Development of biotic indices for establishing and monitoring ecosystem health of the Swan-Canning Estuary

FINAL TECHNICAL REPORT

December 2011

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Prepared for the Swan River Trust, Department of Water and Department of Fisheries

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Chapter 1. General Introduction

The Swan-Canning Estuary is highly valued for its ecological, recreational, commercial and indigenous importance (*e.g.* Seddon 1972, Swan River Trust 2008, 2009). It supports a diverse range of fish species (several of which complete their life cycles in the system and/or are recreationally or commercially important, *e.g.* Loneragan *et al.* 1989, Kanadjembo *et al.* 2001, Hoeksema and Potter 2006), migratory and resident waterbirds (Bamford *et al.* 2003), submerged and fringing vegetation (*e.g.* Hillman *et al.* 1995, Astill and Lavery 2001, McMahon 2001) and a dolphin population (Lo 2009).

The Swan-Canning Estuary and its large (*ca* 125 000 km^2) catchment have been subjected to substantial anthropogenic change since European settlement in the early to mid 1800s, and the system is now classified as highly modified (Commonwealth of Australia 2002). These artificial modifications, combined with the ongoing effects of local population growth and climate change, continue to have a wide range of implications for the water quality of this system. For example, reduced river flow due to damming or diversion of the major tributaries and the effects of climate change, increased tidal exchange through widening and deepening of the estuary mouth and extensive clearing of catchment vegetation, have all contributed to rising salinity throughout this system (Hamilton *et al.* 2001, Thomson *et al.* 2001, Chan *et al.* 2002, CSIRO 2009). Changes in the volumes of marine *vs* riverine flow have also exacerbated the stratification of salinity and dissolved oxygen concentration within the water column, particularly in the upper estuarine reaches where bottom waters become hypoxic during drier periods of the year (Hamilton *et al.* 2001, Thomson *et al.* 2001, http://www.swanrivertrust.wa.gov.au/science/river/Content/plots.aspx). This lack of dissolved oxygen has become so extensive that remedial oxygenation of both the Swan and Canning rivers is now undertaken mechanically (http://www.swanrivertrust.wa.gov.au/science/river/content/oxygenation.aspx). Widespread land clearing, shoreline modification and the growth of surrounding urban and agricultural activity have also resulted in increased surface runoff from the catchment, and thus also of the sediment, nutrient and pollutant loads entering the estuary. These loadings have also risen due to the vast network of drains servicing residential, farming and industrial areas that discharge into the system, and their impacts are further compounded by the reduced flushing of the estuary due to diminishing rainfall (Jakowyna *et al.* 2000, Swan River Trust 2003, 2009, Foulsham 2009). The system, and particularly its upper reaches, is now considered to be eutrophic to hypereutrophic (Swan River Trust 2009), and the levels of various non-nutrient contaminants in the sediment exceed ANZECC and ARMCANZ Interim Sediment Quality Guideline Trigger Values at several locations throughout the estuary (Nice 2009).

The above environmental changes have numerous implications for the biota of the Swan-Canning Estuary. One of the more obvious biotic responses, especially since the early 1990s, has been an increase in the frequency and density of large phytoplankton blooms, particularly in the upper estuarine reaches (Twomey and John 2001, Chan *et al.* 2002, Swan River Trust 2005). Although such blooms may provide a greater direct food source for primary and

subsequently secondary consumers, they may also have a range of adverse effects on biota such as fish, including asphyxiation, poisoning or reductions in their ability to visually locate prey (*e.g.* Potter *et al.* 1983, Lenanton *et al.* 1985, Steckis *et al.* 1995, Deeds *et al.* 2002). Several of the phytoplankton blooms in the upper Swan-Canning Estuary since the 1990s have been associated with fish kills, with those in autumn-winter 2003 and autumn 2006 being among the largest in recent years, resulting in estimated losses of 150 000 and 235 000 fish, respectively (*e.g.* Valesini *et al.* 2005). The increasing prevalence of hypoxic conditions may also have a range of other direct and indirect effects on estuarine biota, such as a reduction in the abundance, diversity and/or biomass of benthic macroinvertebrates, which are the preferred prey of many fish species (*e.g.* Sarre *et al.* 2000, Kanandjembo *et al.* 2001a) and waterbirds (Department of Conservation and Land Management 1999) in the Swan-Canning Estuary.

The influence of the above environmental pressures on the Swan-Canning Estuary led to this nationally-significant system being identified as a "coastal hotspot" by the Australian Government in 2006 (Australian Government 2006). This recognition mirrors the growing concern from the wider community about the environmental health of this system. To address these concerns, several major initiatives have been launched by various levels of government, in conjunction with local management agencies, researchers and community groups, to develop a greater understanding of the environmental problems impacting the system and thus ways of improving its environmental quality. Over the last decade or so, these initiatives have included (i) the Swan-Canning Cleanup Program (SCCP), which commenced in 1994 and resulted in an Action Plan that was implemented in 1999 (Swan River Trust 1999), (ii) Riverplan (Government of Western Australia 2004), which aimed to extend the SCCP and provide a framework for instigating the now defunct *Environmental Protection (Swan and Canning Rivers) Policy 1998* (EPP), (iii) the *Swan and Canning Rivers Management Act 2006*, which replaced the EPP and called for the development of a River Protection Strategy (RPS) and (iv) the Healthy Rivers Action Plan (HRAP; Swan River Trust 2008), which forms part of the RPS and has developed programs for the improvement of water quality (*i.e.* the Swan-Canning Water Quality Improvement Plan [Swan River Trust 2009]), reducing levels of nutrient and non-nutrient contaminants entering the estuary (*i.e.* the Non-Nutrient Contaminants Program [Evans 2009, Foulsham *et al.* 2009, Nice 2009, Nice *et al.* 2009]) and foreshore rehabilitation.

Despite the environmental problems experienced by the Swan-Canning Estuary, and the above-mentioned initiatives to improve them, managers from the major responsible agencies still do not have a reliable, simple and affordable method for (i) evaluating, quantitatively, the "ecological health" of the estuary relative to appropriate reference conditions, (ii) tracking changes in ecological health over time and detecting whether it is likely to deteriorate, or has deteriorated, beyond acceptable limits and (iii) identifying those environmental stressors which are most responsible for changes in ecosystem health. The development of such a method would thus greatly inform the type of management responses needed to mitigate further environmental decline of the Swan-Canning Estuary, and provide an effective way of readily conveying the ecological status of this complex system to the wider community. Note

that, for the purposes of this document, the term "ecological health" is considered to be interchangeable with other similar terms that have variably been used in the relevant literature, such as "ecological condition", "ecological integrity" "or "ecological quality".

Multimetric biotic indices integrate measurements of a suite of characteristics (metrics) of a given community into a single index that is diagnostic of broader ecological health. In doing so, they aim to distil the complex workings of an ecosystem into an easily interpretable signal that quantifies the status of ecosystem structure and function. The main premise underlying these indicators is that any particular community collectively responds to many aspects of their environment, given that they typically comprise species that differ in their physiological tolerances, habitats, trophic levels and life-history stages (Harrison and Whitfield 2004, 2006). Thus, indices constructed from a combination of community metrics are expected to reflect the ecological impacts of a wide array of environmental stressors, ranging from those that are highly localised to those that are diffuse and often difficult to measure (Harrison and Whitfield 2004). By assessing measurements of these metrics against "reference conditions" that are relevant to the community and system of interest, the extent to which ecosystem condition deviates from a "best-attainable" state can be quantified, as can its trends over space and time. Such indices have provided an effective method for evaluating the ecological quality of estuaries in South Africa, Europe and the USA (*e.g.* Deegan *et al.* 1997, Hughes *et al.* 2002, Harrison and Whitfield 2004), and have become such important management tools that they are now required, by legislation, for the environmental management of coastal and transitional waters in those countries, *e.g.* South Africa's National Water Act of 1998 (DWAF 1998), the European Water Framework Directive (European Communities 2000) and the United States Clean Water Act (US Clean Water Act 2002). Biotic indices also have an important advantage over the physico-chemical (*e.g.* water quality) indicators that are often used in estuarine management, in that they provide a measure which directly reflects the ecological consequences of environmental change, rather than one that is indirect and often fails to capture the complexity of the estuarine environment. Fish communities provide particularly good indicators of estuarine health, and their numerous advantages over other biotic groups have been detailed by Whitfield and Elliott (2002) and Harrison and Whitfield (2004).

Numerous workers have also developed biotic indices from the characteristics of a particular species in order to gauge ecosystem health. Although single species are often less effective than communities in reflecting the full suite of environmental stressors impacting on a system, changes in their abundance, distribution and biological characteristics (*e.g.* growth, age, reproduction and diet) can be very informative indicators of ecosystem structure and function. This is particularly so if that species spends its entire life in the ecosystem, is relatively long-lived and, arguably, can readily adapt to environmental change. In the Swan-Canning Estuary, and also many other estuaries in southern Australia, one such fish species is the Black Bream (*Acanthopagrus butcheri*), which completes its life cycle in the estuarine environment, can live to about 30 years (Norris *et al.* 2002) and can survive and reproduce in a wide range of conditions (Sarre and Potter 1999, Sarre *et al.* 2000, Partridge and Jenkins 2002, Hoeksema *et al.* 2006, Hassell *et al.* 2008). This species is also among the most sought

after by recreational fishers in the Swan-Canning Estuary, and thus is of considerable value to the community.

An important final step in developing effective biotic indices is determining the main environmental drivers of trends in index values and, in turn, validating index sensitivity (*i.e.* its ability to discriminate between areas and/or periods of differing environmental integrity) and reliability (*i.e.* the repeatability of index predictions) (Jordan and Vaas 2000, Harrison and Whitfield 2006). This may be achieved by correlating index values with independent measures of environmental condition (*e.g.* water quality) and testing index variability among replicate samples, respectively. However, interpretation of the trends in biotic indices may also be greatly facilitated by a sound understanding of the dietary interrelationships between the various trophic levels in the ecosystem. This information is extremely useful for evaluating the intermediary pathways by which the primary environmental stressors are operating, and is best captured by a quantitative food web that employs a variety of complementary techniques for determining the flow of energy through the ecosystem.

In view of the above, the main aims of this three year (2007-2010) study were as follows.
(1) Use historical and current data to quantify the types and extents of changes that have occurred in the characteristics of the fish fauna of the Swan-Canning Estuary since the late 1970s, and elucidate whether any such changes can be related to concomitant differences in a suite of water quality variables.
(2) Develop a quantitative biotic index, based on a suite of characteristics of the fish community, that is reliable and sufficiently sensitive to detect any significant changes in the ecological health of the Swan-Canning Estuary.
(3) Develop a quantitative biotic index, based on a suite of characteristics derived from fishery-independent data for Black Bream, that is reliable and sufficiently sensitive to detect any significant changes in the health of its stock in the Swan-Canning Estuary.
(4) Identify which fish species are particularly susceptible to mortality during large phytoplankton blooms and determine the movement patterns of those species that are capable of emigrating from bloom-affected areas.
(5) Develop a food web for the upper Swan-Canning Estuary, employing traditional gut content analyses for selected fish species and complementary biochemical analyses of those species and their prey, *i.e.* stable isotope (δ^{13}C and δ^{15}N) and essential fatty acid analyses.

