data were not available from the historical study. The primary purpose of estimating parameters from length frequencies was to create a set of robust starting values for the growth models.

The weighted 1 mm size class length frequency data from the current study were grouped by sampling period and analysed using the Length Frequency Data Analysis 5 (LFDA 5; Kirkwood *et al*., 2001) and Fisheries Stock Assessment Tools II (FiSAT II) packages (Gayanilo *et al*., 2005). In LFDA 5, the Hoenig and Hanumara (1982) and Pauly *et al*. (1992) seasonal version of the von Bertalanffy growth function (VBGF) were fitted to the length frequency data using the ELEFAN (Pauly, 1987) method. A score grid search in LFDA 5 provided the initial parameter estimates, which were then optimised and plotted using the automatic maximisation process. Similarly, in FiSAT II, the ELEFAN I routine was used to directly fit a seasonal VBGF (Pauly *et al*., 1992), by using a response surface analysis and then plotting and optimising the fit by eye.

Parameter estimation from modal progression

The same method for estimating growth parameters by fitting a non-linear least squares (NLS) model to data derived from modal progression was used for the data from 1977-82 and 2013/15. For the earlier data, the modal progression graph recreated in R was used to model and estimate growth parameters. For the 2013/15 data, the means and SD from the finite mixture analyses were plotted separately for female and male prawns in a 26 lunar cycle time series. Modal

progression was used to identify cohorts by tracking each point through time and visually observing its position relative to adjacent cohorts.

The Somers (1988) seasonally oscillating adaption of the VBGF was applied to estimate growth parameters for the cohorts of male and female prawns that could be followed in the carapace length frequency histograms for the longest period.

The Somers adaptation is:

$$L(t) = L_{\infty}\{1 - e^{-[k(t-t_0)+ S(t)- S(t_0)]}\},$$

$$\text{with } S(t) = (Ck/2\pi)\sin 2\pi(t - t_s),$$

$$\text{and } S(t_0) = (Ck/2\pi)\sin 2\pi(t_0 - t_s),$$

where $L(t)$ is the average length at time t, L_{∞} is the asymptotic length, K is the rate at which the model reaches asymptotic length, t_0 is the theoretical time where the average length is 0. The functions $S(t)$ and $S(t_0)$ generate the seasonal oscillation of the growth curve: C controls the amplitude of the growth oscillation during the winter period (if $C = 1$ growth stops or if $C = 0$, growth is continuous, *i.e.* there is no seasonal oscillation), t_s is the start of the curved portion of the first growth oscillation.

The Somers (1988) model was fitted in R using the FSA (Ogle, 2014) and Minpack (Elzhov *et al*., 2013) packages. The former package provided an implementation of the Somers (1988) growth function and the Minpack package was used to implement an NLS function using a modified Levenberg-Marquardt algorithm, which supports lower and upper parameter constraints. The starting values for the NLS model ($L\infty$, K, t_0, C and ts) for the 2013/15 and historical data (1977-82) were estimated by averaging the results of $L\infty$, K, t_0, C and t_s from the length frequency analysis using LFDA 5 and FiSAT II. The following parameters were constrained to optimise model fitting: C between 0 and 1, t_0 between -1 and 0 and t_s between -1 and 1.

The NLS model assumes that the data are homoscedastic and the errors are normally distributed. These assumptions were investigated by (1) plotting the residuals and fitted values for each model and visually verifying the distribution of the plotted points and (2) creating histograms of the residuals and visually checking the distribution for symmetry around the midpoint. A bootstrapping technique using 1,000 resampled data sets was used to create 95% confidence intervals (CI) for each of the estimated parameters.

Estimation of mortality

Instantaneous total mortality (*Z*) was estimated separately for female and male prawns using the weighted 1 mm size class length frequency data from the 2013/15 data set. A catch curve regression was implemented in *R* using length-converted catch curves (LCC; Pauly, 1983b; 1983a, 1984). Both seasonal length-converted catch curves (SLCC; Pauly, 1990) and non-seasonal LCC were fitted to the data. Only the results from the LCC are presented below. The non-seasonal LCC method was implemented with:

$$\log\left(\frac{N_i}{\Delta t_i}\right) = a + b \times t_i, \text{ and}$$

$$t_i = t_0 - \left(\frac{1}{k}\right) \times \log\left(1 - \frac{i}{L_\infty}\right),$$

where N is the number of *M. dalli* in length class i, Δt is the time it takes prawns to grow through length class i, t is the relative age at the mid-length of class i (calculated using the inverse von Bertalanffy growth equation), and the absolute value of b becomes an estimate of Z. The non-seasonal LCC was chosen to estimate Z as a recent study by Hufnagl *et al*. (2013), evaluating eight methods for estimating Z, found that the non-seasonal LCC method was consistently rated among the most accurate of methods for both seasonal and non-seasonal growth scenarios. They also found that when mortality was low (*i.e.* < 5 year^{-1}), the non-seasonal LCC was in general, a suitable choice for estimating Z.

Time and size at maturity

Gravid female prawns, *i.e*. stages 3 and 4 of (Tuma, 1967) and (Crisp *et al*., 2017a), were readily identified macroscopically by the appearance of a distinct green gonad. The maturity schedule assumes that there is a difference between morphologically mature prawns, *i.e.* prawns that have grown to 21 mm CL, the size at which 100% of the population is capable of being mature (see results Fig. 2.1.5), and will cycle between ovigerous and non-ovigerous stages of maturation, and functionally mature prawns, *i.e*. ovigerous individuals, currently appearing gravid.

The relationship between size and the presence of gravid ovaries was examined for female prawns by using data containing all prawns for each sampling month when gravid prawns were present, *i.e.* October to March. A histogram of gravid prawns was constructed using 1 mm CL size classes to identify the smallest and largest length class containing mature prawns. Prawns between these length classes represent an approximate proportion of the female population shifting from an immature to a mature state. This transition was evaluated with logistic regression using a logit transformation in a general linear model (GLM) using *R* and the formula:

$$log\left(\frac{p}{1-p}\right) = a + b_1 X$$

where p is proportion mature and 1 – p is proportion immature; a and b_1 are model parameters and X is the CL. The CL where 50% (CL_{50}) and 90% (CL_{90}) of the female prawn population were gravid was calculated using:

$$X = \frac{log\left(\frac{p}{1-p}\right) - a}{b_1}$$

where p is 0.5 (50% mature) and 0.9 (90% mature), a and b_1 are model parameters. Confidence intervals for CL_{50} and CL_{90} were created by bootstrapping 1,000 samples using the bootCase function from the Car package in R (Ogle, 2014).

Results

Rainfall and environmental data

The total rainfall for the 12 months from October 2013 to September 2014 was 599 mm, with most occurring between May and September and very little to none between December and February (Fig. 2.1.2). The rainfall from October 2014 to September 2015 followed the same pattern as that for 2013/14, but was slightly lower than in the previous 12 months (Fig. 2.1.2). Average maximum air temperatures varied seasonally with the lowest values (18 to 19 °C) recorded in July in 2014 and 2015 and the highest (33 to 34 °C) in January and February. The seasonal patterns of air temperature in 2014/15 followed those of 2013/14 very closely, except that November was 4 °C warmer in 2014/15 (Fig. 2.1.2).

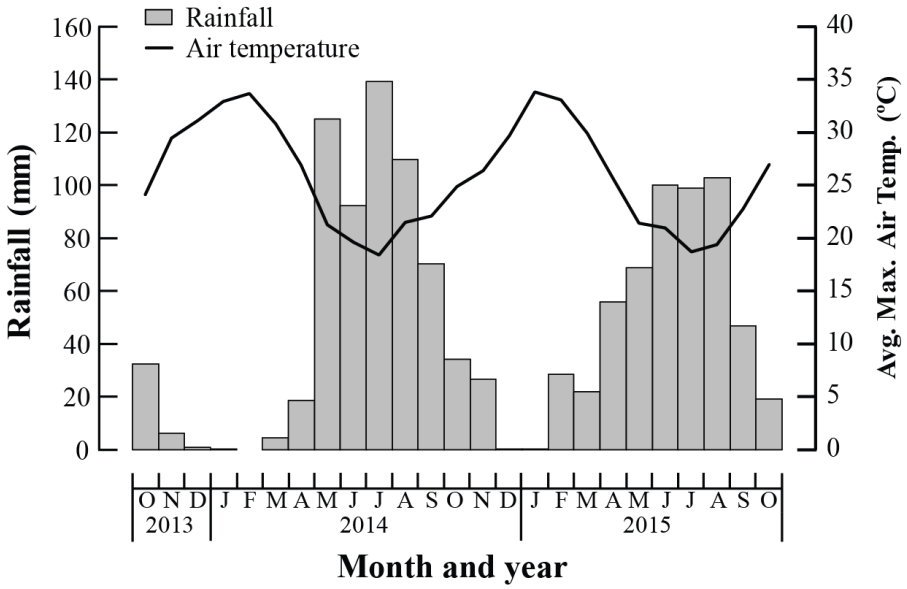


Fig. 2.1.2. Monthly total rainfall (mm, histogram) and average maximum temperature (line) for Perth between October 2013 and October 2015. Data obtained from the Bureau of Meteorology (http://www.bom.gov.au/climate/data/).

The seasonal patterns of change in water temperature were similar in the five regions of the Swan-Canning Estuary and in the two years of the study, with surface temperatures ranging from a minimum of 11.2 °C in the Lower Canning Estuary (LC) during June 2014 to a maximum of 28.5 °C in the Upper Canning Estuary (UC) in January 2014 (Fig. 2.1.3a). The lowest bottom temperatures were 14.4 and 15.1 °C in August 2014 in the UC and Middle Swan Estuary (MS), respectively and highest in the UC in January 2015 (27.8 °C; Fig. 2.1.3b). The lowest range in

surface water temperature (14.0 to 25.3 °C) was recorded in Lower Melville Water (LM), and the highest range in the UC (12.7 to 28.5 °C). Bottom temperatures varied less than those of the surface waters, with the greatest range in the UC (14.4 to 27.8 °C).

Surface salinity ranged from 2.8 in the LC during October 2014 to 37.3 in that same region in March 2014 (Fig. 2.1.3c). With the exception of October 2013, salinities in LM waters were > 22, whereas in all other regions, they declined to ≤ 10. The lowest bottom salinity was 3.7 in the UC in October 2013, while the highest was 37.0 in the LC during April 2014 (Fig. 2.1.3d). The ranges in salinity varied markedly among the regions, from as little as 4.3 in LM (32.8-37.1) to 29.8 (3.7 to 33.5) in the UC.

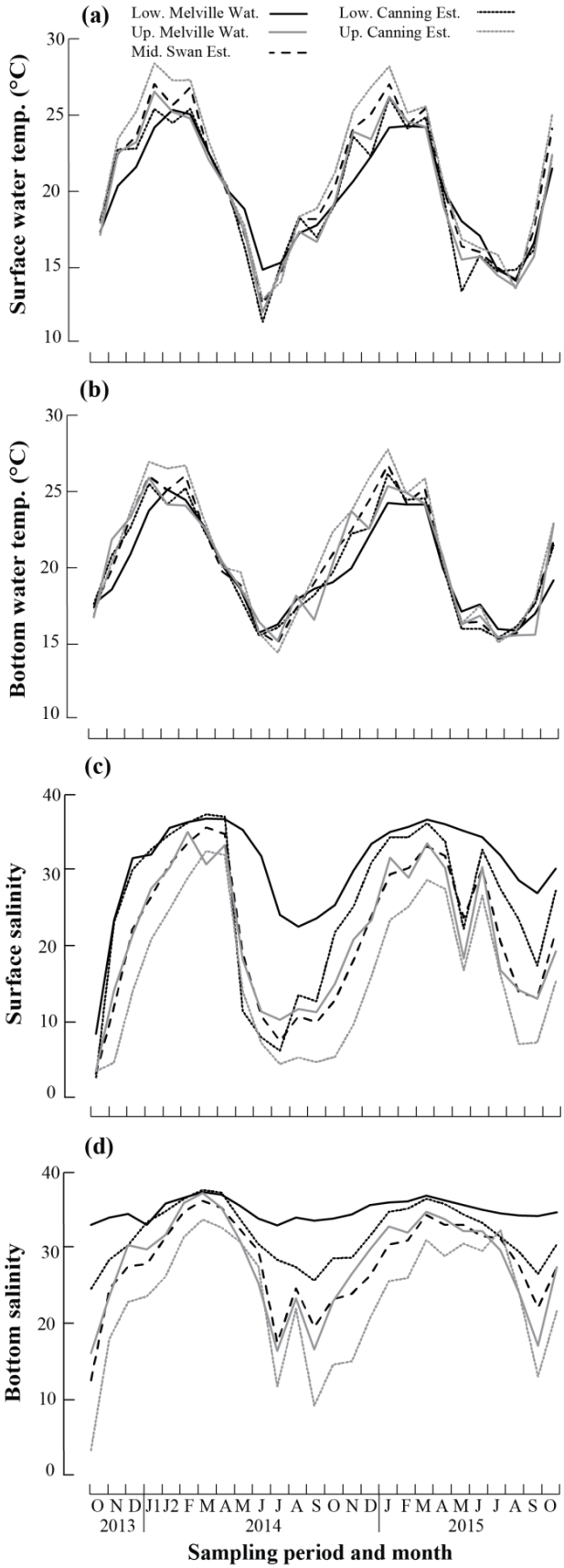


Fig. 2.1.3. Monthly values for (a) surface and (b) bottom water temperature and (c) surface and (d) bottom salinity recorded in each of the five regions of the Swan-Canning Estuary between October 2013 and October 2015. Data sourced from the Department of Water and Environmental Regualtion (http://wir.water.wa.gov.au).

Size structure of the population, time and size at maturity

A total of 10,570 *M. dalli* (5,631 females, 4,939 males) were caught during the 26 consecutive lunar cycles months between October 2013 and October 2015; 1,323 in the hand trawl net and 9,247 in the otter trawl (Fig. 2.1.4). Female prawns ranged from 6.5 to 30.5 mm CL and the males from 6.5 to 24.1 mm CL (Fig. 2.1.4). The smallest prawns, 2.0 mm CL juveniles, were caught in hand trawls in January and February 2015 (Figs 2.1.6, 2.1.7). Two modes were evident in the length frequency distributions for otter trawl nets: one at 11 mm CL and a second, smaller mode at 17-18 mm CL (Fig. 2.1.4). The first mode was also present in the hand trawl nets although greatly reduced in magnitude, while the second mode in the hand trawl nets was slightly smaller (15-16 mm CL) than that in the otter trawls.

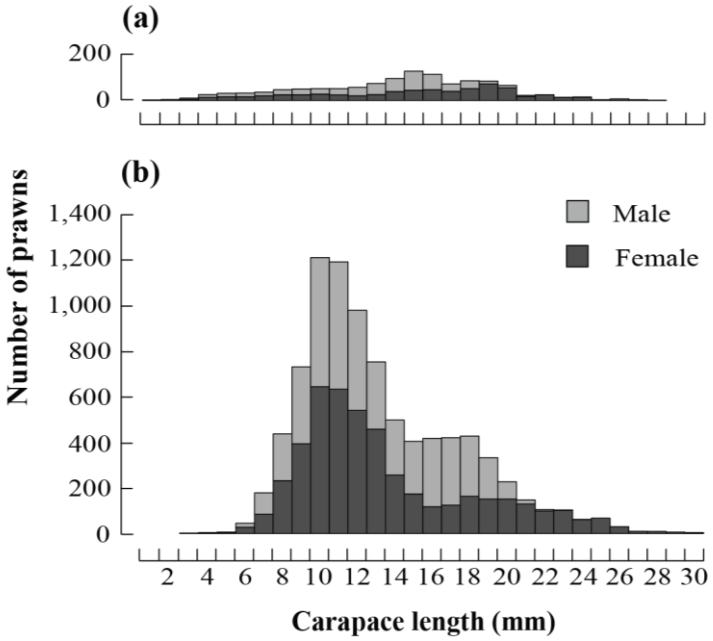


Fig. 2.1.4. The number of female and male *Metapenaeus dalli* caught in each 1 mm carapace length class size from (a) hand and (b) otter trawls samples collected over 26 lunar cycles between October 2013 and October 2015. N = 1,323 for hand trawls, N = 9,247 in otter trawls.

Gravid female *M. dalli* and those carrying a spermatophore were caught in the hand and otter trawls during November 2013 and March 2014 and between October 2014 and March 2015. These females ranged in size from 12.0 to 28.5 mm CL in 2013/14 and 10.0 to 30.0 mm CL in 2014/15. The greatest proportions of gravid females (39 and 54% in 2013/14 and 2014/15, respectively) and those carrying spermatophores (45 and 50% in 2013/14 and 2014/15,

respectively) were recorded in January of both years. The estimated carapace length at 50% maturity (CL_{50}) for females, based on the CL of gravid females, was 16.9 mm CL (95% Confidence Interval [CI] = 16.7 to 17.0 mm CL) (Fig. 2.1.5). The estimated CL_{90} for females was 18.5 mm CL (95% CI = 18.3 to 18.7 mm CL).

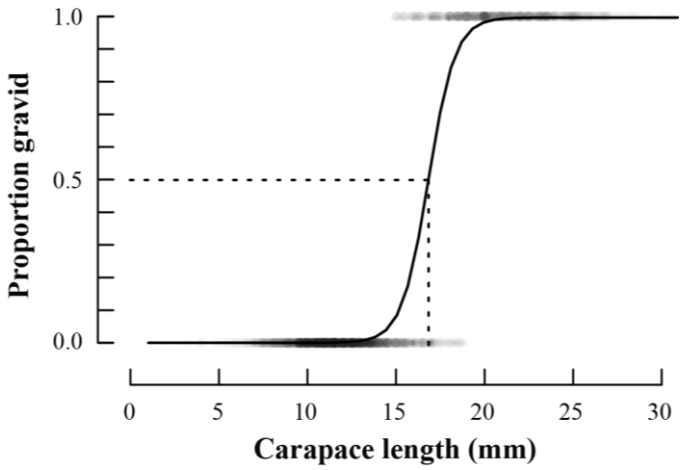


Fig. 2.1.5. The logistic regression fitted to the proportion of gravid females in each 1 mm CL during the breeding season, *i.e.* October to March, (solid line) to estimate the size at maturity for female *Metapenaeus dalli* in the Swan-Canning Estuary. The dashed line represents the carapace length where 50% (CL_{50}) of females are mature.

Growth

The smallest catches of females (n = 43) and males (n = 34) from otter trawls and hand trawl nets were recorded in October 2013 and the largest catch of females in November 2014 (447), while those of males were in December 2013 and April 2015 (326 and 323, respectively; Figs 2.1.6, 2.1.7). Three main cohorts (C, F and J, Fig. 2.1.6) represented about 77% of the total female *M. dalli* catch over the two year sampling period. One cohort (F) could be followed for 17 months from February/March 2014, when the mean size of young female prawns was 10 mm, until May 2015 when it had reached 26 mm CL (Figs 2.1.6, 2.1.8a). Three cohorts of male prawns (A, D and H, Fig. 2.1.7) accounted for nearly 90% of the total male *M. dalli* catch and one cohort (D) was followed for 19 months from February/March 2014 (mean size = 9.5 mm CL) until August 2015 (mean size = 19 mm CL) (Figs 2.1.7, 2.1.8b).

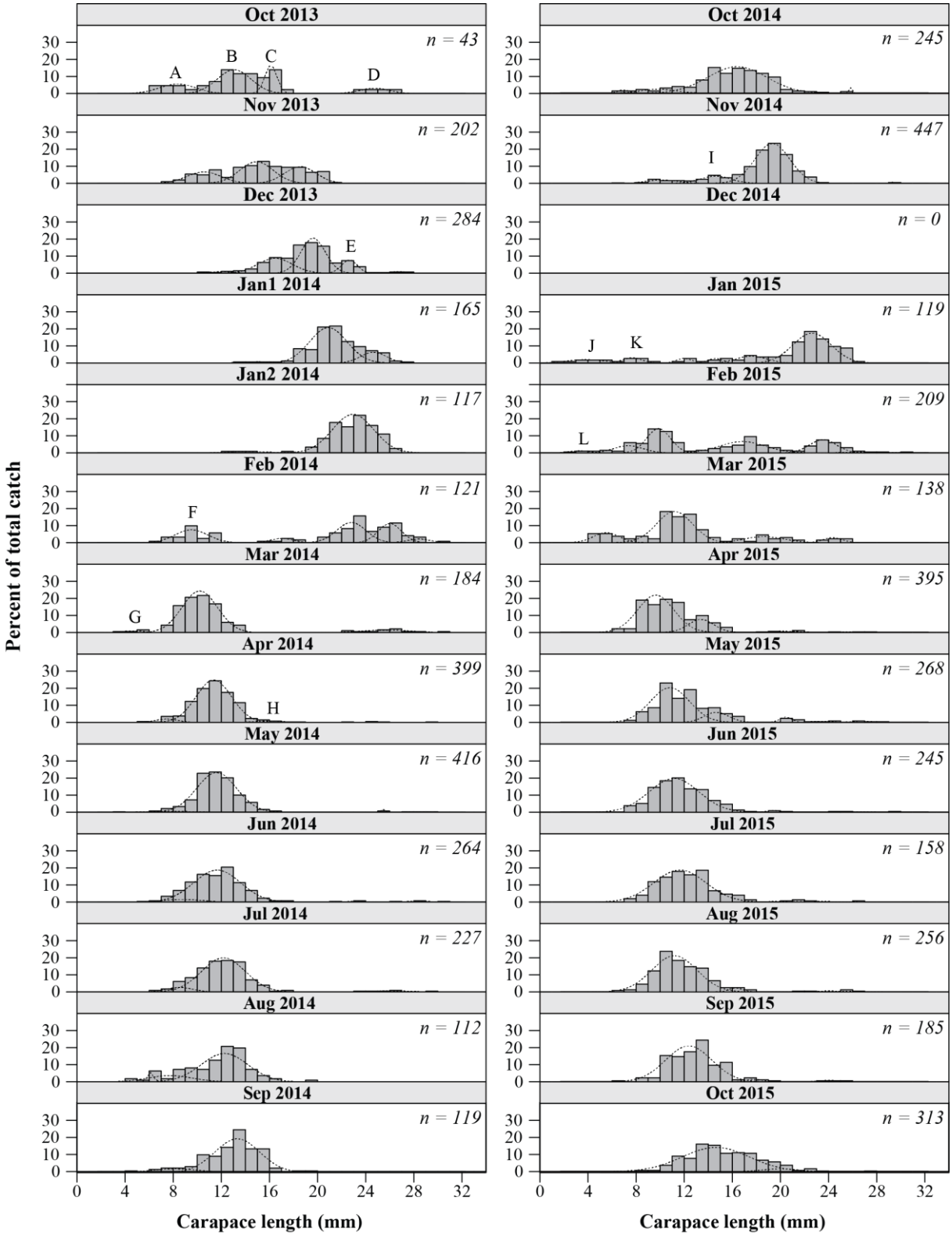


Fig. 2.1.6. Monthly carapace length (mm) frequency histograms for female *Metapenaeus dalli* in 1 mm length classes from hand and otter trawl samples obtained every 28 days (lunar cycle) between October 2013 and September 2015 in the Swan-Canning Estuary. Normal distributions (dashed lines) were fitted to identify the mean, SD, weighting and number of prawns in each cohort (labelled A to F). Note no data are shown for December 2014, when an engine failure prevented sampling with the otter trawl.

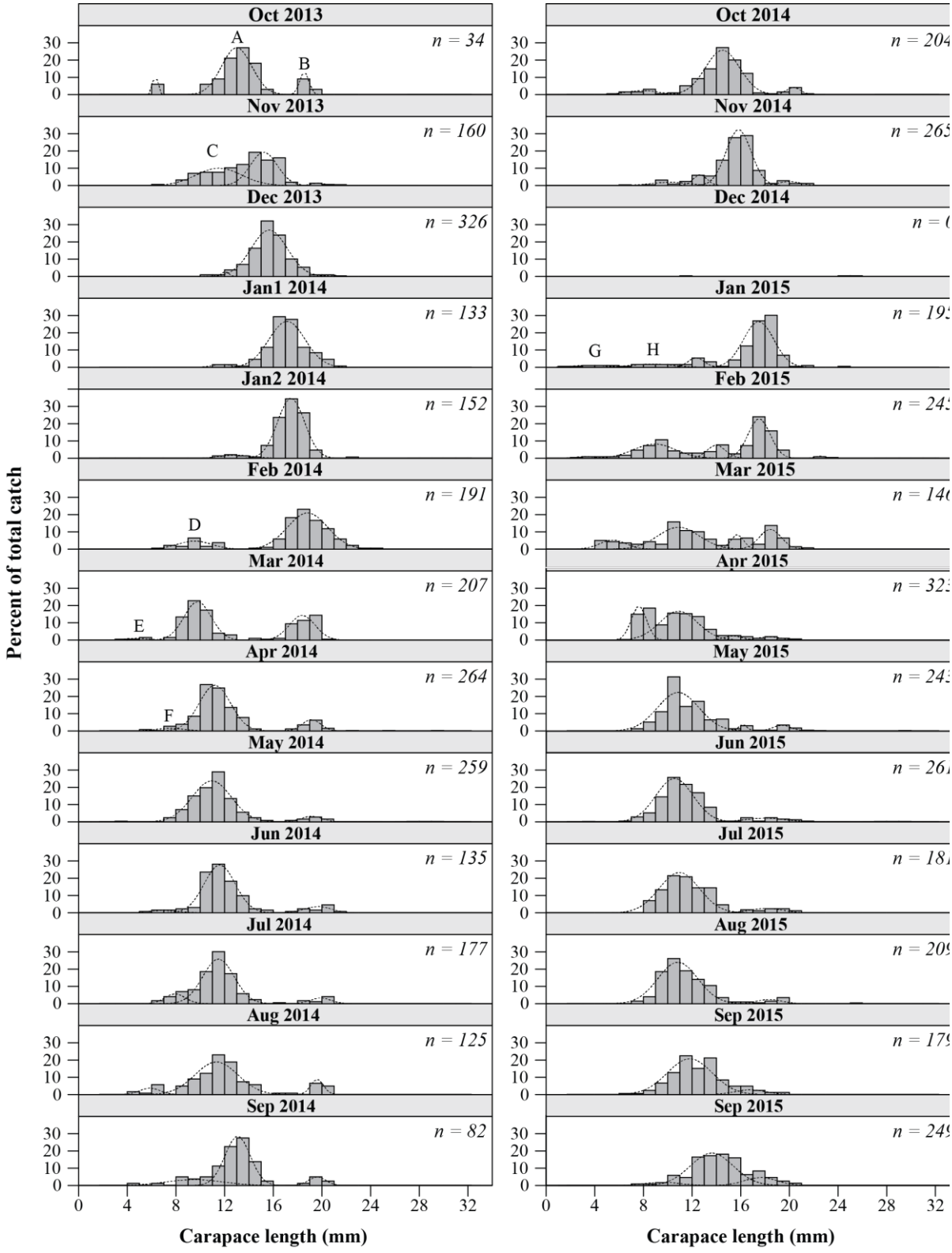


Fig. 2.1.7. Monthly carapace length (mm) frequency histograms for male *Metapenaeus dalli* in 1 mm length classes from hand and otter trawl samples obtained every 28 days (lunar cycle) between October 2013 and September 2015 in the Swan-Canning Estuary. Normal distributions (dashed lines) were fitted to identify the mean, SD, weighting and number of prawns in each cohort (labelled A to F). Note no data are shown for December 2014, when an engine failure prevented sampling with the otter trawl.

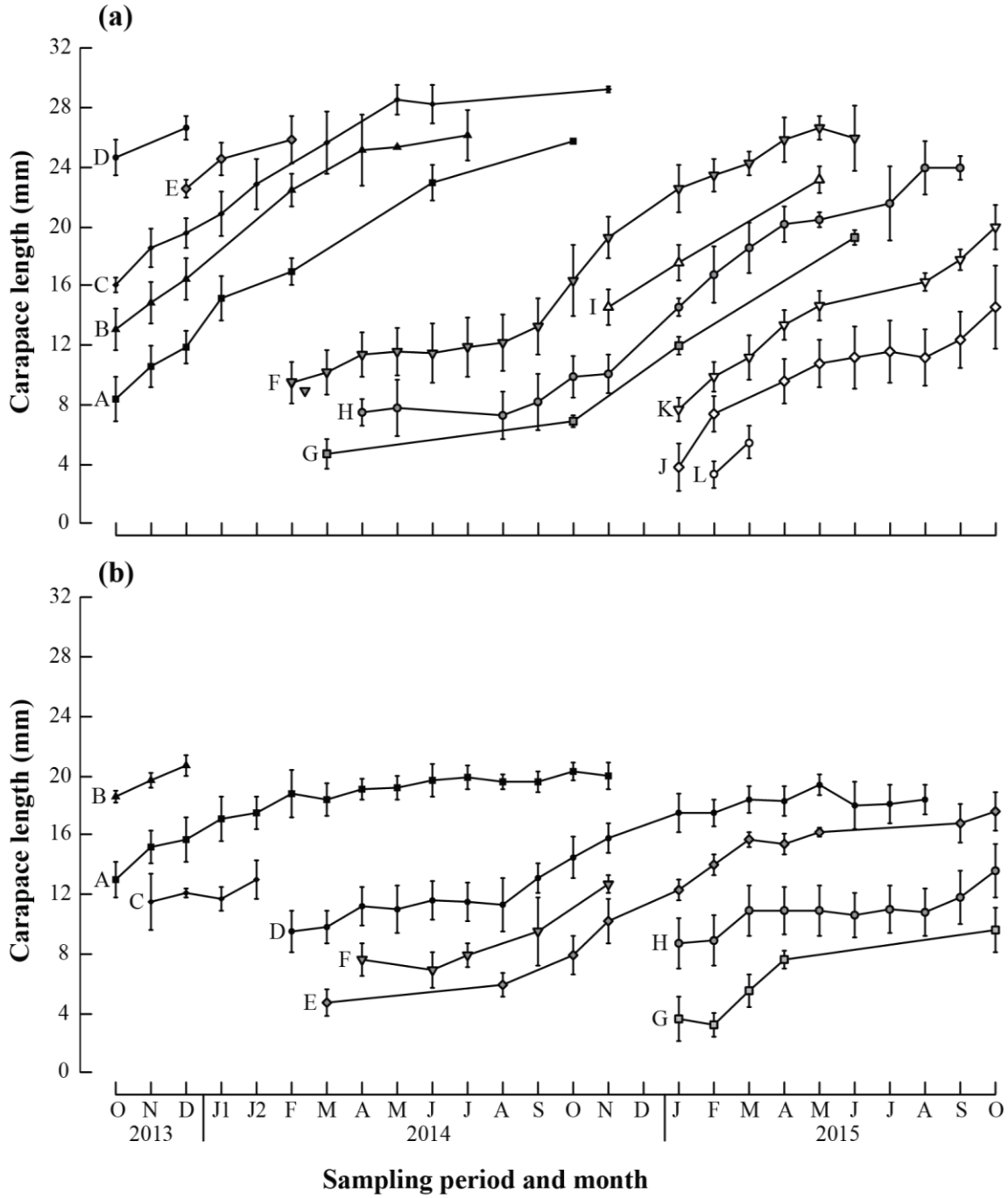


Fig. 2.1.8. The mean carapace length (± 1 SD) for the cohorts identified in the analysis of the carapace length frequency distributions in Fig. 2.1.6 for female and Fig. 2.1.7 for male *Metapenaeus dalli* in each lunar cycle between October 2013 and September 2015 in the Swan-Canning Estuary.

The growth curves derived from the mean carapace length distributions showed a highly seasonal pattern of growth in both years (Fig. 2.1.9a, b). This pattern was also observed when the seasonal growth model was fitted to the historical data collected between 1977 and 1982 (Fig. 2.1.9c, d). Fitting the growth data with non-linear least squares (NLS) growth models gave

similar values of *K* for the current and historical curves for females (0.98 and 1.05, respectively) and males (0.98 and 1.01, respectively; Table 2.1.1). However, the estimated asymptotic mean carapace lengths (L_∞) for females (33.6 mm CL) and males (22.8 mm CL) in the 2013/15 data set were longer than those estimated for the historical values (females = 28.0 mm CL, males = 20.0 mm CL; Table 2.1.1). The value of the C parameter for both females and males was close to 1, indicating that growth almost stops for a period.

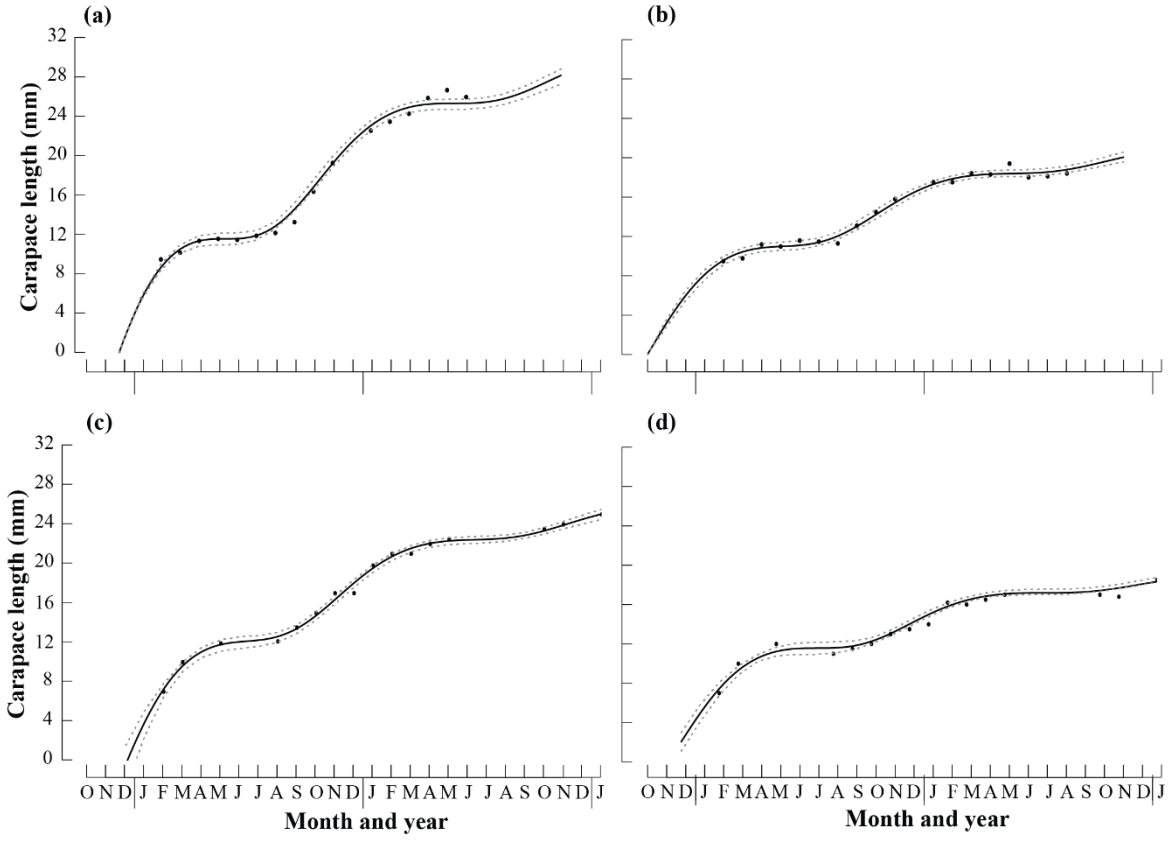


Fig. 2.1.9. Growth models fitted to estimated using Somers' (1988) seasonal adaption of the von Bertalanffy growth model fitted to mean carapace lengths for (a) female and (b) male *Metapenaeus dalli* collected in 2013/15 and (c) female and (d) male *M. dalli* collected in 1977-82. Points for the 2013/15 data show the mean observed values from the length frequency distribution for cohort F for females (Figs 2.1.6, 2.1.8) and D for males (Figs 2.1.7 and 2.1.8). Solid line is the line of best fit, with dashed lines showing 95% confidence intervals.

Table 2.1.1. The von Bertalanffy growth parameters for female and male Metapenaeus dalli estimated using the non-linear least squares (NLS) function from the Minpack package (Gayanilo *et al*., 2005) for the current study and those estimated from the data in Potter *et al*. (1986). CI = 95% confidence Interval. $L(t)$ = average length at time t; $L\infty$ = asymptotic length; K = rate at which length reaches the asymptotic length; t_0 = theoretical time where the average length = 0; t_s = start of the curved portion of the first growth oscillation; C = controls the amplitude of the growth oscillation during the winter period (C = 1 - growth stopped, if C = 0 – continuous growth, *i.e.* no seasonal oscillation).

Years/Sex	L_∞ **(CI)**	K **(CI)**	t_0 **(CI)**	t_s	C
2013-2015					
Female	33.6 (30.9 - 34.4)	0.98 (0.98 - 1.17)	0.15 (-0.01 – 0.21)	0.12	1.00
Male	22.8 (21.6 - 24.4)	0.98 (0.84 - 1.12)	0.00 (-0.06 – 0.06)	0.09	0.90
1977-1982					
Female	28.0 (26.4 - 29.8)	1.05 (0.87 - 1.26)	-0.16 (-0.20 - 0.12)	-0.14	0.89
Male	20.0 (18.8 - 21.7)	1.01 (0.80 - 1.20)	-0.26 (-0.50 - 0.19)	-0.06	1.00

Mortality

The instantaneous total mortality (Z) for female *M. dalli* estimated from the non-seasonal length converted catch curve method (LCC) was 3.57 year^{-1}, about 56% higher than that estimated for males (Z =2.28 year^{-1}, Fig. 2.1.10, Table 2.1.2). These values are equivalent to weekly rates of 0.069 week^{-1} for females and 0.043 week^{-1} for males (Table 2.1.2).

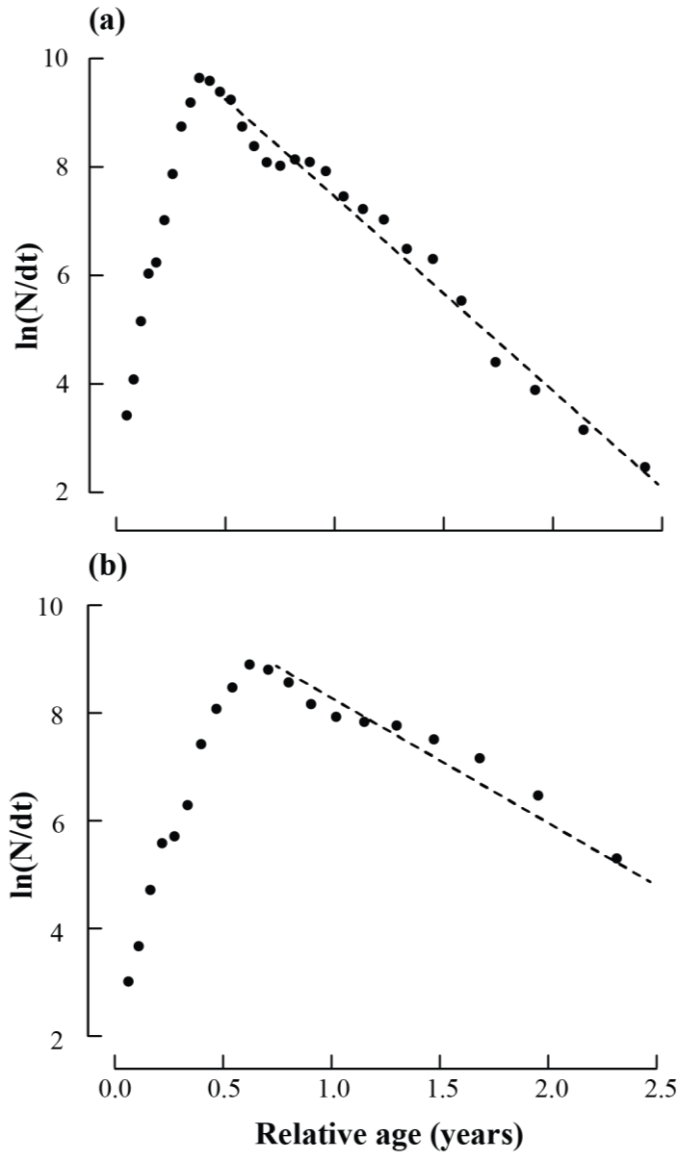


Fig. 2.1.10. Non-seasonal length converted catch curves for (a) female and (b) male prawns showing the relationship between the rate of change in numbers (ln[N/dt]) with the relative age for *Metapenaeus dalli* caught between October 2013 and September 2015 in the Swan-Canning Estuary. Dotted curve shows the catch-curve used to estimate total mortality (see Table 2.1.2).

Table 2.1.2. Annual and weekly instantaneous mortality rates (Z) and 95% confidence intervals (CI) for female and male *Metapenaeus dalli* estimated from data collected in hand trawls and otter trawls from October 2013 to September 2015 using the non-seasonal Length Converted Catch Curve (LCC) method.

Sex	**Instantaneous total mortality *Z* (CI)**	
	***Z* year^{-1} (CI)**	***Z* week^{-1} (CI)**
Female	3.57 (3.28 - 3.86)	0.069 (0.063 - 0.074)
Male	2.28 (1.91 - 2.65)	0.043 (0.037 - 0.051)

Discussion

This study used data collected during a recent comprehensive field study of the Western School Prawn *Metapenaeus dalli* in both nearshore (< 2 m deep) and offshore waters (2 to17 m deep) of the Swan-Canning Estuary to provide the first quantitative estimates of growth and mortality and female size at maturity. Growth and reproduction of *M. dalli* were highly seasonal, with faster growth and mature females recorded in the Austral late spring, summer and early autumn (October to March), when water temperature exceeded 20 °C and virtually no growth in late autumn and winter (May to August). This highly seasonal pattern of growth suggests that releases of aquaculture-raised small prawns in the late autumn and winter months are not likely to grow and as a consequence, may be very vulnerable to predation and much higher mortality than summer releases. Differences were also detected between the growth and mortality of males and females, with females growing to a larger size and experiencing a higher total mortality (*Z*) than males.

Reproduction

Metapenaeus dalli exhibits a strong seasonal cycle of reproduction in the Swan-Canning Estuary with gravid females, and females carrying a spermatophore, first appearing in October or November and last seen in March. During this period the average temperatures in the bottom waters of the five study regions varied from 20 to 28 °C. A strong seasonal cycle of reproduction was also found in a less-intensive, but longer-term, study from 1977 to 1982 by Potter *et al*. (1986b), who recorded gravid *M. dalli* between November and April. This pattern of reproduction defines the period that prawns can be cultured from wild inseminated females, unless the life-cycle is closed or prawns are held in captivity for extended periods of time and spawning is induced in the culture facilities. Thus, under current culture practices for *M. dalli* in Western Australia, where inseminated females are captured in the wild and used to produce eggs and larvae in culture, the time window for releasing cultured prawns is restricted mainly to the period from December to March. The over 30-year gap between sampling in the current

study and Potter *et al*. (1986b) indicates that the seasonal cycle established for *M. dalli* is unlikely related to a short-term events (*e.g.* a single weather event or response to an unusual estuarine condition). Thus, it is more than likely an evolutionary adaption of *M. dalli* to a seasonally-oscillating reproductive cycle in response to longer-term hydrologic and climatic influences, in effect tuning itself with the estuarine environment (Tweedley *et al*., 2016b). A similar conclusion was made by García (1988), while studying environmental effects on the population dynamics of *Penaeus notialis* in coastal waters of the Ivory Coast.

In addition to *M. dalli*, many fish species reproduce during the summer and early autumn in the estuaries of south-western Australian (*i.e.* estuarine species sensu Potter *et al*., 2015a; Tweedley *et al*., 2016b), when freshwater discharge is limited (Hodgkin and Hesp, 1998). It has been suggested that in these microtidal estuaries, water movement via tidal exchange and freshwater discharge is restricted and thus environmental conditions remain fairly stable during summer and autumn and the eggs and larvae are not flushed out of the estuary (Potter *et al*., 2015b; Tweedley *et al*., 2016b).

The initiation of reproductive activity in female *M. dalli* appears to be synchronised with an increase in surface and bottom water temperatures in the Swan-Canning Estuary to temperatures 20 °C. The activity and emergence of benthic invertebrates is strongly influenced by temperature and the emergence time of several species of prawns increase greatly above this temperature (Wassenberg and Hill, 1994). Park and Loneragan (1999) found that this pattern of activity was also demonstrated in two larger species of metapenaeids, *Metapenaeus endeavouri* and *M. ensis*. The months where reproductive activity was greatest (*i.e.* November-February) occur when the surface and bottom temperatures are between 20 and 28 °C (Fig. 2.1.3a,b). This peak reproductive output (*i.e.* November to February) indicates a life history strategy by *M. dalli* to optimise larval survival between 20 and 28 °C. Laboratory studies of the survival and growth of larval *M. dalli* under different temperature and salinity regimes found that survival and growth were greater at approximately 26 °C than either 20 or 32 °C (Crisp *et al*., 2017b). Preston (1985) also found that the survival and development of larval *Metapenaeus*

bennetae, an species breeding in estuaries and marine waters on the east coast of Australia, were greatest in similar environmental conditions to those where the broodstock were collected.

The majority of *M. dalli* reaching reproductive maturity are from the recently maturing 0+ cohort (*i.e.* the prominent cohort in terms of abundance) that are close to 12 months of age, with only small numbers of the 1+ cohort (now nearly 24 months old) found during this time, particularly for females. This indicates that a large percentage of the female population will spawn during one season only, towards the end of their first year of life. However, it is possible that females spawn more than once during a season. The much greater investment in reproduction by female than male *M. dalli* during the time of faster growth is likely to increase their physiological stress and may explain the significant decline in the catch of females after the main period of reproduction (*i.e.* after March, Fig. 2.1.6).

Growth

Like the pattern of reproduction, *M. dalli* also exhibited a strong seasonal growth pattern. Most growth occurred during the warmer months from October to March. In their first five to six months of life, female and male *M. dalli*, on average, grow to approximately 10 to 12 mm carapace length (CL), respectively, and an estimated 0.88 to 1.47 g, respectively, in weight. Growth remained very slow over the colder austral late autumn and winter months (May to August) until the following October when female *M. dalli* grew at a similar rate in length to that during their first six months (≈ 2 mm CL month^{-1}) but much faster in terms of weight (≈ 1 g month^{-1}). Following winter, the growth rate of males also increased but more slowly than that for females, reaching ~19 mm CL by March. Several fish species found in the Swan-Canning Estuary also exhibit the same pattern of highly seasonal growth (Wise *et al*., 1994; Veale *et al*., 2016). Water temperature has been shown to increase moulting frequency and thus increase growth rates in penaeids (Rothlisberg, 1979; Dall *et al*., 1990) and temperature is a key factor effecting their emergence from the sediments (Haywood and Staples, 1993; Wassenberg and Hill, 1994; Park and Loneragan, 1999) and hence vulnerability to fishing.

The von Bertalanffy instantaneous growth parameter, *K*, calculated from the Somers (1988) seasonal model were similar for females and males (both 0.98). However, the asymptotic carapace length (L_∞) was much larger for females (33.6 mm CL, ≈ 23.6 g wet weight) than males (22.8 mm CL, ≈ 9.3 g wet weight). It should be noted, however, that because of the high mortality rates for *M. dalli*, particularly females, few individuals are likely to reach the asymptotic size. The estimated L_∞ may therefore be an artefact of the model estimation process, with little biological meaning and the 95th percentile for length may be a better estimate of the asymptotic size for this species (see Hordyk *et al*., 2015).

The difference in growth patterns between females and males has been recorded in many species of penaeids (*e.g.* Primavera *et al*., 1998; Correa and Thiel, 2003; Callaghan *et al*., 2010; Mehanna *et al*., 2012; Accioly *et al*., 2013). The gender dimorphism recorded in the current study and that by Potter *et al*. (1986b) (see also Fig. 2.1.9), where female *M. dalli* also grew much larger than males, is more than likely a life history strategy to maximise fecundity and optimise reproductive capacity of the population (Ramirez Llodra, 2002). Dall (1958), recorded similar differences in maximum size of female (30.5 mm CL) and male (24.1 mm CL) Greentail Prawns *Metapenaeus mastersii* (now *Metapenaeus ensis*) in the Brisbane river, although the size difference between genders was smaller than that recorded in the current study.

The estimates for the asymptotic length from the current study were 14 to 20% larger than those from over 30 years previously (28.0 mm CL and ≈ 14.44 g for females and 20.0 mm CL and 6.34 g for males) (Fig. 2.1.9; Table 2.1.2 and estimated from figures in Potter *et al*., 1986) when the recreational fishery for school prawns was thriving. These differences in growth could be related to a range of biotic and abiotic factors, including increases in temperature and reductions in rainfall and consequently more persistent high salinities, as well as differences in fishing pressure and the sampling regimes in the thirty years between the studies. Prawns were sampled with greater frequency and at more sites in the current study than 30 years previously, particularly in the offshore waters with otter trawls. Although the mesh size of the otter trawls was similar during both studies, the hand trawl in the current study used smaller mesh (12 mm) than the historical study (19 mm). The difference in mesh size between the hand trawl nets

should not adversely affect the catch of larger prawns (*i.e.* > 10 mm CL) and may not have affected that of small prawns due to the presence of material, such as macroalgae and jellyfish, which clog the mesh and gives the larger mesh net of Potter *et al*. (1986b) a smaller effective net mesh size. An alternative explanation for the difference in L_{∞}, alluded to by Potter *et al*. (1986b), is an increase in recreational fishing during the 1970s and 1980s, leading to increased fishing pressure and potential selection of larger prawns by fishers. Thus, over ≈ 78 nights of sampling in the shallows in the current study, only five groups of recreational prawn fishers were seen, whereas this was a common place activity thirty years ago involving up to 50,000 people each summer (Potter *et al*., 1986b).

Mortality

The total instantaneous mortality rate (Z, assuming fishing mortality $F \sim 0$) estimated using the non-seasonal length-converted catch curve (LCC) method was nearly 60% higher for females (3.57 year^{-1}) than males (2.28 year^{-1}). This higher estimated mortality for females is consistent with the virtual disappearance of larger female *M. dalli* in the 1+ cohort after April, when the spawning season has completed (Fig. 2.1.6). Combined with a greater abundance of female than male prawns in the 0+ cohort, this provides strong evidence to suggest a higher rate of mortality in the female than male *M. dalli*. Because of very low current level of fishing for *M. dalli* (*i.e.* $F \rightarrow 0$), these estimates of total mortality provide an estimate close to those for natural mortality (*M*). The weekly instantaneous rates of mortality (females = 0.069; males = 0.043) are similar to the estimated *M* values for the juvenile stages of other species of penaeids, such as *Penaeus esculentus, P. semisulcatus* (Loneragan *et al*., 1994; Ye *et al*., 2005a; Loneragan *et al*., 2006) and *P. merguiensis* (Haywood and Staples, 1993). The estimates of mortality for *M. dalli* have not taken into account changes in mortality with size, which Lorenzen (2000) has demonstrated can be very significant.

Implications for restocking

The highly seasonal pattern of growth implies that releases during the austral late autumn/winter should be avoided as growth during this time is very slow. The densities of *M. dalli* recorded in the current study, estimates of area covered by each net and assumed *M. dalli* catchability of 0.4 for both hand trawl and otter trawl nets (Joll and Penn, 1990; Loneragan *et al*., 1995), gives an estimated biomass in the Swan-Canning Estuary of about 2.4 tonnes (Broadley, 2014). This estimated biomass for *M. dalli* is only 16% of the maximum commercial catch for prawns (both *M. dalli* and the Western King Prawn *Penaeus* [= *Melicertus*] *latisculatus*) recorded from the system during the peak of the commercial fishery in the 1950s (Smith, 2006). The current biomass of *M. dalli* thus appears to be very low and has not recovered since recreational fishing decreased greatly in the late 1990s, over 15 years ago. Possible explanations of the suppressed *M. dalli* are an allee effect at low population densities (*i.e.* the rate of reproduction decreases at low population densities) and/or a change in environmental conditions in the Swan-Canning Estuary. Since *M. dalli* are not found in the local coastal waters, the population has little chance of recruitment from sources outside the Swan-Canning Estuary (Potter *et al*., 1986b).

Conclusions

This study has established that the *M. dalli* population in the Swan-Canning Estuary exhibits strong seasonal growth and reproductive cycles, similar to those described by Potter *et al*. (1986b) over 30 years ago, when this species was more abundant and supported an iconic recreational fishery. Most of the growth occurs during the warmer months between October and March, with little to no growth in the colder months from May to August. Thus, cultured individuals should be released during the warmer months when prawns grow most rapidly. Reproductively active females were only found between October and March, with most reproduction concentrated in the months from November to February when surface and bottom water temperatures were between 20 and 28 °C. The high mortality of the population and absence of larger, older *M. dalli*, particularly females, is a concern, as it appears that the

majority of females are only spawning for one season, although possibly releasing eggs more than once during this season. Given the low estimated biomass of *M. dalli* in the Swan-Canning Estuary and lack of connectivity with populations in other estuaries to the north and south of this system, restocking has potential to increase the spawning population, provided environmental conditions are suitable.

2.2. Bio-economic evaluation of restocking the Western School Prawn in the Swan-Canning Estuary

This study has been published in an Honours thesis by Andrew Broadley.

Broadley A.D. (2014). Assessing the potential for restocking the Western School Prawn *Metapenaeus dalli* in a temperate Australian estuary. Honours Thesis, Murdoch University.

Summary

The evaluation of the potential benefits of releasing cultured individuals has been identified as an important component of restocking and stock enhancement programs. Bio-economic models provide a mechanism for integrating biological information with fisheries data and economic information to better assess the costs and benefits of release programs. The biological parameters recently estimated for the Western School Prawn *Metapenaeus dalli* (see Section 2.1) were used as inputs in a bio-economic model developed using the EnhanceFish software to evaluate the effectiveness of different stocking levels and sizes of *M. dalli* on the estimated population biomass in the Swan-Canning Estuary. The release sizes were 650,000, 1 million, 2 million and 5 million prawns, and the size-at-release varied from 1 mm carapace length (CL) to 10 mm CL. The results from these simulations were compared with those from a non-restocking scenario. The greatest potential returns were obtained when the 5 million prawns were released at a size of 10 mm CL, however, such an aquaculture effort would require substantial capital expenditure to produce the required number of juveniles. The model results also highlighted that density-dependent processes are likely to be important and decrease the relative effectiveness of releases. At the current low population level, without any restocking, the population biomass was projected to remain virtually unchanged over a five-year period. The model results also highlight the need for empirical information on natural morality, the influence of density on growth and survival and how time-at-release might influence survival.

Rationale and aims

There is little doubt that understanding the population dynamics of a fishery is a critical component of successful restocking programs (*e.g.* Caddy and Defeo, 2003; Lorenzen, 2005; Ye *et al*., 2005a; Leber, 2013). Fishery managers need to make decisions under challenging circumstances, such as the increasing uncertainty around policy, economics and environmental degradation brought about by climate change or other environmental factors (FAO, 2012). It is under these conditions that managers require tools to evaluate the trade-offs in the cost and benefits of different strategies, while also assessing the risks to existing fish stocks and associated ecosystem services.

Although not a panacea, bio-economic theory provides a quantitative framework that allows fishery managers to evaluate the trade-offs of various fishery system dynamics (*i.e.* effort, harvest and stock size) against equilibrium reference points (Anderson and Seijo, 2010; Larkin *et al*., 2011). Bio-economic tools such as *EnhanceFish* or the Excel model produced by Ye *et al*. (2005), provide a cost effective and powerful means to evaluate the viability of a portfolio of fishery management scenarios, such as changes to vessel and gear restrictions, spatial and temporal closures, setting harvest limits and implementing release programs for stock enhancement or restocking (Caddy and Defeo, 2003; Grafton, 2006; Cochrane and Garcia, 2009). For example, a bio-economic assessment undertaken by Hart *et al*. (2013c) using the Greenlip Abalone fishery in south-western Australia to develop a base case from which various enhancement scenarios were applied. This included assessing fishing mortality (at various length classes), density and size of release on their effect on spawning biomass, profit, gross value product and net present value. The model parameters were validated by previous experiments and surveys conducted in south-western Australia (*e.g.* Hart *et al*., 2013b, 2013c). Enhancement scenarios were then extended to the entire Australian Greenlip Abalone fishery, which demonstrated a significant increase in profitability (from $12 to $26 million) could be achieved from annual releases of 6.1 million juveniles at 4 cm in length.

The biological effectiveness of a restocking program is based on the assumption that additional recruits will increase stock production by bypassing the recruitment bottleneck that occurs

during the high mortality stage of larvae through to juveniles (Caddy and Defeo, 2003; Lorenzen, 2005; Bell *et al*., 2008). However, this assumption is constrained by the biological and socio-economic realities surrounding the target species (Johnston *et al*., 2010). Therefore, significant trade-offs exist between the cost and benefits of the various biological and socio-economic restocking scenarios (*e.g.* Caddy and Defeo, 2003; Lorenzen, 2005; Johnston *et al*., 2010; Larkin *et al*., 2011; Leber, 2013).

The aim of this study was to use the biological parameters estimated from the comprehensive sampling program (see above) to evaluate the potential success of restocking *M. dalli* in the Swan-Canning Estuary through the development of a bio-economic model. The biomass of the current population was estimated and the *EnhanceFish* software was used to evaluate the potential increase in population biomass from restocking with different numbers of released prawns and different sizes-at-release.

Methods

In order to develop an understanding of the potential for restocking to increase the biomass of the *M. dalli* population and start rebuilding its stocks, the data collected from the intensive sampling of this species (see Section 1.1) need to be scaled up to the total Swan-Canning Estuary to estimate the biomass of the population. This involved estimating the extent of habitat that the population is likely to occupy and the densities of prawns within this distribution. The population parameters estimated above were then used to examine the population dynamics of the wild population and how restocking of different magnitudes and size classes of prawns might increase the population in the estuary.

Estimation of the population biomass

The area that the population of *M. dalli* is likely to inhabit spans the sampling sites for this species selected as part of a major program on restocking this species in the Swan-Canning

Estuary. These sites cover an area of approximately 35.1 km^2 in the Swan-Canning Estuary. The area has been divided into two depth strata based on the bathymetry of the estuary: shallow waters < 2 m, covering an area of 15.5 km^2 and were sampled using a hand trawl net; deeper waters, *i.e.* 2 to 17 m, cover an area of 19.6 km^2 and were fished using a small otter trawl net.

The biomass of the *M. dalli* population in 2013/14 was calculated in *R* (R Core Team, 2014) using the swept area method. The total weight of the catch was calculated in each month for the otter and hand trawls separately using:

$$C_m = \sum_{i=1}^{n} (w_i \times f_i) ,$$

where C_m is the total weight in kg for each monthly sample, *n* is the number of 1 mm length classes, w_i is the weight in kg of length class *i* (*i.e.* calculated using the length-weight relationship for females and males separately), and f_i is the number of prawns in length class *i*.

The mean catch C_m for the sampling period was used to estimate the biomass for both the otter and hand trawls using:

$$B = \frac{\bar{C}_m}{v} (A/a) ,$$

where $\bar{C}_m$ is the mean monthly sample weight in kg, v is the proportion of prawns caught in the net's area of influence (*i.e.* catchability or efficiency of the net), A is the area occupied by the stock, and a is the monthly trawl area. The biomass, B, for the hand and otter trawls was combined and converted to tonnes in B_0, the estimated total biomass for the *M. dalli* population in the Swan-Canning Estuary.

The vulnerability of prawns (v) to the hand and otter trawl nets has not been determined. However, Joll and Penn (1990) estimated the efficiency of a larger otter trawl net as between 0.30 to 0.51 for the Western King Prawn *Penaeus* (= *Melicertus*) *latisulcatus*. The efficiency of a small beam trawl (Loneragan *et al.*, 1995) was estimated at 0.47 for juvenile *P. semisulcatus*. These values were used to provide a guide to set the vulnerability (v) of *M. dalli* to hand and

otter trawls; a value of 0.4 was chosen for both methods. Empirical estimates of vulnerability would be valuable for refining the estimates of biomass.

Bio-economic modelling

The restocking scenarios were evaluated using the *EnhanceFish* software developed by Lorenzen and Medley (2006). *EnhanceFish* is primarily designed for teleost fish populations that typically live longer than 1 to 2 years, *e.g.* the North Sea Sole (*Solea solea*). However, Lorenzen and Medley (2006) suggest the underlying population dynamics functionality implemented in *EnhanceFish* can be applied to crustacean and mollusc populations. It should be noted that the *EnhanceFish* model does not use a seasonal growth model or have functionality to model the intra-annual timing of release for stocked prawns. The timing of release in *EnhanceFish* is based on yearly time steps, where the numbers released and the size-at-release are the main determinants.

EnhanceFish uses an extended dynamic pool model that has been designed specifically for evaluating stock enhancement and restocking programs. It makes the following four key extensions to conventional dynamic pool models: i) includes parameters for size-dependent mortality, ii) includes density-dependent processes of the wild and hatchery fish, *i.e.* growth, mortality and reproduction, iii) defines the Stock-Recruitment Relationship (SRR) so survival in the pre-recruit phase is known, *i.e.* prawns < 12 mm CL, and iv) splits the population structure into three components, see Fig. 2.2.1, *i.e.* wild phenotype, hatchery phenotype - naturally recruited, hatchery phenotype - stocked (Lorenzen and Medley, 2006).

Recruitment

Currently, the SRR for the *M. dalli* population is unknown and the data collected in Section 2.1 are not extensive enough through time to estimate the SRR using either the Beverton and Holt

(1957) or Ricker (1958) models. *EnhanceFish* offers an alternative method to estimate the SRR using Myers *et al*. (1999) steepness parameter. Myers *et al*. (1999) developed a

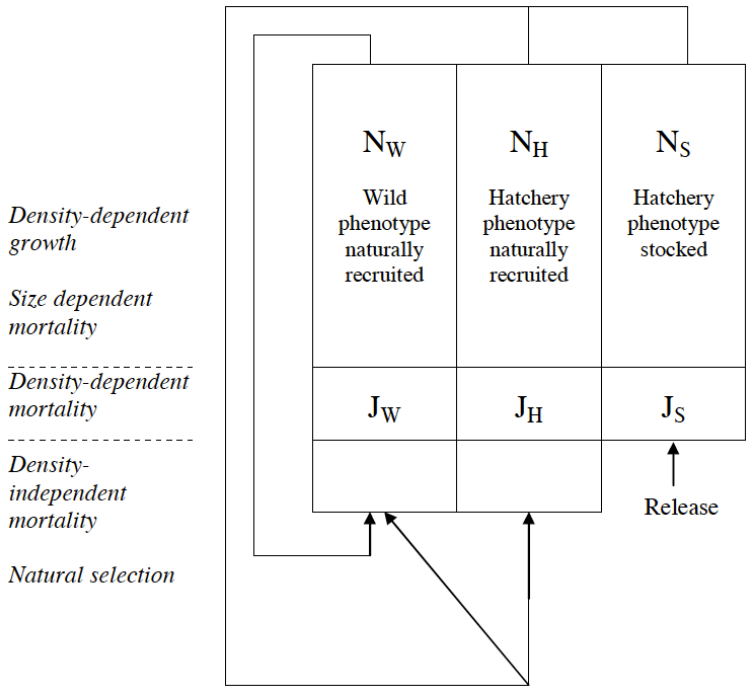


Fig. 2.2.1. Schematic of the density-dependent processes and population structure used in EnhanceFish. The population is divided into fish that have been recruited (N) and those in the pre-recruit phase (J), N_W and J_W – wild phenotype naturally recruited, N_H and J_H – hatchery phenotype naturally recruited and N_S and J_S – hatchery phenotype stocked (source: Lorenzen and Medley, 2006).

standardised annual maximum reproductive rate (*i.e.* between 1 and 7) from a study of 700 spawner-recruit data sets. In data poor situations, *EnhanceFish* employs Myers *et al*. (1999) standardised slope along with an estimate of the current catch, relative effort and length at recruitment to calculate the approximate values for the maximum recruits per unit of spawning stock biomass (SSB) and maximum average recruitment (*i.e.* the parameters a* and b* in the SRR).

The term ‘recruitment’ used throughout this study refers to when prawns become vulnerable to recreational fishing. This occurs from the 1st November each year when the recreational prawn fishery is open. At this time the main cohort of new recruits (*i.e.* the 0+ cohort) reaches a size of between 12 and 18 mm CL after approximately 10 to 12 months of growth (see above).

Natural mortality

A key extension to the dynamic pool model used in *EnhanceFish* is size dependent mortality by defining a function where natural mortality is inversely proportional to length for wild and hatchery prawns (Lorenzen and Medley, 2006). This relationship provides the basis to estimate survival at a given release size. The model outputs in this evaluation are conditional on the following assumptions of natural and fishing mortality: a) the total instantaneous mortality (Z) is estimated at 3.49 year^{-1} for female and 2.69 year^{-1} for male prawns, using the estimates made by the length converted catch curves above (see Section 2.1), b) an estimated catch of 100 kg for 100 recreational fishers, based on anecdotal evidence of historically low levels of fishing for the last 15 years, c) fishing mortality (F) was estimated at 0.042 year^{-1} by configuring *EnhanceFish* with a natural mortality of 3.0 year^{-1}, a catch of 100 kg and adjusting F until the population biomass was equal to 2.37 tonnes, the estimated population biomass.

Given fishing mortality is very low ($\approx 0.014\ M$), natural mortality (M) approximates Z. In this model M has been set to a value of 3.0 year^{-1} with a range of 2.69 to 3.49 year^{-1} for wild and hatchery prawns. The size dependent mortality model in *EnhanceFish* has two parameters (M_{1w} and M_{1s}) representing natural mortality in the wild and stocked prawns. Size dependent mortality was set the same value for both wild and stocked prawns.

Economics of restocking and prawn fishing

This study did not conduct a full assessment of the current socio-economics of the recreational prawn fishery. Furthermore, a literature search into similar studies of small recreational prawn fisheries did not find any information on the economic returns of recreational prawn fishing. Thus, the valuation of recreational utility, including fisher effort dynamics, was not included in the economic evaluation.

The operational costs associated with using the existing aquaculture facilities at the Australian Centre for Applied Aquaculture Research in Fremantle, Western Australia, have been

incorporated into the model (Table 2.2.1). It is assumed these facilities can produce up to 2 million 15-day post larvae (PL15) prawns. However, stocking above 2 million PL15 will require additional infrastructure (*i.e.* tanks, pipes and heating) and therefore require extra capital investment. Any capital costs associated with the project have not been included in the economic assessment.

A base case scenario of stocking 650,000 PL15 (Table 2.2.1) was used to calculate the operational cost of 12 cents per PL15, approximately 1 mm CL. These costs were derived from a trial release of 650,000 PL15 conducted in January 2014. Improvements in techniques to increase larval survival from 11% to 30% will enable the production of 2 million PL15 using a similar sized seed stock of 600 adults that was used to produce 650,000 PL15. It has also been assumed that to culture 5 million PL15, the operational costs will be 250% greater than the cost of producing 2 million PL15, *i.e.* a direct linear increase in the operational costs. This assumption is based on additional labour, prawn feed, power, heating and broodstock collection.

The cost of prawns grown in culture to sizes greater than 1 mm CL (*i.e.* > PL15) was calculated using the growth function created (Broadley *et al*., 2017). A growth rate of 1 mm every 12 days up to a size 10 mm CL was estimated. This rate was reduced by 25%, to 9 days, to allow for optimised growth conditions in culture, *e.g.* temperature, salinity and oxygen (*e.g.* Staples and Heales, 1991). The operational costs for the additional 9 days growth per 1 mm CL were calculated using the aquaculture, consumables, heat and power items only (Table 2.2.1), the broodstock collection was excluded. An operational cost of around 3 cents per prawn for each 1 mm CL in growth post PL15 was calculated, *e.g.* a prawn grown to 2 mm CL would cost 15 cents, this includes the 12 cents to PL15 plus 3 cents to 2 mm CL.

Table 2.2.1. An aggregated summary of the main operational costs of culturing 650,000 prawns to a length of 1 mm CL (*i.e.* PL15). Estimated costs provided by Mr Greg Jenkins, Director of the Centre for Australian Centre for Applied Aquaculture Research in Fremantle, Western Australia.

Item Description	Cost (AUD$)
Aquaculture	64,060
Consumables (*e.g.* vehicles, prawn feed)	2,000
Heat and power	1,500
Broodstock collection	12,000
Total	**79,560**

Model parameters

The model parameters defined in Table 2.2.2 describe the initial configuration of the model used to evaluate various enhancement scenarios (see below). Parameter values for growth, length-weight relationship and maturity were taken from the estimates calculated earlier.

EnhanceFish does not model the population growth and mortality of females and males separately. The estimates of growth and mortality for the separate sexes in calculated earlier were averaged to provide the input to *EnhanceFish* and the range of values from this study used to provide the range (Table 2.2.2).

Model scenarios

Generally, commercial fishery bio-economic assessments are used to estimate the numbers required and economic net present value (NPV) to achieve a target biomass with or without a release program. They do this by looking at various options to reduce or maintain current levels of exploitation (*i.e. F*) or consider closing fisheries until the target biomass is reached with or without stocking.

Model scenarios were developed to evaluate the change in overall biomass of the *M. dalli* population, knowing that there is currently very little fishing pressure and a relatively high natural mortality. The trial release of 650,000 PL15 in January 2014 was used as the lowest level of restocking. Three main options are considered, i) the stocking density (*i.e.* the number

of prawns released), ii) the size-at-release of prawns and iii) a no restocking scenario. This last scenario was evaluated to determine how the wild population's biomass is likely to respond without restocking.

Table 2.2.2. Model parameters used in the *EnhanceFish* bio-economic model to evaluate different restocking scenarios of *Metapenaeus dalli* in the Swan-Canning Estuary.

Parameter	Value	Range	Description
Life history			
L_0	0.1		Length at larval/juvenile transition (mm)
A_0	0.04		Age at larval/juvenile transition (years)
L_r	13	12–14	Length at recruitment (mm)
A_r	1	0.9–1.1	Age at recruitment (years)
h^2	0.2		Heritability of life-history traits
Growth			
L_∞	26	24–33	Asymptotic length at biomass → 0
K	1	0.97–1.06	von Bertalanffy growth rate
g	0		Competition coefficient
α	$1.5x10^{-9}$	$1.1x10^{-9}$–$1.9x10^{-9}$	Coefficient of the length-weight relationship (converted from grams to tonnes)
β	2.79	2.68–2.89	Exponent of the length-weight relationship
Natural Mortality			
M_{1W}	3	2.69–3.46	Mortality of wild phenotype
M_{1S}	3	2.69–3.46	Mortality of hatchery phenotype
Reproduction			
L_m	21		Length at maturity (mm)
p	-1.4		Steepness of maturity curve
r	1		Relative reproductive performance of stocked prawns
Recruitment			
a*	330159		SRR estimates based on growth, mortality and maturity parameters, a catch of 0.01 tonnes and Myers *et al*. (1999) steepness parameter
b*	35437		
r_m	5		Myers *et al*. (1999) steepness parameter
Fishing			
F	0.042		Fishing mortality
L_c	16		Gear selection length (mm)
q	-1.5		Steepness of gear selectivity curve
Economics			
$\gamma 1$	0.12		Cost of hatchery fish at PL15 (AUD$)

Various combinations of numbers released (*i.e.* 650,000, 2 million and 5 million) and size-at-release (*i.e.* 1, 3, 5 and 10 mm CL) were evaluated. This gave 12 scenarios based on different combinations of the number released and size-at-release. All scenarios are run over a 5-year period with a constant starting biomass (B_0).

Sensitivity analyses

Sensitivity analyses were conducted to explore the effects of variation in mortality and SRR on the model results. Probability density functions (PDF) were configured for each of these parameter sets. Input PDFs were defined as normal distributions with the mean set to the values shown in Table 2.2.2. The variances were manually adjusted to extend the range of values by ± 10% of the mean. Monte Carlo simulations were used to sample the input PDFs and generate output PDFs that were displayed graphically and checked visually in *EnhanceFish*.

Results

Population biomass estimate

A total of 4,110 *M. dalli* were caught in the otter trawl and 588 prawns in the hand trawl during the sampling period. The 15 mm CL size class contained the largest number of prawns (69) caught in the hand trawl (Fig. 2.2.2b), while it was the 11 mm CL size class (607) for the otter trawl (Fig. 2.2.2a). The length distribution of prawns in the hand trawl was relatively evenly distributed with a small peak at 11 to 17 mm CL. In contrast, the length distribution of prawns in the otter trawls was bimodal around the 15 and 16 mm CL size classes.

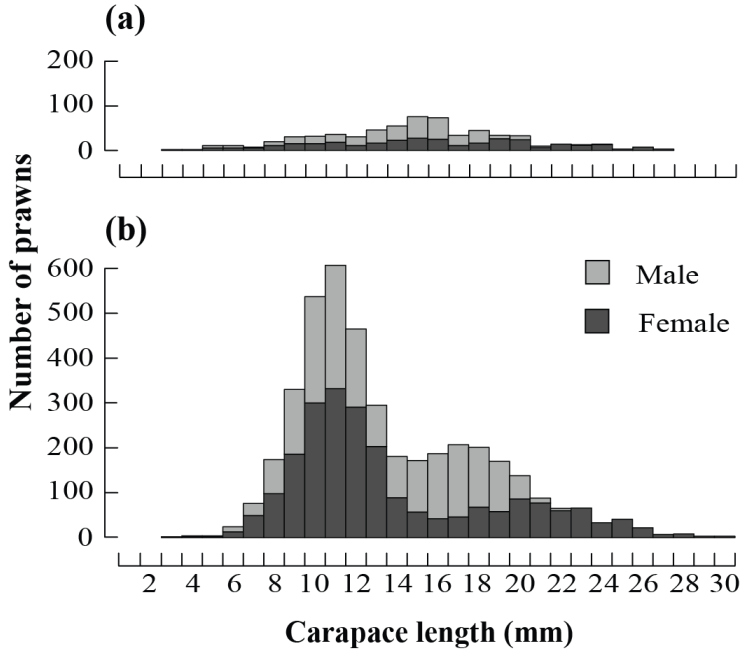


Fig. 2.2.2. The number of female and male *Metapenaeus dalli* caught in each 1 mm carapace length class size from (a) hand and (b) otter trawl samples collected every 28 days between October 2013 and September 2014.

In terms of the total weight of prawns caught over the 12 months, the total hand trawl catch weighed 2 kg and the total otter trawl catch weighed 11.6 kg. The 16 mm CL size class was the heaviest in the hand trawls, with a total weight of 0.24 kg and the 18 mm CL class was the heaviest in the otter trawls (0.97 kg; Fig. 2.2.3a,b). The bimodal distribution in weights of the otter trawl shows an increase in weight of the female population (Fig. 2.2.3b) in size classes 17 to 30, although the numbers in the larger CL classes had decreased greatly (Fig. 2.2.2b).

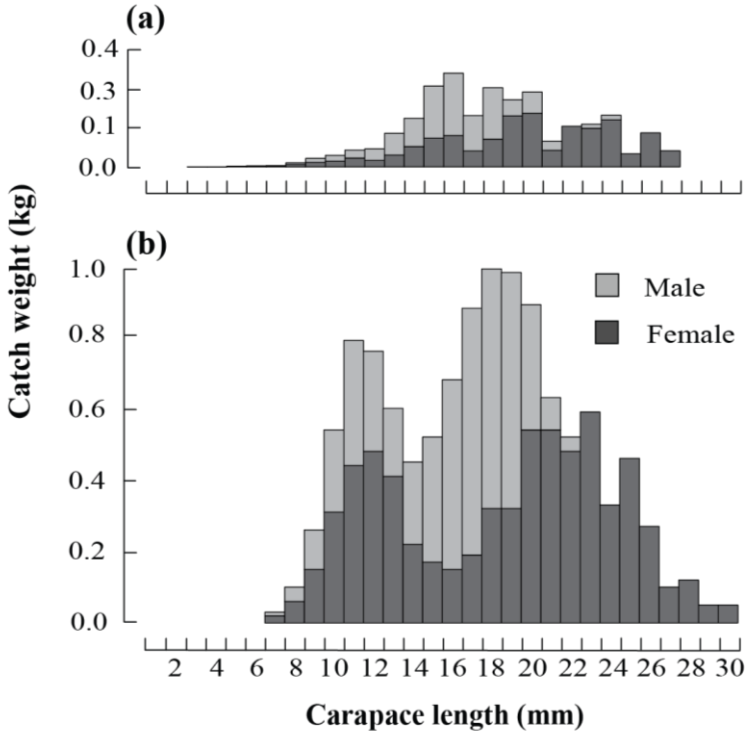


Fig. 2.2.3. The weight (kg) of female and male *Metapenaeus dalli* caught in each 1 mm carapace length class size from (a) hand and (b) otter trawl samples collected every 28 days between October 2013 and September 2014.

The mean weight of prawns caught in hand trawls during the sampling period was 0.17 ± 0.08 kg (1± SE) and 0.97 ± 0.15 kg for the otter trawls. The largest weight of catches for the hand trawl (*i.e.* 1 kg) was recorded in December (Fig. 2.2.4a), while the heaviest weight for the otter trawls (*i.e.* > 1 kg) was caught from December to March (Fig. 2.2.4b). The mean density km^{-2} (± 1 SE) of *M. dalli* caught using the hand trawl was 19.02 ± 9.02 kg km^{-2} and 105.93 ± 16.06 kg/km^2 for the otter trawl (Fig. 2.2.4c,d).

The estimated biomass for the hand trawl area of 15.5 km^2 was calculated at 0.29 tonnes and the otter trawl area covering the shallower water (19.6 km^2) was 2.09 tonnes. The total biomass (B_0) estimated for the combined area of 35.1 km^2 was therefore 2.37 tonnes.

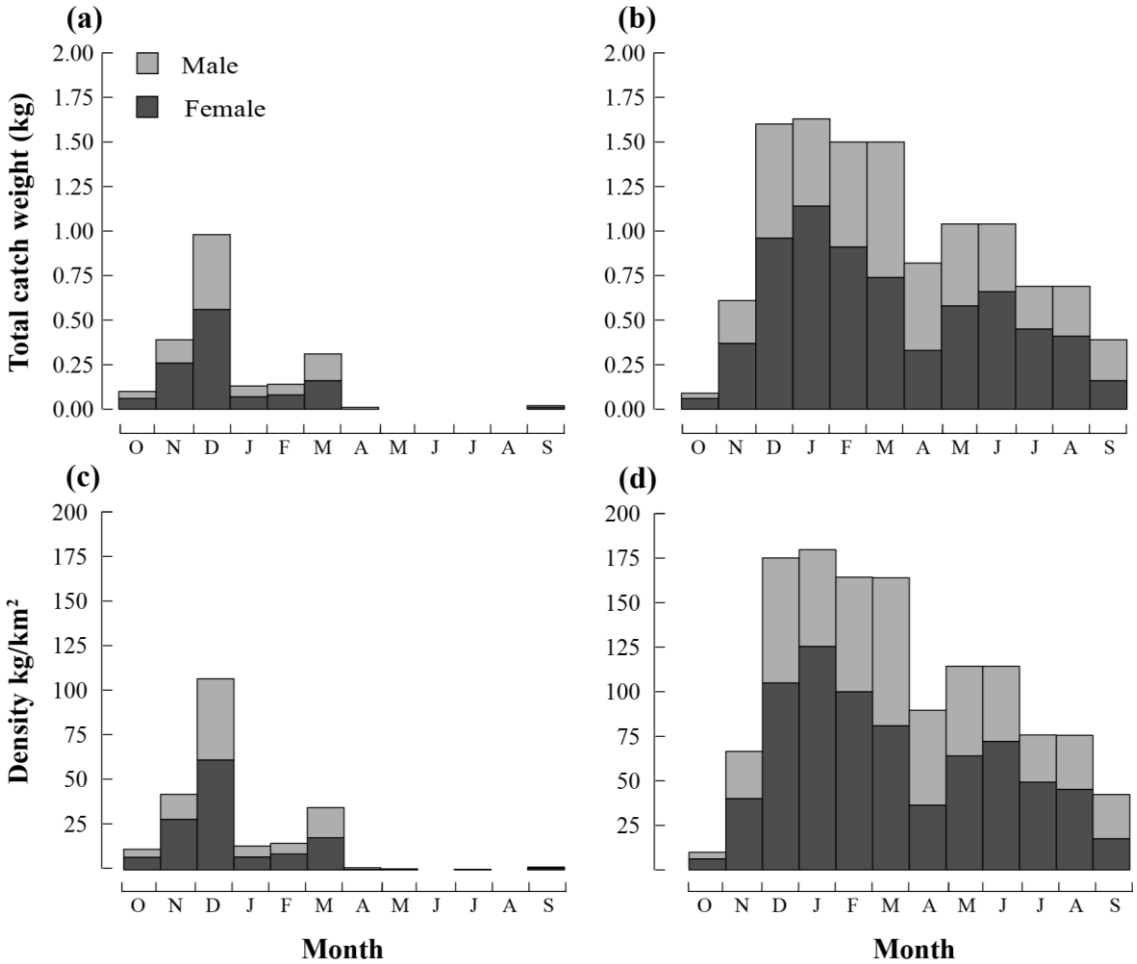


Fig. 2.2.4. Total weight (kg) and density (kg km^{-2}) of female and male *Metapenaeus dalli* caught in (a and b) hand and otter trawl (c and d) samples collected every 28 days between October 2013 and September 2014.

Bio-economic modelling

A release of 650,000 1 mm CL *M. dalli* was estimated to produce the lowest increase in biomass (0.1 tonnes) over a 5-year period (Table 2.2.3). The largest predicted increase in biomass was 4.1 tonnes from the initial population biomass, achieved by stocking 5 million, 10 mm CL *M. dalli*. However, the most notable difference between scenarios is the relatively low increment in biomass increase between releases of 2 to 5 million (*i.e.* a mean of 0.08 ± 0.05 tonnes) in all stocking sizes. A greater relative increase in population biomass was achieved by moving from a release 650,000 to 2 million prawns (*i.e.* 0.44 ± 0.36 tonnes).

Table 2.2.3. An estimate of the total biomass, in tonnes, over a 5-year period for restocking scenarios of *Metapenaeus dalli* in the Swan-Canning Estuary for increasing stocking density and stocking size. Initial estimate of the population biomass was 2.37 tonnes.

Stocking density	Stocking Size (mm CL)			
	1	**3**	**5**	**10**
650,000	2.47	2.70	3.06	4.80
2,000,000	2.51	2.78	3.18	6.33
5,000,000	2.53	2.81	3.22	6.57

The estimated operating costs (Table 2.2.4) have been made by scaling the operational cost per single prawn (*i.e.* 0.12 cents) from the operational costs of producing 650,000 prawns by the Australian Centre for Applied Aquaculture Research in 2013/14. These costs do not include capital or additional costs required to increase the production capacity of the aquaculture facilities needed to generate a stocking density of 5 million prawns. It is therefore no surprise the lowest operational cost (AUD $79,560) is a stocking density of 650,000 1 mm CL prawns. In contrast, the highest cost (AUD $682,500) is associated with a stocking density of 5 million 10 mm CL prawns.

Table 2.2.4. An estimate of total operational costs (AUD$) for restocking scenarios of increasing stocking density and stocking size of *Metapenaeus dalli* in the Swan-Canning Estuary.

Stocking density	Stocking Size (mm)			
	1	**3**	**5**	**10**
650,000	79,560	117,000	162,500	273,000
2,000,000	79,560	117,000	163,500	273,000
5,000,000	198,900	292,500	408,750	682,500

The *EnhanceFish* model also predicts how the wild population will respond to different release strategies. In all cases, it predicts that biomass in the wild population of *M. dalli* will decline over the five years of the simulation. For a release of 1 million prawns, the biomass of the wild population was predicted to decrease to 1.65 tonnes, 1.36 tonnes for 2 million prawns and 1.24 tonnes for a release of 5 million prawns (Fig. 2.2.5).

Changes to the size-at-release (*i.e.* 1, 3, 5 or 10 mm CL) for each of the stocking density scenarios did not influence the rate of decline in the wild population. The greatest increase in biomass, relative to each size-at-release, was found at a density of around 1 million prawns (Fig. 2.2.5). Varying the size-at-release had relatively little impact on the predicted biomass as more than 1 million prawns were released (Fig. 2.2.5).

The sensitivity analysis revealed that changing the mortality and recruitment parameters by ±10% did not have a significant effect on the stability of the model. This analysis was extended to study the effect of changing natural mortality on biomass over a 5-year period (Fig. 2.2.6). At the current level of natural mortality (*i.e.* 3.0 year^{-1}), without restocking, the population biomass will change very little over the next 5 years (Fig. 2.2.6). In contrast, reducing natural mortality to half the current rate (*i.e.* 1.5 year^{-1}) results in a predicted increase in population biomass from 2.37 to 4.2 tonnes, *i.e.* an approximate 180% increase in biomass.

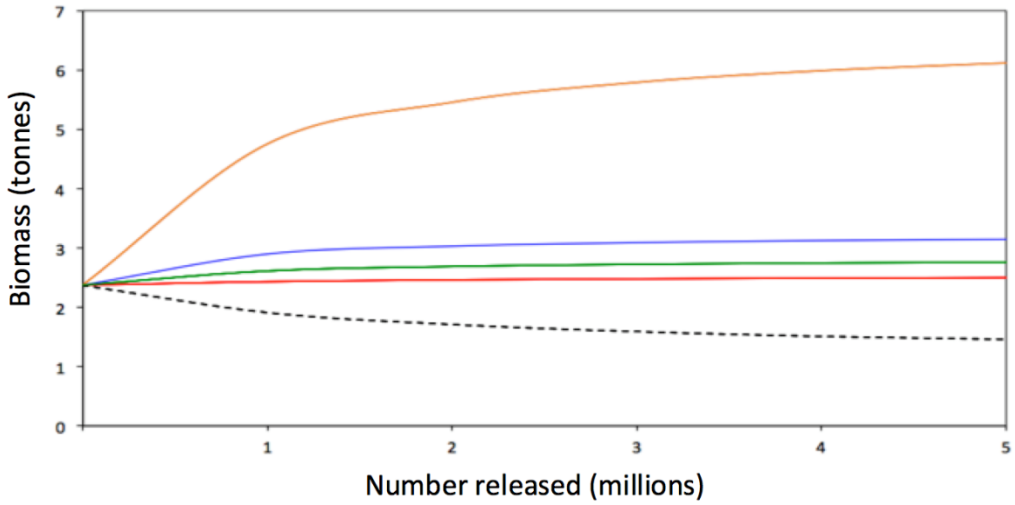


Fig. 2.2.5. Change in the biomass of the *Metapenaeus dalli* population in the Swan-Canning Estuary with the number of prawns released and different sizes-at-release over a 5-year period. Wild population = ----, 1 mm = ■, 3 mm = ■, 5 mm = ■ and 10 mm = ■.

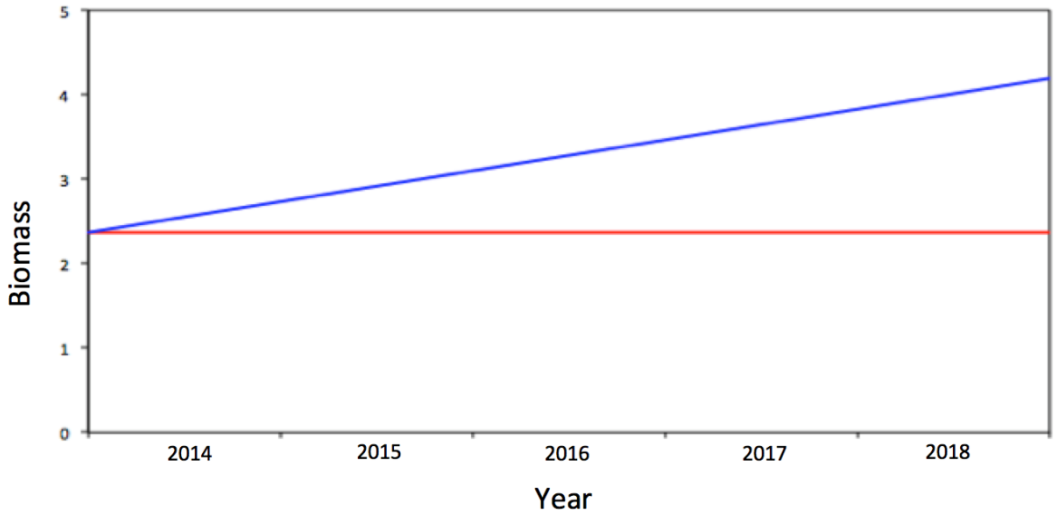


Fig. 2.2.6. The population biomass of *Metapenaeus dalli* in the Swan-Canning Estuary projected forward 5 years without restocking for (a) a natural mortality rate of 3.0 year^{-1} (■) and (b) a natural mortality rate of 1.5 year^{-1} (■).

Discussion

The primary aim of this preliminary bio-economic assessment was to explore the population dynamics and costs associated with restocking *Metapenaeus dalli* in the Swan-Canning Estuary. In general, the model results indicated that the small to moderate potential increases in biomass of the *M. dalli* population would be achieved for releases of 650,000 to 5 million prawns and at a range of sizes (1 to 10 mm CL). The estimated biomass increase for different numbers and sizes-at-release ranged from a 0.1 tonnes (650,000 at 1 mm CL) to 4.2 tonne (5 million at 10 mm CL) over a 5 year period. These preliminary results require further exploration to investigate the behaviour of the *EnhanceFish* model for a short-lived species and compare these predictions with those of a bio-economic model developed specifically for prawns (*e.g.* Ye *et al*., 2005a). Some of the important outcomes from this exploratory modelling exercise and the limitations and assumptions of *EnhanceFish* for short-lived species are discussed below.

Population biomass

The significant decline in *M. dalli* in recent times is supported by anecdotal evidence from recreational prawn fishers who have witnessed a substantial decline in their catches. Unfortunately, there is very little scientific evidence and literature available to quantitatively estimate the decline in catch or create an index of relative abundance. Historical records indicate, however, that the largest commercial prawn catch in the Swan-Canning Estuary was 14 tonnes in 1959 and that the last significant catch of 3 tonnes, was recorded in 1975 (Smith *et al*., 2007). It should be noted that *M. dalli* and *P. latisulcatus* are not distinguished in the commercial catch records so these may be over-estimates of the historical range of *M. dalli* catches. A large recreational fishery continued to target *M. dalli* until the 1990's (Smith *et al*., 2007). Although the annual catch of the recreational fishery is not known, it was very significant given its popularity as a regular summer activity (*i.e.* December to March) until about 20 years ago.