The first two of these aims are addressed in Chapter 2, while the third and fifth aims are addressed in Chapters 3 and 4, respectively. Given that no large fish kills attributable to phytoplankton blooms occurred within the Swan-Canning Estuary during this study, the fourth aim was not addressed in this report. General conclusions and recommendations for management are provided in Chapter 5.

Chapter 2. Changes in the characteristics of the fish fauna of the Swan-Canning Estuary since the late 1970s and development of a fish-based biotic index of ecosystem health

Hallett, C.S. and Valesini, F.J.

Executive Summary

The Swan-Canning Estuary, which has been highly modified since European settlement in the early to mid 1800s, continues to experience a complex range of environmental stressors from the ongoing effects of local population growth, changes in catchment land use and climate change. The extent of such stressors led to this nationally-significant estuary being identified as a "coastal hotspot" by the Australian Government in 2006, mirroring growing concern from the wider community about the ecological health of this system.

Despite the environmental problems experienced by the Swan-Canning Estuary, and the range of initiatives that have been developed to improve them, managers still do not have a reliable, simple and affordable method for (i) evaluating the ecological health of the estuary, (ii) tracking changes in its health over time and (iii) helping to identify those stressors which are most responsible for changes in ecosystem health. This would greatly inform the type of management responses needed to minimise further environmental decline of the Swan-Canning Estuary, and provide a way of easily communicating the health of this complex system to the public.

The fish fauna in the shallow (≤ 2 m in depth) and/or deeper (> 2 m in depth) waters of the Swan-Canning Estuary has been studied during several annual periods between the late 1970s and the mid 2000s by various researchers at the Centre for Fish and Fisheries Research, Murdoch University, *i.e.* 1976-1981, 1993/94, 1995-1997, 1999-2001, 2003/04, 2005-2007. Although the sampling regimes have differed among some of those studies, particularly in the shallows, this historical data set provides a rare and valuable opportunity to examine whether the fish fauna has changed over the last three decades, and to explore how any such changes can be used to measure the ecosystem health of the estuary.

This component of the study had two main roles. The first was to examine whether the fish fauna in the Swan-Canning Estuary has changed significantly between annual periods since the late 1970s and, if so, to test whether those changes are related to water quality. The second role was to develop an "index", based on a range of fish faunal characteristics, which provides a simple and effective way of measuring the overall ecological health of the estuary, both historically and in the future.

To undertake both of the above roles, the fish fauna in the shallow and deeper waters of the estuary was further sampled between 2007 and 2009 using a regime that was designed to complement, as far as possible, those used in all previous studies since the late 1970s. This included sampling on a seasonal basis at various sites in each of the main estuary zones, *i.e.* the Lower Swan-Canning Estuary [LSCE], Middle Swan Estuary [MSE], Upper Swan Estuary [USE] and Canning Estuary/Lower Canning River [CELCR]).

In the shallow waters, however, the type of seine net used to sample the fish fauna differed among some previous studies, with net types ranging widely in length (102-133, 41.5 or 21.5 m) and mesh sizes (3-16 mm in the net pocket). The impact of these sampling biases was minimised, as far as possible, by undertaking a net comparison study in 2008/09 using all three of the above net types. These data were used to calculate "equivalence factors", which were then used to adjust the densities of each fish species in each sample collected since the 1970s, such that they were effectively standardised to just one net type (the 21.5 m seine). Note, however, that it was not possible to adjust the number and identity of species captured (or not captured) in samples from different net types. Given this, and the relatively large confidence intervals for some equivalence factors, some degree of caution must be exercised when interpreting changes in the nearshore shallow fish fauna among annual periods in which different seines were used. The reliability of some comparisons between periods is also compromised by the fact that the same zones of the estuary were not sampled in all studies, and that the spatial and temporal intensity of sampling sometimes differed among studies. The effects of these latter differences have been minimised, as best as possible, by using the same unit of replication across studies and focusing only on comparable samples.

Changes in the characteristics of the fish fauna of the Swan-Canning Estuary since the late 1970s

Shallow nearshore waters
The composition of the fish fauna in the shallow nearshore waters of the Swan-Canning Estuary has changed markedly between the late 1970s and 2009. Differences between annual periods (*i.e.* "inter-period" differences) had a far greater impact on fish faunal composition than did changes between seasons, zones of the estuary or any interaction between those factors.

The overall extent of fish faunal differences among periods was greatest in the MSE and USE and least in the LSCE. In the first two of these estuary zones, very large differences in nearshore fish assemblages were detected between periods from 1978/79-1981/82 and those from 1999/00-2005/06. Moderately large differences were also found between most other periods in the MSE, with the frequent exception of those that were consecutive, *e.g.* 1995/96 *vs* 1996/97. In the CELCR, large to moderate differences in fish faunal composition occurred between periods in the late 1970s/early 1980s and those sampled between 1995/96 and 2005/06, while in the LSCE, the greatest differences occurred between periods in the late 1970s/early 1980s and those sampled from 2003/04 to 2005/06.

The large changes in the nearshore fish faunas of the MSE, USE and CELCR between the late 1970s/early 1980s and more recent periods were often due to the far more abundant catches of Perth Herring (*Nematalosa vlaminghi*) in the earlier periods. *Nematalosa vlaminghi* represented nearly 45% of fish collected during 1978/79-1981/82, but < 4% of those in all other periods. The abundances of Sea Mullet (*Mugil cephalus*) and Yellow-eye Mullet (*Aldrichetta forsteri*) have also declined notably since the late 1970s/early 1980s in the above zones and in the LSCE. In contrast, the abundances of several other species have increased, such as the Southern Black Bream (*Acanthopagrus butcheri*) in the MSE, USE and CELCR and the Banded Toadfish (*Torquigener pleurogramma)* in the LSCE.

Changes in the abundance of the above species since the late 1970s/early 1980s may be due to the influence of (i) increasing salinities in the estuary and/or the presence of higher salinities for longer periods throughout the year, (ii) lower dissolved oxygen levels in the

upper reaches of the estuary, (iii) other reductions in estuarine habitat quality, such as those associated with large phytoplankton blooms in the upper reaches, (iv) fishing pressure in the case of *N. vlaminghi, M. cephalus* and *A. forsteri*, either in the estuary or the local marine waters and/or (v) for those species that spend part of their life at sea (*i.e. N. vlaminghi, M. cephalus, A. forsteri* and *T. pleurogramma*), changes in the environmental conditions of local marine waters.

The average species diversity of the nearshore fish assemblage, as measured by its taxonomic distinctness, has also declined since the late 1970s/early 1980s in the CELCR. This is due mainly to the fish faunas in more recent periods being dominated by closely-related atherinid (Hardyhead) species (*i.e.* from the same genus and/or family), whereas those in the earlier periods contained species from diverse orders and/or families that were almost never recorded in later periods, *e.g.* Estuarine Cobbler (*Cnidoglanis macrocephalus*), Hairy Pipefish (*Urocampus carinirostris*) and Tailor (*Pomatomus saltatrix*).

Lastly, in some zones and seasons, the composition of the nearshore fish assemblages was more variable among replicate samples in later than earlier periods. As increased biotic variability may reflect decreased ecosystem resilience to stress, these findings may provide further indications of greater environmental stress on these fish assemblages.

Deeper offshore waters

Fish in the deeper offshore waters of the estuary have been regularly collected by gill nets in the MSE and USE zones during various periods since the early 1990s, *i.e.* 1993/94, 1995-1997, 2003/04 and 2007-09. Unlike the fish sampling regimes used historically in the shallows, those in the deeper waters of each zone were largely consistent throughout all of the above studies.

The total and mean catch-rates of fish in the deeper waters of the MSE and USE have clearly declined since the early to mid-1990s. The most pronounced inter-period shifts in mean catch-rate occurred in the USE in summer, with values falling from approximately 38 to 8 fish h^{-1} between 1993/94 and 2008/09.

The mean number of species and species diversity in the MSE and USE has also declined from the earlier to later sampling periods. For example, an average of seven species was recorded in the autumn of 1993/94, but only two were recorded in the same season in 2007/08.

The species composition of the offshore fish assemblages has also changed significantly since 1993/94. The overall extent of these inter-period differences was moderate to low, with the greatest differences occurring in the USE. However, in both estuary zones, moderately large differences in fish faunal composition were typically found between the earlier *vs* later periods, *i.e.* 1993/94 and/or 2003/04 *vs* 2007/08 and/or 2008/09.

In both the MSE and USE, the largest differences in fish faunal composition were due mainly to greater catches of *A. butcheri, N. vlaminghi* and/or *M. cephalus* in the earlier than later periods. The trends in the latter two species are similar to those recorded in the shallow waters, where their abundances have also declined over time. In contrast, the reduced catches of *A. butcheri* in the deeper waters oppose the trends found for this species in the shallows, where it has become more abundant over time. Such findings may reflect (i) the movement of *A. butcheri* into the shallows to avoid the low concentrations of dissolved oxygen recorded in the deeper bottom waters of the MSE and USE, (ii) a greater availability of benthic macroinvertebrates, the preferred prey of this species, in the shallows and/or (iii) some differences in the timing of sampling in 1993/94 compared to that in all other studies.

However, given that the catch-rates of *A. butcheri* between 1995 and 2004 were still far higher than those in 2007-09, this last factor is unlikely to be the major cause of the reduced abundance of this species in the offshore waters.

As was the case in the shallows, the composition of the offshore fish fauna was much more variable among replicate samples during 2007-09 than between 1993/94 and 2003/04, particularly in the USE. Again, such findings may reflect greater environmental stress on the offshore fish fauna in more recent periods.

Relationships between fish faunal composition and water quality in nearshore and offshore waters

The trends in the composition of the nearshore and offshore fish fauna from the earliest to latest sampling periods were compared to those in a range of water quality characteristics recorded throughout the estuary over the same time frame. These analyses were used to test whether patterns in the fish fauna could be significantly "explained" by those in water quality. The water quality parameters examined in surface and/or bottom waters included salinity, temperature, concentrations of dissolved oxygen, total nitrogen, total phosphorous and chlorophyll *a* and/or level of turbidity. Most of the measurements for these variables were obtained from the water quality monitoring program that has been undertaken weekly by the Department of Water since 1994.

While the best attempts were made to match the fish and water quality samples in terms of their time and location of collection, the ability to fully correlate these data sets was compromised by the fact that not all of the above water quality parameters were recorded (i) across the range of fish sampling periods and/or (ii) in both the surface and bottom waters. The most comprehensive analyses were thus restricted to those sampling periods between 1995/96 and 2008/09.

Significant correlations between the fish and water quality data recorded between 1995/96 and 2008/09 were detected for most estuary zones and seasons in both the shallow and deeper waters. The overall extent of those correlations was moderate to low. The particular subset of water quality variables that best matched the inter-period trends in fish composition varied among zones, seasons and water depths. However, it was often the case that changes in the fish fauna from earlier to later periods were correlated with (i) decreasing concentrations of total phosphorous, (ii) decreasing concentrations of dissolved oxygen, (iii) increasing salinity and/or (iv) increasing water temperature.