The current population biomass estimate of approximately 2.37 tonnes, derived from the average catches over the 12 months in the nearshore and offshore waters, is low in terms of historical commercial catches in the Swan, *e.g.* the model predicts that the record catch of 14 tonnes in 1959 would have required a population biomass of approximately 34 tonnes with a fishing mortality of 0.6 year^{-1} and natural mortality of 2.4 year^{-1} (*i.e.* assuming the same growth, reproduction and life history model parameters, and a total instantaneous mortality of 3 year^{-1}). The current low population size could potentially affect the rate of population increase. This depensatory behavior, where at low abundance mortality is relatively high, even with very low fishing pressure, suppresses key population processes (*e.g.* growth, mortality and reproduction) and is characterised by a population shifting from one equilibrium to another (Myers *et al*., 1995; Stephens *et al*., 1999; Frank and Brickman, 2000). The *M. dalli* population appears to be exhibiting depensatory behavior; it has a relatively high natural mortality, low biomass and is predicted to grow very little over the next 5 years.

Fortunately, if these model predictions of a very low relative *M. dalli* population size are realistic, restocking has the potential to have a marked positive impact and facilitate rebuilding the population size (Liermann and Hilborn, 1997; Lorenzen, 2005; Cabral *et al*., 2013). One of the most successful restocking programs was carried out in the Northern Japanese Scallop (*Patinopecten yessoensis*) fishery in Hokkaido (Bell *et al*., 2008). In the early half of the twentieth century this fishery had annual harvest of up to 80,000 tonnes per annum. However, in 1945 the fishery collapsed to an annual harvest of only 6,000 tonnes per annum for the next 25 years. During this period, local fishery cooperatives released large numbers of cultured juveniles to habitat selected for its low potential of predation. Since then, the total scallop harvest has increased to around 300,000 tonnes per annum with a value of around ¥37.6 billion (Uki, 2006).

Number released and size-at-release

The results indicate that the population biomass growth over the five-year model period was at a faster rate with releases of up to 1 million prawns and that the population growth rate declined when the numbers released exceeded 1 million. This effect is amplified by also increasing the size of prawns at release, *e.g. EnhanceFish* predicted higher rates of increasing population growth when the size-at-release was larger and approached the population's natural recruitment size. This phenomenon is not unusual in restocking or stock enhancement programs, *e.g.* in the North Sea Sole (*Solea solea*) stock enhancement program, Lorenzen (2008b) found an 81% increase in yield by increasing the same proportional abundance of stocked fish in the recruit life stage rather than at earlier stages in the life-cycle. When releases of larvae or juveniles were simulated, the predicted yield increased by only 4% and 29%, respectively.

As density increases in populations, density-dependence can lead to significant changes in growth, survival and reproductive output (*e.g.* Miller *et al*., 1988; Rose *et al*., 2001; Lorenzen, 2005; Lewin *et al*., 2006). The results from the *EnhanceFish* model show that stocking prawns at densities exceeding 1 million produces the greatest compensatory response in the population, shown by a decrease in population growth rate with an increasing stocking density, possibly caused by density-dependent mortality or reduced growth. Similarly, prawns released at small juvenile sizes (1, 3 and 5 mm CL) elicit strong compensatory responses (*e.g.* juvenile density-dependent mortality). This is evidenced by the relatively low increase in total biomass at these release sizes, *e.g.* a density of 650,000 prawns yields an increase in biomass of 0.1 and 0.69 tonnes in the 1 and 5 mm CL sizes respectively. In contrast to the smaller sizes-at-release, the 10 mm CL prawns (*i.e.* closer to the size of recruitment into the recreational prawn fishery) have a strong positive population growth response, *e.g.* a 2.43 tonne increase at a stocking density of 650,000. These larger prawns appear to circumvent the high level of density-dependent mortality suffered by releases of smaller juveniles (*i.e.* 1 to 5 mm CL). Ye *et al*. (2005a) also estimated that the optimal size-at-release for Brown Tiger Prawns *Penaeus esculentus* was 10 mm CL (≈ 1 g wet weight), similar to the release size for Karuma Prawns *Penaeus japonicas* in Japan (Hamasaki and Kitada, 2006).

In a study of six demersal marine fish species in 17 populations (*i.e.* across the North Sea, Irish Sea, Barents Sea, Baltic Sea and Northwest Atlantic) Myers and Cadigan (1993) found the greatest source of variability in stock sizes was related to density-dependent mortality in the early juvenile life stage. For example, a 100% increase in abundance of Whiting (*Merlangius merlangus*) at age 0 resulted in a 26% increase at age 1. Myers and Cadigan (1993) suggest the primary reasons for this variability are competition for food, habitat availability and predator-prey interactions. Similarly, Ye *et al*. (2005a) found in their bio-economic model, that the greatest source of risk and uncertainty to a large commercial penaeid prawn (*Penaeus esculentus*) enhancement program was post-release mortality and density-dependent mortality caused by the release of 21 million prawns.

Timing of release

It was not possible to vary the time of release using the *EnhanceFish* model. However, the time of release can have a significant impact on the survival of released individuals (*e.g.* Caddy and Defeo, 2003; Bell *et al*., 2005; Leber *et al*., 2008; Gardner *et al*., 2013). For example, Hervas *et al*. (2010) found that the timing of release affected the post-release mortality of stocking White Sea Bass (*Atractoscion nobilis*). White Sea Bass released in spring had the highest chance of survival, while those released in winter had the lowest.

Releases of *M. dalli* close to, or during winter, are likely to be less successful than those during warmer months because of the cessation of growth when water temperatures are less than 21 ºC. It is possible that releases earlier than the normal time of recruitment (*i.e.* around October to November, depending on seasonal variability), when water temperatures are starting to increase, may be more successful than those during the peak of the natural recruitment cycle. The primary benefit of an earlier release time is the potential reduction in competition with the wild population. This strategy could also reduce density-dependent effects within the wild population. However, the earlier releases would only be possible if the prawns could be spawned and cultured outside their breeding season.

Wild population's response to restocking

The mixing of wild and hatchery reared fish can have detrimental effects on both populations if not managed carefully. The scale of the hatchery release and the relative abundance and fitness of the wild population will significantly influence productivity and overall success of restocking (Lorenzen *et al*., 2010, 2012). The *EnhanceFish* model shows that the wild population of *M. dalli* declines by approximately 0.1 tonnes per million prawns released for all restocking scenarios. However, the net population biomass (*i.e.* wild and hatchery prawns combined) increases despite this decline in the wild population.

Restocking genetically and phenotypically fit hatchery prawns carries the risk of displacing the wild genotype. Safeguards to preserve the wild genotype are particularly important for the *M. dalli* population in the Swan-Canning Estuary as this population is at historically low levels. Selection of quality wild seed stock is fundamental to the evolutionary adaption of the broodstock and, as a consequence, the fitness of the released individuals. Careful husbandry and management during aquaculture production is critical to post-release survival, growth, recruitment and reproduction of the hatchery prawns (Lorenzen, 2008a, 2012).

Costs and benefits of restocking

Bypassing the density-dependent early juvenile life stage with larger juveniles, at least 10 mm CL, appears to be an effective strategy for restocking prawns. However, there are clear trade-offs between the size-at-release, the increasing costs of aquaculture production, and negative effects on the wild population. Releasing prawns at larger sizes incurs additional operational costs (*i.e.* around 3 cents per 1 mm increase in CL) associated with labour, prawn feed (*e.g.* algae and rotifers), heating and power. It may also require additional capital costs for the production facilities to grow prawns to the larger size. Indeed, the additional capital costs of investment required to produce the 24 million 1 g *Penaeus esculentus* for 100 tonne enhancement in Exmouth Gulf were one reason this program was discontinued (Loneragan *et*

al., 2004, 2013). Furthermore, there is evidence to suggest that the longer prawns remain in culture the higher, the risk of maladaptation to the wild environment (*e.g.* they will be more exposed to predation and out competed for food) (*e.g.* Masuda and Tsukamoto, 1998; Tomiyama *et al*., 2011; Ochwada-Doyle *et al*., 2012). Thus, this increases the risk and potential costs associated with higher post release mortality.

Currently, very little is known on the benefits of restocking small recreational fisheries, which is particularly apparent for prawns where no obvious information is available, such as *M. dalli* in Western Australia or the Eastern King Prawn (*Penaeus plebejus*) on the east coast of Australia (M. Taylor, NSW Fisheries, pers. comm.). Moreover, recreational fisheries need to include the social benefits from recreational fishing and the economic benefits can be difficult to estimate precisely. This contrasts with commercial fisheries, where there is a substantial amount of information available, in terms of catch, effort, operational costs and revenue. Evaluating the costs and benefits of restocking *M. dalli* requires a complete assessment of the current socio-economics of the recreational prawn fishery. Such a study would provide information on the benefits of restocking by valuing the recreational utility of the fishery. This information could then be used to quantify the benefits in monetary terms, thus enabling a comprehensive analysis of the benefits and costs of restocking *M. dalli*.

Future investigation

These preliminary results indicate the predictions of the potential success of restocking of *M. dalli* from the *EnhanceFish* model require further investigation. A future investigation could explore this potential by: (i) focusing on refining the estimates of mortality used in the model from empirical studies to estimate density-dependent mortality, release mortality and variation in mortality with size of prawns; (ii) investigating the importance of time-at-release on the population biomass, and (iii) comparing the predictions and behaviour of the model with those from a model designed specifically for short-lived penaeid prawns (Ye *et al*., 2005).

Ye *et al*. (2005a) developed a tool to evaluate the economic viability, biological effectivness and risk of a potential stock enhanement program for the Brown Tiger Prawn (*Penaeus esculentus*), which is fished commercially in the Exmouth Gulf Prawn Trawl Fishery (Loneragan *et al*., 2013b; Sporer *et al*., 2013). This bio-economic model was created to include all components of a release program; from the production of prawns in the hatchery, through their culture and release in the Exmouth Gulf to their eventual capture in the fishery. The model uses weekly estimates of the model parameters for growth and mortality and works in weekly time steps. Furthermore, the model design allowed the researchers to explore and quantify the effect of uncertainty for various enhancement scenarios and investigate the influence of time-at-release on prawn survival (Loneragan *et al*., 2004; Ye *et al*., 2005a).

In contrast, *EnhanceFish* is a generalised model, originally designed for teleost fish populations, that can also be configured for a range of release programs, such as small community based programs; *e.g.* Thmorda Reservoir in Cambodia, or large commerical operations *e.g.* North Sea Sole (*Solea solea*), by changing key parameters and data associated with a particular enhancement or restocking program (Lorenzen and Medley, 2006). It was created to provide fishery managers with a tool to quantitatively optimise enhancement scenarios by assessing, impact on yield, wild stock abundance and structure, economic performance, uncertainty and changes in fishing effort (Lorenzen and Medley, 2006).

Both models provide a valuable framework for researchers, managers, and fishers to consider all aspects of a release program and identify important knowledge gaps that are likely to influence the reliability of the model predictions.

Conclusions

Restocking the *M. dalli* population requires stocking densities and sizes-at-release that effectively and economically boost recruitment capacity (Walters and Kitchell, 2001; Lorenzen, 2005). However, it should be noted that it is impossible to avoid a compensatory response in the *M. dalli* population with increasing stocking densities (*e.g.* Myers and Cadigan, 1993, 1995;

Liermann and Hilborn, 1997; Lorenzen, 2005). Furthermore, this preliminary quantitative analysis is the first step in developing an understanding of the *M. dalli* population's compensatory responses. Additional experiments and field studies that test this behaviour will further develop scientific knowledge and optimise the stocking density and size-at-release strategies.

Section 3. Optimise release strategies

This section details research relating to objective 5, *i.e.* optimise release strategies (stocking densities, size and location at release) for Western School Prawns. Five main components were evaluated:

1. Influence of timing-of-release and substrate type on the activity of post-larval Western School Prawns

2. Influence of timing-of-release on predation rates of post-larval Western School Prawns (PhD studies of Brian Poh).

3. A methodology to develop aquaculture-based release strategies with an application to the Western School Prawn in the Swan-Canning Estuary: The Survival Maximisation-At-Release Tool (Honours studies of Kyle Hodson).

3.1. Influence of timing-of-release and substrate type on the activity of post-larval Western School Prawns

Summary

The activity and behaviour of post-larval *Metapenaeus dalli* following release during the day and night into vegetated and unvegtated aquaria were investigated. The vast majority of individuals were found to remain on the benthos throughout the experiment, regardless of photoperiod and/or the presence of aquatic vegetation. Such a result reflects the design of the aquaria used in the experiment and the relatively long duration between counts of active prawns (*i.e.* every minute). Individuals released during the day actively swam to the bottom of the aquarium, whereas at night-time, post-larvae sank as they are negatively buoyant, and no currents were present to resuspend the individuals. However, observations in the holding tanks and experimental aquaria did match those seen during the release of hatchery-reared post-larval *M. dalli* as part of the restocking program. Based on these in-vitro and in-vivo observations, it appears that *M. dalli* released during the day actively swam to the benthos, whereas those released at night, remained in the water column due to lack of downwards swimming, and water movements that resuspend the larvae. These results are important in the context of aquaculture-based enhancement, as predation is a major limiting factor on the success of these programs and individuals are most vulnerable immediately after release. Thus, release strategies could take advantage of the behavioural pattern of *M. dalli* to swim to the benthos during daylight to help reduce predation risk during the critical early stages post release.

Rationale and aims

Penaeid prawns are known to adopt a nocturnal lifestyle; remaining buried during the day, and thus less susceptible to predation; before emerging and foraging at night on the surface of the substratum (Penn, 1976; Stoner, 1991; Abdussamad, 2008; Brito, 2010). This diurnal pattern of behaviour is reflected by the elevated wild catch rates during the night (Yousif, 2003). The association between their activity and photoperiod has been demonstrated in many species under controlled laboratory conditions (Wickham and Minkler, 1975; Hill, 1985; Vance and

Staples, 1992; Wassenberg and Hill, 1994) For example, Primavera and Lebata (1995) observed that juveniles of *Metapenaeus anchistus*, *Metapenaeus* sp., *Penaeus monodon* and *Penaeus merguiensis*), remained burrowed during the day and emerged and were active at night, with the same trend recorded for *Metapenaeus dalli* (Section 1.8). The strong diurnal periodicity was shown to be stronger for species of *Metapenaeus* than *Penaeus*, with members of the former genera spending less time on or above the substrate swimming, walking, feeding or cleaning (Primavera and Lebata, 2000).

Many studies have demonstrated an association between the abundance and distribution of penaeids and the presence of vegetated habitats, including, for example, marshland (Howe *et al*., 1999), mangroves (Sheaves *et al*., 2012), and seagrass meadows (Haywood *et al*., 1995; Loneragan *et al*., 1998). Many penaeids use these habitats as nursery areas, as the vegetation provides shelter for post-larvae and juveniles and makes them less visually and physically accessible to predators (Howe *et al*., 1999). More detailed studies have demonstrated that this habitat preference may vary temporally over a diurnal cycle, with Ochwada-Doyle *et al*. (2009) recording that post-larval *Penaeus plebejus* selected vegetated habitats over less complex habitats of bare sand and mud during the day, but settled randomly at night across vegetated and unvegetated habitats. Such a trend, however, may be species dependent, as, in the nearshore marine waters of Inhaca Island, Mozambique, juvenile *Fenneropenaeus indicus*, *Metapenaeus stebbingi* and *Penaeus japonicas* dominated the catches over sandy sediment, *Metapenaeus monoceros* and *Metapenaeus stebbingi* were most abundant over mudflats and *Penaeus semisulcatus* was almost exclusively caught in seagrass meadows (Macia, 2004).

Understanding the diurnal pattern of burial and emergence, and the influence and associations of habitat on penaeids is crucial in the context of aquaculture-based enhancement, where post-larval prawns are produced in an aquaculture facility and released into the wild. Post-release mortality, the majority of which is due to predation (Hines *et al*., 2008; Støttrup *et al*., 2008), has the potential to severely impact the success of the enhancement (Leber, 2002). Given the diurnal pattern of burial and emergence of penaeids and the different associations with habitat, the aim of the current study was to determine whether hatchery-reared post-larval *M. dalli*

actively seek shelter when released into different habitats, and if there are differences in response depending on photoperiod. The results can then be used in the development of a release strategy for hatchery-reared post-larval *M. dalli* to reduce post-release mortality and maximise the success of the restocking.

Methods

Post-larval *M. dalli* were obtained from the Australian Centre for Applied Aquaculture Research, South Metropolitian TAFE in Fremantle, Western Australia. The larvae were cultured from gravid female *M. dalli* collected from the Swan-Canning Estuary, and spawned in the culture facility for the purposes of a restocking program (Jenkins *et al*., 2015; 2017). Larvae were reared in full-strength seawater (~33 ppt) drawn from a bore accessing nearshore marine water through a limestone filter, aerated constantly and maintained at a temperature of ~26 °C (Appendix 3 for full details of the aquaculture method).

Once the larvae had metamorphosed to post-larvae and reached 10 days old (PL10), the prawns were transferred into large polyethylene bags inflated with oxygen-enriched compressed air and stored in a polystyrene box, and transported from the aquaculture facility to a remotely-controlled temperature room at Murdoch University. Three 70 L aquarium tanks were filled with water from the aquaculture facility and heated to 26°C. The bags were immersed in to a tank for 15 minutes to allow equilibration to ambient water temperatures. Approximately ~1,000 post-larvae were transferred into each tank and provided with constant aeration. Light in the controlled-temperature room was set to a 12:12 light:dark photoperiod to mimic conditions in the hatchery, and the prawns were fed the same diet (Jenkins *et al*., 2017). Water quality was maintained by exchanging 100% of the volume of the tank every 24 h.

Twenty smaller aquaria (10 cm long x 10 cm deep x 30 cm tall) were constructed from aquarium grade glass and silicone sealant. Each small aquarium was filled, to a depth of ~ 1 cm, with sandy substrate (Fig. 3.1.1a) collected from the nearshore waters Deep Water Point on the Swan-Canning Estuary, which at the time of the experiment had relatively high densities of *M. dalli* (Poh, unpublished data). The substrate of ten of the aquaria were 'enhanced' with a small artificial aquarium plant (Fig. 3.1.1b), designed to mimic the seagrass *Halophila ovalis*,

which occurs naturally in the Swan-Canning Estuary (Hillman *et al*., 1995). Each aquarium was filled with ~2.8 L of the same filter seawater used in the hatchery and subjected to constant aeration during experiment set-up.

Twenty aquaria were arranged in two rows, with 10 being unvegetated and 10 being vegetated. At the start of each experiment, the aeration was ceased to minimize the confounding effects of vigorous input of bubbles of air on water circulation, and thus the movements of the post-larvae. Ten live post-larval *M. dalli* were placed simultaneously into the surface most waters of each aquarium. Prawns were scored at each minute over a 10-minute duration based on their position and activity within the aquarium. Individuals that were free-swimming in the water column at any point in time during the 1-minute interval were scored as 'active', whereas individuals on, or in the substrate (or vegetation), were considered as 'resting'. Individuals within the vegetated aquaria that were at rest on the foliage of the artificial grass were also scored as 'resting'. The entire experiment was replicated three times in both light and dark conditions, using fresh *M. dalli* for each experiment.

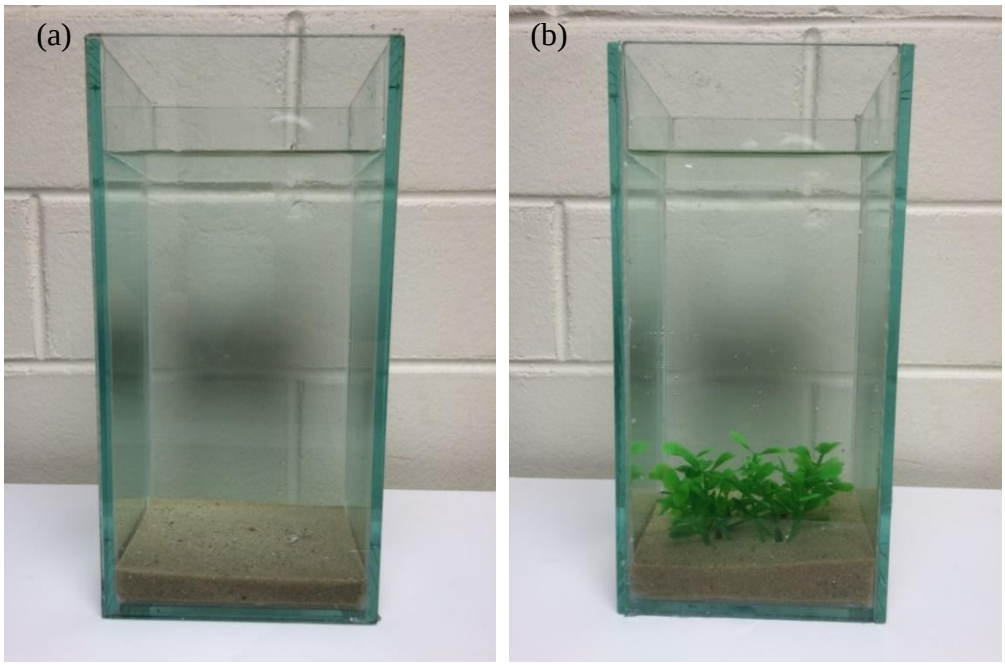


Fig. 3.1.1. Photographs of (a) unvegetated and (b) vegetated 10 x 10 x 30 cm aquaria used in the experiment.

Results

Across all replicates in both the day and night and unvegetated and vegetated tanks, only ~7% of the post-larval *M. dalli* were classified as being in an active state (*i.e.* swimming in the water column) after one minute. In the remaining nine minutes of the experiments, the proportion of active individuals declined further, ranging from 2.17 to 3.17%. No clear differences were observed in the number of active *M. dalli*, in any minute, between the day and night, and over either the unvegetated (Fig. 3.1.2a) or vegetated (Fig. 3.1.2b) substrates. Likewise, the presence nor absence of artificial vegetation had no effect on the number of *M. dalli* active in the water column (*cf.* Fig. 3.1.2a,b). Although not recorded in the experiment due to the one-minute time intervals, post-larval *M. dalli* actively swam downwards to the substrate when the experiment was conducted under daylight conditions. When the experiment was repeated at night, however, *M. dalli* freely drifted to the bottom, based on the fact that they are negatively buoyant.

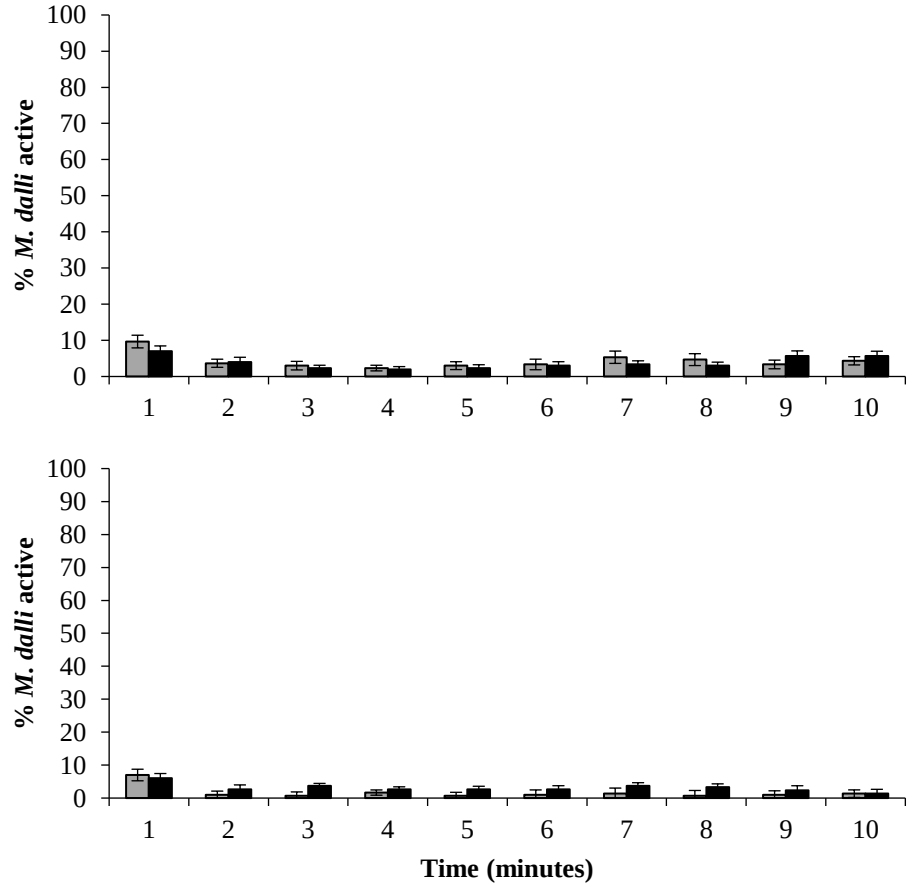


Fig. 3.1.2. Mean percentage of post-larval *M. dalli* actively swimming during the day (■) and night (■) over (a) unvegetated and (b) vegetated substrates in each of ten minutes post-release into the aquaria. Error bars represent ±1 standard error.

Discussion

When released into the experimental aquaria, the vast majority of hatchery-reared post-larval *M. dalli* remained on the benthos throughout the experiment, regardless of photoperiod and/or the presence of aquatic vegetation. This result is consistent with the findings of Chu *et al.* (1996), where the movements of PL5 *M. ensis* were mostly benthic, with small excursions into the water column immediately above the benthos. Likewise, Primavera and Lebata (2000) found that 1-6 mm CL *P. merguiensis* and *M. ensis* spent most of the time stationary or under shelter. Such behaviour may be an adaptation to make the prawns less available to predators, as the post-larvae were too small to burry successfully in sandy sediment and, at this size, would not have a well-developed tail flick response to help evade capture (Arnott *et al*., 1998). The strength of this desire to remain on the benthos may explain why there was no visual difference in the number of active *M. dalli* between the unvegetated or vegetated aquaria. Although it should be noted that not all penaeids exhibit a preference for vegetated over unvegetated habitats (Macia, 2004; Ochwada-Doyle *et al*., 2009).

No clear differences were observed in the number of active post-larval *M. dalli* during the day or night, thus contrasting results from studies conducted in the wild (Yousif, 2003) and in tank experiments focusing on the emergence of larger individuals from the sediment (Wickham and Minkler, 1975; Hill, 1985; Wassenberg and Hill, 1994). It is likely that the lack of a diurnal trend in the behaviour of *M. dalli* is an artefact of the experimental design and not necessarily reflective of 'true' patterns of movement. It is important to note that this experiment was conducted in the absence of movement within the water column, as the aeration was disconnected. This decision was made due to the small diameter of the tank and the refraction of currents. However, in the larger holding tanks, the constant aeration generated a small amount of current without the confounding effects of refraction. Observations made during the preparation of experiment indicated that the post-larval *M. dalli* in these larger tanks exhibited strong diurnal differences in activity levels, with most prawns actively swimming at night and remaining on the bottom during the day. It is noteworthy that when the lights in the controlled-temperature room where turned on out of sequence (*i.e.* turned on during the 12 hour period of

darkness) all *M. dalli* swimming in the water column actively swam to the bottom of the tank. Likewise, when the white lights in the room were turned off during the day, and only dim red light illumination provided, which prawns are unable to detect; refs (Fuss, 1964; Goldsmith and Fernandez, 1968), some postlarval *M. dalli* moved from the bottom of the aquarium, into the water column.

These observations from the holding tanks mirror those recorded during the releases of post-larval *M. dalli* into the nearshore waters of the Swan-Canning Estuary (see Section 3.2.for full details of the methods). Thus, during daytime releases, post-larvae actively swam towards the substrate, whereas at night, they remained in the water column. While this was observed to some extent in the experimental aquaria, the frequency of recordings (every minute) were not sufficient to record this behaviour as most individuals reached the bottom of the aquaria by swimming (day) and sinking (night) within one minute. This is likely due to the lack of water movement, which occurs in the natural environment and resuspends the individuals, and thus, although they are slightly negatively buoyant, post-larvae remain in the water column due to resuspension, unless actively swimming downwards.

The experimental aquaria used in this experiment were designed to enable a small number of post-larval *M. dalli* to be quickly and accurately enumerated within several seconds. This design, however, did not allow currents present in the natural environment to be mimicked and thus the results are not biologically relevant. That being said, the observations in the holding tanks and experimental aquaria did match those seen during the release of hatchery-reared post-larval *M. dalli* as part of the restocking program. On the basis of these in-vitro and in-vivo observations, post-larval *M. dalli* released during the day actively swam down towards to the benthos, whereas those released at night, remained within the water column due to lack of downwards swimming, as well as water movements that resuspend the post-larvae. These results are important in the context of aquaculture-based enhancement as predation is a major limiting factor on the success of these programs, and individuals are most vulnerable immediately after release. Thus, release strategies could take advantage of the behavioural

pattern of *M. dalli* swimming to the benthos during daylight to help reduce predation risk during the critical early stages post release.

If this experiment was re-run in the future, it is recommended that larger tanks be used, with more individuals and over a longer timescale, but with counts being recorded more frequently (10 or 20 seconds). The tanks would require some standardizing circulation of water better mimic the natural environment. Note that in order for the experiment to be feasible, the number of observers would need to be increased dramatically due to the increased intensity of recordings, and *M. dalli* may need to be stained to make them easier to count, particularly at night.

3.2. Influence of timing-of-release on predation rates of post-larval Western School Prawns

This study has been published in Fisheries Research.

Poh, B., Tweedley, J.R., Trayler, K.M., Loneragan, N.R. (2018). Estimating predation rates of restocked individuals: the influence of timing-of-release on metapenaeid survival. *Fisheries Research*. 198: 165-179. DOI: 10.1016/j.fishres.2017.09.019.1.

Summary

The success of aquaculture-based enhancement programs is greatly influenced by the survival of released individuals. Immediate post-release mortality through predation is one of the greatest obstacles to the success of releases, and the choice of a release site or time-of-release can be critical in maximising survival. This paper develops a novel quantitative method of estimating predation rate to inform release programs, and describes its use in determining whether hatchery-reared Western School Prawns *Metapenaeus dalli* should be released into the Swan-Canning Estuary in temperate south-western Australia during the night or day. Fish faunal composition was determined during the day and night, both before and after the release of ~130,000 postlarval *M. dalli*. Far greater numbers of species and individuals were recorded at night. Stomach contents of 16 abundant teleost species were estimated volumetrically (%V) and any postlarval *M. dalli* counted. Although diet varied among species, diel phase and size class, crustaceans (including *M. dalli*) were a key dietary component (>10 %V) of 12 species. The data on the abundance of these fish species and the number of *M. dalli* they consumed were combined and subjected to bootstrapping, to estimate the total relative number of *M. dalli* consumed at the time-of-release. The results indicated that while six species consumed *M. dalli*, two species, *Ostorhinchus rueppellii* (Apogonidae) and *Atherinomorus vaigiensis* (Atherinidae), were responsible for ~99 % of the predation, and that the total number of postlarval prawns consumed was 288 % higher at night than in the day. These findings suggest that releasing *M. dalli* during the day will greatly reduce predation and consequently allow a greater survival rate at this release site. The simple methodology developed here could be readily employed to inform release strategies for other species.

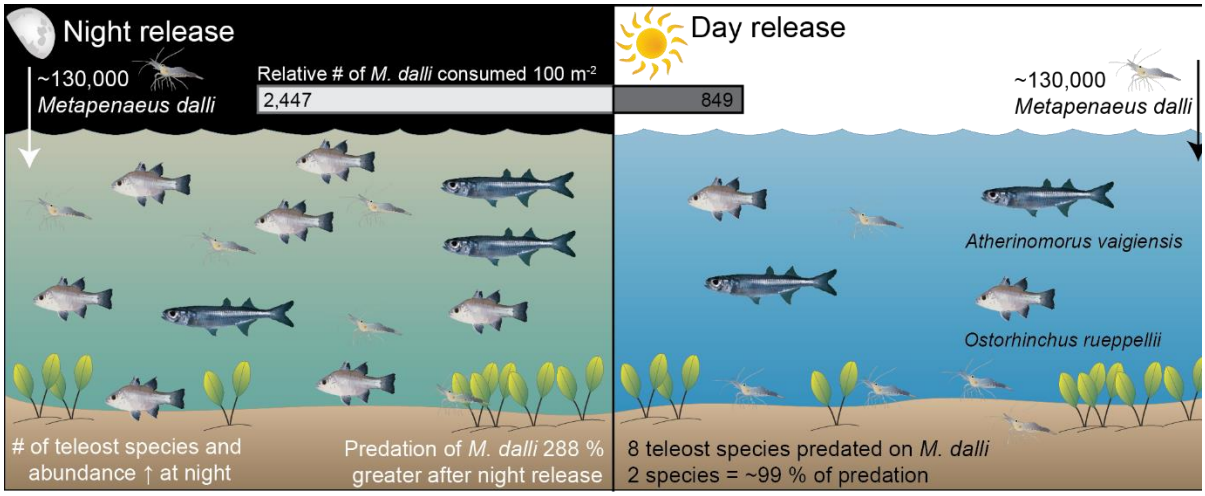


Fig. 3.2.0. Conceptual model of the influence of timing-of-release on predation rates of post-larval *Metapenaeus dalli*.

Rationale and aims

Over the last thirty years, interest in aquaculture-based enhancement (*i.e.* release programs for cultured species) has increased greatly because of the potential for such programs to provide increased food security, socioeconomic benefits and/or restore populations subjected to anthropogenic stress (Taylor *et al*., 2017a). These programs include stock enhancement, restocking and sea ranching (Bell *et al*., 2008; Lorenzen *et al*., 2013). Despite their obvious attraction as a mechanism to increase fisheries production and rebuild fish stocks, the performance of release programs has been mixed and, more often than not, disappointing, with many failing to significantly increase fishery yields or provide economic benefits (Bell *et al*., 2005; Lorenzen, 2005).

Predation is widely understood as a major contributing factor affecting the short-term post-release survival of hatchery-reared juveniles (Stein *et al*., 1981; Støttrup *et al*., 2008), and can contribute >95 % of total mortality (Hines *et al*., 2008). Predation is also considered a major cause of natural mortality in postlarval penaeids (Minello and Zimmerman, 1983; Zimmerman *et al*., 1984). Dall *et al*. (1990) suggested that 25 % of juvenile prawns in coastal inland waters are lost each week, mainly due to predation. This high level of natural mortality may be related to the high energy content of penaeids relative to other benthic

macroinvertebrates, making them attractive prey (Thayer *et al*., 1973). Predation rates, and as a consequence natural mortality, of juvenile penaeids vary greatly among habitat types (Kenyon *et al*., 1995; Haywood *et al*., 1998; Haywood *et al*., 2003), which influences the number of prawns surviving to migrate from juvenile habitats to recruit into fisheries (Ye *et al*., 2005a; Loneragan *et al*., 2006).

Whilst the impact of fish predation on adult penaeids has been well studied (Sheridan *et al*., 1984; Pauly and Palomares, 1987; Salini *et al*., 1990), predation rates on juveniles in estuaries and coastal waters have received less attention. This is possibly because postlarval and juvenile penaeids are rapidly digested in fish stomachs, causing them to be under-represented in stomach content analyses; *e.g.* small penaeids were reduced to ~30 % of their original dry weight just one hour after ingestion by the tetradontid *Monocanthus chinensis* (Haywood, 1995).

A study by Salini *et al.* (1990) found that 37 of the 77 fish species collected in the Embley Estuary fed on juvenile penaeids, which were a significant component of the diets of three of the most abundant predators. Numerous studies have found that many predatory fish are size selective; *i.e.* smaller fish eat smaller prey(Brewer *et al*., 1995; Lek *et al*., 2011), which is important for release programs as, typically, releases comprise a single size-class (cohort). Modelling estimated that the sources of greatest uncertainties in predicting the survival of Brown Tiger Prawns, *Penaeus esculentus*, following their release was in the immediate post-release mortality, natural mortality rates of the juvenile phase and density-dependent effects (Ye *et al*., 2005b). Differences in 'fitness' have also been detected between hatchery-reared and wild postlarval penaeids. For example, Ochwada-Doyle *et al*. (2012), found that wild-caught Eastern King Prawn, *Penaeus (Melicertus) plebejus*, postlarvae out-competed hatchery-raised postlarvae for shelter, resulting in higher predation rates on the hatchery-raised individuals. Thus, in order to estimate the effect of predation on the success of release programs, studies must focus on the predation on a particular size-class(es), *i.e.* that of the hatchery-reared and released individuals.

The abundance of predators is a major contributing factor to predation pressure (Hereu *et al*., 2005). Ichthyofaunal assemblages in estuaries are very dynamic and can vary spatially, and over a range of temporal scales, *e.g.* seasons/years/decades(Ribeiro *et al*., 2008; Potter *et al*., 2016), seasons (Loneragan and Potter, 1990; Veale *et al*., 2014) and diel phase (Young *et al*., 1997; Gray *et al*., 1998). The production and release of hatchery-reared individuals that do not have cultured broodstock available, *i.e.* for species whose life cycle has not been closed, is limited by the temporal availability of wild broodstock. Release strategies are therefore constrained to controlling the site and time-of-day of release, but not season. Although diel changes in fish composition have been recorded in several estuaries in temperate south-western Australia, with different species being more abundant at one time of day/night, the trends are not consistent across estuaries (*cf* Hoeksema and Potter, 2006; Yeoh *et al*., 2017). Generalisations on the diel abundance of predators for the design of a release program are therefore difficult to make.

The dietary compositions of many fish species differ markedly over the diel cycle (Robertson and Howard, 1978; Klumpp and Nichols, 1983; Linke *et al*., 2001), which is often related to changes in the availability of prey species. The diets of fish species also vary significantly with ontogeny, switching from smaller to larger prey with increasing body size (Franco *et al*., 2008; Lek *et al*., 2011). Furthermore, Daly *et al*. (2013) highlighted the importance for investigating both spatial and temporal variation in predation pressure in their study on the stock enhancement of Red King Crab (*Paralithodes camtschaticus*). Thus, understanding the abundance, feeding habitats and dietary composition of predator species is vital when developing release strategies that minimise short-term post-release mortality.

The abundance of the Western School Prawn (*Metapenaeus dalli*), which was once the focus of a small commercial and iconic recreational fishery in the Swan-Canning Estuary, Western Australia, has declined markedly since the 1950s (Smith, 2006; Smith *et al*., 2007). Despite a large reduction in fishing pressure, stocks of *M. dalli* have not recovered, and thus a restocking project was initiated as a possible means of increasing the population size (Broadley *et al*., 2017). The overall aim of this study is to develop a quantitative method for estimating the

immediate post-release effects of predation and determine whether releases of hatchery-reared postlarval *M. dalli* should be conducted during the day or night. In order to achieve this, we aimed to: (i) determine diel changes in the characteristics of the fish fauna at the release site in the Swan-Canning Estuary; (ii) quantify the dietary composition of abundant teleost species prior to and immediately after releases; and (iii) estimate the number of *M. dalli* consumed following releases during the day and night. The results provide the quantitative data required to develop a release strategy that optimises the immediate post release survival of postlarval *M. dalli*.

Methods

Release of post-larval prawns

In each of March 2015 and March 2016, a total of ~130,000 12 day old *M. dalli* post-larvae (PL12) ~ 4 mm TL were released into Matilda Bay in the Swan-Canning Estuary. At this stage of development, the hatchery-reared prawns, grown from fertilised eggs at the Australian Centre for Applied Aquaculture Research, were fully metamorphosed, had adopted a benthic lifestyle, and were better able to shelter and avoid predators than the earlier nauplii, protozeal and mysis stages (Dall *et al*., 1990; Crisp *et al*., 2016).

In the hatchery, post-larvae were collected from 300 L conical base tanks containing full strength seawater (~34) held at ~26 °C and placed in large polyethylene bags that were then inflated with oxygen-enriched compressed air. Each bag was then stored in a polystyrene box and transported by road for 30 minutes from the hatchery to Matilda Bay, upon which the bags were immersed in the nearshore (< 1.5 m deep) waters of the estuary for 15 minutes to allow equilibration to ambient water temperature. The bags were then opened and the hatchery-reared post-larval prawns released along a 50 m stretch of the nearshore waters directly over a bed of the seagrass *Halophila ovalis* (Fig. 3.2.1). The first release (March 2015) occurred at ~12:00 hours, *i.e.* during the day, while the second release (March 2016) occurred at ~19:30 h, *i.e.* at least 30 minutes after sunset, subsequently referred to as 'night'. An onshore breeze was present

during both releases, which ensured that post-larval prawns remained within the release area. Salinity, water temperature and dissolved oxygen concentration were measured using a Yellow Springs International Model 556 water quality meter at three different points along the shore. The water at the release site had an average salinity of 34.48 and 33.61, water temperature of 25.14 and 27.88 °C and dissolved oxygen concentration of 5.42 and 8.81 mg/L in 2015 and 2016, respectively.

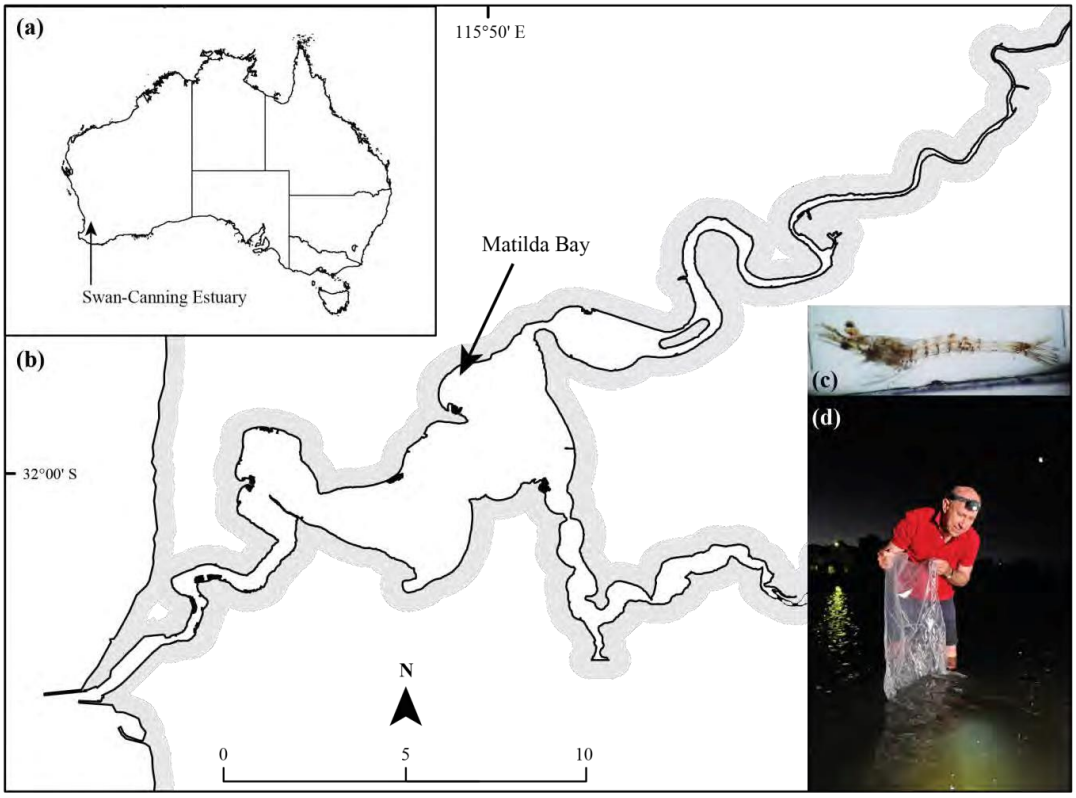


Fig. 3.2.1. Maps showing (a) the location of the Swan-Canning Estuary in temperate south-western Australia and (b) the location of Matilda Bay in the Swan-Canning Estuary where the hatchery-reared post-larval *Metapenaeus dalli* were released and where the fish fauna were sampled, and photographs of (c) a post-larval *M. dalli* ~ 4 mm TL at release size and (d) the former Western Australian Minister for Fisheries, the Hon. Ken Baston, demonstrating the release methodology. Photographs (c) and (d) provided by the Australian Centre for Applied Aquaculture Research and the Department of Biodiversity, Conservation and Attractions, respectively.

Sampling the fish fauna

The fish fauna of a 100 m stretch of the nearshore waters of Matilda Bay was sampled on four occasions in both March 2015 and March 2016 using a 21.5 m seine net. Sampling occurred during the day and night, prior to and after the release of the ~130,000 prawns (Fig. 3.2.2). The first post-release sampling was carried out 2 hours after prawns were released to allow enough

time for the post-larval prawns to be predated upon by the resident fish fauna, but not enough time for the restocked *M. dalli* to become so digested that they could not be accurately identified from the stomach contents (Rosenthal and Paffenhofer, 1972; Klumpp and Nichols, 1983). Prey of similar morphology to *M. dalli* post-larvae include other small crustaceans found in the estuary such as amphipods (*e.g. Corophium minor* and *Caprella scaura*), mysids (*e.g. Gastrosaccus sorrentoensis*) and other small penaeid-like species (*e.g. Palaeomonetes australis* and *Palaeomonetes atrinubes*), which are known to occur in the estuary (Wildsmith *et al*., 2011; Tweedley *et al*., 2016a).

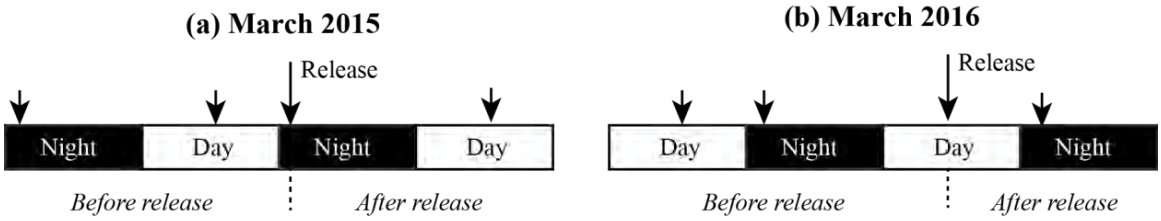


Fig. 3.2.2. Conceptual model showing the design of the sampling regime, with four sampling occasions (represented by arrows) before and after each of the two releases of ~130,000 hatchery-reared post-larval *Metapenaeus dalli* into Matilda Bay in the Swan-Canning Estuary of temperate south-western Australia.

On each of the eight sampling occasions over the two years (four in each year), a minimum of 12 replicate 21.5 m seine net samples were collected, distributed along the 100 m stretch of nearshore waters that encompassed the area where the prawns were released. Thus a minimum of 48 samples were collected in each year. The net comprised of two 10 m long wings (6 m of 9 mm mesh and 4 m of 3 mm mesh) and a 1.5 m bunt (3 mm mesh), fished to a maximum depth of 1.5 m and swept an area of 116 m2. Every fish collected in six of the > 12 samples was retained. The remaining samples were used to supplement the collection of less abundant species, which could potentially still be important predators of post-larval *M. dalli.* The overall objective was to collect 30 individuals, across a wide size range, of each fish species recorded on each of the eight sampling occasions. Once fish were collected, they were euthanized in an ice slurry in accordance with Murdoch University Animal Ethics Permit #RW2664_14 and subsequently frozen. The total number of individuals of each fish species in the six complete samples was recorded and the total length of each individual measured to the nearest 1 mm,

except when a large number of any one species was caught, in which case the lengths of a random subsample of 50 individuals were measured.

Multivariate analyses of fish faunal composition

The variation in fish assemblage composition on the eight sampling occasions was tested using a three-factor PERMANOVA (Year, Diel, Release), with two years (2015, 2016) and two times of day (day, night) and two times either side of the release (before, after). All factors were considered fixed and crossed and the null hypothesis of no significant difference among *a priori* groups rejected if $P < 0.05$.

Prior to statistical analysis, the variability in the numbers of individual species in the replicate samples was used to carry out dispersion weighting for each species, which down-weights the effects of those species whose numbers exhibited large differences among replicate samples due to schooling (Clarke *et al*., 2006). This was achieved by dividing the counts for each species by its mean index of dispersion (*i.e.* average of the variance to mean ratio in replicate samples) and ensures that all species have similar variability structures, and prevents the analyses becoming dominated by large outliers. These data were then subjected to a square-root transformation to down-weight the contributions of species with consistently high values (across replicates within a group) in relation to those with consistently low values (Clarke *et al*., 2014a).

These pre-treated data were then used to construct a Bray-Curtis resemblance matrix and analysed using three-way Permutational Analysis of Variance (PERMANOVA; Anderson *et al*., 2008) described above. Due to the low number of degrees of freedom in each of the factors, Monte Carlo testing, using the asymptotic permutation distribution was used to provide a more robust indicator of the level of significance. Trends in the data were visualised using a centroid non-metric Multidimensional Scaling Ordination plot (Clarke *et al*., 2014a). The plot was constructed from a distance among centroids matrix, which averages the samples representing a

particular *a priori* group (in this case Year × Diel × Release) in full-dimensional space from the Bray-Curtis resemblance matrix.

When a significant difference between the ichthyofaunal compositions of *a priori* group(s) was detected, Similarity Percentages (SIMPER; Clarke *et al*., 2014a) were used to identify those species that typified the ichthyofaunal composition of each group and those that were responsible for distinguishing between the fish compositions in each pair of groups. A shade plot, derived from the dispersion-weighted and square-root transformed data averaged for each year and diel combination, was constructed and used to visualise the trends exhibited by the abundance of all teleost species recorded. This shade plot is a simple visualisation of the frequency matrix, where a white space for a species demonstrates that that teleost was never collected, while the depth of shading from grey to black is linearly proportional to the abundance of the species (Clarke *et al*., 2014b; Valesini *et al*., 2014). Species are clustered based on their Bray-Curtis similarities and placed in optimum serial order, constrained by the cluster dendrogram (Clarke *et al*., 2014a).

Determination of dietary composition

The total length (TL) of each fish, caught on each of the eight sampling occasions, was measured to the nearest mm and weighed to the nearest 0.1 g. The fullness of each stomach was recorded on a scale of 0 (empty) to 10 (fully distended). All dietary items in each stomach were identified to the lowest possible taxonomic level using a dissecting microscope and taxonomic descriptions. The contribution of each dietary item to the total volume of the dietary components (%V) was then estimated visually (Hynes, 1950; Hyslop, 1980). The number of post-larval *M. dalli* in any stomach was counted (Fig. 3.2.3).

Dietary components were identified and allocated to one of 19 different minor dietary categories, which were grouped into seven major dietary categories (Platell and Potter, 2001; Lek *et al*., 2011). When a dietary item had undergone extensive digestion and could not be identified, it was classified as unidentifiable material (UID). This material, which constituted generally < 10% of the overall dietary volume (%V) of each species, was excluded from further

analyses. Sand was also not included in the analyses as presumably it was ingested during the capture of other prey. The percentage frequency of occurrence (%F) for each minor dietary category within each of the species of fish was calculated.

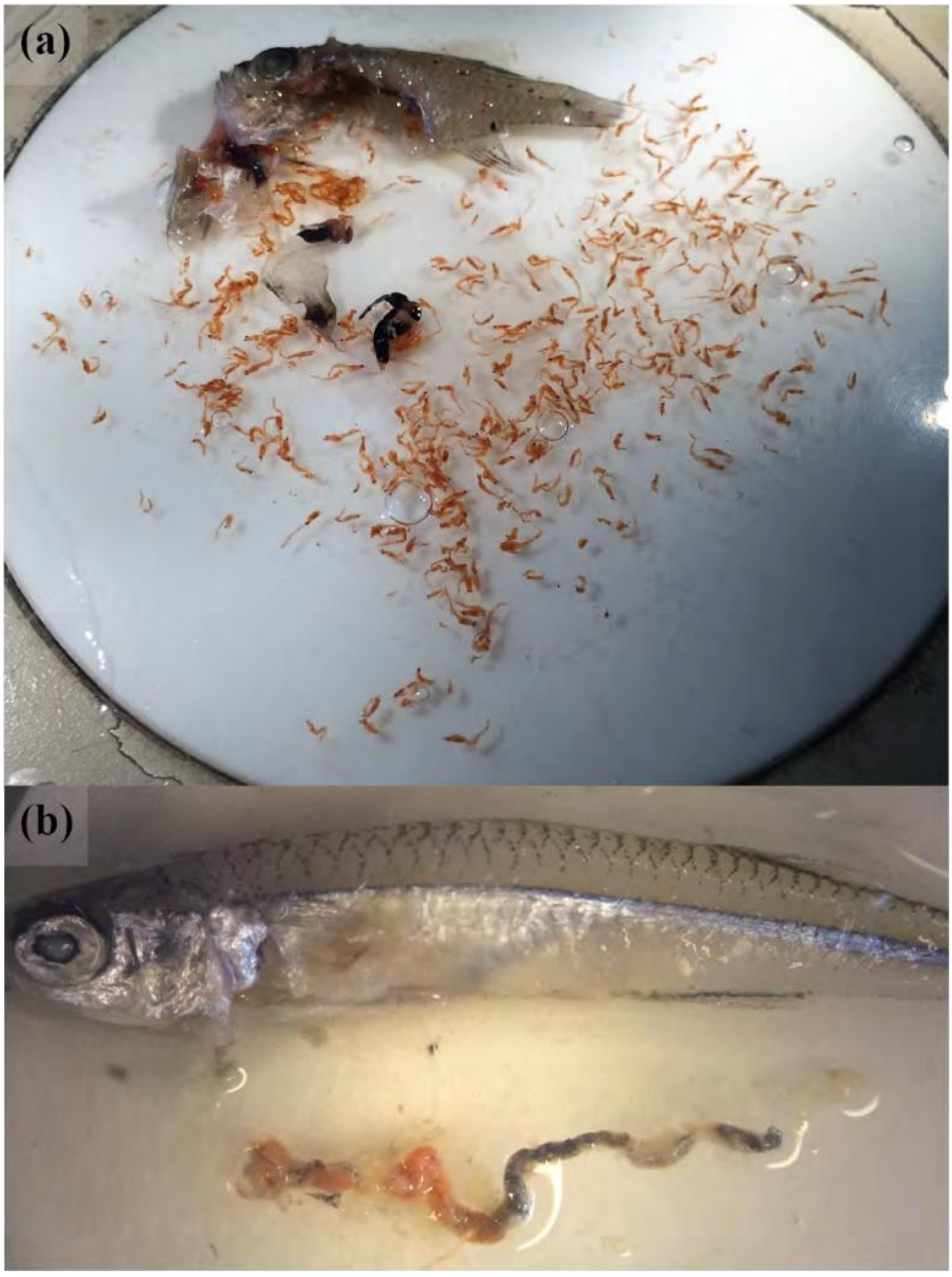


Fig. 3.2.3. Photograph of the stomach contents of (a) a 45 mm total length (TL) *Ostorhinchus rueppellii* showing large numbers of post-larval *Metapenaeus dalli* (n = 300) and (b) a 52 mm TL *Craterocephalus mugiloides*, with much smaller numbers (n = 2).

Stacked bar graphs were constructed to display the mean volumetric contributions of each major dietary category to illustrate whether dietary composition differed (i) among species and within a species (ii) with increasing body size and (iii) between day and night. The focus of this was

to identify which of the numerous fish species present at the time of the releases of 130,000 hatchery-reared post-larval *M. dalli* feed on post-larvae and, if so, at what body size and during what time of day.

Estimating predation

Relative estimates of the number of hatchery-reared *M. dalli* consumed by each of those predator species, identified earlier using the stack bar graphs, on each of the four sampling occasions after the releases (*i.e.* day and night following both a day and night release), were produced by bootstrapping using 1,000 replicates (Fig. 3.2.4). Firstly, the densities of each predator species found in the fish community samples (individuals /100 m^{-2}), excluding the proportion of fish from that species with an empty stomach (see Table 3.2.4), were multiplied by the average number of *M. dalli* consumed by individuals of that species. This latter value was determined by subtracting an estimate of the 'natural' predation from that found in the individual specimen. The estimate of 'natural' predation is the average number of *M. dalli* found in the stomachs of that species collected before the release of hatchery-reared prawns and thus is an approximation of the number of wild-spawned post-larval *M. dalli* consumed. Note that *Acanthopagrus butcheri* longer than 150 mm TL (see later) were excluded from the abundances as these fish did not consume released post-larval *M. dalli* (see Results).

The bootstrapping process generated 1,000 estimates for the number of *M. dalli* consumed by each predator species per 100 m^{-2} during the day and night following both a night and day release (four sampling occasions). The consumption rate for each selected species were then combined to produce a total predation rate immediately after the release and some hours later, enabling a quantitative assessment of the number of restocked *M. dalli* consumed following separate day and night releases of the same number of prawns. As the sampling efficiency of the seine net for each species is unknown, the estimated numbers of prawns consumed are relative.

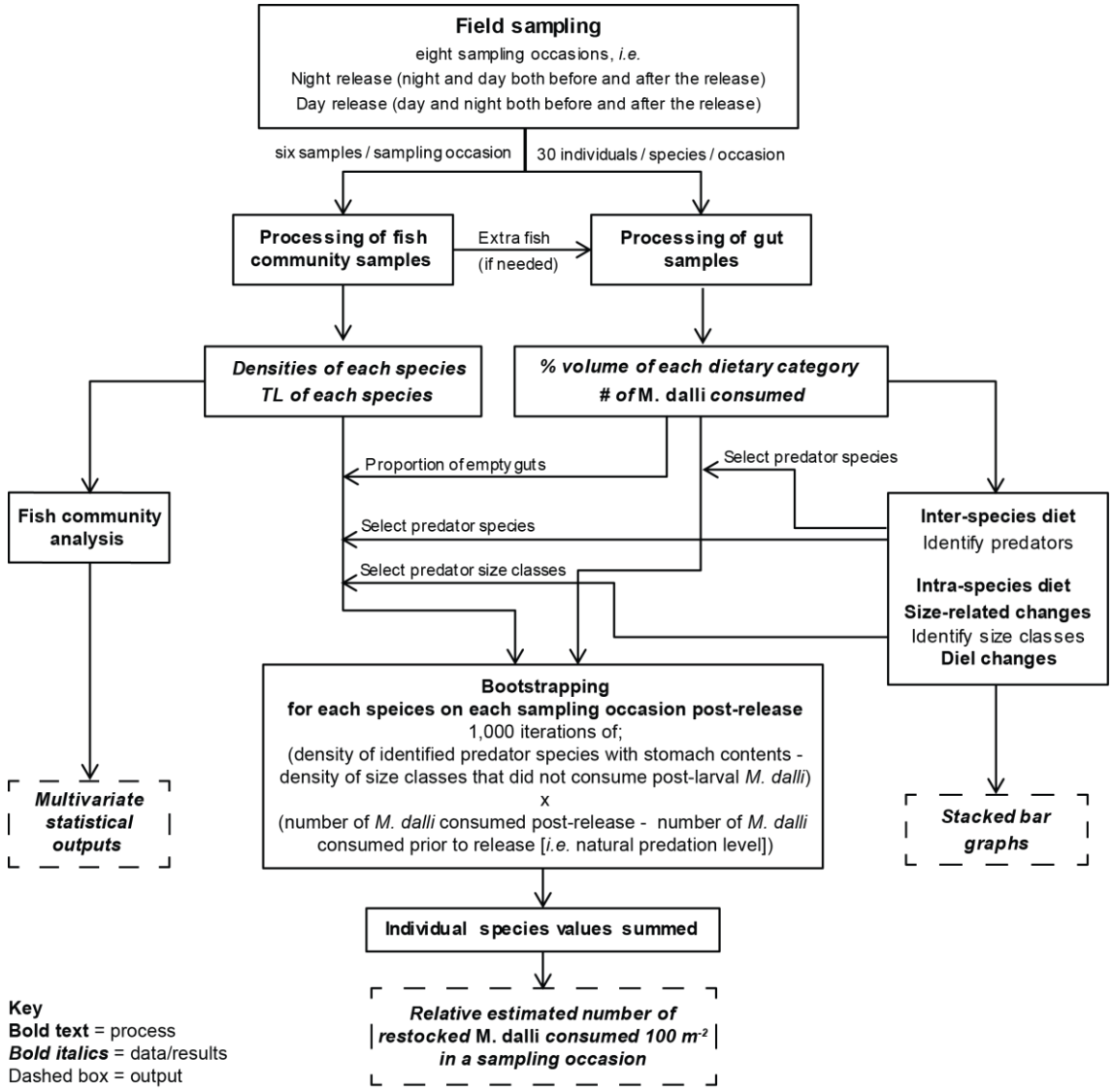


Fig. 3.2.4. Flow chart detailing the methodological approach used to derive the relative estimated number of *Metapenaeus dalli* consumed by teleost predators and confidence limits for consumption, following releases during the day and night.

Results

Fish faunal composition

A total of 15,576 teleosts from 24 species were caught in the samples where the entire fish community collected in the 21.5 m seine net was retained (Table 3.2.1). The total number of species recorded at night (21) was greater than in the day (16) and the total density was over five times greater at night than during the day (*i.e.* 475 vs 84 individuals 100 m-2). Although thirteen species (55%) were recorded at both times of day and, together, represented > 99% of

Table 3.2.1. Mean densities (D; numbers of fish 100 m^{-2}), standard error (SE), percentage contributions to the total catch (%) and rank by numbers (R) of the fish species caught during the day and night at Matilda Bay in the Swan-Canning Estuary during March 2015 and March 2016. Total numbers of species and overall mean density (number of fish 100 m^{-2}) are also shown. Abundant species, *i.e.* those that contributed > 5% to the total abundance highlighted in grey.

	Total				Day				Night			
Species	**D**	**SE**	**%**	**R**	**D**	**SE**	**%**	**R**	**D**	**SE**	**%**	**R**
Leptatherina presbyteroides	114.15	57.98	40.81	1	1.80	1.22	2.13	8	226.51	112.43	47.67	1
Atherinosoma elongata	53.43	25.52	19.10	2	1.65	0.69	1.96	9	105.21	49.28	22.14	2
Ostorhinchus rueppellii	49.14	6.71	17.57	3	21.30	5.89	25.26	1	76.98	9.06	16.20	3
Atherinomorus vaigiensis	24.95	5.12	8.92	4	15.73	6.99	18.65	3	34.16	7.13	7.19	4
Pelates octolineatus	14.19	4.49	5.07	5	20.15	8.56	23.89	2	8.23	2.46	1.73	6
Amniataba caudavittata	9.32	2.49	3.33	6	11.39	4.87	13.50	4	7.26	1.07	1.53	7
Acanthopagrus butcheri	5.39	1.03	1.93	7	2.30	0.55	2.73	6	8.48	1.80	1.78	5
Torquigener pleurogramma	4.85	0.88	1.73	8	5.78	1.20	6.86	5	3.92	1.28	0.82	8
Craterocephalus mugiloides	2.26	0.76	0.81	9	2.26	1.42	2.68	7	2.26	0.59	0.48	9
Favonigobius punctatus	1.35	0.24	0.48	10	1.40	0.38	1.66	10	1.29	0.31	0.27	10
Pseudogobius olorum	0.22	0.06	0.08	11	0.25	0.08	0.30	11	0.18	0.09	0.04	11
Hyperlophus vittatus	0.07	0.04	0.03	12					0.14	0.08	0.03	12
Rhabdosargus sarba	0.07	0.03	0.03	12	0.14	0.07	0.17	12				
Nematalosa vlaminghi	0.05	0.03	0.02	14					0.11	0.06	0.02	13
Sillago schomburgkii	0.05	0.03	0.02	14					0.11	0.06	0.02	13
Gerres subfasciatus	0.05	0.04	0.02	14	0.07	0.07	0.09	13	0.04	0.04	0.01	17
Cnidoglanis macrocephalus	0.04	0.03	0.01	17	0.04	0.04	0.04	14	0.04	0.04	0.01	17
Hippocampus angustus	0.04	0.03	0.01	17					0.07	0.05	0.02	13
Mugil cephalus	0.04	0.04	0.01	17					0.07	0.07	0.02	13
Filicampus tigris	0.02	0.02	0.01	17	0.04	0.04	0.04	14				
Platycephalus endrachtensis	0.02	0.02	0.01	17					0.04	0.04	0.01	17
Istiblennius meleagris	0.02	0.02	0.01	17					0.04	0.04	0.01	17
Arenigobius bifrenatus	0.02	0.02	0.01	17					0.04	0.04	0.01	17
Pseudorhombus jenynsii	0.02	0.02	0.01	17	0.04	0.04	0.04	14				
Number of species		**24**				**16**				**21**		
Overall mean density		**280**				**84**				**475**		

the total number of fish recorded, their densities and percentage contributions differed markedly (Table 3.2.1). In particular, the densities of the small bodied atherinids *Atherinosoma elongata* and *Leptatherina presbyteroides* were ~60 and 120 times greater, respectively, at night. This trend was also exhibited by some of the larger species, as the apogonid *Ostorhinchus rueppellii* and sparid *A. butcheri*, albeit to a lesser extent than the two atherinids. In contrast, the densities of species like the tetraodontid *Torquigener pleurogramma* and the tetrapontids *Pelates octolineatus* and *Amniataba caudavittata* were approximately twice as great during the day than night (Table 3.2.1).

Three-way PERMANOVA demonstrated that the fish fauna present in Matilda Bay differed significantly for Year, Diel and the Year × Diel interaction was also significant, but did not differ significantly before and after Release (Table 3.2.2). The percentage mean square for Diel (34%) was higher than any other term in the model, followed by Year (27%) and then their interaction (17%). The centroid nMDS plot shows the point representing samples collected during the day on the left, clearly separated from night samples on the right hand side of the plot (Fig. 3.2.5). Day samples from 2015 were widely separated from those for 2016, while night samples for the two years were overlain on each over, which explains the significant Diel × Year interaction (Table 3.2.2). Points representing the samples collected before and after the release in each Diel × Year combination where located in close proximity, explaining the lack of a significant difference for any term in the model with Release as a factor (Table 3.2.2; Fig. 3.2.5).

Table 3.2.2. Mean squares (MS), *pseudo-F* ratios (*pF*), components of variation (COV), and significance levels without (*P*) and with Monte Carlo testing (*P* mc) from a three-way PERMANOVA of the fish communities recorded in Matilda Bay in the Swan-Canning Estuary during March 2015 and March 2016. *df* = degrees of freedom. Significant results are highlighted in bold.

Factor	***df***	**MS**	**% MS**	***pF***	**COV**	***P***	***P* mc**
Main effect							
Diel	1	**12917**	**34.0%**	**11.20**	**490.15**	**0.001**	**0.001**
Year	1	**10241**	**27.0%**	**8.88**	**378.67**	**0.001**	**0.001**
Release	1	2409	6.3%	2.08	52.30	0.057	0.069
Interactions							
Diel × Year	1	**6523**	**17.2%**	**5.65**	**447.45**	**0.001**	**0.001**
Diel × Release	1	2221	5.8%	1.93	89.04	0.066	0.078
Year × Release	1	1532	4.0%	1.32	31.56	0.237	0.239
Year × Diel × Release	1	1000	2.6%	0.867	-25.54	0.549	0.534
Residual	40	1153	3.0%		1153		

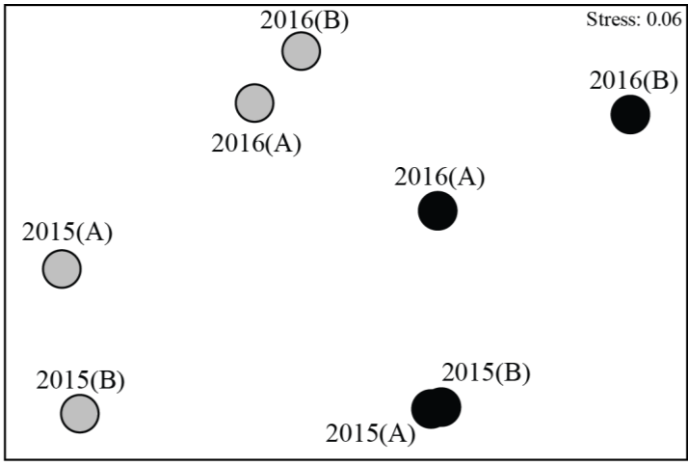


Fig. 3.2.5. Non-metric multidimensional scaling ordination plots of fish community composition constructed from a distance among centroids matrices output from a Bray-Curtis resemblance matrix. Data were derived from the dispersion weighted and square-root transformed abundances of each fish species recorded in Matilda Bay in the Swan-Canning Estuary for each Diel X Year X Release combination. Day (○); Night (●); Before release (B); After release (A).

SIMPER analyses demonstrated that the day samples in both years were typified by *T. pleugoramma*, whilst *O. rueppellii*, *L. presbyteroides* and the atherinids *Atherinomorus vaigiensis* typified the fish fauna at night, with this same suite of species also distinguishing between these two times of day (Table 3.2.3). Although the abundances of some species, such as the highly schooling atherinids *A. vaigiensis*, *Craterocephalus mugiloides, A. elongata* and *L. presbyteroides*, differed among years, clear diel differences were still present, with these species being far more abundant during the night than day (Fig. 3.2.6).

Table 3.2.3. Species identified by SIMPER analysis that typified (shaded) and distinguished (non-shaded) the fish faunas of Matilda Bay during the day and night in both (a) March 2015 and (b) March 2016. The text in superscript denotes the diel period each distinguishing species was most abundant in.

(a) 2015	Day	Night
Day	*Ostorhinchus rueppellii* *Torquigener pleurogramma* *Favonigobius punctatus*	
Night	*Atherinosoma elongata* Night *Leptatherina presbyteroides* Night *Atherinomorus vaigiensis* Night *Torquigener pleurogramma* Day *Ostorhinchus rueppellii* Night	*Ostorhinchus rueppellii* *Amniataba caudavittata* *Atherinosoma elongata* *Atherinomorus vaigiensis* *Leptatherina presbyteroides*

(b) 2016	Day	Night
Day	*Torquigener pleurogramma* *Pelates octolineatus* *Amniataba caudavittata* *Acanthopagrus butcheri*	
Night	*Ostorhinchus rueppellii* Night *Leptatherina presbyteroides* Night *Atherinomorus vaigiensis* Night *Atherinosoma elongata* Night *Pelates octolineatus* Day	*Acanthopagrus butcheri* *Ostorhinchus rueppellii* *Atherinomorus vaigiensis* *Leptatherina presbyteroides*

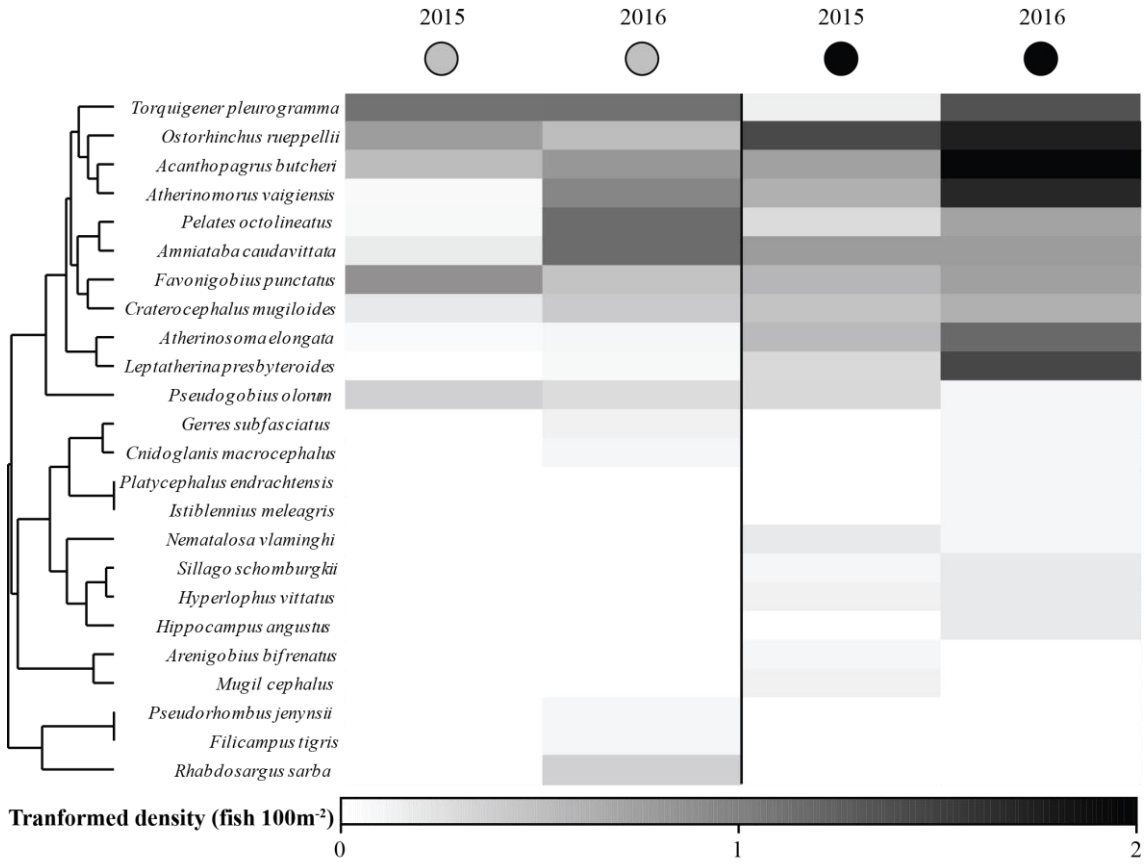


Fig. 3.2.6. Shade plot of the dispersion weighted and square-root transformed densities (100 m^{-2}) of each fish species recorded in Matilda Bay in the Swan-Canning Estuary for each Diel X Year combination. White areas denote the absence and grey scale the abundance of a species. Day (●); Night (●).

Overall dietary composition

The stomach contents of 1,208 individual fish, representing 16 of the most abundant species, were examined both before and after the releases of ~130,000 hatchery-reared post-larval *M. dalli*, with particular focus placed on elucidating dietary composition for the 929 teleosts collected after the releases. The number of stomachs processed for each species differed depending on its abundance at the time of sampling post-release, ranging from ≥ 100 for *O. rueppellii, A. butcheri, T. pleugoramma* and *A. caudavittata* to < 5 for the gobiid *Arenigobius bifrenatus*, clupeid *Nematalosa vlaminghi* and sparid *Rhabdosargus sarba* (Table 3.2.4). These last three species were excluded from further analysis due to the low number of stomachs processed, combined with their minor contribution to the total fish community at the sampling time.

A wide size range of fish was examined, ranging from 17 mm total length (TL) for a *Favonigobius punctatus* to 282 mm TL for an *A. butcheri* (Table 3.2.4). On average, the smallest species was *F. punctatus* (mean TL = 32 mm), and the largest were *G. subfasciatus* and *A. butcheri* (mean TLs = 157 and 141 mm, respectively). The last species also had the greatest size range (> 200 mm – 64 to 282 mm TL), whereas the smallest size range was 12 mm for *L. presbyteroides* (46 to 58 mm TL). All species had > 75% of individuals with food present in their stomach, except for the gobiids and atherinids, with particularly low values recorded for *A. elongata* (28%) and *L. presbyteroides* (11%; Table 3.2.4).

Among the seven major dietary categories, crustaceans were found in 75% of the teleost species (12), followed by molluscs (10), annelids and sipunculids (9) and macrophytes and teleosts (both 7). Crustaceans comprised by far the largest mean volumetric contribution of the stomach contents (42%), with macrophytes (9%) and molluscs (8%) being the next most important contributors to mean volume and the other four categories each contributing < 5% (Table 3.2.4).

In total, individuals of eight of the 13 teleost species (*A. vaigiensis, L. presbyteroides, C. mugiloides, O. rueppellii, F. punctatus, P. olorum, G. subfasciatus* and *A. butcheri*) had

Table 3.2.4. Mean percentage volumetric contribution (%V) and frequencies of occurrence (%F) of the different dietary items and categories (boldface) found in the stomachs of 16 teleost species recorded in Matilda Bay in the Swan-Canning Estuary from samples collected two hours after the release of hatchery-reared post-larval *Metapeaneaus dalli*.

	A. but		*A. cau*		*A. bif*		*A. vai*		*A. elo*		*C. mug*		*F. pun*		*G. sub*	
Dietary item	**%V**	**%F**	**%V**	**%F**	**%V**	**%F**	**%V**	**%F**	**%V**	**%F**	**%V**	**%F**	**%V**	**%F**	**%V**	**%F**
Algae	12.1	29.4	20.3	24.5	100.0	100.0	2.3	10.2								
Seagrass	16.9	44.0	0.1	2.1					3.2	4.5						
Macrophytes	29.0	67.0	20.4	26.6	100.0	100.0	2.3	10.2	3.2	4.5						
Bryozoa	0.0	0.9	0.9	2.1												
Bryozoans	0.0	0.9	0.9	2.1												
Platyhelminthes	0.1	0.9	0.1	1.1									2.6	4.3		
Sipunculids	0.2	0.9														
Annelids	6.1	17.4	1.8	10.6			0.1	1.7					17.0	26.1	10.0	25.0
UID ann./sip.	0.0	0.9														
Annelid/sipunculids	6.4	18.3	1.9	11.7			0.1	1.7					19.6	30.4	10.0	25.0
Metapenaeus dalli	0.7	2.8					46.7	71.2			40.0	40.9	7.8	8.7	2.5	25.0
Penaeus latisulcatus	0.4	4.6														
Alpheus spp.																
Brachyurans	2.3	8.3	0.7	4.3												
Mysids	1.4	2.8														
Isopods	5.5	24.8	31.6	59.6					4.5	4.5	5.0	9.1	0.4	4.3		
Amphipods	3.7	11.0	27.2	64.9			0.9	5.1			0.5	4.5	17.8	26.1	7.5	25.0
Copepods	0.7	0.9	1.0	5.3			26.4	37.3	4.5	4.5	3.2	4.5	7.8	13.0		
UID Crustaceans			0.1	1.1			4.7	5.1	25.9	27.3	22.7	22.7				
Crustaceans	14.7	38.5	60.6	73.4			78.7	86.4	35.0	36.4	71.4	77.3	33.9	47.8	10.0	50.0
Arachnids											0.5	4.5				
Hexapods	0.3	1.8					1.9	3.4			1.4	4.5				
Terrestrial arthropods	0.3	1.8					1.9	3.4			1.8	9.1				
Gastropods	4.3	20.2	2.7	10.6												
Bivalves	13.4	28.4	0.5	3.2					0.2	4.5			3.0	4.3	25.0	50.0
UID molluscs	2.7	5.5	1.1	3.2			0.7	1.7								
Molluscs	20.4	45.0	4.3	16.0			0.7	1.7	0.2	4.5			3.0	4.3	25.0	50.0
Teleosts	9.0	22.9	1.7	5.3			1.9	3.4	28.6	31.8					3.8	25.0
Teleosts	9.0	22.9	1.7	5.3			1.9	3.4	28.6	31.8					3.8	25.0
Detritus	1.4	3.7	5.6	9.6			3.2	3.4	8.9	9.1	9.1	9.1	8.3	17.4	12.5	25.0
Sand	10.7	33.9	0.3	5.3			0.2	1.7	5.9	18.2	8.2	13.6	33.0	65.2	38.8	75.0
UID	8.0	14.7	4.5	9.6			11.1	16.9	18.2	18.2	9.5	13.6	2.2	13.0		
Mean stomach fullness	4.2		5.1		6.0		2.1		0.7		1.1		1.1		3.2	
# stomachs	118		100		1		92		78		57		49		5	
# stomachs full	109		94		1		59		22		22		23		4	
% stomachs full	92.4		94.0		100.0		64.1		28.2		38.6		46.9		80.0	
Mean total length	141		87		78		66		51		47		32		157	
Median total length	130		70		78		66		52		47		33		159	
Total length range	64-282		48-195		78-78		40-104		41-67		34-56		17-50		135-175	

	L. pre		*O. rue*		*P. oct*		*P. olo*		*S. bur*		*T. ple*		*N. vla*		*R. sar*	
Dietary item	**%V**	**%F**	**%V**	**%F**	**%V**	**%F**	**%V**	**%F**	**%V**	**%F**	**%V**	**%F**	**%V**	**%F**	**%V**	**%F**
Algae			1.9	4.1	46.1	57.0					7.6	29.1				
Seagrass					4.4	6.3					4.8	19.8				
Macrophytes			1.9	4.1	50.5	59.5					12.3	43.0				
Bryozoa							3.0	20.0			0.1	1.2				
Bryozoans							3.0	20.0			0.1	1.2				
Platyhelminthes																
Sipunculids																
Annelids			1.2	3.1			14.0	40.0			1.0	4.7				
UID ann./sip.					1.3	1.3										
Annelid/sipunculids			1.2	3.1	1.3	1.3	14.0	40.0			1.0	4.7				
Metapenaeus dalli	26.4	45.5	10.4	12.2			5.0	20.0								
Penaeus latisulcatus																
Alpheus spp.											1.0	1.2				
Brachyurans											2.8	7.0				
Mysids																
Isopods	9.1	9.1	22.3	39.8	11.1	20.3	26.0	40.0			20.2	45.3			60.0	100.0
Amphipods			49.7	64.3	9.0	17.7	22.0	40.0			6.3	17.4			40.0	100.0
Copepods	17.3	27.3	3.2	10.2	5.9	15.2					0.5	5.8				
UID Crustaceans																
Crustaceans	52.7	63.6	85.6	90.8	26.0	30.4	53.0	60.0			30.9	52.3			100.0	100.0
Arachnids																
Hexapods											14.8	24.4				
Terrestrial arthropods											14.8	24.4				
Gastropods											13.8	36.0				
Bivalves									22.0	60.0	12.4	24.4				
UID molluscs			0.7	1.0	0.1	1.3										
Molluscs			0.7	1.0	0.1	1.3			22.0	60.0	26.3	52.3				
Teleosts			3.9	6.1							5.2	9.3				
Teleosts			3.9	6.1							5.2	9.3				
Detritus	18.2	18.2	3.6	4.1	20.6	26.6	20.0	20.0			1.6	3.5	50.0	50.0		
Sand	4.5	9.1	3.1	9.2	0.9	2.5			78.0	100.0	6.4	22.1	50.0	50.0		
UID	24.5	27.3			0.6	2.5	10.0	20.0			1.5	5.8				
Mean stomach fullness	0.2		4.5		2.5		6.0		1.3		1.0		3.4		3.2	
# stomachs	97		2		129		81		7		1		5		107	
# stomachs full	11		2		98		79		5		1		5		86	
% stomachs full	11.3		100.0		76.0		97.5		71.4		100.0		100.0		80.4	
Mean total length	51		179		47		74		34		110		152		106	
Median total length	52		179		45		47		34		110		156		99	
Total length range	46-58		100-258		23-75		22-190		24-43		110-110		139-160		35-165	

A. but, Acanthopagrus butcheri; A. cau, Amniataba caudavittata; A. bif, Arenigobius bifrenatus; A. vai, Atherinomorus vaigiensis; A. elo, Atherinosoma elongata; C. mug, Craterocephalus mugiloides; F. pun, Favonigobius punctatus; G. sub, Gerres subfasciatus; L. pre, Leptatherina presbyteroides; O. rue, Ostorhinchus rueppellii; P. oct, Pelates octolineatus; P. olo, Pseudogobius olorum; S. bur, Sillago burrus; T. ple, Torquigener pleurogramma; N. vla, Nematalosa vlaminghi; R. sar, Rhabdosargus sarba.

consumed post-larval *M. dalli* (Table 3.2.4). The volumetric contribution (%V) of *M. dalli* ranged from 0.7% in *A. butcheri* to > 25% for *L. presbyteroides* (26%), *C. mugiloides* (40%) and *A. vaigiensis* (47%). This prey species was most frequently recorded in the stomachs of *O. rueppellii*, *L. presbyteroides* and *A. vaigiensis* (%F = 12, 46 and 71%, respectively). Other small crustaceans, such as isopods and amphipods, comprised substantial components of the dietary volume of *O. rueppellii* (75.2%), *A. caudavittata* (60.6%) and *P. olorum* (48%; Table 3.2.4).

Macrophytes were consumed in relatively large quantities (*i.e.* > 20% by volume) by four species and, in particular, *P. octolineatus,* where it represented 50% of all stomach contents (Fig. 3.2.7). Gastropods and bivalves made a substantial contribution to the diets of *A. butcheri*, and *T. pleugoramma*, with bivalves also being consumed by *G. subfasciatus* and *Sillago burrus.* Annelids and sipunculids were consumed mainly by the gobiids *F. punctatus* and *P. olorum.* Teleosts were predated on by seven of the species, but only made a notable contribution to the volume of the overall stomach contents of *A. elongata*, with this small-bodied atherinid also feeding on the eggs of other species. Although poorly represented across the teleost species, terrestrial arthropods (*i.e.* arachnids and hexapods) represented 41% by volume of the stomach contents of *T. pleugoramma* (Table 3.2.4; Fig. 3.2.7).

Size related changes in diet

Post-larval *M. dalli* were consumed by all size classes of *O. rueppellii* (20-79 mm TL) and *A. vaigiensis* (40-99 mm TL; Fig. 3.2.8a,b). *Metapenaeus dalli* represented between 4 and 23% by volume of the stomach contents of *O. rueppellii* and, together with other crustaceans, contributed 97% of the diet in all, except the largest size class (> 60 mm TL = 63%), which also fed on teleosts (26%). Other crustaceans and *M. dalli* represented the vast majority of the diet of each size class of A. vaigiensis (*i.e.* 88 to 100%), but with no particular trend with increasing body length (Fig. 3.2.8b). These two dietary groups, and predominantly other crustaceans, also made significant contributions (>50%) to each size class of the gobiid *F. punctatus* (Fig. 3.2.8c).

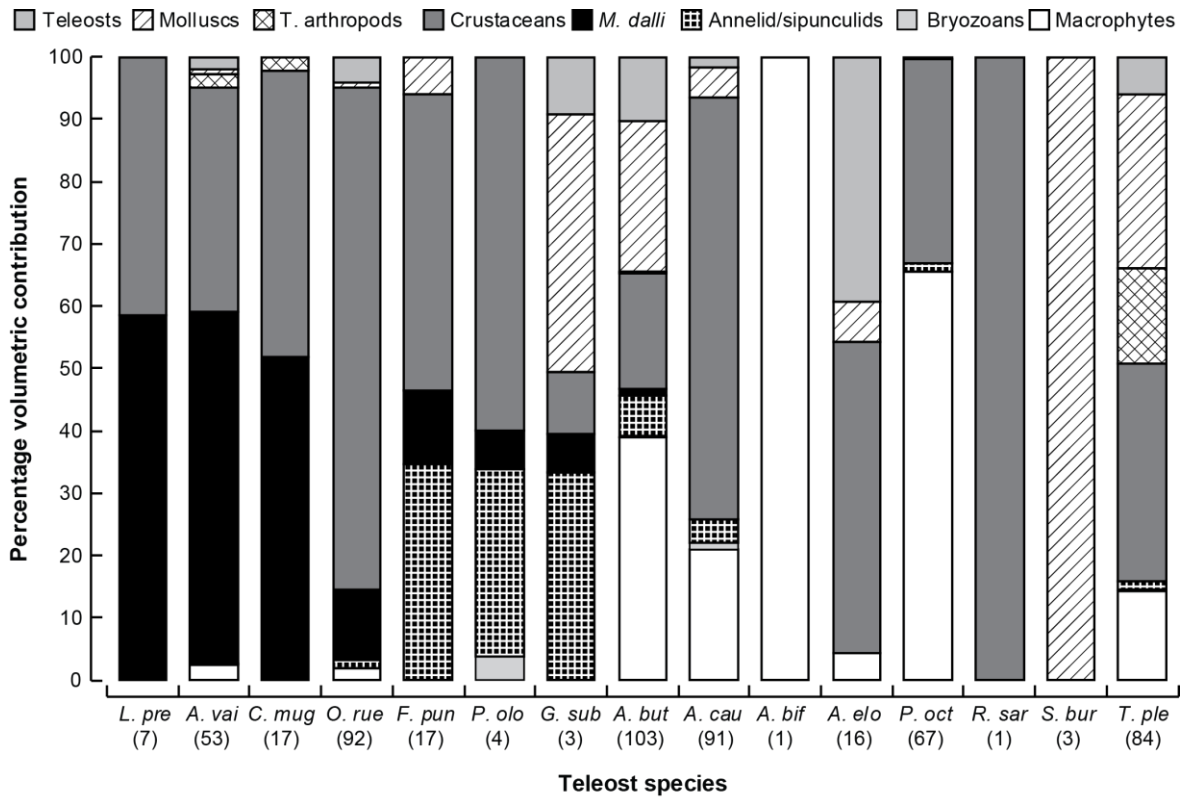


Fig. 3.2.7. The mean percentage volumetric contributions (%V) of various major dietary categories and *Metapenaeus dalli* to the diets of 15 teleost species recorded in Matilda Bay in the Swan-Canning Estuary, temperature south-western Australia. The number of stomachs of each species examined are given in parentheses. *L. pre, Leptatherina presbyteroides; A. vai, Atherinomorus vaigiensis; C. mug, Craterocephalus mugiloides; O. rue, Ostorhinchus rueppellii; F. pun, Favonigobius punctatus; P. olo, Pseudogobius olorum; G. sub, Gerres subfasciatus; A. but, Acanthopagrus butcheri; A. cau, Amniataba caudavittata; A. bif, Arenigobius bifrenatus; A. elo, Atherinosoma elongata; P. oct, Pelates octolineatus; R. sar, Rhabdosargus sarba; S. bur, Sillago burrus; T. ple, Torquigener pleurogramma.*

Post-larval *M. dalli* were only recorded in the 20-39 mm TL *F. punctatus* size class, while benthic prey, such as worms and molluscs, were present in substantial volumes in the larger size classes (80% in the 20-39 mm class, and 83.3% in the 40-59 mm class; Fig. 3.2.8). No size-related shift in dietary composition was detected in the two size classes of *A. elongata*, with both classes feeding largely on crustaceans and teleost eggs (Fig. 3.2.8d).

Among the larger-bodied species, only *A. butcheri* consumed post-larval *M. dalli*, albeit in relatively small amounts. The proportion of this prey item by volume decreased from 3% 50-99 mm TL fish to 1% in 100-149 mm TL fish and was not found in any of the larger fish (Fig. 3.2.8e). This matched a general decrease in the volume of crustaceans and teleosts consumed with increasing size and corresponding increase in molluscs, which represented 91%

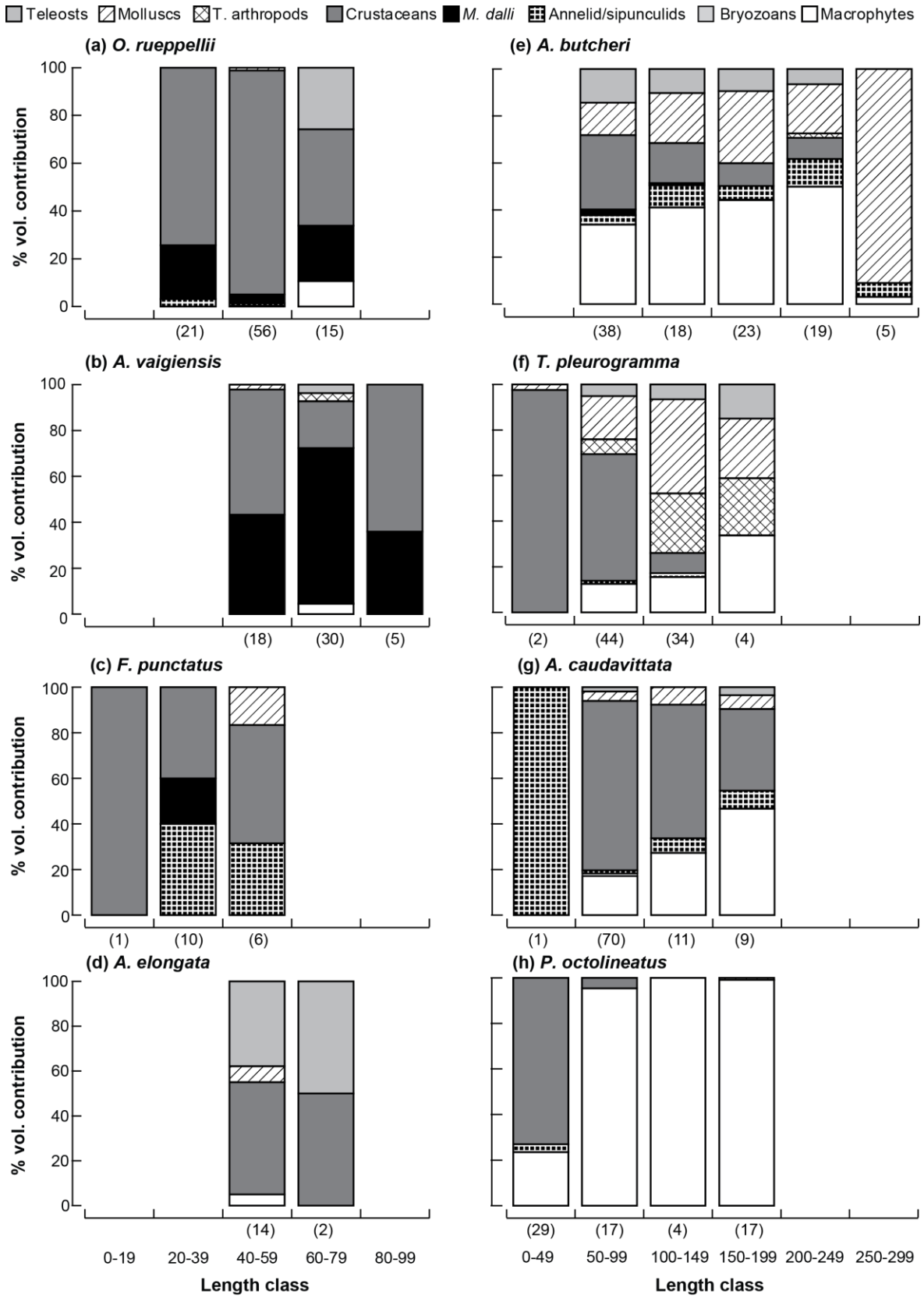


Fig. 3.2.8. The mean percentage volumetric contributions (%V) of various major dietary categories and *Metapenaeus dalli* to the diets of sequential total length (LT) classes of eight abundant teleost species collected after the release of hatchery-reared post-larval *M. dalli* into Matilda Bay. Note that plots for *Craterocephalus mugiloides* and *Leptatherina presbyteroides* are not presented as they were represented by a single size-class (see Fig. 3.2.7). The number of stomachs examined are given in parentheses. Full species names are given in Table 3.2.1.

By volume in the largest size class (Fig. 3.2.8e). The volumetric contributions of crustaceans in *T. pleurogramma* decreased markedly with ontogeny, from 98% in the smallest size class to 9 and 0% in the largest classes (Fig. 3.2.8f). In contrast, the volumetric contributions of macrophytes, terrestrial arthropods, molluscs and teleosts increased with increasing size. The contribution of crustaceans to the diet of the tetrapontids *P. octolineatus* and *A. caudavittata* also declined with increasing size, particularly for *P. octolineatus* (Fig. 3.2.8g,h).

Diel changes in diet

The percentage volumetric contribution of restocked *M. dalli* to the diets differed very little between the day and night for *O. rueppellii* (9 and 13%), *A. vaigiensis* (59 and 56%), *F. punctatus* (10 and 14%) and *A. butcheri* (1 and 1%) (Fig. 3.2.9). In contrast, the volume of *M. dalli* in the diet was greater at night than in the day for the atherinids *L. presbyteroides* and *C. mugiloides*. It should be noted, however, that the sample sizes were low in one of the diel periods for these species because of the number of empty stomachs. Almost the entire remainder of the diet of both species comprised other crustaceans.

The overall dietary composition for *A. elongata* and *T. pleurogramma*, and to a lesser extent *A. caudavittata and A. butcheri*, differed markedly between the day and night. However, *M. dalli* was either absent or represented a minimal proportion of the diet (~1%) in all of these species. *Atherinosoma elongata* fed on crustaceans (100%) during the day, but the proportion of this major prey category declined markedly at night (20%) and was replaced largely by teleost eggs (63%; Fig. 3.2.9). The diet of the tetraodontid *T. pleurogramma* comprised macrophytes, crustaceans, molluscs and teleosts, with substantial volumes of terrestrial arthropods also being consumed during daylight hours, while the volume of teleosts increased at night (Fig. 3.2.9). Similarly, the proportion of teleosts in the stomachs of *A. butcheri* increased from 2% during the day to 18% at night. While crustaceans comprised the majority of the stomach contents in *A. caudavitta* during the day (91%), it declined to 51% at night, while the reverse trend was true for macrophytes (1% in the day and 36% at night; Fig. 3.2.9).

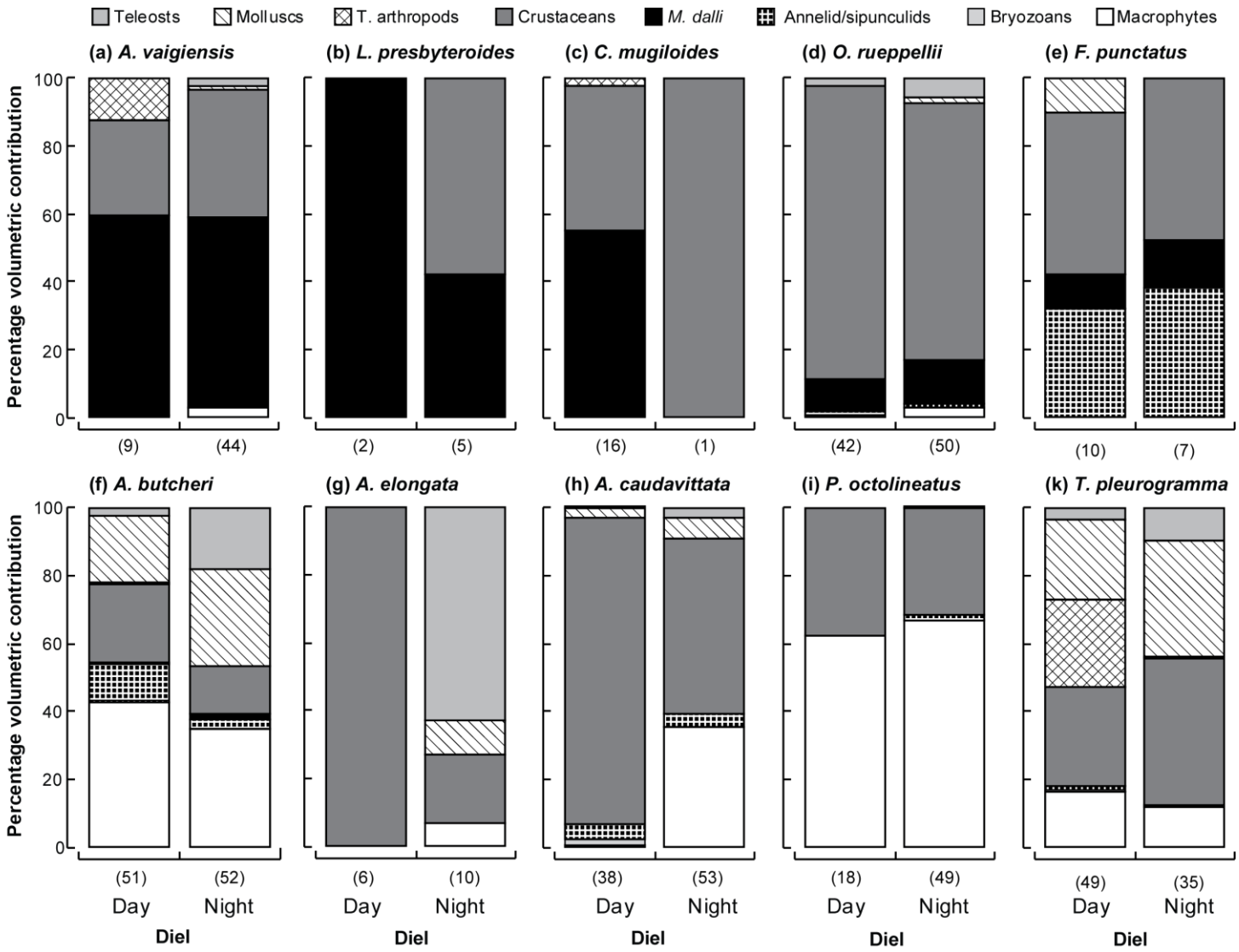


Fig. 3.2.9. The mean percentage volumetric contributions (%V) of various major dietary categories and *Metapenaeus dalli* to the diets of ten teleost species collected during the day and night after the release of hatchery-reared post-larval *M. dalli* into Matilda Bay. The number of stomachs examined are given in parentheses. Full species names are given in Table 3.2.1.

Number of M. dalli consumed

Of the six species found to consume relatively substantial numbers of prawns, *i.e. A. vaigiensis, L. presbyteroides, C. mugiloides, O. rueppellii, F. punctatus* and *A. butcheri*, only *A. vaigiensis* was found to have consumed post-larval *M. dalli* prior to the release of the hatchery-reared prawns, albeit in very small amounts (*i.e.* average of 0.45 prawns per fish with a maximum of 6). Of the fish collected after the releases, the highest mean number of *M. dalli* recorded in the stomachs of any single species, across any of the sampling occasions, was 28 (maximum number = 300; Fig. 3.2.3a) for *O. rueppellii* collected immediately following a night release. The majority of predation on *M. dalli* by *O. rueppellii* was done by individuals in the 40-59 mm TL length class, with all predation occurring after a night release. The atherinid *A. vaigiensis* consumed, on average 18 *M. dalli*, with a maximum of 87, also immediately after a night release. Almost all predation by *A. vaigiensis* was done by individuals in the 40-59 (30%) and 60-79 mm (69%) TL size classes. All predation by the smaller-bodied atherinids *C. mugiloides* and *L. presbyteroides* was undertaken by fish in the 40-59 mm TL size class. All predation by *C. mugiloides* occurred immediately after a night release, with individuals consuming, on average, 23 *M. dalli.* Although *L. presbyteroides* predated on *M. dalli* released during the night and day, most predation occurred following a day release, with individuals consuming 4.2 *M. dalli* immediately after the day release and 3.4 *M. dalli* that night. Very small numbers of *M. dalli* were also consumed by 20-30 mm TL *F. punctatus* during the day following a night release (0.25) and by the 50-99 and 100-149 mm TL size classes of *A. butcheri*, immediately after the day release (0.06 and 0.17, respectively).

Estimating predation rates on M. dalli

The total number of post-larval *M. dalli* consumed by teleost predators was estimated to be 288% greater following a night than day release, *i.e.* 2,447 vs 849 *M. dalli* 100 m^{-2} (Fig. 3.2.11; Table 3.2.5). Predation was greater immediately following a release than during the next diel cycle, regardless of whether the release was in the night or day. Following a night release, *O. rueppellii* consumed by far the greatest estimated proportion *of M. dalli* (91%; 2,080 *M. dalli* 100 m^{-2}), followed by *A. vaigiensis* (9%; Table 3.2.5). By the next day, the total predation had decreased greatly, with *O. rueppellii* still the dominant predator (93%), but the estimated total number of *M. dalli* consumed by all species declined to 149 individuals 100 m^{-2}. Following a day release, *A. vaigiensis* was found to be the main predator, responsible for > 99% of the estimated total number of prawns consumed (594 *M. dalli* 100 m^{-2}). This proportion decreased to 85 % during the night (216 *M. dalli* 100 m^{-2}), with the remaining 15% being predated upon by *L. presbyteroides* (Table. 3.2.5). Thus, although *O. rueppellii* consumed the greatest number of *M. dalli* overall, no *M. dalli* were found in their stomachs following a day release. In contrast, *M. dalli* was found in the stomachs of *A. vaigiensis* regardless of the time-of-release, albeit in lower numbers than for *O. rueppellii*.

Table 3.2.5. Estimated number (X) and 95% confidence limits (CL) of *Metapenaeus dalli* consumed in 100 m^2 of Matilda Bay in the Swan-Canning Estuary by each of the six predator species in the night and day (a) before and release (*i.e.* natural levels of predation), and after a (b) night and (c) day release of 130,000 hatchery-reared post-larvae. % represents the percentage contribution each species made to the total number of *M. dalli* consumed on each sampling occasion.

	(a) Before Release						**(b) After Night Release**						**(c) After Day Release**					
	Night			**Day**			**Night**			**Day**			**Day**			**Night**		
Species	X	±CL	%	X	±CL	%	X	±CL	%	X	±CL	%	X	±CL	%	X	±CL	%
O. rueppellii							2,079.92	57.03	90.94	148.93	6.81	93.32						
A. vaigiensis				2.37	0.15	100.00	207.19	5.87	9.06				594.45	18.23	99.94	215.87	4.56	84.91
L. presbyteroides										0.02	0.00	0.01	0.09	0.008	0.02	38.36	2.53	15.09
C. mugiloides										10.55	0.47	6.61						
A. butcheri													0.25	0.012	0.04			
F. punctatus										0.08	0.01	0.05						
Total	***0.00***			***2.37***			***2,287.11***			***159.58***			***594.79***			***254.23***		
Grant total				**2.37**						**2,446.69**						**849.02**		

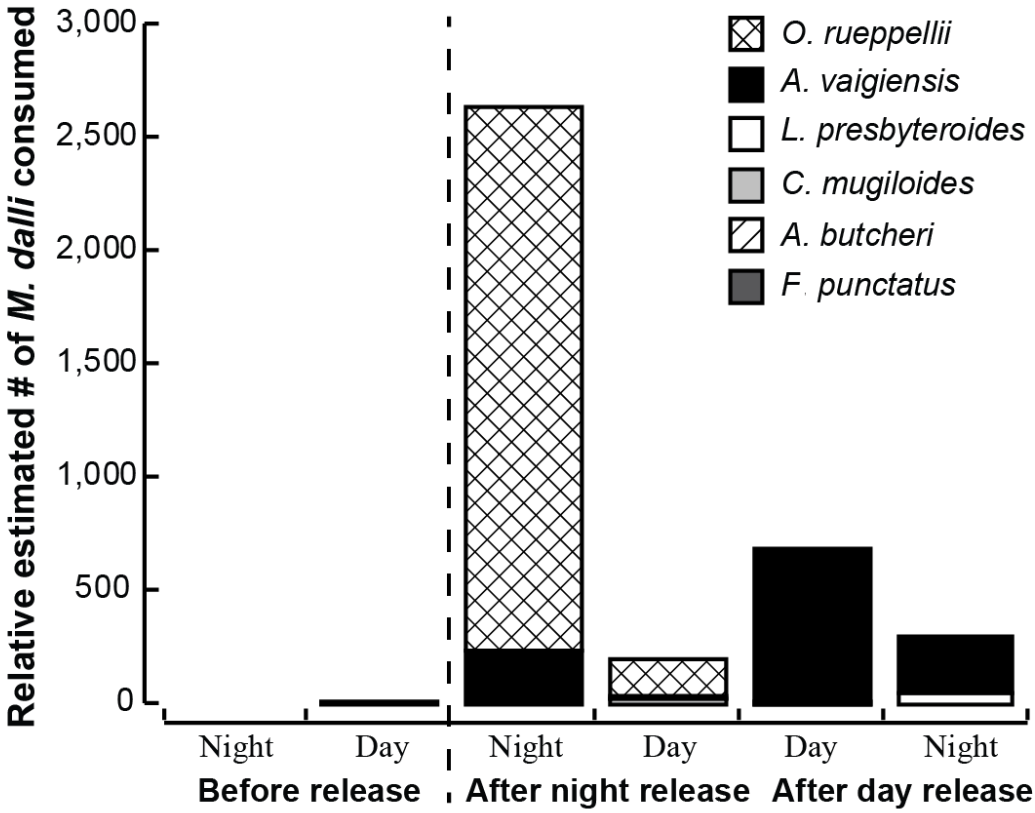


Fig. 3.2.10. The relative estimated number of post-larval *Metapenaeus dalli* consumed in 100 m^{-2} of Matilda Bay during the night and day, before and after the release of ~130,000 hatchery-reared *M. dalli*. Full species names are given in Table 3.2.1.

Discussion

This study developed and tested a method of estimating predation rates of newly released hatchery-reared postlarval Western School Prawns *Metapenaeus dalli* to identify the optimal time-of-day for their release into the Swan-Canning Estuary. The approach to estimating predation was to determine the relative density of the fish fauna before and after releases, examine the gut contents of likely predators and estimate the total relative number of postlarvae consumed follow a night and a daytime release. Although there was no replication of the night and day releases of prawns, the estimated relative predation rates of postlarval M. dalli were much greater when released during the night than the day. This was due mainly to large amounts of predation by *Ostorhincus rueppellii* immediately after the night release. As such, releasing the postlarval *M. dalli* during the day would improve success of the restocking.

Fish faunal composition

The largest influence on the composition of the fish fauna was found to be diel differences, with the total number of species and total density of fish at the release site being greater during the night (*i.e.* 21 species, 475 fish 100 m^{-2}) than the day (*i.e.* 16 species, 84 fish 100 m^{-2}). This increase in both number of species and total density of fish at night is consistent with that recorded in other estuaries (Gray *et al*., 1998; Griffiths, 2001; Arévalo-Frías and Mendoza-Carranza, 2015), as well as in littoral and pelagic zones in marine and freshwater ecosystems (Gibson *et al*., 1996; Pessanha and Araújo, 2003; Azzurro *et al*., 2007; Říha *et al*., 2015). Such a movement is typically been related to a number of factors including predator– prey interactions and feeding-related movements (Yeoh *et al*., 2017).

Atherinids dominated the teleost fauna in the nearshore waters of the release site, as they typically do in south-western Australian estuaries (Prince *et al*., 1982; Loneragan *et al*., 1989; Hoeksema *et al*., 2009; Valesini *et al*., 2014), and microtidal systems throughout the world (Franco *et al*., 2006; James *et al*., 2007; Tweedley *et al*., 2016b). The densities of three species of atherinids increased during the night, with those for *L. presbyteroides* and *A. elongata* being particularly marked, increasing by 126 and 64 times, respectively. The comparative absence of these small-bodied and highly schooling species during the day has also been recorded in the upper reaches of the Swan-Canning and in the nearby Moore River Estuary and is thought to occur in response to the threat of predation by piscivorous birds (Young *et al*., 1997; Hoeksema and Potter, 2006), such as the Little Black Cormorant (*Phalacrocorax sulcirostris*). This visual predator typically feeds in waters < 2 m deep and a study found that atherinids accounted for 92% of the prey items in their stomachs (Trayler *et al*., 1989).

The apogonid *O. rueppellii* also made a significant contribution to the teleost fauna (17.6%), with its densities also increasing by a factor of 3.6 during the night. These findings are consistent with those of Kapoor and Khanna (2004), who described members of this family as being nocturnal, and Chrystal *et al*. (1985) who recorded lower abundances of this species in the deep waters of the Swan-Canning Estuary at night, indicating that they immigrated to

shallower waters at this time. The reduction in the densities in shallow water is thought to reduce the likelihood of predation from larger predatory and avian predators (Říha *et al*., 2015). Trayler *et al*. (1989) found that *O. rueppellii* represented 61 and 20% of the number of individuals found in the stomachs of the cormorants *Phalacrocorax varius* and *Phalacrocorax melanoleucos* in the Peel-Harvey Estuary. Given the substantial diel changes in diet that occur in this species, this movement may also be food-related, due to emergence of crustaceans, such as amphipods, at night (Chrystal *et al*., 1985; Linke *et al*., 2001).

Although not a major focus of the current study, significant differences in fish composition were detected among years, albeit to a lesser extent than between diel phases. These differences were largely explained by the greater abundances of the, *A. elongata, L. presbyteroides*, and *A. vaigiensis* in 2016 than 2015. Species from this family are highly schooling, often forming extremely large mixed species schools in the shallow water of south-western Australian estuaries (Prince *et al*., 1982; Potter *et al*., 1986a).

Dietary composition

Overall dietary composition of the 16 fish species in Matilda Bay varied greatly, indicating that, these teleosts partition dietary resources in an effort to reduce competition (Prince *et al*., 1982; Gill and Potter, 1993; Humphries, 1993; Humphries and Potter, 1993; Platell *et al*., 2006). Crustaceans were identified as a major contributor to dietary composition, occurring in the stomachs of all 14 of the 16 species, (*i.e.* all except *A. bifrenatus* and *S. burrus*), which may be due to high calorific value of crustaceans (Thayer *et al*., 1973) and the increased abundance of small crustaceans in seagrass beds (*e.g.* Stoner, 1983). In the case of almost all species, greater proportions of this dietary category were consumed by individuals of a smaller size. This is especially evident in *P. octolineatus*, where crustaceans contributed 73% of the overall diet in fish ≤ 49 mm TL, but 4.6 and 0% in fish 50-99 and > 99 mm TL; which instead consumed macrophytes. This finding supports the work of (Bessey and Heithaus, 2015) in that fish > 120 mm TL are herbivores, but suggests juveniles feed on small crustaceans as does the congener

Pelates sexlineatus in seagrass beds in coastal waters of New South Wales (Sanchez-Jerez *et al*., 2002).

Of the 13 species that consumed crustaceans, eight were found to have ingested *M. dalli*, with this dietary item making a very substantial proportion of the several species of atherinids. *Atherinids* are highly abundant in marine, estuarine and freshwater environments worldwide and represent over 90% of total number of fish in the nearshore waters of estuaries in south-western Australia (Hoeksema *et al*., 2009; Tweedley *et al*., 2016b). Their high densities are thought to be achieved via resource partitioning, for example, Prince *et al*. (1982) found that when multiple species of atherinid co-occur, there were apparent differences in food preference. This is reflected in our data, to some extent, due to *A. elongata* consuming large quantities of teleost eggs and no *M. dalli*, which is the opposite of *L. presbyteroides, A. vaigiensis*, and *C. mugiloides*. The lack of marked differences in dietary composition among the last three atherinid species may be due to the fact that the dietary composition of the atherinids is considered reflective of the relative abundance of prey in the environment (Humphries and Potter, 1993) and *M. dalli* comprised > 50% of the diet of each species.

The dietary composition for *Ostorhinchus rueppellii* did not change greatly between day and night, with their diet largely comprising crustaceans (95.71% in the day, 88.36% at night), however, size-related changes were evident with copepods being consumed by small individuals and teleosts by larger fish. This latter finding mirrors that of Chrystal *et al*. (1985), which these authors associated with larger fish having larger mouths. Linke (2011) observed a similar trend in *O. rueppellii* found over bare sand in Shark Bay, with diets dominated by crustaceans, and increasing dietary contribution of teleosts with fish size. This dietary pattern was also attributed to their larger mouth size, allowing a corresponding increase in prey item size.

The stomach contents of *A. butcheri* were particularly diverse, comprising items from 19 different minor dietary categories, paralleling the findings for this species (Chuwen *et al*., 2007) and the opportunistic feeding strategies of sparids in general (Blaber, 1973; Buxton and Clarke,

1991; Booth and Buxton, 1997). A previous study of the diet of *A. butcheri* in the Swan-Canning Estuary by Sarre *et al*. (2000) demonstrated that this sparid consumed small volumes of *M. dalli* (1.1%), which was very similar to the value of 0.7% obtained in the current study. Moreover, this earlier study also recorded a marked shift in dietary composition with increasing size, *i.e.* decline in the contribution of crustaceans and increase in large bivalves, which is presumably due to larger fish having a bigger mouth and the ability to crush more robust prey (Sarre *et al*., 2000; Chuwen *et al*., 2007).

Predation on post-larval Metapenaeus dalli

On the basis of percentage volume, six of the 16 abundant teleost species collected at Matilda Bay were found to consume post-larval *M. dalli*, namely the apogonid *O. rueppellii,* the atherinids *A. vaigiensis, L. presbyteroides,* and *C. mugiloides*, the sparid *A. butcheri* and the gobiid *F. punctatus*. Of these species only one, *A. vaigiensis,* was found to consume post-larval *M. dalli* before either of the release events but did so in very small amounts. This indicates that these six teleost species will opportunistically feed on post-larval *M. dalli* and that the standing stock of these prawns in the sampling area was substantially lower before than after the release of ~130,000 hatchery-reared individuals. The view that individuals of these six teleost species will opportunistically feed on post-larval *M. dalli* when present at high densities is consistent with the generally broad array of dietary items that these species were found to consume and that fact that *M. dalli* were not specifically listed in previous dietary studies of these teleost species from the Swan-Canning Estuary (Prince *et al*., 1982; Chrystal *et al*., 1985; Gill and Potter, 1993), except in the case of *A. butcheri* where they made a very small volumetric contribution (Sarre *et al*., 2000). It is also relevant that the release of hatchery-reared post-larval *M. dalli* occurred at the same time similar sized wild individuals are present in this estuary (Broadley *et al*., 2017).

The volumetric contribution of post-larval *M. dalli* in the diets of the teleosts caught after the releases, were far greater in the atherinids *L. presbyteroides*, *A. vaigiensis*, and *C. mugiloides*

(58.6%, 56.4%, and 51.8%, respectively) than in *O rueppellii* (10.2%). However, in terms of number of prawns eaten, the vast majority were consumed by *O. rueppellii*. This indicates that the percentage volumetric contribution is not a good measure of the extent to which a species may predate on *M. dalli*, as it standardises across species morphology, size and amount of food an individual consumes. It is relevant that the contribution of *M. dalli* to the diet of *O. rueppellii* was similar during the day and night (9 and 13%, respectively), as was the average gut fullness (both 2.5%) yet substantially greater numbers of *M. dalli* were consumed at night than in the day (12.5 vs 3.4). This thus suggests that this species feeds during the night and day, but that *M. dalli* may be more susceptible to predation during darkness (see below).

Having accounted for the abundance of the predator species, and the number of *M. dalli* they consumed, an estimated ~99% the post-release predation on *M. dalli* was attributed to *O. rueppellii* (67.6 %) and *A. vaigiensis* (30.9 %). The estimated number of *M. dalli* consumed by *O. rueppellii*, after a night release, was 14 times greater immediately after the release at night than the following day. This difference can be attributed to the diurnal behaviour of *M. dalli*, like many other penaeids, are active during the night, and remain buried in the substrate during the day (Kutty and Murugapoopathy, 1968; Ruello, 1973; Wassenberg and Hill, 1994; Park and Loneragan, 1999; Bennett, 2014). As the post-larval *M. dalli* are nocturnal, they remain within the water column, rather than hiding on the benthos, when released during the night (B. Poh, personal observation) and are thus highly assessable to predators such as *O. rueppellii*. This predation risk is compounded by the nocturnal behaviour of *O. rueppellii*, whose individuals migrate into the shallow waters from deeper, offshore waters during the night (Chrystal *et al*., 1985). The following day, the amount of predation was reduced as the remaining post-larval *M. dalli* may have acclimated to the conditions at the release site, buried in the substratum and individuals of *O. rueppellii* would have migrated back into the offshore waters. The reduced predation by *O. rueppellii* following a daytime release of post-larval *M. dalli* can similarly be explained, as by night-time *M. dalli* had had time to adjust to the environment and avoid predation.

The presence of *M. dalli* in the diets of *Atherinomorus vaigiensis* before the release of hatchery-raised postlarvae indicates that *A. vaigiensis* are an opportunistic feeder, and not discerning *M. dalli* from other similar crustaceans on the benthos. The variation in predation by *A. vaigiensis* appears to be simply associated with the abundance of post-larval *M. dalli* in the area; predation levels are higher when the post-larvae are first introduced to the site, and are reduced in the following time period, where post-larval densities are reduced. Although the species targeted by the coastal populations were different (Tweedley *et al*., 2015), Hourston *et al*. (2004) also found *A. vaigiensis* to be similarly opportunistic in their diets in the coastal environment, their dietary composition changing with the seasons, and corresponding abundance of different prey items available.

Regardless of the time of release, predation was greatest immediately after the release and was substantially less during the next diel period. This is due to the combined effects of; (i) prawns being dispersed away from the exact point of release by currents/swimming and also being predated on, both of which reduce their abundance and make them less susceptible to opportunistic predators; and (ii) recovering from the stress of the release, adjusting to their environment and become less venerable.

In the current study, only fish below 150 mm total length were found to consume post-larval *M. dalli*, with most predation undertaken by teleosts in the 40-59 mm size class. Such a trend is likely due to the post-larval prawns being ~4 mm in total length (Jenkins *et al*., 2015) and the fact that the dietary composition of fish species changes with increasing body size (Bennett, 1989; Veale *et al*., 2015). In the case of the suction feeding *O. rueppellii* individuals of a large size would have greater jaw protrusion and the ability to generate greater inhalant currents and ingest larger prey with a greater calorific value (Barnett *et al*., 2006), in this case teleosts. Similarly, as *A. butcheri* attain greater body lengths their mouth size and ability to crush and consume larger prey, such as bivalves (Clifton and Motta, 1998; Sarre *et al*., 2000). If, however, as found for release programs of *Penaeus orientalis* in China and *P. japonicas* in Japan, increasing the size-at-release would alter the range of species and the size of individual fish that could predate on the hatchery-reared prawns (*i.e.* ~30 mm total length; Liu, 1990). It is thus

relevant that stocking of juvenile rather than post-larval *P. orientalis* resulted in an increase in the fishery yield, which Bell *et al*. (2005) attributed to better predator avoidance.

Conclusions

This study detected marked diel changes in the abundance and diet of teleost predators, and the number of hatchery-reared postlarval *M. dalli* consumed, indicating that releasing hatchery-reared postlarval *M. dalli* into vegetated nearshore waters during the day is likely to result in much less predation than at night. Although daytime releases potentially expose postlarvae to increased predation from *A. vaigiensis*, they would greatly reduce the predation risk posed by *O. rueppellii*, which consumed by far the greatest numbers of *M. dalli*. The diel variation in abundance and diet of individual fish species at the release site makes it difficult to select a release time when predation by all fish species is reduced. The methodological approach developed here helps to resolve this dilemma as it combines (i) the effect of predator abundance and (ii) the magnitude of predation on the target prey species, allowing quantitative comparisons to be made across species and times-of-release. Thus, a sound judgement, based on empirical evidence, can be made in determining the best time to release hatchery-reared *M. dalli*.

The method devised in the current study could readily be applied to determine whether alternative sites-of-release would provide a more suitable environment with lower predation pressure. While the abundance and survival of some penaeids, such as *P. esculentus*, is related to the presence of aquatic macrophytes (Loneragan *et al*., 2013b), this relationship has not been established for *M. dalli*. Moreover, the abundance of *O. rueppellii* and *A. vaigiensis*, which were responsible for 68 and 31% of the total predation at the release site, fluctuated in the nearby Peel-Harvey Estuary commensurate with changes in the extent and biomass of macrophytes (Potter *et al*., 2016). Therefore, releasing hatchery-reared postlarval *M. dalli* over unvegetated substrates may facilitate greater survival, due to reduced abundances of its key teleost predators over bare substratum during the day. However, as the abundance, feeding behaviour and diet of

fish differ in different habitats (Linke *et al*., 2001; Schafer *et al*., 2002), and unvegetated areas offer less shelter, further investigation will be required to determine the suitability of the bare sandy areas as potential release sites.

3.5. A methodology to develop aquaculture-based release strategies with an application to the Western School Prawn in the Swan-Canning Estuary: the Survival Maximisation-At-Release Tool (SMART)

This study has been published in an Honours thesis by Kyle Hodson.

Hodson, K. (2016). A methodology to develop aquaculture-based release strategies with an application to *Metapenaeus dalli* in the Swan-Canning Estuary: the Survival-Maximisation-at-Release Tool. Honours Thesis, Murdoch University.

Summary

The aim of this study was to create a tool to inform the development of an optimal release strategy, by evaluating site selection and time of release for the release of post-larval Western School Prawns (*Metapenaeus dalli*) in the Swan-Canning Estuary. This was achieved by developing the Survival-Maximisation-at-Release-Tool (SMART), a quantitative tool that collates variables considered to affect the survival of released *M. dalli* at potential sites around the estuary and determines a SMART score (0-100) for each potential release site and time (Month, Year, Day/Night). The major factors incorporated in SMART were water quality (salinity and water temperature), sediment composition and densities of conspecifics (total *M. dalli* and gravid *M. dalli*), competitors (*Penaeus* (= *Melicertus*) *latisulcatus*) and teleost predators. The scores for each factor were given equal weighting and the average of these used to calculate an overall SMART score for each site and time. Statistical analyses on the SMART scores determined that region of release was the most influential factor on the survival of released prawns, followed by year and then month, and that the salinity, sediment composition and predation variables had the most influence on overall SMART score. The optimal site of release identified was at Deep Water Point in the Lower Canning Estuary during the night in January 2014. Further enhancements to the SMART are identified and mechanisms for adapting this tool for its application to other species and/or ecosystems are discussed. An output of the SMART is also presented showing how the tool can be readily conveyed to diverse audiences to enhance discussions on optimal release strategies.

Rationale and aims

Global landings from wild capture fisheries have plateaued since the 1990s (Pauly *et al*., 2002), with many stocks suffering from overexploitation and the adverse effects of habitat degradation. This, coupled with an expected increase in demand for fish protein as a result of increase in global population (FAO, 2012), highlights the need for immediate management intervention in order to reduce fisheries impact and increase the sustainability of global fisheries.

One method currently used to address this need to reduce fisheries impact is the control of fishing effort (*e.g.* input controls such as spatial and temporal closures and gear restrictions, and output controls such as size limits, catch quotas and habitat protection and/or restoration). However, such interventions have the potential to place significant social and economic hardship on businesses and communities that previously had less restricted access to fish stocks (Mascia *et al*., 2010; Bennett and Dearden, 2014). Another potential intervention that is becoming increasingly popular is the use of aquaculture-based enhancement, a method that involves the release of cultured juveniles into an environment to enhance, conserve, or restore fisheries (Bell *et al*., 2005; Lorenzen, 2008a; Lorenzen *et al*., 2010; Taylor *et al*., 2017a). This method can be separated into three major categories: (i) stock enhancement, the release of hatchery seed to improve self-sustaining populations; (ii) restocking, the release of hatchery seed to rebuild severely depleted fish stocks; and (iii) sea ranching, the release of hatchery seed in put and take operations (Bell *et al*., 2008; Lorenzen *et al*., 2013).

Success in aquaculture-based enhancement programs, known hereon as release programs, has the potential to yield significant social, economic and ecological benefits for fisheries and their users. They can increase productivity beyond the level achievable by harvest management alone, create economic opportunities for fishery-livelihoods and provide incentives for active management of fisheries resources (Lorenzen and Garaway, 1998; Lorenzen, 2005; Pinkerton, 2011). Despite these potential benefits, along with significant investment in research and recent advances in the science of release programs, successes in establishing a viable population have been variable, often due to high mortality of released individuals shortly after release (Bell *et al*., 2005; Lorenzen, 2005; Armstrong and Seddon, 2008; Villemey *et al*., 2013). In order to

reduce this post-release mortality, a more informed approach to the development and implementation of release programs is required.

Multiple examples of release programs have linked the reduction of post-release mortality to significant improvements in success (Leber, 2002; Loneragan *et al*., 2006; Taylor and Suthers, 2008). For example for the release of Chesapeake Bay Blue Crab *Callinectes sapidus* in Northern America, it was determined that the survival of released crabs, their growth rate to sexual maturity, and likelihood of successful integration into the spawning stock was a direct function of the well researched and well developed release program (Zohar *et al*., 2008). Thus, optimising the success of a release program is vital to ensuring the post-release survival of released organisms (Blankenship and Leber, 1995; Lorenzen *et al*., 2010).

Despite an extensive search of the literature (Hodson, 2016), only a single study was identified that had attempted to use an objective, quantitative approach for selecting release sites for individuals. In this study, Carvalho and Gomes (2003) developed a tool to identify the optimal release site for the European Wild Rabbit *Oryctolagus cuniculus*, a species of conservation significance on the Iberian Peninsula, based predominantly on the attributes of habitat type, *i.e.* tall scrub, rocky terrain, grassland. The lack of similar studies is surprising as there has been an increase in the popularity of release programs around the world over the last thirty years (Taylor *et al*., 2017a). Given (i) the considerable financial costs in producing hatchery-reared individuals, (ii) the fact that many release programs are not successful (Bell *et al*., 2005; Lorenzen, 2005) and (iii) the lack of tools to aid in the selection of release sites and times, there is thus a need to develop a tool to enable the quantitative selection of appropriate release sites and times to maximise the survival of hatchery-reared individuals.

In light of the above, the broad aim of this study is to develop the Survival-Maximisation-At-Release-Tool (SMART), a quantitative tool to aid in the selection of the most appropriate release sites and times for hatchery-reared *M. dalli* in the Swan-Canning Estuary. In order to achieve this, the specific aims of this study are to:

1. Determine those factors associated with release site that are likely to affect the survival of released *M. dalli*.
2. Determine the spatial and temporal variation in these factors within the Swan-Canning Estuary.
3. Develop an approach for scaling the values for these factors at each site onto a common scale for inclusion into the final model.
4. Critically review the output of the model and its effectiveness in selecting a release site as well as identifying improvements that could be made to the model and its underlying data layers.

Methods

Site description

The Swan-Canning Estuary is a shallow, permanently open system located in the Perth metropolitan region of south-western Australia (Fig. 3.5.1). The estuary is ~50 km long, covers ~55 km^2 in area and comprises of a narrow entrance channel, two basins (Melville and Perth Waters) and the tidal portions of the Swan and Canning Rivers (Valesini *et al*., 2014). The majority of the estuary is shallow, *i.e.* < 2 m in depth, with extensive sand flats of ~0.5 m deep, however, it reaches a maximum depth of ~20 m in the entrance channel. Estuaries in south-western Australia are microtidal (tidal range <2 m) and experiences a typical Mediterranean climate, with hot, dry summers and cooler, wet winters (Gentilli, 1971), leading to pronounced seasonal variations in environmental conditions in the estuary (Tweedley *et al*., 2016b).

The estuary flows through the greater Perth metropolitan area, which supports ~78% of the 2.6 million people in the state of Western Australia (Australian Bureau of Statistics, 2015). The system has been extensively modified by anthropogenic activity (Commonwealth of Australia, 2002) and, as a result, has led to multiple stressors on the system, such as increased delivery of sediments and nutrients, in addition to changes in salinity and hydrological regime, including periodic hypoxia (Stephens and Imberger, 1996; Tweedley *et al*., 2016a). Despite these

perturbations, the estuary is highly valued for its aesthetic, cultural and social significance to residents and tourists of the area and recreational fisheries (Malseed and Sumner, 2001).

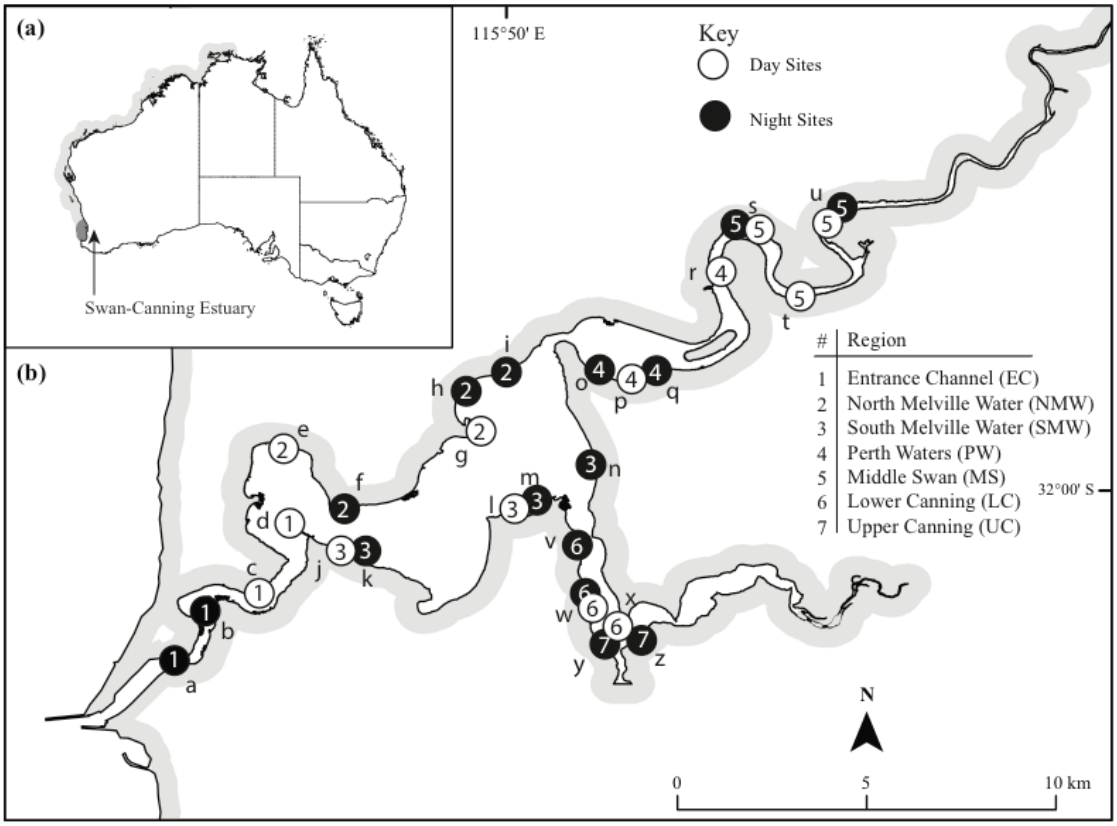


Fig. 3.5.1. Map showing (a) Australia and the distribution of *Metapenaeus dalli* in inshore marine waters (light grey) and solely in estuaries (dark grey) and the location of the Swan-Canning Estuary in south-western Australia and (b) location of the sites sampled during the night (●). The number inscribed in each site symbol denotes the region of the estuary that sites belongs to. Sites and abbreviations used later are as follows; a = Stirling Bridge (SB), b = Leeuwinn Barracks (LB), c = Chidley Point (CP), d = Point Walter (PTW), e = Claremont (C), f = Dalkeith (DK), g = Pelican Point (PP), h = Matilda Bay (MB), i = Kings Park (KP), j = Attadale (A), k = Point Walter (PTW), l = Heathcoate (H), m = Applecross (A), n = Como (CO), o = South Perth (SP), p = Perth Water (PW), q = Coode St. (CS) r = Windan Bridge (W), s = Maylands, (ML) t = Belmont (B), u = Garratt Rd. Bridge (GRB), v = Canning Bridge (CB), w = Deep Water Point (DWP), x = Mount Henry Bridge (FW), y = Freeway (FW), z = Rossmoyne. (R).

Rationale for selecting variables

To minimise the number of variables, only those that were deemed to be influential (positive or negative), on the survival of hatchery-reared post-larval *M. dalli*, were incorporated into SMART. The following section describes the rationale for the choice of variables included in the model, which were selected in conjunction with stakeholders and experts at that time from the Department of Parks and Wildlife, Department of Fisheries and the Australian Centre for Applied Aquaculture Research at South Metropolitan TAFE.

Water quality

Laboratory experiments investigating the influence of salinity and water temperature on the survival of cultured *M. dalli* demonstrated that both variables have a significant effect on the survival of the protozoal and mysis stages of *M. dalli* (Crisp *et al*., 2017b). Whilst this experiment was conducted using larval stages, the results are likely to also apply, to some extent, to the post-larvae. In addition to direct effects on survival, adverse salinities and water temperatures will slow growth due to increased energetic cost of homeostasis, and also impair foraging, slow movement and therefore increase the risk of predation.

In addition, due to changes in the volume of freshwater discharge and air temperature, salinity and water temperature vary spatially throughout the Swan-Canning Estuary and temporally throughout the year (Hoeksema and Potter, 2006; Tweedley *et al*., 2016b). Fauna living with the system, thus respond to these changes. For example, the growth of *M. dalli* is much faster when water temperatures were >20˚C, which was also the time when female prawns started to become gravid and spawning occurs (Broadley *et al*., 2017). As a result, it is important to account for spatial and temporal changes in water physico-chemistry within SMART and score positively sites that have optimal salinities and water temperatures to promote survival of hatchery-reared post-larval *M. dalli*.

Note that dissolved oxygen concentration was recorded during the major sampling program along with salinity and water temperature (see later) and that crustaceans are particularly sensitive to this physico-chemical variable (Wu *et al*., 2002; Dauvin and Ruellet, 2007; Tweedley *et al*., 2016a). However, while this variable was considered influential for the survival hatchery-reared post-larval *M. dalli*, no major differences in dissolved oxygen concentration were detected among spatially or temporally and no hypoxic events were recorded in the shallow waters. This variable was therefore excluded from the model.

Sediment composition

The proportion of particulate organic matter and inorganic grain sizes were found to affect the distribution and abundance of *M. dalli* in the Swan-Canning Estuary during summer, *i.e.* the time when hatchery-reared individuals would be released (Bennett and Dearden, 2014; Section 1.7). Moreover, laboratory experiments demonstrated that this species preferred sediments with a higher proportion of finer and/or lower portion of larger grain sizes and that prawns were better able to bury rapidly in finer sediment, thus reducing their exposure to predators (Bennett, 2014). Given their strong association with the benthos, it is not surprising that, in addition to *M. dalli*, other penaeid species have also been shown to exhibit a preference for particular sediments (Ruello, 1973; Branford, 1980; Somers *et al*., 1987).

Abundance of competitors

Competition among species occurs on one of more of three axes, *i.e.* food, space and time (Ross, 1986). Thus, competition for food and shelter may negatively affect the post-release survival of cultured individuals (Støttrup and Sparrevohn, 2007; Ochwada-Doyle *et al*., 2012). For example, it was observed that an increase in weight and yield of Brook Trout *Salvelinus fontinalis* was inversely correlated with the occurrence of White Sucker *Catostomus commersonii*, attributed to the competition caused by a strong dietary overlap between these two species (Tremblay, 1991). Along with *M. dalli*, the Western King Prawn (*Penaeus latisulcatus*) and Linda's Velvet Prawn (*Metapenaeopsis lindae*) are also found in the shallow waters of the Swan-Canning Estuary (Manning, 1988; Potter *et al*., 1991). Due to the similar morphology and likely also the diets of these species, it is possible these other species may compete for resources with the hatchery-reared *M. dalli* and lower their survival. While densities of up to 12 individuals 500 m^{-2} of *P. latisulcatus* were recorded at some sites over the study period, only low numbers of *M. lindae* (*i.e.* up to 1 individuals 500 m^{-2} and < 1% of samples) were recorded and thus the density of the latter competitor species was excluded as a variable from the model.

Abundance of conspecifics

A study on the hatching and survival of decapod species concluded that each species exhibits a preference for a particular range of salinities and water temperatures and that above or below these conditions larval mortality increased (Roberts, 1971). Likewise, Preston (1985) demonstrated that the larval survival of the Greentail Prawn (*Metapenaeus bennettae*) was greatest when the larvae were raised in conditions that mimicked the water temperature and salinity conditions present at the time of spawning. Both the total density of *M. dalli* (male and females) and the density of gravid females were considered a positive influence for predicting the survival of released post-larvae. This was because a higher density of gravid female *M. dalli* was thought to indicate the location of a preferred spawning ground and the preferred time of year for spawning in the natural population, thus one with optimal conditions for the hatching and survival of larval as well as likely juvenile *M. dalli*.

Predation by teleost and scyphozoan species

Predation by fish species has been identified as potentially the single greatest hurdle in short-term post-release survival (Hines *et al*., 2008; Støttrup *et al*., 2008). For example, (Buckmeier *et al*., 2005) found that ~27.5% of released Largemouth Bass (*Micropterus salmoides*) were lost to predation after just 12 hours and Dall *et al*., (1990) suggested that 25% of juvenile prawns in coastal inland waters are lost each week, mainly due to predation. Stomach content analysis of teleost species collected from the site of a release of hatchery-reared post-larval *M. dalli* (Poh *et al*., 2018), along with previous published studies of the diet of fish in the Swan-Canning Estuary, identified 19 species that may predate on hatchery-reared post-larval *M. dalli* (described in more detail below). In order to minimise the threat that these species pose on the survival of released *M. dalli*, the effect of predation from teleost species was included in the model to assist in selecting a site where their abundance and thus the influence of predation would be least.

When scyphozoans occur in high numbers, they collectively have a large clearance rates (*i.e.* the rate at which food particles can be ingested from the water column), significantly affecting the population size of zooplankton organisms (Hansson *et al*., 2005; Hosia *et al*., 2012). They have even been implicated in the collapse of fisheries, due to killing the larval stages (Hansson *et al*., 2005). As a result, the abundance of local scyphozoan species was included in the preliminary analysis of the abundance and magnitude of predators.

Sources of data

Metapenaeus dalli exhibit a strong seasonal cycle of reproduction in the Swan-Canning Estuary, with gravid females first appearing in the shallow, nearshore waters in November and disappearing in March (Broadley *et al*., 2017). A similar pattern of seasonality was also recorded in a separate study, in the same estuary, from 1977-1982, with gravid *M. dalli* recorded between November and early April (Potter *et al*., 1986b). Current aquaculture practices for *M. dalli* require heavily gravid females to be captured from the wild (*i.e*. broodstock collection), rather than conditioning broodstock populations held in the hatchery (Jenkins *et al*., 2015; 2017). Thus, the production of *M. dalli* in the hatchery is limited by the timing and duration of natural spawning. Given that larval development lasts for ~12 days and the post-larvae are being released 10-15 days after they metamorphose into post-larvae (Crisp *et al*., 2016), there is a one month lag between broodstock collected and the subsequent release of post-larvae. Although, some of the data used below were collected monthly over 36 months, only data from November to March in each of 2013/14, 2014/15 and 2015/16 were included in the model as this coincides with the production and release of hatchery-reared *M. dalli* (see Annex 3.5.1).

Water quality

Measurements of water physico-chemistry (*i.e.* salinity, water temperature and dissolved oxygen concentration) were recorded during the night at sixteen sites in the nearshore (< 1.5 m

deep) waters of the Swan-Canning Estuary (Fig. 3.5.1), ranging from the Stirling Bridge, in the entrance channel, as far up the Swan River as Garratt Road Bridge and as far upstream in the Canning River as Rossmoyne. Sampling was conducted every 28 days on a new moon on 31 occasions between October 2013 and March 2016. At each site, on each sampling occasion, salinity, water temperature and dissolved oxygen concentration were recorded at a depth of 1 m using a Yellow Spring International 556 Handheld Multiparameter Instrument.

Sediment composition

Two replicate sediment samples were collected from each of the 16 sites sampled during the night (Fig. 3.5.1) on a single occasion during the month of February 2014. Full details of the sampling and laboratory protocols are described in Bennett (2014), but a brief summary of the methodological approach is provided here.

Samples were collected using a cylindrical corer, which was 3.57 cm in diameter and sampled to a depth of 10 cm. In the laboratory, the percentage contribution of particulate organic matter (POM) was calculated using the Loss of Ignition method (Heiri *et al*., 2001), and converted to a percentage (Hourston *et al*., 2009). Fine sediment (*i.e.* particles <63 µm in diameter) was removed from the inorganic portion of the sediment by wet-sieving through a 63 µm sieve before drying and re-weighing. Finally, the remaining sediment was wet-sieved through six mesh sizes corresponding to the Wentworth Scale for grain size, *i.e.* 63, 125, 250, 500, 1000 and 2000 µm (Wentworth, 1922). The inorganic fraction for each grain size was then dried, weighed and their percentage contribution by weight determined for each sample.

While the current study only utilised the nearshore sediment composition data from February 2014 (summer), Bennett (2014) also collected sediment from the same sites in August 2014 (winter) and found that there was no statistical difference in composition. Given this lack of temporal changes in sediment composition in the nearshore waters, the data from summer 2014 was used throughout the model, *i.e.* in each month between November and March in 2013/14, 2014/15 and 2015/16. Although sediment composition was not expected to undergo a diel change, not all the day sites matched those sampled at night and thus the quantitative sediment

maps produced by Bennett (2014) were used to estimate the sediment composition at sites where no empirical data were available.

Abundance of penaeids, teleosts and scyphozoans

Faunal sampling at each of the 16 sites sampled during the night (Fig. 3.5.1) occurred on each of the 31 sampling occasions (see above) and was conducted using a hand trawl net that was 4 m in width and constructed from 9 mm mesh. Although the net was 4 m wide when fully stretched, its 'functional' width when trawling was, on average, 2.85 m and thus, when dragged for 200 m, covered an area of ~570 m^2. Two replicate trawls were conducted at each site, on each sampling occasion.

Upon completion of the drag, the contents of the net were emptied and the faunal identified and enumerated. Penaeids were immediately identified to species, sexed (*i.e.* females identified by presence of a thelycum and males by the presence of a petasma) and counted. In additional to being counted as part of the total number of *M. dalli*, gravid female prawns that were readily identified macroscopically by the appearance of a distinct green gonad (Crisp *et al*., 2017a) were also counted separately. After processing, all penaeids were returned alive to the water. The number of individuals of each teleost and scyphozoan species recorded, except in the case of the Spotted Hardyhead (*Craterocephalus mugiloides*), Elongate Hardyhead (*Atherinosoma elongata*) and Presbyter's Hardyhead (*Leptatherina presbyteroides*), which were grouped together as 'Athernidae' as, due to their large abundances and similar morphology could not be identified quickly enough at night in the field to enable them to be returned to the water alive. As with any penaeids, all teleosts and scyphozoans were returned to the water alive as per the instructions in Murdoch University Animal Ethics Committee permit #RW2566.

Scaling of data and inclusion in SMART

The aims of SMART are to objectively combine those variables, *i.e.* salinity, water temperature, sediment composition and the abundance of penaeids, teleosts and scyphozoans, that are thought to influence the survival of hatchery-reared post-larval *M. dalli* and thus should be considered in the selection of a suitable release site within the Swan-Canning Estuary. As many of these variables are measured on different scales, standardisation is needed to place all data on a common scale and thus allow comparability.

The first part of this section will explain how the data for each variable was standardised, with the second focusing on the combination of the variables and development of the SMART model. Focus is mainly placed on the production of SMART for data collected at night, as this was the original aim of the Thesis, and the diel period with the most comprehensive data. However, the changes in the development of the day and/or day vs night models will be highlighted.

Data standardisation

This section details the steps taken to standardise each of the variables included in SMART (Table 3.5.1). These steps are simplified in a flow chart below (Fig. 3.5.2), with additional detail provided in the text. Table 3.5.1 indicates those values that were included in the tool, and those that were removed after being deemed not to have a large enough effect on the post-release survival of *M. dalli* as explained in the text.

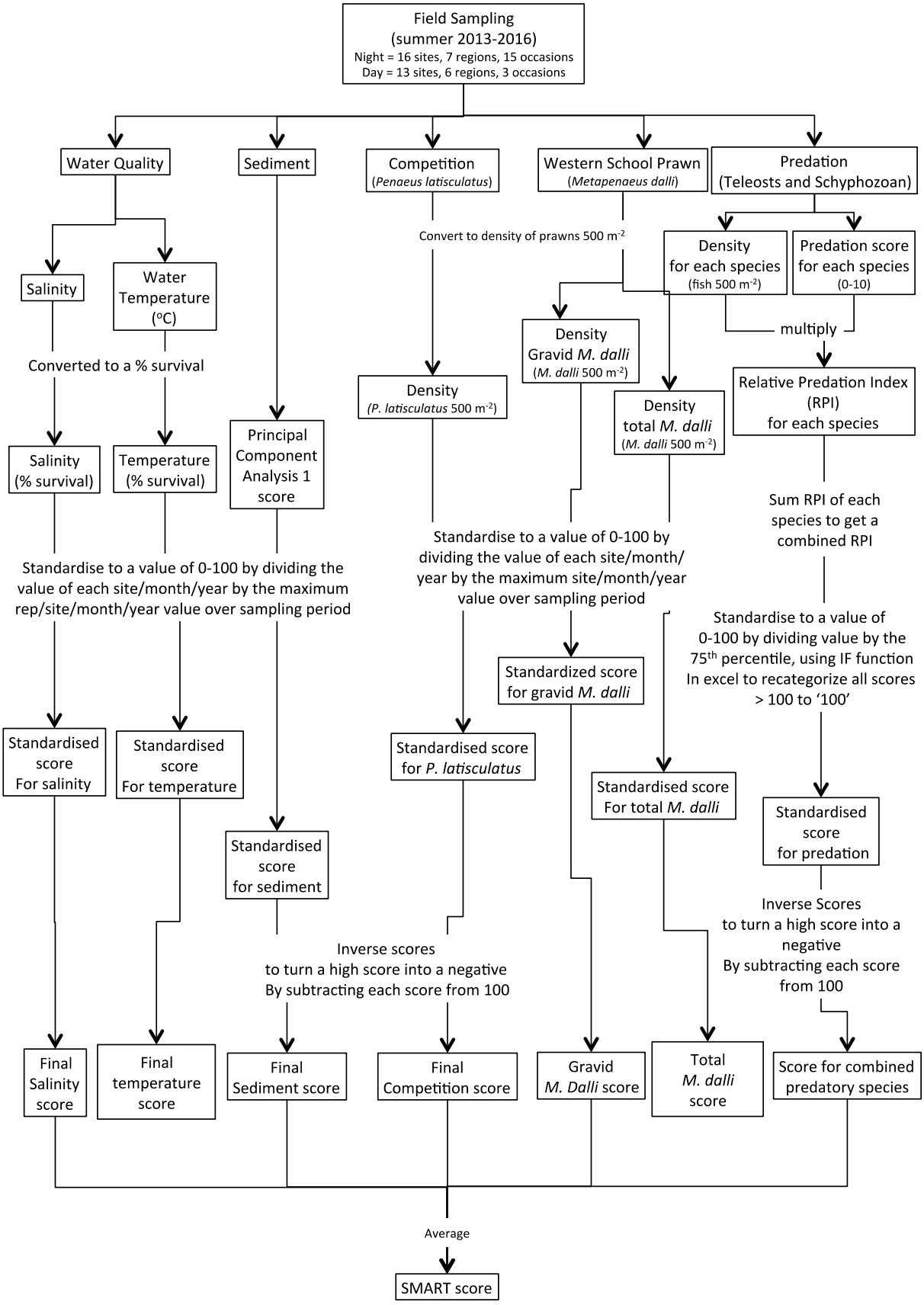


Fig. 3.5.2. Flowchart outlining all of the data and process used to produce SMART.

Table 3.5.1. Factors and variables considered for inclusion in the SMART.

Factors and Variables				
Category	**Variable**	**Units**	**Hypothesised Pos/Neg Effect**	**Included**
Water quality	Water temperature	ºC	+/-	Y
	Salinity	‰	+/-	Y
	Dissolved oxygen concentration	mg/L	+/-	N
Sediment	Sediment composition	PC1 Score	+/-	Y
Competitors	*Penaeus latisculcatus*	Density (500 m^{-2})	-	Y
Conspecifics	Total *Metapenaeus dalli*	Density (500 m^{-2})	+	Y
	Gravid female *Metapenaeus dalli*	Density (500 m^{-2})	+	Y
Predators	*Atherinosoma elongata*	Density (100 m^{-2})	-	Y
	Craterocephalus mugiloides	Density (100 m^{-2})	-	Y
	Leptatherina presbyteroides	Density (100 m^{-2})	-	Y
	Ostorhinchus rueppellii	Density (100 m^{-2})	-	Y
	Atherinomorus vaigiensis	Density (100 m^{-2})	-	Y
	Favonigobius punctatus	Density (100 m^{-2})	-	Y
	Acanthopagrus butcheri	Density (100 m^{-2})	-	Y
	Leptatherina wallacei	Density (100 m^{-2})	-	N
	Pseudogobius olorum	Density (100 m^{-2})	-	N
	Engraulis australis	Density (100 m^{-2})	-	N
	Amniataba caudavittata	Density (100 m^{-2})	-	N
	Torquigener pleurogramma	Density (100 m^{-2})	-	N
	Gambusia holbrooki	Density (100 m^{-2})	-	N
	Pelates octolineatus	Density (100 m^{-2})	-	N
	Afurcagobius suppositus	Density (100 m^{-2})	-	N
	Sillago burrus	Density (100 m^{-2})	-	N
	Haletta semifasciata	Density (100 m^{-2})	-	N
	Rhabdosargus sarba	Density (100 m^{-2})	-	N
	Urocampus carinirostrus	Density (100 m^{-2})	-	N
	Elops machnata	Density (100 m^{-2})	-	N
	Hyperlophus vittatus	Density (100 m^{-2})	-	N
	Sillago schomburgkii	Density (100 m^{-2})	-	N
	Pseudorhombus jenynsii	Density (100 m^{-2})	-	N
	Stigmatopora nigra	Density (100 m^{-2})	-	N
	Spratelloides robustus	Density (100 m^{-2})	-	N
	Mugil cephalus	Density (100 m^{-2})	-	N
	Hyporhamphus melanochir	Density (100 m^{-2})	-	N
	Gerres subfasciatus	Density (100 m^{-2})	-	N
	Phyllorhiza punctata	Density (100 m^{-2})	-	N
	Aurelia aurata	Density (100 m^{-2})	-	N

Water quality

Salinity

Controlled laboratory experiments demonstrated that salinity significantly influenced the survival of larval *M. dalli* (Crisp *et al*., 2017b). Larval *M. dalli* are likely to be more susceptible to the adverse effects of water physico-chemical conditions than the post-larval prawns that would be released (Pechenik, 1999), however similar studies on post-larvae *M. dalli* have not

been conducted. While hatchery-reared *M. dalli* may be more resistant to variable salinity, indirect consequences of salinity, such as slow growth due to the metabolic costs of osmoregulation, inability to forage efficiently or become slower moving would still result in more exposure to predation and thus increased mortality. The study by Crisp *et al*. (2017b) demonstrated that a salinity of ~35 was optimal for larval *M. dalli*, with both lower (30) and higher (40) salinities decreasing survival (Fig. 3.5.3).

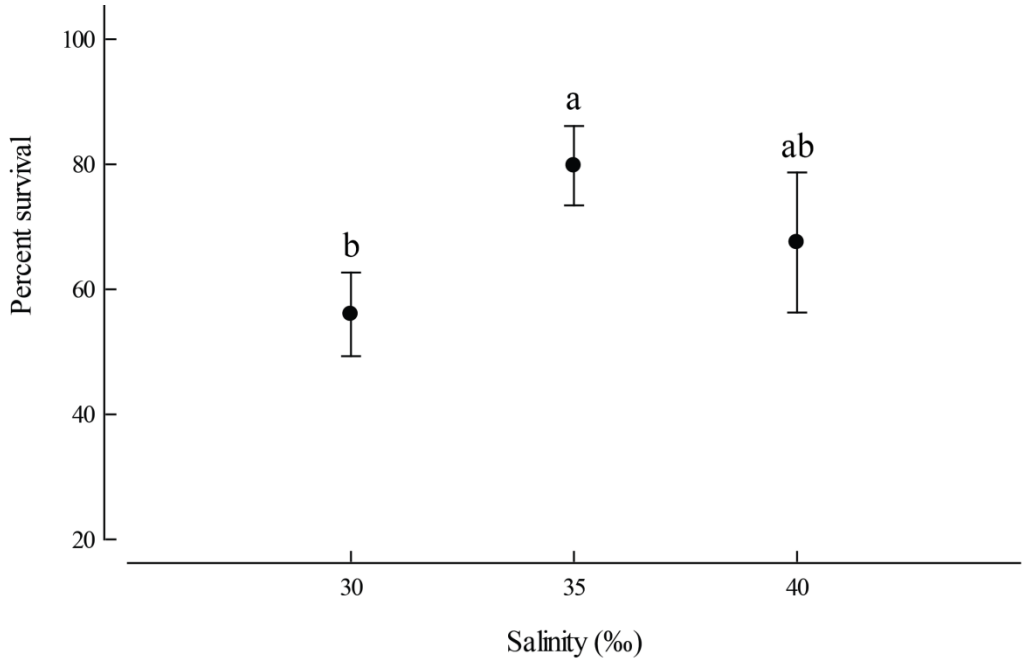


Fig. 3.5.3. Mean percentage survival (±95% CL) of *Metapenaeus dalli* larvae over a 48 h period from N VI sub-stage at three different salinities. Letters above error bars denote groups of samples identified by Tukey's HSD (p = < 0.05). Taken from Crisp *et al*. (2017b).

These data were used to estimate the influence salinity recorded at each site, on every sampling occasion, had on *M. dalli* percentage survival. This was done by extrapolating the results from Crisp *et al*. (2017b) to determine a percentage survival of *M. dalli* in the salinity at each site based on the salinity recorded at that site. To assign a percentage survival for salinities outside of those experimented by Crisp *et al*. (2017b) a linear line was followed to extrapolate, whilst this is likely not an accurate representation of the actual percentage survival it was only a small amount of the data that was outside of the known salinity percentage survival rates (~17% when November of 2013 is not included). These percentage values were then standardised onto the

common scale of 0-100 by dividing each value by the maximum percentage survival and multiplying by 100. This formula produced the final score for salinity used in the model.

Water temperature

Similarly to salinity, controlled laboratory experiments undertaken by Crisp *et al*. (2017b) showed that temperature significantly influenced the percentage survival of larval *M. dalli*. Survival rates were greatest at a temperature of 25.8 ˚C, with lower (22.6 ˚C) and slightly higher temperatures (29.4 ˚C), lowering survival albeit not significantly, while considerably higher temperature (32.6 ˚C) markedly reduced survival (Fig. 3.5.4). Data from this study was used in an identical manner to that in salinity to convert temperature recorded for each site into an expected percentage survival of *M. dalli*, which was then standardised. This standardised value was that representing temperature in the model.

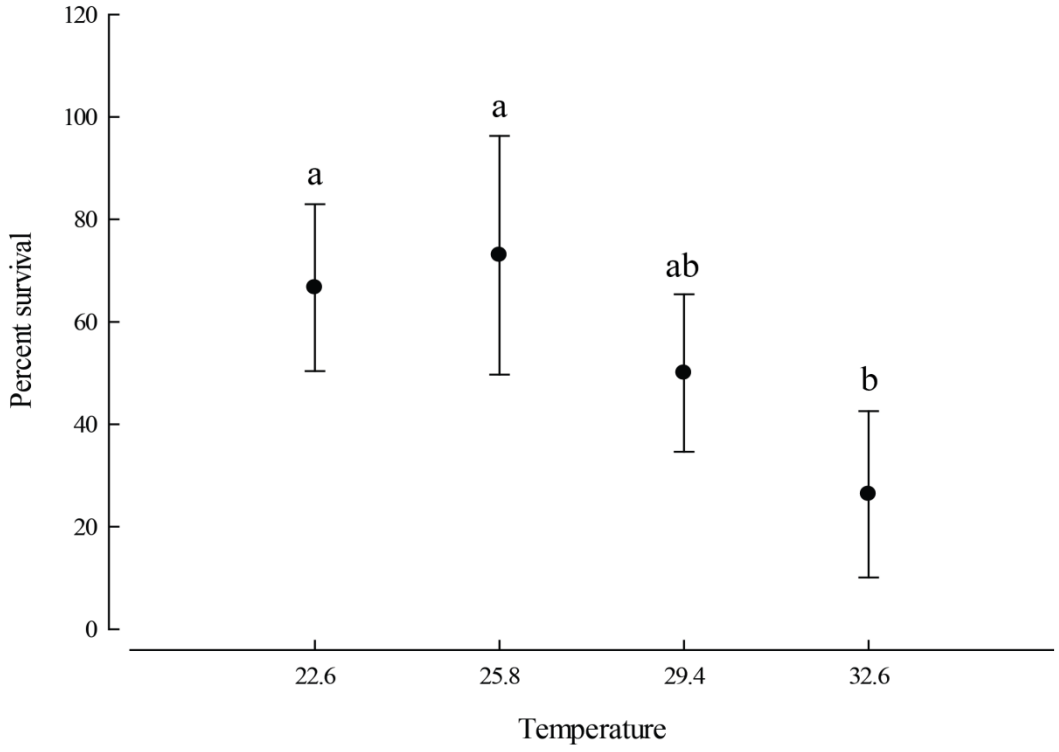


Fig. 3.5.4. Mean percentage survival (±95% CL) of *Metapenaeus dalli* larvae during to development from Nauplius VI to Mysis I at four different water temperatures. Letters above error bars denote groups of samples identified by Tukey's HSD (p = < 0.05). Taken from Crisp *et al*. (2017b).

Sediment composition

To test whether juvenile *M. dalli* prefer different sediment types, tank experiments were conducted, under controlled laboratory conditions, in which prawns were exposed to two different sediments types from the Swan-Canning Estuary (Garratt Road Bridge and Dalkeith; Fig. 3.5.1). Chi-square tests demonstrated that *M. dalli* preferred the sediment composition of Dalkeith over that at Garratt Road Bridge (see Bennett, 2014 for full details). As it is hard to construct and test an *a priori* hypothesis for the response of post-larval *M. dalli* to POM and each of the Wentworth grain sizes, the data for each sediment composition variable were square-root transformed and subjected to Principal Component Analysis. This test was used to objectively determine sites with similar sediment composition and place them on a linear scale, *i.e.* a principal component, PC1, (Leonard *et al*., 2006; Tweedley *et al*., 2015). The PC1 scores for each site range from ~40 to ~140 (see later) with the scores for Daliketh (~-25) and Garratt Road Bridge (~50) used to determine the orientation of the scores, *i.e.* that sites with negative scores considered to have a good sediment composition for post-larval *M. dalli*. These PC1 scores were inversed, so that positive scores indicated a good release site, and standardised to produce a score of 0-100 for each site. This standardised value was that representing sediment composition in the model.

Abundance of conspecifics and competitors

The abundances of (i) all individuals of *M. dalli*, (ii) solely gravid female *M. dalli* and (iii) all *P. latisulcatus* were converted to a density 500 m^2. Each of these variables was standardised (0-100) by dividing the density for a replicate by the maximum value recorded for the variable and multiplying by 100. While the densities of *M. dalli* were considered to be a positive metric (as densities had not reached the point where density-dependent effects are influential; see Broadley *et al*., 2017), densities of *P. latisulcatus* were considered a negative metric due to the density-dependent competition they impose. Thus, values for this variable were inversed by subtracting each score by 100, so that species with high abundances now had a score closer to

0 and *vice versa*, with these scores forming the basis of the competitor factor, while the scores for total *M. dalli* and gravid female *M. dalli* were used in the conspecific factor in the model.

Abundance of teleosts and scyphozoan predators

Each of the teleost and scyphozoan species considered likely to predate on post-larval *M. dalli* based on dietary analysis were assigned a 'predation score' ranging between 1, *i.e.* rarely likely to predate on post-larval *M. dalli* and, if so, only consume low numbers, and 10, *i.e.* likely to be a significant predator on post-larval *M. dalli* and able to consume larger numbers (Tables 3.5.2, 3.5.3). To reduce the number of variables in the model, only those teleost species with a predation score ≥3 were included, while the two scyphozoan predators were excluded as they would be less able to target the benthic post-larval than pelagic larval stages. Thus, the predatory species included in the model were the apogonid Western Gobbleguts (*Ostorhinchus rueppellii*), the atherinids Common Hardyhead (*Atherinomorus vaigiensis*), *A. elongata*, *C. mugiloides* and *L. presbyteroides* (noting in the samples collected at night the last three species were unable to be distinguished and recorded as 'Atherindae'), the sparid Black Bream *Acanthopagrus butcheri* and the gobiid Yellowspotted Sandgoby (*Favonigobius punctatus*).

The replicate densities of each of these species (fish 100 m^{-2}) in each site and month combination were averaged and multiplied by the predation scores of that species (Tables 3.5.2, 3.5.3) to calculate the Relative Predation Index (RPI), which aimed to determine the potential impact that species in that sample may have on the survival of hatchery-reared post-larval *M. dalli.* This quantitative index aimed to remove the bias of a species such as atherinids that may not, individually, predate on large amounts on post-larval *M. dalli*, but can occur in huge densities (Hoeksema *et al*., 2009; Valesini *et al*., 2009) and likewise those species that may occur in lower numbers, such as *O. rueppellii*, but have been recorded consuming large number of post-larval *M. dalli* (*i.e.* the 300 prawns recorded in the stomach of one *O. rueppellii*; Poh *et al*., 2018).

The RPI of each of the seven species was combined to give an overall teleost predator RPI before weighting took place. This method of weighting species was based on their potential predation impact, rather than giving each an equal contribution to the model. Thus, it circumvented the problem of not all species being found in each sample, which as the absence of a predator results in a positive score, could produce an artificially high score.

Whereas maximum score was used for the standardisation of the other variables in the model, it was believed that due to the highly schooling nature of fish, that standardisation using the 75^{th} percentile value would be a more appropriate method. This would prevent standardising all scores to potentially an outlier in the data and was deemed preferable over a power transformation, *e.g.* square-root, fourth-root or Log(x+1). The 75^{th} percentile of the combined RPI was calculated and used in the standardisation process detailed above. Note that as this resulted in some values being > 100 these were modified to read 100, *i.e.* the maximum possible score. Finally, like the effect of competition by *P. latisulcatus*, predation was deemed to have a negative effect on the survival of post-larval *M. dalli* and thus the standardised combined RPI scores were inverted and used in the model to represent the predation factor.

Calculation of the SMART score

As mentioned above, this section focuses predominantly on the production and development of the SMART using the night data, as this is the most comprehensive data set. Differences in the day model and also a comparison of sites where both day and night sampling overlapped will be highlighted at the end of the section.

The standardised (and in some cases inversed) data for each of the five factors, *i.e.* water quality (salinity and water temperature), sediment composition (PC1 score), competitors (density of *P. latisulcatus*), conspecifics (density of all *M. dalli* and gravid *M. dalli*) and predation (the combined RPI of the seven teleost species) were averaged to give a final SMART score which ranged from 0-100 (0 being totally unsuitable for the survival of hatchery-reared post-larval *M. dalli* and 100 being optimal) for each site and time. Note that in the case of water quality

and conspecifics where the factor is comprised of more than one variable, the average of the variables for a site x month combination was calculated and used as the value for the factor.

Analysis and interpretation of the SMART score

Although the study aimed to be able to distinguish among individual sites in a given month, there were not enough replicates (*i.e.* 1-2) at that level to enable a robust statistical interpretation of the results. This is because the development of SMART was not anticipated when the requisite sampling regimes were devised and, in any case, would have required the collection and processing of far larger numbers of samples, which may not have been financially viable. As a result, sites were pooled into regions based on their location in the estuary (see Fig. 3.5.1), which provided enough replicates for analysis at various levels.

Prior to undertaking statistical analysis, the SMART scores were assessed using the R software package (R Core Team, 2014) to ascertain the type of transformation required, if any, to meet the test assumptions. The extent of the linear relationship between $\log_e$ (mean) and $\log_e$ (standard deviation) of all groups of replicate samples was determined and then using slope criteria provided by Clarke and Warwick (2001) an appropriate level of transformation was selected. This analysis indicated that, in all cases, the values for the SMART scores did not require transformation.

Each of the following statistical analyses was performed using PRIMER v7 multivariate software package (Clarke and Gorley, 2015), with the PERMANOVA+ add on module (Anderson *et al*., 2008). Although region was the main factor of interest, differences among months (November to March) and summers (2013/14, 2014/15 and 2015/16) were accounted for so that their confounding influence could be quantified.

The final SMART scores for the night sites, used to make a Euclidean distance matrix, which was, in turn, subjected to a three-way Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson, 2001) to test whether the SMART scores differed significantly among Region (7 levels; Entrance Channel-Upper Canning), Month (6 levels; November-March) and Year (3 levels; 2013/14, 2014/15 and 2015/16). The null hypothesis of no

significant differences among each term was rejected if the significance level (p) was < 0.05 and the relative influence of each factor in the model was quantified using the magnitude of the mean squares. In the event that a significant difference was detected in a main effect or interaction term, a pairwise PERMANOVA was conducted to elucidate the levels of the term on the model that were responsible for the differences. The extent of any significant differences among *a priori* groups were determined by the magnitude of the test statistic (t).

Bar and line graphs were produced to provide a visual representation of the change in SMART scores among significant factors and/or interactions. Shade plots (Clarke *et al*., 2014b) were produced using PRIMER v7 to visually display the SMART scores, in combination with the values for each factor and its component variables to illustrate why samples received a good or bad SMART score. These plots are a simple visualisation of the frequency matrix, where a white space for a score/factor/variable demonstrates that the score/factor/variable had a score of 0 and thus the site/region/month/year was totally unsuitable for the release of hatchery-reared post-larval *M. dalli* and the depth of shading from grey to black is linearly proportional to the score for the score/factor/variable. Black cells indicate that the score was 100 and thus that site/region/month/year was the optimal place or time to release the cultured prawns. Note that although the PERMANOVA tests were conducted at the region level, the main interest in the SMART score is at the site level and thus some of the shade plots (which are a data visualisation tools and not a statistical test) show data at this finer spatial scale, albeit from fewer replicates. Note also that the values for all factors and variables are those used in the model except for the individual components of the predation factors, these have been standardised to place them on a common scale with the other variables (*i.e.* 0-100).

During the day, the SMART score was calculated using only the water quality, sediment composition and predation factors, as abundance data for penaeids, which are used in the competitor and conspecific factors, were not recorded. As data for the variables in these factors was only collected in one month that overlapped with the night sites and in a single replicate, there were only three scores per site over the three years. To enable statistical analysis, the data for the regions were pooled across the three years to be able to test for differences in SMART score among regions.

Finally, a comparison between the SMART scores for those eight sites where data was collected both during the night and day was undertaken. The aim of this was to indicate whether day or night releases would facilitate better survival of post-larval *M. dalli*. As the focus of this analysis was to determine differences in optimal release site during day and night, rather than spatially across the estuary only the predation factor was included. This decision was made because, as mentioned above, the abundance of penaeids was not recorded during the day. Moreover, sediment composition was considered unlikely to undergo a diel change and, although water quality to would change during a 24 hour cycle, this factor was to be more effective for describing spatial variation across the estuary rather than fine scale diel differences, especially as *M. dalli* released during the day would be exposed to the night-time temperatures in a few hours and *vice versa*.

Results

The results have been written in two major sections. The first summarises the results for each of the variables included in the model to establish the context for understanding the results of the SMART and the second refers to the results of the SMART.

Variation in individual factors

Water quality

Water temperature followed a similar monthly pattern during each of the three years rising to a peak in January and February and typically declining in March (Fig. 3.5.5). In the summer of 2015/16, the temperatures were slightly warmer than those in the preceding year, while those regions located in Melville Water and the Entrance Channel were usually 2°C cooler than those further upstream (*i.e.* 21-25; Fig. 3.5.5a *vs* 23-27°C; Fig. 3.5.5b).

Salinity increased progressively from November to March in most regions during each of the three years, except in Perth Water between the November (~25) and December (~9) of 2013, when salinity declined markedly, before returning to ~34 in January (Fig. 3.5.6). A similar, albeit, less pronounced trend occurred in the Entrance Channel in 2015/16. Typically, salinity

remained fairly consistent in this region and the two in Melville Water ranging 28 to 37, whereas in the other regions it ranged from 15 in November to 37 in March (Fig. 3.5.6). In almost all months, salinity was lowest in the Middle Swan Estuary and sometimes markedly so.

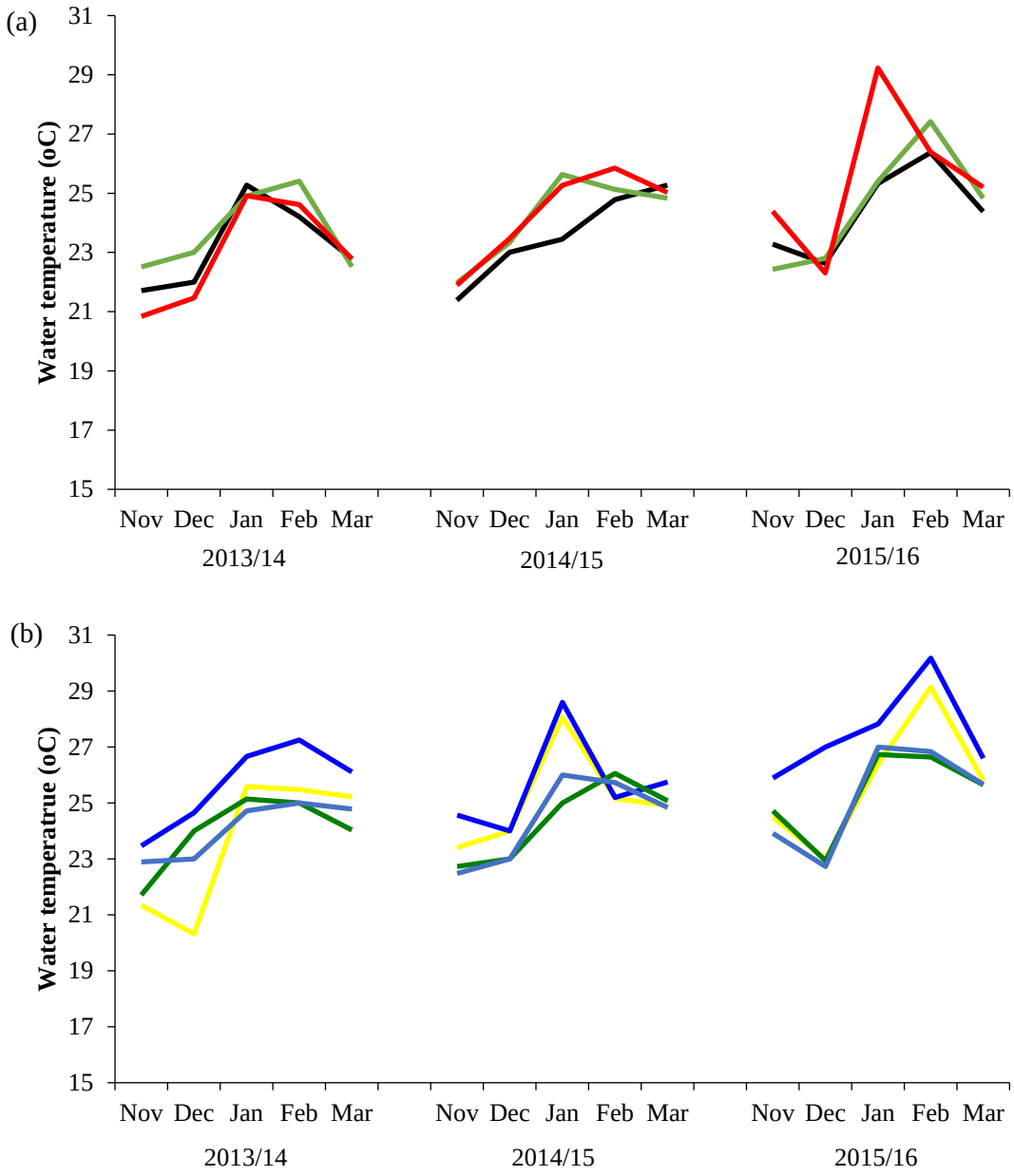


Fig. 3.5.5. Mean water temperature (°C) recorded at night in the nearshore waters of each of the seven regions of Swan-Canning Estuary between November and March in three consecutive years. Regions; Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●).

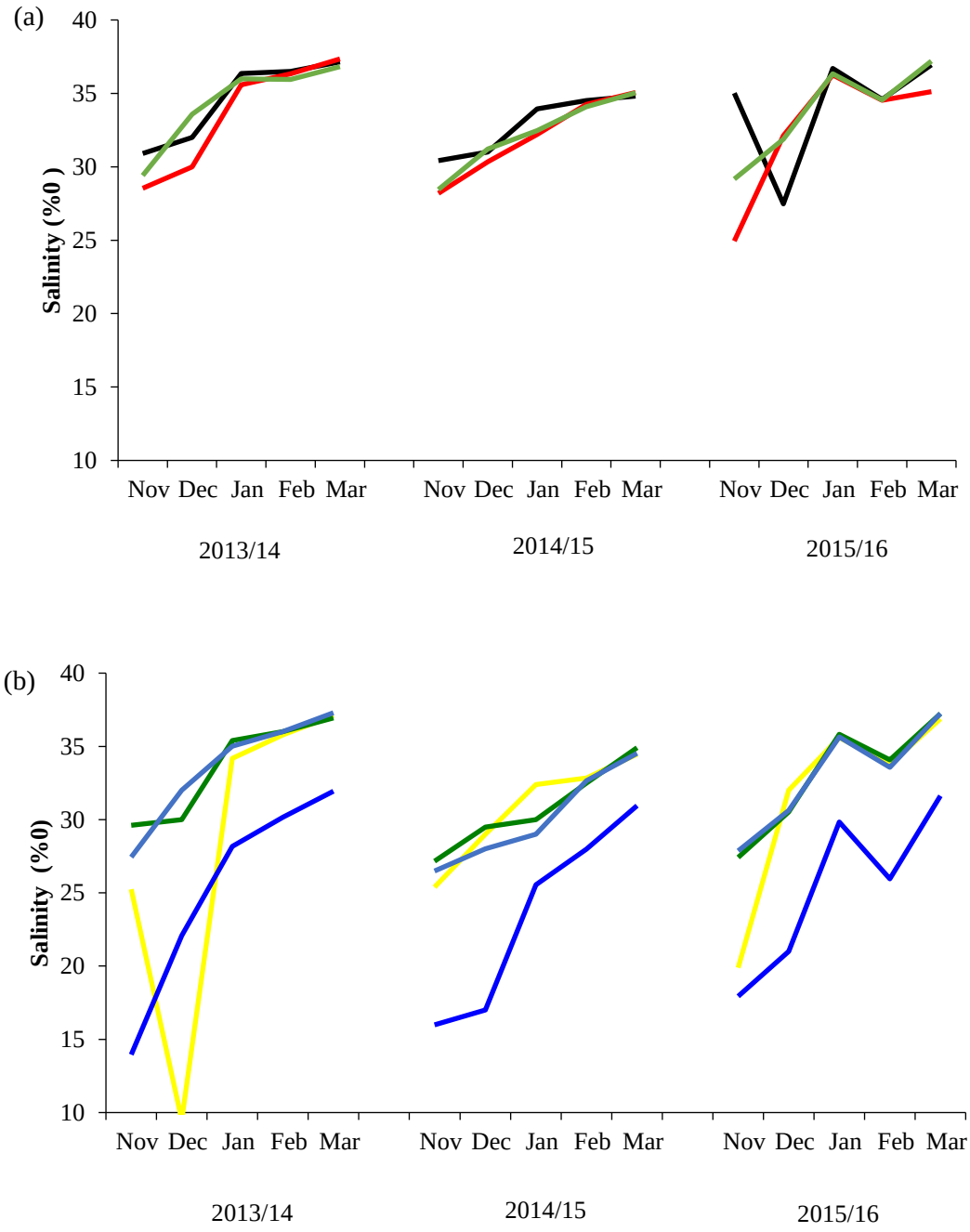


Fig. 3.5.6. Mean salinity (‰) recorded at night in the nearshore waters of each of the seven regions of Swan-Canning Estuary between November and March in three consecutive years. Regions; Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●).

Sediment composition

Principal Component Analysis of the sediment composition data for the 16 nearshore sites in the Swan-Canning Estuary demonstrated that 55% of the variation was explained by PC1. Those sites in the Entrance Channel had the greater PC1 scores (90-125), which was due to this

region exhibiting the largest percentage of particulate organic material (POM) and proportion of the 125, 63 and <63 µm inorganic grain sizes (Figs 3.5.7, 3.5.8). Similarly, the sites with the next greatest PC1 values were those in the Middle Swan Estuary, due to large percentage contributions of POM. The remaining regions, had a more similar sediment composition, being dominated by the 500 and 250 µm grain sizes, with those sites in Perth Water typically containing large contributions of the former grain size. The PC1 axis provided good separation of the Garratt Road Bridge (39) and Dalkeith (-34) sites, which were the two sediments types that Bennett (2014) used for sediment preference experiments. The sediment at Garratt Road Bridge was characterised by a large amount of POM, while that at Dalkeith, comprised greater proportions of the 500 µm grain size (Fig. 3.5.8).