Development of a biotic index of estuarine health using fish assemblages

Indices of ecosystem health were developed for the shallow and deeper waters of the Swan-Canning Estuary using a suite of characteristics ("metrics") of its fish communities. These so-called "multimetric biotic indices" are the first such tool to be developed for an estuary in Western Australia. They provide a reliable, practical and cost-effective way of "summarising" the complex ecological condition of an ecosystem by using changes in a particular animal or plant community to provide indications of the collective state of the environment. Fish have many characteristics that make them well-suited to this purpose, including their broad range of feeding and life history strategies and the ease with which they can be sampled and identified. Fish-based indices of ecosystem health have therefore been used successfully to measure the ecological condition of many aquatic ecosystems worldwide. These indices also readily lend themselves to producing "report cards" for a given

ecosystem, indicating whether environmental health is good, fair, poor or very poor. They thus provide an excellent basis for communicating those findings to the general public and stakeholders.

The development and testing (validation) of a multimetric biotic index for assessing ecosystem health requires several complex stages. However, once these stages have been completed, final use of the index as a monitoring tool is a relatively simple task that does not require those earlier stages to be revisited. This study focused on the development and initial validation of fish-based multimetric indices for the Swan-Canning Estuary. Each of the stages that were undertaken during this process are summarised in the following flow chart and outlined further below.

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┌─────────────────────────────────────────────────────────────┐
│                          Identify appropriate                │
│                          candidate fish metrics              │
│                                   │                          │
│                          Select best subset of               │
│                          metrics sensitive to                │
│                          temporal changes                    │
│                                   │                          │
│   Net comparison study            │                          │
│          │                Establish best available           │    INDEX
│   Standardisation of      reference conditions         DEVELOPMENT
│   historical data    ──▶  for each metric                    │
│                                   │                     2007-2010
│                          Calculate metric scores for each    │
│                          sample obtained over time           │
│                          (scores reflect deviation from      │
│                          reference conditions)               │
│                                   │                          │
│                          Calculate index score               │
│                          for each sample                     │
│                                   │                          │
│                          Validate index performance          │
│                            • Index sensitivity               │
│                            • Inter-annual variability        │
│                            • Inter-seasonal variability      │
│                            • Effect of sampling variability  │
└─────────────────────────────────────────────────────────────┘
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Identifying appropriate candidate metrics and selecting the best subset
A suite of about 30 candidate fish community metrics, including measures of species composition, diversity and abundance, trophic (*i.e.* feeding) structure and life history characteristics, were first tested for their suitability in building reliable indices of ecosystem health for the nearshore and offshore waters of the Swan-Canning Estuary. The aim of this stage of the process was to select those fish metrics that were most responsive to changes in ecosystem health.

The initial approach focused on identifying those fish metrics that best reflected differences in the quality of physical habitat at 71 local-scale sites throughout the estuary. Thus, at each site, physical habitat quality was first quantified using a novel and independent measure of habitat degradation (*i.e.* a rapid visual assessment that involved scoring each site for various

habitat quality characteristics), and the fish fauna were then sampled and metrics calculated. However, this approach failed to confirm the predicted responses of any of the candidate fish metrics to physical habitat degradation, and was thus unsuccessful in selecting appropriate metrics. This may be because, in the Swan-Canning Estuary, habitat quality acts to structure fish communities at a broader scale than that assessed in this study.

The second approach to metric selection, which was also entirely unique to this study, sought to identify those metrics which best reflected annual (period) changes at the ecosystem level, and thus those which are most sensitive to longer-term changes in ecosystem condition. This approach used a combination of statistical techniques to assess not only how sensitive each of the candidate metrics were to ecosystem condition, but also how necessary they were to include in the process (*i.e.* their level of redundancy). Metrics were selected based on the "weight of evidence" from multiple analyses of the nearshore and offshore fish data sets collected between 1976/77 and 2008/09 (see earlier). Subsets of eleven and seven fish metrics were ultimately selected to construct the multimetric indices for the nearshore and offshore waters, respectively.

Metric reference conditions
Reference conditions were established for each selected nearshore and offshore metric using the above 30-year fish assemblage data sets that had previously been standardised for differences in sampling regime, *i.e.* net type and/or sampling intensity (see earlier). These reference conditions represented the "best-available" values recorded for each fish metric in each zone of the Swan-Canning Estuary during each season. They thus provide a benchmark against which any previous, current or future values of the fish metrics can be compared to measure how the health of the estuary has changed from the "best-available" state.

Calculating metric and index scores
The metric values for all fish samples collected between the late 1970s and 2009 were then "scored" according to how far they deviated (negatively or positively) from their appropriate reference condition, *e.g.* a value of six species for the metric "*Number of trophic specialist taxa*" in an autumn sample from the CELCR zone received a score of 7.5 out of 10, given the best available reference condition of eight species. The final scores for both the nearshore and offshore health indices were then calculated by simply summing the metric scores, then adjusting the value by the number of component metrics to produce an easily interpretable number ranging from 0 (poorest ecosystem health) to 100 (best ecosystem health). This range of health index scores was also divided into four equal classes to more easily reflect the health status of the system, *i.e.* Good (100-75), Fair (74-50), Poor (49-25), Very poor (24-0). Index scores and their corresponding health status can be reported for the estuary as a whole, or for individual zones and/or seasons.

When calculated for the whole estuary in each period from 1976/77 to 2008/09, the mean health index scores for the shallow nearshore waters of the Swan-Canning Estuary showed a moderate degree of variation, although the resulting health status remained as "fair" throughout this time. There is, however, evidence to suggest that the health of the shallows has increased in more recent years, *i.e.* from approximately 58 in 2005/06 to 64 in 2008/09. It is suggested that such findings could reflect the onshore movement of particular fish species to avoid the poorer ecological quality of the deeper bottom waters (see earlier and below).

In contrast, the mean offshore index score for the entire estuary has decreased consistently from 56.5 ("fair") in the late 1970s to 47 ("poor') in 2008/09. The scores for the offshore

index also varied more among replicate sites than those for the nearshore index in most seasons during the two years of the current study. Variability in index scores between seasons in 2007-09 was also greater for offshore than nearshore sites, particularly at sites of poorest ecological quality. Such findings provide further indications that the offshore waters are in poorer health than the nearshore waters, most notably in the USE.

Initial validation of index performance
Index scores in both the nearshore and offshore waters were the least variable in summer and autumn, indicating that, dependent on further examination, these seasons may represent the best period for monitoring the ecological health of the Swan-Canning Estuary in the future. The spatial and temporal variability in the indices produced in this study were comparable to those of other biotic multimetric indices used in the USA and Europe. While the precision was lower and the bias greater for offshore than nearshore index scores (indicating that the former is less robust to random sampling variability), we were able to demonstrate that classification of the health status of the estuary was not affected by natural fish faunal variability or sampling error.

Although index responses to changes in specific environmental stressors could not be demonstrated in this study, the consistent decrease in offshore health index scores over the last three decades suggests that this index is capable of detecting the widely-perceived, long-term decline in the condition of the offshore waters of the Swan-Canning Estuary.

Future index validation and implementation as a monitoring tool
Further work is needed to (i) validate the precision and sensitivity of the indices and (ii) design a robust and cost-effective annual fish monitoring regime for the Swan-Canning Estuary so that its health can be assessed into the future.

It is important to reiterate that any future implementation of these fish-based indices would not require the technical stages of index development and validation detailed in the current study to be performed again. Implementation of the index would require only a conceptual understanding of the rationale behind each of the development stages, and could be done by anyone supplied with basic training and the spreadsheet-based tools developed in the current project to calculate metric and index values.

2.1 Introduction

The extreme difficulty of measuring the numerous and complex impacts of anthropogenic stressors on aquatic ecosystems has led many workers since the late 1980s to develop "indicators" for assessing and monitoring environmental condition (otherwise referred to as environmental health, integrity or quality; *e.g.* Ramm 1988, Cooper *et al.* 1994, Engle *et al.* 1994). Such indicators aim to distil the complex workings of an ecosystem into easily interpretable signals that quantify its health, and their role has taken precedence in the "DPSIR" (drivers, pressures, status, impact, response) approach adopted by many environmental agencies worldwide for identifying ecosystem change and the most appropriate management response (Elliott 2002). Although indicators can be developed from different facets of the ecosystem, such as social or physico-chemical aspects, those developed from biotic characteristics, and particularly from entire communities, often provide the most comprehensive and ecologically-relevant measures of ecosystem health. That is, biota react to all parts of their environment, and the species comprising a community often range widely in their responses due to differences in physiological tolerance, habitat, life history and interactions with other biota. Thus, integrated measurements of a suite of community characteristics, such as species composition and the proportions of different trophic or life-history guilds, can provide an effective "summary" of the condition of ecosystem components and their complex functional interactions. Biotic indices thus directly reflect the "ecological consequences" of a particular environmental state, unlike the indirect measures provided by other types of indicators.

Fish communities often provide very effective indicators for assessing estuarine health. This is due, firstly, to the fact that they usually occupy a diverse range of trophic levels and thus require a whole suite of ecosystem components, functions and processes to be intact for their survival, growth and reproduction (Karr 1981, Deegan *et al.* 1997, Hughes *et al.* 2002). Secondly, different fish species typically use estuaries in a variety of ways throughout their life (see Potter and Hyndes 1999, Elliott *et al.* 2007). The numerous other advantages of using these organisms for this purpose, such as their being relatively long-lived and thus providing a longer term record of environmental condition, are provided in detail by Whitfield and Elliott (2002). Indices of ecosystem health based on characteristics of fish communities have proven to be very effective and sensitive tools for measuring the ecological health of estuaries (*e.g.* Ramm 1988, Deegan *et al.* 1997, Coates *et al.* 2007) and freshwater environments worldwide (*e.g.* Karr 1981, Lyons *et al.* 1995, Pont *et al.* 2007).

Biotic indices that integrate a variety of characteristics (metrics) of fish communities for measuring estuarine health have developed mainly from that produced by Karr (1981) for assessing environmental degradation in North American freshwater streams, *i.e.* the Index of Biotic Integrity (IBI). This quantitative index was originally based on 12 metrics of fish community structure and function, *i.e.* those representing species richness, indicator taxa, trophic guild structure and the incidence of disease. However, the specific metrics chosen should be those that are relevant to the bioregion in which the IBI is applied (Belpaire *et al.*

2000). The flexibility, and thus applicability, of the IBI is evidenced by the fact that it, or related schemes, have been employed worldwide (Hughes and Oberdorff 1999) and modified for use in different types of aquatic environments, including estuaries (*e.g.* Carmichael *et al.* 1992, Quinn *et al.* 1999, Breine *et al.* 2007).