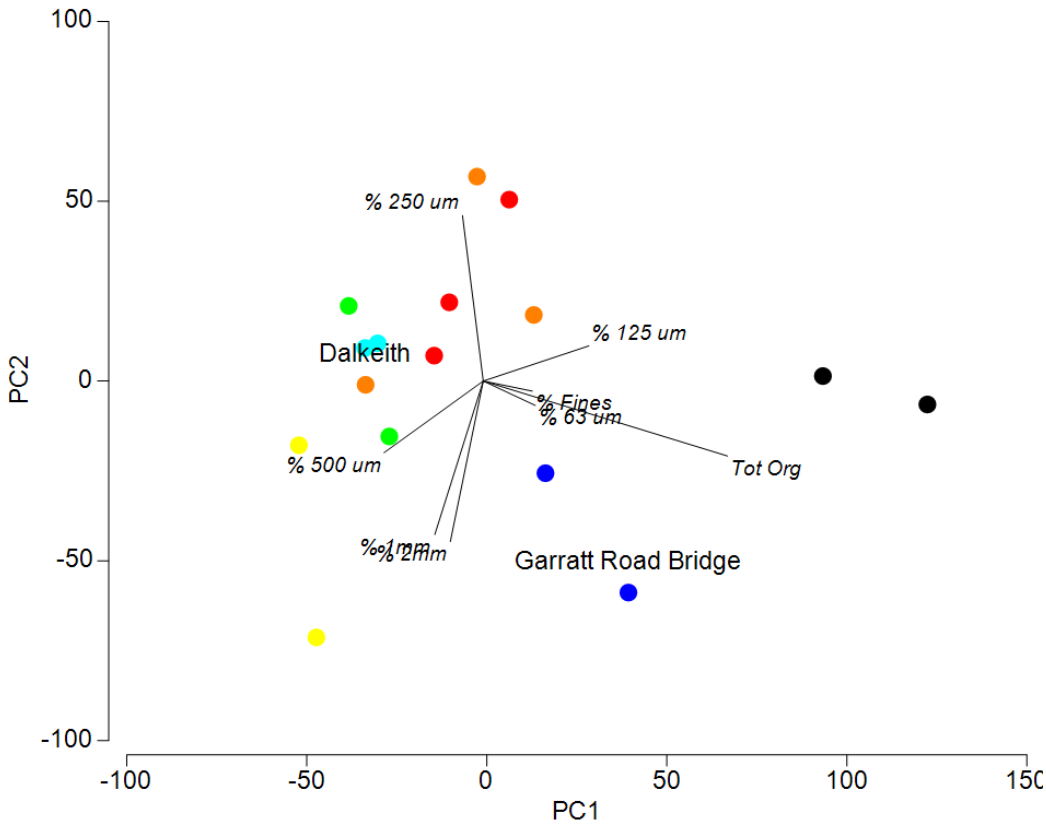


Fig. 3.5.7. Principal Component Analysis plot of the mean sediment composition at each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in February 2014. Vectors have been overlaid showing trends in the percentage contribution of particulate organic matter (Tot Org) and each of the inorganic grain sizes (*i.e.* 2 mm, 1 mm, 500 µm, 250 µm, 125 µm and 63 µm and < 63 µm [fines]). Sites coded for region, *i.e.* Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●). Data for the analysis taken from Bennett (2014).

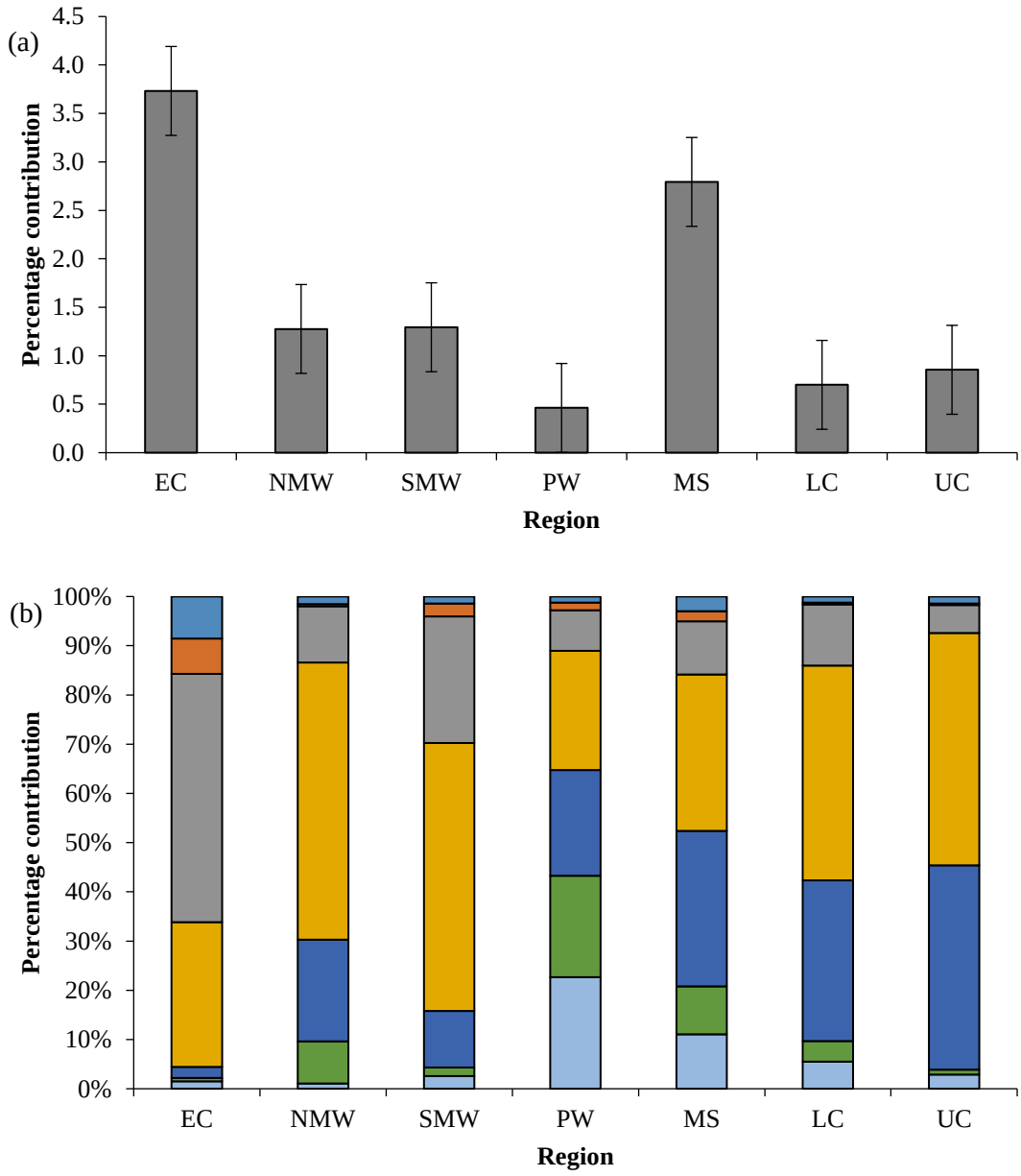


Fig. 3.5.8. Mean percentage contribution of (a) particulate organic matter (b) and various inorganic grain sizes to the sediment at each of the seven regions in the nearshore waters of the Swan-Canning Estuary. Inorganic grain sizes; 2 mm (), 1 mm (), 500 µm (), 250 µm (), 125 µm (), 63 µm () and < 63 µm [fines] (). Regions; Entrance Channel (EC), South Melville Water (SMW), North Melville Water (NMW), Perth Water (PW), Lower Canning Estuary (LC), Upper Canning Estuary (UC) and the Middle Swan Estuary (MS). Data for the analysis taken from Bennett (2014).

Densities of penaeids

The Western King Prawn *Penaeus latisculatus* was found predominantly in the Entrance Channel and North and South shores of Melville Water, with its densities greatest in the first region (up to 12 individuals 500 m^{-2}; Fig. 3.5.9). This species was only infrequently recorded and, if so, in low densities in Perth Water and the Lower Caning Estuary (maximum density of 2 individuals 500 m^{-2}), thus its density and frequency of occurrence declined with increased distance upstream. No clear patterns were evident in either among month in each year or between years (Fig. 3.5.9).

The densities of *M. dalli* showed a different spatial pattern to those of *P. latisulcatus*, with the regions further upstream of Melville Water, such as the Lower and Upper Canning Estuary and Perth Water harbouring the greatest densities of *M. dalli* (Fig. 3.5.10). Densities fluctuated within each year, typically exhibiting two peaks, with the first in November/December and the second in February. Densities of gravid *M. dalli* exhibited a similar pattern to the total population, with the exception that relatively larger numbers were found at sites in North Melville Water, and particularly so during 2015/16 (Fig. 3.5.11). While two peaks in the densities of gravid *M. dalli* were also recorded in each year, in some cases, *e.g.* Lower Canning Estuary in 2013/14 and North Melville Water in 2015/16, the first peak (*i.e.* ~8 and ~7 individuals 500 m^{-2}, respectively) was considerably larger than the second (*i.e.* ~2 and ~1 individuals 500 m^{-2}, respectively; Fig. 3.5.11).

Densities of teleost and scyphozoan predators

Nineteen of the 41 fish species recorded during night-time sampling in the nearshore waters of the Swan-Canning Estuary were identified as predators, or considered likely to be predators of post-larval *M. dalli,* based on stomach content analysis (Table 3.5.2; Fig. 3.5.13). The Atherinidae (grouped *A. elongata*, *C. mugiloides* and *L. presbyteroides*) were the most abundant species, comprising ~89% of all fish recorded. Other abundant fish included the Banded Toadfish (*Torquigener pleurogramma*) and the Western Gobbleguts (*Ostorinchus*

rueppellii), which represented 5 and 3% of the total number of fish. The Brown Jellyfish (*Phyllorhiza punctata*) represented 80% of the total abundance of scyphozoans (~12 individuals 100 m^{-2}), with the Moon Jellyfish (*Aurelia aurata*) comprising the remaining 20% (~4 individuals 100 m^{-2}; Table 3.5.2).

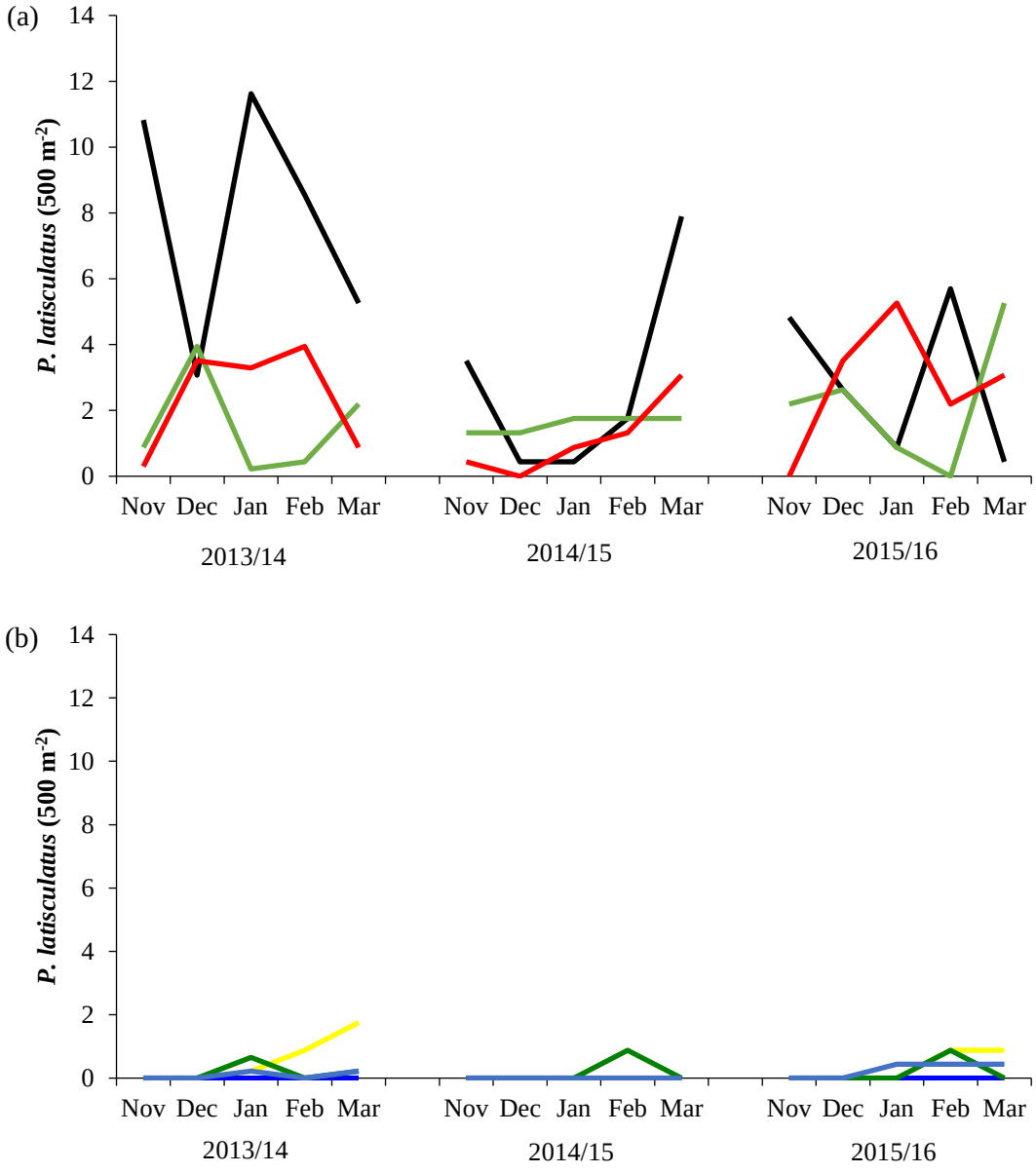


Fig. 3.5.9. Density of the Western King Prawn *Penaeus latisulcatus* (individuals 500 m^{-2}) to the sediment at each of the seven regions in the nearshore waters of the Swan-Canning Estuary monthly between November and March of 2013/14, 2014/15 and 2015/16. Regions; Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●).

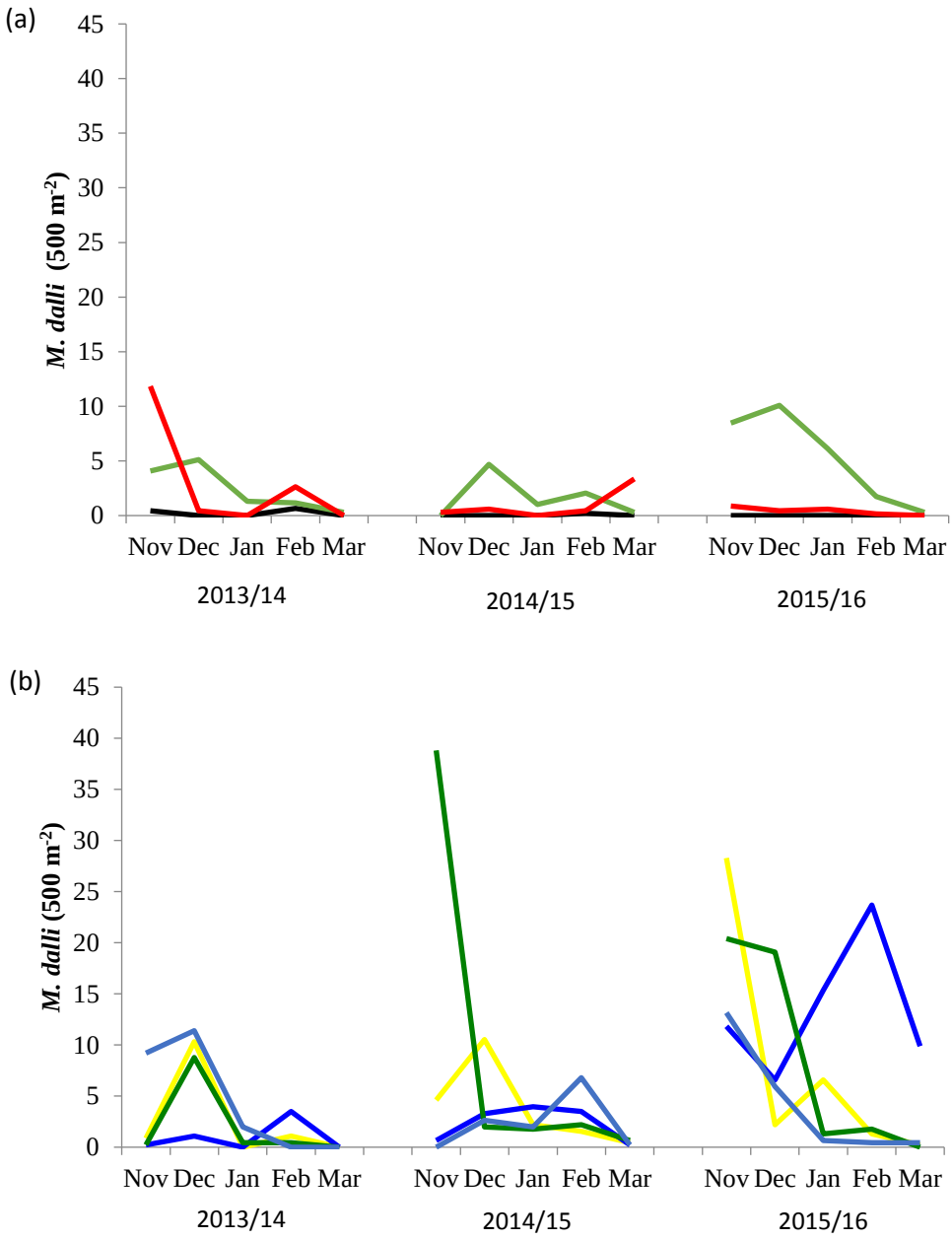


Fig. 3.5.10. Density of the Western School Prawn *Metapenaeus dalli* (individuals 500 m^{-2}) to the sediment at each of the seven regions in the nearshore waters of the Swan-Canning Estuary monthly between November and March of 2013/14, 2014/15 and 2015/16. Regions; Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●).

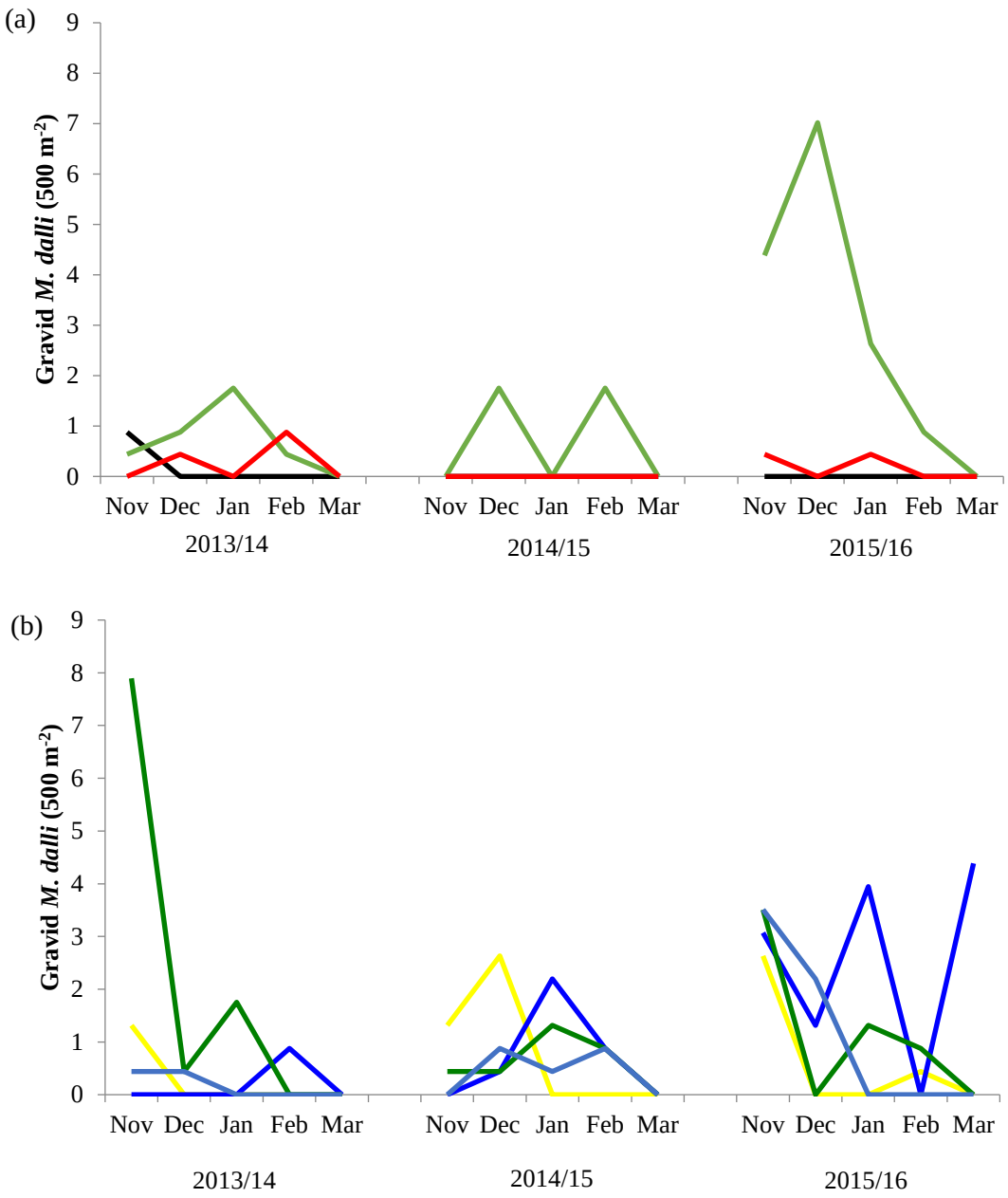


Fig. 3.5.11. Density of the Western School Prawn *Metapenaeus dalli* (individuals 500 m-2) to the sediment at each of the seven regions in the nearshore waters of the Swan-Canning Estuary monthly between November and March of 2013/14, 2014/15 and 2015/16. Regions; Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●).

The scoring of predators for the model are based on the dietary studies in Section 3.2, where the gut contents of numerous teleost species following the release of post-larval *M. dalli* at Matilda Bay. *Ostorinchus rueppellii* was the predator that contained the largest number of

prawns (up to 300 post-larval *M. dalli* in a single fish) and fed on them most consistently and thus was identified as the most significant threat to released *M. dalli* and assigned a predation score of 10 (Table 3.5.2). This species was consistently present in high densities within each month for all regions, except the Entrance Channel (Fig. 3.5.12).

The second highest predation score (6) was assigned to the Atherinid (*Atherinomorus vaigiensis*). Whilst consistent in its predation of *M. dalli* during the study period, *A. vaigiensis* was not observed to have predated as heavily on the released prawns as *O. rueppellii*. This species was not particularly abundant for long periods throughout the any region apart from in the Lower Canning Estuary, where it was present in each month of each year (Fig. 3.5.12).

The Yellowspotted Sandgoby *Favonigobius punctatus* was assigned a predation score of 3 as it was found to predate on *M. dalli* in small amounts, as well as other small crustaceans. Likewise, *Acanthopagrus butcheri* was assigned a score of 3, observed to have directly predated on *M. dalli*, albeit only fish of this species <100 mm in total length were found with *M. dalli* in their stomachs and only in small amounts. Both of these species were most abundant in the Perth Water and the Middle Swan Estuary (Fig. 3.5.12).

The final group to be assigned a predation score > 3 were the Atherinidae. While predating predominantly on *M. dalli* and other small crustaceans, a high percentage of stomach contents from this family were empty, therefore not contributing to the percentage composition of stomach contents. Further, when *M. dalli* was recorded in stomach contents of members of this group, they occurred in small numbers (*i.e.* 1 or 2). As a result, although their densities were very high, they were assessed as unlikely to have a large impact on survival of released *M. dalli*. Like *O. rueppellii*, the atherinids were present throughout the estuary in all sampling periods, except in the Middle Swan Estuary where they were not found in high abundance in any month (Fig. 3.5.12). All other species were assigned a predation score of ≤2 based on the fact that their diet only included a small percentage of *M. dalli* and/or other small crustaceans.

Table 3.5.2. Density 100 m-2 (D) and percentage contribution (%) to total density of all teleost and scyphozoan species deemed to predate on released *M. dalli* or have the potential to predate on them. A predation score (P) ranging between 1 (low) and 10 (very high) is assigned to each species based on the risk that species presents for predation on released post-larval *Metapenaeus dalli*. Species with a predation score ≥3 are shaded in grey. Atherinidae is a combination of *A. elongata*, *C. mugiloides* and *L. presbyteroides* as these species require laboratory classification.

Teleost species	Common name	P	Total		2013/14		2014/15		2015/16	
			D	%	D	%	D	%	D	%
Atherinidae	Hardyheads	3	936.5	89.8	395	89.9	170.8	87.2	370.6	90.5
Torquigener pleurogramma	Banded Toadfish	1	50.6	4.9	25.3	5.8	10.2	5.2	15.1	3.7
Ostorhinchus rueppellii	Western Gobbleguts	10	33.2	3.2	7.1	1.6	8.1	4.1	18	4.4
Spratelloides robustus	School Whiting	2	4.5	0.4			3.6	1.8	0.9	0.2
Favonigobius punctatus	Sandgoby	3	3.7	0.4	2.5	0.6	0.5	0.3	0.7	0.2
Atherinomorus vaigiensis	Common Hardyhead	6	3.3	0.3	1.9	0.4	0.7	0.4	0.7	0.2
Pelates octolineatus	Striped Grunter	1	2.9	0.3	2.6	0.6	0.1	0.1	0.2	>0.1
Favonigobius lateralis	Southern Longfin Goby	1	2.6	0.2	2.5	0.6	0.1	0.1	0.1	>0.1
Acanthopagrus butcheri	Black Bream	3	1.8	0.2	1	0.2	0.2	0.1	0.6	0.1
Pseudogobius olorum	Swan River Goby	2	1.4	0.1	0.2	0	0.5	0.3	0.7	0.2
Amniataba caudavittata	Yellowtail Grunter	1	0.7	0.1	0.3	0.1	0.2	0.1	0.2	>0.1
Mugil cephalus	Sea Mullet	1	0.6	> 0.1	0.1	>0.1	0.7	0.4	1.3	0.3
Engraulis australis	Australian Anchovy	1	0.5	> 0.1	0.3	0.1	0.2	0.1	0.1	>0.1
Rhabdosargus sarba	Tarwhine	2	0.3	> 0.1	0.3	0.1				
Hyporhamphus melanochir	Southern Sea Garfish	1	0.2	> 0.1	0.2	> 0.1				
Haletta semifasciata	Blue Weed Whiting	1	0.2	> 0.1	0.2	> 0.1				
Hyperlophus vittatus	Sandy Sprat	2	0.1	> 0.1					0.1	>0.1
Gerres subfasciatus	Common Silver Belly	1	0.1	> 0.1					0.1	>0.1
Sillago burrus	Trumpeter Whiting	1	0.1	> 0.1					0.1	>0.1
	Number of Species		**19**		**15**		**13**		**15**	
	Total density		**1,043**		**440**		**196**		**410**	
	Total no. fish		**232,806**		**122,310**		**35,712**		**74,784**	

Scyphozoan Species		D	%	D	%	D	%	D	%
Phyllorhiza punctata	Brown Jellyfish	11.6	80	0.6	40	1.4	80	9.6	80
Aurelia aurata	Moon Jellyfish	3.8	20	0.8	60	0.3	20	2.8	20
	Total density	**15.4**		**1.4**		**1.7**		**12.4**	
	Total no. scyphozoans	**2,929**		**371**		**305**		**2,253**	

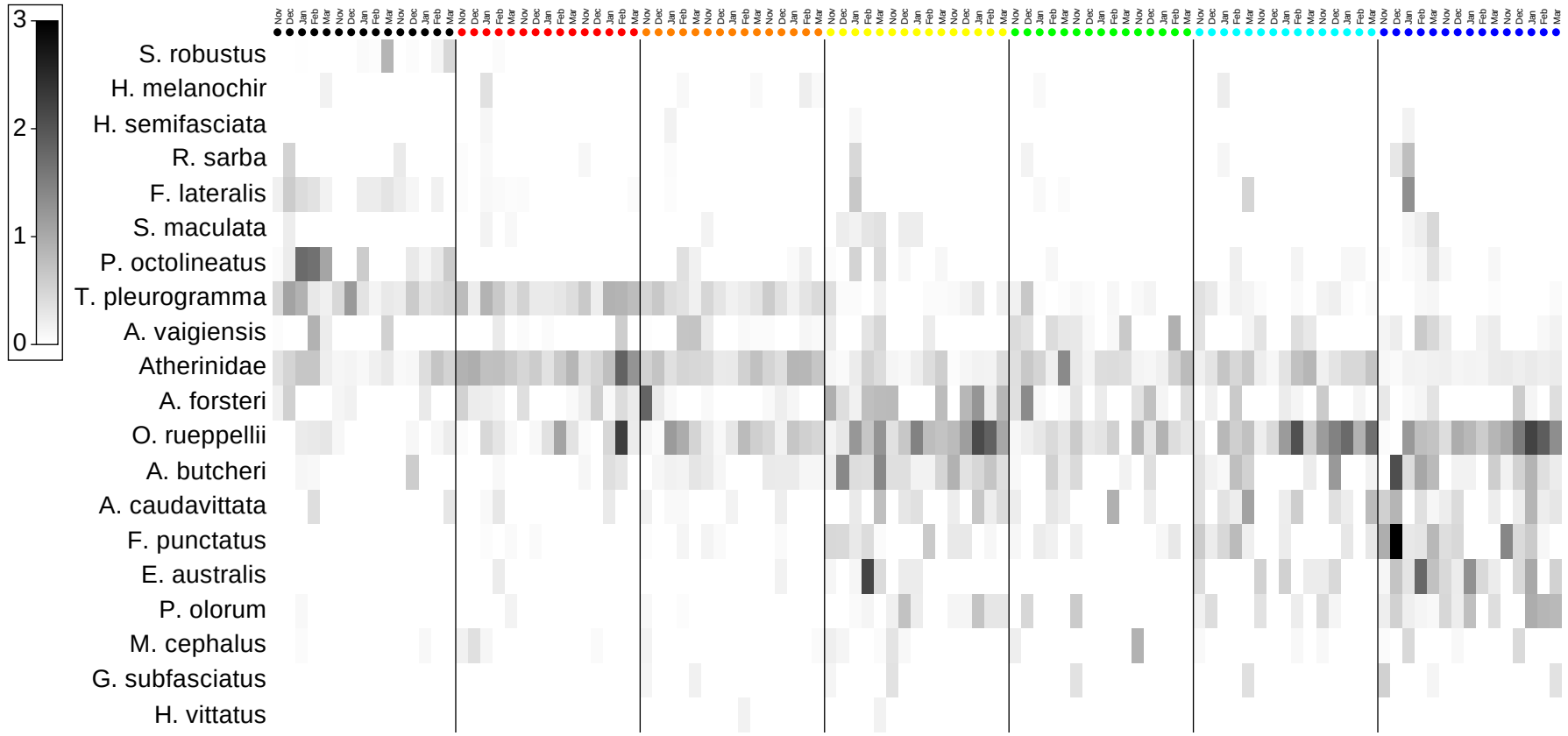


Fig. 3.5.12. Shade plot dispersion weighted and square-root transformed densities of each of the 19 species identified to predate or potentially predate on *Metapenaeus dalli* in each of the seven regions during each month (November-March) of each year (2013/14, 2014/15 and 2015/16) in the Swan-Canning Estuary. White space denotes the absence of a species, with the grey scale representing the pretreated abundances. Regions; Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●).

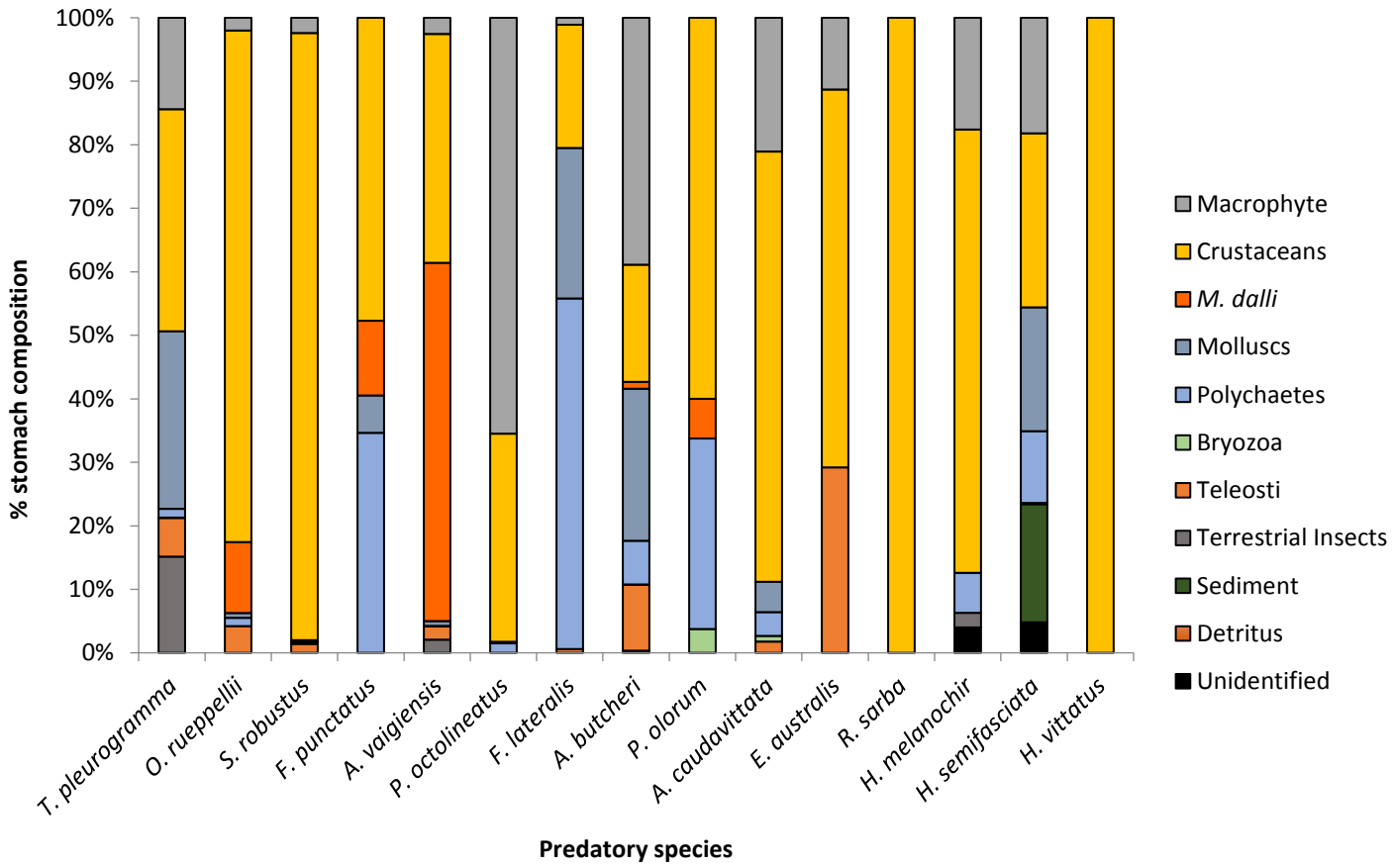


Fig. 3.5.13. Percentage contribution of dietary items to the stomachs of the 19 teleost species identified to predate or potentially predate upon *Metapenaeus dalli*. * indicates those species that were sampled by Poh (Section 3.2) after the release of *M. dalli* into the area, separating *M. dalli* from other crustaceans in the stomach contents. Data taken from Poh *et al*. (2018); Humphries and Potter (1993); Coull *et al*. (1995); Hyndes *et al*. (1997); MacArthur and Hyndes (2007); Dube and Kamusoko (2013); Rao and Babu (2013).

SMART results

More detailed results for all variables, *i.e.* scores in each region during each month of each year, are summerised in a table (Annex 3.5.2) and provided as shade plots (Annex 3.5.3-3.5.17). Three-way PERMANOVA identified significant differences in SMART score among Region, Month and Year (*i.e.* the breeding season, Nov-March of 2013/14, 2014/15 and 2015/16) and all two way interaction terms (Table 3.5.3). The mean squares for Region (2,721) was by far the greatest and over five times greater than that for the next most influential terms in the model (*i.e.* Year, 518 and Month, 431). As the proportion of the variance explained by each of the main effects was markedly greater than any of the interaction terms, post-hoc test focused on differences in SMART among Region, Months and Year (Table 3.5.3).

Table 3.5.3. Mean squares (MS), pseudo F-ratios (*pF*) and significance levels (*p*) from a three-way PERMANOVA test on the SMART scores among the seven regions in the Swan-Canning Estuary, between November and March in each of three year. Data obtain during the night. *df* = degrees of freedom. Significant results are highlighted in bold.

Main effects	***df***	**MS**	***pF***	***p***
Region	6	**2721.3**	**53.38**	**0.001**
Month	4	**431.4**	**8.46**	**0.001**
Year	2	**518.3**	**10.17**	**0.001**
Interactions				
Region × Month	24	**83**	**1.63**	**0.03**
Region × Year	12	**123.4**	**2.42**	**0.002**
Month × Year	5	**158.5**	**3.11**	**0.01**
Region × Month × Year	30	34.4	0.68	0.91
Residual	396	51		

A pairwise PERMANOVA test conducted on the Region main effect, detected significant differences in 18 of the 21 comparisons (Table 3.5.4a). t-values were greatest for comparison involving the Entrance Channel, which was due to SMART scores for sites in this region being significantly lower (49) than all other regions (58-69; Fig. 3.5.14). The next highest t-values were found in comparisons involving Perth Water (69) and South Melville Water (58), due to these regions having high and low SMART scores, respectively The highest scores were found in the Lower Canning Estuary (also 69), and thus statistically similar to those in Perth Water and the Upper Canning Estuary (65).

Although the range of SMART scores among months (*i.e.* 59-63) was less than that among regions (49-69), significant differences were detected among months, with the SMART scores for November and December being different to those in all other months (Table 3.5.4b). Values in these two months (~63) were higher than that in the remaining months and SMART scores declined progressively from 62 in January to 61 in February and 59 in March (Fig. 3.5.15). Pairwise PERMANOVA also detected differences in SMART scores among years, with those for 2013/14 (60) being significantly lower than both 2014/15 (63) and 2015/16 (62; Table 3.5.4c; Fig. 3.5.16). No significant difference was detected between the last two years.

Table 3.5.4. T-statistic values derived from a pairwise PERMANOVA tests on the SMART scores for the (a) Region, (b) Months and (c) Year main effects. Significant pairwise comparisons are highlighted in grey ($p<0.05$). EC = Entrance Channel, NMW = North Melville Water, SMW = South Melville Water, PW = Perth Water, LC = Lower Canning, UC = Upper Canning, MS = Middle Swan.

(a) Region	EC	NMW	SMW	PW	MS	LC
NMW	10.81					
SMW	6.83	4.74				
PW	13.46	3.17	7.77			
MS	7.59	3.07	1.32	6.33		
LC	13.58	3.87	8.30	0.82	6.77	
UC	12.75	1.99	6.69	1.33	5.30	2.10

(b) Month	Mar	Feb	Jan	Dec
Feb	1.24			
Jan	1.50	0.22		
Dec	4.82	3.44	3.35	
Nov	4.48	3.13	4.02	0.43

(c) Year	3 (2015/16)	2 (2014/15)
2 (2014/15)	0.27	
1 (2013/14)	3.92	3.99

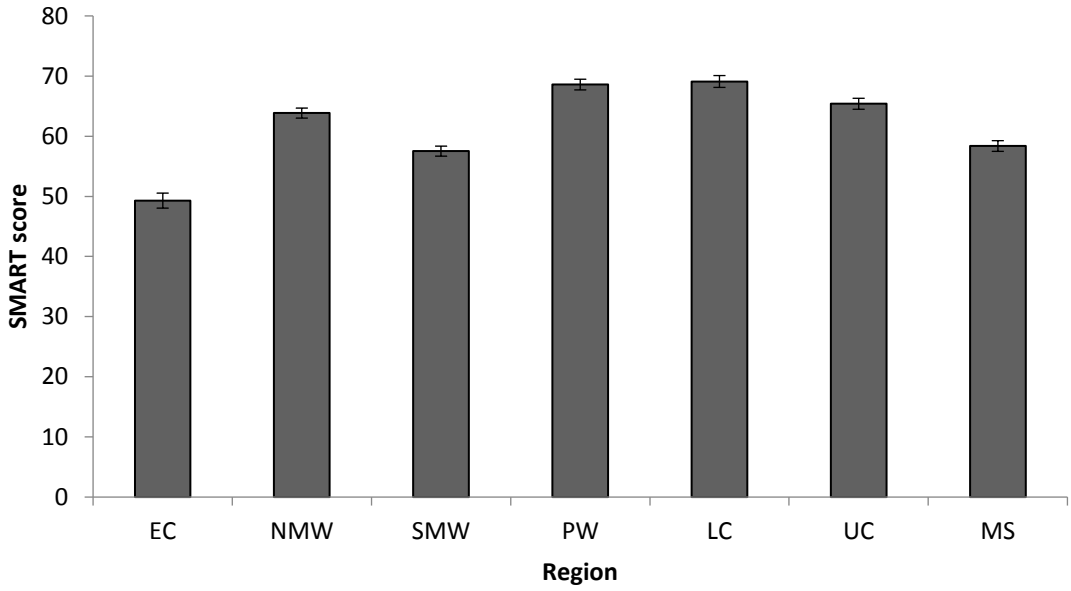


Fig. 3.5.14. Average SMART scores for each of the seven regions in the Swan-Canning Estuary at night (pooled across Month and Year). Error bars represent ± 1 standard error. Regions; Entrance Channel (EC), North Melville Water (NMW), South Melville Water (SMW), Perth Water (PW), Lower Canning Estuary (LC), Upper Canning Estuary (UC) and Middle Swan Estuary (MS).

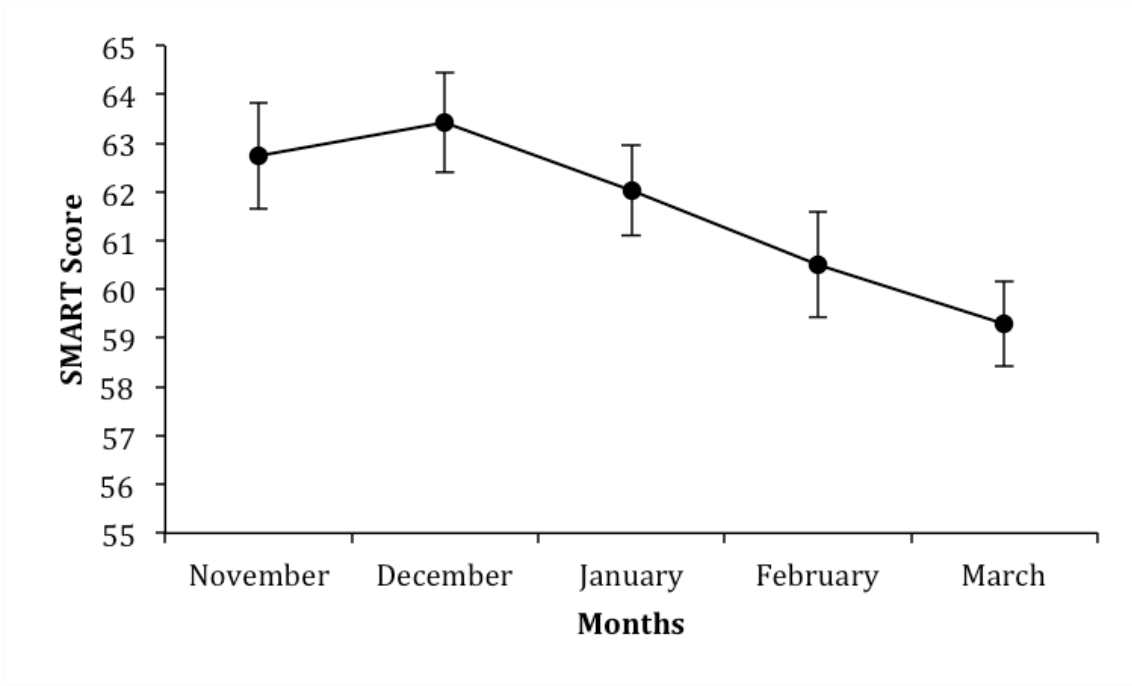


Fig. 3.5.15. Average SMART score at night in the Swan-Canning Estuary in each month between November and March (pooled across Region and Year). Error bars represent ± 1 standard error.

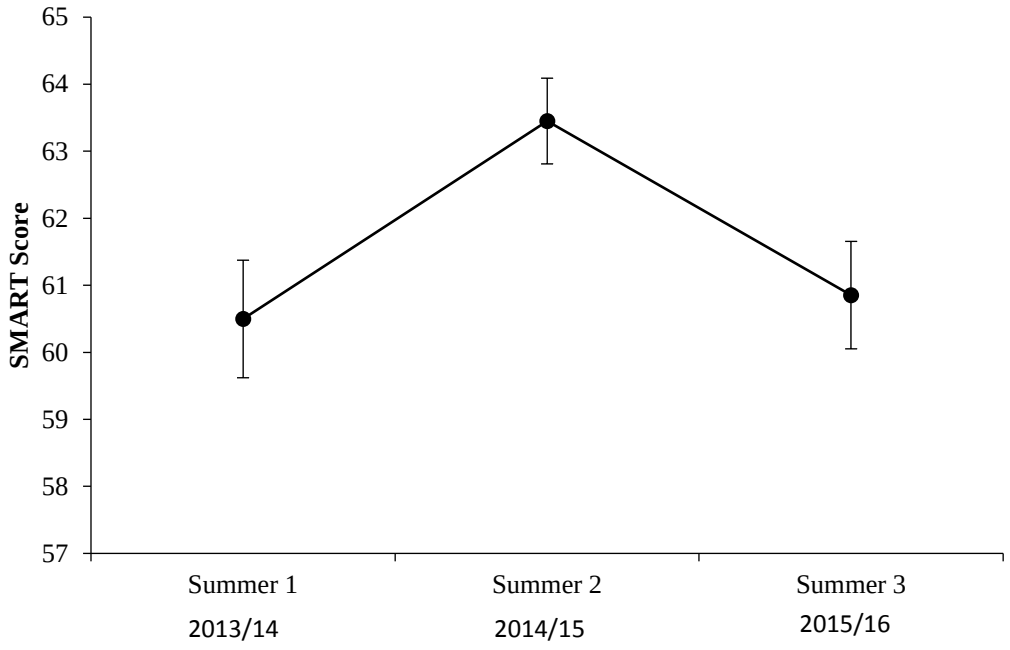


Fig. 3.5.16. Average SMART score at night in the Swan-Canning Estuary in each year between 2013/14 and 2015/16 (pooled across Region and Month). Error bars represent ± 1 standard error.

Shade plots provide a visual indication as to the reason for a high or low overall SMART score as they denote the score of each factor and its component variables. Note that in the case of Region, the shade plot has been constructed at the site level to showcase the variability among sites within a region, as it is at this spatial level that a release strategy would operate.

Among individual sites, the highest scores were recorded at Dalkeith South Perth and Deep Water Point. Each of these sites featured relatively high scores across all factors (Fig. 3.5.17). In contrast the lowest overall scores for a site was recorded at Stirling Bridge and Leeuwin Barracks and these featured a high scores for water quality and predation, however, a very low score for sediment composition and conspecifics.

Shadeplots of the SMART score of the combined regions over months (November – March of each year) showed a relatively similar score over all months, however the makeup of this score from variables differed (Fig. 3.5.18). For example, January, February and March all had considerably higher scores for water quality than November and December, however, these month had a considerably lower score for predation mainly due to the increase densities of

O. rueppellii. Not all the factors differed markedly among month, with sediment composition and the densities of competitor and conspecifics remaining fairly similar (Fig. 3.5.18).

The slightly lower SMART score in 2013/14 than in both 2014/15 and 2015/16 is explained by the fact the water quality measures, particularly salinity, were significantly higher in the last two summers than 2013/14, this is despite the fact that the predation score was slightly higher score in 2013/14, due to the increased densities of *O. rueppellii* in the latter two summers (Fig. 3.5.19).

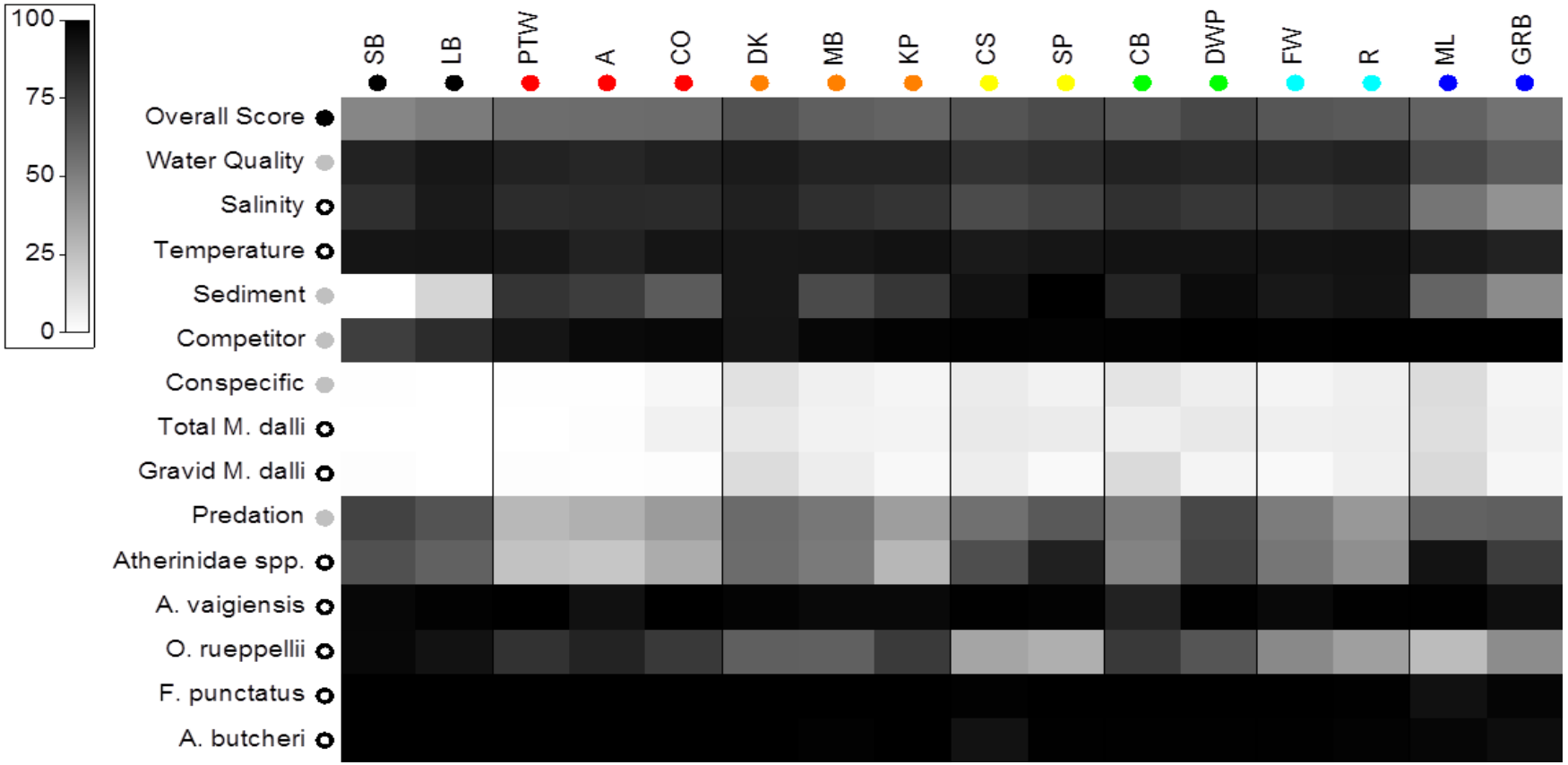


Fig. 3.5.17. Shade plot, constructed using the raw overall SMART score (□) and the raw scores for each factor (□) and variable (○), except in the case of the abundances of each predator taxa which have been standardised to place them on a common scale with the other variables, among sites (pooled across Month and Year). The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.

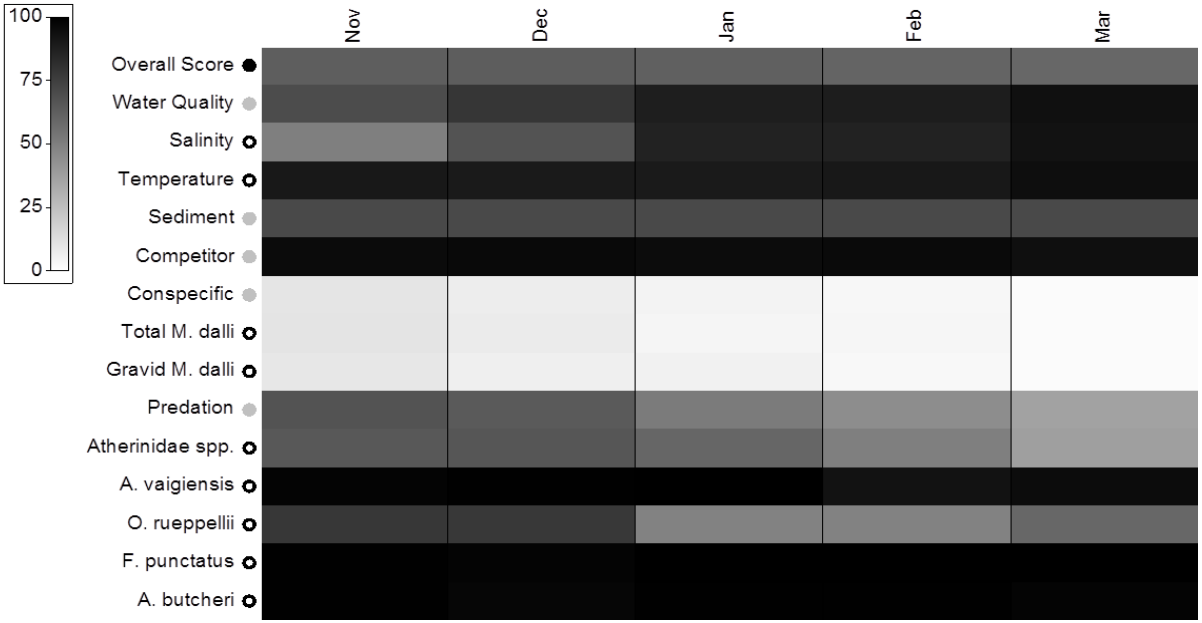


Fig. 3.5.18. Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○), except in the case of the abundances of each predator taxa which have been standardised to place them on a common scale with the other variables, among months (pooled across Region and Year). The greyscale from white to black denotes increasing SMART scores and thus a better release month for hatchery-reared *M. dalli*.

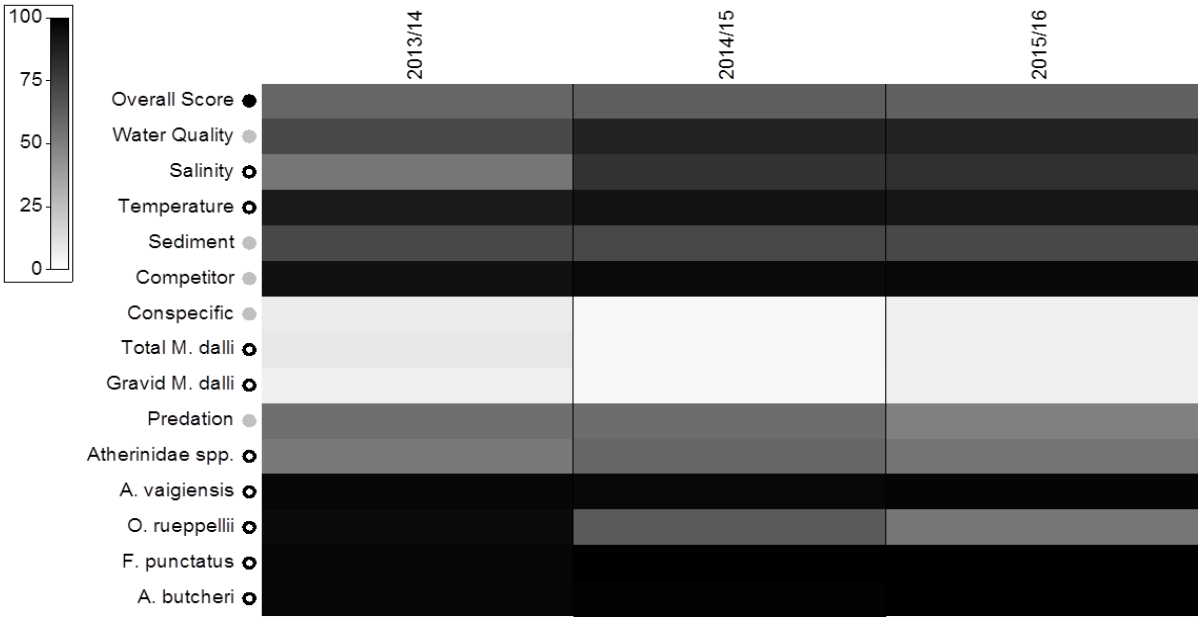


Fig. 3.5.19. Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○), except in the case of the abundances of each predator taxa which have been standardised to place them on a common scale with the other variables, among years (pooled across Region and Month). The greyscale from white to black denotes increasing SMART scores and thus a better release month for hatchery-reared *M. dalli*.

Discussion

The primary focus of this study was to develop and test a quantitative methodology for evaluating potential sites and times of release for post-larval *Metapenaeus dalli* in the Swan-Canning Estuary. This was achieved examining a suite of abiotic and biotic variables considered as likely to influence the post-release survival of *M. dalli* in the Swan-Canning Estuary using the Survival-Maximisation-At-Release-Tool (SMART). These variables were selected through an extensive literature search and stakeholder engagement and evaluated via the collation of published and unpublished data held at Murdoch University to produce a final SMART score for each site (region) in each month and year as well as a comparison of scores between day and night releases.

The only study found to use a similar tool to that developed here was undertaken to determine suitable sites for the release of the European Wild Rabbit (*Oryctolagus cuniculus*) in the Iberian Peninsula (Carvalho and Gomes, 2003). In that earlier study, the tool focused predominantly on a single variable, habitat, using Geographical Information Systems to select a release site with the highest quantity of optimal habitat within 200 m, *i.e.* the predetermined extent that released *O. cuniculus* travel to settle. Potential release sites were graded from 0 to 100 based on their suitability, with a score of 100 being optimal. The tool developed here is much more comprehensive and takes into account a range of environmental and biological variables.

The ensuing discussion has been written in four sections. The first interprets the results of the SMART, focusing on the variation of results during the night, with less comprehensive discussion of the more limited results for the day. The second section evaluates ways in which the tool may be enhanced in the future to better guide the selection of release sites and times of release, followed by a discussion of how the tool may be adapted for the release of other species and other water bodies. The fourth and final section explains how the outputs of the SMART could be displayed in order to facilitate discussions among different groups in developing an optimal release strategy based on selecting the best sites and times for release.

Selection of best release sites

Statistical analyses of the SMART outputs determined that Region of the Swan-Canning Estuary had the greatest influence on the potential to maximise the post-release survival of hatchery-reared post-larval *M. dalli* during the night. Year (*i.e.* 2013/14, 2014/15 and 2015/16) was the next most influential factor, followed by Month (*i.e.* November-March). Although some of the interactions between release Region, Year and Month were also significant, they accounted for a far smaller proportion of the variation in the SMART scores any of the main effects, and particularly that of Region. Combining each site, month and year, the best release site and time for juvenile *M. dalli* in the Swan-Canning Estuary was Deep Water Point in the Lower Canning Estuary at night in January 2014 (Annex 3.5.10). This site had high scores for water quality, sediment composition, low abundances of competitor and predatory species and relatively high scores for the abundance of conspecifics.

Regional differences

Across all months and years, the best regions for release were the Lower Canning Estuary and Perth Water, with the former region having the highest average SMART score across the estuary. Both of these regions scored highly for water quality, sediment composition and competitors, as well as receiving relatively high scores for conspecifics and predation. Variance in water quality scores was mainly due to the influence of salinity, with very high to optimal scores for water temperature recorded throughout much of the estuary. Perth Water had a lower overall water quality score than the Lower Canning Estuary, probably due to the slightly lower salinity in the former region, which is due to the catchment of the Swan River being much larger than that of the Canning and the fact that stop boards are placed into Kent Street Weir between September/October, thus preventing freshwater discharge entering the Canning axis of the estuary (Swan River Trust, 2009). As the optimal value of salinity used to determine SMART score in this experiment is close to that of full strength seawater (Crisp *et al.*, 2017b), the fresher

nearshore waters of the Perth Water region are less suitable for the release of *M. dalli*, explaining the lower score for salinity than that recorded in the Lower Canning Estuary.

While scores for sediment composition, competitors and conspecifics were similar for the two regions, those for predation differed. Predation scores for *A. vaigiensis*, *F. punctatus* and *A. butcheri* were very high in both of the Lower Canning Estuary and Perth Water, however, scores for atherinids and *O. rueppellii* varied. Predation by atherinids was the most influential on the overall predation score in the Lower Canning Estuary. Atherinids were present in this region throughout the sampling period, a trend consistent with studies on the fish assemblages of the Swan-Canning Estuary by Loneragan and Potter (1990), in which it was observed that the atherinids *A. elongata* and *C. mugiloides* dominated percentage contribution to the overall density of fish in the Melville Waters and Lower Canning Estuary. *Ostorhinchus rueppellii* was observed in high abundance in the Perth Waters across the sampling period, consistent with observations by Loneragan and Potter (1990). This apogonid, which is classified as a marine & estuarine species (Potter *et al*., 2015a), migrates to the shallows of the upper estuary during the early summer to spawn (Chrystal *et al*., 1985), explaining the low scores for predation by this species in the summer months.

The Entrance Channel was identified as being the worst region for the release of post-larval *M. dalli*. While this region received high scores for water quality and predation, it had very low scores for sediment composition and conspecifics as well as the lowest score for competitors. The inorganic portion of the sediment in this region were typical of those recorded in nearshore coastal waters of south-western Australia (Wildsmith *et al*., 2005), with grain dominated by the ~125-249 μm size fraction, smaller than that observed in most other regions throughout the estuary and considered optimal for *M. dalli*, *i.e.* 249-500 μm (Bennett, 2014). Moreover, this region, likely due to its seagrass beds, had the largest amount of particulate organic matter, which inhibits burying in small *M. dalli* (Bennett, 2014). Sites in the Entrance Channel received very low scores for conspecifics due to the low numbers of adult and gravid *M. dalli*. This trend is consistent with observations of *M. dalli* abundance between 1977 and 1982, where this species was mainly recorded from North Melville Water and further upstream, which was

attributed to the annual migration of adult *M. dalli* from the deeper, offshore waters of the estuary into the shallow waters of the upstream regions to spawn in early summer (Potter *et al*., 1986b). This trend is also consistent with patterns in distribution of *M. dalli* in summer in the Peel-Harvey Estuary, 100 km south of the Swan-Canning Estuary, prior to the construction of the Dawesville Cut (Potter *et al*., 1989).

Relatively large densities of *Penaeus latisulcatus* in this region resulted in the Entrance Channel receiving a low score for competitors. This penaeid is regarded as a marine estuarine-opportunist (Potter *et al*., 2015b) and has a lifecycle similar to many other marine penaeids, spawning in the marine environment and post-larvae/juveniles recruiting to sheltered coastal environments and/or estuaries (Dall *et al*., 1990; Potter *et al*., 1991; Bailey-Brock and Moss, 1992). This life history strategy, the abundance of suitable habitat and the maintenance of high salinities year round in the lowermost regions of the Swan-Canning Estuary are likely to be the key factors limiting the distribution of *P. latisulcatus* predominantly to the Entrance Channel, and thus explaining why all other regions recorded very high scores for competitors.

Interannual differences

Although variation in SMART score across years was low in comparison to that across regions, significant differences were detected. The highest average SMART score was recorded in 2014/15, due to higher scores for water quality and predation than those in the other two years. Scores for all other factors were similar across the three years. Variation in the predation across all three years was predominantly due to the scores for atherinids and *O. rueppellii.* Scores for *O. rueppellii* were highest during 2013/14, likely due to the lower salinity precluding the migration of this apogonid from the more saline offshore waters into the nearshore areas of the estuary.

Monthly differences

Although the overall SMART scores were relatively consistent across months, scores for the factors of each month varied considerably. For example, water quality scores were lowest in November and December, due to the lingering influence of freshwater discharge from the upper river in late spring/ early summer. Discharge decreases as summer progresses, leading to the intrusion of saltwater further upstream (Tweedley *et al*., 2016b; Broadley *et al*., 2017), resulting in salinities across the estuary becoming closer to full strength seawater and ideal for the release of *M. dalli* (Thompson, 2001; Crisp *et al*., 2017b). Score for conspecifics (both total and gravid *M. dalli*) were highest in November and decreased progressively throughout summer, likely attributable the movement of individuals back to offshore waters after spawning and, in the case of females, mortality (Potter *et al*., 1986b; Potter *et al*., 1989; Broadley *et al*., 2017). Similarly, predation scores were highest in November and December, before decreasing gradually and sequentially over summer. This variation in predation score was influenced by changes in the abundances of atherinids. These species, like most estuarine residents in the Swan-Canning Estuary, spawn in the early summer, *i.e.* December (Prince and Potter, 1983), resulting in the subsequent recruitment of juveniles increasing the risk of predation on post-larval *M. dalli* as summer progresses.

Improvements to the SMART

As the SMART is the first objective and quantitative tool developed to combine multiple variables considered to affect the selection of release site for a hatchery-reared population, and it has been developed over a relatively short time period, there are a number of improvements that can be made. Through the first run of the SMART, three areas for improvement became apparent. Firstly, the inclusion of optimal release size and density for release of *M. dalli* into the tool. Secondly, revision of the variables to be incorporated into the tool and the standardisation of these variables onto a single scale. Finally, the procedure for weighting and

scaling of variables and factors when determining the score for the factors and final SMART scores.

Variables and standardisation

The variables selected for the model were chosen based on an extensive survey of the literature on studies of survival at release sites and times (Hodson, 2016), and through discussions in 2016 with staff at Murdoch University, and the then Departments of Parks and Wildlife; Fisheries WA and Water (now Departments of: Biodiversity, Conservation and Attractions; Primary Industries and Regional Development; Water and Environmental Regulation, respectively) and the Australian Centre for Applied Aquaculture Research. Each variable selected for the inclusion into model was one considered to have a significant effect on the post-release survival of *M. dalli*.

In order to increase the effectiveness of the SMART, some variables require further study to better understand either the range of their values throughout the estuary or their effect on *M. dalli*. For example, it was assumed that the effects of water temperature and salinity on post-larval *M. dalli* are similar to those on post-larval *M. dalli* (Crisp *et al*., 2017b). However, studies on other penaeids suggest that this may not be the case. For example, whilst changes in salinity had an adverse effect on the survival of larval Brown Shrimp *Farfantepenaeus aztecus* (Saoud and Davis, 2003), in studies on post-larval *F. aztecus*, survival over 24 h was not impacted over a wide range of water temperature and salinities. Moreover, post-larval survival in *F. aztecus* was still high after 28 days in a range of water temperature and salinity conditions (Zein-Elden and Aldrich, 1965; Zein-Elden and Renaud, 1986). A wide tolerance in salinity, *i.e.* 1-40, was also recorded in post-larval Northern White Shrimp *Litopenaeus setiferus* and four other species of prawns from Mexico (Mair *et al*., 1982). While these studies found that prawns could survive in a broad range of water temperatures and salinities, it was noted that growth, behaviour and function were not optimal across the whole range, which could increase their predation risk. Further study on the effect of salinity and water temperature on post-larval *M. dalli* is required

to determine the optimal releases conditions for this stage in the life-cycle and whether it differs from that of the larvae.

More extensive and detailed sediment data would improve the resolution of SMART. Although the sediment data collected by Bennett (2014) is the most in-depth to date, it was based on only two replicates per site, did not cover a sufficiently large number of sites in the different regions and did not study sediment changes in these regions over a long period of time. Thus, to determine how the variability of sediment composition changes spatially and temporally in the Swan-Canning Estuary and the effect this has on *M. dalli*, a more robust sampling regime addressing these issues is required. In the current iteration of SMART, the sediment composition factor is based on a single variable, *i.e.* the PC1 score, despite the fact that data on the contribution of individual grain size fractions were available. These were not used as laboratory studies had not been conducted on the preference of *M. dalli*, and particularly post-larval individuals, to sediments comprised of a single grain size. Moreover, as the Swan-Canning Estuary has been exposed to large amounts of anthropogenic activity over a substantial period of time, the sediments in some areas may contain a range of contaminants, which could potentially affect the post-release survival of *M. dalli*. The effect of contaminants could be quantified and included in the model, provided laboratory studies and sediment contaminant data were also available. The provision of these data is important when undertaking aquaculture-based enhancements in highly urbanised area, such as estuaries which can be heavily degraded (Jackson *et al*., 2001; Tweedley *et al*., 2015).

Further examination of the methodology for standardisation of the scores within the SMART would also be beneficial, *e.g.* the Relative Predation Index (RPI), which was calculated by multiplying the density of a species by its predation score. The aim of developing the RPI was to quantify the level at which a species predated on released *M. dalli* and to remove the bias of abundance of a species when determining the score for the predation variable. This was mainly done to scale down the predation effect of atherinids, which are extremely numerous, and scale up that of *O. rueppellii*, which occur in far lower densities. Whilst stomach content analyses identified atherinids to predate predominantly on released *M. dalli* and other small crustaceans,

this was based on a small proportion of these fish containing items in their stomachs (*i.e.* ~28% of *A. elongata*, ~38% of *C. mugiloides* and ~11% of *L. presbyteroides*; Poh *et al*., 2018). Thus, the predation potential of atherinids was assigned a much lower predation score than that of *O. rueppellii*, which had a much greater percentage of full guts (~76%) which also consisted of greater numbers of released *M. dalli*, with up to 300 found in a single fish stomach. The current scoring of predation may underestimate the predation by *O. rueppellii* and should be reviewed to ensure that the predation potential of each predatory species is accurately accounted for in the tool based more predominantly on the dietary studies of Poh *et al*. (2018) once completed rather than the current scoring method.

Enhancing the standardisation of scores for the conspecifics and competitor factors would also be valuable for enhancing SMART. Neither of these variables varied greatly in SMART scores and therefore did not have a large influence on the overall score, even though they were identified as being important to the selection of release site and time for juvenile *M. dalli* by Hodson (2016). Further exploration of the effect of these variables is required to effectively determine the extent to which abundance of *M. dalli* or *P. latisulcatus* effect post-release survival of juvenile *M. dalli*. This would be enhanced by empirical experimental studies, similar to those carried out on the Eastern King Prawn *Penaeus plebejus* (Ochwada-Doyle *et al*., 2012) and Brown Tiger Prawn *Penaeus esculentus* (Loneragan *et al*., 2001).

Scaling and weighting of factors and variables

Where factors were comprised of more than one variable, *e.g.* water quality or conspecifics, both of which had two variables, each variable was given an equal weighting, except for predation where the RPI of each species was added together and the total standardised. The equal weighting of variables in a factor may dampen variation in the overall factor score, as seen for example in the water quality factor where temperature was similar across all sites, but salinity varied. As a result, a positive score for temperature accounted for 50% of the weighting in the water quality factor, which reduced the effect that variation in salinity had on the overall

score and made differentiation between sites less pronounced. Differential weighting of factors, such as temperature and salinity so that that variation in one, in this case salinity, has more influence on the final score could yield better results. In the case of conspecifics, both total *M. dalli* and gravid *M. dalli* scores were assigned the same weight. However, as the tool was designed interested mostly in identifying the spawning grounds of the *M. dalli* population, a better approach may be to weight the score for the abundance of gravid female *M. dalli* more than the total abundance.

In the current version of the SMART, each factor was given equal weighting for contribution to the final score, which may not be representative of their importance on post-release survival. For example, predation is often the single greatest obstacle for short-term post-release survival of hatchery-reared juveniles in the wild (Hines *et al*., 2008; Støttrup *et al*., 2008). It may therefore by more realistic to weight predation more highly than the other factors in the SMART. Further study is required to determine the relative effect of each of the factors on the selection of release site and time in order to understand the most effective procedure for weighting factors in the tool.

Adaptation of the SMART to other release programs

The main objective of this Thesis was to use the SMART to determine the optimal release site and time for *M. dalli* in the Swan-Canning Estuary. However, the aim of designing the SMART was to develop a tool that could be adapted for other release programs, *i.e.* for other species in different aquatic systems. Many ongoing release programs include a robust post-release monitoring regime, therefore sufficient data is likely to be available for these programs to modify the SMART for their use and assist in optimising their release strategy. In order to adapt the SMART to be applied to evaluate release strategies for other species and environments, the factors or variables included in the calculation need to be re-evaluated in consultation with the stakeholders for the release. These stakeholders should include researchers/aquaculturists, managers, beneficiaries of the release and the broader community. The following section

explores the variables that may need to be included, removed or altered in order to adapt the SMART for selection of a release site for other species.

Water quality

In our study the only water quality variables identified as having a large impact on the survival of released *M. dalli* were water temperature and salinity. Other environmental variables may have an effect on other species and/or on the water body they are being released into. For example, pH, although not included in our study, may have a significant effect on the release of molluscs. Exposure of molluscs to even slightly acidic pH can result in shell dissolution, which may decrease shell strength and thus increase predation on these organisms (Gazeau *et al*., 2013). The abundance of phytoplankton as a food source may also be a significant factor for releases of filter feeding organisms such as bivalves (Arapov *et al*., 2010). While the concentrations of dissolved oxygen were not low during our study in the Swan-Canning Estuary, they have been in the past (Hamilton *et al*., 2001; Tweedley *et al*., 2016a) and have the potential to kill large number of released (and wild) individuals. Therefore, dissolved oxygen concentration should be included in future SMART models, and particularly for release strategies in water bodies where periodic hypoxia and anoxic conditions may occur.

Availability of habitat

Sediment composition is an important habitat factor for benthic species, such as *M. dalli*, but is less important for pelagic species. It is therefore necessary to identify habitat preferences or requirements specific to the target species and to include the relevant variable. For example, a study on the habitat preference of juvenile Mulloway *Argyrosomus japonicas* determined that the presence of deep holes influenced the residence times and dispersal of released fish; with individuals' resident for much longer in deeper holes than in shallower release sites (Taylor *et al*., 2006). Therefore, the presence of, or distance to deep holes, should be included as a variable to select an appropriate release site for *A. japonicas*. For the release of *P. esculentus* in Exmouth

Gulf, (Loneragan *et al*., 2004) determined that a density of at least 5 gm^{-2} of seagrass bed was needed for juvenile settlement, survival and growth. Modeling results based on empirical studies (Haywood, 1995; Loneragan *et al*., 1998; Loneragan *et al*., 2001) predicted that much greater numbers of prawns survive in high biomass seagrass beds than those with low biomass or bare substratum (Loneragan *et al*., 2006). Therefore, seagrass density at each site would be an important factor to include in the model if adapting the SMART for the release of *P. esculentus* in the Exmouth Gulf.

Abundance of competitors

In this study, the only competitor identified as having a potential negative effect on *M. dalli* survival post-release was *Penaeus latisulcatus*. However, the number of competitor species may be larger or the distribution of competitors throughout the estuary may be higher for the release of other species or in other water bodies. For example, competition for space can clearly play a pivotal role in the survival of a species in rocky intertidal communities (Cornell, 1961; Paine, 1966). Whilst *M. dalli* and *P. latisulcatus* showed a high degree of spatial segregation in the Swan-Canning Estuary, teleosts may have a lesser degree of segregation due to their greater movement ability. A possible example of interspecific competition between fish is shown by the release of Brook Trout *Salvelinus fontinalis* in lakes across the Laurentian Shield, Canada. The growth and yield of Brook Trout were inversely correlated with the density of White Sucker *Catostomus commersonii* (Tremblay, 1991), suggesting that interspecific competition may have been having affected trout growth.

Abundance of conspecifics

The presence of conspecifics was considered as positive metric for the release of *M. dalli*. However, this may not be the case for all species and thus it is important to understand the lifecycle of the target species and density-dependent response to determine whether conspecifics are positive or negative influence on the release. For example, the presence of

adults may not be the best place to release juveniles of all species. If releasing juvenile *P. latisulcatus,* a species that spawns in marine waters and the post-larvae migrate to inshore waters or estuaries, releasing juveniles where adults are found is unlikely to be successful. In eastern Australia, releases of post-larval *P. plebejus* have been made in shallow lagoons distant from the oceanic breeding grounds (Ochwada-Doyle *et al*., 2009; Taylor and Ko, 2011). If the wild population of the target species is large, density-dependent effects on growth and mortality may become important (Lorenzen, 2005). The abundance of conspecifics may also become a negative factor if the carrying capacity of the site is reached, with larger adults outcompeting smaller released individuals, however, it is unlikely that release of hatchery-reared individuals would be required in such a situation. The point at which density-dependent effects is an important consideration for establishing release densities. For example, field experiments demonstrated the growth of small juvenile Green Tiger Prawns *Penaeus semisulcatus* is not adversely affected until the stocking density exceeds 10 prawns m^{-2}, a density much greater than high natural densities of 1 to 2 prawns m^{-2} in high biomass seagrass beds (Loneragan *et al*., 2001). Cannibalism by conspecifics may also become a factor. For example, juvenile Blue Crabs *Canninectes sapidus* are cannibalised by adults and peak mortality coincides with the maximum abundance of adults (Zmora *et al*., 2005; Zohar *et al*., 2008). Thus, releasing juveniles in areas with high adult abundance may therefore be a negative for releases of this species (Hines and Ruiz, 1995; Johnson *et al*., 2008).

Prey abundance

The abundance of prey was not included in the SMART as little is known of the diet of *M. dalli,* though they are thought to operate low in the food web, *i.e.* mainly primary consumers and detritivores. For release of predatory teleosts such as *A. japonicus* or Barramundi *Lates calcarifer,* however, prey availability should be included in the model. When identifying whether a site was appropriate for the release of juvenile *A. japonicas,* Taylor *et al*. (2006) used the abundance of prey items as one of the determining factors to ensure that there was enough food available to support the density of the released population. This would not be applicable

to all species, but is more important for higher trophic level species where abundance of prey items are more likely to be a limiting factor.

Abundance of predator species and fishing pressure

In our experiment the only predation considered was that by teleosts (although scyphozoans were considered in the preliminary analyses), however, predation by other animals should also be considered for other species. For example, in a release program for Murray Cod, *Maccullochella peelii*, predation from piscivorous birds had a significant effect on post-release survival (Hutchison *et al*., 2012). Therefore, predation by birds should be incorporated into the model alongside other predators when designing a release strategy for *M. peelii*. This applies for all species that may have predators other than teleosts.

The level of fishing effort on released populations is another source of mortality that may affect the success of a release program, and could potentially be included as a factor. For example, if the selection of a release site was on a larger scale such as that between lakes, then the effect of fishing pressure may differ based on the accessibility of that lake. If the aim of a release program were to rebuild stocks for conservation, then the level of fishing pressure would be an important factor for selecting which lake to release in. Changes in management, such as reductions in fishing effort or introducing seasonal closures to fishing, may also be beneficial to the success of releases for restocking purposes.

Visualisation of the SMART results

Once the SMART has been refined based on the improvements suggested above, the output should be presented in a way that is simple and easy to interpret for researchers/aquaculturists, managers, recreational fishers and the broader community. A visual output of the SMART scores for each site, such as that shown in Fig. 3.5.20, is likely to facilitate discussions about the selection of the optimal release site(s). This example was created for January 2014, the time

period assessed as the best for release across the three years of available data. At this time, the best release site based on the overall SMART Score was Deep Water Point (80), with several other sites having scores of ≥ 70 (*i.e.* Coode St, Rossmoyne, Dalkeith and Matilda Bay, Fig. 3.5.20). These figures provide valuable background for informed decision making on optimising the release strategies through the selection of the best release site for cultured *M. dalli* when a batch is ready for release. All the data required to population the model, with the exception of sediment composition, which is readily available from Bennett (2014), comes from a faunal monitoring regime. The entire sampling regime to provide the prerequisite data takes three evenings with researchers spending about ~20 minutes at each site. As all data is collected in the field, there is no laboratory processing of samples, and thus the SMART calculation could be calculated immediately to provide fast advice on optimal release site to stakeholders at any time.

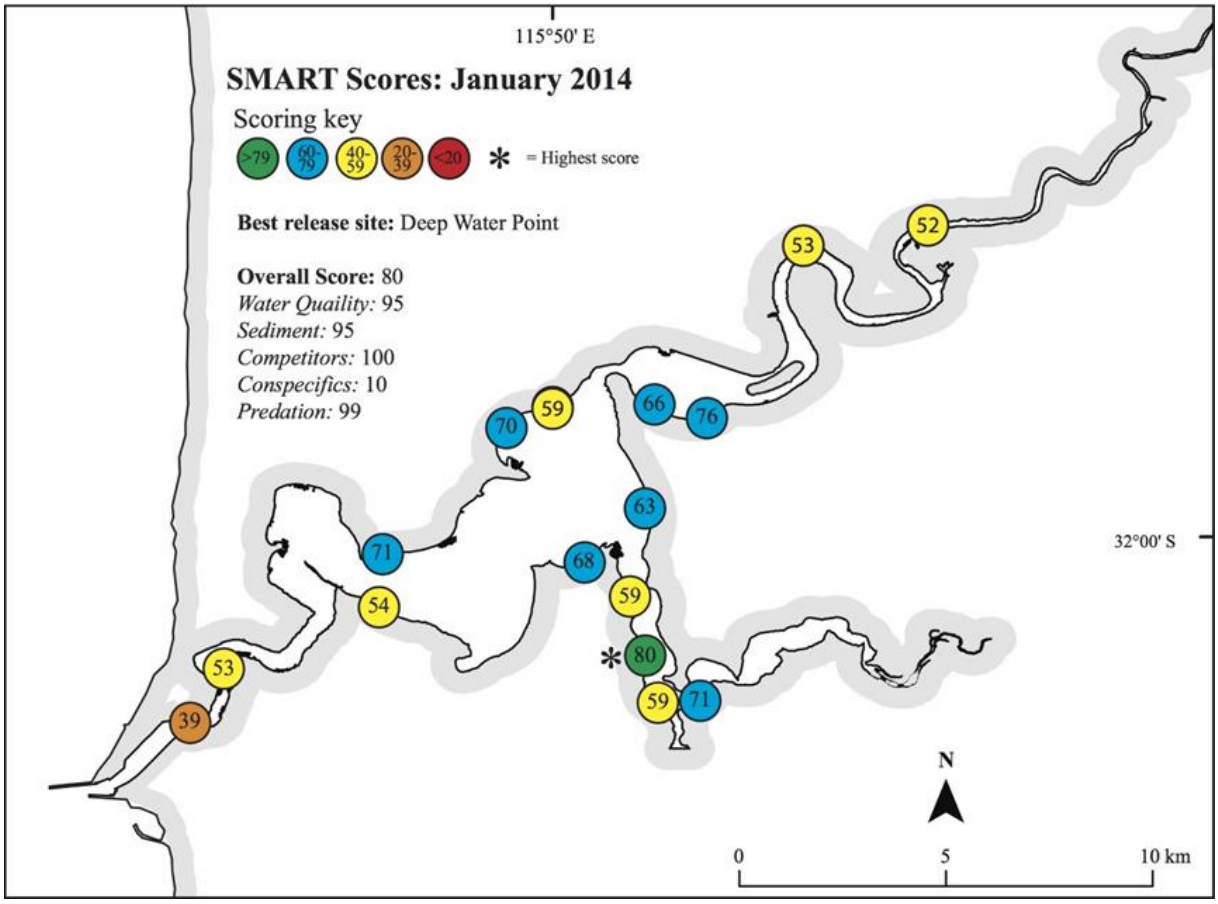


Fig. 3.5.20: Map showing the overall SMART scores for each of the 16 nearshore sites in the Swan-Canning Estuary for a night time release in January 2014.

Conclusions

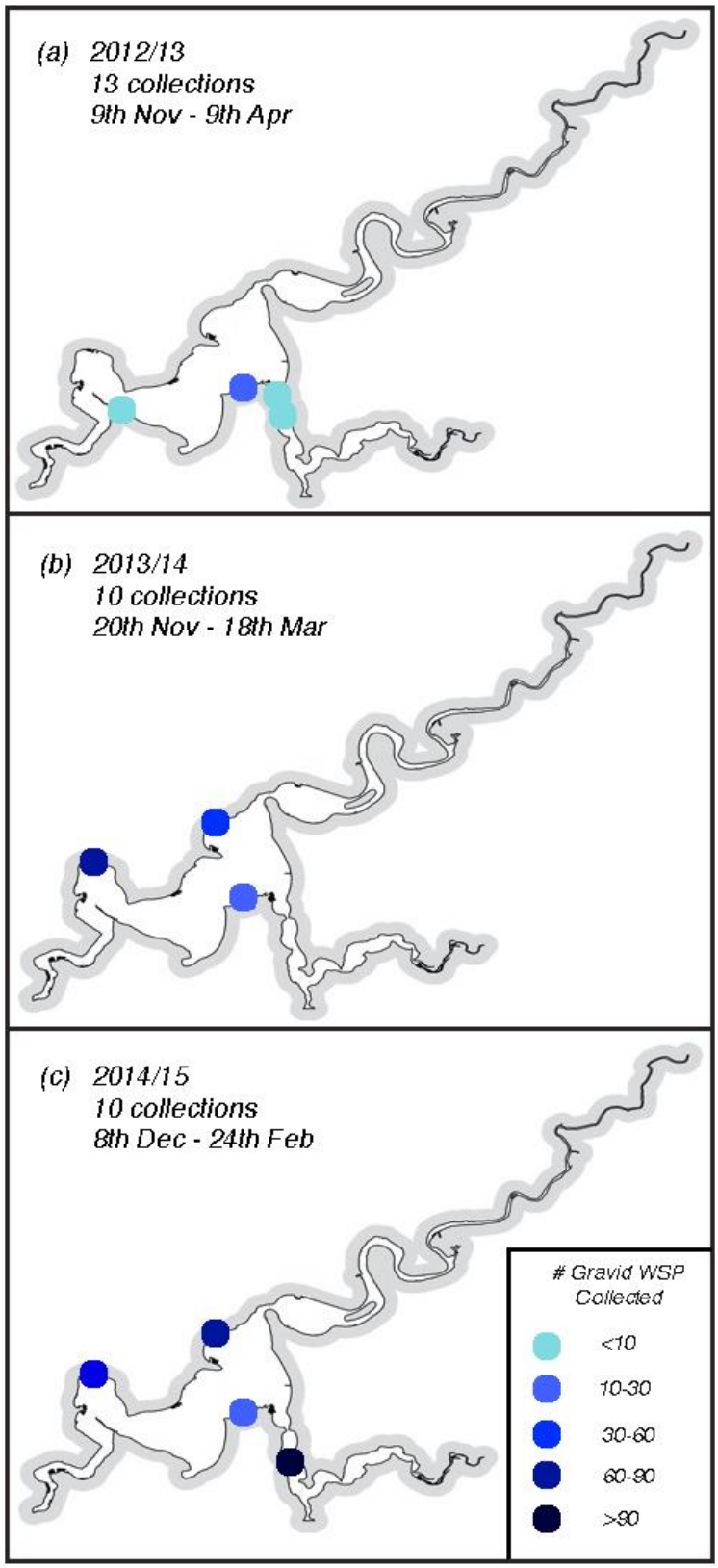
The SMART is, to the best of our knowledge, the first tool to quantitatively evaluate all data pertaining to the selection of an optimal release site and time. Through an extensive literature search, only a single study was found that employed a quantitative approach to facilitate release site selection, however, it used data only on habitat composition. Within the SMART, a comprehensive suite of biotic and abiotic variables were assessed at each site and time on their potential impact to the post-release survival of released *M. dalli* and standardised onto a common scale of 0-100. For example, the effect of salinity on the survival of prawns (Crisp *et al*., 2017b) was used to convert salinity recorded at each site into a percentage survival of *M. dalli* based on salinity at that site at the time of sampling. Where needed, scores were inverted (*e.g.* as for predators, so that a large abundance of predators had lower scores) so that a score of 100 was always optimal for the survival of released *M. dalli*. These scores were then averaged to record an overall SMART score for each potential site and time, with the highest score identifying the optimal site or time.

Region was found to exert the greatest influence on the survival of hatchery-reared *M. dalli,* with regions in the middle of the estuary (*i.e.* Lower Canning Estuary and Perth Waters) recording the highest scores. The next most influential factor was years, followed by months. The variables used in the calculation of SMART that varied the most among regions and/or over time (months and year) were salinity, sediment composition and teleost predation. The optimal site and time of the year and day for release was during the night, at Deep Water Point in the Lower Canning in January 2014. This site, at the time, had good water quality (*i.e.* a water temperature of ~ 26 °C and salinity ~ 36) and sediment composition (*i.e.* relatively low amounts of particulate organic matter and large contributions of fine sand particles) the complete absence of any competitor (*P. latisculatus*), as well as relatively high scores for conspecific *M. dalli* including those that were gravid and low abundances of teleost predators.

The result from this initial run of the SMART supports the view that an objective, quantitative approach for selecting release sites and times will facilitate the development of informed release strategies that help maximise the success of aquaculture-based enhancements. The tool showed significant difference in the variability of scores both spatially and temporally within the Swan-Canning Estuary. While this first iteration of the SMART shows promise, further modifications are likely to improve its reliability. The following section identifies future considerations for the development of the SMART that will enhance its power in selecting an appropriate release sites and times and thus the development of a sound release strategy.

Annexes

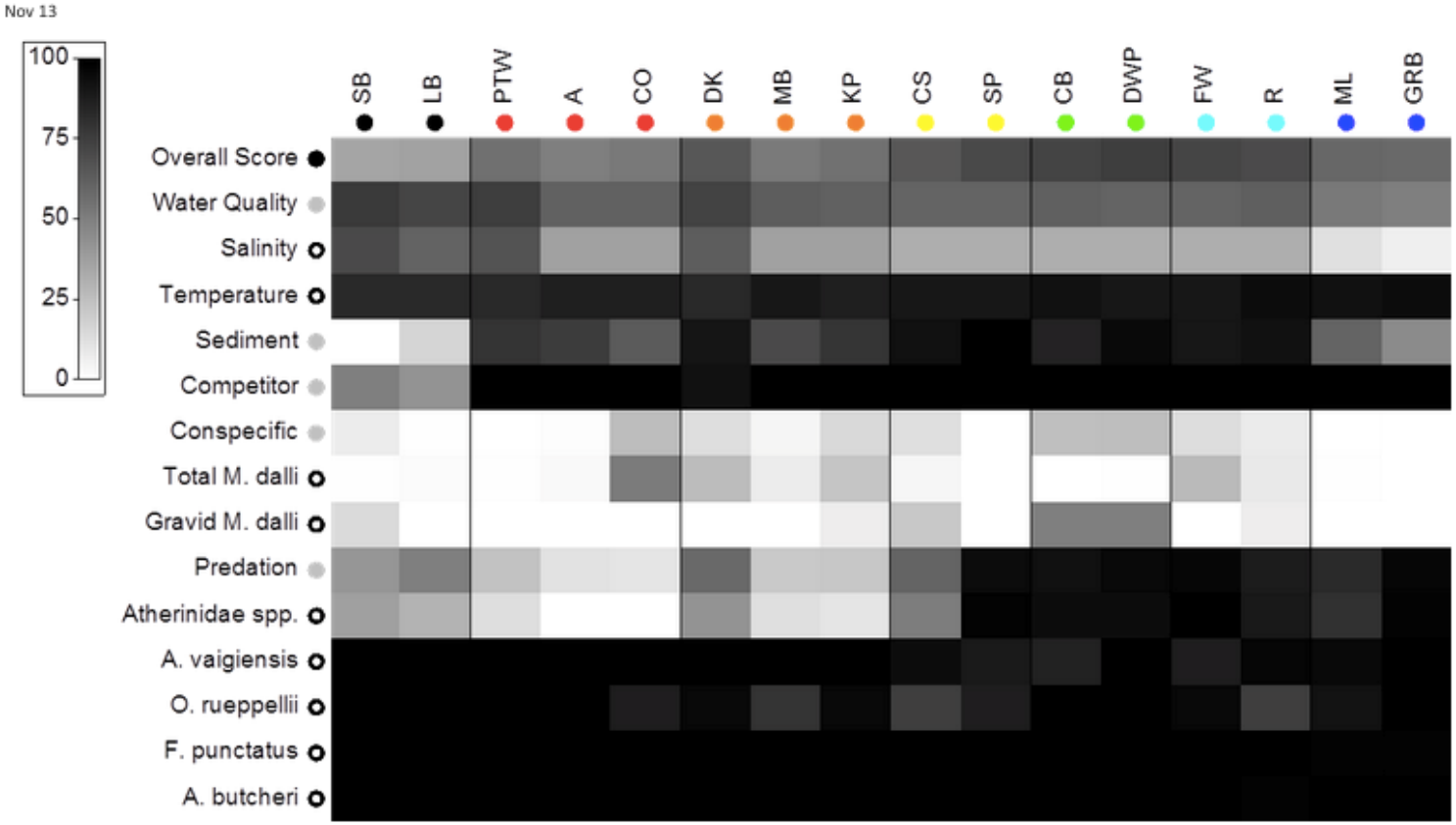
Annex 3.5.1. Map of the Swan-Canning Estuary indicating areas where broodstock collection took place each of the three year of the pilot study. Coloured circles indicate the approximate number of gravid female *M. dalli* collected. Data taken from Jenkins *et al*. (2015).



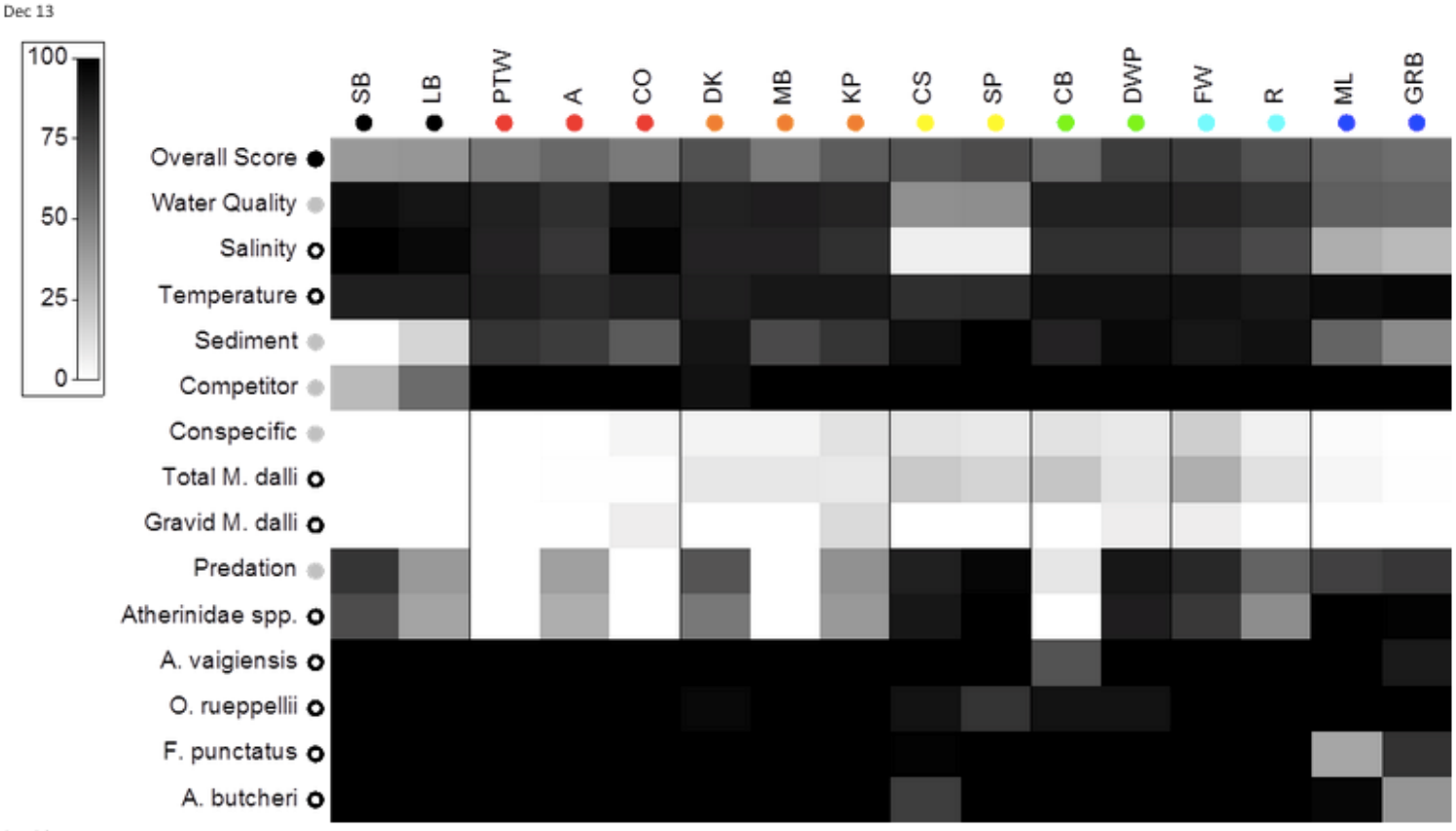
Annex 3.5.2. SMART Scores for each month/year at each site during the night-time. Colouring in each cell represents the SMART score, *i.e.* ● <40, ● 41-49, ● 51-60, ● 60-69, ● 70-79, ● 80+

Sites	Months/Years														
	Nov-13	Dec-13	Jan-14	Feb-14	Mar-14	Nov-14	Dec-14	Jan-15	Feb-15	Mar-15	Nov-15	Dec-15	Jan-16	Feb-16	Mar-16
Stirling Bridge	35	40	38	44	42	53	56	58	55	50	52	50	49	34	52
Leeuwin Barracks	36	41	51	42	60	55	57	61	59	48	61	55	54	40	51
Point Walter	56	53	52	55	53	58	68	72	53	51	69	68	55	43	51
Attadale	50	59	66	58	60	58	56	66	64	63	58	58	49	48	52
Como	52	52	61	69	57	59	59	58	66	55	66	54	57	52	52
Dalkeith	66	68	69	76	73	60	74	71	65	75	66	76	63	58	59
Matilda Bay	52	53	68	68	60	63	70	70	66	53	68	65	64	68	52
Kings Park	55	64	57	62	60	58	61	61	56	58	58	64	76	65	58
Coode Street	65	67	74	62	55	71	75	68	64	64	75	74	64	66	61
South Perth	71	70	64	76	75	76	78	63	73	66	75	69	66	61	70
Canning Bridge	73	59	58	70	64	60	71	63	63	65	80	74	75	56	64
Deep Water Point	75	76	80	76	61	76	75	72	72	74	70	76	71	66	58
Freeway	72	76	57	73	63	71	73	65	57	58	68	72	62	61	61
Rossmoyne	70	68	69	57	60	70	73	67	63	59	75	57	65	63	58
Maylands	59	60	51	66	57	62	59	67	68	66	68	61	60	52	67
Garratt Road Bridge	58	57	50	57	61	56	57	55	63	59	50	47	49	52	57

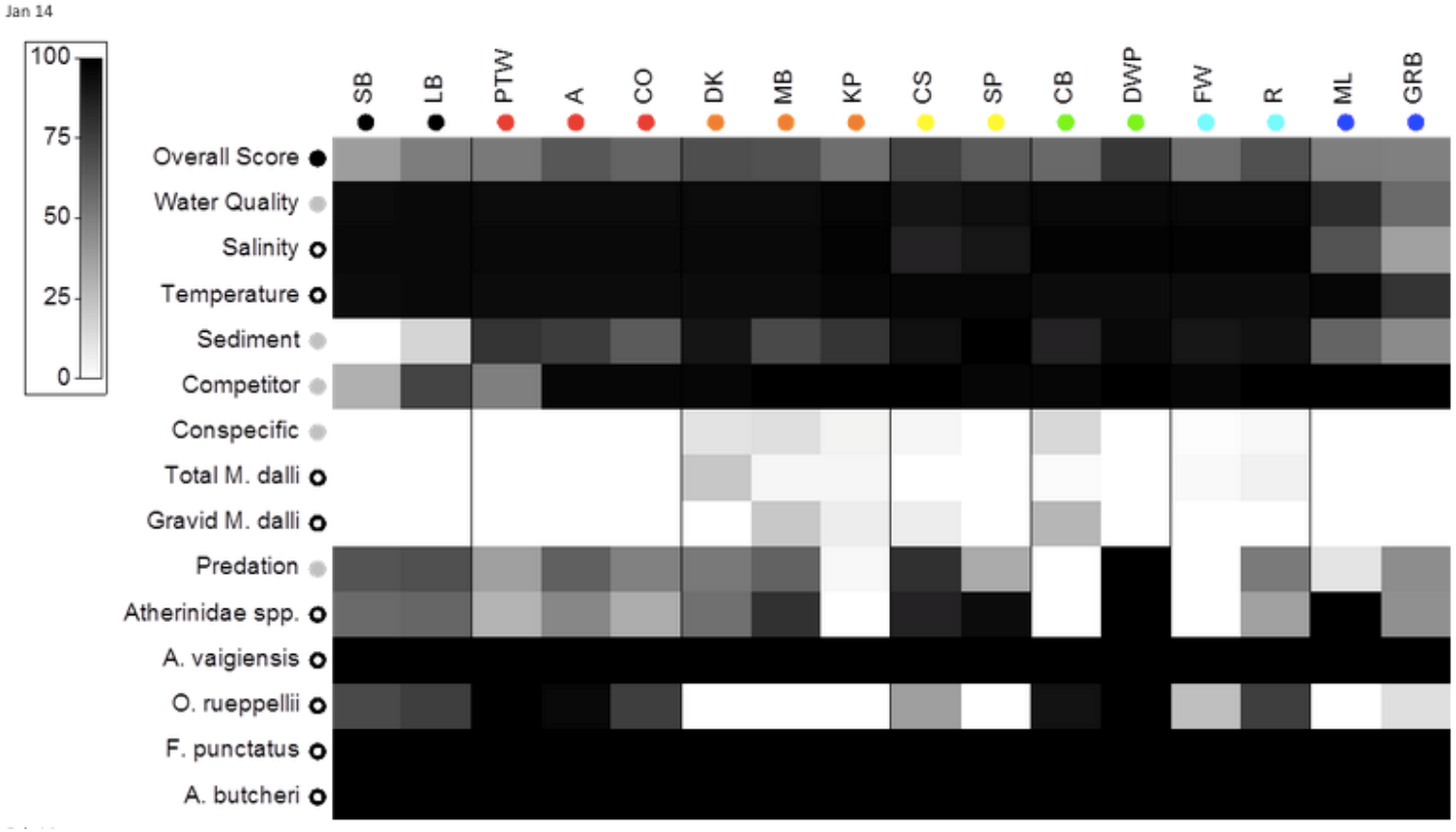
Annex 3.5.3. Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in November 2013. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.5.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.



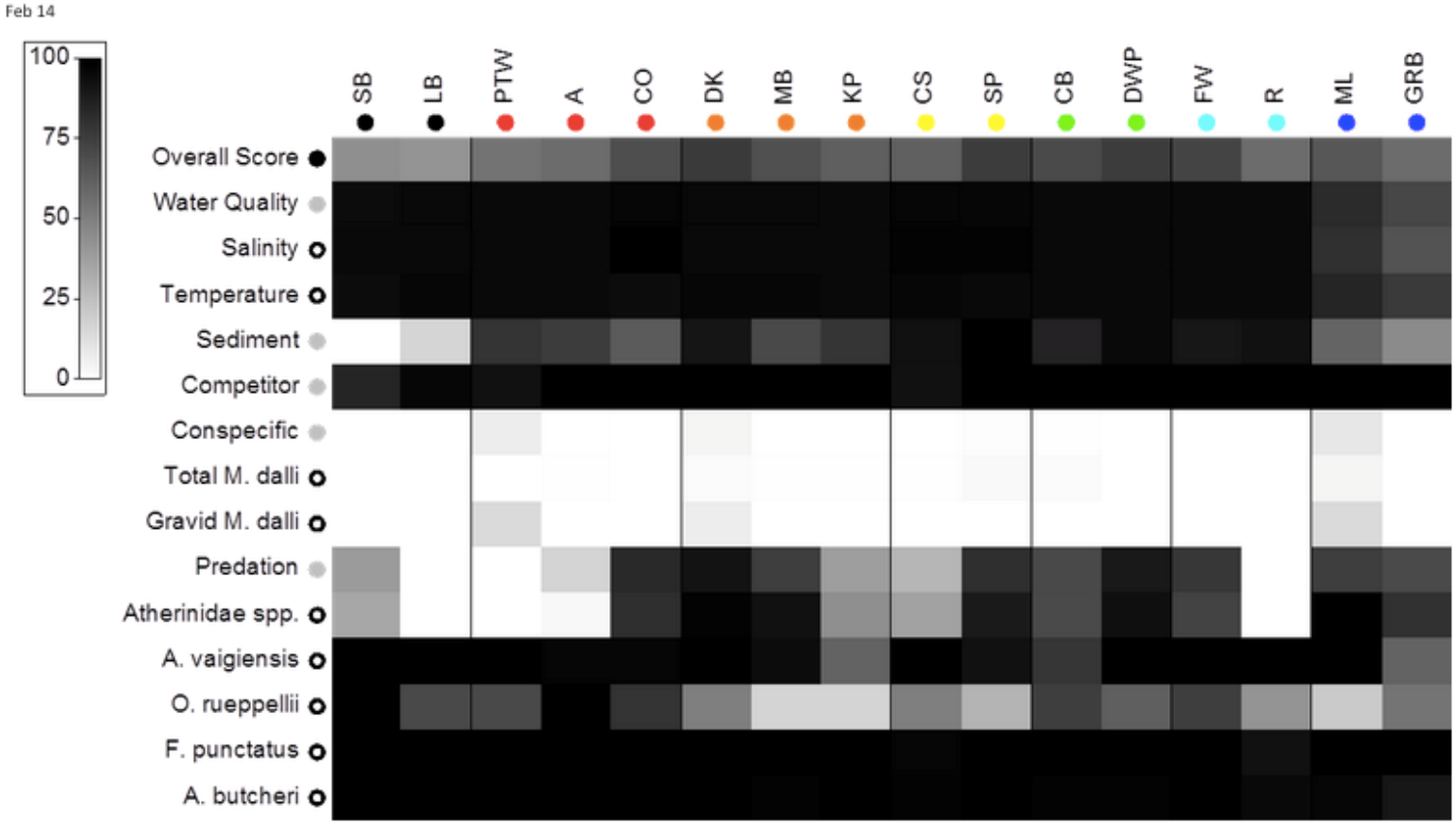
Annex 3.5.4. Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in December 2013. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.5.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.



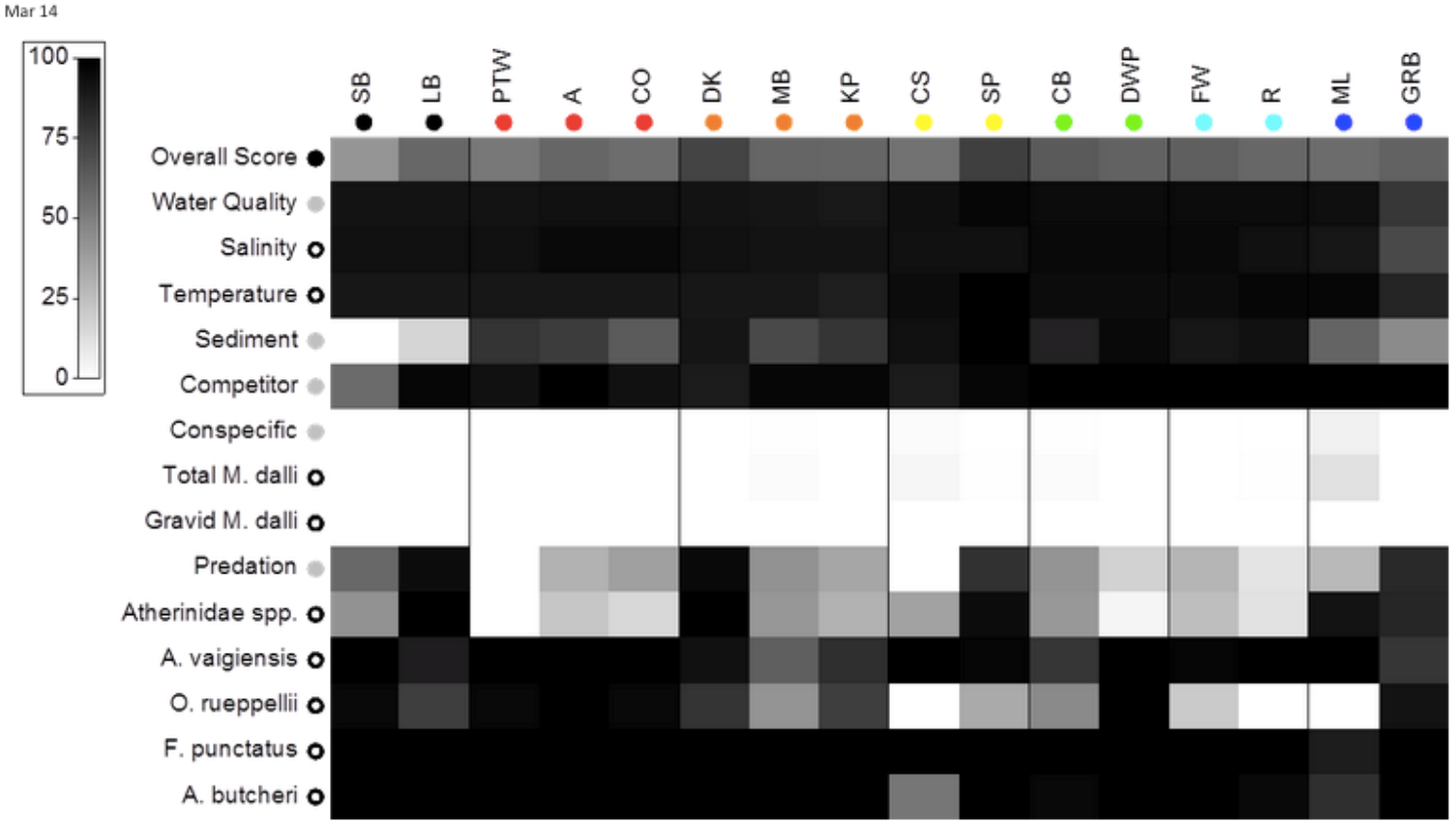
Annex 3.5.5. Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in January 2014. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.5.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.



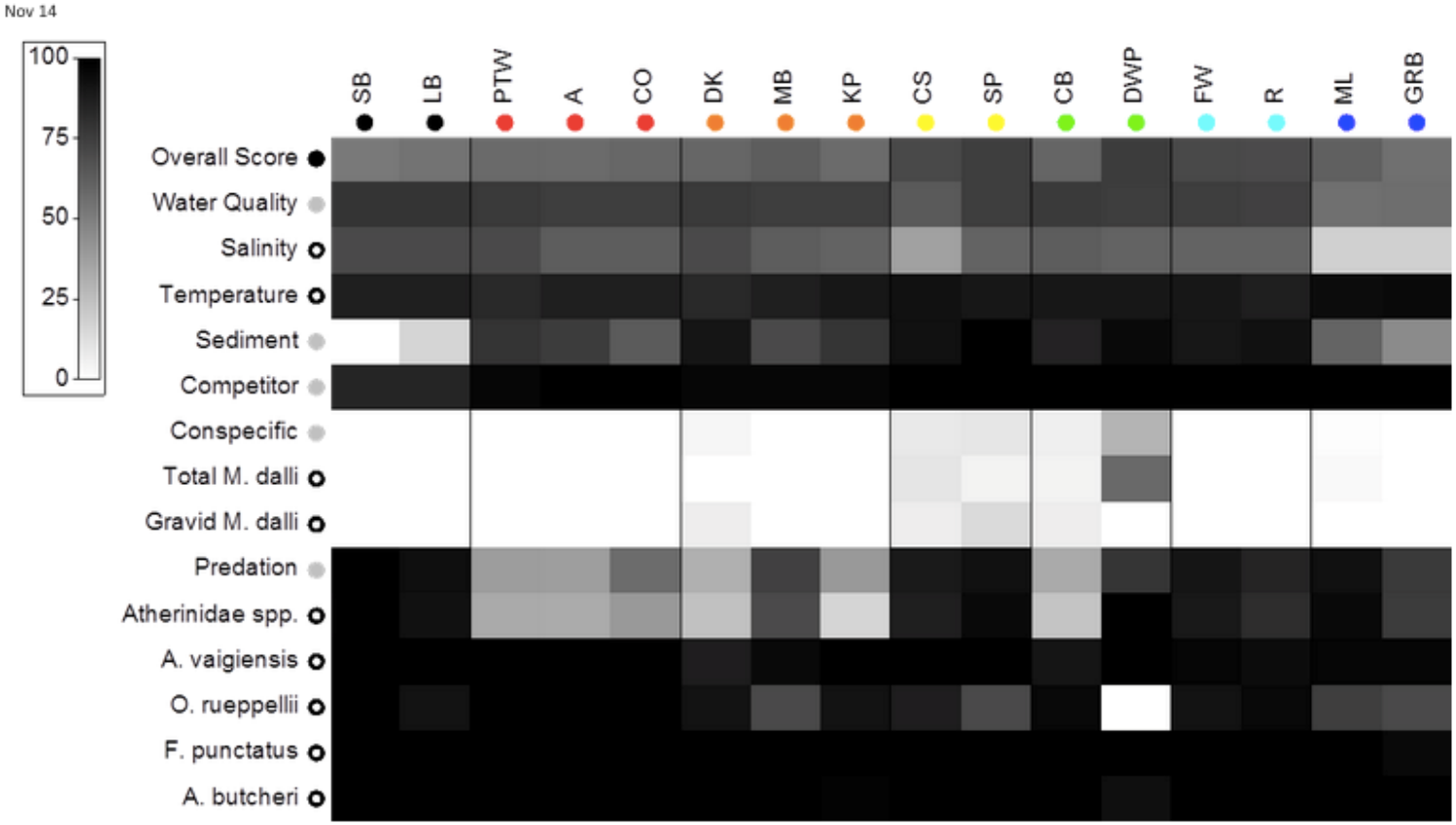
Annex 3.5. 6. Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in February 2014. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.5.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.



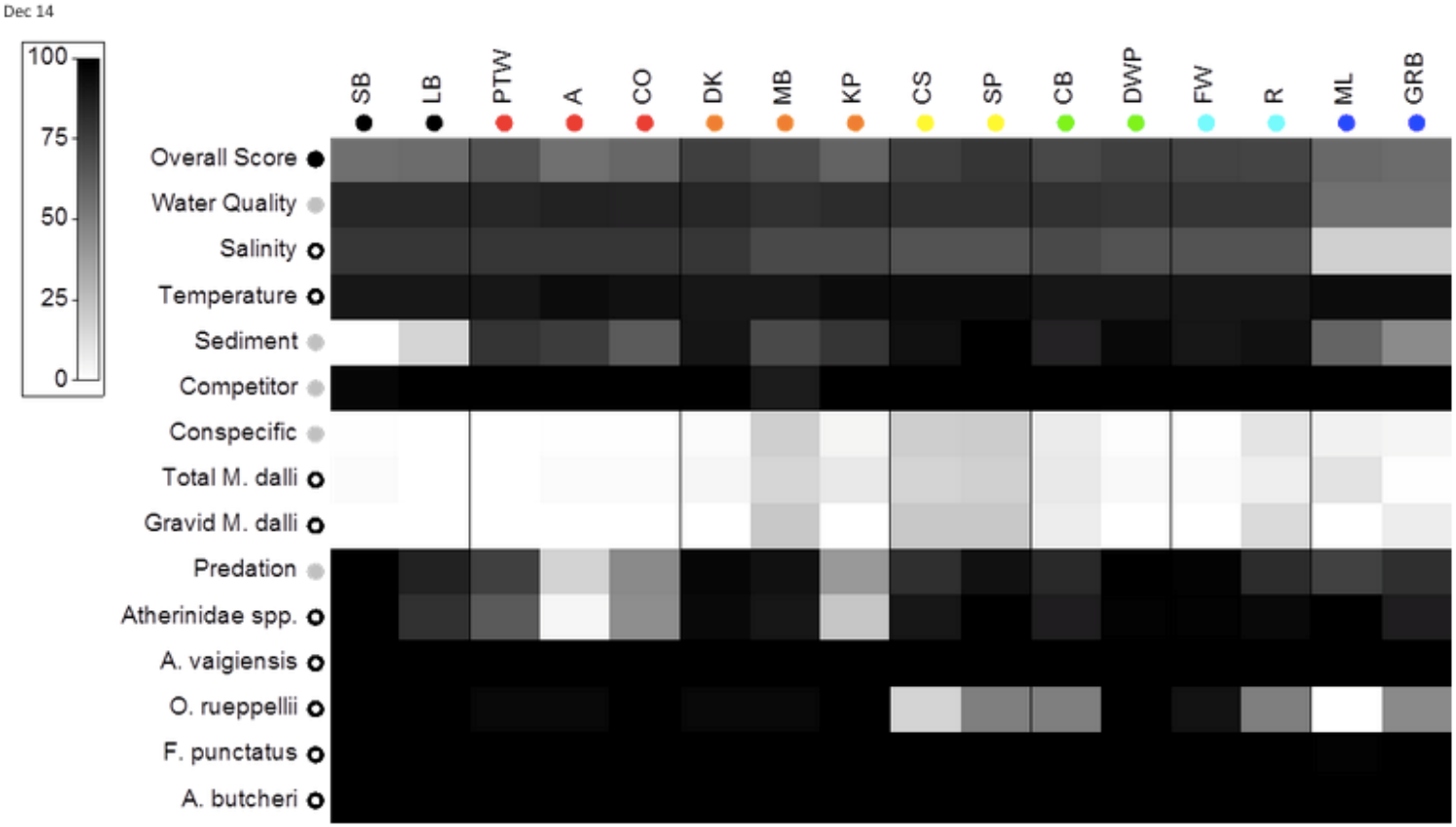
Annex 3.5.7. Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in March 2014. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.5.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.



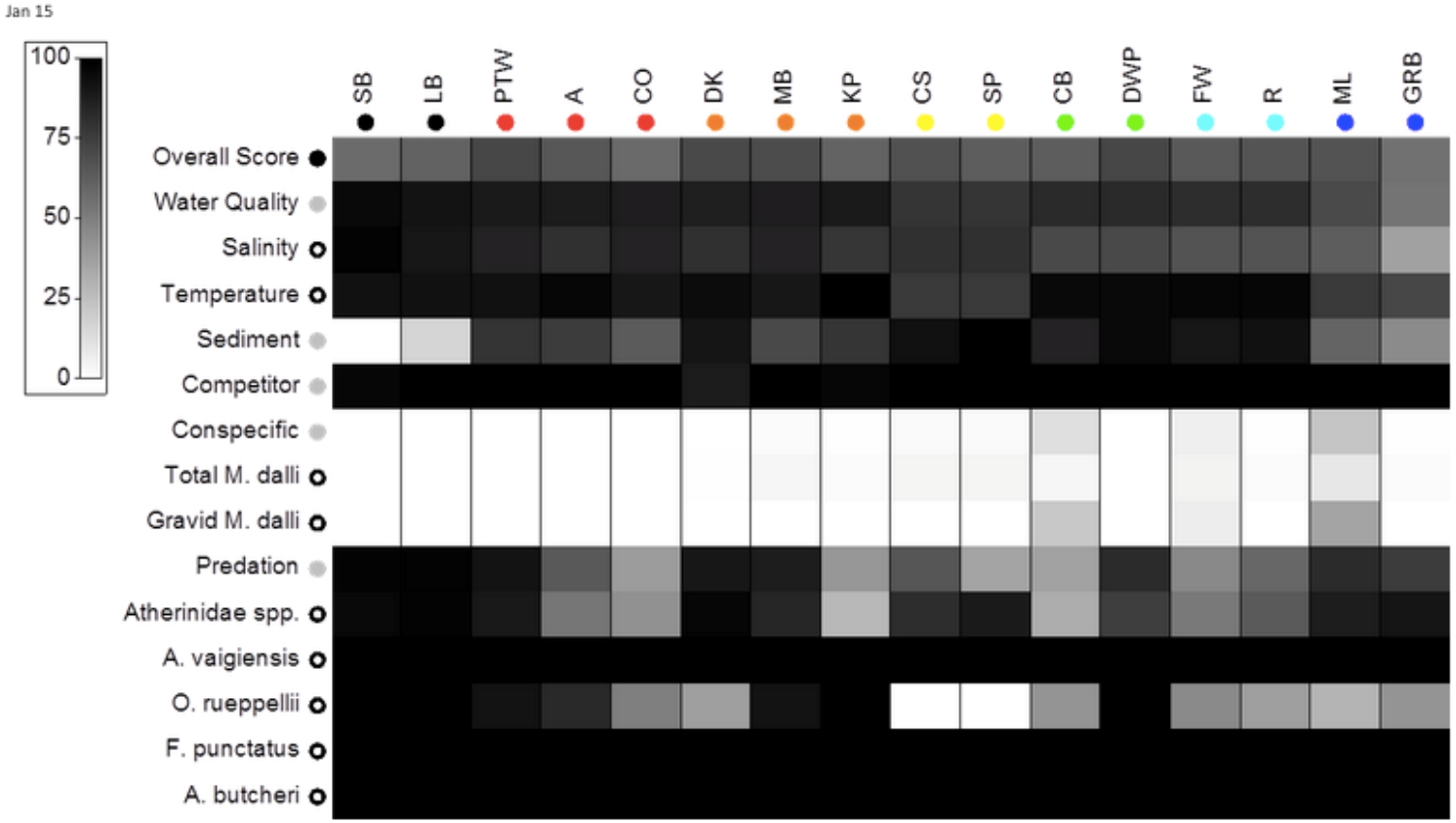
Annex 3.5.8. Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in November 2014. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.5.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.



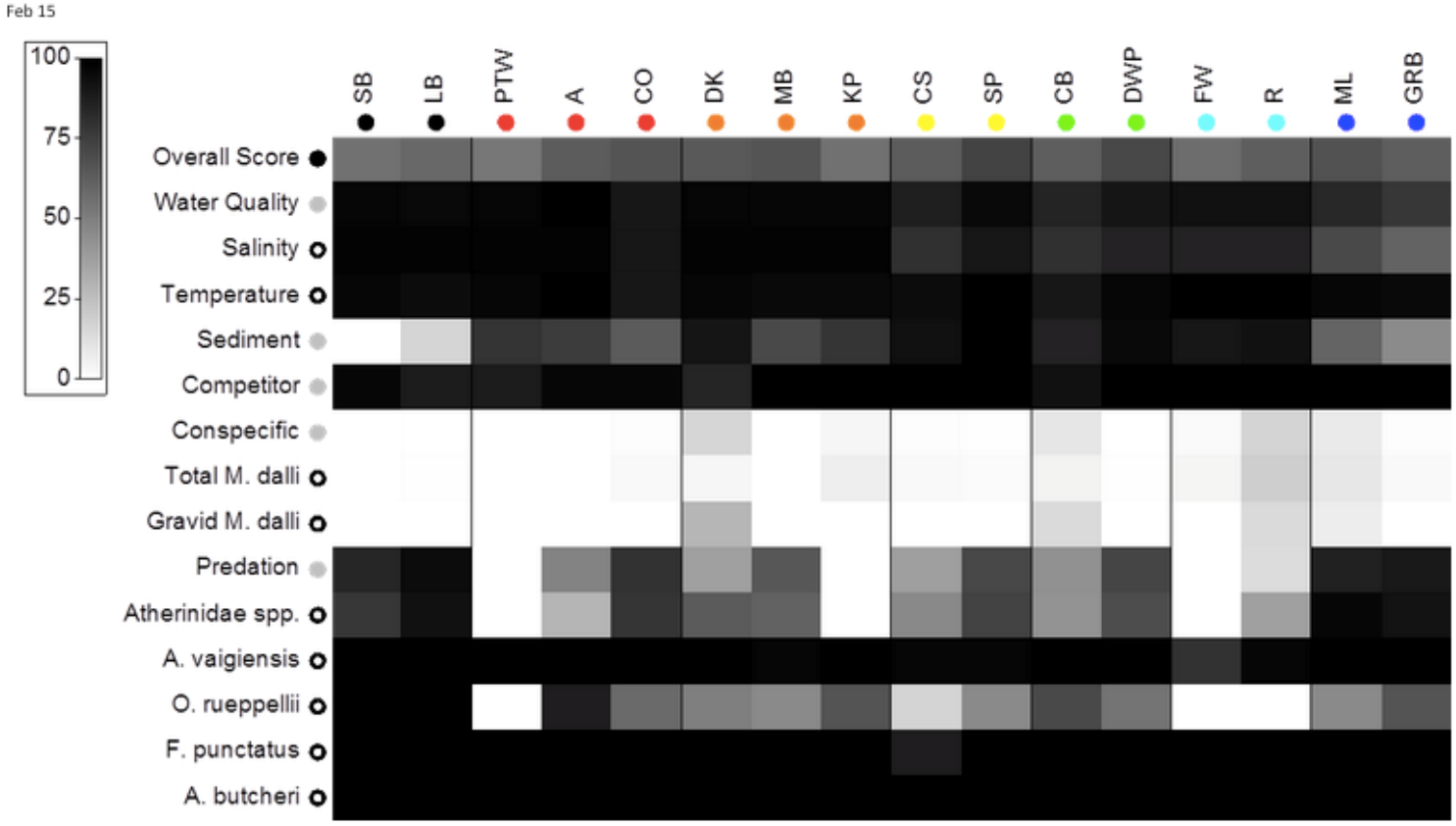
Annex 3.5.9. Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in December 2014. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.5.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.



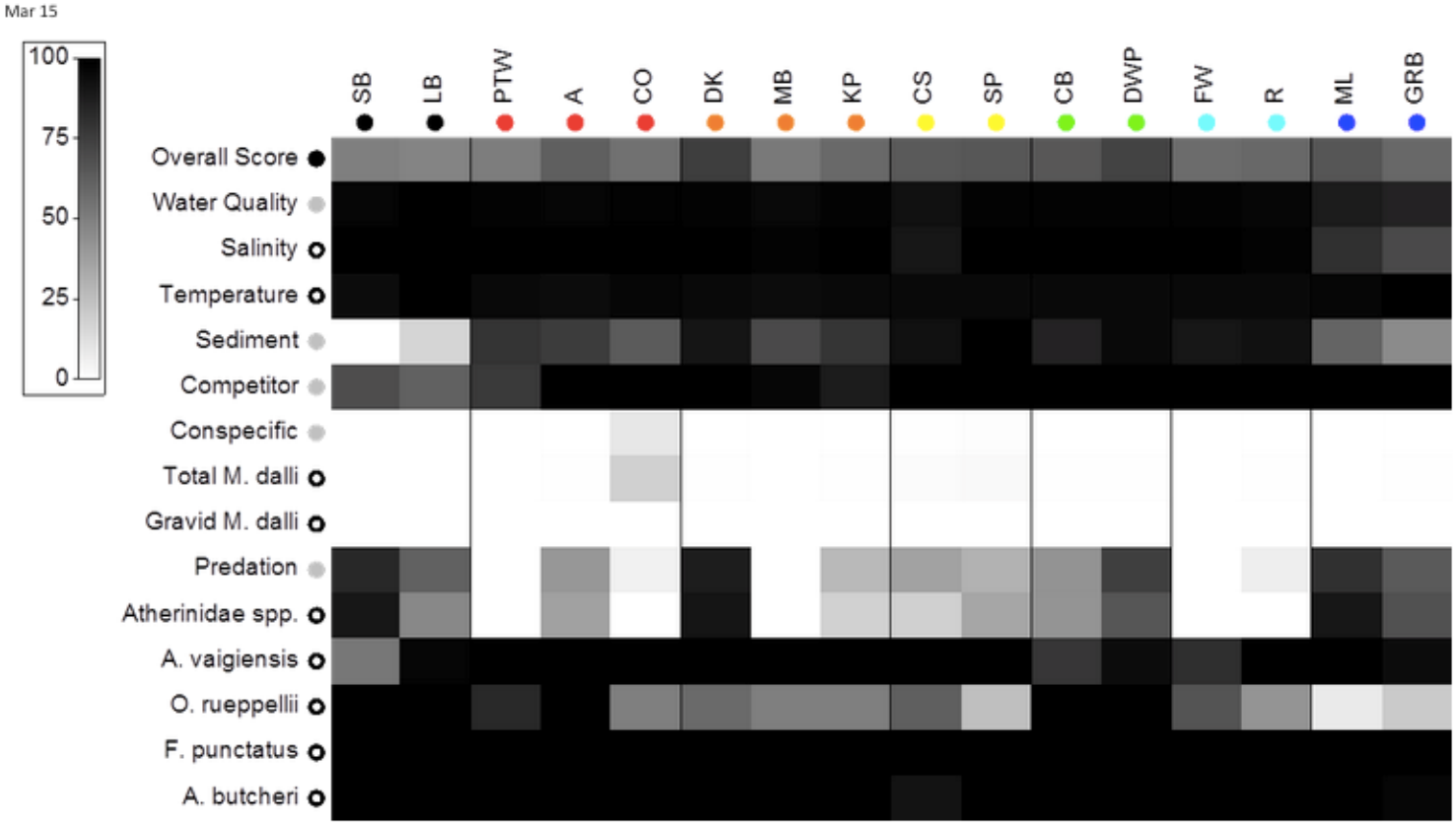
Annex 3.5.10. Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in January 2015. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.5.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.



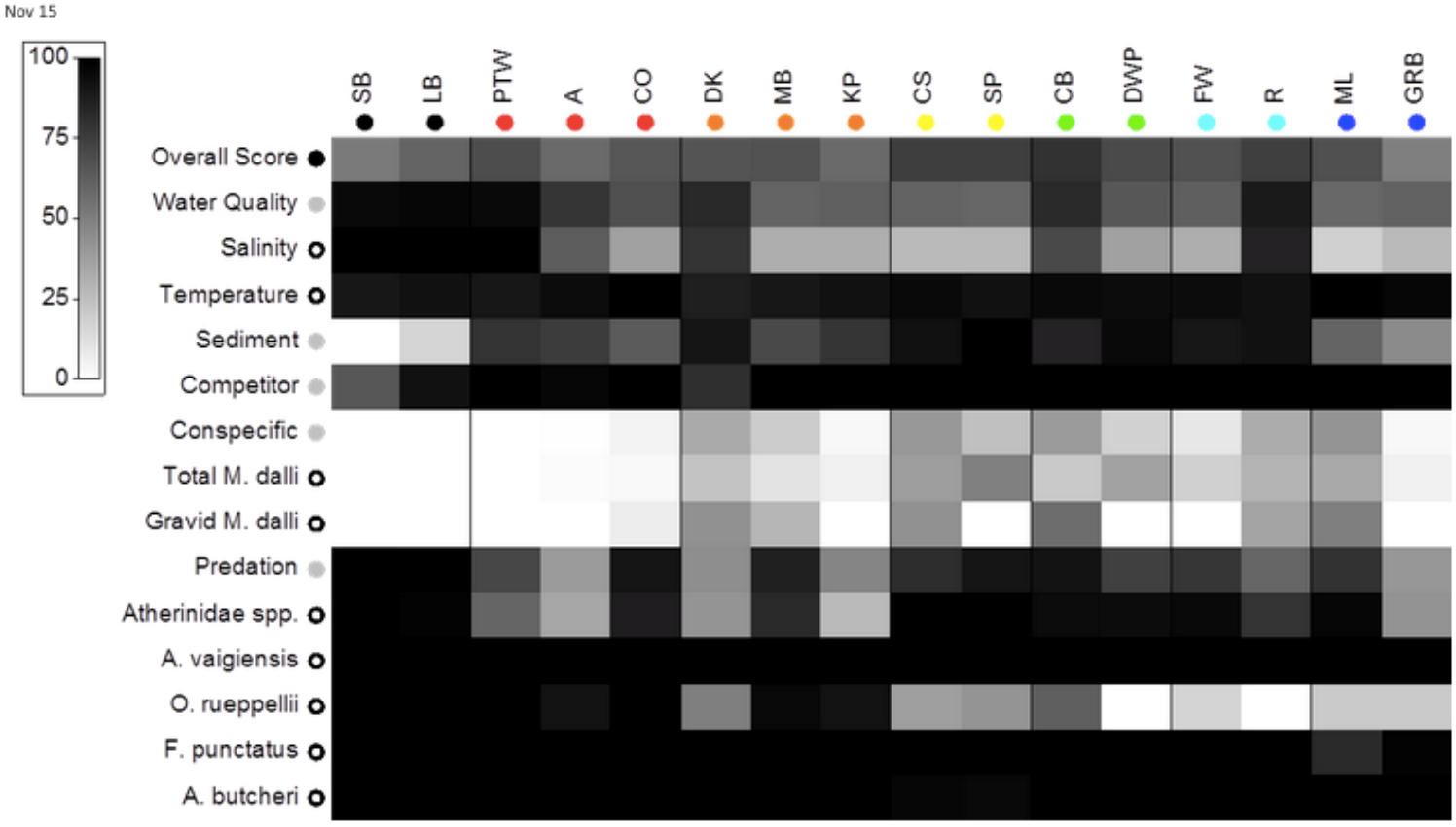
Annex 3.5.11. Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in February 2015. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.5.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.



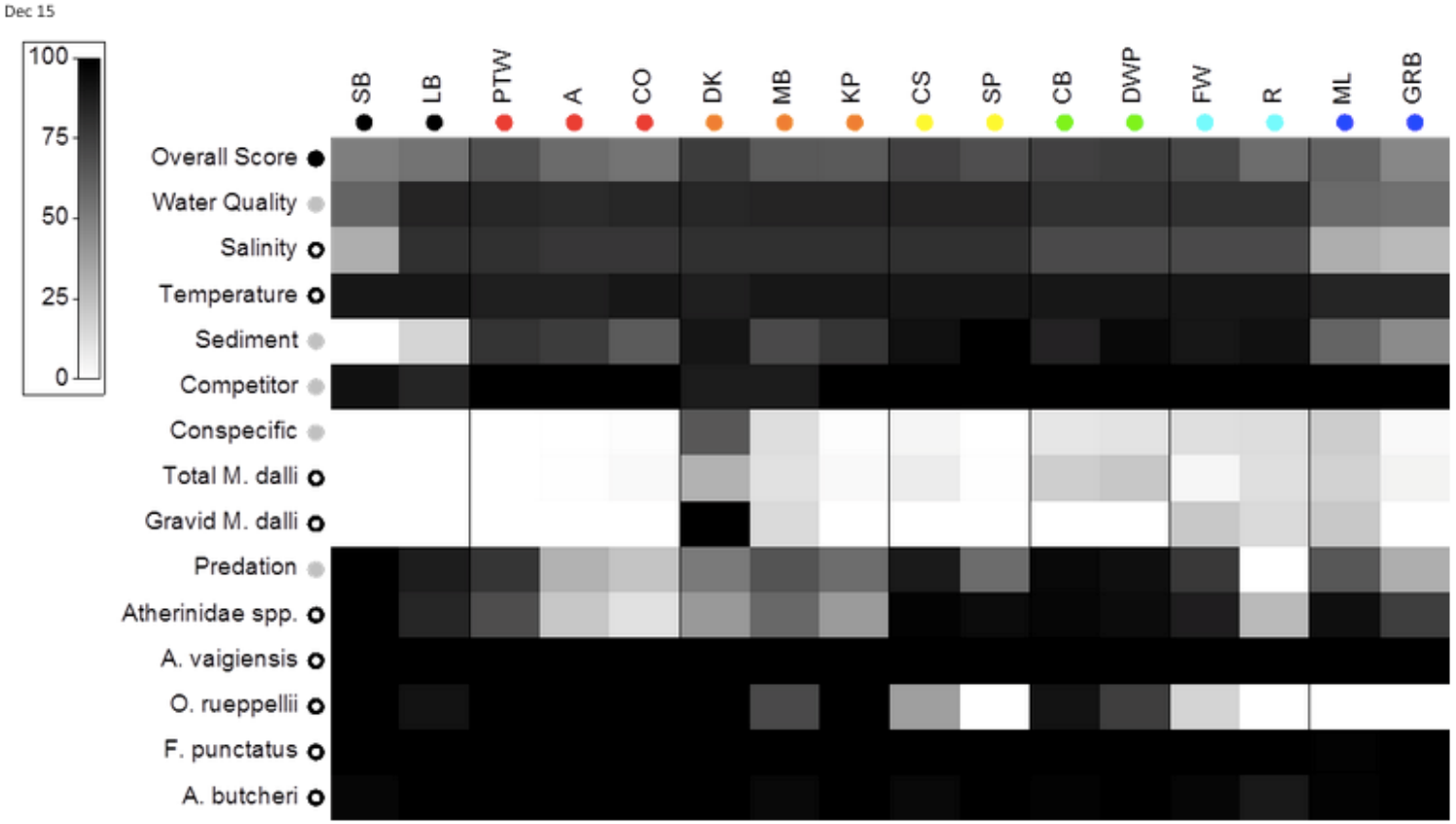
Annex 3.5.12. Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in March 2015. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.5.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.



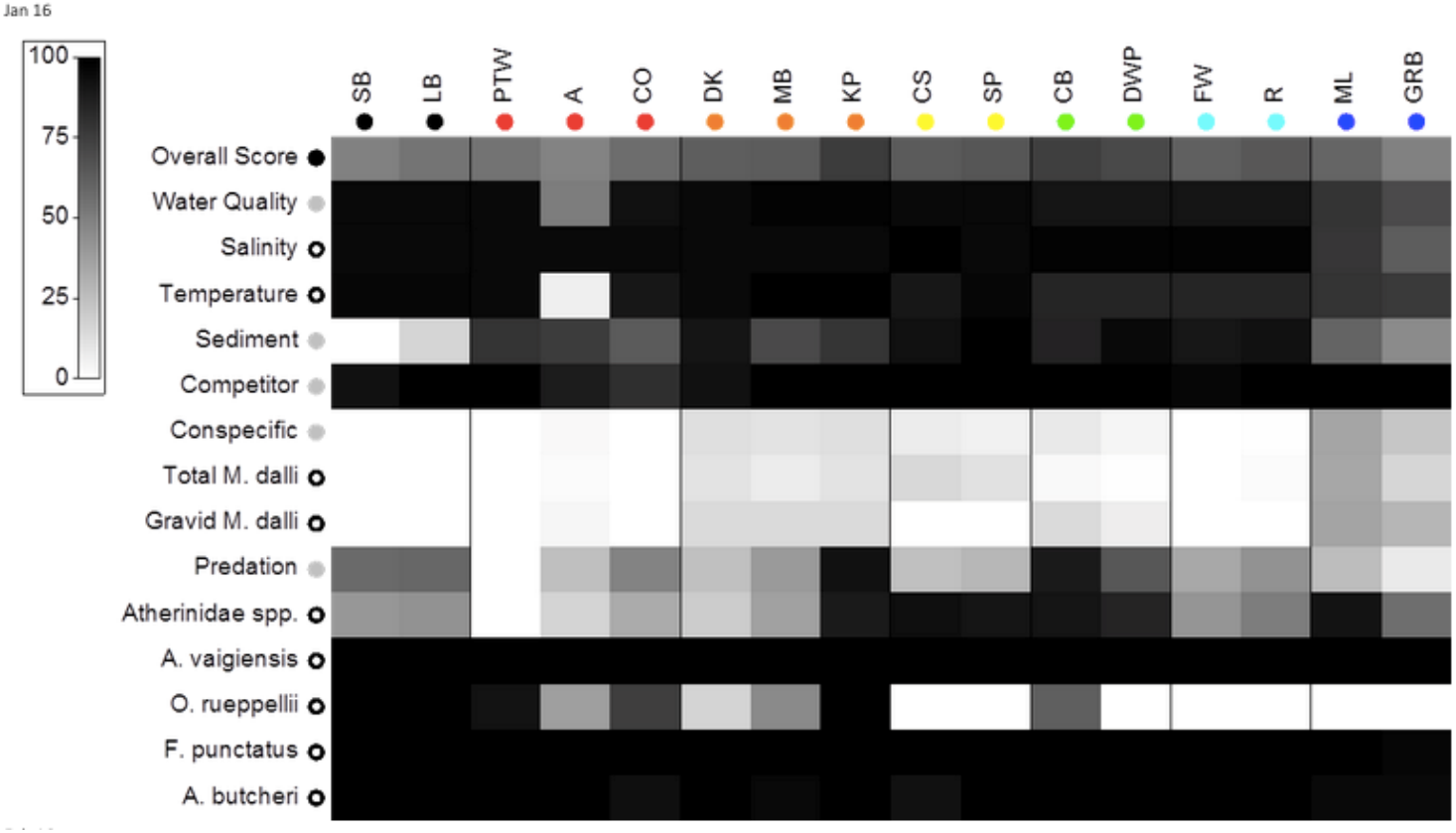
Annex 3.5.13. Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in November 2015 The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.5.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.



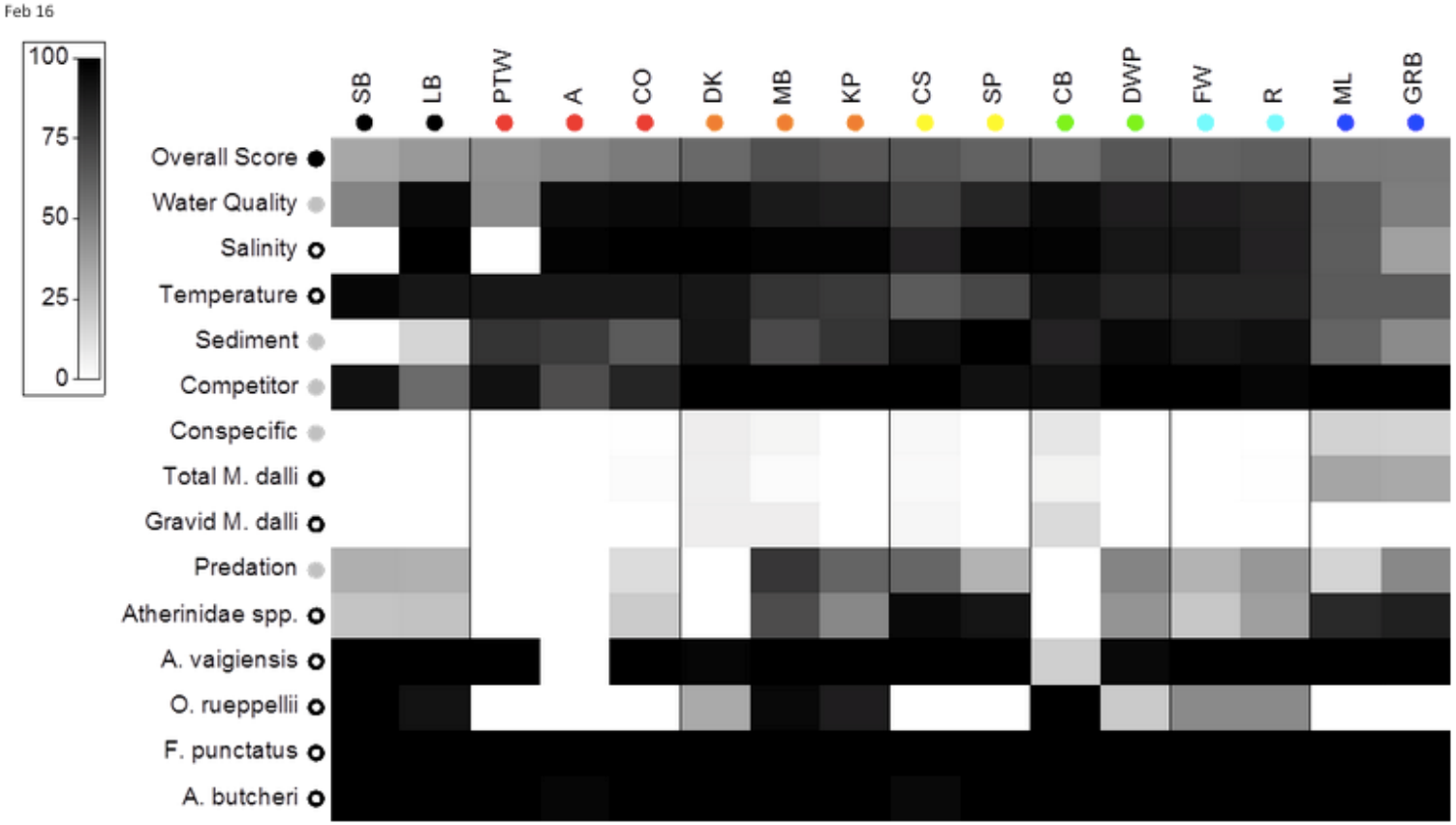
Annex 3.5.14. Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in December 2015. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.5.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.



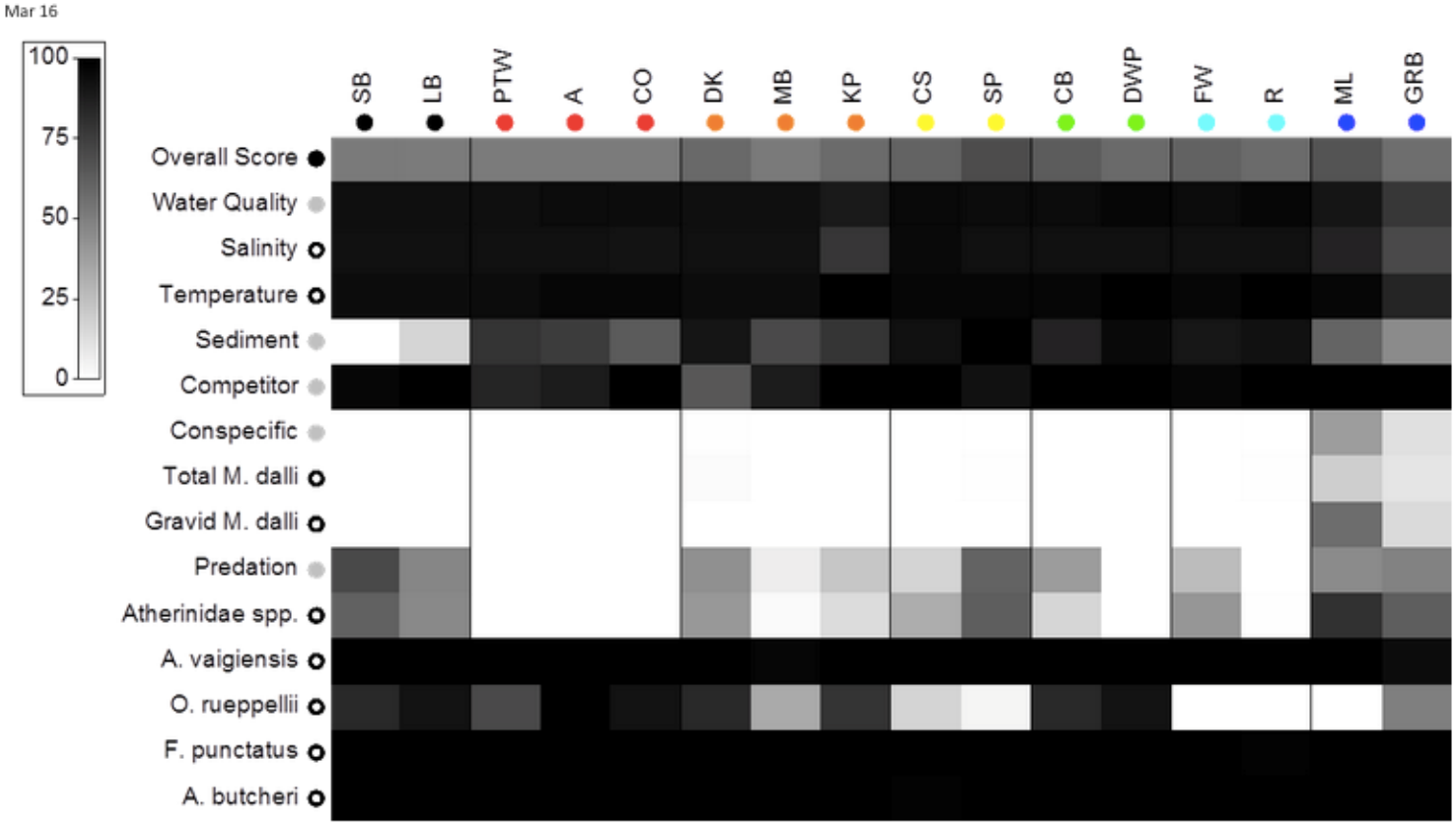
Annex 3.5.15. Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in January 2016. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.5.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.



Annex 3.5.16. Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in February 2016. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.5.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.



Annex 3.5.17. Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in March 2016. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.5.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.



Section 4. Community engagement and stewardship

This section details research relating to objective 6, *i.e.* contribute to the improved understanding of the Western School Prawn, improved stewardship of the fishery and the Swan-Canning Riverpark and objective 7, *i.e.* contribute to a citizen science program that is complementary to scientific investigation. These objectives were address in three components.

1. Prawn Watch: engaging community in science, restocking and sustainable management of an iconic species that has declined markedly in abundance (The work detailed in this component was led by the Department of Parks and Wildlife between 2012 and 2016 and supported by James Tweedley).

2. Evaluation of the effectiveness of data collected by recreational prawn fishers to provide low cost monitoring of Western School Prawns (This study was an extension of the citizen science monitoring developed in Prawn Watch and funded, in part, with the Recreational Fishing Initiatives Fund and led by Kerry Trayler and James Tweedley).

3. Recommendations for a sustainable Western School Prawn fishery.

4.1 Prawn Watch: engaging community in science, restocking and sustainable management of an iconic species that has declined markedly in abundance



Summary

Prawn Watch was initiated by the Swan River Trust and continued through that organization's merger into the Department of Parks and Wildlife (now Department of Biodiversity, Conservation and Attractions). The program is aimed at engaging the community in the sustainable management of the Swan Canning Riverpark and the Western School Prawn fishery. As a citizen science project, Prawn Watch was linked to a restocking project through the involvement of volunteers in broodstock collection and prawn monitoring. It was also linked to research, through this project and the desire to better understand the ecology of *Metapeneus dalli* and factors that were limiting their natural recruitment. Researchers were engaged to share their results with community at regular feedback forums and training events. Other means of information transfer about research outcomes included River Guardians emails, Facebook, media events and video. The project effectively engaged both the public and the media thereby increasing awareness of the value of the recreational prawn fishery and the Riverpark. Through data collection, information sharing, training and awareness raising, the project has shown improvements in community understanding of the fishery, sustainable fishing practice and river management issues. Prawn Watch also provided support for prawn restocking outcomes through the involvement of volunteers in broodstock collection.

Rationale and aims

Prawn Watch is a citizen science/community engagement developed as a component of the Department of Parks and Wildlife (formerly Swan River Trust) River Guardians community engagement framework (now within the Department of Biodiversity, Conservation and Attractions). The program was initiated in 2012 and had a number of goals.

- ***Short-term:*** facilitate improved understanding of an important iconic species and the river system through data collection, information sharing and awareness raising about river issues, the prawn fishery and its management.

- ***Intermediate-term:*** contribute to improved stewardship of the prawn fishery and the Riverpark by: promoting river friendly activities; encouraging sustainable fishing practice; and contributing to improve fishing policy.

- ***Long-term:*** Contribute to an improved fishery and Riverpark environment.

These goals were outlined in a communications strategy established at the start of the project and carried through into a strategic media plan developed in 2015. The outcomes of the overall project are reported here as well as deliverables under the media plan.

Approach

Established in November 2012, with the implementation of a communications plan, Prawn Watch fostered partnerships with universities; technical institutions, government departments and natural resource management organisations. It was recognised that Prawn Watch was integral to, and one element of, a much bigger project that included: the culture and release of *Metapenaeus dalli*; and a university based research and monitoring project (Sections 1-3). As such communications outputs, products and engagement activities acknowledge the desired messages and required outcomes of partner organizations.

Prawn Watch worked to engage with community, local businesses, media outlets, scientific and government sectors across four areas of activity that are described further below that include: (i) engagement and awareness raising (ii) communications, (iii) citizen science monitoring and (iv) influencing sustainable management and policy.

Engagement and awareness raising

Prawn Watch was established with a small number of volunteers engaged early in the project to help develop communications tools and to identify prawning sites based on historical catch information to support broodstock collection. These volunteers became part of an ongoing Prawn Watch Reference Group. In order to boost volunteer numbers the project was "soft launched" through a range of networks in time for a broodstock collectors' workshop on 17 October 2013. The project was later, formally launched by the then Western Australian Minister for Fisheries Hon. Ken Baston on 6 February 2014 (Fig. 4.1.1).

Four volunteer training workshops were held over the course of this project. The first volunteer induction workshop (held on 17th October 2013 at the Royal Perth Yacht Club) attracted 80 community members. The event was targeted at engaging and training people interested in helping with broodstock collection. Subsequently, 44 volunteers registered as broodstock collectors. A second event (held on 6th February 2014 at the South Perth Yacht Club) was aimed at engaging the broader community into being involved with Prawn Watch. One hundred people attended this event, with 80 new members signing up, bringing the total number of volunteers to 124.



Fig. 4.1.1. Photograph taken in 2015 of former Western Australian Minister for Fisheries, Hon. Ken Baston MLA (second from right), with some members of the project team from left Kevin Reid (Prawn Watch Reference Group), Dr Kerry Trayler (then Parks and Wildlife), Greg Jenkins (ACAAR), Will Smithwick (Prawn Watch Reference Group), Jen Elliot (then Parks and Wildlife), Dr James Tweedley, Professor Neil Loneragan (Murdoch University), Mark Pagano (then Department of Fisheries) and Dr Andrew Rowland (Recfishwest).

The third training event (held on the 15th October 2014 at Murdoch University) was targeted at engaging and training students interested in helping with broodstock collection and the university based monitoring project. Twelve people were inducted as broodstock collectors following that event bring total volunteers to 135. The fourth and final training event (held at South Perth Yacht Club on November 13th 2014) was again open to the broad community (Fig. 4.1.2). Following this event, membership stood at 179 people. The project maintained over 75% of its members, with 135 still involved two year later in November 2016.

Each of the volunteer workshops (2013-2016) included presentations from the Swan River Trust/Department of Parks and Wildlife, Australian Centre for Applied Aquaculture (South Metropolitan TAFE), Murdoch University, with additional presentation and training provided by the Department of Fisheries and community members. Topics included information on the overall project and its aims, river issues, prawn biology and ecology, aquaculture techniques, recreational fisheries regulations, safety issues, how to fish for prawns and broodstock, as well as sustainable fishing and RiverWise messages.



Fig. 4.1.2. Composite photograph of theory and practical components of training events.

As part of the volunteer workshops, community attitudes and understandings were surveyed both before and after every event. At each of the four training sessions, participants showed an increased understanding of the daily recreational bag limit for prawns after training, with 100% of respondents answering correctly in the last three events (Fig. 4.1.3). In general, participants also showed increased understanding of prawn ecology, issues facing the river and of River-wise practices. All participants at the 2015 forum were surveyed after the event and 100% of participants indicated that their understanding of Western School Prawns and/or the Riverpark had been improved.

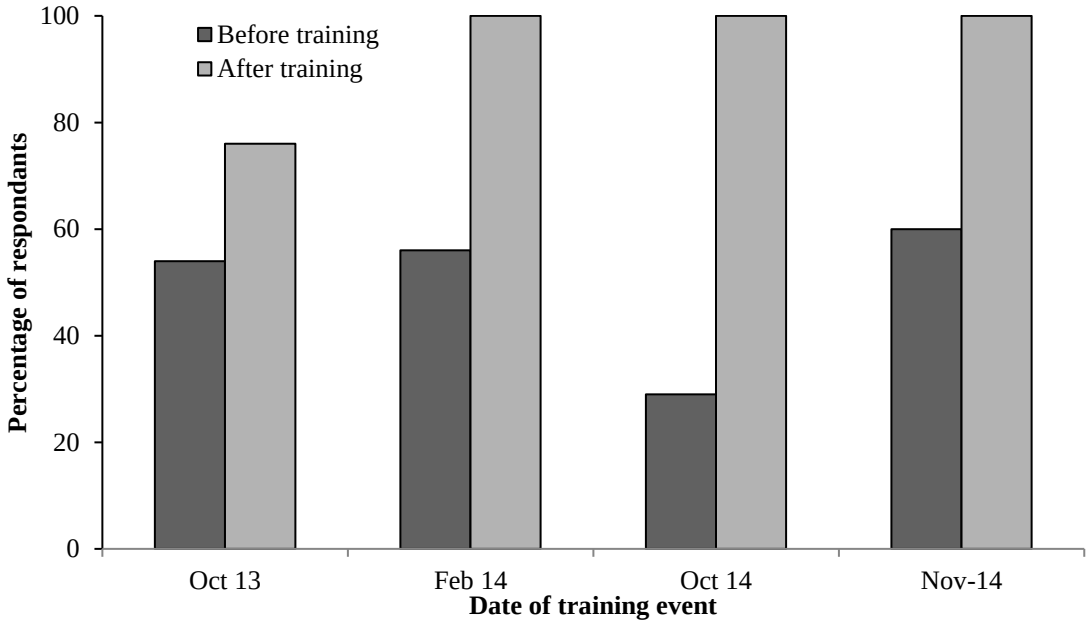


Fig. 4.1.3. Proportion of participants that knew the correct recreational bag limit for prawns in the Swan-Canning Estuary before and after training events. Return rate 8-30%.

Annual feedback forums were held in September 2014 and 2015 and a final forum, "*The Secret Life of Prawns*", was held in October 2016. These events were intended to celebrate the action of participants in Prawn Watch as well as the associated aquaculture and research and monitoring projects. They enabled researchers and managers to summarise their findings and activities across the year as well as to provide information on community monitoring, aquaculture and restocking results (Annex 4.1.8). The forums also provided an avenue to thank the community for their efforts in broodstock collection and citizen science monitoring and prizes were awarded to members for their efforts. In recognition of the action of the volunteers, awards were presented to the Citizen Scientist of the Year and Prawn Watcher of the Year (Table 4.1.1).

Table 4.1.1. Volunteer awards and winners over the course of the project.

Award	Winner	Rationale
2014 Citizen Scientist	Will Smithwick	Awarded to the volunteer who contributed the best citizen scientist observations.
2014 Prawn Watcher of the year	Damien Mansfield	Awarded to the volunteer who contributed the most amount of monitoring time.
2015 Citizen Scientist	Kevin Reid	Awarded for his valuable contribution to Prawn Watch through observation and reporting
2015 Prawn Watcher of the year	Darren Hamley	Awarded for his valuable contribution to Prawn Watch through monitoring and activity
2016 Citizen Scientist	Mel Turner	Awarded for her valuable contribution to Prawn Watch through reporting and extension
2016 Prawn Watcher of the year	Jeevarayan Rao	Awarded for their valuable contribution to Prawn Watch through monitoring and reporting

In December 2015, the Department of Parks and Wildlife held their annual Volunteer of the Year and Outstanding Service Awards. The 2014-15 was a record year, with 4,636 individuals contributing more than 600,000 hours to Parks and Wildlife environmental and recreational projects. Outstanding Service awards were presented by the former Minister for the Environment, Hon. Albert Jacob, to Prawn Watch volunteers, Will Smithwick and Kevin Reid for their ongoing contribution to the project (Fig. 4.1.4).



Fig. 4.1.4. Photograph taken in 2015 of the former Western Australian Minister for the Environment, Hon. Albert Jacob MLA (left), to Prawn Watch volunteers, Will Smithwick and Kevin Reid (right).

Communications

Prawn Watch web pages were established in November 2013 as part of the River Guardians site (http://www.riverguardians.com/projects/prawn-watch) to provide information on the

project, as well as facts about *M. dalli* and where to catch them. The web pages also tell people how they can get involved and encourages them to share their own stories as well as citizen science data. The content of the pages was updated in 2015 and 2016 in order to ensure information was current based on the ongoing research project and to update the data input pages based on feedback and improvements in technology. An evaluation of hits to the River Guardians Website conducted in 2016, showed that between 1 July 2015 and 30 June 2016, the "Catching River Prawns" page had 3,697 hits and was second only in popularity to the landing page for that site.

A fact sheet on the Western School Prawns was made available through the Prawn Watch web pages as well as the Parks and Wildlife website (now Department of Biodiversity, Conservation and Attractions) and will be updated based on the findings of the various research projects. The information in that fact she*et al*so provided the basis for a "Creature Feature" article as part of the Swan River Trust's Riverview magazine (Issue 2, Spring 2013). That magazine issue also contained a feature article on the restocking of prawns. A third article, focussed on the "Communities Role in the River Prawn Comeback" was published in Riverview magazine (Issue 3, Summer 2014). A smaller update to the community on the prawn restocking was provided in Riverview (Issue 4, Autumn/Winter 2014). Community involvement in the project was also highlighted as a feature story in the 2013-14 Annual Report of the Swan River Trust. Restocking successes were reported in the River Protection Strategy Community Update in 2015 and then later in the June 2016 winter edition of Landscope Magazine (see Annex 4.1.1) and the Parks and Wildlife Annual Report 2015/16. Further updates and linkages to video footage are being prepared as part of future Landscope articles.

Prawn Watchers were kept up to date with the project through regular emails as part of the River Guardians mail out. This provided opportunities to promote broodstocking activities they could partake in, share sustainability messages and invite the community to other events where Prawn Watch was showcased. An example of these is provided in Annex 4.2.2. In addition, messages promoting sustainable prawning were posted to the River Guardians Facebook page (Fig. 4.1.5). The Facebook page also provided an opportunity to engage Prawn Watchers in other River Guardians events, such as larger community festivals and river clean-up days.



Fig. 4.1.5. Facebook post encouraging improved prawning practices in the Riverpark.

Prawn Watch was promoted at a range of events on and about the Riverpark, including the Autumn Rivers Festival (a festival held on the banks of the Swan River in Bassendean and Belmont) in April 2014 and March 2015 and March 2016 (Fig. 4.1.6), SwanFish 2015 and 2016 (a recreational fishers event), and the Science Week – Patterns in Science festival (a celebration of science in the community), August 2015 (Fig. 4.1.7). At each of these events, a series of storyboards describing Prawn Watch, the culture of prawning in Perth and research outputs were displayed along with hands on material, such as prawning equipment and aquaria

containing different species of prawns. The storyboards were updated in 2016 (Annex 4.2.3). Oral presentations were also provided to audiences at all these events, with the exception of Swanfish. In particular, the Autumn Rivers Festival regularly attracts over 7,000 people and new River Guardians / Prawn Watchers were signed up at these events. Prawn Watch also featured in promotional activity at the 2016 Blessing of the River Festival.

A flyer promoting sustainable fishing was added to a suite of the educational materials available at the display stands in 2015 and was updated in 2016 (Annex 4.1.4). A promotional video, prepared in 2014, was used at all events where facilities were available. See: https://www.youtube.com/watch?v=CmHktABVy0A). A new 6 minute video describing the project and its outcomes was developed in time for the 2016 feedback forum (link to video here).

Print, audio and visual media played a key role in raising awareness of the Prawn Watch project and the wider restocking project. An extensive media campaign was launched in association with the formal launch of Prawn Watch through the Western Australian Minister for Fisheries in February 2014. The campaign was based around a collaborative media statement released by all partner organisations connected to this project. The aim of the release was to informing the public about the project and engaging their interest and involvement. Media analyses (in the period associated with the launch (5th February to 18th March 2014) indicated that our release was promoted in 26 media items across five different types and reaching a cumulative audience of over 761,000 people with an estimated advertising value of $64,545 (Table 4.1.2 and see Annex 4.1.5 for full details).



Fig. 4.1.6. Sustainable fishing displays and engagement at the Autumn Rivers Festival in 2015.



Fig. 4.1.7. Community engagement officers and Gaia Resources staff with displays and interacting with community at the Patterns in Science Festival in August 2015.

Table 4.1.2. The number of media articles, audience and advertising space rate for media associated with Prawn Watch launch.

Media Type	Number	Audience	Advertising space rate
AM Radio	6	115,000	$8,505
FM Radio	1	42,000	$255
On-line	10	55	$12,353
Print	7	430,543	$7,393
TV	2	174,000	$36,039
Total	**26**	**761,598**	**$64,535**

The release of the Prawn Watch smartphone application (app) by the Western Australian Ministers for Fisheries and Environment in January 2015, generated further media interest with follow-up Channel 9 New 'Special Report', two radio interviews on ABC and 6PR and a number of follow up articles. A further media release by the Fisheries and Environment Ministers in February 2015 announced the news that more than one million prawns had been restocked during the 2014/15 breeding season. Channel 9 then did a second report that resulted in follow up stories. The prawn restocking story was linked to stories about the health of the Swan River on a number of occasions.

The project also sparked other media interest, with project officers being asked to take part in educational documentaries including:

- Destinations WA: Catching Swan River Prawns (https://www.youtube.com/watch?v=K0CJKeYRB0M)
- Destinations WA: Drunken Prawns (https://www.youtube.com/watch?v=WS4jMDb-8Xc).
- What's the catch (http://www.sbs.com.au/programs/whats-the-catch) hosted by the Gourmet Farmer Matthew Evans (Fig. 4.1.8). Note that while video footage about prawning and the restocking project was recorded it was not used in the final series.



Fig 4.1.8 Project officers Dr Kerry Trayler and Dr James Tweedley discussing sustainability of prawning with "What's the Catch" presenter Matthew Evans.

In March 2016, a joint media release was made by the former Western Australian Minister for Fisheries Hon. Ken Baston and former Minister for Environment Hon. Albert Jacob (Annex 4.1.6). This marked the release of the 4 millionth *M. dalli* into the Swan-Canning Estuary since the project began and sparked another flurry of media including video footage on Channel 9, 10 and 7, as well as online at WA today and the Sydney Morning Herald.

- https://www.youtube.com/watch?v=C2cV2-7Eziw
- http://tenplay.com.au/news/perth/2016/3/2/a-special-prawn
- https://au.news.yahoo.com/thewest/video/watch/30996269/swan-river-shrinking-for-stadium-footbridge/#page1
- http://www.smh.com.au/video/video-news/video-wa-news/swan-river-is-prawn-again-20160302-4bfas
- http://www.watoday.com.au/wa-news/400000-western-school-prawns-released-into-the-swan-and-canning-rivers-20160302-gn8wco.html

Media was also used to promote the findings of the research undertaken from the broader project. The example below was used to breakdown the paradigm that blowies (*Torquigener pleurogramma*) were the major predator of *M. dalli*, presumably as they are abundant in the estuary and are infamous for striping bait from recreational fishers. Research showed that the small apogonid comnmonly called the "Gobbleguts" (*i.e. Ostorhinchus rueppellii*) was a more

significant predator (See Section 3.2); an inportant finding in relation to determining appropriate release strategies. Murdoch University released a media statement in March 2016 regarding the findings of the research and this was picked up by ABC online (Fig. 4.1.9).

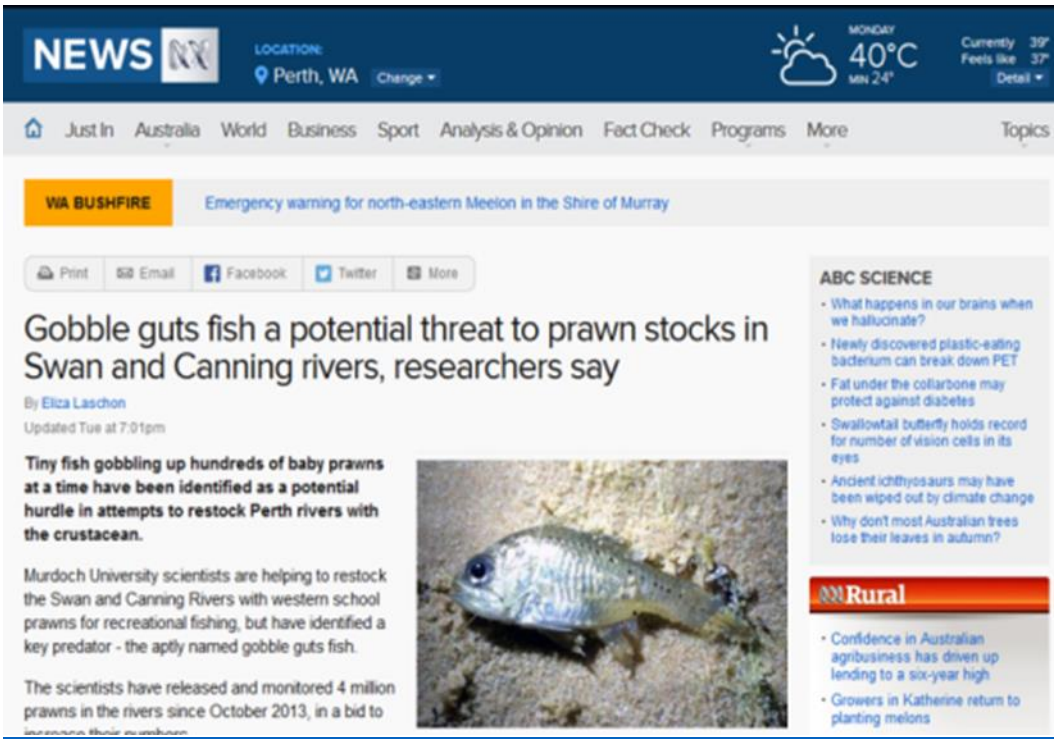


Fig. 4.1.9. Excerpt from ABC online media about predation on restocked *Metapenaeus dalli* by fish. See http://media.murdoch.edu.au/science-maximises-prawn-restocking-success and http://www.abc.net.au/news/2016-03-08/gobble-guts-potential-threat-to-prawn-stocks-swan-canning-rivers/7231216.

Citizen science monitoring

Community members were supported in undertaking monitoring of prawn populations in the Swan-Canning Estuary through the provision of waterproof logbooks, a web based database and the Prawn Watch smartphone app. The Prawn Watch logbook was developed to enable community members to recorded their data in the field and printed in time for the first training workshop in October 2013. This edition was constructed from waterproof paper and the front cover featured former Western Australian Minister for Fisheries Hon. Ken Baston. A further update is in production to ensure the logbook aligns with phone app and to reflect changes in management over time. Once the data are collected, members are able to enter the information from the logbook into a web-based database (URL) or into a smartphone app (see Fig. 4.1.10).

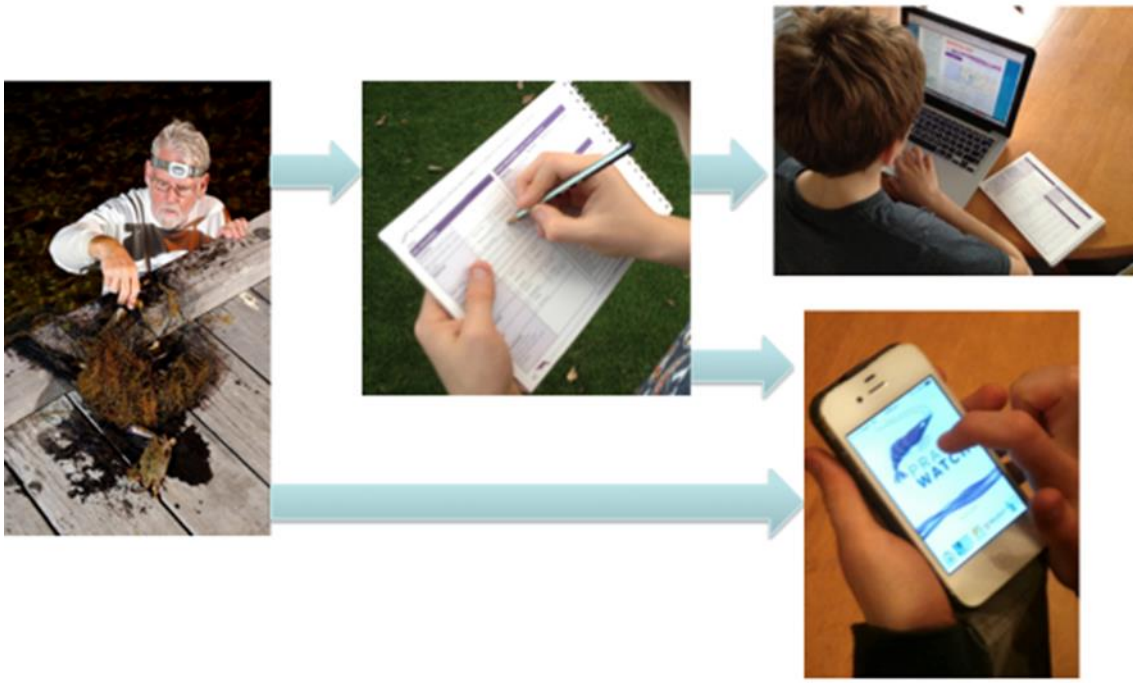


Fig. 4.1.10. Schematic showing the ways in which Prawn Watchers can collect data using their logbooks and submit data through a web based database and smartphone app. Photos: Stewart Allen and Kerry Trayler.

The Prawn Watch smartphone app was developed by Gaia Resources, for both Apple and Android platforms, to coincide with the November 2014 training and the opening of the prawning season. The app framework was based on the successful Dolphin Watch smartphone app. The approach to logging data on the app follows the same sequence in the logbook, with location data, prawn catch data and by-catch data all being recorded. Version, *i.e.* 1.03, which was released in May 2015, and updated in 2016 enables displays of both individual records and a broad summary of the number of prawns being recorded in different zones of the Swan-Canning Estuary (Fig. 4.1.11). Specific training sessions to support community in the use of the app were provided through the River Guardians program and in collaboration with Gaia Resources. These sessions occurred as part of the Patterns in Science Festival in 2015 and 2016 and targeted special events in 2016 (see Fig. 4.1.7).

Community members were supported in undertaking monitoring of prawn populations in the Swan-Canning Estuary through the provision of waterproof logbooks, a web based database and the Prawn Watch smartphone app. The Prawn Watch logbook was developed to enable community members to recorded their data in the field and printed in time for the first training

workshop in October 2013. This edition was constructed from waterproof paper and the front cover featured former Western Australian Minister for Fisheries Hon. Ken Baston. A further update is in production to ensure the logbook aligns with phone app and to reflect changes in management over time. Once the data is collected, members are able to enter the information from the logbook into a web-based database (URL) or into a smartphone app (see Fig. 4.1.10).



Fig. 4.1.11. Screenshot of the Prawn Watch app showing both individual records (purple prawn) and total number of prawns caught by prawn watchers in four different regions of Riverpark Catch is shown as a heat map overlaid on the regions.

The web based database, which was originally established in November 2013, was brought into line with the smartphone app in May 2015, to allow for sites to be located using a map interface (Fig. 4.12). Data entry boxes were replaced with drop down menus to improve the speed and ease of data entry.

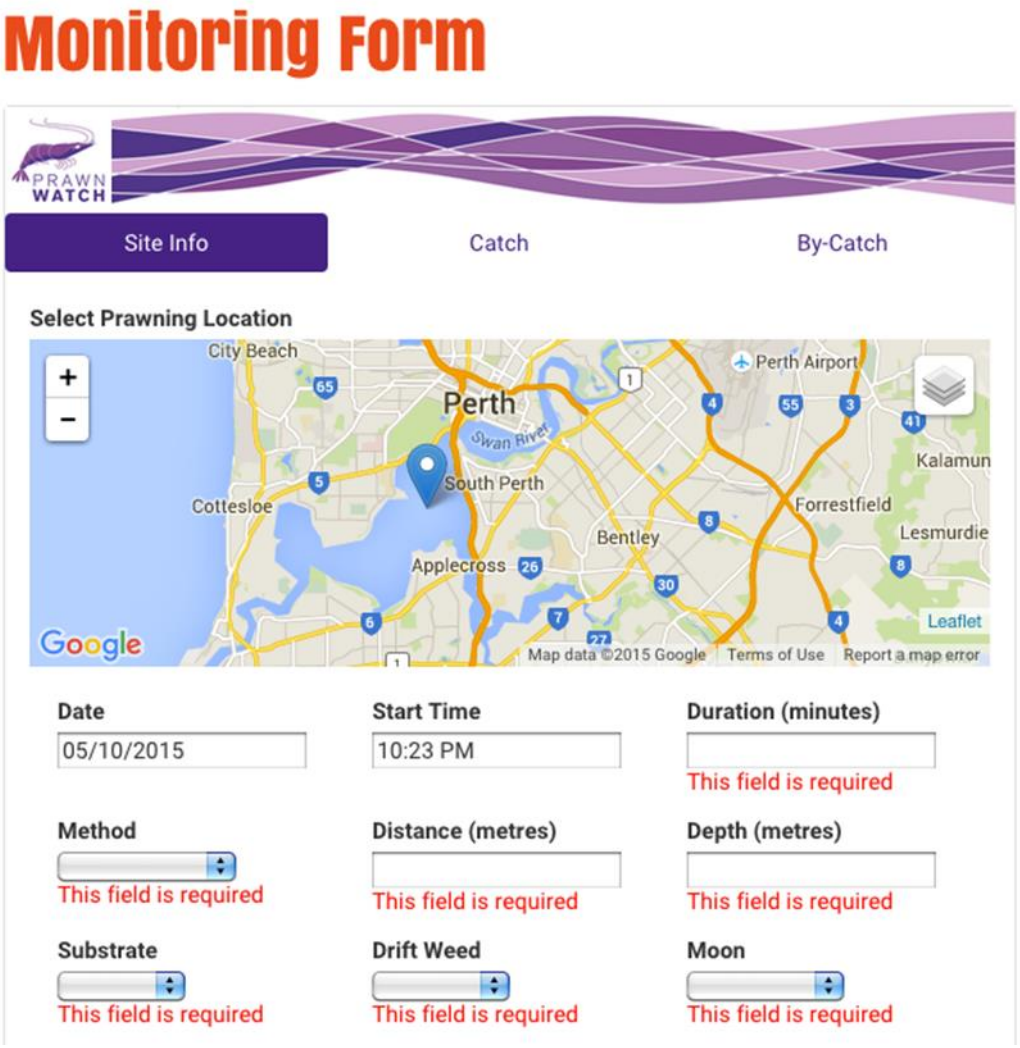


Fig. 4.1.12. Screenshot of the updated monitoring data entry form on Prawn Watch website.

In addition to independently logging their own catch into a database via a web interface or smartphone app, a group of dedicated volunteers were engaged in the collection of broodstock to support the aquaculture effort (Fig. 4.1.12). Since 2012, these volunteers contributed over 980 hours of time with an additional 300 hours provided by partner organisations in this project. Volunteers were encouraged to log the information collected at the broodstock events on-line. These volunteers were also involved in the evaluation of the effectiveness of citizen science data to provide low-cost monitoring of *M. dalli* (see Jenkins *et al*., 2017). In addition, a plethora of undergraduate students from Murdoch University were also engaged in this project through Prawn Watch training and promotion via Facebook and have volunteered over 3,600 hours of time to support the university based research and monitoring project connected to this project (see Sections 1-3).



Fig.4.1.13. Photographs of broodstock collection events held in 2015.

A total of 213 records were logged by Prawn Watchers over the course of the project, with 96 entries in the web based database over the 2013/14 season, 102 in 2014/15, (20 via the web-based database and 82 by the smartphone app) and 15 in 2015/16. Data collected in the 2013/14 season shows the activity of Prawn Watchers across the Melville Water and the lower portions of the Canning Estuary and that most prawners surveyed the shallow, nearshore waters using a hand trawl or scoop net (Fig. 4.1.14).

In the 2014/15 prawning season the 20 records in the web-based database again showed a range of methods being undertaken to record prawns in the river (Fig. 4.1.15. Unfortunately, due to an error in version 1.0 of the Prawn Watch app, the method of survey was not recorded. Data that were recorded using the app in 2014/15 show a spread of survey information across the river, with sites at Matilda Bay, Canning Bridge and Freshwater Bay being preferred locations for Prawn Watchers (Fig. 4.1.15).

Entries into the database using the app and the web pages dropped significantly over the prawning season in 2015-16, with only 15 records identified (Fig. 4.1.16). The decline has been attributed to the cessation of targeted training for community prior to the start of the season.

Community monitoring data were never been formally validated as the program was intended as an engagement tool in the first instance and not as the prime mechanism for monitoring prawns in the estuary. The community data were seen as supplementary to an extensive university-led monitoring project, which from 2013-2016 (see Section 1.1). A comparison of

species data collected by the community in 2013/14 (Fig. 4.1.18) with data collected by Murdoch University over a period, showed similar patterns of distribution of *M. dalli* and *P. latisulcatus*, with the former species found throughout Melville Water and the lower reaches of the Canning Estuary and the latter species located in the more saline areas further downstream (Fig. 4.1.17). This improved confidence in the community's capacity to distinguish between the two large penaeid species occurring in the estuary. However, some inaccuracies remain in the dataset. Key amongst these were incomplete records and the accuracy of catch/unit effort data. The latter was problematic as it was apparent that many fishers would log their entire effort over an evening (*i.e.* sometimes 3 hours), rather than logging each hand trawl separately. Other issues around the identification of the location of record were resolved through the mapping interface available on the Smartphone app. and database updates. In 2015 it was recommended that if community monitoring was to be used for ongoing monitoring purposes then an approach must be standardised and validated against independent data (see Further development).