Multimetric indices, such as the IBI and its derivatives, have typically been developed by a common process that has several main stages (Simon 2000). These are illustrated and then further explained below.

```
┌──────────────────────────────┐
│  Compile candidate metrics   │
└──────────────────────────────┘
              ↓
┌──────────────────────────────┐
│  Select appropriate metrics  │◄─┐
└──────────────────────────────┘  │
              ↓                    │
┌──────────────────────────────┐  │
│ Establish reference conditions│  │   Reassess index
└──────────────────────────────┘  │   design in light of
              ↓                    │   validation results
┌──────────────────────────────┐  │
│   Set scoring thresholds     │  │
└──────────────────────────────┘  │
              ↓                    │
┌──────────────────────────────┐  │
│   Calculate index scores     │  │
└──────────────────────────────┘  │
              ↓                    │
┌──────────────────────────────┐  │
│      Validate index          │──┘
└──────────────────────────────┘
```
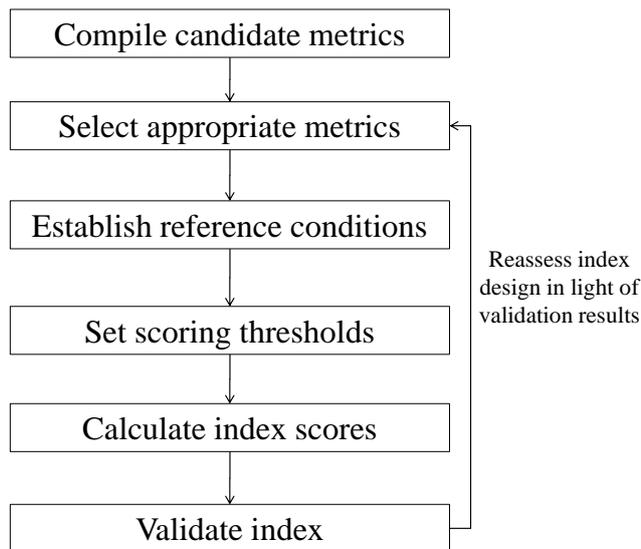
(1) Identify a suite of candidate metrics for potential inclusion in the index. This should be achieved by reviewing relevant existing biotic indices and/or using expert knowledge of the system. Each candidate metric should be ecologically meaningful and, together, the suite should reflect all major aspects of ecosystem structure and function. Metrics reflecting assemblage composition and trophic, habitat and life-history guilds are commonly employed in fish-based biotic indices, as are those reflecting the abundance of sentinel species (Noble *et al.* 2007).

(2) Metric selection: Candidate metrics selected for inclusion in the final biotic index should be those that respond the most sensitively and consistently to environmental degradation, are practical to measure and are not highly correlated with other metrics (Barbour *et al.* 1995, USEPA 2006, Noble *et al.* 2007, Roset *et al.* 2007, Niemeijer and de Groot 2008). Metrics selected by rigorous statistical techniques that test their efficiency, reliability and sensitivity in detecting environmental decline in the system of interest, as opposed to those selected solely by expert judgement (*e.g.* Karr 1981, Belpaire *et al.* 2000), will invariably comprise the most effective indices (Seegert 2000, Breine *et al.* 2007).

(3) Establishing reference conditions: Benchmark or reference conditions must be set for each selected metric, against which their observed values can be compared to quantify

deviation from an "ideal" state (Hughes 1995). Given that few aquatic systems are free from human impacts, many studies have selected "best available" sites or times as a reference (*e.g.* Gibson *et al.* 2000, Breine *et al.* 2007, Qadir and Malik 2009). It is essential that the reference conditions for each metric account for natural spatio-temporal variability, such that the true ecological effects of anthropogenic stressors (signal) can be distinguished from background variability (noise).

(4) Metric scoring and index calculation: For every sample collected, observed values of each metric are next allocated a score, based on the extent of their deviation from the reference condition, *e.g.* 1 (within 50% of reference) to 5 (within 90% of reference, *e.g.* Harrison and Whitfield 2004, Coates *et al.* 2007). The final index value for each sample is then calculated by summing its scores for all component metrics, with larger scores reflecting greater similarity to the "best attainable" state.

(5) Index validation: An important final step is the validation of index sensitivity (*i.e.* its ability to discriminate between levels of environmental integrity) and reliability (*i.e.* the repeatability of index predictions). The former can be achieved by determining how well index values are correlated with independent measures of environmental condition (*e.g.* water quality; Jordan and Vaas 2000, Harrison and Whitfield 2006), while the latter may be determined by comparing observed index values to those derived from repeated samples. However, the best validation of index efficacy is a demonstration of its ability to track changes in ecosystem health in response to documented ecological degradation or rehabilitation.

Since the late 1980s, numerous workers have developed fish-based biotic indices for assessing the health of estuaries, primarily in North America (*e.g.* Deegan *et al.* 1997, Jordan and Vaas 2000, Meng *et al.* 2002), Europe (*e.g.* Goethals *et al.* 2002, Coates *et al.* 2007, Borja *et al.* 2008) and South Africa (*e.g.* Ramm 1988, Harrison and Whitfield 2004). The usefulness of these indices in monitoring and communicating estuarine health is reflected by their incorporation into major environmental management initiatives and/or legislation, and the large scales over which they have been applied. For example, fish-based biotic indicators are now commonly employed throughout Europe under the European Union Water Framework Directive in response to legislation requiring regular monitoring of the health of all transitional (estuarine) waters (Borja *et al.* 2008). Moreover, the index developed by Harrison and Whitfield (2004) has been used to assess the condition of 190 estuaries across South-Africa (Harrison and Whitfield 2006).

In contrast to the above, few such indicators have been established for assessing the ecological integrity of Australian estuaries (Deeley and Paling 1998, Scheltinga and Moss 2007). Indeed, in a global review, Borja *et al.* (2008) highlighted an alarming lack of direction in Australia's approach to ecological health assessment of its aquatic systems, compounded by confusion over state and federal responsibilities and a widespread deficit of biotic indicator schemes to undertake this task. Given the well-documented environmental decline in many Australian estuaries, there is thus a clear need to develop integrated

assessment schemes that incorporate biotic indicators to understand, monitor and communicate the ecological health of these systems. This need clearly extends to the Swan-Canning Estuary, which is exhibiting many signs of environmental stress (see Chapter 1). Moreover, the quantitative fish assemblage data collected throughout the estuary by various researchers from Murdoch University's Centre for Fish and Fisheries Research during several periods since the late 1970s (*i.e.* Loneragan *et al.* 1989, Kanadjembo *et al.* 2001, Valesini *et al.* 2005, 2009, Hoeksema and Potter 2006, Sarre unpubl.) provides a rare and excellent basis for establishing sound reference conditions for a fish-based biotic index for this system.

Given the above, the aims of this component of the current study were as follows.

(1) Quantify the nature and extent of any changes in the characteristics of the fish fauna in the Swan-Canning Estuary since the late 1970s, and elucidate whether they can be related to concomitant changes in a suite of water quality variables.

(2) Develop a sensitive, statistically rigorous and easily interpretable biotic index of ecosystem health for the Swan-Canning Estuary, based on a suite of characteristics of its fish assemblage.

(3) Evaluate the sensitivity and reliability of the resultant index.

(4) Identify key considerations for the future development of a cost effective and scientifically robust fish monitoring regime to enable this index to be used for assessing the ongoing ecological health of the Swan-Canning Estuary.

The biotic index developed in this component of the study will employ stages 1-4 outlined above, and some validation of the index (stage 5) will be undertaken. While more rigorous validation of the index is beyond the scope of this study, it is envisaged to be the subject of future work. Moreover, although the current index has been developed for the Swan-Canning Estuary, the approaches employed here (several of which are unique) can be applied to any other estuary.

2.2 Materials and Methods

2.2.1 Changes in the characteristics of the fish fauna of the Swan-Canning Estuary since the late 1970s

2.2.1.1 Fish sampling regime

The nearshore and offshore fish sampling regime adopted in this study was designed to complement, as far as possible, those employed in all fish assemblage studies that have been undertaken in the Swan-Canning Estuary since the 1970s, *i.e.* Loneragan *et al.* 1989 (1976-1981), Sarre unpubl. (1993-1994), Kanadjembo *et al.* 2001 (1995-1997), Valesini *et al.* 2005 (2003-2004), 2009 (2005-2007) and Hoeksema and Potter 2006 (1999-2001). However, it is important to note that, particularly for the nearshore waters, the sampling regimes varied markedly among those historical studies, reflecting differences in their overarching aims. This included differences in net type and the frequency and location of sampling, which are summarised in Tables 2.2.1.1 and 2.2.1.2 and Fig. 2.2.1.1 (see each of the above publications for full descriptions of the historical sampling regimes). Thus, while the current study has attempted to replicate the collective sampling regimes of all of the above studies, and also to standardise the various data sets wherever possible to maximise their comparability (see subsections 2.2.1.1.1 and 2.2.1.3.1), one must be mindful of these differences when interpreting (i) the comparisons of the nearshore fish fauna between the late 1970s and 2009 and (ii) temporal trends in the nearshore biotic index over that period (see subsections 2.2.2, 2.3.2 and 2.4.2).

The spatial classification of the Swan-Canning Estuary that has been adopted in this component of the study reflects the Ecological Management Zones developed by the Swan River Trust in conjunction with the Department of Water (DoW) (Swan River Trust 2009). The zones of that classification that were applicable to the current study included the Lower Swan-Canning Estuary (LSCE), the Middle Swan Estuary (MSE), the Upper Swan Estuary (USE), the Canning Estuary (CE) and the Lower Canning River (LCR). Note that the last two zones were considered to be a single zone for the purposes of this study, and are subsequently referred to as the CELCR. The number and location of replicate nearshore and offshore sites sampled within each of these zones during the previous and current fish faunal studies are shown in Fig. 2.2.1.1.

Fish collected during the current study were immediately placed in an ice slurry to euthanase all individuals. In each replicate sample, all fish were identified to species and the total number of individuals of each species was recorded. The total length of each fish was measured to the nearest 1 mm, except when a large number of a species was caught, in which case the lengths of 100 randomly selected individuals were measured.

Table 2.2.1.1: Summary of the nearshore fish sampling regimes undertaken during the historical and current studies in the Swan-Canning Estuary (periods sampled provided in brackets). Codes used to denote zone of the estuary are as follows: LSCE=Lower Swan-Canning Estuary; MSE=Middle Swan Estuary; USE=Upper Swan Estuary; CELCR=Canning Estuary/Lower Canning River. The location of these zones, and their replicate sites, is shown in Fig. 2.2.1.1. ●=fish samples collected and data included in analyses; ○=fish samples collected but data not included in analyses (see subsection 2.2.1.1.1). * Net length only. Full dimensions are given in subsection 2.2.1.1.1. [†] some additional sampling was undertaken by these workers in periods outside of those stated, which has not been included due to the extensive spatial and temporal inconsistencies in data collection (see subsection 2.2.1.3.1).

Zone	Site	Loneragan *et al.* 1989[†] (1978-81)	Kanandjembo *et al.* 2001a (1995-97)	Hoeksema & Potter. 2006 (1999-01)	Valesini *et al.* 2005 (2003-04)	Valesini *et al.* 2009[†] (2005-06)	Current (2007-09)
LSCE	1	●				●	●
LSCE	2					○	
LSCE	3					●	●
LSCE	4	●				●	●
LSCE	5					○	
LSCE	6					○	
LSCE	7					○	
LSCE	8					●	●
LSCE	9					○	
LSCE	10	●			●	●	●
LSCE	11					○	
LSCE	13					○	
LSCE	14				●		●
LSCE	15					○	
MSE	16	●			●	●	●
MSE	17					●	
MSE	18		●		●	●	●
MSE	19	●	●		●	●	●
MSE	20		●		●		●
MSE	21		●	●	●	●	●
MSE	22		●	●	●		●
USE	23	●	●	●	●		●
USE	24			●		●	●
USE	25	●		●	●		●
USE	26			●			●
USE	27			●			●
USE	28			●		●	●
USE	29			●			●
CELCR	30	●	●		●		●
CELCR	31					●	
CELCR	32					●	
CELCR	33					●	
CELCR	34		●		●		
CELCR	35	●	●		●		●
CELCR	36	●			●		●
Sampling frequency		2-weekly to bimonthly	seasonally	monthly	seasonally	seasonally	seasonally
Net type*		66.5, 102.5 or 133m	41.5m	21.5m	41.5m	21.5m	21.5 and 41.5m

Table 2.2.1.2: Summary of the offshore fish sampling regimes undertaken during the historical and current studies in the Swan-Canning Estuary (periods sampled provided in brackets). Codes used to denote zone of the estuary are as follows: LSCE=Lower Swan-Canning Estuary; MSE=Middle Swan Estuary; USE=Upper Swan Estuary; CELCR= Canning Estuary/Lower Canning River. The locations of these zones, and their replicate sites, are shown in Fig. 2.2.1.1. ●=fish samples collected and data included in analyses; ◦=fish samples collected but data not included in analyses (see subsection 2.2.1.1.2). All fish were collected using gill nets (see subsection 2.2.1.1.2 for net dimensions).

Zone	Site	Loneragan et al. (1977-80)	Sarre (unpubl.) (1993/94)	Kanandjembo et al. 2001a (1995-97)	Valesini et al. 2005 (2003/04)	Current (2007-09)
LSCE	7	◦				
LSCE	10	◦				◦
LSCE	12					◦
MSE	16					◦
MSE	18		●	●	●	●
MSE	19		●	●	●	●
MSE	20		●	●	●	●
MSE	21		●	●	●	●
MSE	22		●	●	●	●
USE	23	◦	●	◦	●	●
USE	24		●		●	●
USE	25		●		●	●
USE	26		●		●	●
USE	27				●	●
USE	28				●	●
USE	29				●	●
CELCR	30	◦				◦
CELCR	32					◦
CELCR	34					◦
		annually to bimonthly	monthly	seasonally	seasonally	seasonally
		3 hrs	3 hrs	2.5 hrs	3 hrs	3 hrs
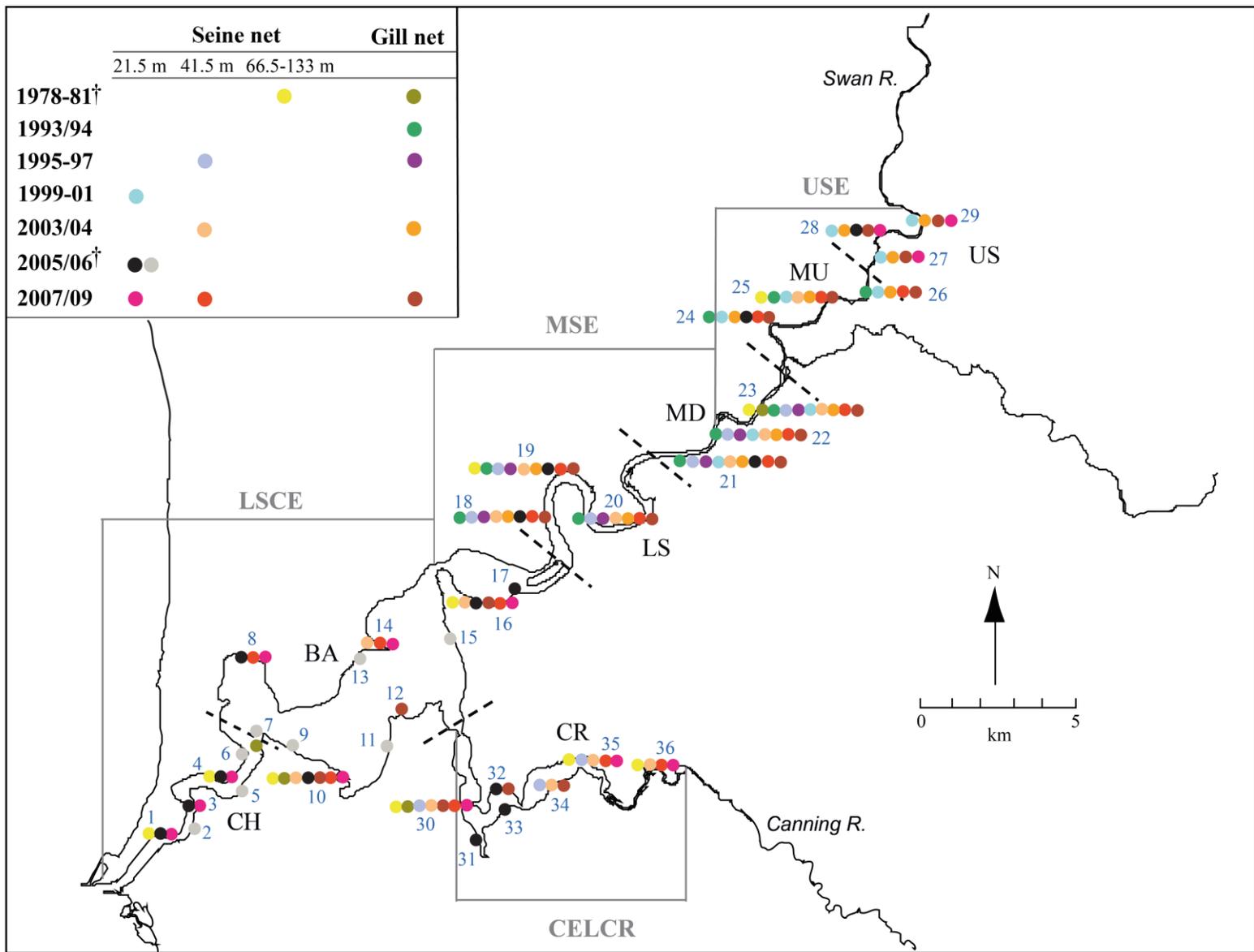
Figure 2.2.1.1: Location of the nearshore and offshore sites (blue numbers) in each of the historical and current fish sampling regimes undertaken in the Swan-Canning Estuary between 1978/79 and 2008/09. The gear types used to sample fish at each site are also provided. The codes used to denote zone of the estuary are: LSCE=Lower Swan-Canning Estuary; MSE=Middle Swan Estuary; USE=Upper Swan Estuary; CELCR=Canning Estuary/Lower Canning River. The codes used to denote region of the estuary are: CH=Channel; BA=Basin; LS=Lower Swan River; MD=Middle-downstream Swan River; MU=Middle-upstream Swan River; US=Upper Swan River; CR=Canning River. ⬤ = fish samples collected by Valesini *et al.* (2009) but data not included in analyses. † some additional sampling was undertaken in periods outside of those stated, which has not been included due to the extensive spatial and temporal inconsistencies in data collection (see subsections 2.2.1.1.2 and 2.2.1.3.1).

2.2.1.1.1 Nearshore waters

<u>*Main sampling regime*</u>

In the current study, fish in the nearshore waters (*i.e.* ≤ 2 m in depth) were sampled during the day in each season between winter 2007 and autumn 2009 at 22 sites throughout the Swan-Canning Estuary (Fig. 2.2.1.1). At each of these sites, fish were collected using one or both of two different seine nets which replicated or approximated those used in the nearshore waters by Loneragan *et al.* (1989), Kanandjembo *et al.* (2001a), Valesini *et al.* (2005, 2009) and/or Hoeksema and Potter (2006) (see Table 2.2.1.1 and Fig. 2.2.1.1). Note that there were some nearshore sites sampled historically that were not sampled in the current study, which predominantly included several in the LSCE sampled only by Valesini *et al.* (2009). This was considered reasonable as (i) the above zone was already sufficiently spatially-replicated and (ii) sampling resources were better allocated to the MSE, USE and CELCR zones of the estuary, which have received greater focus in most historical studies (*i.e.* and thus provide a stronger basis for comparison) and have been the most heavily affected by environmental degradation in recent years.

The largest of the nets used in the current study was 41.5 m long and 2 m deep and comprised two 20 m long wings made of 25 mm mesh and a 1.5 m wide central bunt made of 9 mm mesh. This net, which swept an area of 274 m^2, was laid in a semi-circle from the bank by boat and then hauled on to the beach. The same net was also used seasonally by Kanandjembo *et al.* (2001a) and Valesini *et al.* (2005) at each of the sites shown in Fig. 2.2.1.1. The second of the seine nets was 21.5 m long and 1.5 m deep and consisted 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 made of 3 mm mesh. This net, which swept an area of 116 m^2, was laid parallel to the shore and then hauled on to the beach, and was the same one employed on a monthly and seasonal basis by Hoeksema and Potter (2006) and Valesini *et al.* (2009), respectively, at the sites shown in Fig. 2.2.1.1.

The seine net predominantly used by Loneragan *et al.* (1989) on a twice monthly to bimonthly basis was 133 m long, 2 m deep and contained 25.4 mm mesh in the wings and 15.9 mm mesh in the bunt. These workers also occasionally employed nets that were 102.5 or 66.5 m long, and which had the same height and mesh sizes as the 133 m long net. The areas swept by these nets were 2815, 1670 and 704 m^2, respectively. However, during the current study, it was not possible to consistently use a seine net as long as this throughout the estuary, due mainly to the presence of submerged snags and the narrowness of the banks in the middle to upper reaches. Thus, the 41.5 m seine was instead used to collect fish at each of the nearshore sites previously sampled by Loneragan *et al.* (1989) in order to approximate samples of the fish fauna that would have been obtained using the above three larger nets. The extent to which fish samples collected with the 41.5 m net could be reliably compared with those collected using the 133 m net, and thus how the data acquired using different seine nets needed to be standardised to maximise their comparability, was formally examined using a net comparison experiment, which is described below.

Net comparison study

A net comparison study was undertaken to (i) ascertain the extent of sampling bias attributable to differences in the types of seine nets used to sample the nearshore fish fauna of the Swan-Canning Estuary since the late 1970s and (ii) derive, statistically, equivalence factors for standardising the abundances of each fish species across all historical and current seine net samples, such that the effects of sampling bias on the resultant data are minimised. This study compared the three seine nets that have been used most consistently by the various researchers in the Swan-Canning Estuary between 1978/79 and 2008/09 (*i.e.* the 21.5, 41.5 and 133 m nets), and relied on the assumption that the relative biases of these nets have not changed markedly over that period.