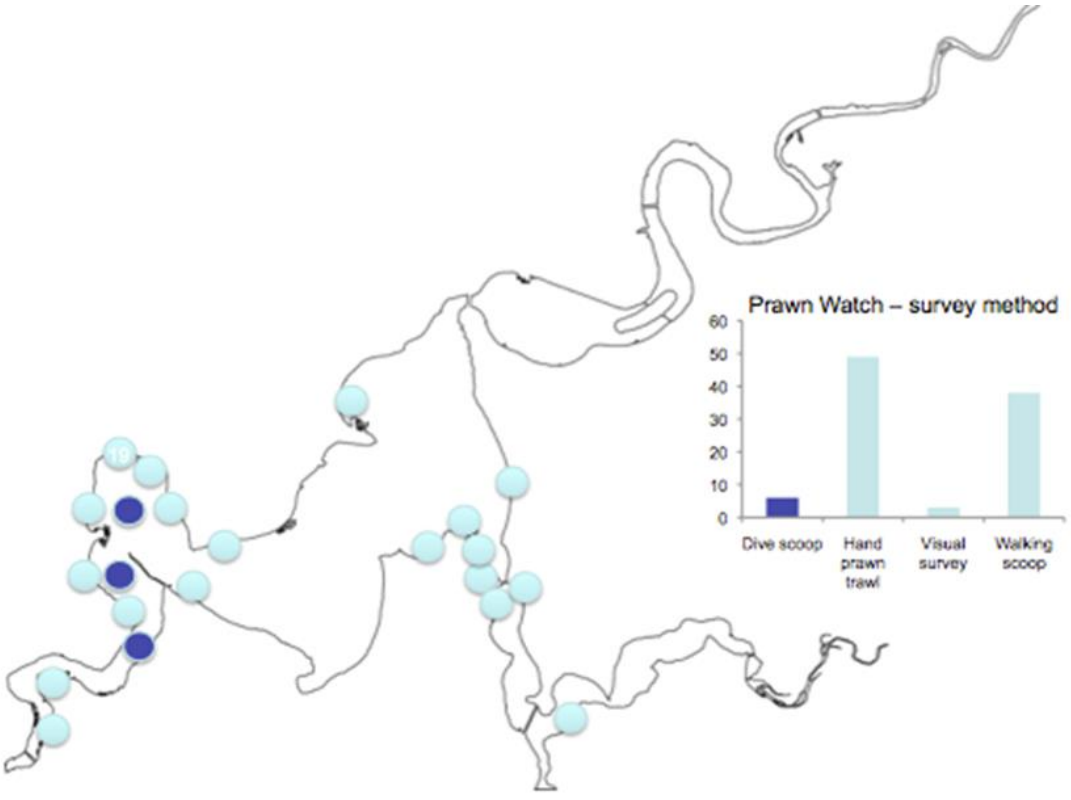


Fig. 4.1.14. Map showing the locations where Prawn Watchers recorded data between November 2013 and June 2014 and their survey method. ● shore-based prawning methods and ● SCUBA-based methods. n = 96.



Fig. 4.1.15. Map showing the locations where Prawn Watchers recorded data between November 2014 and June 2015 and their survey method. Data obtained from the web-based database. n = 20.

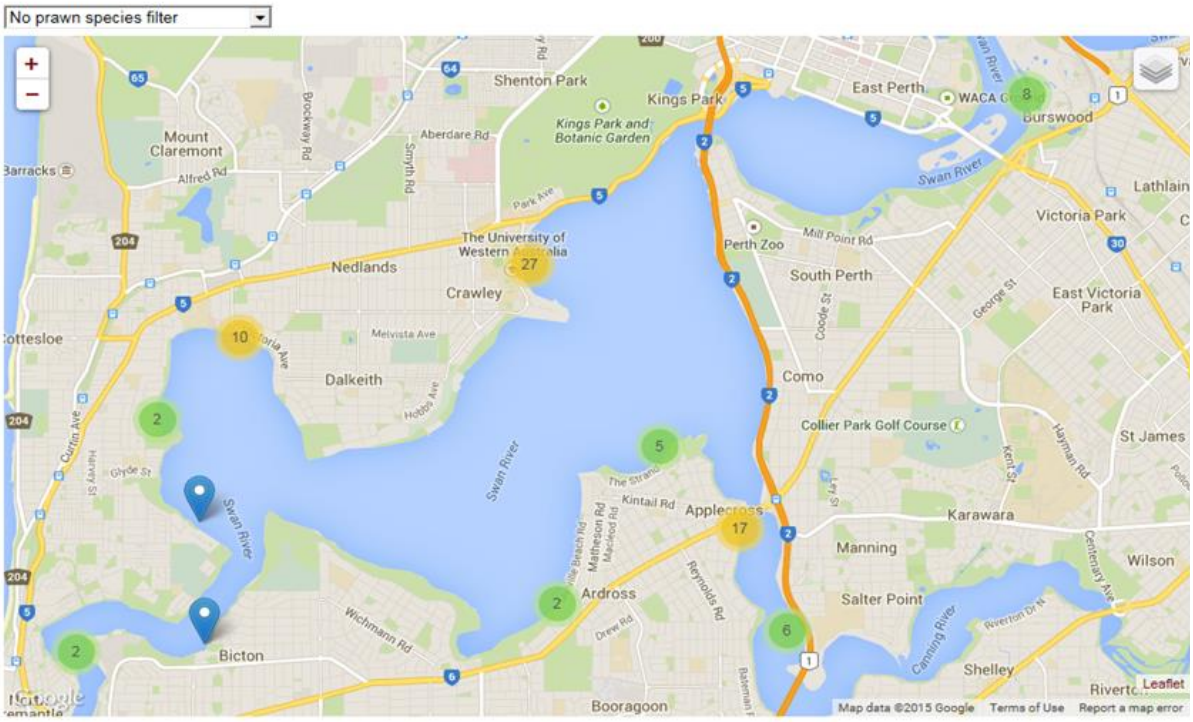


Fig. 4.1.16. Map showing the locations where Prawn Watchers recorded data between November 2014 and June 2015 and the number recorded at each site. Data obtained from the smartphone app. n = 82.



Fig. 4.1.17. Map showing the location of Prawn Watch data collection between November 2015 and July 2016. n = 15.

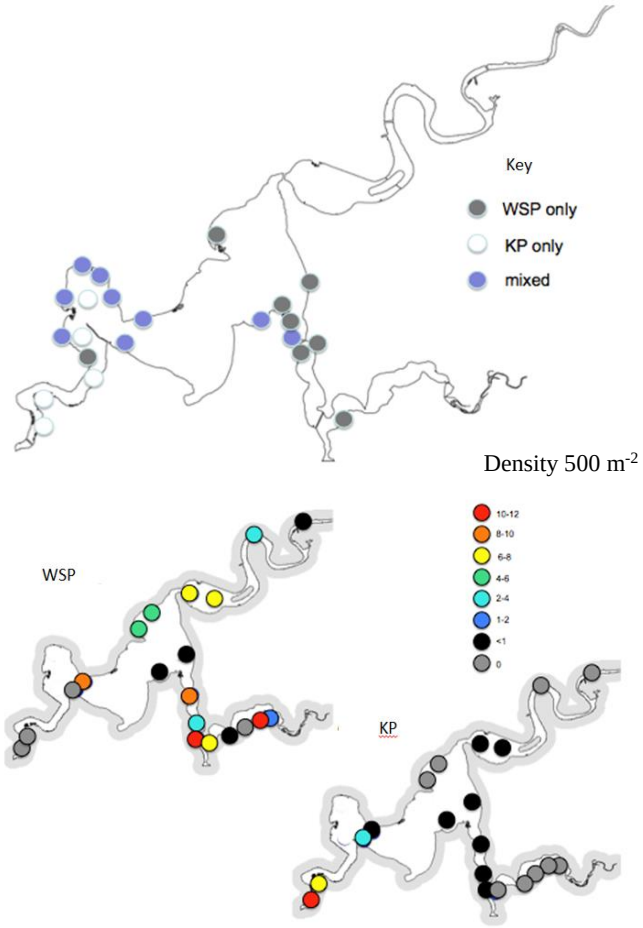


Fig. 4.1.18. Map showing the sites at which *Metapenaeus dalli* (WSP) and *Peaneus latisulcatus* (KP) were recorded by the Prawn Watchers (top) and University-led monitoring project (bottom) in December 2013 and February 2014.

Influencing policy

Through training events, project communications, *i.e.* website, flyers, logbooks, media statements and forums, this project has promoted key messages about sustainability including:

- Returning all by-catch (*e.g.* Weeping Toadfish [*Torquigener pleurogramma*] and jellyfish [*Phyllorhiza punctata* and *Aurelia aurita*]) to the estuary.
- Returning gravid and/or small prawns in the estuary.
- Taking all rubbish home.
- Abiding by fisheries regulations set by the Western Australian Department of Fisheries (now Department of Primary Industries and Regional Development).
- Avoiding prawning within 100m of Swan Canning Marine Reserves.
- Avoiding trampling vegetation when assessing prawning sites.

Furthermore, the Parks and Wildlife Service at the Department of Biodiversity, Conservation and Attractions has continued to raise issues about the sustainability of fishing practices with the Department of Fisheries (now Department of Primary Industries and Regional Development, Fisheries Division). In recognition the restocking component of the project was coming to an end and results of associated research were being compiled the department initiated discussions with key stakeholders to discuss approaches to manage the fishery moving forward.

Discussion

In 2015, a strategic media campaign set out to update the communication outputs of Prawn Watch and contribute against the overall objectives of that project, established in 2012. Existing products (*i.e.* logbooks, website, flyers, display boards and the Smartphone App) were updated through this campaign and a series of events and forums were held that enabled the communication of sustainable fishing approaches and Prawn Watch (*i.e.* Blessing of the Rivers festival, Autumn Rivers Festival, Patterns in Science festival, Clean-up days). In addition, the community continued to be engaged in sharing their data through Prawn Watch and

contributing to broodstock collection events. Information was shared with the community through River Guardians emails, facebook, media events and video and celebrated through feedback forums. The media campaign is ongoing and being delivered through the prawn season of 2016/17 as this is the most appropriate time to connect with fishers.

The Prawn Watch initiative is a good example of a contributory citizen science – community engagement project. The initiative engaged many people from all walks of life including fishers, businessmen and women, local government representatives and children. In part this may have been because many people had a personal connection with the rivers and prawning – typically from childhood experiences that they held dear. Training events provided people with understandings about sustainable fishing and confidence in technique. Many people brought their children along to training events and broodstock collection nights and were keen to share with them their experience and pass of their passion for prawning and connection to the river.

The community were able to demonstrate learnings as part of the project and contribute to a genuine effort to try and improve the prawn population. The project linked closely with university based monitoring to provide feedback to the community as part of the community engagement program. Community monitoring was reported back to community, along with the findings from the university based monitoring and research.

The benefits of an ongoing citizen science approach to monitoring prawns include, but are not limited to:

- A more informed and engaged community;
- An inexpensive monitoring approach;
- Individuals able to share knowledge about prawns, the river and sustainable fishing with their communities;
- Social capital –building on good will for productive purpose;
- Behaviour and attitude change;
- Providing connection to the river.

The community based data from Prawn Watch were not used as a primary mechanism for monitoring prawns. However, provided steps are in place to deal with validation and data integrity, the existing tools of the log books, database and smartphone app could be applied to such a purpose.

Annexes

Annex 4.1.1. Landscope Magazine (Winter 2016) and Parks and Wildlife Annual Report 2015/16.



LANDSCOPE Winter 2016 | pages: 6–7 / 56

Bush telegraph by Karla Graham

READERS' PIC

Argyle cycad (*Cycas pruinosa*)

Words and photo by Don Stallard

"Argyle cycad (*Cycas pruinosa*) supposedly represents "the earliest form of plant life" on the Earth's surface.

This particular stand is a fine example of this species distributed throughout the central and north-east Kimberley. For me, the location of this stand thrives at the core of the Kimberley where the mighty King Leopold and Durack ranges meet. Where the higher peaks of Mt Wells, Bedford and King are concentrated. Where the source of the Ord and Fitzroy rivers rise and flow in opposite directions but are separated by few kilometres by what I call the Kimberley Divide. This divide continues north-west to further separate the sources of the Little Fitzroy River from the Durack and Chamberlain rivers. Adding to these attractions are the nearby Teronis Gorge, old Elgee Cliff's homestead and cave paintings, all of which highlight the importance of this location."

Have you got a fantastic nature photograph you would like to see published in *LANDSCOPE*? Send it, along with a 100-word description of the species or how and where you took the shot, to landscope@dpaw.wa.gov.au.

Snap shot

Prawn project boasts 4.5 million milestone

A project to address the decline of western school prawns in the Swan and Canning rivers has seen more than 4.5 million prawns released back into the river system in the past three years.

The restocking project works with the community through the citizen science program *Prawn Watch*, to collect gravid (pregnant) female prawns from the river each year during the November–April breeding season. They are transferred to tanks and allowed to spawn naturally in a carefully controlled environment, where chances of the young's survival are much higher than in the river. The juvenile prawns are later released into the Swan Canning Riverpark, boosting the population and helping to reinstate prawning as a recreational activity for Perth families.

The historically popular activity of drag netting for prawns has all but disappeared as prawn numbers have declined since the 1950s.

Restocking began in 2013, using recreational fishing licence fees to partly fund the project. In March 2016, 674,000 juvenile prawns were released along the Como foreshore to bring the project's total above the 4.5 million mark.

The project is a joint effort between the Department of Parks and Wildlife, the Australian Centre for Applied Aquaculture Research, Murdoch University, Recfishwest, the Department of Fisheries, the WA Fish Foundation and the Fisheries Research and Development Corporation.

Below Discover more about *Prawn Watch*. *Video – Parks and Wildlife*

Camera watch program calls on volunteers

People all around the world now have the opportunity to take part in *Western Shield*, the biggest conservation project ever undertaken in Australia, with the launch of an online citizen science program.

Hosted on the collaborative volunteer research website Zooniverse, the *Western Shield Camera Watch* project draws on the help of more than two million volunteers to identify native and introduced animals captured by 90 remote cameras around Western Australia.

Volunteers don't need any scientific knowledge, just a keen eye and a willingness to learn. The website provides a step-by-step guide to correctly identifying the animal by shape, size, colour and markings.

The identifications received will help *Western Shield* scientists at the Department of Parks and Wildlife measure the success of its broadscale feral cat and fox baiting program.

More than 200,000 images are already loaded onto the site for identification. To get involved, visit www.zooniverse.org, register, and search for '*Western Shield Camera Watch*'.

6 LANDSCOPE

Case study
Government outcome: The ecological health and community benefit of the Swan and Canning rivers is protected.

Prawn project restocks rivers

2015–16 saw the 4.5 millionth western school prawn (*Metapenaeus dalli*) released into the Swan Canning Riverpark as part of a multi-agency project to increase the species' population.

The western school prawn restocking and monitoring program sees Parks and Wildlife work with six project partners to research, monitor, culture and release western school prawns with the aim of bringing recreational prawning back as a popular pastime for Perth residents.

The project began in 2012, led by the former Swan River Trust, in response to declining numbers of western school prawns in the river since the 1950s. Historically, both commercial and recreational fishers targeted this species. Commercial landings peaked at 14 tonnes in 1959 but declined to three tonnes by 1975. While the commercial fishery no longer targets prawns, recreational fishers continue to take part in drag netting for the crustaceans as a treasured part of river culture.

Environmental factors, and not fishing pressures, are thought to be responsible for the decline in western school prawn abundance. The restocking project aims to re-establish prawning in the Riverpark and encourage people to have a stronger engagement with the river environment.

Three main components make up the project: the production and release of prawns into the Riverpark; engaging the community through stock release and monitoring; and evaluating stock status, release strategies and factors affecting population recruitment and survival.

The restocking aspect of the program began in 2014, with Challenger Institute releasing 635,000 western school prawns. Almost two million were released in 2015 and a further 1.8 million during 2016. By April 2016 the project celebrated a total of 4.5 million western school prawns released.

The project's restocking success was largely made possible by scientists at the Australian Centre for Applied Aquaculture Research (ACAAR) when they successfully cultured school prawns in 2012.

The culturing process involves collecting wild female egg-carrying (gravid) prawns from the river. They are transferred to tanks and allowed to spawn naturally in a carefully controlled environment, where chances of the young's survival are much higher than in the river. The juvenile school prawns are released back into the river when they grow past the main predation stage.

March 2016 also marked another project milestone – the completion of monitoring over three full breeding seasons, allowing researchers to gather a robust data set that can be used to determine the biological characteristics of the prawn population in the Swan Canning Riverpark. Preliminary information has indicated that restocking has had an impact on prawn populations. Monitoring will be extended into the 2016–17 breeding season, promising even more clarity on stock status and changes over time.

Scientists also continued to undertake research into the salinity and temperature tolerance of western school prawns. In addition, a survey into the diets of potential predators revealed one of the prawns' major predators to be the aptly named gobble guts fish. A Murdoch University researcher found about 300 juvenile prawns in the stomach of a single gobble guts that was only 45mm long.

Results of all the research are being processed to determine the best release sites in the Riverpark, taking into account the availability of suitable substrate, the presence of juvenile or adult prawns at the site at the time, the environmental conditions such as prevailing wind, and easy access to the site.

Getting members of the community involved is a vital aspect of the project. Under the Parks and Wildlife *Prawn Watch* project, volunteers take part by helping collect gravid prawns, reporting details of prawning catches to help inform research, and releasing juvenile prawns into the river.

Over the project's three-year history, 179 volunteers formally joined the *Prawn Watch* project, taking part in collection and release events, training sessions and workshops. In addition, university students have contributed more than 3000 hours to support monitoring.

Community members also continued to contribute to citizen science research by logging details of their prawning catches in the *Prawn Watch* logbook, smartphone app and website.

Project partners include Parks and Wildlife, Murdoch University, the Department of Fisheries, the WA Fish Foundation, Recfishwest, ACAAR at Challenger Institute of Technology, and the Fisheries Research and Development Corporation.



Drag netting for prawns. *Photo – Stewart Allen*



A western school prawn. *Photo – Stewart Allen*



Volunteers are an important aspect of the project. *Photo – Stewart Allen*



Returning by-catch to the river alive. *Photo – Stewart Allen*



Gravid (egg-carrying) prawn. *Photo – Mark Thornley/Parks and Wildlife*

Annex 4.1.2. Example of communication material sent to community through the Department of Parks and Wildlife's River Guardians program (now within the Department of Biodiversity, Conservation and Attractions).

RIVER GUARDIANS

May 2016

Welcome to all our new members who have become River Guardians over the last couple of months.

This newsletter is an update on everything to do with the Swan Canning Riverpark.

If there is anything you would like to see more or less of in this quarterly newsletter please let us know at guardians@dpaw.wa.gov.au.



Prawning in the rivers

With thanks to our Prawn Watch volunteers and world-first prawn culturing techniques, western school prawn numbers in the Swan and Canning rivers have been boosted by another 1.5 million animals across 2015–16.

More than 2.5 million juvenile school prawns were restocked between May 2013 and March 2015 and a further two million school prawns are targeted for release by June 2016 under the current Recreational Fishing Initiatives Fund (RFIF) supported by Recfishwest.

If you're out prawning, please use the Prawn Watch App, online monitoring form or log book to record your catch. Community input is vital to help monitor the western school prawn and to help us plan and re-establish the experience and pleasure of prawning.

iPhone
Android
online monitoring form
or logbook

For more information visit the River Guardians website.
More details on this and other RFIF projects are available on the Recfishwest website.

Warm regards,

Marnie, Rachel and Carol
River Guardians Team
Department of Parks and Wildlife

17 Dick Perry Avenue, Technology Park, Western Precinct, Kensington WA 6151
P: 08 9219 9000

Web: www.swanrivertrust.wa.gov.au |
www.riverguardians.com
CARING FOR THE SWAN CANNING RIVERPARK

Annex 4.1.3. Story boards produced as part of Prawn Watch that were displayed at community engagement events.

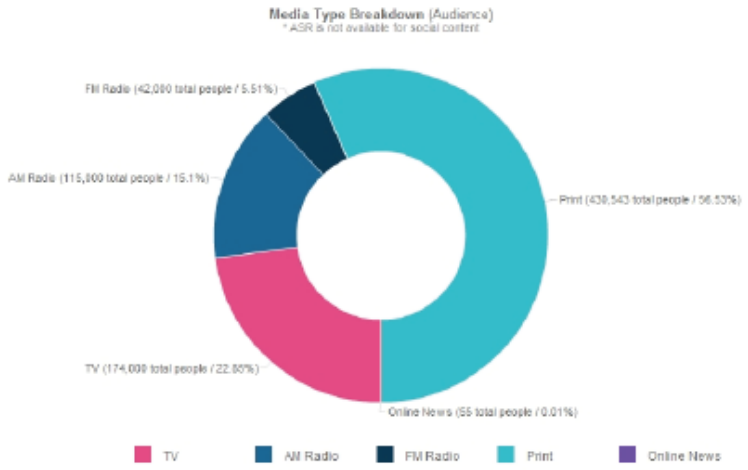


Annex 4.1.4. Sustainable fishing flyer that was produced by the Department of Parks and Wildlife.



Annex 4.1.5. Media analytics associated with Prawn Watch launch.

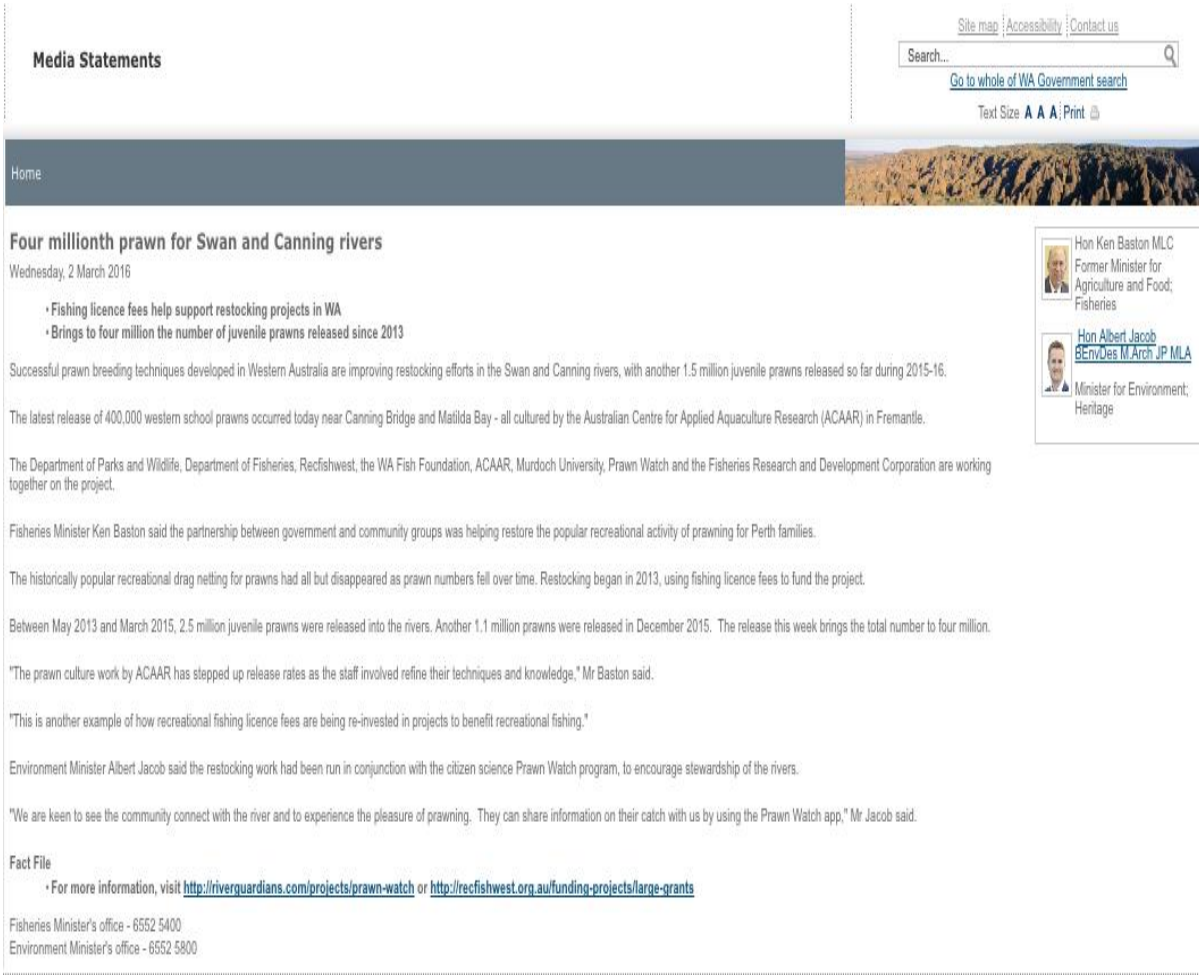
Total media types	Total items	Total audience	Total ASR (in AUD)
5	26	761,598	$64,545



An analysis of coverage produced in the 42 days between 05 Feb 2014 and 18 Mar 2014 from 1 folders (Prawn Watch) found 26 items. This coverage reached a cumulative audience of 761,598 and had an advertising space rate of AUD 64,545.
* Online News had the highest volume of coverage (10 items or 38% of the total volume of coverage)
* Print reached the highest cumulative audience (430,543 items or 56% of the cumulative audience)
* TV had the highest advertising space rate (AUD 36,039 or 55% of the total advertising space rate)

Media Type	Volume	Audience	ASR (in AUD)
AM Radio	6	115,000	$8,505
FM Radio	1	42,000	$255
Online	10	55	$12,353
Print	7	430,543	$7,393
TV	2	174,000	$36,039

Annex 4.1.6. Joint media statement in March 2016 from the former Western Australian Ministers for Fisheries and Environment.



Media Statements

Site map | Accessibility | Contact us

Search...

Go to whole of WA Government search

Text Size A A A | Print

Home

Four millionth prawn for Swan and Canning rivers

Wednesday, 2 March 2016

- **Fishing licence fees help support restocking projects in WA**
- **Brings to four million the number of juvenile prawns released since 2013**

Successful prawn breeding techniques developed in Western Australia are improving restocking efforts in the Swan and Canning rivers, with another 1.5 million juvenile prawns released so far during 2015-16.

The latest release of 400,000 western school prawns occurred today near Canning Bridge and Matilda Bay - all cultured by the Australian Centre for Applied Aquaculture Research (ACAAR) in Fremantle.

The Department of Parks and Wildlife, Department of Fisheries, Recfishwest, the WA Fish Foundation, ACAAR, Murdoch University, Prawn Watch and the Fisheries Research and Development Corporation are working together on the project.

Fisheries Minister Ken Baston said the partnership between government and community groups was helping restore the popular recreational activity of prawning for Perth families.

The historically popular recreational drag netting for prawns had all but disappeared as prawn numbers fell over time. Restocking began in 2013, using fishing licence fees to fund the project.

Between May 2013 and March 2015, 2.5 million juvenile prawns were released into the rivers. Another 1.1 million prawns were released in December 2015. The release this week brings the total number to four million.

"The prawn culture work by ACAAR has stepped up release rates as the staff involved refine their techniques and knowledge," Mr Baston said.

"This is another example of how recreational fishing licence fees are being re-invested in projects to benefit recreational fishing."

Environment Minister Albert Jacob said the restocking work had been run in conjunction with the citizen science Prawn Watch program, to encourage stewardship of the rivers.

"We are keen to see the community connect with the river and to experience the pleasure of prawning. They can share information on their catch with us by using the Prawn Watch app," Mr Jacob said.

Fact File

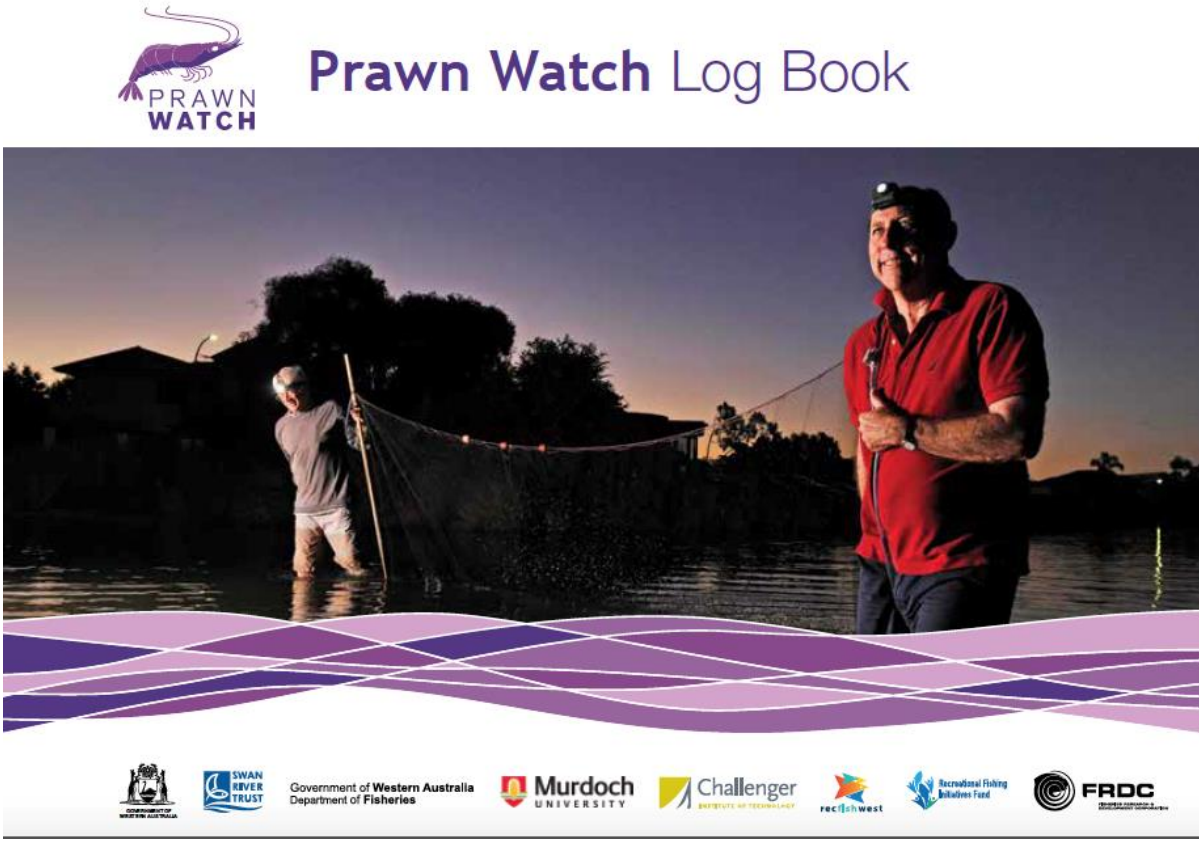
- **For more information, visit http://riverguardians.com/projects/prawn-watch or http://recfishwest.org.au/funding-projects/large-grants**

Fisheries Minister's office - 6552 5400
Environment Minister's office - 6552 5800

Hon Ken Baston MLC
Former Minister for Agriculture and Food; Fisheries

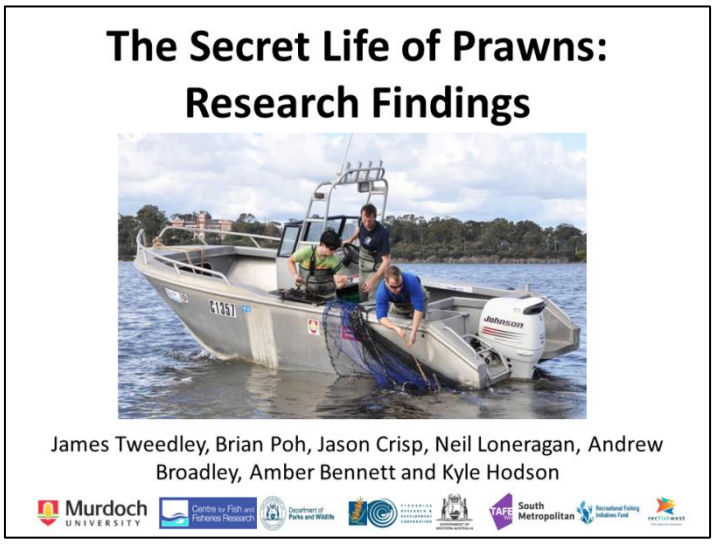
Hon Albert Jacob BEnvDes M.Arch JP MLA
Minister for Environment; Heritage

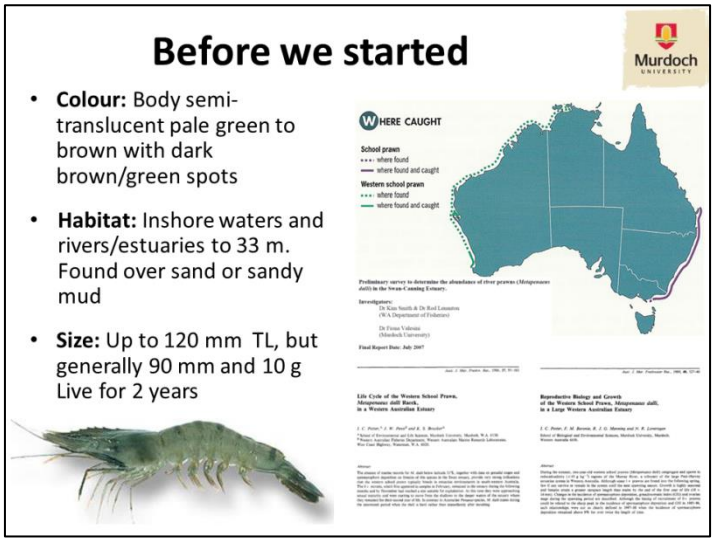
Annex 4.1.7. Prawn Watch logbook with former Fisheries Minister on cover (top) and most recent version (bottom).

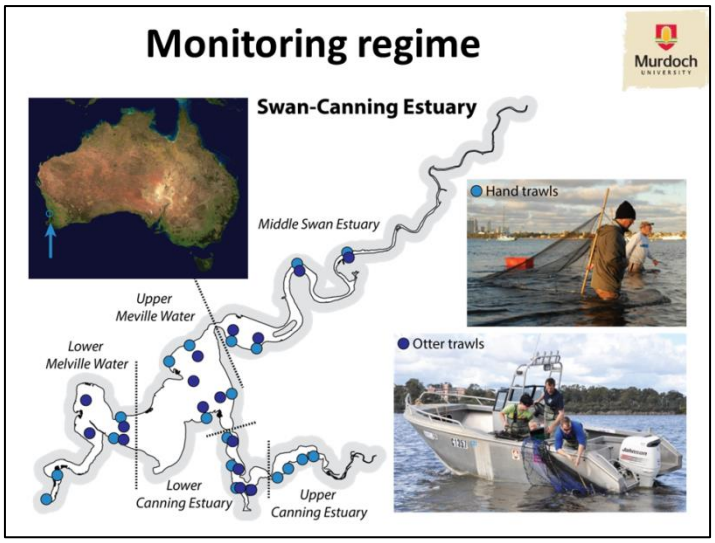




Annex 4.1.8. Slides presented by Dr James Tweedley on the Western School Prawn research program at the Prawn Watch wrap-up event "*The Secret Life of Prawns*" in September 2016.







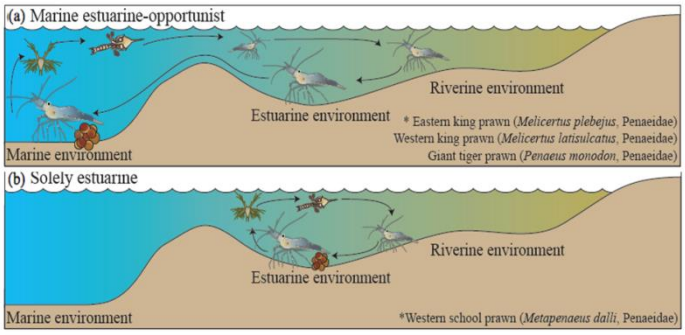
Monitoring regime

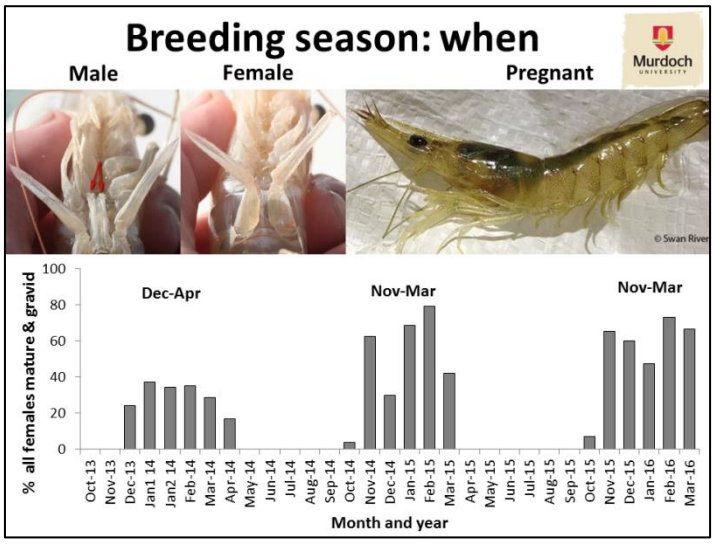


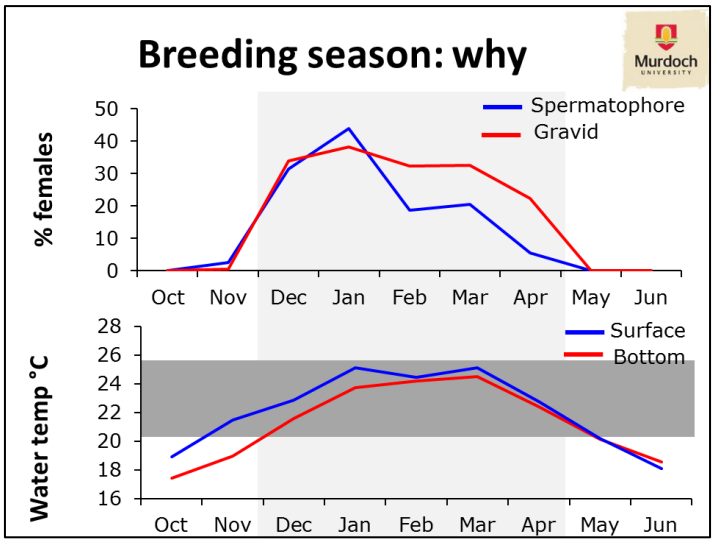
Our job; to investigate……

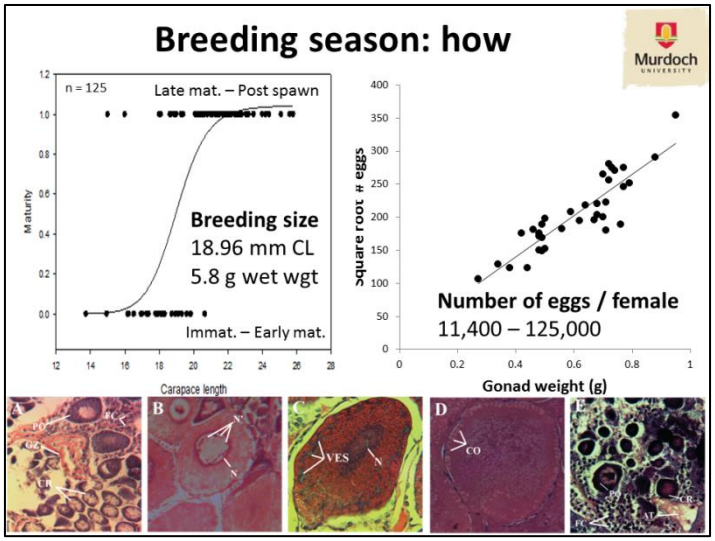
- A year in the life of a prawn (sex, birth, growth & death)
- Where prawns live and how many there are
- What sediments do prawns prefer
- What eats prawns
- Where do we release restocked prawns
- Determine whether the restocking has worked

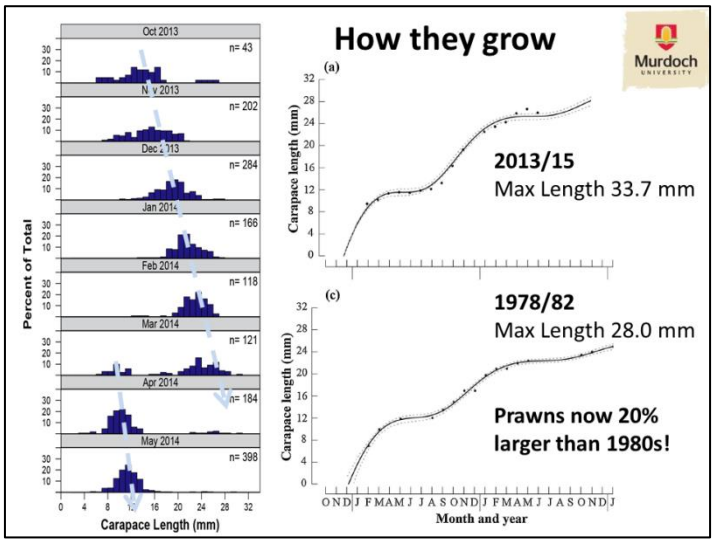
A year in the life of a prawn

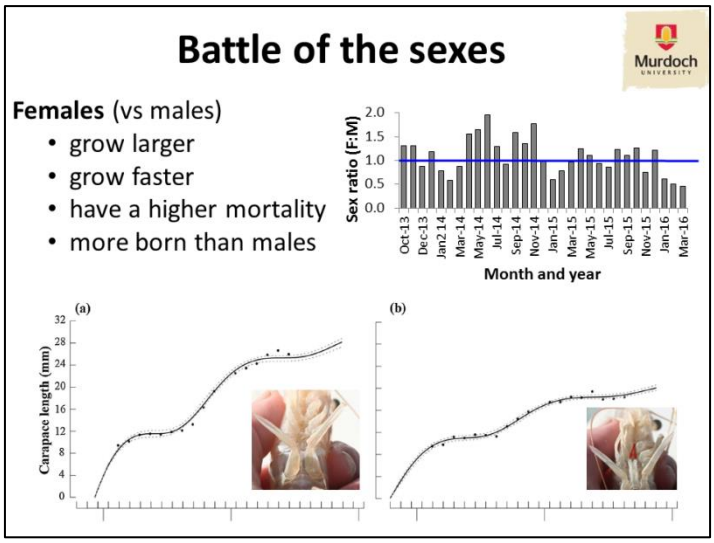


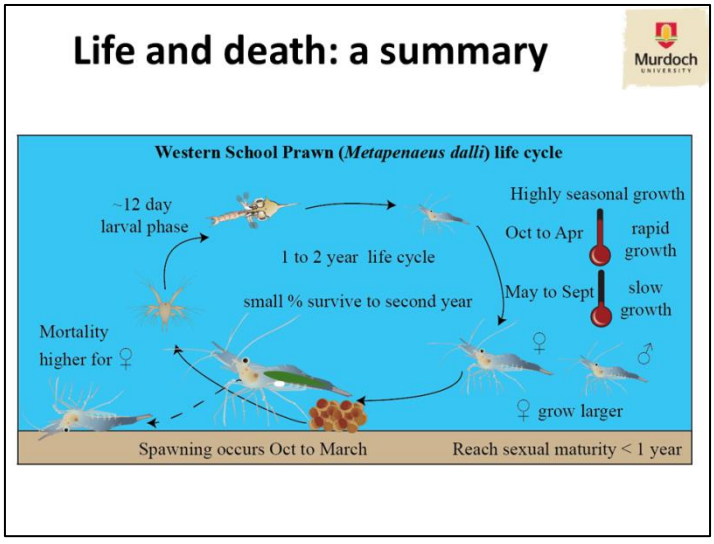

Breeding season: when
Male
Female
Pregnant
Murdoch
© Swan River
Dec-Apr
Nov-Mar
Nov-Mar
% all females mature & gravid
Month and year


Breeding season: why
Murdoch
% females
Spermatophore
Gravid
Oct Nov Dec Jan Feb Mar Apr May Jun
Water temp °C
Surface
Bottom
Oct Nov Dec Jan Feb Mar Apr May Jun


Breeding season: how
Murdoch
n = 125
Late mat. – Post spawn
Maturity
Breeding size
18.96 mm CL
5.8 g wet wgt
Immat. – Early mat.
Carapace length
Square root # eggs
Number of eggs / female
11,400 – 125,000
Gonad weight (g)
A
B
C
D
E


How they grow
Murdoch
Oct 2013
n= 43
Nov 2013
n= 202
Dec 2013
n= 284
Jan 2014
n= 166
Feb 2014
n= 118
Mar 2014
n= 121
Apr 2014
n= 184
May 2014
n= 398
Percent of Total
Carapace Length (mm)
(a)
Carapace length (mm)
2013/15
Max Length 33.7 mm
(c)
1978/82
Max Length 28.0 mm
Prawns now 20% larger than 1980s!
O N D J F M A M J J A S O N D J F M A M J J A S O N D J
Month and year

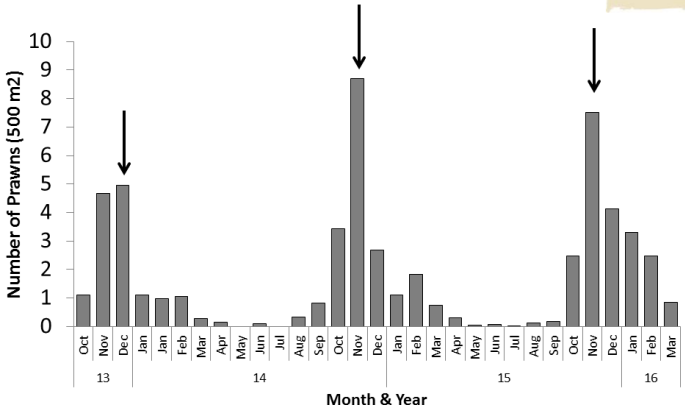

Battle of the sexes
Murdoch
Females (vs males)
• grow larger
• grow faster
• have a higher mortality
• more born than males
Sex ratio (F:M)
Oct-13
Dec-13
Jan2 14
Mar-14
May-14
Jul-14
Sep-14
Nov-14
Jan-15
Mar-15
May-15
Jul-15
Sep-15
Nov-15
Jan-16
Mar-16
Month and year
(a)
(b)
Carapace length (mm)


Life and death: a summary
Murdoch
Western School Prawn (Metapenaeus dalli) life cycle
~12 day larval phase
1 to 2 year life cycle
small % survive to second year
Mortality higher for ♀
Highly seasonal growth
Oct to Apr
rapid growth
May to Sept
slow growth
♀ grow larger
Spawning occurs Oct to March
Reach sexual maturity < 1 year

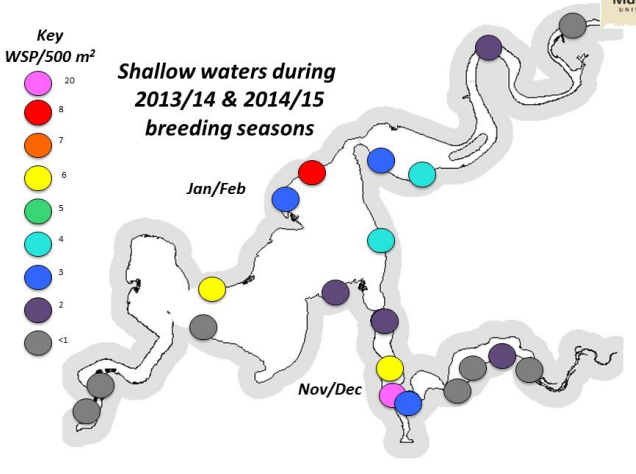
Where do they live?



When are they in the shallows?



Where can I catch them?



How many are there?

- Estimated total biomass in 2013/14 calculated
- Current biomass = **2.37 T**
- Very low compared to the record catch of 15 T in 1959

Shallow waters < 2 m

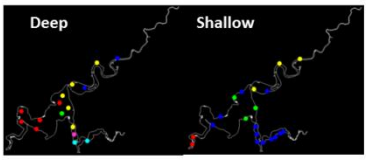
Deep waters > 2 m

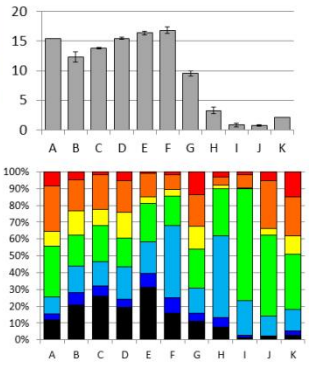
- Likely higher now

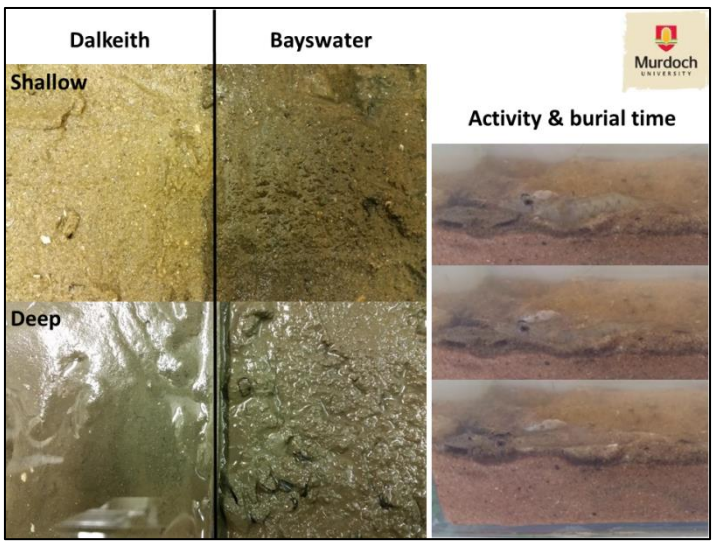


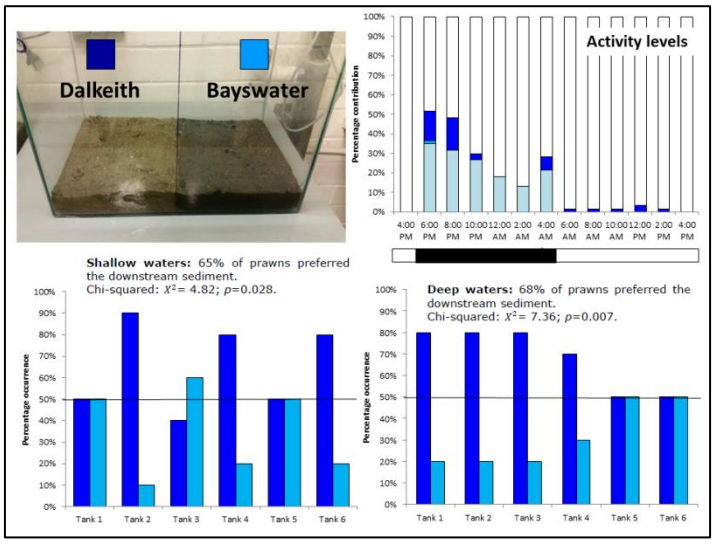
Influence of sediment

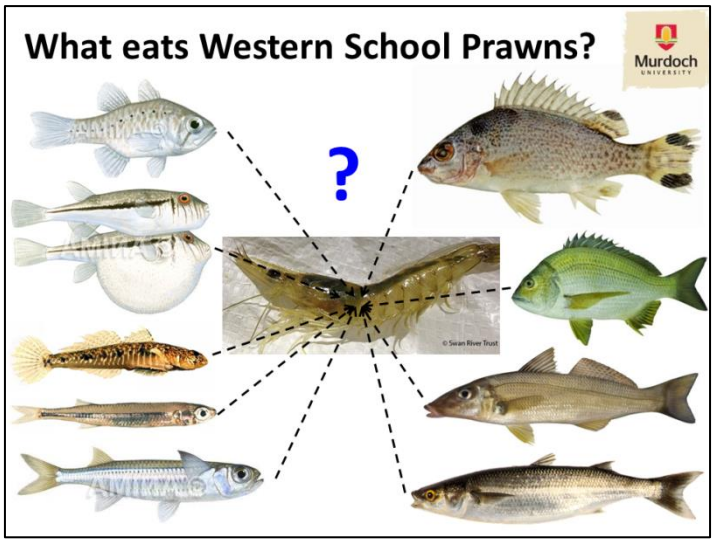


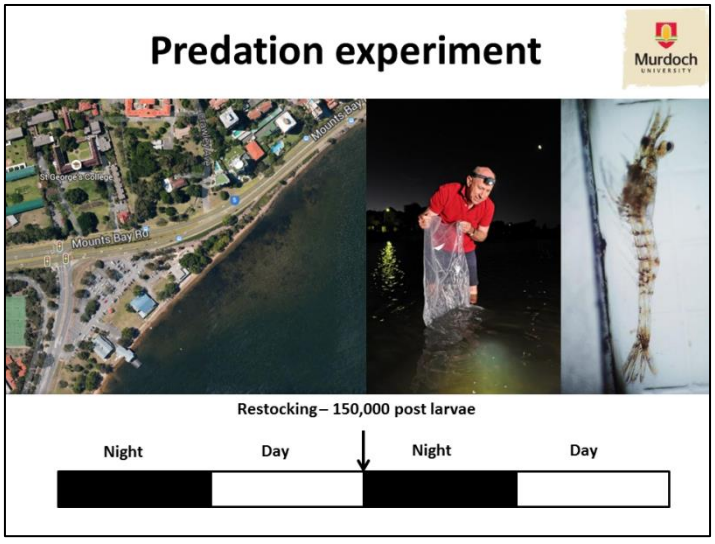


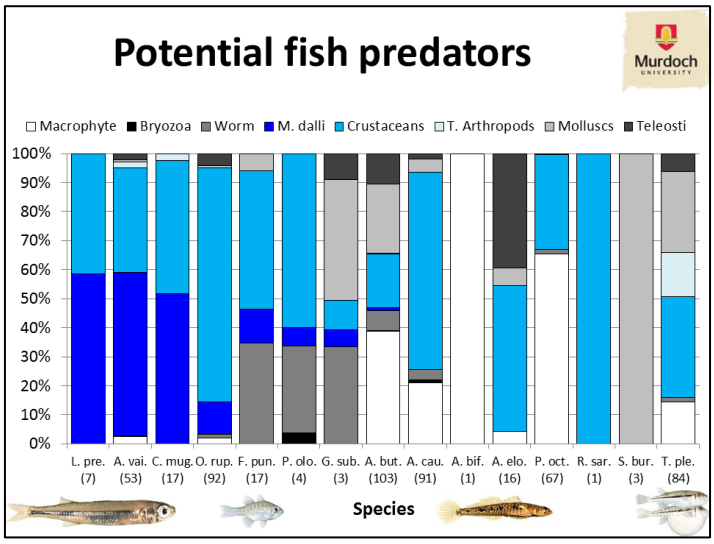




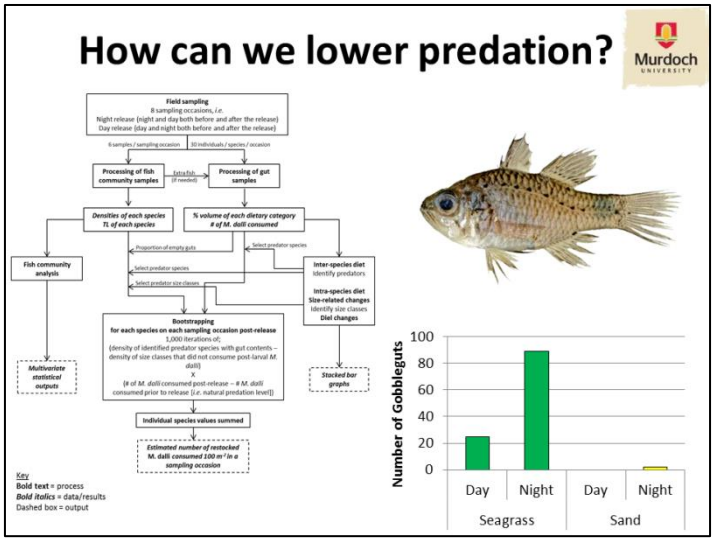

Dalkeith
Bayswater
Activity levels
Percentage contribution
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
4:00 PM
6:00 PM
8:00 PM
10:00 PM
12:00 AM
2:00 AM
4:00 AM
6:00 AM
8:00 AM
10:00 AM
12:00 PM
2:00 PM
4:00 PM
Shallow waters: 65% of prawns preferred the downstream sediment.
Chi-squared: X^2= 4.82; p=0.028.
Deep waters: 68% of prawns preferred the downstream sediment.
Chi-squared: X^2= 7.36; p=0.007.
Percentage occurrence
Tank 1
Tank 2
Tank 3
Tank 4
Tank 5
Tank 6

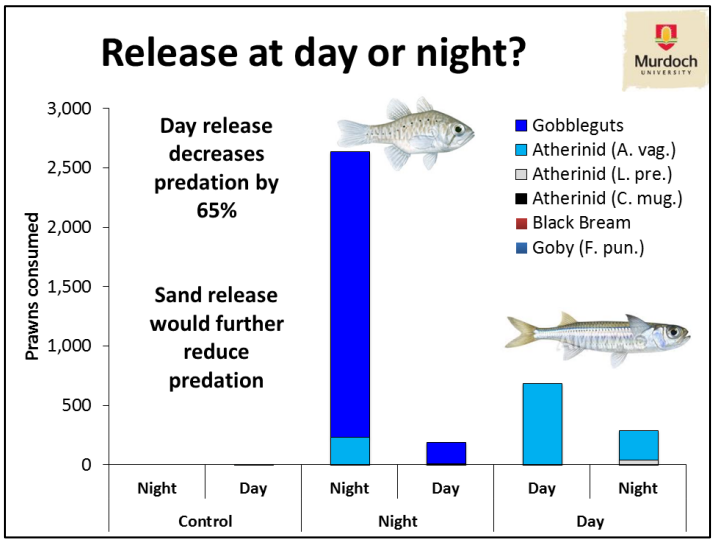

What eats Western School Prawns?
Murdoch
UNIVERSITY
?
© Swan River Trust


Predation experiment
Murdoch
UNIVERSITY
St George's College
Mounts Bay Rd
Mounts Bay
Restocking– 150,000 post larvae
Night
Day
Night
Day


Potential fish predators
Murdoch UNIVERSITY
Macrophyte
Bryozoa
Worm
M. dalli
Crustaceans
T. Arthropods
Molluscs
Teleosti
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
L. pre. (7)
A. vai. (53)
C. mug. (17)
O. rup. (92)
F. pun. (17)
P. olo. (4)
G. sub. (3)
A. but. (103)
A. cau. (91)
A. bif. (1)
A. elo. (16)
P. oct. (67)
R. sar. (1)
S. bur. (3)
T. ple. (84)
Species


Scale of the problem
Murdoch UNIVERSITY


How can we lower predation?
Murdoch UNIVERSITY
Field sampling
8 sampling occasions, i.e.
Night release (night and day both before and after the release)
Day release (day and night both before and after the release)
6 samples / sampling occasion
10 individuals / species / occasion
Processing of fish community samples
Extra fish (if needed)
Processing of gut samples
Densities of each species
TL of each species
% volume of each dietary category
# of M. dalli consumed
Proportion of empty guts
Select predator species
Select predator species
Select predator size classes
Fish community analysis
Inter-species diet
Identify predators
Intra-species diet
Size-related changes
Identify size classes
Diel changes
Bootstrapping
for each species on each sampling occasion post-release
1,000 iterations of;
(density of identified predator species with gut contents –
density of size classes that did not consume post-larval M. dalli)
x
(# of M. dalli consumed post-release – # M. dalli consumed prior to release (i.e. natural predation level))
Multivariate statistical outputs
Stacked bar graphs
Individual species values summed
Estimated number of restocked M. dalli consumed 100 m-2 in a sampling occasion
Key
Bold text = process
Bold italics = data/results
Dashed box = output
Number of Gobbleguts
100
80
60
40
20
0
Day
Night
Day
Night
Seagrass
Sand

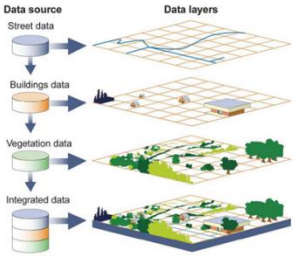

Release at day or night?
Murdoch
Prawns consumed
3,000
2,500
2,000
1,500
1,000
500
0
Day release decreases predation by 65%
Sand release would further reduce predation
Gobbleguts
Atherinid (A. vag.)
Atherinid (L. pre.)
Atherinid (C. mug.)
Black Bream
Goby (F. pun.)
Night
Day
Night
Day
Day
Night
Control
Night
Day


Jellyfish: the great unknown
Murdoch


Where to release prawns?
Murdoch

Choosing a good release spot

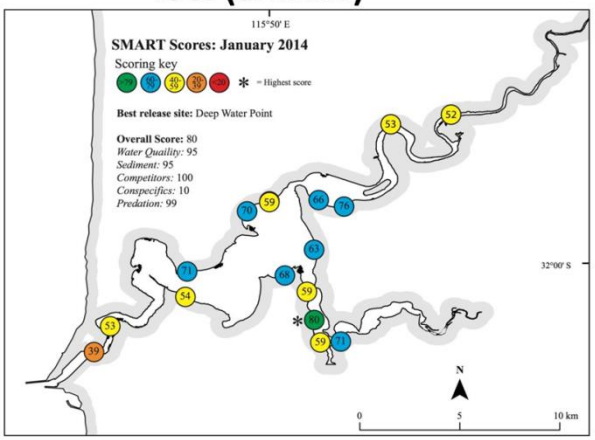
Theory: Combine data together to calculate the best release site in an unsubjective way



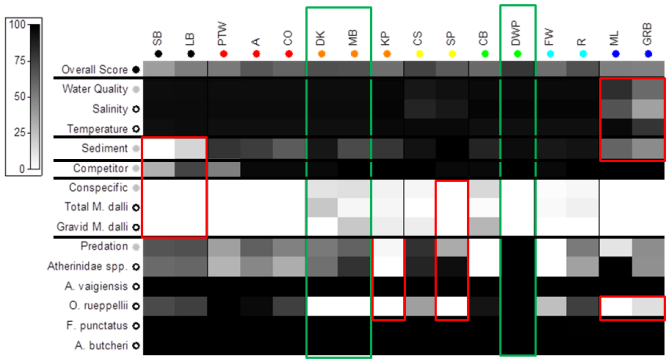
Data needed:

- Water quality
- Sediment type
- Competitors (King Prawns)
- Western School Prawns
- Fish predators

Survival Maximisation At Release Tool (SMART)



SMART Restocking

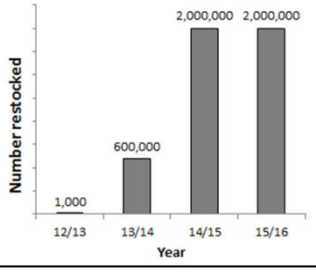


Darker colour = better for prawns

Evaluating the restocking

Australian Centre for Applied Aquaculture Research

- Developed culture methods
- Gravid females collected in the wild
- Post-larvae (PL10 to PL15) released

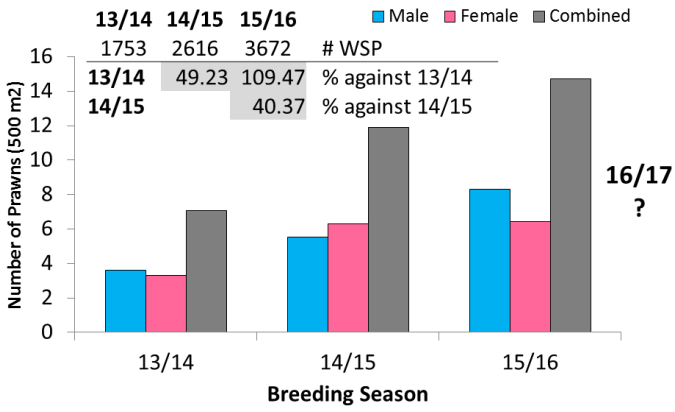




Has the restocking worked?

Catches from the deeper waters

	13/14	14/15	15/16	
	1753	2616	3672	# WSP
13/14	49.23	109.47		% against 13/14
14/15		40.37		% against 14/15



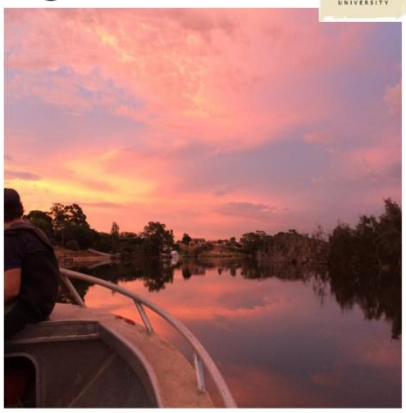
Acknowledgements

Funding bodies

- Dept. Parks & Wildlife, FRDC, RFIF/Recfishwest & Murdoch Uni.

Support

- Greg Jenkins and team at South Metro TAFE
- Dr Kerry Trayler and colleagues (DPaW)
- Recreational prawners
- The many student volunteers who assisted with sampling!



4.2. Evaluation of the effectiveness of data collected by recreational prawn fishers to provide low cost monitoring of Western School Prawns

Summary

A trial citizen science monitoring project to determine the abunbdance of the Western School Prawns (*Metapenaeus dalli*) in the Swan-Canning Estuary was undertaken in February 2015 and the results compared to a complementary university-led scientific sampling program. Analysis of the results suggested that it would be possible to establish a refined community monitoring program *i.e.* Prawn Patch Watch, which could provide sound quantitative data on prawn populations. It is envisaged that such a program would involve ~20 keen volunteers who would monitor their own site (Prawn Patch) on the closest weekend for the 1st of each month between October to March (*i.e.* the prawn breeding and main recreational fishing season). On each sampling occasion, four 50 m trawls would be conducted each of the 10 sites to produce reliable data. Having received training, after each trawl the volunteers would record the total number of *M. dalli* and the number of gravid females, potentially also with the abundance of key teleost predators and salinity and water temperature data. Data could be recorded into existing logbooks, database or the Prawn Watch smartphone app. It is recommended that this focussed citizen science project could sit within the existing Prawn Watch project, which would continue to engage a wide audience. Importantly it should be run alongside a university-led monitoring program for the first year to ensure it provide robust results that can be validated.

Rationale and aims

The aim of this study is to evaluate the effectiveness of data collected by recreational prawn fishers to provide low cost monitoring of Western School Prawns (*Metapenaeus dalli*) in the Swan-Canning Estuary into the future. This was done by trialling a citizen science monitoring program involving skilled volunteers against that conducted by university-based scientists.

Materials and methods

This study employed two sampling regimes, the first of which was the university-led sampling the second that that designed by University researchers, but operated by citizen scientists. The University-led sampling was that described in detail in Section 1.1. In brief, it employed a modified recreational prawn net (*i.e.* same dimensions), but with a heavier lead line sowed into the net and constructed from a finer mesh (9 mm). The net was dragged for 200 m twice at each of the 20 sites (Fig. 4.2.1), with the sampling conducted at least 30 minutes after sunset and over three days of the new moon phase, namely 8, 10 and 11 February 2016. The citizen science sampling regime was carried out by 11 teams of two people at a subset of seven sites (Fig. 4.2.1). Six of these sites overlapped with the university-led sampling, with the remaining site chosen for a broodstock collection event which occurred on the same night. To reduce temporal bias, the citizen science sampling occurred on 9 February 2016 and thus during the same period as the university-led sampling. Each team of citizen scientists was given a detailed set of instructions and data sheets (Annex 4.2.1,-2) and a recreational hand trawl net. This net was the same dimensions, *i.e.* 4 m wide and 1.5 m tall, as the net used in the University-led sampling, but was constructed from 16 mm mesh, as this is the smallest mesh allowed under the recreational fishing regulations and that used in most prawn nets sold in tackle stores in Perth. At each site, 10 samples hand trawl samples of 50 m in length were conducted and the number of Western School Prawns (*Metapenaeus dalli*) and Western King Prawns (*Penaeus* (=*Melicertus*) *latisulcatus*) in each sample recorded.

Maps denoting the presence and absence of both *M. dalli* and *P. latisulcatus* at each of the sites were produced together with line plots showing the running mean of the abundance against the number of samples to determine how many replicates were needed to produce a reliable result.

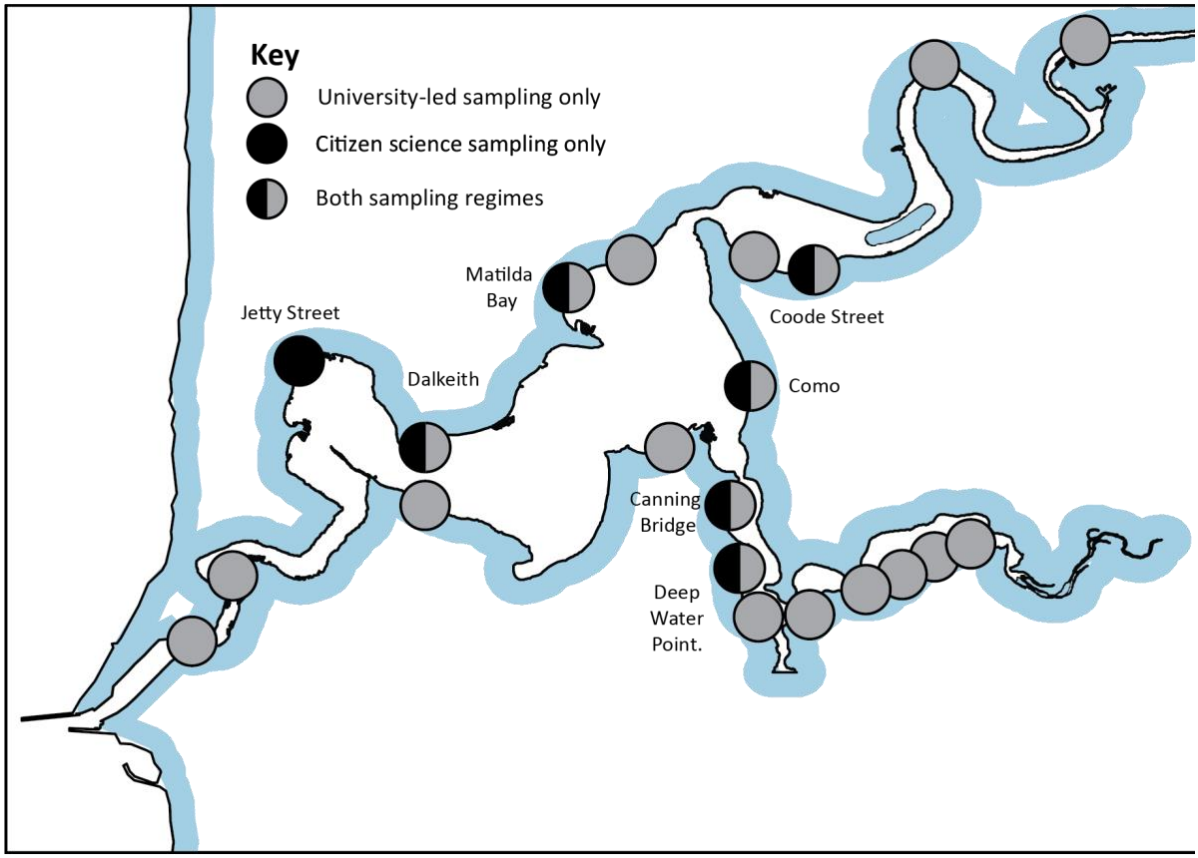


Fig. 4.2.1. Sites in the Swan-Canning Estuary at which sampling for *Metapenaeus dalli* was conducted by university-based and citizen scientists in February 2016. The names of the sites at which the citizen science sampling occurred at are provided.

Results

A total of 480 *M. dalli* and 204 *P. latisulcatus* were recorded during the citizen science sampling program on 9 February 2016, with the former species being recorded at every site and, in particular, Matilda Bay and Canning Bridge. Individuals of *P. latisulcatus* were recorded at four of the seven sites, being most abundant at Jetty Street in the lower parts of Melville Water (Fig. 4.2.2).

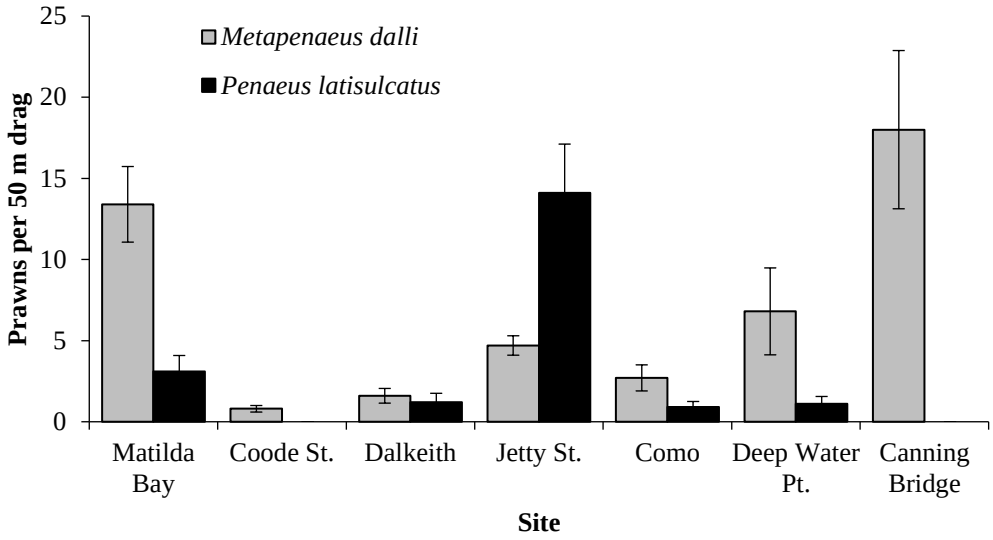


Fig. 4.2.2. Mean number of Western School Prawns (*Metapenaeus dalli*) and Western King Prawns (*Penaeus latisulcatus*) recorded in a 50 m drag at seven sites in the nearshore waters of the Swan-Canning Estuary in February 2016. Locations of the sites are shown in Fig. 4.2.1.

The trends exhibited by the running means, at each of the individual sites, showed that the number of *M. dalli* recorded in after each 50 m drag sample was relatively consistent. When the running mean data were expressed as percentage variation away from the final mean, which visually maximises differences, after four samples the running mean was within 50% of the value for the final mean, *i.e.* that after ten drags had been completed (Fig. 4.2.3). This suggests that four replicates would provide a good measure of the abundance of *M. dalli.* The only exception was Deep Water Point, on the Canning Estuary, where two replicates (numbers 6 and 7), contained far larger number of *M. dalli* (25 and 18, respectively) than the other samples (average of 3). The trend for *P. latisulcatus* was similar with relatively consistent catches at most sites and running mean after four replicates falling within 50% of the final mean at five of the seven sites (Fig. 4.2.4).

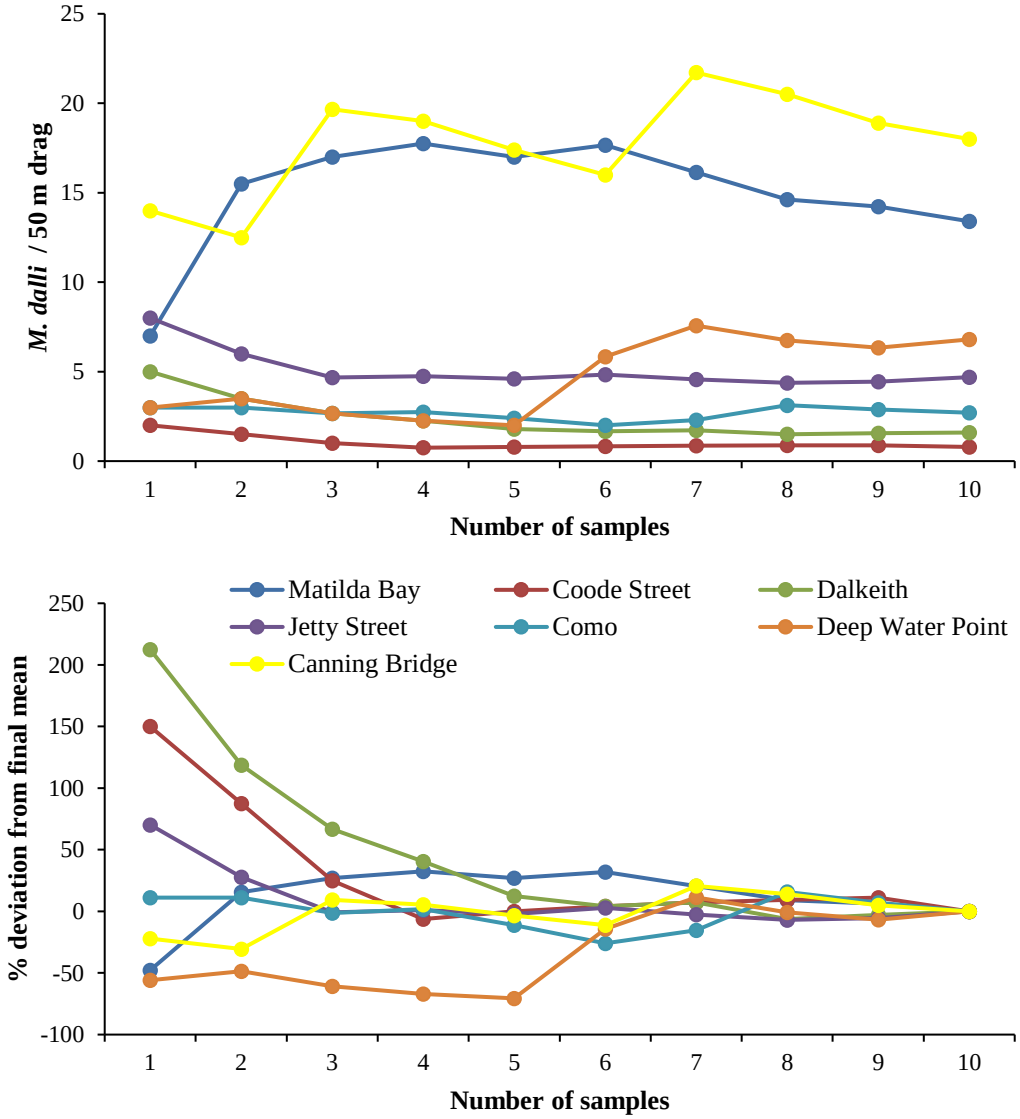


Fig. 4.2.3. (Top) Running mean of the number of *Metapenaeus dalli* recorded in a 50 m drags at seven sites in the nearshore waters of the Swan-Canning Estuary in February 2016. (Bottom) Percentage deviation of each running mean to the final mean recorded after ten drags at each of the seven sites. Locations of the sites are shown in Fig. 4.2.1.

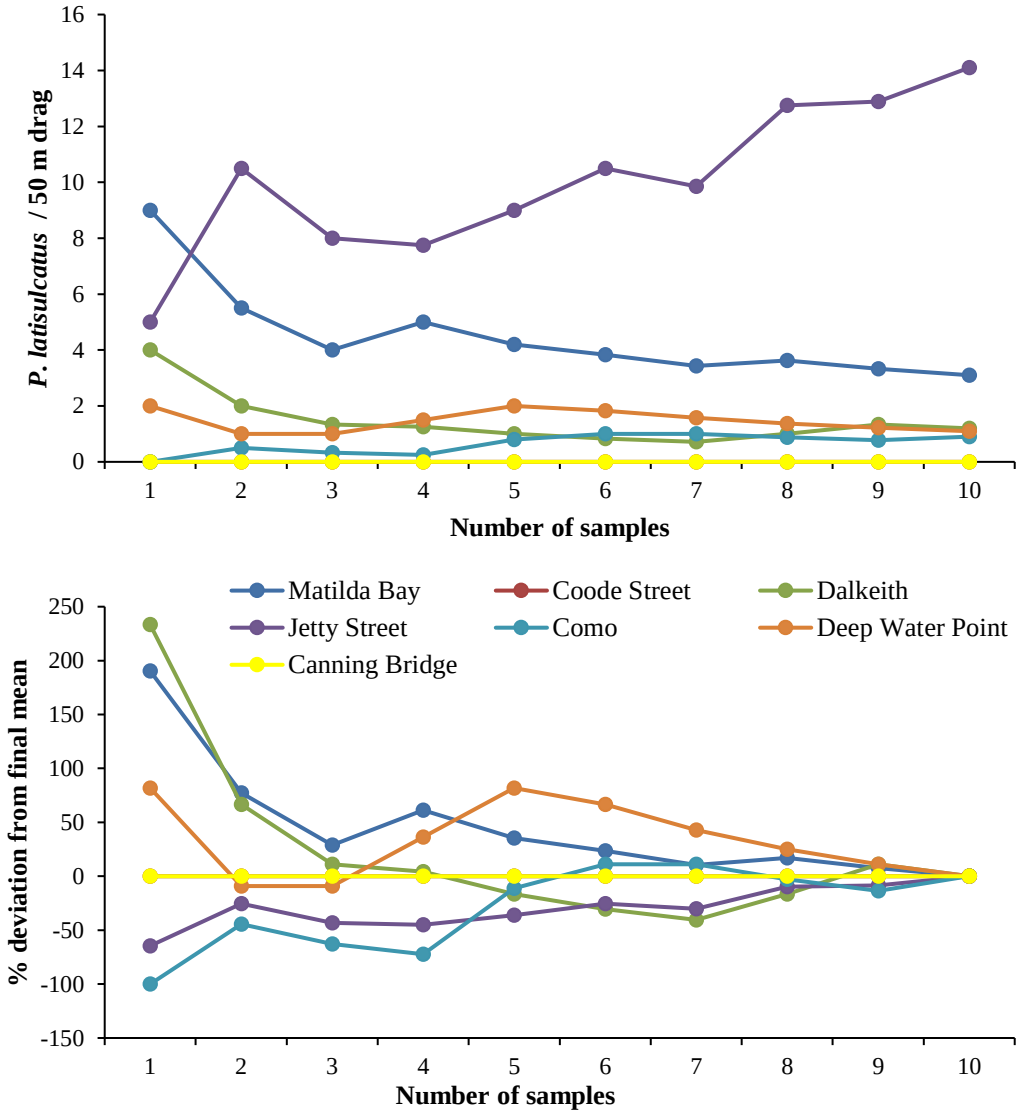


Fig. 4.2.4. (Top) Running mean of the number of *Penaeus latisulcatus* recorded in a 50 m drags at seven sites in the nearshore waters of the Swan-Canning Estuary in February 2016. (Bottom) Percentage deviation of each running mean to the final mean recorded after ten drags at each of the seven sites. Locations of the sites are shown in Fig. 4.2.1.

A comparison of the presence/absence of *M. dalli* and *P. latisulcatus* at the sites sampled by university and citizen scientists indicated that the patterns of distribution of each prawn species were broadly similar in both sampling regimes (Fig. 4.2.5). Generally both species were recorded throughout Melville Water, with the *M. dalli* occurring upstream of the Narrows Bridge. Although it is hard to standardise the two methodologies (*i.e.* 2 x 200 m *vs* 10 x 50 m drags) when the catches were standardised to individuals per 200 m drag far larger number of prawns were captured using the recreational net. This equated to 18 and 27 times greater catches of *M. dalli* and *P. latisulcatus.*

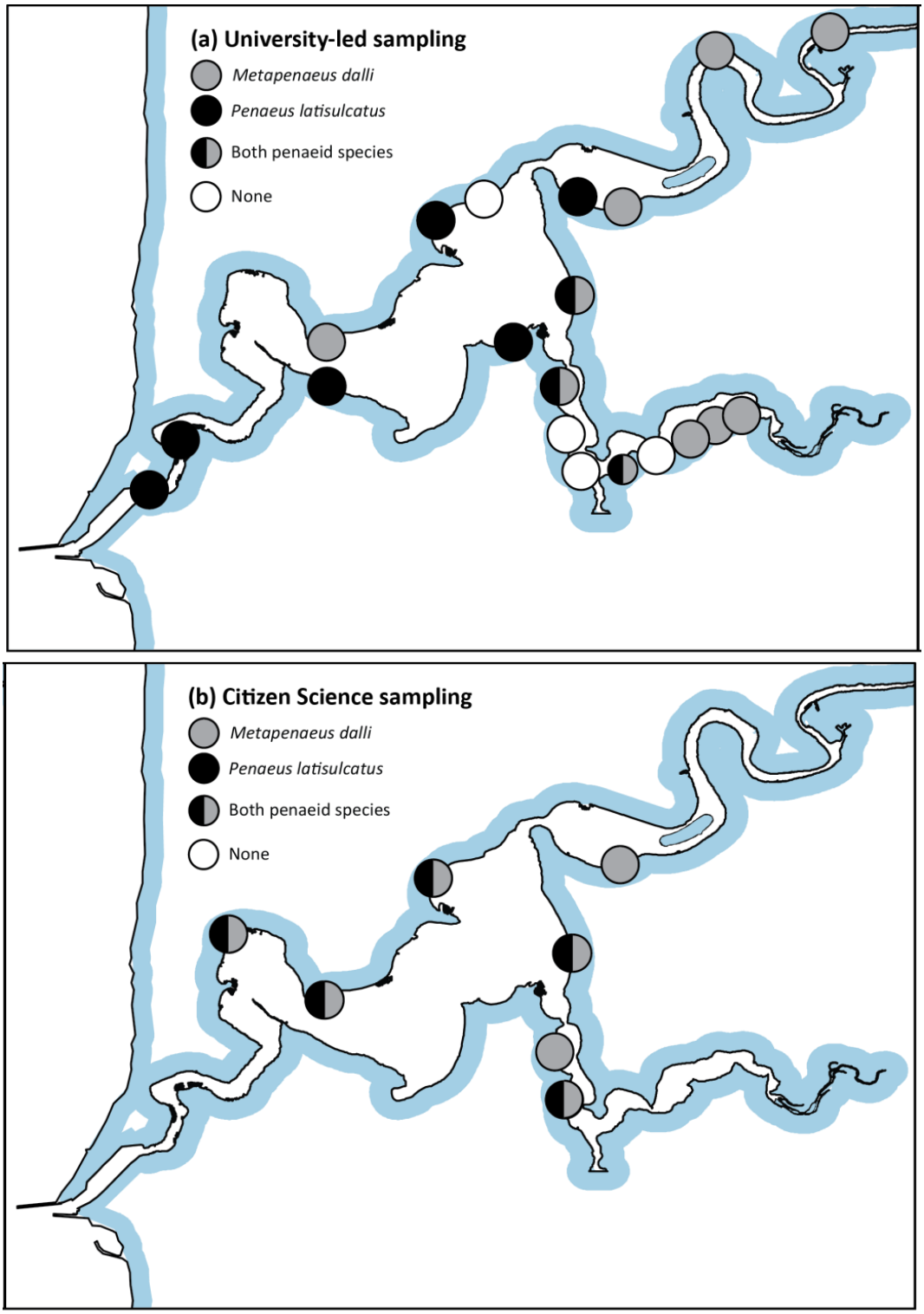


Fig. 4.2.5. Map comparing the presence/absence of *Metapenaeus dalli* and *Penaeus latisulcatus* at the sites sampled by university-based and citizen scientists in February 2016. Note two replicate 200 m drags were conducted at each site by the university-based scientist, while ten drags of 50 m in length were conducted by the citizen scientists.

Discussion

Comparison between University-led and citizen science monitoring

This study aimed to determine whether experienced Prawn Watchers could be utilised as part of a citizen science program to monitor populations of *M. dalli* in the Swan-Canning Estuary into the future. A comparison of the results of the university-led *vs* citizen science monitoring program demonstrated that the community could distinguish between *M. dalli* and *P. latisulcatus* and that both sampling regimes showed similar patterns of distribution for each species across the Swan-Canning Estuary. Where differences did occur, it was generally due to one of the species not being recorded in the scientific monitoring. This reflects the fact that a lower number of replicate samples were collected at each site, on each sampling occasion, during the University (2) than citizen (10) programs, ala the species-area curve relationship (Cain, 1938).

It is interesting that far larger number of prawns were collected using the recreational hand trawl net. Such a finding may be due to the mesh size being larger in the recreational than scientific net (16 *vs* 9 mm), which would enable less debris to be retained in the net thus increasing the speed at which it could be dragged through the water, thereby reducing the chance of prawns escaping or avoiding the net, *i.e.* increasing it fishing efficiency. As such, there would be value in conducting a depletion experiment to determine the catchability of the net (Loneragan *et al*., 1995). The rationale behind the use of the smaller mesh size in the scientific nets was to allow the potential to detect post-larval and early juvenile *M. dalli* during winter and spring, however, this sampling occurred during summer, at a time when induvial would be at or approaching their maximum size (Broadley *et al*., 2017), the reduced mesh size afforded no advantage.

The lower catch rates in the scientific net may also reflect the fact that drags were longer, which may also decrease fishing efficiency and that given the increased number of drags it is possible that some of the prawns collected in the recreational net may have been collected in a previous drag. One modification that should be made to the recreational net for any future citizen science

monitoring is the addition of weight to the leadline, this was included on the nets used in the current study and, to the best of our knowledge, does increase catches of this benthic species.

Analysis of the running means suggested that four replicate samples would be sufficient to obtain a robust estimate of the density of both *M. dalli* and *P. latisulcatus* (*i.e.* < 50% deviation from the mean). Given our experience with community Prawn Watchers during the current study, four 50 m drags is not seen as too onerous for volunteers, to the point where they would be unwilling to participant in the sampling or drop out of any future monitoring program. While the trends were consistent here it is worth considering that the *M. dalli*, is relatively mobile and thus conducting a lower number of replicates would reduce the quality of the results.

In summary, the results in this section indicate that highly trained citizen scientist could produce sound monitoring results for *M. dalli* and *P. latisulcatus* using a recreational prawn net providing at least four replicate drags of 50 m are conducted at each site on each sampling occasion. Examples of the training materials are provided in Annex 4.2.1 and 4.2.2.

Recommendations for a future citizen science prawn monitoring project

The existing Prawn Watch project is a citizen science/community engagement project. The information that community have provided (as outlined in Section 4.1) has complemented the university-led monitoring of prawn populations. Monitoring was not the sole purpose of the current Prawn Watch project and, as such, rigour in the methodology of sampling, and data provided, prevents the citizen data being used confidently for the purpose of providing sound information on prawn population size and dynamics.

Prawn Watch (2012-2016) achieved many societal outcomes as outlined in its objectives. Through the associated university-led monitoring it was also able to provide quality information on prawn populations and influence management (see Section 4.3). Moving forward, if it is intended that a citizen science project monitors and provides sound scientific information on prawn population size and dynamics, then it is important to build more rigour into the data collection, validation and quality assurance. In addition, it is important to have clearly

articulated goals for this kind of citizen science project (Tweddle *et al*., 2012). While they were clearly articulated and achieved for Prawn Watch (2012-12016), these may change and may encompass:

- Programmatic outcomes: population information to influence fishery policy/ management;
- Societal outcomes; social capital and engagement; *capacity building; behaviour change*;
- Individual outcomes: skill development; attitude change; learning.

The investigation reported in this section of the report has shown that a comparatively small number (*i.e.* 4) trawl samples of 50 m in length could provide sound data on the abundance of prawns at a range of sites. This gives confidence that it is possible to create a more rigorous monitoring regime with fishers that could complement a broader scale engagement program.

It is, therefore proposed that a small number of fishers (~20) are inducted as Prawn Patch Watchers. These fishers could be engaged to monitor between October and March each year at 10 patches in total across a broad area of the Swan-Canning Estuary, known to support populations of *M. dalli* (Fig. 4.2.6).

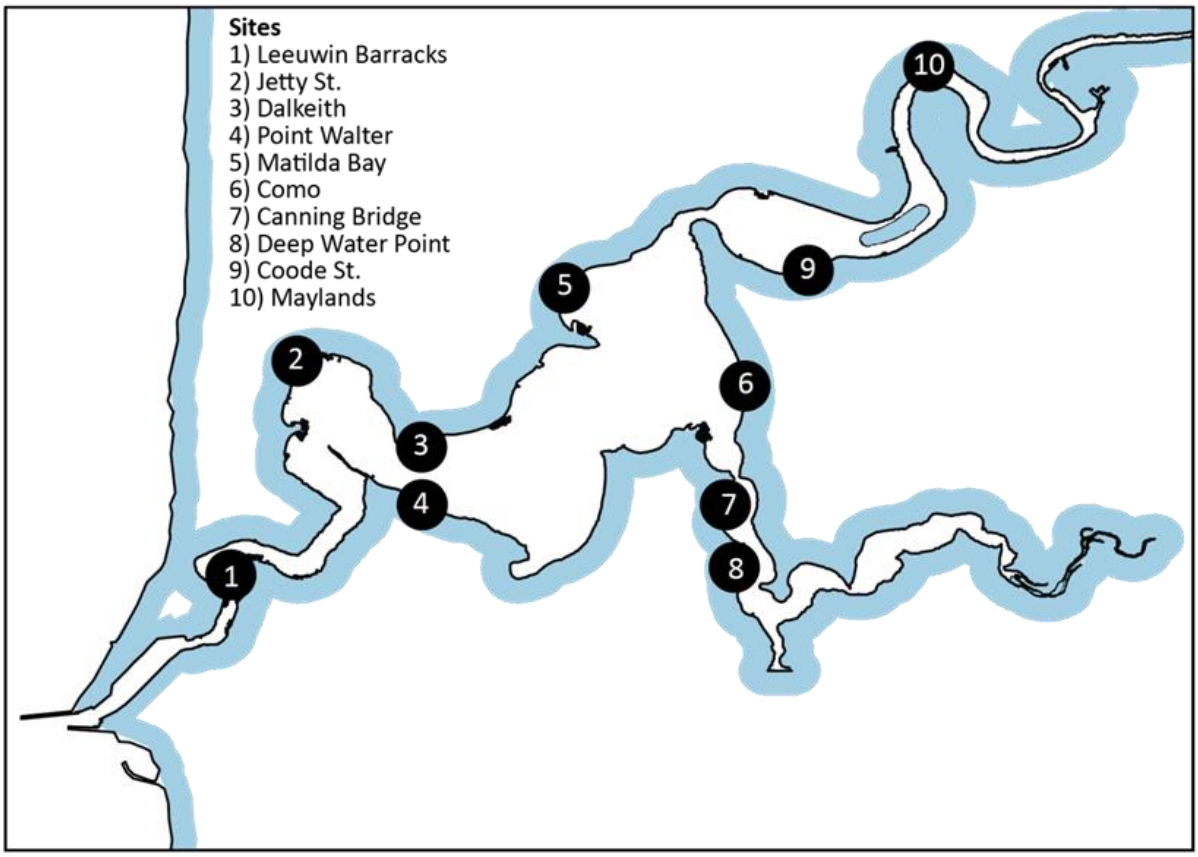


Fig. 4.2.6. Suggested sites to be sampled in any future citizen science monitoring project for *Metapenaeus dalli* in the Swan-Canning Estuary.

It is recommended that Prawn Patch Watchers are trained to use a standard technique and approach. This should involve taking four independent 50 m trawls at each site (*i.e.* their own patch) using a recreational prawn net. These would be undertaken monthly (on closest weekend to 1st of the month). After each trawl, the number of all *M. dalli* and the number of gravid (stage 4) *M. dalli* would be recorded (Fig. 5.7; Crisp *et al.*, 2017a). Prawn Patch Watchers could also record the number of key predator (*i.e.* Gobbleguts; *Ostorhinchus rueppellii*) in each trawl sample, as well as salinity and temperature using simple, inexpensive equipment (*e.g.* a manual [glass] thermometer and a refractometer). Data could be recorded into existing logbooks, database or the Prawn Watch smartphone app.



Fig. 4.2.7. Photograph showing a gravid (stage 4) *Metapenaeus dalli* from the Swan-Canning Estuary.

This dedicated citizen science monitoring approach should be complemented in its first year with a University-led monitoring program in order to validate the results of the citizen science monitoring. Thereafter, providing there is proven rigour in the approach, then it could continue. The existing logbooks, database and smartphone app were designed to be flexible and can support this approach as well as broader scale community monitoring, provided Prawn Patch Watchers had a dedicated identifier. The app would need to be updated to enable Prawn Patch Watch data to be provided back to the community in a meaningful way.

Citizen science monitoring of fisheries using targeted volunteers is not without precedent in Western Australia. An example of this type of approach has been used by the Department of Fisheries Volunteer Angling program (now within the Department of Primary Industries and Regional Development to provide annual recruitment information on juvenile Tailor (*Pomatomus saltatrix).* However, it must be recognized that, in order to succeed in the longer term such programs must be resourced.

Advice provided by the Fisheries Division, Department Primary Industries and Regional Development (K. Smith, pers. comm.) suggests that the success of maintaining a volunteer base for these kinds of programs hinges on a number of factors including:

- One-on-one engagement; personal connection; sense of belonging; feedback; incentives and celebration;
- Scientist involvement (rigour / engagement); Volunteer coordinators;
- Specialist skills in community engagement and recruitment.

A summary of components of the proposed Prawn Patch Watch *vs* the existing Prawn Watch are provided in Table 4.2.1.

Table 4.2.1. Comparison of the components in the existing Prawn Watch and proposed Prawn Patch Watch for a targeted number of participants.

Components of Prawn Watch	Components of Prawn Patch Watch
Community engagement strategy	Community engagement strategy and data management plan
Pre-season engagement and training in approaches to sustainable fishing and monitoring	Pre-season engagement and training in specific techniques for patch monitoring and data recording. Provision of equipment.
Ad-hoc in-season app training	One-on-one training on site at start of monitoring period. Complementary university based monitoring for one year.
On-going communications through River Guardians messages	One on one communications through season and incentives
App based display of individual data and composite data across zones	App based display of individual data and composite Prawn Patch data across zones. Additional analyses of datasets for each site and zone. Comparisons to University dataset
Feedback forum	Report development. Feedback forum and celebration bbq.

In order to support a dedicated citizen science monitoring project the project would need to be adequately resourced. Indicative resourcing required for these two components of Prawn Watch would include;

- DPaW principal scientist 0.1 FTE
- DPaW environmental officer 0.2 FTE
- DPaW community engagement officer 0.5 FTE
- DPaW marketing officer 0.1 FTE
- University research scientist (1st year) 0.2 FTE
- University research assistant (1st year) (0.2 FTE
- University sampling budget (*e.g.* car hire and minor equipment)
- App revision to improve data discovery
- 10 nets and associated monitoring equipment for participants
- Incentive resources for volunteers
- Engagement budget (*e.g.* venue hire, travel and catering).

Prawn Watch is currently a contributory citizen science project. Community provided data that was analysed and combined with university results before being provided back to the community via the app and/or feedback forums. A more focussed project, with a smaller number of key contributors involved in the collection of data would enable increased involvement of citizens in the analyses and interpretation of data, and enable volunteers to be involved in the coordination of the project, thereby creating a more collaborative project and enhancing both societal and individual outcomes.

Annex 4.2.1. Instruction sheet provided to the citizen scientists during the citizen science monitoring trial.

recfish west

Recreational Fishing Initiatives Fund

Government of Western Australia
Department of Fisheries

PRAWN WATCH

Western School Prawn – Citizen Science Monitoring

Overview

Thank you for taking part in this trail to help develop a citizen science methodology suitable for monitoring populations of the Western School Prawn.

The aim of tonight's sampling is to record prawn numbers at a range of sites across the Swan-Canning Estuary.

You will be spilt into small teams and assigned to a particular site. At each site, we require **10 drags of 50 m** to be conducted. Note that there may be more than one team of fishers at a site. You will be provided with a data sheet to fill out after each drag, which also includes some information on how to distinguish between the Western School and Western King prawns.

Method

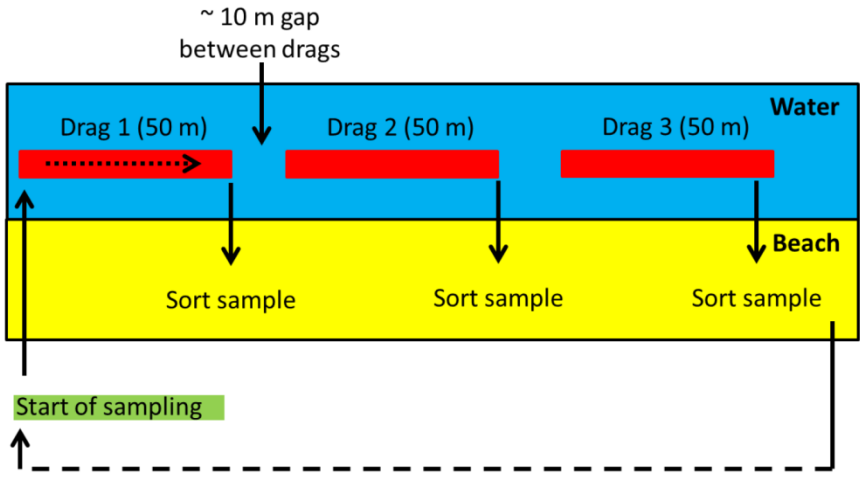
- Start at one end of the beach.
- Together with your partner, enter the water to a depth of ~ 1m (waist deep).
- Drag the net for ~ 50 m (this should be around 80 steps and could be marked out on the beach beforehand).
- Bring net onto shore, shake contents into the cod-end and empty on to a tarp.
- Sort and return all fish to the water alive immediately.
- Count the number of Western School Prawns and Western King Prawns and record on the data sheet.
- Keep gravid female School Prawns for broodstock collection and return other prawns to water.
- Prepare net for next drag (tie net and remove any plant material).
- Repeat up to 10 times for each site (note there may be more than one team per site).
- Please leave ~ 10 m between drags (to avoid overlap/disturbance).
- If you run out of beach, walk back to the beginning and start again.
- See overleaf for diagram.

recfishwest

Recreational Fishing Initiatives Fund

Government of Western Australia
Department of Fisheries

Sampling diagram



If you run out of beach return to the start of the first drag and continue

THANK YOU!

Annex 4.2.2. Data sheet provided to the citizen scientists during the citizen science monitoring trial.

recfish west

Recreational Fishing Initiatives Fund

Government of Western Australia
Department of Fisheries

PRAWN WATCH

Western School Prawn – Citizen Science Monitoring

Site: _____

Date: _____

Start time: _____ End time: _____

Drag #	# School Prawns	# King Prawns
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

recfish west

Recreational Fishing Initiatives Fund

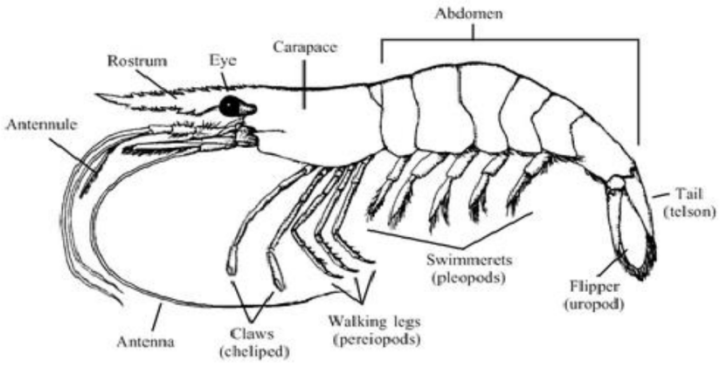
Government of Western Australia
Department of Fisheries

Useful information

Identification guide

	Western School Prawn	Western King Prawn
		
Size	85 mm total length	200 mm total length
Antennal filaments	Comparatively long and visibly bifurcate (2 tips)	Small and indistinct
Rostrum	Pale and straight. No spine	Ridges are dark brown. Visible spine at base of rostrum
Eyes	Eyes stalks are small and eyes held close to the head. Eyes are rounded and dark in colour	Eye stalks are long. Eyes are somewhat bean shape and appear compound (*i.e.* 'fly-like')
Walking Legs	Pale yellow-green	Light blue
Tail (Telson & Uropod)	Pale yellow-green	Light blue

Prawn anatomy



4.3. Recommendations for a sustainable Western School Prawn fishery

Prawning was an integral part of Perth culture in the 1970-1990s, engaging up to 50,000 people each year, however, once the stocks of *Metapenaeus dalli* declined, interest in the fishery waned. Over the course of two associated projects to develop aquaculture techniques for *M. dalli* and pilot the release of hatchery-reared individuals (Jenkins *et al*., 2015; 2017), 4.5 million post-larval *M. dalli* were released into the Swan-Canning Estuary over a four year period between 2013 and 2016. The publicity generated from this study, and particularly the Prawn Watch component, increased interest in prawning. Anecdotal information suggests that this resulted in an increase in recreational fishing effort, with participants reporting their best catches in years and some even decades.

The estimated biomass for *M. dalli* in the Swan-Canning Estuary was calculated by Broadley *et al*. (2017) is 2.37 tonnes, and is thus only ~16% of the maximum commercial catch for prawns (both *M. dalli* and the Western King Prawn *Penaeus* [= *Melicertus*] *latisculatus*) recorded from the system during the peak of the commercial fishery in the 1950s (Smith, 2006). It is apparent therefore that the current biomass of *M. dalli* is very low and has not recovered since recreational fishing decreased greatly in the late 1990s, over 15 years ago.

This pilot restocking project has likely had a significant positive effect on the populations of *M. dalli* in the Swan-Canning Estuary, with the abundance of this species in the deeper waters of the estuary during the breeding season (Oct-Mar) being 49 and 110% greater in 2014/15 and 2015/16, respectively, than after the first year of restocking in 2013/14 (Annex 4.1.9). Note that monitoring was only initiated in October 2013, which was after the release of 1,000 prawns in May 2013. However, with the restocking now finalised and anecdotal evidence of an increase in fishing pressure, attention must turn to ways to ensure the sustainability of the current *M. dalli* population. A large amount of research and monitoring has been completed through this project, to support management in decision making. The project steering committee therefore sought to engage with stakeholders and policy makers to present the knowledge obtained during the project and discuss ways of improving the sustainability of the fishery in the longer term.

On 9 June 2016, the Department of Parks and Wildlife (now Department Biodiversity, Conservation and Attractions, Parks and Wildlife Service) facilitated a meeting of stakeholders to discuss the sustainable management of this recreational fishery. At that time, representatives of the Parks and Wildlife, Department of Fisheries (both research and policy divisions), Murdoch University, Recfishwest, Australian Centre for Applied Aquaculture Research and two experienced recreational fishers from the Prawn Watch Reference Committee were present. At that meeting, the historical and existing prawning regulations were summarised (see Table 4.3.1) and it was acknowledged that in the past, fishery regulations had included a 50 mm length limit and seasonal closures and that over time the recreational fishing regulations of the *M. dalli* fishery in the Swan-Canning Estuary had been simplified such that seasonal and size limits had been removed. The recreational fishery guidelines (2016) permit the taking of prawns year round in the estuary. A daily bag limit of 9L applies. Gear is restricted to hand- dip, scoop and trawl nets. The use of hand-throw net is not permitted. The use of hand-trawls is excluded within 100m of the Pelican Point and Milyu sections of the Swan Estuary Marine Park. This exclusion does not, however, include the Alfred Cove section of the marine park.

Table 4.3.1. History of fishery regulations for the recreational Western School Prawn fishery in the Swan-Canning Estuary.

Year	Management regulations				
	Size limit	**Bag limit**	**Gear**	**Season**	**Location in Swan-Canning Estuary**
1988	50 mm from eye to tip of tail	9L person day^{-1}	Scoop net, hand trawl net or hand dip net. Hand trawl. Nets must not exceed 4 m in width, nor contain any mesh < 16 mm.	Not mentioned	Not mentioned
1990	None	9L person day^{-1}	Scoop net, hand trawl net or hand dip net. Hand trawl. Hand trawl nets must not exceed 4 m in width, contain any mesh < 16 mm and must not be attached to a boat or set.	Swan-Canning Estuary: closed from 31 July to 1 November each year. Serpentine, Murray, Dandalup, and Harvey Rivers are closed from 1 May to a date in November announced each year in the papers.	Not mentioned
2013	None	9L person day^{-1}	Hand dip net, hand scoop net, hand throw net and hand trawl net. Hand trawl nets must not exceed 4 m in width, contain any mesh < 16 mm and must not be attached to a boat or set.	Swan-Canning Estuary: Closed to drag trawl nets 1 August – 31 October Peel-Harvey Estuary and rivers: closed all year to trawl nets	With 100 m of any part of the Pelican Point Nature Reserve or within 100 m of Milyu Nature Reserve
2015	None	9L person day^{-1}	Hand dip net, hand scoop net, hand throw net and hand trawl net. Hand trawl nets must not exceed 4 m in width, contain any mesh < 16 mm and must not be attached to a boat or set.	Swan-Canning Estuary: None. Peel-Harvey Estuary and rivers: closed all year to trawl nets	With 100 m of any part of the Pelican Point Nature Reserve or within 100 m of Milyu Nature Reserve

Information pertaining to the biology and ecology of *M. dalli* was also presented at that meeting (Annex 4.3.1). These demonstrated that individuals of this species in the Swan-Canning Estuary represent an isolated population, as they complete their life cycle with the estuary and are not found in adjacent marine waters (Potter *et al*., 1986). They are also a relatively small species, with the larger females reaching a maximum size (L_{∞}) of 33.6 mm CL and 23.6 g wet weight, but also suffering a higher natural mortality than males. Although the fecundity of *M. dalli* is similar to that of other metapenaeids in temperate waters, this value is far smaller than for congeners inhabiting tropical environments and *Penaeus* spp. in any environment. Breeding in *M. dalli* occurs in the shallow waters of the estuary between October/November, when water temperature rise to > 20 °C, and March.

These biological characteristics have implications for the management of this because:

(i) There is unlikely to be significant immigration of *M. dalli* individuals into the Swan-Canning Estuary from other nearby estuaries (*e.g.* Peel-Harvey) and/or nearshore marine waters, *i.e.* the population is isolated.

(ii) Females suffer a higher natural mortality than males with most dying after spawning.

(iii) Females obtain a far larger size and weight and thus may preferentially be the target of recreational fishers.

(iv) Individuals move from offshore water refuges into the nearshore waters to breed.

(v) The breeding and peak recreational fishing seasons co-occur and as such they are subject to fishing pressure throughout the breeding period.

A range of potential management options were discussed to improve the sustainability of the *M. dalli* fishery in the Swan-Canning Estuary. Each option was discussed and comments made on its likelihood of success, feasibility and maintenance of social values (Table 4.3.2).

Table 4.3.2. Potential management options to improve the sustainability of the recreational Western School Prawn fishery in the Swan-Canning Estuary.

Options	Comments
Size limits; *e.g.* 50 mm length used in 1980s	Too hard to for fishers to measure each prawn captured. Difficult to enforce. Best to be managed through code on conduct.
Return females or gravid female; *e.g.* as with Blue Swimmer Crabs (*Portunus armatus)*	Most community members are unable to distinguish between male and female prawns and also gravid and non-gravid females. Best to be managed via community education.
Bag limits; currently 9L per person per day (mixed M. dalli and P. latisculatus limit)	Current bag limit too high, especially as hand trawl nets require two people to operate. However, any reduction needs to consider effects on scuba divers who target P. latisculatus. Community find it hard to distinguish prawn species and so separate M. dalli and P. latisculatus limits unlikely to be successful.
Spatial closure in marine reserves	Currently prawning allowed in two of the three marine reserves in the Swan-Canning Estuary. Banning in all three would simplify regulations and prevent bird disturbance.
Spatial closure in other areas	Confusing for the community and may result in a loss of amenity as fishers may have to travel away from their local area to prawn.
Full closure	Not supported. Would result in the loss of an iconic fishery and pastime.
Temporal closure; *e.g.* closure from 1st August – 31st October as enforced previously.	Potential option that could be used to reduce/prevent fishing pressure at the start of the breeding seasons. However, prawning should be allowed to occur at Christmas to maintain social benefits.
Fishing gear restrictions	Hand scoop only fishery not supported.

Following discussions during the meeting, a number of recommendations were produced.

(i) A code of conduct and/or community education program (*e.g.* Prawn Watch) were best placed to promote sustainable fishing practices, such as the release of sexually immature prawns, female prawns and/or gravid females.

(ii) Any future management should focus on maintaining stocks of *M. dalli* and the social value of the fishery. Thus, full closure, a hand scoop/dip net only fishery and spatial closures outside of marine reserves were not supported.

(iii) A bag limit per person of 5L per day should be considered subject to considerations of the implications for recreational fishing of *P. latisulcatus*. Temporal closures considered at the start of the breeding season to allow a proportion of the population to spawn.

(iv) There is a need for the population of *M. dalli* in the Swan-Canning Estuary to be monitored in the future.

(v) Recreational prawning regulations should be reassessed in the near future if monitoring indicates that stocks are increasing.

In early August 2016, staff from Parks and Wildlife met again with Recfishwest, with the view to trying to reach common agreement around bag limits, partial closure and exclusion zones. Additional information on the fecundity of adult females during breeding seasons between October 2013 and March 2016 was prepared for that meeting and cumulative fecundity plotted (Fig. 6.1) to show the impact of reinstating a season closure on spawning and thereby the future stock.

Estimates of cumulative egg production were variable within a month in each year (Fig. 4.3.1), reflecting differences in the timing and duration of reproduction (see Section 1.3 for full details of the methodology) and therefore some caution has to be exercised in using these for policy formation. However, they did at least provide some information for discussion. Without a closed season all gravid females, and thereby future stock, are vulnerable to fishing pressure as they

move into the shallow waters to breed. The implementation of a seasonal closure delaying the starting of the prawning season until 1 of November, 1 December and 1 January would have the potential to protect 0.5%, 4-57 and 17-70% of future stock (measured here as eggs produced by the gravid females; Fig. 4.3.1).

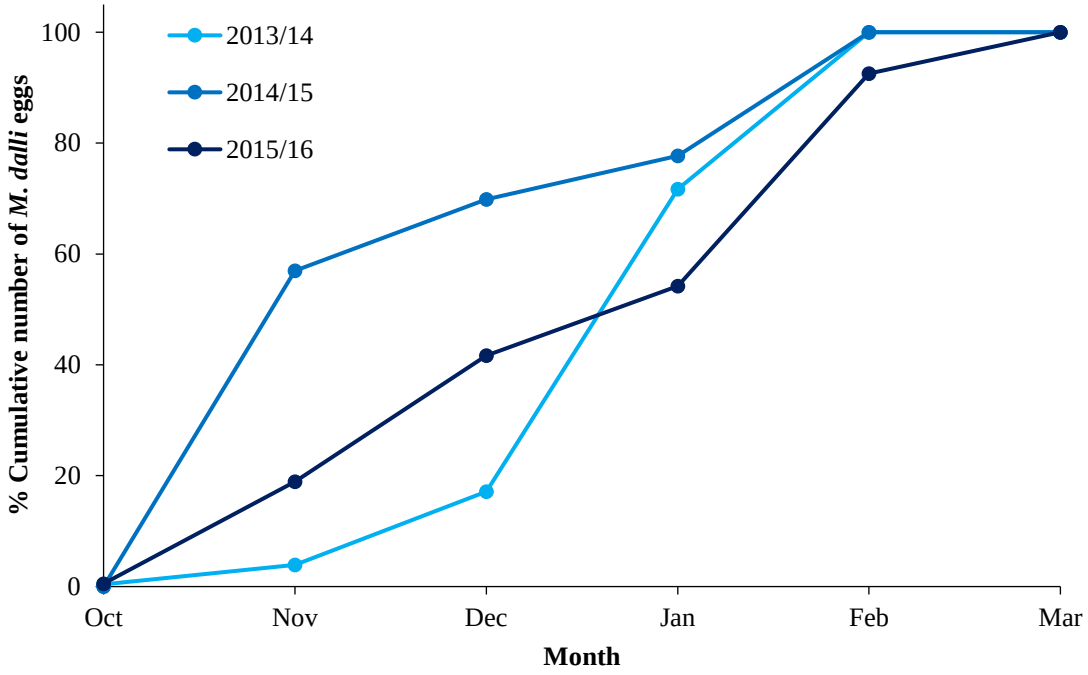


Fig. 4.3.1. Percentage cumulative number of eggs produced by female *Metapenaeus dalli* in each month between October and March in three breeding seasons, *i.e.* 2013/14, 2014/15 and 2015/16. Note sampling was conducted on the new moon and thus specific dates vary among months and years. Number of eggs was estimated by summing the fecundity of each gravid female recorded in a month, using a length to fecundity relationship (Section 1.3). These data make the assumptions that (i) females spawn once and (ii) that each gravid female will spawn before the next sampling occasion ~ 28 days later.

At the meeting it was agreed that a reduced daily bag limit (from 9 to 5L) and a partial closure between mid-October and mid-December would be beneficial to the sustainability of the school prawn fishery. No agreement was reached regarding exclusion zones. Based on those discussions the Parks and Wildlife then wrote (CEO2818/16) to the Department of Fisheries requesting their consideration for a review of the recreational fisheries guidelines for prawning to include:

(i) a reduced daily bag limit (from 9L to 5L);

(ii) introduction of a partial closure to hand-trawling between 15 October to 15 December inclusive;

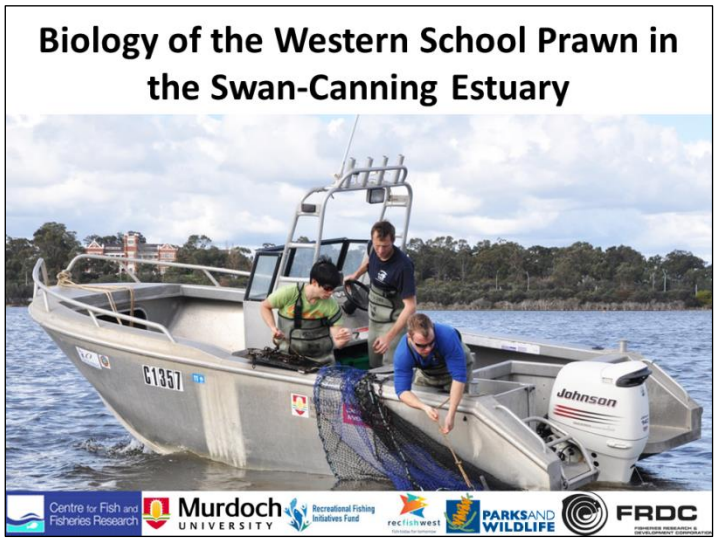
(iii) continuation of the existing zone restrictions in relation to throw and hand-trawl nets;

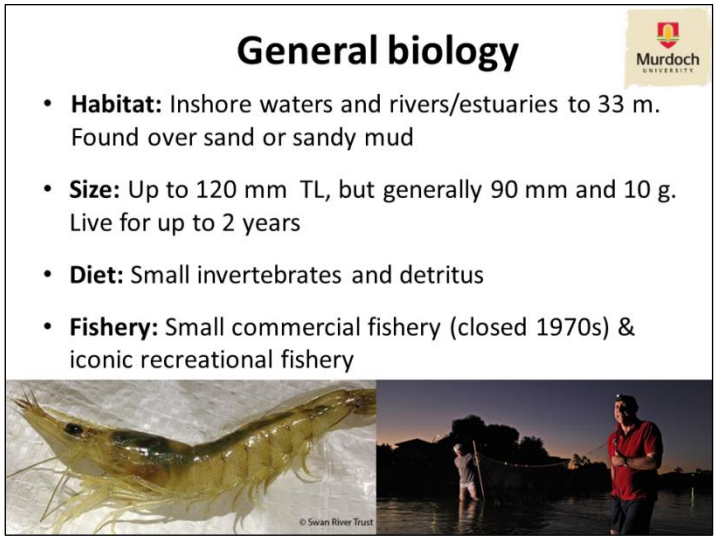
(iv) continuation of existing gear prescriptions and expectation of by-catch return.

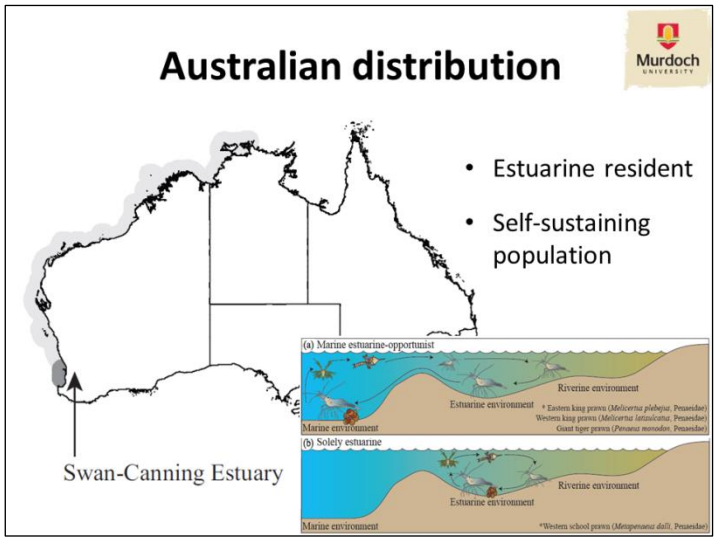
In that request, the Parks and Wildlife recognised the importance of prawning as a cultural activity and a community benefit of the Swan-Canning Estuary. It also recognized that the current Recreational Fisheries Guidelines (2016) provided some exclusion zones. These prevent the use of throw nets across the waterway and prevent the use of hand-trawl nets within 100 metres of any part of the Pelican Point and Milyu Nature Reserves. While Parks and Wildlife noted that this regulation did not protect the Alfred Cove Nature Reserve from the potential impact of trawling, it acknowledged that the regulation does provide some protection to habitat values within the Swan Estuary Marine Park, and should therefore remain in place.

Recfishwest has since formally advised the Department of Fisheries (now within the Department Primary Industries and Regional Development) that it is supportive of the measures outlined in the Parks and Wildlife letter (CEO2818/16) requesting a review of the regulations relating to prawn fishing. It is understood that changes will now be considered by the Department in the preparation of the Aquatic Resources Management Regulations.

Annex 4.3.1. Slides presented by researchers from Murdoch University about the biology and ecology of the Western School Prawn at the prawn fishery management meeting.





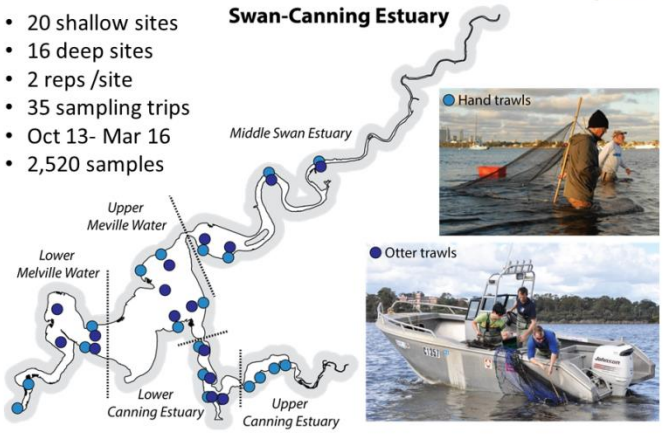


MU Research component

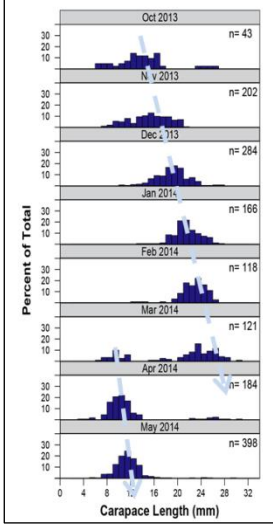
- ***Overall aim: Optimizing the release strategies***
- *Specific aims:*
  - *Larval development and ecology*
  - *Genetic implications of restocking*
  - Biological parameters
  - Spatial and temporal abundance/distributions of prawns
  - *Influence of environmental variables*
  - Impact of predation
  - *Bio-economic model for restocking*

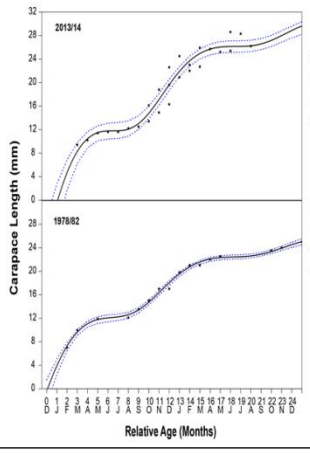
Monitoring regime

- 20 shallow sites
- 16 deep sites
- 2 reps /site
- 35 sampling trips
- Oct 13- Mar 16
- 2,520 samples



Growth

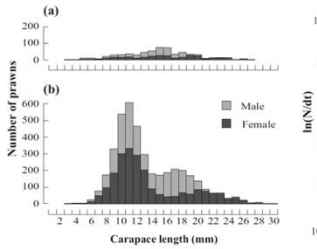


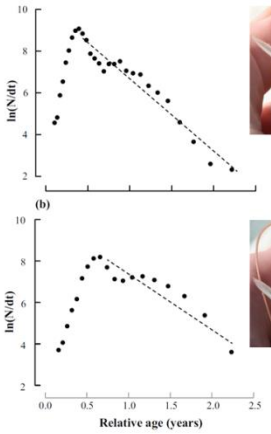


2013/14
- L_{∞} = 33.7 mm
- *k* = 1.06
- T_0 = -0.16

1978/82
- L_{∞} = 28.0 mm
- *K* = 1.05
- T_0 = -0.16

Growth and mortality



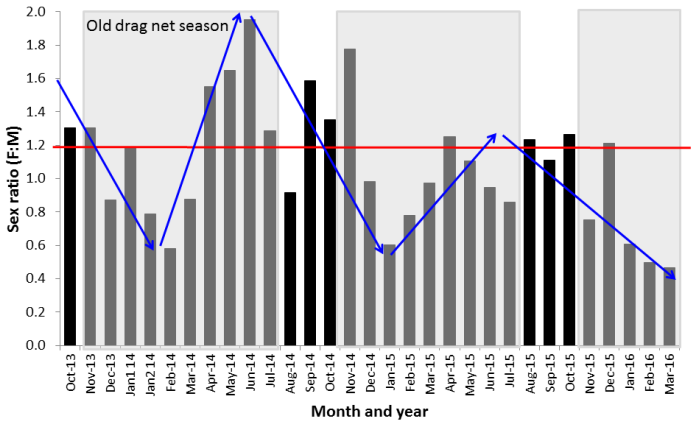




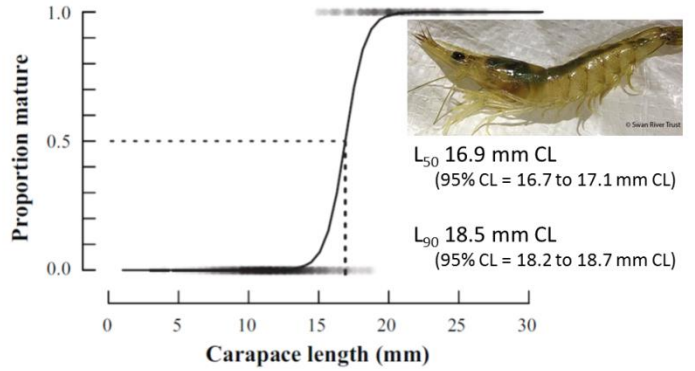


Sex	Instantaneous total mortality Z (CI)	
	Z year^{-1} (CI)	Z week^{-1} (CI)
Female	3.46 (3.03-3.89)	0.067 (0.058-0.075)
Male	2.69 (2.00-3.38)	0.052 (0.038-0.065)

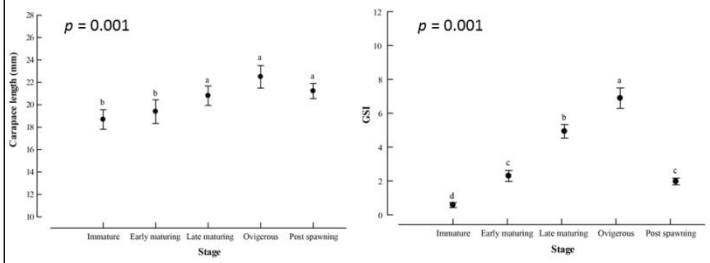
Sex ratio

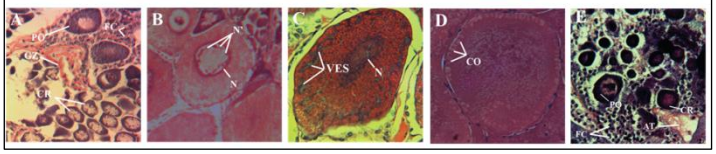


Size at maturity

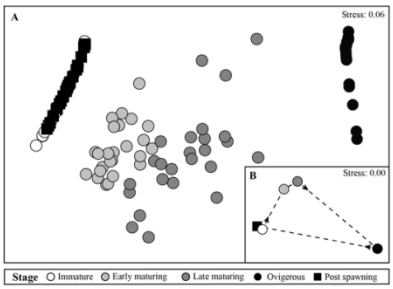


Gonadal development

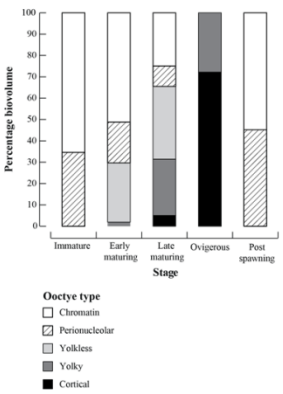




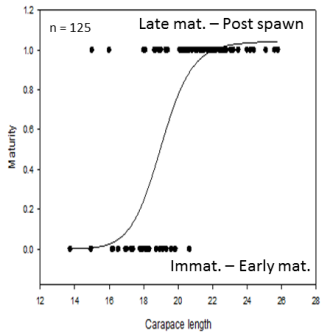
Gonadal development

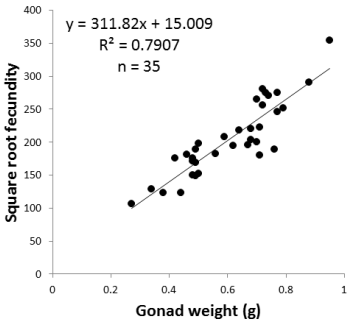


Global $R = 0.787$, $p = 0.001$



Histological size at maturity



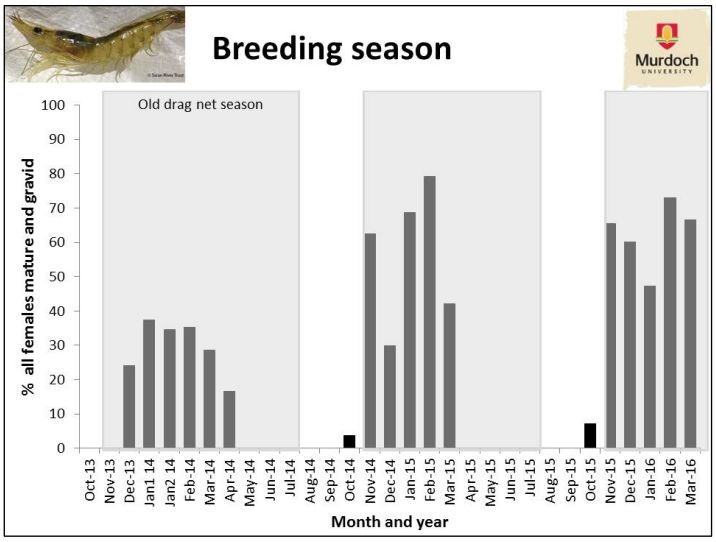


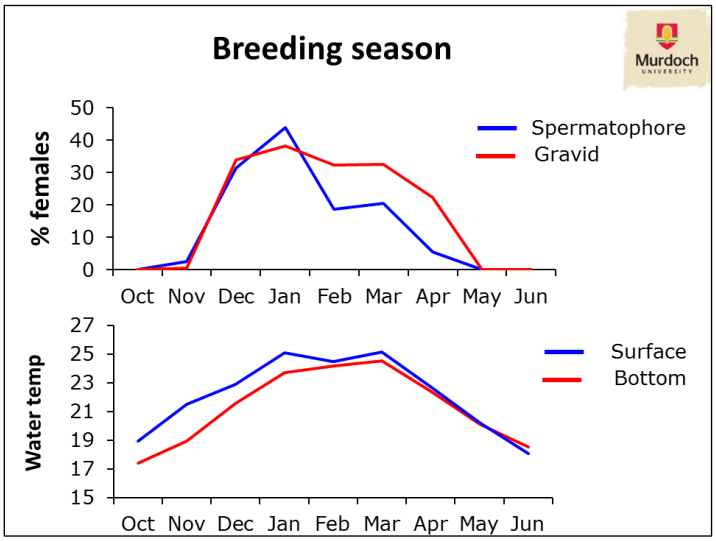
L_{50}

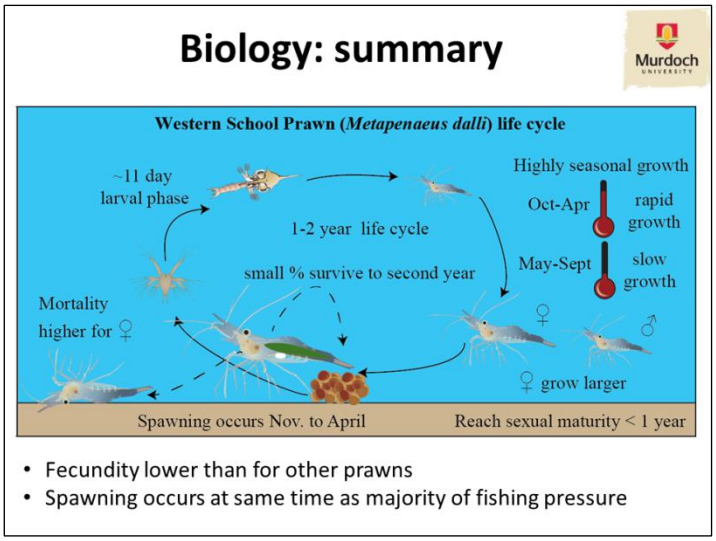
- 18.96 mm CL
- 5.8 g wet weight

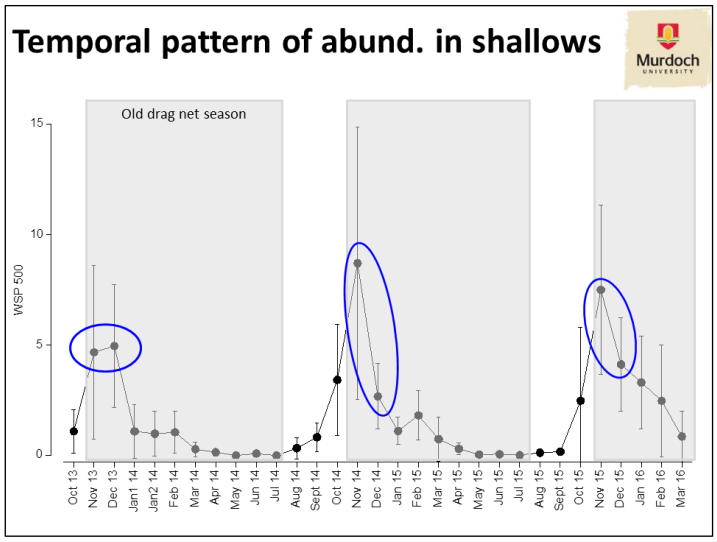
Fecundity

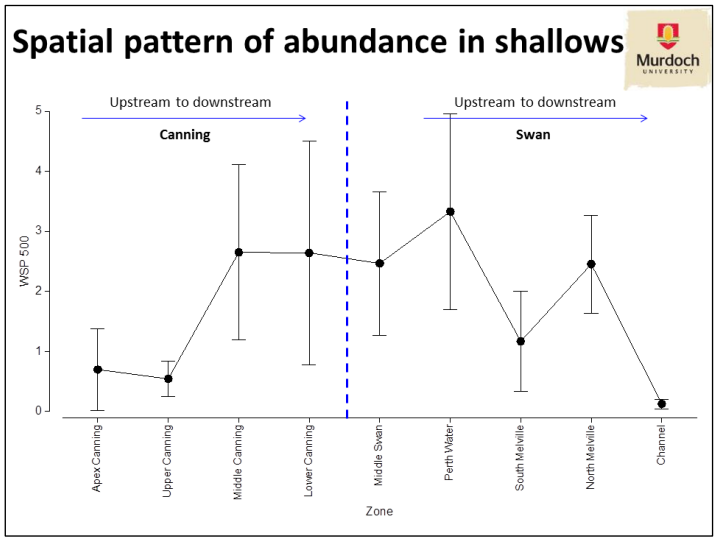
- 11,400 – 125,000 ova

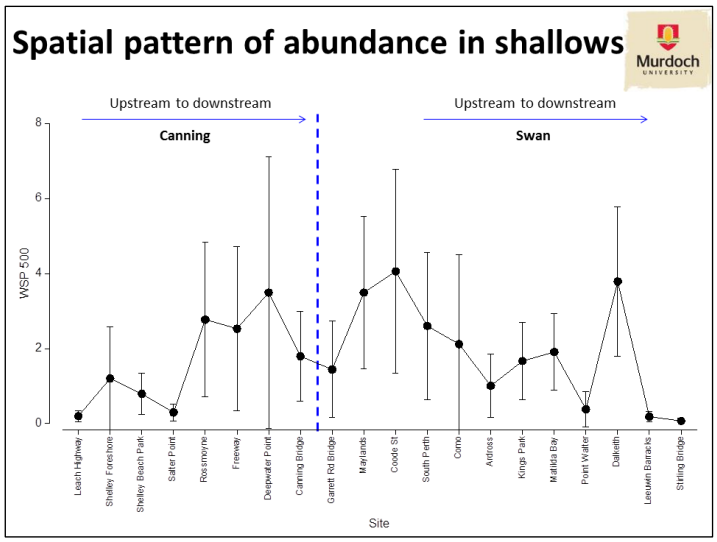

Breeding season
Murdoch
Old drag net season
% all females mature and gravid
100
90
80
70
60
50
40
30
20
10
0
Oct-13
Nov-13
Dec-13
Jan1 14
Jan2 14
Feb-14
Mar-14
Apr-14
May-14
Jun-14
Jul-14
Aug-14
Sep-14
Oct-14
Nov-14
Dec-14
Jan-15
Feb-15
Mar-15
Apr-15
May-15
Jun-15
Jul-15
Aug-15
Sep-15
Oct-15
Nov-15
Dec-15
Jan-16
Feb-16
Mar-16
Month and year


Breeding season
Murdoch
% females
50
40
30
20
10
0
Spermatophore
Gravid
Oct Nov Dec Jan Feb Mar Apr May Jun
Water temp
27
25
23
21
19
17
15
Surface
Bottom
Oct Nov Dec Jan Feb Mar Apr May Jun


Biology: summary
Murdoch
Western School Prawn (Metapenaeus dalli) life cycle
~11 day larval phase
1-2 year life cycle
small % survive to second year
Mortality higher for ♀
Highly seasonal growth
Oct-Apr rapid growth
May-Sept slow growth
♀ grow larger
Spawning occurs Nov. to April
Reach sexual maturity < 1 year
• Fecundity lower than for other prawns
• Spawning occurs at same time as majority of fishing pressure

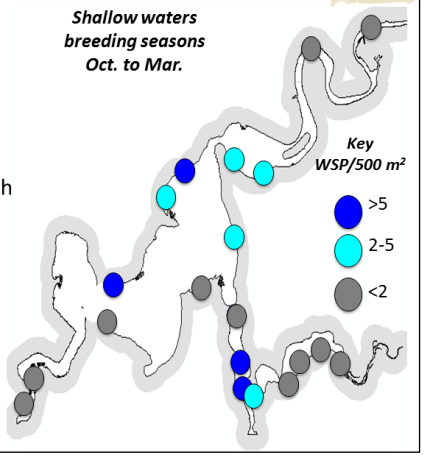

Temporal pattern of abund. in shallows
Murdoch UNIVERSITY
Old drag net season
WSP 500
15
10
5
0
Oct 13
Nov 13
Dec 13
Jan1 14
Jan2 14
Feb 14
Mar 14
Apr 14
May 14
Jun 14
Jul 14
Aug 14
Sept 14
Oct 14
Nov 14
Dec 14
Jan 15
Feb 15
Mar 15
Apr 15
May 15
Jun 15
Jul 15
Aug 15
Sept 15
Oct 15
Nov 15
Dec 15
Jan 16
Feb 16
Mar 16


Spatial pattern of abundance in shallows
Murdoch UNIVERSITY
Upstream to downstream
Canning
Upstream to downstream
Swan
WSP 500
5
4
3
2
1
0
Apex Canning
Upper Canning
Middle Canning
Lower Canning
Middle Swan
Perth Water
South Melville
North Melville
Channel
Zone


Spatial pattern of abundance in shallows
Murdoch UNIVERSITY
Upstream to downstream
Canning
Upstream to downstream
Swan
WSP 500
8
6
4
2
0
Leach Highway
Shelley Foreshore
Shelley Beach Park
Salter Point
Rossmoyne
Freeway
Deepwater Point
Canning Bridge
Garrett Rd Bridge
Maylands
Coode St
South Perth
Como
Ardross
Kings Park
Matilda Bay
Point Walter
Dalkeith
Leeuwin Barracks
Stirling Bridge
Site

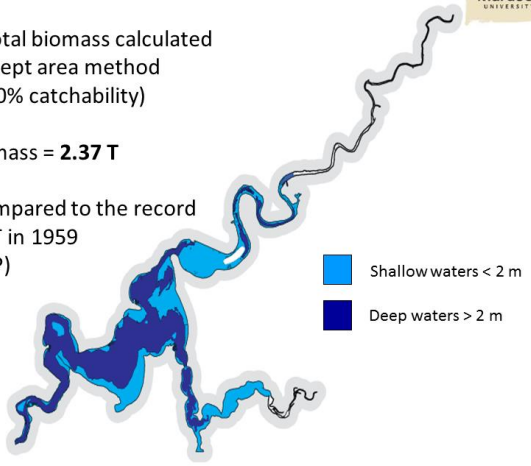
Spatial patterns in prawn abundance

- Most numerous in middle reaches of estuary
- 'Hotspots' of abundance
- Highest densities occur with accumulations of (live) macroalgal drift
- Highly mobile
- Spatial patterns different from Western King Prawn *Melicertus latisculatus*



Population biomass in 13/14

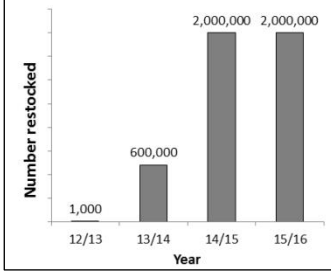
- Estimated total biomass calculated using the swept area method (assuming 40% catchability)
- Current biomass = **2.37 T**
- Very low compared to the record catch of 15 T in 1959 (WKP & WSP)



Restocking program

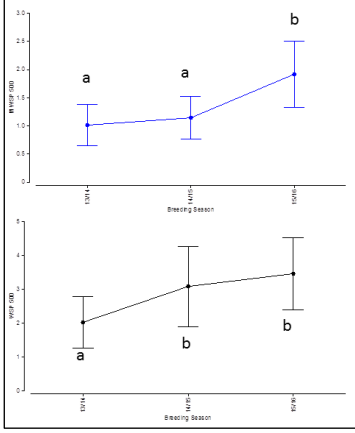
Australian Centre for Applied Aquaculture Research

- Developed culture methods
- Gravid females collected in the wild
- Post-larvae (PL10 to PL15) released



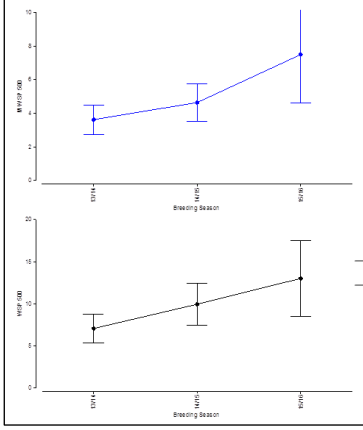


Abundance during breeding season shallows



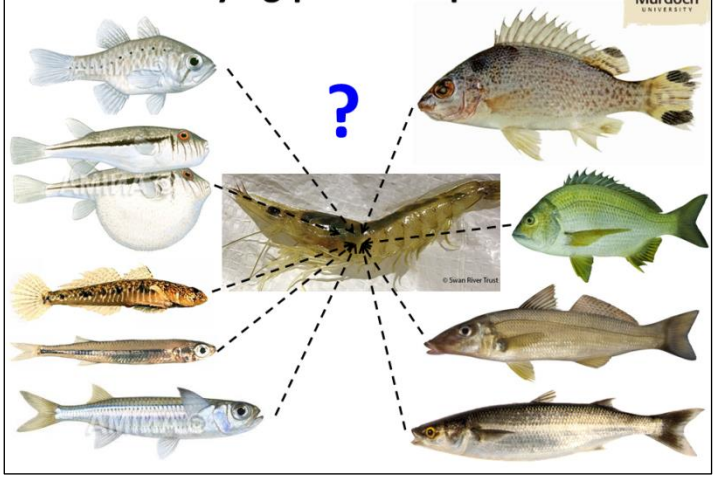
- Male: $p = 0.004$
- Female: $p = 0.029$
- All: $p = 0.008$

Abundance during breeding season deeper waters



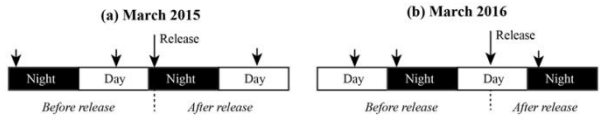
13/14	14/15	15/16	
1753	2616	3672	# WSP
	49.23	109.47%	against 13/14
		40.37%	against 14/15

Identifying potential predators

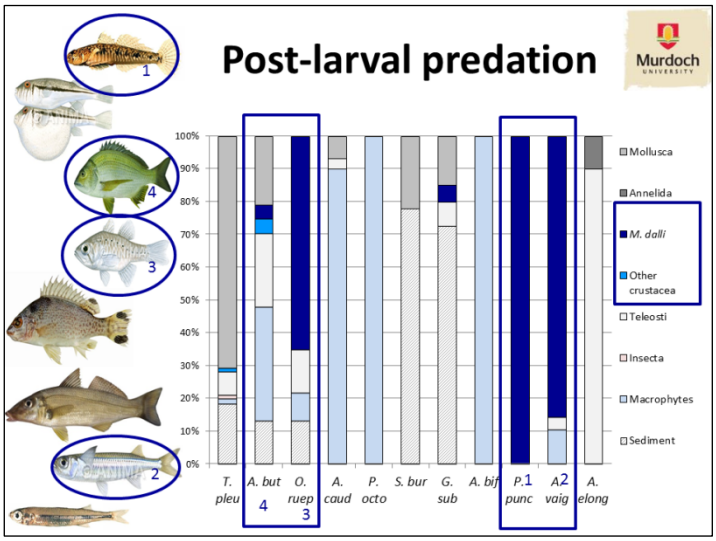


Predation experiment





Post-larval predation



Post-larval predation



Summary: Fishery implications

- **Prawn biology**
  - Estuarine species so no recruitment from outside
  - Small species (12 cm long & 16 g)
  - Short-lived, but relatively low fecundity
  - Females larger and suffer higher natural mortality
  - Move into shallows to spawn between Oct and April
- **Potential issues for discussion**
  - Fishing activity occurs during breeding season
  - Fishers select larger prawns (females)
  - Project exposure has increased fishing activity

Where to from here?



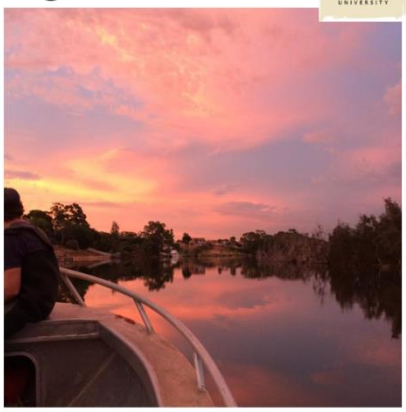
Acknowledgements

Funding bodies

- Dept. Parks & Wildlife, FRDC, RFIF/Recfishwest & Murdoch Uni.

Support

- Greg Jenkins and team at South Metro TAFE
- Dr Kerry Trayler and colleagues (DPaW)
- Recreational prawners
- The many student volunteers who assisted with sampling!



Conclusion

The Western School Prawn (*Metapenaeus dalli*) and the Western King Prawn *Penaeus* (=*Melicertus*) *latisulcatus* (a marine species) were the focus of a small commercial and iconic recreational fishery in the Swan-Canning Estuary before catches, of particularly *M. dalli*, declined significantly. Although the reasons for the decline are unclear, restocking was seen as a possible means of increasing the population size of *M. dalli* in the estuary. With this in mind the objectives of the project were to evaluate the current stock status, factors affective natural recruitment of *M. dalli*, the cost and benefits of a restocking program, devise an optimal release strategy and engage community and increase stewardship of the prawn fishery in the Swan-Canning Estuary.

The completion of an extensive monitoring program and range of laboratory and desktop studies have produced the following key findings.

- Clear spatial and temporal patterns of abundance and distribution of *M. dalli* in the Swan-Canning Estuary; including the marked seasonality in their presence in the shallow, nearshore waters during the summer breeding season (Oct-Mar) and a migration into offshore waters (> 2 m deep) during the rest of the year (autumn/winter). As *Metapenaeus dalli* were not found in the entrance channel and females were found in spawning condition within the main body of the system, confirms that this species complete its life-cycle in the Swan-Canning Estuary, unlike more northern populations (*e.g.* Shark Bay).

- Larval *M. dalli* exhibit the best growth and survival in a water temperature of 26 °C, salinity of 35 and when fed a mixed algal feed comprising the diatom *Chaetoceros muelleri* and chlorophyte *Tetraselmis suecica.*

- Both male and female *M. dalli* exhibit a highly seasonal pattern of growth, with rapid growth in late spring and summer and virtually no growth in winter. Females grow about 25% longer than males and have a higher natural mortality due to the dual demands of rapid somatic and reproductive growth.

- Reproduction can occur between October and March, but predominantly happens from November to February, when water temperatures are >18 °C.

- The post-release mortality of hatchery-reared post-larval *M. dalli* through predation by small fish species can be significant. Two key teleost predators were identified, namely the apogonid *Ostorinchus rueppellii* and the atherinid *Atherinomorous vaigiensis*. Releasing *M. dalli* during the day (rather than night) was estimated to facilitate greater survival of the post-larvae.

- *Metapenaeus dalli* was found to have a preference for fine sediments, which facilitate fast and easy burial.

- A structured, quantitative approach for evaluating the potential success of different release sites and times was developed, *i.e.* the **S**urvival **M**aximization-**A**t-**R**elease **T**ool [SMART]. Releases in the Lower Canning River and Perth Water during early to mid-summer (December to January) were predicted to lead to maximum post-release survival. This tool could readily be applied to other species.

- The preliminary bio-economic model evaluated releases ranging from 650,000 to 5 million *M. dalli* and size-at-release from 1 mm carapace length (CL) to 10 mm CL. The greatest potential returns were obtained when the 5 million prawns were released at a size of 10 mm CL, however, such an aquaculture effort would require substantial capital expenditure to produce the required number of juveniles. At the current low population level, without any restocking, the population biomass was projected to remain virtually unchanged over a five-year period.

- The findings from this research have been presented to the broader community at numerous training workshops for Prawn Watchers and community feed-back forums and promoted in the media. The quantitative biological and ecological information on *M. dalli* derived from this study and their implications for management of the *M. dalli* recreational fishery in the Swan-Canning Estuary have been discussed with representatives from Recfishwest, the Departments of Fisheries WA and Parks and Wildlife (now Department of Primary

Industries and Regional Development, Fisheries Division and Department of Biodiversity, Conservation and Attractions, Parks and Wildlife Service, respectively.

References

Abdussamad, E.M., 2008. Emigration dynamics of three species of penaeid prawn from backwaters and tidal ponds of Cochin, India. Bangladesh Journal of Fisheries Research 12, 81-88.

Abraham, J., Manisseri, M.K., 2012. Histological and morphological changes associated with ovarian development of Speckeled Shrimp *Metapenaeus monoceros* (Fabricius, 1798). Indian Journal of Fisheries 59, 119-124.

Accioly, I.V., Lima-Filho, P.A., Santos, T.L., Barbosa, A., Campos, L.B.S., Souza, J.V., 2013. Sexual dimorphism in *Litopenaeus vannamei* (Decapoda) identified by geometric morphometrics. Pan-American Journal of Aquatic Sciences 8, 276-281.

Ackermann, F., 1980. A procedure for correcting the grain size effect in heavy metal analyses of estuarine and coastal sediments. Environmental Technology Letters 1, 518-527.

Akaike, H., 1973. Maximum likelihood identification of Gaussian autoregressive moving average models. Biometrika 60, 255-265.

Aktas, M., Cavdar, N., 2012. The combined effects of salinity and temperature on the egg hatching rate, incubation time, and survival until protozoeal stages of *Metapenaeus monoceros* (Fabricius) (Decapoda: Penaeidae). Turkish Journal of Zoology 37, 249-253.

Anderson, L.G., Seijo, J.C., 2010. Bioeconomics of fisheries management. John Wiley & Sons, New Jersey, USA.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26, 32-46.

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth, UK.

Anger, K., 2003. Salinity as a key parameter in the larval biology of decapod crustaceans. Invertebrate Reproduction & Development 43, 29-45.

Arapov, J., Ezgeta-Balić, D., Peharda, M., Ninčević Gladan, Z., 2010. Bivalve feeding - how and what they eat? Ribarstvo 68, 105-116.

Araujo, M.A.V.C., Teixeira, J.C.F., Teixeira, S.F.C.F., 2010. Physical characterisation of estuarine sediments in the northern coast of Portugal. Journal of Coastal Research 26, 301-311.

Arévalo-Frías, W., Mendoza-Carranza, M., 2015. Influence of temporal and spatial factors on abundance and richness of fish early stages in shallow tropical estuaries. Environmental Biology of Fishes 98, 891-904.

Armstrong, D.P., Seddon, P.J., 2008. Directions in reintroduction biology. Trends in Ecology and Evolution 23, 20-25.

Arnott, S., Neil, D., Ansell, A., 1998. Tail-flip mechanism and size-dependent kinematics of escape swimming in the brown shrimp *Crangon crangon*. Journal of Experimental Biology 201, 1771-1784.

ASEAN, 1978. Manual on pond culture of penaeid shrimp, Ministry of Foreign Affairs. Manila, Philippines, p. 132.

Australian Bureau of Statistics, 2015. Australian Demographic Statistics. http://www.abs.gov.au/ausstats.

Ayub, Z., Ahmed, M., 2002. A description of the ovarian development stages of penaeid shrimps from the coast of Pakistan. Aquaculture Research. 33, 767-776.

Aziz, K.A., Greenwood, J.G., 1981. A laboratory investigation of temperature and salinity tolerances of juvenile *Metapenaeus bennettae* Racek and Dall (Crustacea: Penaeidae). Journal of Experimental Marine Biology and Ecology 54, 137-147.

Aziz, K.A., Greenwood, J.G., 1982. Response of juvenile *Metapenaeus bennettae* Racek and Dall, 1965 (Decapoda, Penaeidae) to sediments of differing particle size. Crustaceana 43, 121-126.

Azzurro, E., Pais, A., Consoli, P., Andaloro, F., 2007. Evaluating day–night changes in shallow Mediterranean rocky reef fish assemblages by visual census. Marine Biology 151, 2245-2253.

Bailey-Brock, J.H., Moss, S.M., 1992. Penaeid taxonomy, biology and zoogeography, in: Fast, A.W., Lester, L.J. (Eds.), Marine Shrimp Culture: Principles and Practices. Developments in in Aquaculture and Fisheries Science. Elsevier Science Publisher, The Netherlands, pp. 9-27.

Barclay, M., Dall, W., Smith, D., 1983. Changes in lipid and protein during starvation and the moulting cycle in the tiger prawn, *Penaeus esculentus* Haswell. Journal of Experimental Marine Biology and Ecology 68, 229-244.

Barko, J.W., Gunnison, D., Carpenter, S.R., 1991. Sediment interactions with submersed macrophyte growth and community dynamics. Aquatic Botany 41, 41-65.

Barnett, A., Bellwood, D.R., Hoey, A.S., 2006. Trophic ecomorphology of cardinalfish. Marine Ecology Progress Series 322, 249-257.

Baum, J.K., Worm, B., 2009. Cascading top-down effects of changing oceanic predator abundances. Journal of Animal Ecology 78, 699-714.

Beachler, M.M., Hill, D.F., 2003. Stirring up trouble? Resuspension of bottom sediments by recreational watercraft. Lake and Reservoir Management 19, 15-25.

Beck, M.W., Heck Jr, K.L., Able, K.W., Childers, D.L., Eggleston, D.B., Gillanders, B.M., Halpern, B., Hays, C.G., Hoshino, K., Minello, T.J., Orth, R.J., Sheridan, P.F., Weinstein, M.P., 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. BioScience 51, 633-641.

Becker, S.L., Finn, J.T., Danylchuk, A.J., Pollock, C.G., Hillis-Starr, Z., Lundgren, I., Jordaan, A., 2016. Influence of detection history and analytic tools on quantifying spatial ecology of a predatory fish in a marine protected area. Marine Ecology Progress Series 562.

Bell, J.D., Rothlisberg, P.C., Munro, J.L., Loneragan, N.R., Nash, W.J., Ward, R.D., Andrew, N.R., 2005. Restocking and stock enhancement of marine invertebrate fisheries. Advances in Marine Biology 49, 1-374.

Bell, J.D., Leber, K.M., Blankenship, H.L., Loneragan, N.R., Masuda, R., 2008. A new era for restocking, stock enhancement and sea ranching of coastal fisheries resources. Reviews in Fisheries Science 16, 1-9.

Bell, T.A.L., Bell, D.V.T.A., Lightner, D.V., 1988. A handbook of normal penaeid shrimp histology. World Aquaculture Society, Baton Rouge, United States.

Benaglia, T., Chauveau, D., Hunter, D.R., Young, D., 2009. Mixtools: an R package for analyzing finite mixture models. Journal of Statistical Software 32, 1-29.

Bennett, A.L., 2014. The influence of sediment compositions on the Western School Prawn *Metapenaeus dalli* in a temperate Australian estuary. Murdoch University, Perth, Australia.

Bennett, B.A., 1989. The diets of fish in three south-western Cape estuarine systems. South African Journal of Zoology 24, 163-177.

Bennett, N.J., Dearden, P., 2014. Why local people do not support conservation: community perceptions of marine protected area livelihood impacts, governance and management in Thailand. Marine Policy 44, 107-116.

Bessey, C., Heithaus, M.R., 2015. Ecological niche of an abundant teleost *Pelates octolineatus* in a subtropical seagrass ecosystem. Marine Ecology Progress Series 541, 195-204.

Bianchi, T.S., 2006. Biochemistry of Estuaries. Oxford University Press, Cary, North Carolina, USA.

Bindhuja, M.D., Gopal, C., Meenakshi, M., Revathi, K., 2013. Effect of salinity on ovarian development of Indian white shrimp *Fenneropenaeus Indicus* (H. Milne Edwards, 1837). Journal of Aquaculture in the Tropics 28, 11.

Blaber, S.J.M., 1973. Population size and mortality of juveniles of the marine teleost *Rhabdosargus holubi* (Pisces: Sparidae) in a closed estuary. Marine Biology 21, 219-225.

Blaber, S.J.M., Blaber, T.G., 1980. Factors affecting the distribution of juvenile estuarine and inshore fish. Journal of Fish Biology 17, 143-162.

Blankenship, H.L., Leber, K.M., 1995. A responsible approach to marine stock enhancement. American Fisheries Society Symposium 15, 167-175.

Boos, K., Gutow, L., Mundry, R., Heinz-Dieter, F., 2010. Sediment preference and burrowing behaviour in the sympatric brittlestars *Ophiura albida* (Forbes, 1839) and *Ophiura ophiura* (Linnaeus, 1758) (Ophiuroidea, Echinodermata). Journal of Experimental Marine Biology and Ecology 393, 176-181.

Booth, A.J., Buxton, C.D., 1997. The biology of the panga, *Pterogymnus laniarius* (Teleostei: Sparidae), on the Agulhas Bank, South Africa. Environmental Biology of Fishes 49.

Bradley, M.P., Stolt, M.H., 2005. Landscape-level seagrass–sediment relations in a coastal lagoon. Aquatic Botany 84, 121-128.

Branford, J.R., 1980. Penaeid shrimps in the Tokar Delta Region of the Red Sea, Proceedings of the symposium on the coastal and marine environment of the Res Sea, Gulf of Aden and Tropical West Indian Ocean, University of Khartoum, Sudan.

Branford, J.R., 1981. Sediment and the distribution of penaeid shrimp in the Sudanese Red Sea. Estuarine, Coastal and Shelf Science 13, 349-354.

Bravington, M., Ward, R., 2004. Microsatellite DNA markers: evaluating their potential for estimating the proportion of hatchery-reared offspring in a stock enhancement programme Molecular Ecology 13, 1287-1297.

Brewer, D.T., Blaber, S.J.M., Salini, J.P., Farmer, M.J., 1995. Feeding ecology of predatory fishes from Groote Eylandt in the Gulf of Carpentaria, Australia, with special reference to predation on penaeid prawns. Estuarine, Coastal and Shelf Science 40, 577-600.

Brito, A., 2010. Lunar cycles, catchability of penaeid shrimps and implications for the management of the shrimp fishery on Sofala Bank in Mozambique. Western Indian Ocean Journal of Marine Science 9, 75-89.

Broadley, A.D., 2014. Assessing the potential for restocking the Western School Prawn *Metapenaeus dalli* in a temperate Australian estuary Murdoch University, Perth, Australia.

Broadley, A.D., Tweedley, J.R., Loneragan, N.R., 2017. Estimating biological parameters for penaeid restocking in a temperate Australian estuary. Fisheries Research 186, 488-501.

Brown, M., Jeffrey, S., Volkman, J., Dunstan, G., 1997. Nutritional properties of microalgae for mariculture. Aquaculture 151, 315-331.

Brown, M.R., 1991. The amino-acid and sugar composition of 16 species of microalgae used in mariculture. Journal of Experimental Marine Biology and Ecology 145, 79-99.

Buckmeier, D.L., Betsill, R.K., Schlechte, J.W., 2005. Initial predation of stocked fingerling largemouth bass in a Texas reservoir and implications for improving stocking efficiency. North American Journal of Fisheries Management 25, 652-659.

Bureau of Meteorology, 2017. Australian Government, Bureau of Meteorology. http://www.bom.gov.au.

Buxton, C.D., Clarke, J.R., 1991. The biology of the white musselcracker *Sparodon durbanensis* (Pisces: Sparidae) on the Eastern Cape coast, South Africa. South African Journal of Marine Science 10, 285-296.

Cabral, R.B., Aliño, P.M., Lim, M.T., 2013. A coupled stock-recruitment-age-structured model of the North Sea cod under the influence of depensation. Ecological Modelling 253, 1-8.

Caddy, J.F., Defeo, O., 2003. Enhancing or restoring the productivity of natural populations of shellfish and other marine invertebrate resources. Food and Agriculture Organization of the United Nations, Vol. 448. Rome, Italy.

Cahu, C., Guillaume, J.C., Stephan, G., Chim, L., 1994. Influence of phospholipid and highly unsaturated fatty acids on spawning rate and egg and tissue composition in *Penaeus vannamei* fed semi-purified diets. Aquaculture 126, 159-170.

Cahu, C.L., Cuzon, G., Quazuguel, P., 1995. Effect of highly unsaturated fatty acids, α-tocopherol and ascorbic acid in broodstock diet on egg composition and development of *Penaeus indicus*. Comparative Biochemistry and Physiology Part A: Physiology 112, 417-424.

Cain, S.A., 1938. The species-area curve. The American Midland Naturalist 19, 573-581.

Callaghan, T.R., Degnan, B.M., Sellars, M.J., 2010. Expression of sex and reproduction-related genes in *Marsupenaeus japonicus*. Marine Biotechnology 12, 664-677.

Carvalho, J.C., Gomes, P., 2003. Habitat suitability model for European Wild Rabbit (*Oryctolagus cuniculus*) with implications for restocking. Game and Wildlife Science 20, 287-301.

Ch, S.B., Shailender, M., 2013. Effect of salinity and temperature on larval growth and survival of Black Tiger Shrimp *Penaeus monodon* (Fabricius) in laboratory conditions. International Journal of Bio-Pharma Research 2, 72-77.

Cha, H.K., Oh, C., Hong, S.Y., Park, K.Y., 2002. Reproduction and population dynamics of *Penaeus chinensis* (Decapoda: Penaeidae) on the western coast of Korea, Yellow Sea. Fisheries Research 56, 25–36.

Cha, H.K., Choi, J.H., Oh, C.W., 2004. Reproductive biology and growth of the Shiba Shrimp, *Metapenaeus joyneri* (Decapoda: Penaeidae), on the western coast of Korea Journal of Crustacean Biology 24, 93-100.

Chan, J.T., Sherwin, W.B., Taylor, M.D., 2014. A tool for tracking genetic contributions of wild *Penaeus* (*Melicertus*) *plebejus* broodstock to hatchery populations. Animal genetics 45, 888-892.

Chan, T.U., Hamilton, D.P., 2001. Effect of freshwater flow on the succession and biomass of phytoplankton in a seasonal estuary. Marine and Freshwater Research 52, 869-884.

Chapman, P.M., Wang, F., 2001. Assessing sediment contamination in estuaries. Environmental Toxicology and Chemistry 20, 3-22.

Chrystal, P.J., Potter, I.C., Loneragan, N.R., Holt, C.P., 1985. Age structure, growth rates, movement patterns and feeding in an estuarine population of the cardinalfish *Apogon rueppellii*. Marine Biology 85, 185-197.

Chu, K.H., Chen, Q.C., Huang, L.M., Wong, C.K., 1995. Morphometric analysis of commercially important penaeid shrimps from the Zhujiang Estuary, China. Fisheries Research 23, 83-93.

Chu, K.H., Sze, C.C., Wong, C.K., 1996. Swimming behaviour during the larval development of the shrimp *Metapenaeus ensis* (De Haan, 1844) (Decapoda, Penaeidae). Crustaceana 69, 368-378.

Chuwen, B., Platell, M., Potter, I., 2007. Dietary compositions of the sparid *Acanthopagrus butcheri* in three normally closed and variably hypersaline estuaries differ markedly. Environmental Biology of Fishes 80, 363-376.

Chuwen, B.M., Hoeksema, S.D., Potter, I.C., 2009. The divergent environmental characteristics of permanently-open, seasonally-open and normally-closed estuaries of south-western Australia. Estuarine, Coastal and Shelf Science 85, 12-21.

Clarke, A., 1987. Temperature, latitude and reproductive effort. Marine Ecology Progress Series 38, 89-99.

Clarke, K.R., Green, R.H., 1988. Statistical design and analysis for a 'biological effects' study. Marine Ecology Progress Series 46, 213-226.

Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology **18**, 117-143.

Clarke, K.R., Ainsworth, M., 1993. A method of linking multivariate community structure to environmental variables. Marine Ecology Progress Series 92, 205-219.

Clarke, K.R., Warwick, R.M., 2001. Change in marine communities: An approach to statistical analysis and interpretation, 2nd edition ed. PRIMER-E, Plymouth.

Clarke, K.R., Chapman, M.G., Somerfield, P.J., Needham, H.R., 2006. Dispersion-based weighting of species counts in assemblage analyses. Marine Ecology Progress Series 320, 11-27.

Clarke, K.R., Gorley, R.N., 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth.

Clarke, K.R., Somerfield, P.J., Gorley, R.N., 2008. Testing of null hypotheses in exploratory community analyses: Similarity profiles and biota-environment linkage. Journal of Experimental Marine Biology and Ecology 366, 56-69.

Clarke, K.R., Gorley, R.N., Somerfield, P.J., Warwick, R.M., 2014a. Change in Marine Communities: an Approach to Statistical Analysis and Interpretation, 3 ed. PRIMER-E Ltd, Plymouth, UK.

Clarke, K.R., Tweedley, J.R., Valesini, F.J., 2014b. Simple shade plots aid better long-term choices of data pre-treatment in multivariate assemblage studies. Journal of the Marine Biological Association of the United Kingdom 94, 1-16.

Clarke, K.R., Gorley, R.N., 2015. PRIMER v7: User Manual/Tutorial. PRIMER-E, Plymouth.

Clifton, K.B., Motta, P.J., 1998. Feeding morphology, diet, and ecomorphological relationships among five Caribbean labrids (Teleostei, Labridae). Copeia 1998, 953-966.

Cochrane, K.L., Garcia, S.M., 2009. A fishery manager's guidebook, 2nd Edition, in: Cochrane, K.L., Garcia, S.M. (Eds.). Wiley-Blackwell, Rome, Italy.

Coles, R.G., Greenwood, J.G., 1983. Seasonal movement and size distribution of three commercially important Australian prawn species (Crustacea: Penaeidae) within an estuarine system. Australian Journal of Marine and Freshwater Research 34, 727-743.

Coman, G.J., Crocos, P.J., 2003. Effect of age on the consecutive spawning of ablated *Penaeus semisulcatus* broodstock. Aquaculture 219, 445-456.

Commonwealth of Australia, 2002. Australian Catchment, River and Estuary Assessment 2002. National Land and Water Resources Audit, Canberra.

Conceição, L.E., Yúfera, M., Makridis, P., Morais, S., Dinis, M.T., 2010. Live feeds for early stages of fish rearing. Aquaculture Research. 41, 613-640.

Conklin, D.E., Devers, K., Shleser, R.A., 1975. Initial development of artificial diets for the lobster, *Homarus americanus*. Journal of the World Aquaculture Society 6, 235-248.

Cooper, J.A.G., 2001. Geomorphological variability among microtidal estuaries from the wave-dominated South African coast. Geomorphology 40, 99-122.

Cornell, J.H., 1961. The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. Ecology of Freshwater Fish 42, 710-723.

Correa, C., Thiel, M., 2003. Mating systems in caridean shrimp (Decapoda: Caridea) and their evolutionary consequences for sexual dimorphism and reproductive biology. Revista Chilena de Historia Natural 76, 187-203.

Costello, J.H., Collin, S.P., 1995. Flow and feeding by swimming scyphomedusae. Marine Biology 124, 399-406.

Cottingham, A., Hesp, S.A., Hall, N.G., Hipsey, M.R., Potter, I.C., 2014. Changes in condition, growth and maturation of *Acanthopagrus butcheri* (Sparidae) in an estuary reflect the deleterious effects of environmental degradation. Estuarine, Coastal and Shelf Science 149, 109–119.

Coull, B.C., Greenwood, J.G., Fielder, D.R., Coull, B.A., 1995. Subtropical Australian juvenile fish eat meiofauna: experiments with Winter Whiting *Silago maculata* and observations on other species. Marine Ecology Progress Series 125, 13-19.

Courtney, A.J., Dredge, M., Masel, J.M., 1989. Reproductive biology and spawning periodicity of endeavour shrimps *Metapenaeus endeavouri* (Schmitt, 1926) and *Metapenaeus ensis* (de Haan, 1850) from a central Queensland (Australian) fishery. Asian Fisheries Science 3, 133-147.

Courtney, A.J., Masel, J.M., Die, D.J., 1995. Temporal and spatial patterns in recruitment of three penaeid prawns in Moreton Bay, Queensland, Australia. Estuarine, Coastal and Shelf Science 41, 377-392.

Courtney, A.J., Masel, J.M., 1997. Spawning stock dynamics of two penaeid prawns, *Metapenaeus bennettae* and *Penaeus esculentus*, in Moreton Bay, Queensland, Australia. Marine Ecology Progress Series 148, 37-47.

Creighton, C., Boon, P.I., Brookes, J.D., Sheaves, M., 2015. Repairing Australia's estuaries for improved fisheries production – what benefits, at what cost? Marine and Freshwater Research 66, 493-507.

Crisp, J.A., Tweedley, J.R., D'Souza, F.M.L., Partridge, G.J., Moheimani, N.R., 2016. Larval development of the western school prawn *Metapenaeus dalli* Racek, 1957 (Crustacea: Decapoda: Penaeidae) reared in the laboratory. Journal of Natural History 50, 1699-1724.

Crisp, J.A., D'Souza, F.M.L., Tweedley, J.R., Partridge, G.J., Moheimani, N.R., 2017a. Quantitative determination of ovarian development in penaeid prawns (Decapoda: Penaeidae). Journal of Crustacean Biology 37, 81-89.

Crisp, J.A., Partridge, G.J., D'Souza, F.M.L., Tweedley, J.R., Moheimani, N.R., 2017b. Effects of temperature and salinity on larval survival and development of the western school prawn *Metapenaeus dalli*. International Aquatic Research 9, 1-10.

Crocos, P.J., Kerr, J., 1983. Maturation and spawning of the banana prawn *Penaeus merguiensis* de Man (Crustacea: Penaeidae) in the Gulf of Carpentaria, Australia. Journal of Experimental Marine Biology and Ecology 69, 37-59.

Crocos, P.J., 1987. Reproductive dynamics of the tiger prawn *Penaeus esculentus*, and a comparison with *P. semisulcatus*, in the north-western Gulf of Carpentaria, Australia. Marine and Freshwater Research 38, 91-102.

Crocos, P.J., Coman, G.J., 1997. Seasonal and age variability in the reproductive performance of *Penaeus semisulcatus* broodstock: optimising broodstock selection. Aquaculture 155, 55-67.

Crocos, P.J., Park, Y.C., Die, D.J., Warburton, K., Manson, F., 2001. Reproductive dynamics of endeavour prawns, *Metapenaeus endeavouri* and *M. ensis*, in Albatross Bay, Gulf of Carpentaria, Australia. Marine Biology 138, 63-75.

D'Souza, F.M., Loneragan, N.R., 1999. Effects of monospecific and mixed-algae diets on survival, development and fatty acid composition of penaeid prawn (*Penaeus* spp.) larvae. Marine Biology 133, 621-633.

D'Souza, F.M.L., Kelly, G.J., 2000. Effects of a diet of a nitrogen-limited alga (*Tetraselmis suecica*) on growth, survival and biochemical composition of tiger prawn (*Penaeus semisulcatus*) larvae. Aquaculture 181, 311–329.

D'Souza, F.M.L., Lecossois, D., Heasman, M.P., Diemar, J.A., Jackson, C.J., Pendrey, R.C., 2000. Evaluation of centrifuged microalgae concentrates as diets for *Penaeus monodon* Fabricius larvae. Aquaculture Research. 31, 661-670.

D'Souza, F.M.L., Knuckey, R., Hohmann, S., Pendrey, R., 2002. Flocculated microalgae concentrates as diets for larvae of the tiger prawn *Penaeus monodon* Fabricius. Aquacult. Nutr. 8, 113-120.

Dall, W., 1958. Observations on the biology of the greentail prawn, *Metapenaeus mastersii* (Haswell)(Crustacea Decapoda: Penaeidae). Marine and Freshwater Research 9, 111-134.

Dall, W., 1967. Hypo-osmoregulation in Crustacea. Comparative Biochemistry and Physiology 21, 653-678.

Dall, W., Hill, B.J., Rothlisberg, P.C., Sharple, D.J., 1990. The biology of the penaeidae. Advances in Marine Biology 27, 1-489.

Daly, B., Eckert, G.L., White, T.D., 2013. Predation of hatchery-cultured juvenile red king crabs (*Paralithodes camtschaticus*) in the wild. Canadian Journal of Fisheries and Aquatic Sciences 70, 358–366.

Dankers, P.J.T., 2002. The behaviour of fines released due to dredging – a literature review., http://www.vliz.be/en/imis?module=ref&refid=234338/adssn.

Dauvin, J.C., Ruellet, T., 2007. Polychaete/amphipod ratio revisited. Marine Pollution Bulletin 55, 215-224.

De Croos, M.D.S.T., Palsson, S., Thilakarathna, R.M.G.N., 2011. Sex ratios, sexual maturity, fecundity, and spawning seasonality of *Metapenaeus dobsoni* off the western coastal waters of Sri Lanka. Invertebrate Reproduction and Development 55, 110-123.

De Freitas, A.J., 1986. Selection of nursery areas by six southeast African Penaeidae. Estuarine and Coastal Marine Science 23, 901-908.

De Grave, S., Fransen, C., 2011. Carideorum catalogus: the recent species of the dendrobranchiate, stenopodidean, procarididean and caridean shrimps (Crustacea: Decapoda). Zoologische Mededelingen 85, 195.

De Grave, S., 2014. *Metapenaeus* Wood-Mason in Wood-Mason & Alcock, 1891a. World Register of Marine Species.

Department of Fisheries, W.A., 2017. Recreational fishing guide, Perth, Western Australia.

Department of Water Western Australia Water Information Reporting, 2017. http://wir.water.wa.gov.au/Pages/Water-Information-Reporting.aspx.

Dichmont, C.M., Deng, A.R., Punt, A.E., Venables, W., Haddon, M., 2006. Management strategies for short lived species: The case of Australia's Northern Prawn Fishery: 3. Factors affecting management and estimation performance. Fisheries Research 82, 235-245.

Dube, A., Kamusoko, R., 2013. An investigation of fish species diversity, abundance and diets of selected predator fish in Insukamini Dam, Zimbabwe. Journal of Animal Science Advances 3, 121-128.

Elzhov, T.V., Mullen, K.M., Spiess, A., Bolker, B., 2013. Minpack.lm: R interface to the Levenberg-Marquardt nonlinear least-squares algorithm found in MINPACK, R package version 1.1-8.

FAO, 1978. Manual on pond culture of penaeid shrimp. ASEAN National Coordinating Agency of the Philippines, Ministry of Foreign Affairs, Manila, Philippines.

FAO, 2007. Improving *Penaeus monodon* hatchery practices. Manual based on experience in India. FAO, Rome.

FAO, 2012. The State of World Fisheries and Aquaculture 2012. Food and Agriculture Organization of the United Nations, Rome.

FAO, 2016. The State of World Fisheries and Aquaculture 2016. Food and Agriculture Organization of the United Nations, Rome.

FAO, 2017. FishStat Plus - Universal software for fishery statistical time series data retreival. http://www.fao.org/fishery/statistics/software/fishstat/en.

Field, A., 2009. Discovering Statistics Using SPSS. SAGE Publications Inc., London, England.

Franco, A., Franzoi, P., Malavasi, S., Riccato, F., Torricelli, P., Mainardi, D., 2006. Use of shallow water habitats by fish assemblages in a Mediterranean coastal lagoon. Estuarine, Coastal and Shelf Science 66, 67-83.

Franco, A., Elliott, M., Franzoi, P., Torricelli, P., 2008. Life strategies of fishes in European estuaries: the functional guild approach. Marine Ecology Progress Series 354, 219-228.

Frank, K.T., Brickman, D., 2000. Allee effects and compensatory population dynamics within a stock complex. Canadian Journal of Fisheries and Aquatic Sciences 57, 513-517.

Friedman, G.M., Sanders, J.E., 1978. Principles of Sedimentology. Wiley, New York.

Fuss, C.M., 1964. Observations on burrowing behaviour of the pink shrimp *Penaeus duorarum*. Bulletin of Marine Science 14, 62-73.

Fuss, C.M., Ogren, L.H., 1966. Factors affecting activity and burrowing habits of the pink shrimp *Penaeus duorarum*. Biological Bulletin. 130, 170-191.

Galindo-Bect, M.S., Hernández-Ayón, J.M., Lavín, M.F., Huerta Diaz, M.A., Hinojosa, F.D., Zavala, J.A.S., 2010. Distribution of penaeid shrimp larvae and postlarvae in the upper Gulf of California. Crustaceana 83, 809-819.

Gallegos, C.L., Jordan, T.E., Hines, A.H., Weller, D.E., 2005. Temporal variability of optical properties in a shallow, eutrophic estuary: Seasonal and interannual variability. Estuarine, Coastal and Shelf Science 64, 156-170.

Gamboa-Delgado, J., Le Vay, L., 2009. Artemia replacement in co-feeding regimes for mysis and postlarval stages of *Litopenaeus vannamei*: Nutritional contribution of inert diets to tissue growth as indicated by natural carbon stable isotopes. Aquaculture 297, 128-135.

García, S., 1988. Tropical penaeid prawns, in: Gulland, J.A. (Ed.), Fish population dynamics: the implications for management. Sydney, Australia, pp. 219-249.

Gardner, M.J., Cottingham, A., Hesp, S.A., Chaplin, J.A., Jenkins, G.I., Phillips, N.M., Potter, I.C., 2013. Biological and genetic characteristics of restocked and wild *Acanthopagrus butcheri* (Sparidae) in a southwestern Australian estuary. Reviews in Fisheries Science 21, 441-453.

Garlock, T.M., Lorenzen, K., 2017. Marine angler characteristics and attitudes toward stock enhancement in Florida. Fisheries Research 186, 439-445.

Gayanilo, F.C., Sparre, P., Pauly, D., 2005. FAO-ICLARM stock assessment tools II (FiSAT II). WorldFish Center, Food and Agriculture Organization of the United Nations, Rome, Italy.

Gazeau, F., Parker, L.M., Comeau, S., Gattuso, J., O'Connor, W.A., Martin, S., Pörtner, H., Ross, P.M., 2013. Impacts of ocean acidification on marine shelled molluscs. Marine Biology 160, 1-39.

Gendron, L., Ouellet, P., 2009. Egg development trajectories of early and late-spawner lobsters (*Homarus americanus*) in the Magdalen Islands, Québec. Journal of Crustacean Biology 29, 356-363.

Gentilli, J., 1971. World Survey of Climatology. Volume 13. Climate of Australia and New Zealand. Elsevier Publishing Company., Amsterdam.

Gerritse, R.G., Wallbrink, P.J., Murray, A.S., 1998. Accumulation of phosphorus and heavy metals in the Swan-Canning Estuary, Western Australia. Estuarine, Coastal and Shelf Science 47, 165-179.

Geyer, W.R., Farmer, D.M., 1989. Tide-induced variation of the dynamics of a salt wedge estuary. Journal of Physical Oceanography 19, 1060-1072.

Gibson, R.N., Robb, L., Burrows, M.T., Ansell, A.D., 1996. Tidal, diel and longer term changes in the distribution of fishes on a Scottish sandy beach. Marine Ecology Progress Series 130, 1-17.