Two broad areas of the Swan-Canning Estuary, denoted as 'Basin' (Melville and Perth waters) and 'River' (the estuarine portions of the Swan and Canning rivers), were chosen for undertaking the net comparison study. Differences in gear-induced sampling bias were expected between these broad regions due to their geomorphological differences, *i.e.* the former is often shallower with a gently sloping substrate, while the latter is typically deeper and has a steeper substrate. Ten nearshore sites were selected systematically across each of these regions to encompass their range of habitats (Fig. 2.2.1.2). At all 20 sites, the fish community was sampled once with each of the 21.5, 41.5 and 133 m seine nets, which were deployed in a randomised order over no more than three consecutive days in both spring 2008 and autumn 2009. At several of these sites, submerged snags or the narrowness of the waterway prevented deployment of the 133 m seine to its full extent (see Fig. 2.2.1.2), and thus only half of that net was used. This was accounted for in the standardisation process described in subsection 2.2.1.3.1.
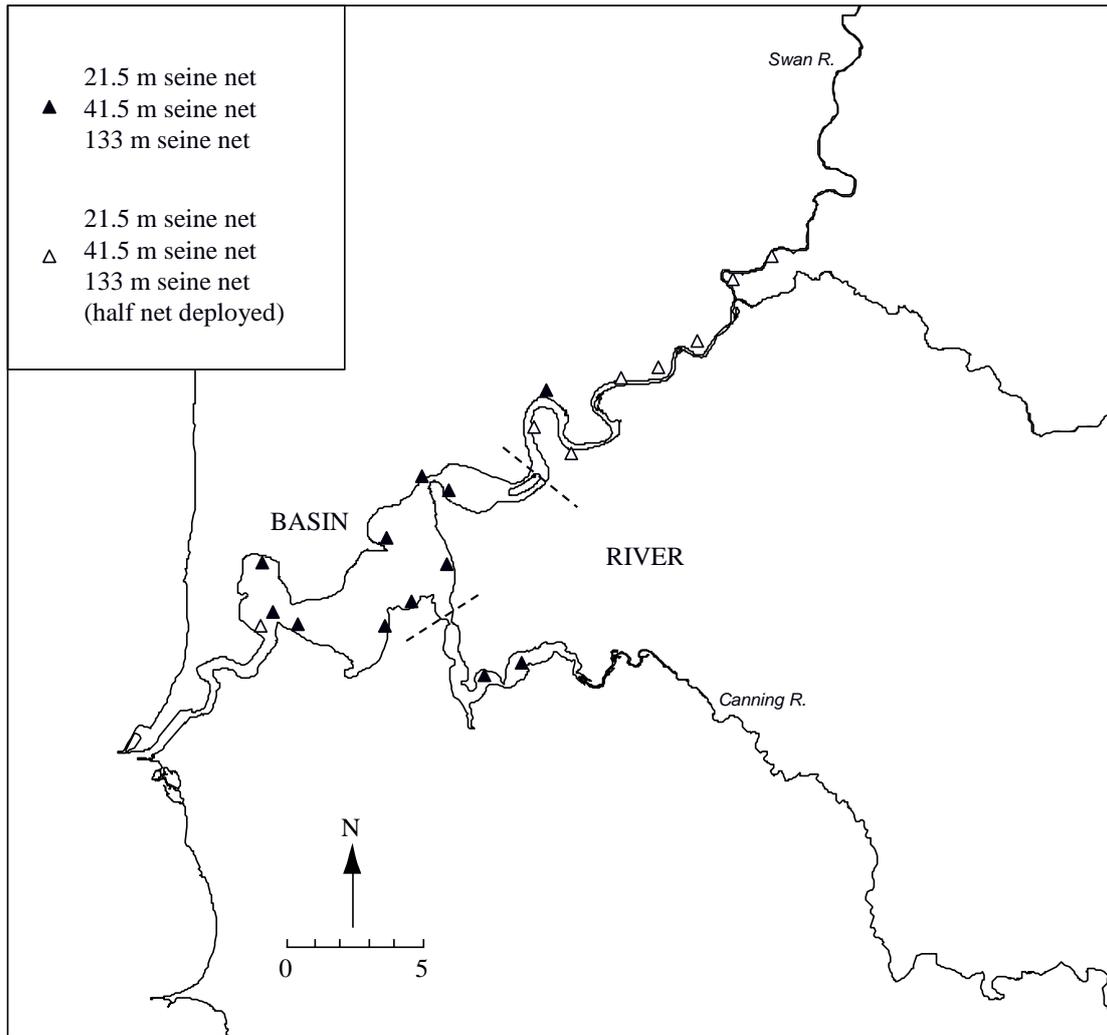
Figure 2.2.1.2: Locations of nearshore sites throughout the Swan-Canning Estuary at which three seine nets of different sizes were used to sample the fish community during spring 2008 and autumn 2009.

2.2.1.1.2 Offshore waters

Fish in the deeper offshore waters were sampled at 18 sites throughout the Swan-Canning Estuary in each season between winter 2007 and autumn 2009. Up to 12 of these sites were also sampled seasonally by Kanadjembo *et al.* (2001) and/or Valesini *et al.* (2005) and/or monthly by Sarre (unpubl.) (Table 2.2.1.2, Fig. 2.2.1.1). Data from the remaining six sites, which were located mainly in the LSCE and CELCR (Table 2.2.1.2), were not employed in the comparative analyses among all studies (see subsection 2.2.1.4), as those sites were never sampled historically. Moreover, while Loneragan *et al.* (1989) also sampled the offshore fish fauna at a small number of sites throughout the estuary between 1977 and 1980 (Table 2.2.1.2 and Fig. 2.2.1.1), that sampling regime was highly inconsistent, particularly from a temporal perspective, and lacked sufficient spatial replication within zones. It was thus not feasible to replicate that offshore sampling regime in the current study, or to include that data in the analyses of changes in the offshore fish assemblages among periods (subsection 2.2.1.4). However, both of these extraneous offshore data sets were able to be used in developing the offshore index of estuarine health (subsection 2.2.2).

The offshore fish fauna was sampled using bottom-set, multimesh gill nets with same length, height and mesh dimensions in each of the above historical studies and in the current study. These nets were 120 m long, 2 m high and comprised six 20 m long panels of varying stretched mesh size, *i.e.* 38, 51, 63, 76, 89 and 102 mm. At each site, one net was laid parallel to the shoreline at dusk and retrieved after three hours in all studies except for that by Kanandjembo *et al.* (2001a), in which the nets were retrieved after 2.5 hours. This difference in net setting time was accounted for by the data standardisation methods described in subsection 2.2.1.4.1.

2.2.1.2 Water quality parameters

A range of water quality parameters were measured concurrently with the collection of fish during the various sampling periods between the late 1970s and 2008/09. Measurements were made at the water surface at nearshore sites and at the surface and bottom of the water column at the offshore sites. Salinity (‰) and water temperature (°C) were measured in all sampling periods, while dissolved oxygen concentration (mg L^{-1}) was recorded in those studies carried out after 1994, *i.e.* Kanandjembo *et al.* 2001a (1995-1997), Valesini *et al.* 2005 (2003-2004), 2009 (2005-2006), Hoeksema and Potter 2006 (1999-2001).

The DoW have also undertaken weekly measurements of numerous water quality parameters at a range of routine monitoring sites throughout the Swan-Canning Estuary since 1994 (Fig. 2.2.1.3). Mean seasonal measurements of those parameters that were considered likely to influence the distribution of fish, either directly or indirectly, were derived from DoW records for 13 of those sites for the periods in which fish were sampled between 1994 and 2009 (see Fig. 2.2.1.3). The sites selected were those that lay in the same zones as the fish sampling sites, and at which water quality data was recorded consistently over the above time frame. The water quality parameters selected included salinity (‰), water temperature (°C), dissolved oxygen concentration (mg L^{-1}), chlorophyll *a* concentration (mg L^{-1}), the concentrations of both total nitrogen (N) and total phosphorous (P) (mg L^{-1}) and turbidity (NTU). Measurements recorded within the top 0.5 m of the water column were considered to be representative of surface waters, while those recorded within 0.5 m above the substrate were considered representative of bottom waters. Note that turbidity and chlorophyll *a* concentration were not recorded consistently during the above time period in the surface and bottom waters, respectively. Analyses of surface water data thus excluded the former variable, while those of surface and bottom water data excluded both of these variables.

2.2.1.3 Statistical analyses – nearshore fish fauna

The fish species abundance data recorded in the nearshore waters were subjected to a range of univariate and multivariate analyses to determine, primarily, the extent to which various characteristics of the fish assemblage differed among the various years in which samples were collected between 1978 and 2009. Given that the various studies differed in the time of year at which they commenced, and that samples collected during summer typically ranged over months belonging to two consecutive years, the different years have been treated as the
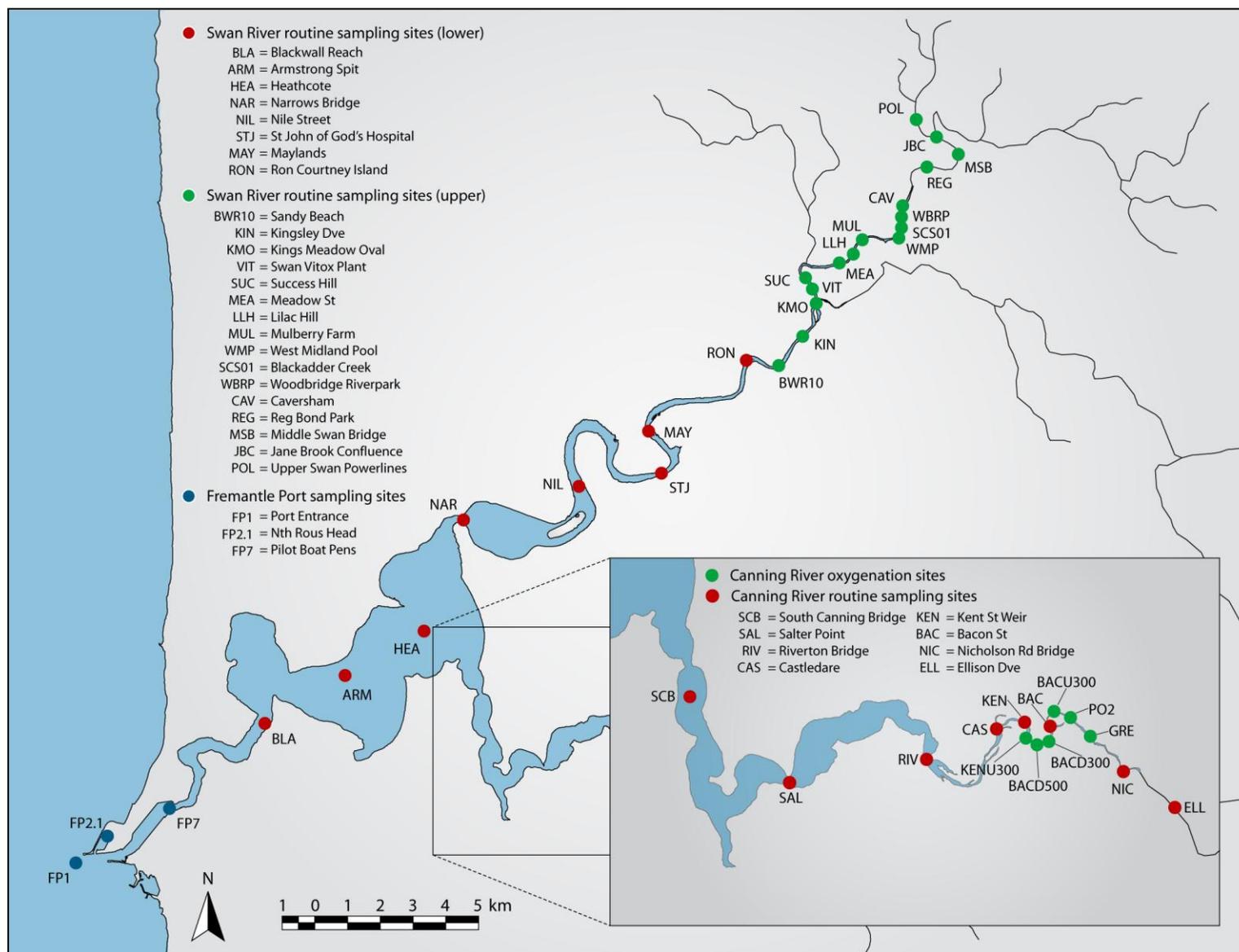
Figure 2.2.1.3: Location of the sites monitored by the Department of Water for water quality throughout the Swan-Canning Estuary. Data from only the following routine sampling sites were employed in the current study: BLA, ARM, NAR, NIL, STJ, MAY, RON, KIN, SUC, SAL, RIV, CAS and KEN. Figure provided by the Swan River Trust (2010).

following "periods" in the subsequent analyses; 1978-79, 1979-80, 1980-81, 1981-82 (*i.e.* those sampled by Loneragan *et al.* 1989), 1995-96, 1996-97 (*i.e.* those sampled by Kanadjembo *et al.* 2001), 1999-2000, 2000-01 (*i.e.* those sampled by Hoeksema and Potter 2006), 2003-04 (*i.e.* that sampled by Valesini *et al.* 2005), 2005-06 (*i.e.* that sampled by Valesini *et al.* 2009), 2007-08 and 2008-09 (*i.e.* those sampled in the current study). Where necessary, investigations of inter-period differences were carried out separately for each zone of the estuary and/or season to remove the confounding influence of one or both of those factors.

2.2.1.3.1 Data standardisation
The following standardisations were applied to the nearshore fish species abundance data collected between 1978/79 and 2008/09 to overcome, as much as possible, the inconsistencies attributable to differences in (i) net type and (ii) spatial and temporal sampling intensity employed over that 30 year time frame.

<u>*(i) Corrections for net type*</u>
The data recorded during the net comparison study (see subsection 2.2.1.1.1) were employed to establish equivalence factors for quantitatively standardising fish species abundances in all historical and current samples collected using either the 41.5 or 133 m seine, to those that would have most likely been recorded if the 21.5 m seine was used consistently throughout all studies. Note that, rather than deriving equivalence factors for individual species, they were instead calculated for each of five representative "habitat guilds" (*i.e.* small pelagic, small benthic, benthopelagic, pelagic and demersal), which reflected similarities in species size, schooling behaviour, movement responses and position in the water column, and thus their tendency to be captured and retained by any particular net type (see Table 2.2.2.1 and subsection 2.2.2.1.1). The abundances of individual species were then corrected on the basis of the guild to which they were assigned. This approach was adopted as (i) not all species were collected in every net type during the net comparison study, thus making it impossible to derive an equivalence factor across all methods and (ii) it was considered beneficial to employ a consistent method of standardisation for species with similar morphological and behavioural traits. The latter is supported by several other studies that have examined differences in seine net efficiency among fish species (*i.e.* Lyons 1986, Parsley *et al.* 1989, Allen *et al.* 1992).

The counts of the species in each sample collected in the net comparison study were thus summed by habitat guild and, for each of those guilds, the resulting data were subjected to a Poisson regression analysis to assess the influence of net type and other confounding factors, namely sampling occasion, region of the estuary and season (*i.e.* predictor variables), on fish counts (the response variable). The null hypothesis for any given habitat guild was that the mean count of all constituent species obtained with either the 41.5 or 133 m net was equal to that obtained with the 21.5 m net. Various alternative linear models, each of which contained different combinations of the above predictor variables, were employed in the regression analysis to test this hypothesis. These candidate models, which are listed below, were fitted using the generalised linear model (glm) procedure in the R statistical package (R

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Development Core Team 2009). Note that each of these models (i) expressed the natural logarithm of fish counts (*c*) and (ii) contained an offset variable, namely the natural logarithm of an area adjustment factor (*A*=area swept by net (m^2) / 100), to compensate for differences in the area of substrate swept by the three net types, *i.e.* by adjusting all fish counts to densities (number of fish per 100 m^2).

$$c \sim O + offset(log_e[A])$$

$$c \sim O + N + offset(log_e[A])$$

$$c \sim R * S + offset(log_e[A])$$

$$c \sim R * S * N + offset(log_e[A])$$

where *O, N, R* and *S* are categorical variables relating to sampling occasion, net type, region, and season, respectively.

The effects of net type and all other predictor variables were tested for significance using the Wald test, which used the parameter estimate and associated standard error for the predictor variable to construct a *z*-statistic with an asymptotically normal distribution (Faraway 2006). The fit of the candidate models was compared using the Akaike Information Criterion (AIC), and the best model was considered to be that with the lowest AIC.

The results of the best model were then examined to assess whether the predicted counts exhibited overdispersion. The residual deviance should be approximately equal to the residual degrees of freedom (*i.e.* the dispersion parameter φ=residual deviance/residual degrees of freedom=1) if the assumption of the Poisson distribution (*i.e.* that the variance is equal to the mean) is satisfied (McCullagh and Nelder 1989). As there was evidence of overdispersion in each habitat guild (see subsection 2.3.1.1), the above candidate models were then re-fitted to the count data using the glm.nb function in the MASS library of R (Venables and Ripley 2002), assuming that the data had a negative binomial distribution (*i.e.* and thus allowing for a variance that exceeds the mean). The re-fitted models were again compared using the AIC to determine the best model.

For each of the five habitat guilds, equivalence factors (*β*) were derived from the best model by exponentiation of the statistically significant (*P*<0.05) estimates of the parameter coefficients for the 41.5 and 133 m nets, and 95% confidence intervals for those equivalence factors were determined as exp(*β*±2 x SE) (Maki *et al.* 2006). The equivalence factors and confidence intervals for the 133 m net were adjusted by swept area for application to those historical samples collected using the 102.5 and 66.5 m nets (see subsection 2.2.1.1.1). The appropriate equivalence factors were then applied to all historical and current counts of fish species in samples collected using the 41.5, 66.5, 102.5 or 133 m nets to obtain a nearshore

data set that was standardised to counts per 21.5 m net. Where no significant effect of a given net on a specific guild was identified, the historical count data were left unadjusted.

It is important to note that, although the above technique was effective in standardising species densities to account for differences in net type, it had no effect on the number or type of species, *i.e.* it is not possible to remove species which were previously recorded, or to add species that were never recorded. Note also that the use of rarefaction, a method which enables estimates of species richness to be adjusted for differences in sample size, was not valid in the present case as the schooling behaviour of many fish species violates the independence assumption on which this technique depends (Clarke and Warwick 2001).

(ii) Corrections for spatio-temporal sampling intensity
Several of the historical nearshore fish assemblage data sets were also corrected to account for differences in the spatial and/or temporal intensity with which samples were collected. Thus, following any required corrections for differences in net type, all data sets were standardised such that (i) sites represented replicates of the zones (*i.e.* with no further replication within sites) and (ii) each season was represented in each study period. This was achieved by calculating site and/or seasonal averages of fish species abundances for each zone and period in those studies in which samples were collected at finer spatio-temporal resolutions, *i.e.* Loneragan *et al.* (1989), Hoeksema and Potter (2006) and Valesini *et al.* (2009).

Further modifications were also made to particular nearshore data sets to harmonise spatio-temporal sampling intensity as much as possible across the different studies. Thus, as mentioned in subsection 2.2.1.1.1, data recorded at eight of the 12 sites in the LSCE by Valesini *et al.* (2009) (*i.e.* those which had never been sampled in any other study) were excluded from analysis to reduce sampling imbalance in that zone of the estuary (see Table 2.2.1.1 and Fig. 2.2.1.1). Secondly, for those sites at which samples were collected using both the 21.5 and 41.5 m seine nets in the main sampling regime of the current study (see Fig. 2.2.1.1), data derived from only the second of those net types was employed. Lastly, the data recorded by Loneragan *et al.* (1989) and Valesini *et al.* (2009) in 1976-77 and 2006/07, respectively, was excluded from the following analyses due to spatial and/or temporal inconsistencies in sample collection. However, all of the above extraneous nearshore data sets were employed in the development of the nearshore index of estuarine health (see subsection 2.2.2).

2.2.1.3.2 Data analysis
The standardised nearshore fish species abundance data was subjected to the following analyses to determine, primarily, the extent and/or cause of any significant differences in overall fish density, quantitative average taxonomic distinctness (a measure of diversity based on the taxonomic relatedness of species; Warwick and Clarke 1995) and assemblage composition among the 12 periods sampled between 1978/79 and 2008/09. Note that, while estuarine zone and season have also been included as factors in the following analyses to segregate their potential confounding influences, the only factor that has been interpreted in

detail is that for inter-period differences. All analyses were carried out using the PRIMER v6 software (Clarke and Gorley 2006) with the PERMANOVA+ add-on module (Anderson *et al.* 2008).

It is important to note that, even following the above data standardisation techniques, there was considerable imbalance in the number of replicate sites per zone among the various studies and, in some cases, particular zones were not sampled (Table 2.2.1.1). Moreover, the location of replicate sites within a zone also frequently differed among studies (Fig. 2.2.1.1). While the latter point is less problematic, and is accounted for by treating each of the following analyses as completely randomised crossed designs (*i.e.* with period, season and/or zone as factors), the former has greater implications for analysis and interpretation. These effects were minimised in the following ways.

(i) Fish assemblage data for the LSCE zone was analysed separately from that for the remaining three zones, as it was not sampled during several of the periods in which the other zones were sampled (*i.e.* those between 1995 and 1997 and between 1999 and 2001; Table 2.2.1.1). Data from the LSCE were thus subjected to tests containing period (*i.e.* a limited suite) and season as factors, while that from the remaining three zones were subjected to tests containing period, zone and season as factors.

(ii) The following permutational ANOVA and MANOVA (PERMANOVA; Anderson 2001) tests were carried out using two different types of sums of squares (Type III and I, or fully partial and sequential, respectively) to ascertain the effects of the unbalanced sampling design on the partitioning of variation across the model, and thus the test results. While Type III is typically recommended for unbalanced designs, Type I, repeated for several tests that each contain a different ordering of the model terms, is also considered useful for assessing the influence of design imbalance (Anderson *et al.* 2008). In all cases, the influence of these different types of sums of squares was small, thus indicating that the design imbalance was not unduly compromising test results. All PERMANOVA results presented in the following Results section are those derived from using a Type III sums of squares.

Univariate analyses

Three-way crossed PERMANOVA was used to test whether, for the MSE, USE and CELCR zones of estuary, the (i) overall density of fish and (ii) quantitative average taxonomic distinctness of the fish assemblage differed significantly among periods, zones and seasons. Data for these two dependent variables in the LSCE was also subjected to a two-way crossed PERMANOVA to ascertain the extent of their differences among periods and seasons. The second of the above dependent variables, which was calculated for each replicate sample using the DIVERSE routine, is a measure of species diversity that accounts for the relatedness of individuals from different species based on their taxonomic separation through the hierarchical levels of the Linnaean tree (Warwick and Clarke 1995). Note that PERMANOVA rather than standard parametric ANOVA was employed to test for differences in the above univariate dependent variables, since the former permutational test does not make any assumptions about the distribution of the underlying data (Anderson

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2001). Also note that differences in the overall number of species were not tested in the manner described above, as species richness could not be standardised among samples collected using different seine net types (see subsection 2.2.1.3.1).