Gill, H.S., Potter, I.C., 1993. Spatial segregation amongst goby species within an Australian estuary, with a comparison of the diets and salinity tolerance of the two most abundant species. Marine Biology 117, 515-526.

Gill, H.S., Humphries, P., 1995. An experimental evaluation of habitat choice in three species of goby (Pisces: Gobiidae). Records of the Western Australian Museum 17, 231-233.

Glaister, J., 1978. The impact of river discharge on distribution and production of the school prawn *Metapenaeus macleayi* (Haswell)(Crustacea: Penaeidae) in the Clarence River region, northern New South Wales. Marine and Freshwater Research 29, 311-323.

Goldsmith, T.H., Fernandez, H.R., 1968. Comparative studies of crustacean spectral sensitivity. Zeitschrift für vergleichende Physiologie 60, 156-175.

Grafton, R.Q., 2006. Economics for fisheries management. Ashgate Publishing, Ltd., Aldershot, England.

Gray, C.A., Chick, R.C., McElligott, D.J., 1998. Diel changes in assemblages of fishes associated with shallow seagrass and bare sand. Estuarine, Coastal and Shelf Science 46, 849-859.

Gray, J.S., 1974. Animal-sediment relationships. Oceanography and Marine Biology: An Annual Review 12, 223-261.

Gray, J.S., Elliott, M., 2009. Ecology of Marine Sediments: From Science to Management, 2nd ed. Oxford University Press, Oxford.

Gray, P., Wess, G., 1950. The use of polyvinyl alcohol and its derivatives as microscopical mounting media. Part I. water miscible mounting media Journal of the Royal Microscopical Society 70, 287–291.

Green, C., Coco, G., 2014. Review of wave-driven sediment resuspension and transport in estuaries. Reviews of Geophysics 52, 77-117.

Grey, D.L., Dall, W., Baker, A., 1983. A guide to the Australian penaeid prawns, A guide to the Australian penaeid prawns. Northern Territory Government Printing Office, Darwin, Australia, p. 140.

Gribble, N.A., Wassenberg, T.J., Burridge, C., 2007. Factors affecting the distribution of commercially exploited penaeid prawns (shrimp) (Decapod:Penaeidae) across the northern Great Barrier Reef, Australia. Fisheries Research 85, 174-185.

Griffiths, S.P., 2001. Diel variation in the seagrass ichthyofaunas of three intermittently open estuaries in south-eastern Australia: implications for improving fish diversity assessments. Fisheries Management and Ecology 8, 123-140.

Guerin, A.J., Neil, D.M., 2015. Escape trajectories of the Rockpool Prawn (*Palaemon elegans*) in response to visual and mechanosensory stimuli. Marine and Freshwater Behaviour and Physiology 48, 145-161.

Gueroun, S.K.M., Kéfi-Daly Yahia, O., Deidun, A., Fuentes, V., Piraino, S., Daly Yahia, M.N., 2015. First record and potential trophic impact of *Phyllorhiza punctata* (Cnidaria: Scyphozoa) along the north Tunisian coast (South Western Mediterranean Sea). Italian Journal of Zoology 82, 95-100.

Guillard, R.R.L., Ryther, J.H., 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. Canadian Journal of Microbiology 8, 229-239.

Hamasaki, K., Kitada, S., 2006. A review of Kuruma Prawn *Penaeus japonicus* stock enhancement in Japan. . Fisheries Research 80, 80-90.

Hamilton, D.P., Chan, T., Robb, M.S., Pattiaratchi, C.B., Herzfeld, M., 2001. The hydrology of the upper Swan River Estuary with focus on an artificial destratification trial. Hydrological Processes 15, 2465-2480.

Hansson, L.J., Moeslund, O., Kiørboe, T., Riisgård, H.U., 2005. Clearance rates of jellyfish and their potential predation impact on zooplankton and fish larvae in a neritic ecosystem (Limfjorden, Denmark). Marine Ecology Progress Series 304, 117-131.

Harris, P.T., Heap, A.D., Bryce, S.M., Porter-Smith, R., Ryan, D.A., Heggie, D.T., 2002. Classification of Australian clastic coastal depositional environments based on a quantitative analysis of wave, tide and river power. Journal of Sedimentary Research 72, 858-870.

Hart, A.M., Fabris, F., Strain, L.W.S., Davidson, M., Brown, J., 2013a. Stock enhancement in greenlip abalone part II: population and ecological effects. Reviews in Fisheries Science 21, 310-320.

Hart, A.M., Strain, L.W.S., Fabris, F., Brown, J., Davidson, M., 2013b. Stock enhancement in greenlip abalone part I: long-term growth and survival. Reviews in Fisheries Science 21, 299-309.

Hart, A.M., Strain, L.W.S., Hesp, S.A., 2013c. Stock enhancement in greenlip abalone part III: bioeconomic evaluation. Reviews in Fisheries Science 21, 354-374.

Hartnoll, R.G., 1985. Growth, sexual maturity and reproductive output. Crustacean issues 3, 101-128.

Hassan, H., 1980. Early developmental stages of *Metapenaeus affinis* (Decapoda, Penaeidae) reared in a laboratory. ICES Journal of Marine Science 39, 30-43.

Haywood, M.D.E., Staples, D.J., 1993. Field estimates of growth and mortality of juvenile Banana Prawns (*Penaeus merguiensis*). Marine Biology 116, 407-416.

Haywood, M.D.E., 1995. Rates at which post-larval prawns are digested by a small predatory fish and the implications for predation studies. Journal of Fish Biology 47, 337-340.

Haywood, M.D.E., Vance, D.J., Loneragan, N.R., 1995. Seagrass and algal beds as nursery habitats for tiger prawns (*Penaeus semisulcatus* and *P. esculentus*) in a tropical Australian estuary. Marine Biology 122, 213-223.

Haywood, M.D.E., Heales, D.S., Kenyon, R.A., Loneragan, N.R., Vance, D.J., 1998. Predation of juvenile tiger prawns in a tropical Australian estuary. Marine Ecology Progress Series 162, 201-214.

Haywood, M.D.E., Manson, F.J., Loneragan, N.R., Toscas, P.J., 2003. Investigation of artifacts from chronographic tethering experiments—interactions between tethers and predators. Journal of Experimental Marine Biology and Ecology 290, 271-292.

Heiri, O., Lotter, A.F., Lemcke, G., 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. Journal of Paleolimnology 25, 101-110.

Hereu, B., Zabala, M., Linares, C., Sala, E., 2005. The effects of predator abundance and habitat structural complexity on survival of juvenile sea urchins. Marine Biology 146, 293-299.

Hervas, S., Lorenzen, K., Shane, M.A., Drawbridge, M.A., 2010. Quantitative assessment of a White Seabass (*Atractoscion nobilis*) stock enhancement program in California: post-release dispersal, growth and survival. Fisheries Research 105, 237-243.

Hill, B.J., 1985. Effect of temperature on duration of emergence speed of movement, and catchability of the prawn *Penaeus esculentus*, in: Rothlisberg, P.C., Hill, B.J., Staples, D.J. (Eds.), 2nd Australian National Prawn Seminar, Kooralbyn, Queensland, Australia, pp. 77-83.

Hillebrand, H., Dürselen, C.D., Kirschtel, D., Pollingher, U., Zohary, T., 1999. Biovolume calculation for pelagic and benthic microalgae. Journal of phycology 35, 403-424.

Hillman, K., McComb, A.J., Walker, D.I., 1995. The distribution, biomass and primary production of the seagrass *Halophila ovalis* in the Swan-Canning Estuary, Western Australia. Aquatic Botany 51, 1-54.

Hines, A., Johnson, E.G., Young, A.C., Aguliar, R., Kramer, M.A., Goodison, M., Zmora, O., Zohar, Y., 2008. Release strategies for estuarine species with complex migratory life cycles: stock enhancement of Chesapeake Blue Crabs (*Callinectes sapidus*). Reviews in Fisheries Science 16, 175-185.

Hines, A.H., Ruiz, G.M., 1995. Temporal variation in juvenile Blue Crab mortality: nearshore shallows and cannabilism in Chesapeake Bay. Bulletin of Marine Science 57, 884-901.

Hodgkin, E.P., Hesp, P., 1998. Estuaries to salt lakes: Holocene transformation of the estuarine ecosystems of south-western Australia. Marine and Freshwater Research 49, 183-201.

Hodson, K.P., 2016. A methodology to develop aquaculture-based release strategies with an application to *Metapenaeus dalli* in the Swan-Canning Estuary: the Survival-Maximisation-at-Release Tool (SMART). Murdoch University, Perth, Australia.

Hoeksema, S.D., Potter, I.C., 2006. Diel, seasonal, regional and annual variations in the characteristics of the ichthyofauna of the upper reaches of a large Australian microtidal estuary. Estuarine, Coastal and Shelf Science 67, 503-520.

Hoeksema, S.D., Chuwen, B.M., Potter, I.C., 2009. Comparisons between the characteristics of ichthyofaunas in nearshore waters of five estuaries with varying degrees of connectivity with the ocean. Estuarine, Coastal and Shelf Science 85, 22-35.

Hoenig, N., Hanumara, R.C., 1982. A statistical study of a seasonal growth model for fishes. Department of Computer Sciences and Statistics, University of Rhode Island, Rhode Island, USA.

Hordyk, A., Ono, K., Sainsbury, K., Loneragan, N.R., Prince, J., 2015. Some explorations of the life history ratios to describe length composition, spawning-per-recruit, and the spawning potential ratio. ICES Journal of Marine Science 72, 204-216.

Hosia, A., Granhag, L., Katajisto, T., Lehtiniemi, M., 2012. Experimental feeding rates of gelatinous predators *Aurelia aurita* and *Mnemiopsis leidyi* at low northern Baltic Sea salinity. Boreal Environmental Research 17, 473-483.

Hourston, M., Platell, M.E., Valesini, F.J., Potter, I.C., 2004. Factors influencing the diets of four morphologically divergent fish species in nearshore marine waters. Journal of the Marine Biological Association of the United Kingdom 84, 805-817.

Hourston, M., Potter, I.C., Warwick, R.M., Valesini, F.J., Clarke, K.R., 2009. Spatial and seasonal variations in the ecological characteristics of the free-living nematode assemblages in a large microtidal estuary. Estuarine, Coastal and Shelf Science 82, 309-322.

Hourston, M., Potter, I.C., Warwick, R.M., Valesini, F.J., 2011. The characteristics of the nematode faunas in subtidal sediments of a large microtidal estuary and nearshore coastal waters differ markedly. Estuarine, Coastal and Shelf Science 94, 68-76.

Howe, J.C., Wallace, R.K., Rikard, F.S., 1999. Habitat utilization by postlarval and juvenile penaeid shrimps in Mobile Bay, Alabama. Estuaries 22, 971-979.

Hufnagl, M., Huebert, K.B., Temming, A., 2013. How does seasonal variability in growth, recruitment, and mortality affect the performance of length-based mortality and asymptotic length estimates in aquatic resources? ICES Journal of Marine Science 70, 329-341.

Hughes, G.M., 1966. The dimensions of fish gills in relation to their function. Journal of Experimental Biology 45, 177-195.

Humphries, P., Potter, I.C., Loneragan, N.R., 1992. The fish community in the shallows of a temperate Australian estuary: relationships with the aquatic macrophyte *Ruppia megacarpa* and environmental variables. Estuarine, Coastal and Shelf Science 34, 325-346.

Humphries, P., 1993. A comparison of the mouth morphology of three co-occurring species of atherinid. Journal of Fish Biology 42, 585-593.

Humphries, P., Potter, I.C., 1993. Relationship between the habitat and diet of three species of atherinids and three species of gobies in a temperate Australian estuary. Marine Biology 116, 193-204.

Hutchison, M., Stewart, D., Chilcott, K., Butcher, A., Henderson, A., McLennan, M., Smith, P., 2012. Strategies to improve post release survival of hatchery reared threatened fish species. Murray–Darling Basin Authority, Canberra, p. 62.

Hyndes, G.A., Platell, M.E., Potter, I.C., 1997. Relationships between diet and body size, mouth morphology, habitat and movements of six sillaginid species in coastal waters: implications for resource partitioning. Marine Biology 128, 585-598.

Hynes, H.B.N., 1950. The food of fresh-water sticklebacks (*Gasterosteus aculeatus* and *Pygosteus pungitius*), with a review of methods used in studies of the food of fishes. Journal of Animal Ecology 19, 36–58.

Hyslop, E.J., 1980. Stomach content analysis – a review of methods and their application. Journal of Fish Biology 17, 411–439.

IBM, 2013. SPSS Statistics for Windows, Version 22.0. IBM Corp., Armonk, NY.

Jackson, C.J., Rothlisberg, P.C., 1994. Larval ecology and reproductive activity of *Metapenaeus ensis* and *M. endeavouri* in the Gulf of Carpentaria, Australia, assessed from the distribution and abundance of the protozoeal stages. Journal of Plankton Research 16, 219-231.

Jackson, C.J., Burford, M.A., 2003. The effects of temperature and salinity on growth and survival of larval shrimp *Penaeus semisulcatus* (Decapoda: Penaeoidea). Journal of Crustacean Biology 23, 819-826.

Jafri, A.W., 1997. Life history and energy relations of *Phyllorhiza punctata* (Cnidaria: Rhizostomae). Curtin University, Perth, Western Australia.

James, N.C., Cowley, P.D., Whitfield, A.K., Lamberth, S.J., 2007. Fish communities in temporarily open/closed estuaries from the warm- and cool-temperate regions of South Africa: a review Reviews in Fish Biology and Fisheries 17, 565-580.

Jenkins, G.I., Trayler, K.M., Tweedley, J.R., Stagles, I., Loneragan, N.R., 2015. Production and release of Western School Prawns into the Swan-Canning River park over a three year period to enhance the community values of the recreational prawn fishery. Western Australian Fish Foundation, Perth, Western Australia, p. 192.

Jenkins, G.I., Tweedley, J.R., Trayler, K.M., 2017. Re-establishing recreational prawning in the Swan-Canning Estuary. Western Australian Fish Foundation, Perth, Western Australia, p. 128.

Jenkins, G.P., Conron, S.D., Morison, A.K., 2010. Highly variable recruitment in an estuarine fish is determined by salinity stratification and freshwater flow: implications of a changing climate. Marine Ecology Progress Series 417, 249-261.

Jerry, D.R., Preston, N.P., Crocos, P.J., Keys, S.J., Meadows, J.R.S., Li, Y., 2004. Parentage determination of Kuruma Shrimp *Penaeus* (*Marsupenaeus*) *japonicus* using microsatellite markers (Bate). Aquaculture 235, 237-247.

Johnson, E.G., Hines, A.H., Kramer, M.A., Young, A.C., 2008. Importance of season and size of release to stocking success for the blue crab in Chesapeake Bay. Reviews in Fisheries Science 16, 243-253.

Johnston, F.D., Arlinghaus, R., Dieckmann, U., 2010. Diversity and complexity of angler behaviour drive socially optimal input and output regulations in a bioeconomic recreational-fisheries model. Canadian Journal of Fisheries and Aquatic Sciences 67, 1507-1531.

Joll, L., Penn, J., 1990. The application of high-resolution navigation systems to Leslie-DeLury depletion experiments for the measurement of trawl efficiency under open-sea conditions. Fisheries Research 9, 41-55.

Jones, D., Kumlu, M., Le Vay, L., Fletcher, D., 1997. The digestive physiology of herbivorous, omnivorous and carnivorous crustacean larvae: a review. Aquaculture 155, 285-295.

Jones, M.B., Simons, M.J., 1981. Habitat preferences of two estuarine burrowing crabs *Helice crassa* Dana (Grapsidae) and *Macrophthalmus hirtipes* (Jacquinot) (Ocypodidae). Journal of Experimental Marine Biology and Ecology 56, 49-62.

Kalejta, B., Hockey, P.A.R., 1991. Distribution, abundance and productivity of benthic invertebrates at the Berg River Estuary, South Africa. Estuarine, Coastal and Shelf Science 33, 175-191.

Kanandjembo, A.N., Platell, M.E., Potter, I.C., 2001. The benthic macroinvertebrate community of the upper reaches of an Australian estuary that undergoes marked seasonal changes in hydrology. Hydrological Processes 15, 2481-2501.

Kapoor, B.G., Khanna, B., 2004. Ichthyology Handbook, 1 ed. Springer-Verlag, Berlin.

Kenyon, R.A., Loneragan, N.R., Hughes, J.M., 1995. Habitat type and light affect sheltering behaviour of juvenile tiger prawns (Penaeus esculentus Haswell) and success rates of their fish predators. Journal of Experimental Marine Biology and Ecology 192, 87-105.

Kenyon, R.A., Loneragan, N.R., Manson, F.J., Vance, D.J., Venables, W., 2004. Allopatric distribution of juvenile Red-legged Banana Prawns (*Penaeus indicus* H. Milne Edwards, 1837) and juvenile White Banana Prawns (*Penaeus merguiensis* De Man, 1888), and inferred extensive migration, in the Joseph Bonaparte Gulf, northwest Australia. Journal of Experimental Marine Biology and Ecology 309, 79-108.

Kesarcodi-Watson, A., Kaspar, H., Lategan, M.J., Gibson, L., 2008. Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. Aquaculture 274, 1-14.

Keys, S.J., Crocos, P.J., 2006. Domestication, growth and reproductive performance of wild, pond and tank-reared Brown Tiger Shrimp *Penaeus esculentus*. Aquaculture 257, 232-240.

Khorshidian, K., 2002. Biological Characteristics of Commercially Exploited Penaeidae Shrimp (*Penaeus Semisulcatus*) in the North-western Part of the Persian Gulf.

Kienzle, M., Sterling, D.J., 2016. Rising temperatures increased recruitment of Brown Tiger Prawn (*Penaeus esculentus*) in Moreton Bay (Australia). ICES Journal of Marine Science.

King, J.E., 1948. A study of the reproductive organs of the common marine shrimp, *Penaeus setiferus* (Linnaeus). Biological Bulletin. 94, 244-262.

Kinne, O., 1963. The effects of temperature and salinity on marine and brackish water animals: I. Temperature. Oceanography and Marine Biology an Annual Review 1, 301-340.

Kinne, O., 1964. The effects of temperature and salinity on marine and brakish water animals. II. Salinity and temperature-salinity combinations. Oceanography and Marine Biology: An Annual Review 2, 281-339.

Kirkwood, G.P., Aukland, R., Zara, S.J., 2001. Length Frquency Distribution Analysis (LFDA), Version 5.0. MRAG Ltd, London, UK.

Kittaka, J., Kudo, R., Onoda, S., Kanemaru, K., Mercer, J.P., 2001. Larval culture of the European Spiny Lobster *Palinurus elephas*. Marine and Freshwater Research 52, 1439-1444.

Kittaka, J., Stevens, B., Teshima, S., Ishikawa, M., 2002. Larval culture of the king crabs *Paralithodes camtschaticus* and *P. brevipes*, in: Paul, A.J., Dawe, E.G., Elner, R., Jamieson, G.S., Kruse, G.H., Otto, R.S., Sainte-Marie, B., Shirley, T.C., Woodby, D. (Eds.), Crabs in Cold Water Regions: Biology, Management, and Economics, Anchorage, AK,, pp. 189-209.

Klumpp, D.W., Nichols, P.D., 1983. Nutrition of the Southern Sea Garfish *Hyporhamphus melanochir*: gut passage rate and daily consumption of two food types and assimilation of seagrass components. Marine Ecology Progress Series 12, 207-216.

Kompas, T., Dichmont, C.M., Punt, A.E., Deng, A., Che, T.N., Bishop, J., Gooday, P., Ye, Y., Zhou, S., 2010. Maximizing profits and conserving stocks in the Australian Northern Prawn Fishery. Australian Journal of Agricultural and Resource Economics 54, 281-299.

Kumlu, M., Eroldogan, O., Aktas, M., 2000. Effects of temperature and salinity on larval growth, survival and development of *Penaeus semisulcatus*. Aquaculture 188, 167-173.

Kumlu, M., Eroldogan, O., Aktas, M., Sağlamtimur, B., 2001. Larval growth, survival and development of *Metapenaeus monoceros* (Fabricius) cultured in different salinities. Aquaculture Research. 32, 81-86.

Kurmaly, K., Jones, D.A., Yule, A.B., East, J., 1989. Comparative analysis of the growth and survival of *Penaeus monodon* (Fabricius) larvae, from protozoea 1 to postlarva 1, on live feeds, artificial diets and on combinations of both. Aquaculture 81, 27-45.

Kutty, M.N., Murugapoopathy, G., 1968. Diurnal activity of the prawn *Penaeus semisulcatus* De Haan. Journal of the Marine Biological Association of India 10, 95-98.

Lakra, W.S., Goswami, M., Singh, A.S.P., Mishra, P., Gayathri, N., Nagpure, N.S., 2010. Genetic characterization of *Metapenaeus affinis* (H.M. Edwards, 1837) using RAPD markers. Molecular Biology Reports 37, 3757-3761.

Larkin, S.L., Harte, M., Sylvia, G., Alvarez, S., 2011. Practical considerations in using bioeconomic modelling for rebuilding fisheries, OECD Food, Agriculture and Fisheries Papers, No. 38. OECD Publishing, Paris.

Laubier-Bonichon, A., Laubier, L., 1976. Reproduction contrôlée chez la crevette Penaeus japonicus, FAO Technical Conference on Aquaculture No. FIR:AQ/Conf/76/E. 38, i-ii, , pp. 1-6.

Leber, K.M., 2002. Advances in marine stock enhancement: shifting emphasis to theory and accountability, in: Stickney, R.R., McVey, J.P. (Eds.), Responsible marine aquaculture. CABI Publishing, New York, pp. 79-90.

Leber, K.M., Kitada, S., Blankenship, H.L., Svasand, T., 2008. Stock Enhancement and Sea Ranching: Developments, Pitfalls and Apportunities. John Wiley & Sons, New Jersey.

Leber, K.M., 2013. Marine fisheries enhancement: coming of age in the new millennium, in: Christou, P., Savin, R., Costa-Pierce, B.A., Misztal, I., Whitelaw, C.B.A. (Eds.), Sustainable Food Production. Springer, New York.

Lee, B., Lee, T., 1968. Larval development of the penaeidean shrimp *Metapenaeus joyneri* (Miers) Publications of the Haewundae Marine Laboratory 1, 1-18.

Lek, E., Fairclough, D.V., Platell, M.E., Clarke, K.R., Tweedley, J.R., Potter, I.C., 2011. To what extent are the dietary compositions of three abundant, co-occurring labrid species different and related to latitude, habitat, body size and season? Journal of Fish Biology 78, 1913-1943.

Lellis-Dibble, K.A., McGlynn, K.E., Bigford, T.E., 2008. Estuarine fish and shellfish species in U.S. commercial and recreational fisheries: economic value as an incentive to protect and restore estuarine habitat. U.S. Department of Commerce, NOAA Technical Memo. NMFSF/SPO-90, p. 94.

Lemos, D., Phan, V., Alvarez, G., 2001. Growth, oxygen consumption, ammonia-N excretion, biochemical composition and energy content of *Farfantepenaeus paulensis* Pérez-Farfante (Crustacea, Decapoda, Penaeidae) early postlarvae in different salinities. Journal of experimental marine biology and ecology 261, 55-74.

Lenanton, R., Potter, I., 1987. Contribution of estuaries to commercial fisheries in temperate Western Australia and the concept of estuarine dependence. Estuaries and Coasts 10, 28-35.

Leonard, D.R.P., Clarke, K.R., Somerfield, P.J., Warwick, R.M., 2006. The application of an indicator based on taxonomic distinctness for UK marine biodiversity assessments. Journal of Environmental Management 78, 52-62.

Leong, P.K.K., Chu, K.H., Wong, C.K., 1992. Larval development of *Metapenaeus ensis* (de Haan) (Crustacea: Decapoda: Penaeidae) reared in the laboratory. Journal of Natural History 26, 1283-1304.

Lewin, W.-C., Arlinghaus, R., Mehner, T., 2006. Documented and potential biological impacts of recreational fishing: insights for management and conservation. Reviews in Fisheries Science 14, 305-367.

Liermann, M., Hilborn, R., 1997. Depensation in fish stocks: a hierarchic Bayesian meta-analysis. Canadian Journal of Fisheries and Aquatic Sciences 54, 1976-1984.

Linke, T.E., Platell, M.E., Potter, I.C., 2001. Factors influencing the partitioning of food resources among six fish species in a large embayment with juxtaposing bare sand and seagrass habitats. Journal of Experimental Marine Biology and Ecology 266, 193-217.

Linke, T.E., 2011. Trophic interactions among abundant members of the fish fauna in a permanently-open and seasonally-open estuary in south-western Australia. Murdoch University, Perth, Australia.

Liu, J.Y., 1990. Resource enhancement of Chinese Shrimp, *Penaeus orientalis*. Bulletin of Marine Science 47, 124-133.

Liu, Z.J., Cordes, J., 2004. DNA marker technologies and their applications in aquaculture genetics. Aquaculture 238, 1-37.

Loneragan, N., Potter, I., 1990. Factors influencing community structure and distribution of different life-cycle categories of fishes in shallow waters of a large Australian estuary. Marine Biology 106, 25-37.

Loneragan, N.R., Potter, I.C., Lenanton, R.C.J., Caputi, N., 1986. Spatial and seasonal differences in the fish fauna in the shallows of a large Australian estuary. Marine Biology 92, 575-586.

Loneragan, N.R., Potter, I.C., Lenanton, R.C.J., Caputi, N., 1987. Influence of environmental variables on the fish fauna of the deeper waters of a large Australian estuary. Marine Biology 94, 631-641.

Loneragan, N.R., Potter, I.C., Lenanton, R.C.J., 1989. Influence of site, season and year on contributions made by marine, estuarine, diadromous and freshwater species to the fish fauna of a temperate Australian estuary. Marine Biology 103, 461-479.

Loneragan, N.R., Kenyon, R.A., Haywood, M.D.E., Staples, D.J., 1994. Population dynamics of juvenile tiger prawns in seagrass habitats of the north-western Gulf of Carpentaria, Australia. Marine Biology 119, 133-143.

Loneragan, N.R., Wang, Y.-G., Kenyon, R.A., Staples, D.J., Vance, D.J., Heales, D.S., 1995. Estimating the efficiency of a small beam trawl for sampling tiger prawns *Penaeus esuclentus* and *P. semisulcatus* in seagrass by removal experiments. Marine Ecology Progress Series 118, 139-148.

Loneragan, N.R., Kenyon, R.A., Staples, D.J., Poiner, I.R., Conacher, C.A., 1998. The influence of seagrass type on the distribution and abundance of postlarval and juvenile tiger prawns (*Penaeus esculentus* and *P. semisulcatus*) in the western Gulf of Carpentaria, Australia. Journal of Experimental Marine Biology and Ecology 228, 175-195.

Loneragan, N.R., Bunn, S.E., 1999. River flows and estuarine ecosystems: Implications for coastal fisheries from a review and a case study of the Logan River, southeast Queensland. Austral Ecology 24, 431-440.

Loneragan, N.R., Heales, D.S., Haywood, M.D.E., Kenyon, R.A., Pendrey, R.C., Vance, D.J., 2001. Estimating the carrying capacity of seagrass for juvenile Tiger Prawns (*Penaeus semisulcatus*): enclosure experiments in high and low biomass seagrass beds. Marine Biology 139, 343-354.

Loneragan, N.R., Crocos, P.J., Barnard, R., McCulloch, R., Penn, J.W., Ward, R.D., Rothlisberg, P.C., 2004. An approach to evaluating the potential for stock enhancement of Brown Tiger Prawns (*Penaeus esculentus* Haswell) in Exmouth Gulf, Western Australia, in: Leber, K.M., Kitada, S., Blankenship, H.L., Svasand, T. (Eds.), Stock Enhancement and Sea Ranching: Developments, Pitfalls and Opportunities, 2nd Edition. Wiley-Blackwell, Oxford, UK, pp. 444-464.

Loneragan, N.R., Ye, Y., Kenyon, R.A., Haywood, M.D.E., 2006. New directions for research in prawn (=shrimp) enhancement and the use of models in providing directions for research. Fisheries Research 80, 91-100.

Loneragan, N.R., Jenkins, G.I., Taylor, M.D., 2013a. Marine stock enhancement, restocking and sea ranching in Australia: future directions and a synthesis of two decades of research and development. Reviews in Fisheries Science 21, 222-236.

Loneragan, N.R., Kangas, M., Haywood, M.D.E., Kenyon, R.A., Caputi, N., Sporer, E., 2013b. Impact of cyclones and aquatic macrophytes on recruitment and landings of Tiger Prawns *Penaeus esculentus* in Exmouth Gulf, Western Australia. Estuarine, Coastal and Shelf Science 127, 46-58.

Lorenzen, K., Garaway, C.J., 1998. How predictable is the outcome of stocking? FAO Fisheries Technical Paper 374, 133-152.

Lorenzen, K., 2000. Allometry of natural mortality as a basis for assessing optimal release size in fish-stocking programmes. Canadian Journal of Fisheries and Aquatic Sciences 57, 2374-2381.

Lorenzen, K., 2005. Population dynamics and potential of fisheries stock enhancement: practical theory for assessment and policy analysis. Philosophical Transactions of the Royal Society B: Biological Sciences 360, 171.

Lorenzen, K., Medley, P., 2006. Enhancefish manual (beta release). Imperial College, London, UK.

Lorenzen, K., 2008a. Understanding and managing enhancement fisheries systems. Reviews in Fisheries Science 16, 10-23.

Lorenzen, K., 2008b. Fish population regulation beyond "stock and recruitment": the role of density-dependent growth in the recruited stock. Bulletin of Marine Science 83, 181-196.

Lorenzen, K., Leber, K.M., Blankenship, H.L., 2010. Responsible approach to marine stock enhancement: an update. Reviews in Fisheries Science 18, 189-210.

Lorenzen, K., Beveridge, M.C., Mangel, M., 2012. Cultured fish: integrative biology and management of domestication and interactions with wild fish. Biological reviews of the Cambridge Philosophical Society 87, 639-660.

Lorenzen, K., Agnalt, A.-L., Blankenship, H.L., Hines, A.H., Leber, K.M., Loneragan, N.R., Taylor, M.D., 2013. Evolving context and maturing science: aquaculture-based enhancement and restoration enter the marine fisheries management toolbox. Reviews in Fisheries Science 21, 213-221.

Lorenzen, K., K. M. Leber and H. L. Blankenship, 2010. Responsible approach to marine stock enhancement: an update. Reviews in Fisheries Science 18, 189-210.

Lovett, D.L., Felder, D.L., 1990. Ontogenetic change in digestive enzyme activity of larval and postlarval white shrimp *Penaeus setiferus* (Crustacea, Decapoda, Penaeidae). Biological Bulletin. 178, 144-159.

Lui, H., Loneragan, N.R., 1997. Size and time of day affect the response of postlarvae and early juvenile Grooved Tiger Prawns *Penaeus semisulcatus* De Haan (Decapoda: Penaeidae) to natural and artificial seagrass in the laboratory. Journal of Experimental Marine Biology and Ecology 211, 263-277.

MacArthur, L.D., Hyndes, G.A., 2007. Varying foraging strategies of Labridae in seagrass habitats: Herbivory in temperate seagrass meadows? Journal of Experimental Marine Biology and Ecology 340, 247-258.

Macia, A., 2004. Juvenile penaeid shrimp density, spatial distribution and size composition in four adjacent habitats within a Mangrove-Fringed Bay on Inhaca Island, Mozambique. Western Indian Ocean Journal of Marine Science 3, 163-178.

Maher, J., 2002. Where have all the Swan-Canning prawns gone. Western Fisheries 2002, 20-21.

Mair, J.M., Watkins, J.L., WIlliamson, D.I., 1982. Factors affecting the immigration of post-larval penaeid shrimp into a Mexican lagoon system, Proceedings of an International Symposium on Coastal Lagoons. Oceanologica Acta, Bordeaux, France:, pp. 339-345.

Malseed, B.E., Sumner, N.R., 2001. A 12-month survey of recreational fishing in the Swan-Canning Estuary Basin of Western Australia during 1998-99. Department of Fisheries, Western Australia, Perth, Australia, p. 44.

Manning, R., 1988. Two new species of *Metapenaeopsis* (Crustacea Decapoda Penaeidae) from south western Australia. Western Australian Museum Records and Supplements 14, 91-103.

Marsden, G., Mather, P., Richardson, N., 2007. Captivity, ablation and starvation of the prawn *Penaeus monodon* affects protein and lipid content in ovary and hepatopancreas tissues. Aquaculture 271, 507-515.

Mascia, M.B., Claus, C.A., Naidoo, R., 2010. Impacts of marine protected areas on fishing communities. Conservation Biology 24, 1424-1429.

Masselink, G., Pattiaratchi, C.B., 1998. Sea breeze climatology and nearshore processes along the Perth Metropolitan coastline, Western Australia. Proceedings of the International Coastal Engineering Conference, Copenhagen, Denmark, 3165-3177.

Masuda, R., Tsukamoto, K., 1998. Stock enhancement in Japan: review and perspective. Bulletin of Marine Science 62, 337-358.

McLusky, D.S., Elliott, M., 2004. The Estuarine Ecosystem: Ecology, Threats and Management, 3rd ed. Oxford University Press, Oxford.

Meade, R.H., 1969. Landward transport of bottom sediments in estuaries of the Atlantic coastal plain. Journal of Sedimentary Petrology 39, 222-234.

Meager, J.J., Vance, D.J., Loneragan, N.R., I., W., 2003. Seasonal variation and environmental influences on juvenile Banana Prawn (*Penaeus merguiensis*) abundance in a subtropical estuary (Logan River) of eastern Australia. Estuarine, Coastal and Shelf Science 57, 569–576.

Medina, A., Vila, Y., Mourente, G., Rodríguez, A., 1996. A comparative study of the ovarian development in wild and pond-reared shrimp, *Penaeus kerathurus* (Forskål, 1775). Aquaculture 148, 63-75.

Mehanna, S.F., Al-Mamary, J., Al-Kharusi, L., 2012. Fishery characteristics and population dynamics of Indian White Shrimp, *Fenneropenaeus indicus* from Arabian Sea, Sultanate of Oman. Turkish Journal of Fisheries and Aquatic Sciences 12, 239-246.

Menasveta, P., Aranyakanonda, P., Rungsupa, S., Moree, N., 1989. Maturation and larviculture of penaeid prawns in closed recirculating seawater systems. Aquacultural Engineering 8, 357-368.

Miller, T.J., Crowder, L.B., Rice, J.A., Marschall, E.A., 1988. Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Canadian Journal of Fisheries and Aquatic Sciences 45, 1657-1670.

Minello, T.J., Zimmerman, R.J., 1983. Fish predation on juvenile brown shrimp, *Penaeus aztecus* Ives: the effect of simulated *Spartina* structure on predation rates. Journal of Experimental Marine Biology and Ecology 72, 211-231.

Mishra, P.S., Chaudhari, A., Krishna, G., Kumar, D., Lakra, W.S., 2009. Genetic diversity in *Metapenaeus dobsoni* using RAPD analysis. Biochemical Genetics 47, 421-426.

Mitchener, H., Torfs, H., 1996. Erosion of mud/ sand mixtures. Coastal Engineering 29, 1-25.

Mohamed, K., Muthu, M., Pillai, N., George, K., 1979. Larval development - *Metapenaeus monoceros* (Fabricius). Central Marine Fisheries Research Institute Bulletin 28, 50-59.

Moller, T.H., Jones, D.A., 1975. Locomotory rhythms and burrowing habits of *Penaeus semisulcatus* (de Haan) and *P. monodon* (Fabricius) (Crustacea: Penaeidae). Journal of Experimental Marine Biology and Ecology 18, 61-77.

Montgomery, S.S., 2010. Biology and Life Cycles of Prawns. New South Wales Department of Primary Industrie, Orange, Australia.

Mosha, E.J., Gallardo, W.G., 2013. Distribution and size composition of penaeid prawns, *Penaeus monodon* and *Penaeus indicus* in Saadan estuarine area, Tanzania. Ocean & Coastal Management 82, 51-63.

Motoh, H., 1985. Biology and ecology of *Penaeus monodon*, Proceedings of the First International Conference on the Culture of Penaeid Prawns/Shrimps, 4-7 December 1984, Iloilo City, Philippines. Aquaculture Department, Southeast Asian Fisheries Development Center, pp. 27-36.

Munga, C.N., Mwangi, S., Ong'anda, H., Ruwa, R., Manyala, J., Groeneveld, J.C., Kimani, E., Vanreusel, A., 2013. Species composition, distribution patterns and population structureof penaeid shrimps in Malindi-Ungwana Bay, Kenya, based on experimental bottom trawl surveys. Fisheries Research 147, 93-102.

Muthu, M., Pillai, N., George, K., 1979. Larval development - *Metapenaeus dobsoni* (Miers). Central Marine Fisheries Research Institute Bulletin 28, 30-39.

Myers, R., Barrowman, N., Hutchings, J., Rosenberg, A., 1995. Population dynamics of exploited fish stocks at low population levels. Science 269, 1106-1108.

Myers, R.A., Cadigan, N.G., 1993. Density-dependent juvenile mortality in marine demersal fish. Canadian Journal of Fisheries and Aquatic Sciences 50, 1576-1590.

Myers, R.A., Bowen, K.G., Barrowman, N.J., 1999. Maximum reproductive rate of fish at low population sizes. Canadian Journal of Fisheries and Aquatic Sciences 56, 2404-2419.

Myers, R.A., Worm, B., 2003. Rapid worldwide depletion of predatory fish communities. Nature 423, 280-283.

Nandakumar, G., Pillai, N.N., Telang, K.Y., K., B., 1989. Larval development of *Metapenaeus moyebi* (Kishinouye) reared in the laboratory. Journal of the Marine Biological Association of India. Cochin 31, 86–102.

Ochwada-Doyle, F., Loneragan, N.R., Gray, C.A., Suthers, I.M., Taylor, M.D., 2009. Complexity affects habitat preference and predation mortality in post-larval *Penaeus plebejus*: implications for stock enhancement. Marine Ecology Progress Series 380, 161-171.

Ochwada-Doyle, F., Gray, C.A., Loneragan, N.R., Suthers, I.M., Taylor, M.D., 2012. Competition between wild and captive-bred *Penaeus plebejus* and implications for stock enhancement. Marine Ecology Progress Series 450, 115-129.

Ogle, D.H., 2014. FSA: Fisheries Stock Analysis. R package version 0.4.14

Önal, H., McCarl, B.A., Griffin, W.L., Matlock, G., Clark, J., 1991. A bioeconomic analysis of the Texas shrimp fishery and its optimal management. American Journal of Agricultural Economics 73, 1161-1170.

OzCoasts, 2017. Australian Online Coastal Information. Geoscience Australia, Canberra. www.ozcoasts.gov.au.

Paine, R.T., 1966. Food web complexity and species diversity. American Naturalist 100, 65-75.

Palma, M.B., da Costa, K.G., Magalhães, A., Flores Montes, J., Pereira, L.C.C., da Costa, R.M., 2013. Zooplankton spatial and temporal distribution in an equatorial estuary (Amazon littoral, Brazil). Journal of Coastal Research 65, 1182-1187.

Parado-Estepa, F.D., 1998. Survival of *Penaeus monodon* post larvae and juveniles at different salinity and temperature levels. The Israeli Journal of Aquaculture-Bamidgeh 50, 174-183.

Park, Y., Loneragan, N.R., 1999. The effect of temperature on the activity of the endeavour prawns, *Metapenaeus endeavouri* (Schmitt) and *Metapenaeus ensis* (De Haan) (Decapoda: Penaeidae). Marine and Freshwater Research 50, 431-440.

Pasternack, G.B., Brush, G.S., 2002. Biogeomorphic controls on sedimentation and substrate on a vegetated tidal freshwater delta in upper Chesapeake Bay. Geomorphology 43, 293-311.

Patchineelam, S.M., Kjerfve, B., Gardner, L.R., 1999. A preliminary sediment budget for the Winyah Bay estuary, South Carolina, USA. Marine Geology 162, 133-144.

Pauly, D., 1983a. Length-converted catch curves: a powerful tool for fisheries research in the tropics (part 2). Fishbyte 2, 17-19.

Pauly, D., 1983b. Length-converted catch curves: a powerful tool for fisheries research in the tropics (part 1). Fishbyte 1, 9-13.

Pauly, D., 1984. Length-converted catch curves: a powerful tool for fisheries research in the tropics (III: conclusion). Fishbyte 2, 9-10.

Pauly, D., 1987. A review of the ELEFAN system for analysis of length-frequency data in fish and aquatic invertebrates. t,, International conference on the theory and application of length-based methods stock assessment. ICLARM conference proceedings, Mazzara del Vallo, Sicily, Italy, pp. 7-34.

Pauly, D., Palomares, M.L.D., 1987. Shrimp consumption by fish in Kuwait waters: a methodology, preliminary results and their implications for management and research. Kuwait. Bulletin of Marine Science 9, 101-125.

Pauly, D., 1990. Length-converted catch curves and the seasonal growth of fishes. Fishbyte 8, 33-38.

Pauly, D., Christensen, V., Guénette, S.P., T., Sumaila, U.R., Walters, C.J., Watson, R., Zeller, D., 2002. Towards sustainability in world fisheries. Nature 418, 689-695.

Pauly, S., Soriano-Bartz, M., Moreau, J., Jarre-Teichmann, A., 1992. A new model accounting for seasonal cessation of growth in fishes. Marine and Freshwater Research 43, 1151-1156.

Peixoto, S., Cavalli, R.O., D'Incao, F., Milach, Â.M., Wasielesky, W., 2003. Ovarian maturation of wild *Farfantepenaeus paulensis* in relation to histological and visual changes. Aquaculture Research. 34, 1255-1260.

Peixoto, S., Cavalli, R.O., Wasielesky, W., 2005a. Recent developments on broodstock maturation and reproduction of *Farfantepenaeus paulensis*. Brazilian Archives of Biology and Technology 48, 997-1006.

Peixoto, S., Coman, G. , Arnold, S., Crocos, P., Preston, N., 2005b. Histological examination of final oocyte maturation and atresia in wild and domesticated *Penaeus monodon* (Fabricius) broodstock. Aquaculture Research. 36, 666-673.

Penn, J.W., 1976. Tagging experiments with Western King Prawns *Penaeus latisulcatus* Kishinouye II. Estimation of population parameters. Australian Journal of Marine and Freshwater Research 27, 239-250.

Penn, J.W., 1980. Spawning and fecundity of the Western King Prawn, *Penaeus latisulcatus* Kishinouye, in western Australian waters. Marine and Freshwater Research 31, 21-35.

Perkins-Visser, E., Wolcott, T.G., Wolcott, D.L., 1996. Nursery role of seagrass beds: enhanced growth of juvenile Blue Crabs (*Callinectes sapidus* Rathbun). Journal of Experimental Marine Biology and Ecology 198, 155-173.

Pessanha, A.L.M., Araújo, F.G., 2003. Spatial, temporal and diel variations of fish assemblages at two sandy beaches in the Sepetiba Bay, Rio de Janeiro, Brazil. Estuarine, Coastal and Shelf Science 57, 817-828.

Pillai, B.R., Diwan, A., 2002. Effects of acute salinity stress on oxygen consumption and ammonia excretion rates of the marine shrimp *Metapenaeus monoceros*. Journal of Crustacean Biology 22, 45-52.

Piña, P., Voltolina, D., Nieves, M., Robles, M., 2006. Survival, development and growth of the Pacific white shrimp *Litopenaeus vannamei* protozoea larvae, fed with monoalgal and mixed diets. Aquaculture 253, 523-530.

Pinkerton, E.W., 2011. Local fisheries co-management: a review of international experiences and their implications for salmon management in British Columbia. Canadian Journal of Fisheries and Aquatic Sciences 51, 2363-2378.

Pinn, E.H., Ansell, A.D., 1993. The effect of particle size on the burying ability of the Brown Shrimp *Crangon crangon*. Journal of the Marine Biological Association of the United Kingdom 73, 365-377.

Pinto, U., Maheshwari, B.L., 2012. Impacts of water quality on the harvest of School Prawn (*Metapenaeus macleayi*) in a peri-urban river system. Journal of Shellfish Research 31, 847-853.

Platell, M.E., Potter, I.C., 2001. Partitioning of food resources amongst 18 abundant benthic carnivorous fish species in marine waters on the lower west coast of Australia. Journal of Experimental Marine Biology and Ecology 261, 31–54.

Platell, M.E., Orr, P.A., Potter, I.C., 2006. Inter- and intraspecific partitioning of food resources by six large and abundant fish species in a seasonally open estuary. Journal of Fish Biology 69, 243-262.

Poh, B., Tweedley, J.R., Trayler, K.M., Loneragan, N.R. (2018). Estimating predation rates of restocked individuals: the influence of timing-of-release on metapenaeid survival. *Fisheries Research*. 198: 165-179.

Ponce-Palafox, J., Martinez-Palacois, C.A., Ross, L.G., 1997. The effects of salinity and temperature on the growth and survival rates of juvenile white shrimp, *Penaeus vannamei*, Boone, 1931. Aquaculture 157, 107-115.

Postma, H., 1967. Sediment transport and sediment in the estuarine environment. The American Association for the Advancement of Science 83, 158-179.

Potter, I., Baronie, F., Manning, R., Loneragan, N., 1989. Reproductive biology and growth of the Western School Prown, *Metapenaeus dalli*, in a large Western Australian Estuary. Marine and Freshwater Research 40, 327-340.

Potter, I.C., Ivantsoff, W., Cameron, R., Minnard, J., 1986a. Life cycles and distribution of atherinids in the marine and estuarine waters of southern Australia. Hydrobiologia 139, 23-40.

Potter, I.C., Penn, J.W., Brooker, K.S., 1986b. Life cycle of the Western School Prawn, *Metapenaeus dalli* Racek, in a Western Australian estuary. Marine and Freshwater Research 37, 95-103.

Potter, I.C., Manning, R.J.G., Loneragan, N.R., 1991. Size, movements, distribution and gonadal stage of the Western King Prawn (*Penaeus latisulcatus*) in a temperate estuary and local marine waters. Journal of Zoology 223, 419-445.

Potter, I.C., Hyndes, G.A., 1999. Characteristics of the ichthyofaunas of southwestern Australian estuaries, including comparisons with holarctic estuaries and estuaries elsewhere in temperate Australia: a review. Austral Ecology 24, 395-421.

Potter, I.C., Chuwen, B.M., Hoeksema, S.D., Elliott, M., 2010. The concept of an estuary: a definition that incorporates systems which can become closed to the ocean and hypersaline. Estuarine, Coastal and Shelf Science 87, 497-500.

Potter, I.C., Tweedley, J.R., Elliott, M., Whitfield, A.K., 2015a. The ways in which fish use estuaries: a refinement and expansion of the guild approach. Fish and Fisheries 16, 230-239.

Potter, I.C., Warwick, R.M., Hall, N.G., Tweedley, J.R., 2015b. The physico-chemical characteristics, biota and fisheries of estuaries, in: Craig, J. (Ed.), Freshwater Fisheries Ecology. Wiley-Blackwell, pp. 48-79.

Potter, I.C., Veale, L.J., Tweedley, J.R., Clarke, K.R., 2016. Decadal changes in the ichthyofauna of a eutrophic estuary following a remedial engineering modification and subsequent environmental shifts. Estuarine, Coastal and Shelf Science 181, 345-363.

Preston, N., 1985. The combined effects of temperature and salinity on hatching success and the survival, growth, and development of the larval stages of *Metapenaeus bennettae* (Racek & Dall). Journal of Experimental Marine Biology and Ecology 85, 57-74.

Primavera, J., Lebata, J., 1995. Diel activity patterns in *Metapenaeus* and *Penaeus* juveniles. Hydrobiologia 295, 295-302.

Primavera, J., Parado-Estepa, F., Lebata, J., 1998. Morphometric relationship of length and weight of Giant Tiger Prawn *Penaeus monodon* according to life stage, sex and source. Aquaculture 164, 67-75.

Primavera, J.H., Lebata, M.J.H.L., 2000. Size and diel differences in activity patterns of *Metapenaeus ensis*, *Penaeus latisulcatus* and *P. merguiensis*. Marine and Freshwater Behaviour and Physiology 33, 173-185.

Prince, J., Potter, I., Lenanton, R., Loneragan, N., 1982. Segregation and feeding of atherinid species (Teleostei) in south-western Australian estuaries. Marine and Freshwater Research 33, 865-880.

Prince, J., Potter, I., 1983. Life-cycle duration, growth and spawning times of five species of atherinidae (Teleostei) found in a Western Australian estuary. Marine and Freshwater Research 34, 287-301.

Purcell, J.E., Uye, S., Lo, W., 2007. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: a review. Marine Ecology Progress Series 350, 153-174.

Quintero, M.E.S., Gracia, A., 1998. Stages of gonadal development in the Spotted Pink Shrimp *Penaeus brasiliensis*. Journal of Crustacean Biology 18, 680-685.

R Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Racek, A., 1957. Systematic position of the school prawn from Western Australia. Fisheries Bulletin, Western Australia 6, 1-13.

Rainer, S., 1981. Temporal patterns in the structure of macrobenthic communities of an Australian estuary. Estuarine, Coastal and Shelf Science 13, 597-620.

Ramirez Llodra, E., 2002. Fecundity and life-history strategies in marine invertebrates. Advances in Marine Biology 43, 87-170.

Rao, G.S., 1979. Larval development-*Metapenaeus brevicornis* (H. Milne Edwards). Central Marine Fisheries Research Institute Bulletin 28, 60-64.

Rao, P.V., 1973. Studies on the larval development of the commerically important penaeid prawns of India. Journal of the Marine Biological Association of India 15, 95-124.

Rao, R.K., Babu, K.R., 2013. Studies on food and feeding habits of *Mugil cephalus* (Linnaeus 1758) east coast off Andhra Pradesh, India. Canadian Journal of Pure and Applied Sciences 7, 2499-2504.

Rate, A.W., Robertson, A.E., Borg, A.T., 2000. Distribution of heavy metals in near-shore sediments of the Swan River Estuary, Western Australia. Water, Air, and Soil Pollution 124, 155-168.

Rhoads, D.C., Young, D.K., 1970. The influence of deposit-feeding organisms on sediment stability and community trophic structure. Journal of Marine Research 28, 150-178.

Rhoads, D.C., 1974. Organism-sediment relations on the muddy sea floor. Oceanography and Marine Biology: An Annual Review 12, 263-300.

Ribeiro, J., Monteiro, C.C., Monteiro, P., Bentes, L., Coelho, R., Gonçalves, J.M.S., Lino, P.G., Erzini, K., 2008. Long-term changes in fish communities of the Ria Formosa coastal lagoon (southern Portugal) based on two studies made 20 years apart. Estuarine, Coastal and Shelf Science 76, 57-68.

Říha, M., Ricard, D., Vašek, M., Prchalová, M., Mrkvička, T., Jůza, T., Čech, M., Draštík, V., Muška, M., Kratochvíl, M., Peterka, J., Tušer, M., Seďa, J., Blabolil, P., Bláha, M., Wanzenböck, J., Kubečka, J., 2015. Patterns in diel habitat use of fish covering the littoral and pelagic zones in a reservoir. Hydrobiologia 747, 111-131.

Roberts, M., 1971. Larval development of *Pagrus longlcarpus* reared in the laboratory: effects of reduced salinity on larval development. Biological Bulletin. 140, 104-116.

Roberts, S.D., Dixon, C.D., Anderacchio, L., 2012. Temperature dependent larval duration and survival of the Western King Prawn, *Penaeus (Melicertus) latisulcatus* Kishinouye, from Spencer Gulf, South Australia. Journal of Experimental Marine Biology and Ecology 411, 14-22.

Robertson, A.I., Howard, R.K., 1978. Diel trophic interactions between vertically-migrating zooplankton and their fish predators in an eelgrass community. Marine Biology 48, 207-213.

Rönnbäck, P., Macia, A., Almqvist, G., Schultz, L., Troell, M., 2001. Do penaeid shrimps have a preference for mangrove habitats? Distribution pattern analysis on Inhaca Island, Mozambique. Estuarine, Coastal and Shelf Science 55, 427-436.

Ronquillo, J.D., Saisho, T., 1993. Early developmental stages of Greasyback Shrimp, *Metapenaeus ensis* (de Haan, 1844) (Crustacea, Decapoda, Penaeidae). Journal of Plankton Research 15, 1177-1206.

Rose, K.A., Cowan, J.H., Winemiller, K.O., Myers, R.A., Hilborn, R., 2001. Compensatory density dependence in fish populations: importance, controversy, understanding and prognosis. Fish and Fisheries 2, 293-327.

Rosenthal, H., Paffenhofer, G.A., 1972. On the digestion rate and calorific content of food and faeces in young garfish. Naturwissenschaften 59, 274-275.

Ross, S.T., 1986. Resource partitioning in fish assemblages: a review of field studies. Copeia, 352-388.

Rothlisberg, P.C., 1979. Combined effects of temperature and salinity on the survival and growth of the larvae of *Pandalus jordani* (Decapoda: Pandalidae). Marine Biology 54, 125-134.

Rothlisberg, P.C., Jackson, C.J., Pendrey, R.C., 1983. Specific identification and assessment of distribution and abundance of early penaeid shrimp larvae in the Gulf of Carpentaria, Australia. Biological Bulletin. 164, 279–298.

Roy, P.S., Williams, R.J., Jones, A.R., Yassini, I., Gibbs, P.J., Coates, B., West, R.J., Scanes, P.R., Hudson, J.P., Nichol, S., 2001. Structure and function of south-east Australian estuaries. Estuarine, Coastal and Shelf Science 53, 351-384.

Ruello, N.V., 1973. Burrowing, feeding, and spatial distribution of the School Prawn *Metapenaeus macleayi* (Haswell) in the Hunter River region, Australia. Journal of Experimental Marine Biology and Ecology 13, 189-206.

Ruppert, E.E., Barnes, R.D., 1994. Invertebrate Zoology 6th edition. Saunders College Publishing, Tokyo.

Ryan, K.L., Hall, N.G., Lai, E.K., Smallwood, C.B., Taylor, S.M., Wise, B.S., 2015. State-wide survey of boat-based recreational fishing in Western Australia 2013/14, Fisheries Research Report No. 268, Department of Fisheries, Western Australia, p. 208.

Ryther, J.H., Guillard, R.R., 1962. Studies of marine planktonic diatoms: III. Some effects of temperature on respiration of five species. Canadian Journal of Microbiology 8, 447-453.

Sakaji, H., Tsuchiya, K., Segawa, S., 2000. Oocyte development of *Metapenaeopsis dalei* (Penaeidae, Decapoda, Crustacea). Invertebrate Reproduction & Development 38, 7-12.

Salini, J.P., Blaber, S.J.M., Brewer, D.T., 1990. Diets of piscivorous fishes in a tropical Australian estuary, with special reference to predation on penaeid prawns. Marine Biology 105, 363-374.

Sanchez-Jerez, P., Gillanders, B.M., Kingsford, M.J., 2002. Spatial variation in abundance of prey and diet of trumpeter (*Pelates sexlineatus*: Teraponidae) associated with *Zostera capricorni* seagrass meadows. Austral Ecology 27, 200-210.

Sanders, H.L., 1958. Benthic studies in Buzzards Bay. I. animal-sediment relationships. Limnology and Oceanography 3, 245-258.

Sanford, L.P., 1994. Wave-forced resuspension of upper Chesapeake Bay muds. Estuaries 17, 148–165.

Saoud, I.P., Davis, D.A., 2003. Salinity tolerance of Brown Shrimp *Farfantepenaeus aztecus* as it relates to post-larval and juvenile survival, distribution and growth in estuaries. Estuaries and Coasts 26, 970-974.

Sardá, R., Foreman, K., Valiela, I., 1995. Macroinfauna of a southern New England salt marsh: Seasonal dynamics and production. Marine Biology 121, 431-445.

Sarre, G.A., Potter, I.C., 1999. Comparisons between the reproductive biology of black bream *Acanthopagrus butcheri* (Teleostei: Sparidae) in four estuaries with widely differing characteristics. International Journal of Salt Lake Research 8, 179-210.

Sarre, G.A., Platell, M.E., Potter, I.C., 2000. Do the dietary compositions of *Acanthopagrus butcheri* in four estuaries and a coastal lake vary with body size and season and within and amongst these water bodies? Journal of Fish Biology 56, 103-122.

Schafer, L.N., Platell, M.E., Valesini, F.J., Potter, I.C., 2002. Comparisons between the influence of habitat type, season and body size on the dietary compositions of fish species in nearshore marine waters. Journal of Experimental Marine Biology and Ecology 278, 67-92.

Schneider, G., Behrends, G., 1998. Top-down control in a neritic plankton system by *Aurelia aurita* medusae- a summary. Ophelia 48, 71–82.

SEAFDEC, 2012. The Southeast Asian State of Fisheries and Aquaculture. Southeast Asian Fisheries Development Center, Bangkok, Thailand, p. 130.

Sheaves, M., Johnston, R., Connolly, R.M., Baker, R., 2012. Importance of estuarine mangroves to juvenile banana prawns. Estuarine, Coastal and Shelf Science 114, 208-219.

Sheridan, P.F., Trimm, D.L., Baker, B.M., 1984. Reproduction and food habits of seven species of northern Gulf of Mexico fishes. Contributions in Marine Science 27, 175-204.

Slack-Smith, R.J., 1967. The prawn fishery of Shark Bay, Western Australia. FAO.

Smith, K., Lenanton, R., Valesini, F., 2007. Preliminary survey to determine the abundance of river prawns (*Metapenaeus dalli*) in the Swan-Canning Estuary. Department of Fisheries, Western Australia, Perth, Australia, p. 11.

Smith, K.A., 2006. Review of fishery resources and status of key fishery stocks in the Swan-Canning Estuary. Department of Fisheries, Western Australia, Perth, Australia, p. 86.

Smithwick, W., Reid, K., Ensor, R., 2011. Black Water Prawning: Drag Netting in the Swan River. Arts Naked Publications, Perth, Australia.

Snelgrove, P.V.R., Butman, C.A., 1994. Animal-sediment relationships revisited: cause versus effect. Oceanography and Marine Biology: An Annual Review 32, 111-177.

Somerfield, P.J., Clarke, K.R., 2013. Inverse analysis in non-parametric multivariate analyses: distinguishing groups of associated species which covary coherently across samples. Journal of Experimental Marine Biology and Ecology 449, 261-273.

Somers, I., 1988. On a seasonally oscillating growth function. Fishbyte 6, 8-11.

Somers, I., Kirkwood, G., 1991. Population ecology of the grooved tiger prawn, *Penaeus semisulcatus*, in the north-western Gulf of Carpentaria, Australia: growth, movement, age structure and infestation by the bopyrid parasite *Epipenaeon ingens*. Marine and Freshwater Research 42, 349-367.

Somers, I.F., 1987. Sediment type as a factor in the distribution of commercial prawn species in the western Gulf of Carpentaria, Australia. Australian Journal of Marine and Freshwater Research 38, 133-149.

Somers, I.F., Crocos, P.J., Hill, B.J., 1987. Distribution and abundance of the tiger prawns *Penaeus esculentus* and *P. semisulcatus* in the north-western Gulf of Carpentaria, Australia. Marine and Freshwater Research 38, 63-78.

Spencer, R., 1956. Studies in Australian estuarine hydrology. II. The Swan River. Marine and Freshwater Research 7, 193-253.

Sporer, E., Kangas, M., Brown, S., 2013. Exmouth Gulf Prawn Managed Fishery Status Report., in: Fletcher, W.J., Santoro, K. (Eds.), Status Reports of the Fisheries and Aquatic Resources of Western Australia 2012/13: The State of the Fisheries. Department of Fisheries, Western Australia, pp. 132-138.

Staples, D., Heales, D., 1991. Temperature and salinity optima for growth and survival of juvenile Banana Prawns *Penaeus merguiensis*. Journal of Experimental Marine Biology and Ecology 154, 251-274.

Stein, R.A., Carline, R.F., Hayward, R.S., 1981. Largemouth bass predation on stocked tiger muskellunge. Transactions of the American Fisheries Society 110, 604-612.

Stephens, P.A., Sutherland, W.J., Freckleton, R.P., 1999. What is the allee effect? Oikos 87, 185-190.

Stephens, R., Imberger, J., 1996. Dynamics of the Swan River Estuary: the seasonal variability. Marine and Freshwater Research 47, 517-529.

Stevens, B.G., 1990. Temperature-dependent growth of juvenile Red King Crab (*Paralithodes camtschatica*) and its effects on size-at-age and subsequent recruitment in the eastern Bering Sea. Canadian Journal of Fisheries and Aquatic Sciences 47, 1307-1317.

Stoecker, D.K., Michaels, A.E., Davis, L.H., 1987. Grazing by the jellyfish, *Aurelia aurita*, on microzooplankton. Journal of Plankton Research 9, 901-915.

Stoner, A.W., 1983. Distributional ecology of amphipods and tanaidaceans associated with three seagrass species. Journal of Crustacean Biology 3, 505-518.

Stoner, A.W., 1991. Diel variation in the catch of fishes and penaeid shrimps in a tropical estuary. Estuarine, Coastal and Shelf Science 33, 57-69.

Støttrup, J.G., Overton, J.L., Paulsen, H., Möllmann, C., Tomkiewicz, J., Pedersen, P.B., Lauesen, P., 2008. Rationale for restocking the eastern Baltic cod stock. Reviews in Fisheries Science 16, 58-64.

Støttrup, L.G., Sparrevohn, C.R., 2007. Can stock enhancement enhance stocks? Journal of Sea Research 57, 104-113.

Subramanian, S.P., 1990. Chwaka Bay (Zanzibar, East Africa) as nursery grounds for penaeid prawns. Hydrobiologia 208, 111-122.

Sutherland, D.A., O'Neill, M.A., 2016. Hydrographic and dissolved oxygen variability in a seasonal Pacific Northwest estuary. Estuarine, Coastal and Shelf Science 172, 47-59.

Swan River Trust, 2009. Swan Canning Water Quality Improvement Plan. Swan River Trust, Perth, Western Australia, p. 114.

Tan-Fermin, J.D., Pudadera, R.A., 1989. Ovarian maturation stages of the wild Giant Tiger Prawn, *Penaeus monodon* Fabricius. Aquaculture 77, 229-242.

Taylor, M., Laffan, W.S.D., Fielder, D.S., Suthers, I.M., 2006. Key habitat and home range of Mulloway *Argyrosomus japonicas* in a south-east Australian estuary: finding the estuarine niche to optimise stocking. Marine Ecology Progress Series 328, 237-247.

Taylor, M., Ko, A., 2011. Monitoring acoustically tagged King Prawns *Penaeus* (*Melicertus*) *plebejus* in an estuarine lagoon. Marine Biology 158, 835-844.

Taylor, M.D., Suthers, I.M., 2008. A predatory impact model and targeted stock enhancement approach for optimal release of Mulloway (*Argyrosomus japonicus*). Reviews in Fisheries Science 16, 125-134.

Taylor, M.D., 2017. Preliminary evaluation of the costs and benefits of prawn stocking to enhance recreational fisheries in recruitment limited estuaries. Fisheries Research 186, Part 2, 478-487.

Taylor, M.D., Chick, R.C., Lorenzen, K., Agnalt, A.-L., Leber, K.M., Blankenship, H.L., Haegen, G.V., Loneragan, N.R., 2017a. Fisheries enhancement and restoration in a changing world. Fisheries Research 186, Part 2, 407-412.

Taylor, M.D., Fry, B., Becker, A., Moltschaniwskyj, N., 2017b. Recruitment and connectivity influence the role of seagrass as a penaeid nursery habitat in a wave dominated estuary. Science of The Total Environment.

Teng, S.K., 1971. Observations on certain aspects of the biology of *Metapenaeus brevicornis* (H. Milne-Edwards) and *Penaeus merguiensis* (de Man) in the Brunei Estuarine System. University of Singapore, Singapore.

Teske, P.R., Wooldridge, T.H., 2001. A comparison of the macrobenthic faunas of permanently open and temporarily open/closed South African estuaries. Hydrobiologia 464, 227-243.

Teske, P.R., Wooldridge, T.H., 2003. What limits the distribution of subtidal macrobenthos in permanently open and temporarily open/closed South African estuaries? Salinity vs. sediment partical size. Estuarine, Coastal and Shelf Science 57, 225-238.

Thayer, G.W., Schaaf, W.E., Angelovic, J.W., LaCroix, M.W., 1973. Caloric measurements of some estuarine organisms. Fishery Bulletin 71, 289-296.

Thomas, M., Kathirvel, M., Pillai, N., 1974. Spawning and rearing of the penaeid prawn, *Metapenaeus affinis* (H. Milne Edwards) in the laboratory. Indian Journal of Fisheries 21, 543-566.

Thompson, P.A., 2001. Temporal variability of phytoplankton in a salt wedge estuary, the Swan–Canning Estuary, Western Australia. Hydrological Processes 15, 2617-2630.

Thrush, S.F., Hewitt, J.E., Cummings, V.J., Ellis, J.I., Hatton, C., Lohrer, A.M., Norkko, A., 2004. Muddy waters: elevating sediment input to coastal and estuarine habitats. Frontiers in Ecology and the Environment 2, 299–306.

Tirmizi, N., Ul Hasan, M., Kazmi, Q., 1981. The larval development and spawning of *Metapenaeus affinis* (H. Milne Edwards) under laboratory conditions. Pak. J. Zool. 13, 141-155.

Tobias-Quinitio, E., Villegas, C.T., 1982. Growth, survival and macronutrient composition of *Penaeus monodon* Fabricius larvae fed with *Chaetoceros calcitrans* and *Tetraselmis chuii*. Aquaculture 29, 253-260.

Tomiyama, T., Watanabe, M., Kawata, G., Ebe, K., 2011. Post-release feeding and growth of hatchery-reared Japanese Flounder *Paralichthys olivaceus*: relevance to stocking effectiveness. Journal of Fish Biology 78, 1423-1436.

Trayler, K.M., Brothers, D.J., Wooller, R.D., Potter, I.C., 1989. Opportunistic foraging by three species of cormorants in an Australian estuary. Journal of Zoology 218, 87-98.

Tremblay, P.M., 1991. Interactions between two distinctly related species, Brook Trout (*Salvelinus fontinalis*) and White Sucker (*Catostomus commersoni*). Canadian Journal of Fisheries and Aquatic Sciences 48, 857-867.

Tuma, D.J., 1967. A description of the development of primary and secondary sexual characters in the Banana Prawn, *Penaeus merguiensis* de Man (Crustacea:Decapoda: Penaeidae). Australian Journal of Marine and Freshwater Research 18, 73-88.

Turner, A., Millward, G.E., Tyler, A.O., 1994. The distribution and chemical composition of particles in a macrotidal estuary. Estuarine, Coastal and Shelf Science 38, 1-17.

Tweddle, J.C., Robinson, L.D., Pocock, M.J.O., Roy, H.E., 2012. Guide to citizen science: developing, implementing and evaluating citizen science to study biodiversity and the environment in the UK, Natural History Museum and NERC Centre for Ecology & Hydrology for UK-EOF.

Tweedley, J.R., 2011. The Relationships Between Habitat Types and Faunal Community Structure in Broke Inlet, Western Australia. Murdoch University, Australia, Perth, p. 259.

Tweedley, J.R., Warwick, R.M., Valesini, F.J., Platell, M.E., Potter, I.C., 2012. The use of benthic macroinvertebrates to establish a benchmark for evaluating the environmental quality of microtidal, temperate southern hemisphere estuaries. Marine Pollution Bulletin 64, 1210-1221.

Tweedley, J.R., Warwick, R.M., Potter, I.C., 2015. Can biotic indicators distinguish between natural and anthropogenic environmental stress in estuaries? Journal of Sea Research 102, 10-21.

Tweedley, J.R., Hallett, C.S., Warwick, R.M., Clarke, K.R., Potter, I.C., 2016a. The hypoxia that developed in a microtidal estuary following an extreme storm produced dramatic changes in the benthos. Marine and Freshwater Research 67, 327-341.

Tweedley, J.R., Warwick, R.M., Potter, I.C., 2016b. The contrasting ecology of temperate macrotidal and microtidal estuaries. Oceanography and Marine Biology: An Annual Review 54, 73-171.

Tweedley, J.R., Loneragan, N.R., Crisp, J.A., Poh, B., Broadley, A.D., Bennett, A.L., Hodson, K.P., Trayler, K.M., Jenkins, G.I., Chaplin, J.A., 2017. Stock enhancement of the Western School Prawn (*Metapenaeus dalli*) in the Swan-Canning Estuary; evaluating recruitment limitation, environment and release strategies. Report for the Fisheries Research and Development Corporation, Murdoch University, Perth, Western Australia, p. 546.

Twomey, L., John, J., 2001. Effects of rainfall and salt-wedge movement on phytoplankton succession in the Swan–Canning Estuary, Western Australia. Hydrological Processes 15, 2655-2669.

Uki, N., 2006. Stock enhancement of the Japanese Scallop *Patinopecten yessoensis* in Hokkaido. Fisheries Research 80, 62-66.

Valesini, F.J., Coen, N.J., Wildsmith, M.D., Hourston, M., Tweedley, J.R., Hallett, C.S., Linke, T.E., Potter, I.C., 2009. Relationships between fish faunas and habitat type in south-western Australian estuaries. Project 2004/045. Draft Final Report for Fisheries Research and Development Corporation. Murdoch University, Perth.

Valesini, F.J., Hourston, M., Wildsmith, M.D., Coen, N.J., Potter, I.C., 2010. New quantitative approaches for classifying and predicting local-scale habitats in estuaries. Estuarine, Coastal and Shelf Science 86, 645-664.

Valesini, F.J., Tweedley, J.R., Clarke, K.R., Potter, I.C., 2014. The importance of regional, system-wide and local spatial scales in structuring temperate estuarine fish communities. Estuaries and Coasts 37, 525-547.

Vance, D.J., Staples, D.J., 1992. Catchability and sampling of three species of juvenile penaeid prawns in the Embley River, Gulf of Carpentaria, Australia. Marine Ecology Progress Series 87, 201-213.

Vance, D.J., Haywood, M.D.E., Heales, D.S., Kenyon, R.A., Loneragan, N.R., Pendrey, R.C., 1996. How far do prawns and fish move into mangroves? Distribution of juvenile Banana Prawns *Penaeus merguiensis* and fish in a tropical mangrove forest in northern Australia. Marine Ecology Progress Series 131, 115–124.

Vance, D.J., Haywood, M.D.E., Heales, D.S., Kenyon, R.A., Loneragan, N.R., 1998. Seasonal and annual variation in abundance of postlarval and juvenile Banana Prawns *Penaeus merguiensis* and environmental variation in two estuaries in tropical northeastern Australia: a six year study. Marine Ecology Progress Series 163, 21–36.

Vance, D.J., Haywood, M.D.E., Heales, D.S., Kenyon, R.A., Loneragan, N.R., Pendrey, R.C., 2002. Distribution of juvenile penaeid prawns in mangrove forests in a tropical Australian estuary, with particular reference to *Penaeus merguiensis*. Marine Ecology Progress Series 228, 165–177.

Vaseeharan, B., Rajakamaran, P., Jayaseelan, D., Vincent, A.Y., 2013. Molecular markers and their application in genetic diversity of penaeid shrimp. Aquaculture International. 21, 219-241.

Veale, L., Tweedley, J.R., Clarke, K.R., Hallett, C.S., Potter, I.C., 2014. Characteristics of the ichthyofauna of a temperate microtidal estuary with a reverse salinity gradient, including inter-decadal comparisons. Journal of Fish Biology 85, 1320-1354.

Veale, L., Coulson, P., Hall, N., Hesp, A., Potter, I.C., 2015. Age and size compositions, habitats, growth and reproductive characteristics of a terapontid (*Pelates octolineatus*) in coastal waters. Marine and Freshwater Research 66, 535-548.

Veale, L.J., Coulson, P.G., Hall, N.G., Potter, I.C., 2016. Recruitment, emigration and seasonal growth of a marine estuarine-opportunist fish species in a microtidal estuary, including comparisons with coastal waters and among decades. Marine and Freshwater Research.

Villegas, C.T., Kanazawa, A., 1979. Relationship between diet composition and growth rate of the zoeal and mysis stages of *Penaeus japonicus* Bate. Fisheries Research Journal of the Philippines 4, 32-40.

Villemey, A., Besnard, A., Grandadam, J., Eidenschneck, J., 2013. Testing restocking methods for an endangered species: effects of predator exclusion and vegetation cover on common hamster (*Cricetus cricetus*) survival and reproduction. Biological Conservation 158, 147-154.

Walters, C., Kitchell, J.F., 2001. Cultivation/depensation effects on juvenile survival and recruitment: implications for the theory of fishing. Canadian Journal of Fisheries and Aquatic Sciences 58, 39-50.

Wang, Q., Zhuang, Z., Deng, J., Ye, Y., 2006. Stock enhancement and translocation of the shrimp *Penaeus chinensis* in China. Fisheries Research 80, 67-79.

Wassenberg, T., Hill, B., 1994. Laboratory study of the effect of light on the emergence behaviour of eight species of commercially important adult penaeid prawns. Marine and Freshwater Research 45, 43-50.

Watson, E.B., Pasternack, G.B., Gray, A.B., Goñi, M., Woolfolk, A.M., 2013. Particle size characterization of historic sediment deposition from a closed estuarine lagoon, Central California. Estuarine, Coastal and Shelf Science 126, 23-33.

Webster, I., Ford, P., Hodgson, B., 2002. Microphytobenthos contribution to nutrient-phytoplankton dynamics in a shallow coastal lagoon. Estuaries 25, 540-551.

Wells, J.T., 1995. Tide-dominated estuaries and tidal rivers, in: Perillo, G.M.E. (Ed.), Developments in Sedimentology. Elsevier, Amsterdam, Netherlands, pp. 179-205.

Wentworth, C.K., 1922. A scale of grade and class terms for clastic sediments. Journal of Geology 30, 377-382.

Whittaker, R.H., Likens, G.E., 1975. The biosphere and man, in: Lieth, H., Whittaker, G.E. (Eds.), Primary Production of the Biosphere. Springer-Verlag, New York, pp. 305-328.

Wickham, D.A., 1967. Observations on the activity patterns in juveniles of the Pink Shrimp, *Penaeus duorarum*. Bulletin of Marine Science 17, 769-786.

Wickham, D.A., Minkler, F.C., 1975. Laboratory observations on daily patterns of burrowing and locomotor activity of Pink Shrimp, *Penaeus duorarum*, Brown Shrimp, *Penaeus aztecus* and White Shrimp, *Penaeus setiferus*. Contributions in Marine Science 19, 21-35.

Wildsmith, M.D., Potter, I.C., Valesini, F.J., Platell, M.E., 2005. Do the assemblages of the benthic macroinvertebrates in nearshore waters of Western Australia vary among habitat types, zones and seasons? Journal of the Marine Biological Association of the United Kingdom 85, 217-232.

Wildsmith, M.D., 2007. Relationships between benthic macroinvertebrate assemblages and habitat types in nearshore marine and estuarine waters along the lower west coast of Australia. Murdoch University, Perth.

Wildsmith, M.D., Rose, T.H., Potter, I.C., Warwick, R.M., Clarke, K.R., 2011. Benthic macroinvertebrates as indicators of environmental deterioration in a large microtidal estuary. Marine Pollution Bulletin 62, 525-538.

Williams, A.B., 1958. Substrates as a factor in shrimp distribution. Limnology and Oceanography 3, 283-290.

Wise, B., Potter, I., Wallace, J., 1994. Growth, movements and diet of the terapontid *Amniataba caudivittata* in an Australian estuary. Journal of Fish Biology 45, 917-931.

Wu, R.S.S., Lam, P.K.S., Wan, K.L., 2002. Tolerance to, and avoidance of, hypoxia by the penaeid shrimp (*Metapenaeus ensis*). Environmental Pollution 118, 351-355.

Yano, I., 1985. Induced ovarian maturation and spawning in Greasyback Shrimp, *Metapenaeus ensis*, by progesterone. Aquaculture 47, 223-229.

Ye, Y., Loneragan, N., Die, D., Watson, R., Harch, B., 2005a. Bioeconomic modelling and risk assessment of Tiger Prawn (*Penaeus esculentus*) stock enhancement in Exmouth Gulf, Australia. Fisheries Research 73, 231-249.

Ye, Y., Loneragan, N.R., Die, D.J., Watson, R.A., Harch, B., 2005b. Bioeconomic modelling and risk assessment of Tiger Prawn (*Penaeus esculentus*) stock enhancement in Exmouth Gulf, Australia. Fisheries Research 73, 231-249.

Yeoh, D.E., Valesini, F.J., Hallett, C.S., Abdo, D.A., Williams, J., 2017. Diel shifts in the structure and function of nearshore estuarine fish communities. Journal of Fish Biology.

Young, G.C., Potter, I.C., Hyndes, G.A., de Lestang, S., 1997. The ichthyofauna of an intermittently open estuary: implications of bar breaching and low salinities on faunal composistion. Estuarine, Coastal and Shelf Science 45, 53-68.

Young, G.C., Potter, I.C., 2003. Induction of annual cyclical changes in the ichthyofauna of a large microtidal estuary following an artificial and permanent increase in tidal flow. Journal of Fish Biology 63, 1306-1330.

Yousif, A., 2003. Diel variability of size and catch rate of three fish species and three penaeid prawns in the NW Red Sea trawl fishery. Fisheries Research 63, 265-274.

Zacharia, S., Kakati, V., 2004. Optimal salinity and temperature for early developmental stages of *Penaeus merguiensis* de Man Aquaculture 232, 373-382.

Zein-Elden, Z.P., Aldrich, D.V., 1965. Growth and survival of post-larval *Penaeus aztecus* under controlled conditions of temperature and salinity. Biological Bulletin. 129, 199-216.

Zein-Elden, Z.P., Renaud, M.L., 1986. Inshore environamental effects on Brown Shrimp, *Penaeus aztecus*, and White Shrimp, *P. setiferus*, populations in coastal waters, particularly of Texas. Marine Fisheries Review 48, 9-19.

Zimmerman, R.J., Minello, T.J., Zamora, G., 1984. Selection of vegetated habitat by Brown Shrimp, *Penaeus aztecus*, in a Galveston Bay salt marsh. Fishery Bulletin 82, 325-336.

Zmora, O., Findiesen, A., Stubblefield, J., Frenkel, V., Zohar, Y., 2005. Large-scale juvenile production of the Blue Crab (*Callinectes sapidus*). Aquaculture 244, 129-139.

Zohar, Y., Hines, A.H., Zmora, O., Johnson, E.G., Lipcius, R.N., Seitz, R.D., Eggleston, D.B., Place, A.R., Schott, E.J., Stubblefield, J.D., Chung, J.S., 2008. The Chesapeake Bay Blue Crab (*Callinectes sapidus*): a multidisciplinary approach to responsible stock replenishment. Reviews in Fisheries Science 16, 24-34.

Appendices

Appendix 1. Publications arising from this study

Papers

Crisp, J.A., Tweedley, J.R., D'Souza, F.M.L., Partridge, G.J., Moheimani, N.R. (2016). Larval development of *Metapenaeus dalli* (Racek) (Crustacea: Decapoda: Penaeidae) reared in the laboratory, including comparisons with other metapenaeids. *Journal of Natural History*. 50(27-28): 1699-1724. DOI: 10.1080/00222933.2016.1155669.

Broadley, A.D., Tweedley, J.R., Loneragan, N.R. (2017). Estimating biological parameters for potential penaeid restocking in a temperate estuary. *Fisheries Research*. 186(2): 488-501. DOI: 10.1016/j.fishres.2016.09.007. Special edition following the 5th International Symposium on Stock Enhancement and Sea Ranching.

Crisp, J.A., D'Souza, F.M.L., Tweedley, J.R., Partridge, G.J., Moheimani, N.R. (2017). Quantitative determination of ovarian development in penaeid prawns (Decapoda: Penaeidae). *Journal of Crustacean Biology*. 37(1): 81-89. DOI: 10.1093/jcbiol/ruw001.

Crisp, J.A., D'Souza, F.M.L., Tweedley, J.R., Partridge, G.J., Moheimani, N.R. (2017). Effects of temperature and salinity on larval survival and development of the Western School Prawn *Metapenaeus dalli* (Racek, 1957). *International Aquatic Research*. 9: 1-10. DOI: 10.1007/s40071-016-0151-0.

Crisp, J.A., D'Souza, F.M.L., Tweedley, J.R., Partridge, G.J., Moheimani, N.R. (2017). Performance of mixed species and mono-specific algal diets for culture of larval western school prawns *Metapenaeus dalli*. *Journal of the World Aquaculture Society*. DOI: 10.1111/jwas.12484.

Poh, B., Tweedley, J.R., Trayler, K.M., Loneragan, N.R. (2018). Estimating predation rates of restocked individuals: the influence of timing-of-release on metapenaeid survival. *Fisheries Research*. 198: 165-179. DOI: 10.1016/j.fishres.2017.09.019.1.

Crisp, J.A., Tweedley, J.R., D'Souza, F.M.L. Poh, B. & Loneragan, N.R. (In press). Environmental factors influencing the reproduction of an estuarine penaeid population and implications for management. *Fisheries Management and Ecology*.

Conference presentations

Bennett, A., Tweedley, J.R., Loneragan, N.R., Poh, B. (2014). Influence of sediment conditions on the decline of the Western School Prawn (*Metapenaeus dalli*) in a temperate Australian Estuary. 12th Australian Marine Science Association (AMSA) Marine Science Student Workshop 2014. Rottnest Island, Australia.

Crisp, J., Moheimani, N., Tweedley, J.R., D'Souza, F., Partridge, G. (2014). Larval development and rearing of the Western School Prawn *Metapenaeus dalli* (Racek) in the laboratory. 12th Australian Marine Science Association (AMSA) Marine Science Student Workshop 2014. Rottnest Island, Australia.

Poh, B, Tweedley, J.R., Chaplin, J.A., Loneragan, N.R., Trayler, K.M. (2014). Monitoring the restocking of the Western School Prawn (*Metapenaeus dalli*) in the Swan-Canning Estuary. 12th Australian Marine Science Association (AMSA) Marine Science Student Workshop 2014. Rottnest Island, Australia.

Tweedley. J.R., Loneragan, N.R., Jenkins, G., Trayler, K.M., Chaplin, J.A. (2014). Assessing the decline in western school prawn (*Metapenaeus dalli*) abundance in a temperature Australian estuary: evaluating the effectiveness of prawn releases and the impacts of environmental change. 2014 Annual Symposium of the Fisheries Society of the British Isles. Hull, United Kingdom.

Broadley, A., Tweedley, J.R., Loneragan, N.R. (2015). Bio-economic evaluation of restocking the Western School Prawn (*Metapenaeus dalli*) in an Australian estuary. 5th International Symposium on Stock Enhancement and Sea Ranching. Sydney, Australia.

Trayler, K., Onton, K., Giroud, M., Smith, K. (2015). Snapshots of Citizen Science in the Swan Canning. National Estuaries Network Forum: Mini-Workshop on Barriers and Bridges to Citizen Science in Estuaries: Managing volunteers, data, expectations and outcomes. Perth, Australia.

Trayler, K., Tweedley, J.R., Jenkins, G.I. (2015). "Prawn Watch: Engaging fishers in science, restocking and sustainable management of an iconic species that has declined in abundance. Australian Citizen Science Conference: Maximising the Capacity of Citizen Science for Science and Society. Canberra, Australia.

Trayler, K., Giroud, M., Shedwari, G. (2015). What makes good citizen science projects 2015 Department of Parks and Wildlife Conference. Perth, Australia.

Tweedley, J.R., Poh, B., Broadley, A.D., Crisp, J.A, Loneragan, N.R. (2015). Understanding the ecology of the Western School Prawn to maximize restocking success. 5th International Symposium on Stock Enhancement and Sea Ranching. Sydney, Australia.

Crisp, J.A., Tweedley, J.R., D'Souza, F.M.L., Partridge, G.J., Moheimani, N.R. (2016). Reproductive factors affecting the culturing and restocking of the Western School Prawn Metapenaeus dalli. Asian-Pacific Aquaculture 2016. Surabaya, Indonesia.

Crisp, J.A., Tweedley, J.R., D'Souza, F.M.L., Partridge, G.J., Moheimani, N.R. (2016). Reproductive factors affecting the aquaculture of the Western School Prawn. 14th Australian Marine Science Association (AMSA) Marine Science Student Workshop 2016. Rottnest Island, Australia.

Poh, B., Tweedley, J.R., Loneragan, N.R. (2016). Maximising restocking success by reducing predation. Australian Marine Science Association (AMSA) Marine Snapchat. Perth, Australia.

Poh, B., Tweedley, J.R., Trayler, K.M., Loneragan, N.R. (2017). Calculation of predation rates of restocked individuals: the influence of timing-of-release on survival. 15th Australian Marine Science Association (AMSA) Marine Science Student Workshop 2017. Rottnest Island, Australia.

Crisp, J.A., D'Souza, F.M. L., Partridge, G.J., Tweedley, J.R., Moheimani, N.R. (2017). Developing larval aquaculture practices for restocking the Western School Prawn Metapenaeus dalli. 15th Australian Marine Science Association (AMSA) Marine Science Student Workshop 2017. Rottnest Island, Australia.

Conference posters

Bennett, A., Tweedley, J.R., Loneragan, N.R., Poh, B. (2014).The influence of sediment conditions on the decline of the western school prawn (*Metapenaeus dalli*) in a temperate Australian estuary. 2014 Annual Symposium of the Fisheries Society of the British Isles. Hull, United Kingdom.

Broadley, A., Tweedley, J.R., Poh, B., Loneragan, N.R. (2014). Estimation of biological parameters for evaluating the success of western school prawn (*Metapenaeus dalli*) releases in a temperate Australian estuary. 2014 Annual Symposium of the Fisheries Society of the British Isles. Hull, United Kingdom.

Broadley, A., Tweedley, J.R., Loneragan, N.R. (2015). Estimation of biological parameters for the bio-economic evaluation of restocking the Western School Prawn (*Metapenaeus dalli*) in an Australian estuary. 5th International Symposium on Stock Enhancement and Sea Ranching. Sydney, Australia.

Bennett, A.L., Tweedley, J.R., Poh, B., Loneragan, N.R. (2015). Assessing the sediment preference of a penaeid prawn to inform release strategies. 5th International Symposium on Stock Enhancement and Sea Ranching. Sydney, Australia.

Theses

Bennett, A.L. (2014). The influence of sediment compositions on the Western School Prawn *Metapenaeus dalli* in a temperate Australian estuary. Honours Thesis, Murdoch University. *Awarded 1st class.*

Broadley A.D. (2014). Assessing the potential for restocking the Western School Prawn *Metapenaeus dalli* in a temperate Australian estuary. Honours Thesis, Murdoch University. *Awarded 1st class.*

Hogan-West, K. (2015). Biology and ecology of the non-indigenous goby *Acentrogobius pflaumii* (Bleeker 1853) in the Swan-Canning Estuary. Honours Thesis, Murdoch University. *Not directly related to this project, however, the samples of* A. pflaumii *were collected during the sampling regime for* Metapenaeus dalli. *Awarded 1st class.*

Hodson, K. (2016). A methodology to develop aquaculture-based release strategies with an application to *Metapenaeus dalli* in the Swan-Canning Estuary: the Survival-Maximisation-at-Release Tool. Honours Thesis, Murdoch University. *Awarded 1st class.*

Crisp, J.A. (Submitted in 2017). Aspects of reproduction, larval development and rearing for the aquaculture-based enhancement of the Western School Prawn Metapenaeus dalli. *PhD Thesis, Murdoch University.*

*Poh, B. (In preparation due to be submitted in 2018). Evaluating the population genetics, recruitment limitations and release strategies of Western School Prawns (*Metapenaeus dalli*) during a restocking program in the Swan-Canning Estuary. PhD Thesis, Murdoch University.*

Independent Study Contracts

Hodson, K.P. (2015). Determining which fish species in the Swan-Canning Estuary predate on restocked post-larval Western School Prawns. Awarded High distinction.

Richards, S. (2016). Maximising survival of restocked prawns in the Swan-Canning Estuary. Awarded High distinction.

Appendix 2. Introduced goby *Acentrogobius pflaumii*

During the monitoring program for the Western School Prawns relatively large catches of an introduced goby native to Japan, the Striped Sand Goby *Acentrogobius pflaumii* were recorded. Samples of *A. pflaumii* were retained and a study on the biology and ecology of this species undertaken. The abstract from this work is provided below.

Keyley Hogan-West Honours Thesis: Biology and ecology of the non-indigenous goby *Acentrogobius pflaumii* (Bleeker 1853) in the Swan-Canning Estuary

Abstract

Non-indigenous species can have significant deleterious impacts on the ecosystems in which they become established. Following the recent establishment of the Striped Sandgoby *Acentrogobius pflaumii* in the Swan-Canning Estuary, south-western Australia, a study was initiated to determine its spatial and temporal distribution and biological characteristics. Although *A. pflaumii* was not recorded in the coarse sandy sediment present in the nearshore, shallow waters of the estuary, substantial numbers were recorded on soft muddy sediments in the deeper waters, where it comprised 55% of the total number of gobies. While *A. pflaumii* dominated the gobiid fauna in Lower Melville Water (~98%), its contributions declined progressively upstream, indicating a preference for waters with a salinity close to that of full strength sea water. Size and age compositions determined that the oldest individual was 3.9 years old and 89 mm in total length, but that the population is dominanted by 1+ individuals. Population mortality and turn-over rates are therefore likely to be very high. Both males and females attained > 87% of their asymptotic lengths (L_∞) of 74.9 and 69.3 mm, respectively, during the first year of life, which is characteristic of smaller, shorter-lived species of fish. The results from gonadosomatic indices and the histological examination of gonads suggest that *A. pflaumii* is able to spawn throughout most of the year, with a peak from November to February. The presence of mature, spawning and depleted gonads in *A. pflaumii* suggests that this species spawns within the Swan-Canning Estuary. *Acentrogobius pflaumii* can be thus considered an estuarine & marine species like *Favonigobius lateralis*. As *A. pflaumii* attains

high densities over a relatively large part of the estuary and can breed within the system, it is likely to be a permanent resident and further work is needed to determine its impact on the native gobiid fauna.

A full copy of the thesis can be freely downloaded from the Murdoch University Research Repository at http://researchrepository.murdoch.edu.au/29594/.



Photographs: (Top) Will Smithwick and Kevin Reid about to prawn. (Bottom) staff from the partner organisations the former Western Australian Minister for Fisheries the Hon. Ken Baston inspecting the resultant catch. Photos taken by Parks and Wildlife Service, DBCA.