Prior to undertaking the above PERMANOVA tests, the replicate data for each dependent variable was examined to ascertain the type of transformation required, if any, to approximate the test assumption of homogeneous sample dispersions among groups. This was achieved by determining the slope of the linear relationship between the $\log_e$(mean) and $\log_e$(standard deviation) of groups of replicate samples, then applying the criteria provided by Clarke and Gorley (2001). This showed that the overall density of fish and taxonomic distinctness of the fish assemblage required a $\log_e(x+1)$ and square-root transformation, respectively. These transformed data were then used to construct separate Euclidean distance matrices for each dependent variable, which provided the basis of the information required for the above PERMANOVA tests.

All factors in the above analyses were considered to be fixed, and the null hypothesis of no significant differences among groups was rejected if the significance level (P) was ≤0.05. The components of variation for each term in the PERMANOVA model were used to ascertain their relative importance to differences in the dependent variable. The main causes of any significant differences detected by PERMANOVA were determined by examining plots of the marginal means of the dependent variable, back-transformed where necessary, with associated 95% confidence intervals.

Multivariate analyses
The fish species abundance data recorded in the MSE, USE and CELCR zones, and that recorded in the LSCE zone, were subjected to the same three- and two-way PERMANOVA tests described above, respectively, to ascertain the extent of any significant differences in fish composition among periods. Prior to undertaking these analyses, the replicate fish species abundance data was subjected to dispersion weighting (Clarke *et al.* 2006) to downweight the contributions of those species that exhibited large and erratic differences in abundance within groups of replicate samples (*i.e.* within each zone x season x period combination). The dispersion weighted data was then square-root transformed to balance the contributions of highly abundant species with those that were less abundant. The pretreated replicate data were then used to construct a Bray-Curtis similarity matrix, which was then subjected to the above PERMANOVA tests.

When PERMANOVA detected significant differences among the period main effect or an interaction involving period, particular sub-matrices of the above Bray-Curtis matrix (*i.e.* those containing only samples from a selected zone, for example) were subjected to one-way or two-way crossed Analysis of Similarities tests (ANOSIM; Clarke and Green 1988) to examine the inter-period differences in more detail. The particular sub-matrices and factors employed in each of these ANOSIM tests are described fully in subsections 2.3.1.2.3 and 2.3.1.3.3 of the Results. In each ANOSIM test, the null hypothesis that there were no significant differences in fish assemblage composition among groups was rejected if the P value was ≤5%, and the relative extent of any significant differences was determined by the

magnitude of the associated R-statistic, *i.e.* values close to 0 indicate little difference between groups, while those close to +1 indicate large differences between groups (Clarke and Green 1988). The same Bray-Curtis sub-matrices as those employed in the ANOSIM tests were also subjected to Multidimensional Scaling (MDS) ordination, and the samples on the resultant plots were coded to illustrate the nature of inter-period differences in fish composition. When ANOSIM detected a significant result, complementary one-way or two-way crossed Similarity Percentages analyses (SIMPER; Clarke and Green 1988) were used to identify which species best typified the fish assemblage in each period, and those that best distinguished the assemblages of each pair of periods. In these and all subsequent SIMPER analyses, emphasis was placed on those typifying and distinguishing species that (i) had relatively high similarity to standard deviation and dissimilarity to standard deviation ratios, respectively, and (ii) were relatively abundant.

2.2.1.4 Statistical analyses – offshore fish fauna

2.2.1.4.1 Data standardisation
The number of fish collected in each gill net sample was corrected to a catch-rate of number of fish h^{-1} to overcome the differences in the length of time that gill nets were set between particular studies. Moreover, as Sarre (unpubl.) collected samples of the offshore fish fauna on a monthly basis, whereas those in all other studies were collected seasonally, data from the former study were averaged for each season.

2.2.1.4.2 Data analysis
The standardised offshore fish species catch-rate data were subjected to similar univariate and multivariate analyses as those described above for the nearshore fish fauna (subsection 2.2.1.3.2) to determine the extent and/or cause of any significant differences in overall catch-rate, species richness, quantitative average taxonomic distinctness and species composition among the various periods sampled between 1993/94 and 2008/09. However, given that, unlike the nearshore sampling regime, the same offshore sites were sampled consistently by (i) Sarre (unpubl.), Valesini *et al.* (2005) and in the current study in the MSE and USE (*i.e.* nine common sites), (ii) Sarre (unpubl.), Kanadjembo *et al.* 2001, Valesini *et al.* (2005) and in the current study in the MSE (*i.e.* five common sites) and (iii) Valesini *et al.* (2005) and in the current study in the USE (*i.e.* seven common sites; see Table 2.2.1.2), an approach that accounted for the effect of "site" was employed for each of these data subsets in the following analyses. Moreover, also unlike the nearshore data set, there was no imbalance in each of the above three data subsets.

Univariate analyses
Prior to analysis by PERMANOVA, the data for overall catch-rate, number of species and taxonomic distinctness in each replicate sample were subjected to the transformation test described in subsection 2.2.1.3.2. This test showed that the first and last of the above dependent variables required a $\log_e(x+1)$ and square-root transformation, respectively, to

approximate the test assumption of homogeneous sample dispersions among groups. The data for each of these variables were then used to construct separate Euclidean distance matrices.

For the first of the above data subsets, a four-way PERMANOVA containing period, season, zone and site nested within zone (hereafter denoted as site[zone]) was employed to test for significant differences in each of the above three dependent variables. The first three of these factors were considered to be fixed, while the last was considered random. Note that the bottom-level interaction term among all four factors was excluded from the PERMANOVA design (*i.e.* after Anderson *et al.* 2008). For the latter two of the above data subsets, a three-way PERMANOVA containing period, season (both fixed) and site (random) was employed to test for differences in the above three dependent variables. Again, the bottom-level interaction term was excluded from the model. As for the nearshore analyses, emphasis was placed only on interpreting the period component of the PERMANOVA tests. The null hypothesis and method of interpretation for these tests was the same as that described in subsection 2.2.1.3.2.

Multivariate analyses
The offshore fish species catch-rates in data subset (i) were subjected to the same four-way PERMANOVA as described above to ascertain, primarily, the extent of any significant differences in fish composition among periods. Prior to undertaking this analysis, the data was pre-treated using the same procedures as described in subsection 2.2.1.3.2, and then used to construct a Bray-Curtis similarity matrix for input into the above test. In contrast to the approach adopted with the offshore univariate data (*i.e.* in which data subsets [ii] and [iii] were subjected to three-way PERMANOVA), the results of this four-way PERMANOVA test were used as the basis for determining the most appropriate multivariate approach for examining significant inter-period differences in fish composition in more detail. Thus, as this test detected a significant three-way interaction among periods, zones and seasons (see subsection 2.3.1.3.3), the data in each zone were separately subjected to MDS ordination and two-way crossed period x season ANOSIM and SIMPER analyses to elucidate the extent and cause of ichthyofaunal differences among periods without any confounding influences. Note that, for the MSE, these analyses employed data subset (ii) above, while for the USE, they were carried out firstly for those four sites that were common to the Sarre (unpubl.), Valesini *et al.* (2005) and current studies, and then secondly for data subset (iii) above. The methods for interpreting these ANOSIM and SIMPER analyses were the same as those described in subsection 2.2.1.3.2.

2.2.1.5 Relationships between fish faunal composition and water quality in nearshore and offshore waters
The seasonal averages of salinity, water temperature and the concentrations of dissolved oxygen, total N, total P and/or chlorophyll *a* recorded by the DoW at their monitoring sites in each fish sampling period since 1994, supplemented by those for salinity and water temperature recorded prior to 1994 by Loneragan *et al.* (1989) and Sarre (unpubl.) at their fish sampling sites, were used to examine the following.

(1)   The extent of any significant differences in each water quality parameter among those periods in which fish were sampled between 1995/96 and 2008/09 or, in the case of salinity and temperature, between 1978/79 and 2008/09.

(2)   Whether the relative differences among periods in fish faunal composition were significantly correlated with those displayed by the suite of water quality variables and, if so, which of those variables provided the best match.

The former was examined using PERMANOVA, with separate tests carried out for each of the above water quality variables recorded consistently throughout the estuary in (i) the surface waters, which were considered to be representative of conditions in the nearshore areas and (ii) surface and bottom waters, which were considered representative of conditions in deeper offshore areas. Note that the latter suite of tests did not include that for chlorophyll *a* concentration, which was not measured consistently in the bottom waters.

Given the inconsistencies among the different nearshore fish studies in the zones of the estuary that were sampled (see Table 2.2.1.1 and subsection 2.2.1.3.2), the analyses of the surface water quality data recorded in the LSCE were, as for the fish fauna, undertaken separately from those for the other three zones. Thus, the extent to which surface salinity and temperature each differed significantly among the periods in which the nearshore fish were sampled since the late 1970s was tested using a two-way crossed period x season PERMANOVA in the LSCE and a three-way crossed period x zone x season PERMANOVA in the remaining zones. Inter-period differences in the other surface water quality variables measured since 1994 in the LSCE and in the remaining three zones were each tested using the same two-way and three-way PERMANOVA designs, respectively. All factors in these tests were considered to be fixed.

The extent of any inter-period differences in the salinity and temperature of the surface and bottom waters in the MSE and USE between 1993/94 and 2008/09 (*i.e.* those zones and periods in which the offshore fish were sampled) was examined using a four-way crossed period x depth (*i.e.* surface or bottom) x zone x season PERMANOVA, with each of these factors being considered as fixed. These tests required the use of some data collected by Sarre (unpubl.) at his fish sampling sites and some recorded by the DoW at their fixed monitoring sites. Inter-period differences in surface and bottom dissolved oxygen, total N and total P concentrations between 1995/96 and 2008/09, which were recorded consistently by the DoW at the same monitoring sites on all sampling occasions, were each analysed separately for the MSE and USE using a period x depth x season x site PERMANOVA (excluding the bottom-level interaction term; Anderson *et al.* 2008). The first three of these factors were considered fixed, while the last was considered random.

Prior to undertaking each of the above PERMANOVA tests, Draftsman plots (*i.e.* scatterplots of samples between every pair of variables) were used to ascertain the type of transformation required, if any, to approximate the test assumption of homogeneous sample dispersions among groups. These plots, which were constructed separately for the surface and surface *vs* bottom water quality data, allowed visual detection of whether the data distribution for any

variable was notably skewed, and thus provided a basis for selecting an appropriate transformation to ameliorate any such effect. These plots showed that, for the surface water quality data, the concentrations of total N and P each required a fourth-root transformation, while that for chlorophyll a required a $\log_e(x+0.001)$ transformation. For the surface vs bottom water quality data, the first two of the above variables required a $\log_e(x)$ transformation. The data for each water quality variable in each of these two main data sets, transformed where necessary, were used to construct separate Euclidean distance matrices, which were then subjected to the above PERMANOVA tests. The null hypothesis and method of interpretation for these tests was the same as that described in subsection 2.2.1.3.2.

The second of the above two queries was addressed, for both the nearshore and offshore waters, using the Biota and Environment matching routine (BIOENV; Clarke and Ainsworth 1993). This test was used to correlate the underlying pattern of rank order resemblances between complementary fish and water quality matrices to determine which subset of water quality variables "best explained" any inter-period differences in fish composition. Note that separate BIOENV tests were undertaken for each zone and season to remove the confounding influence of those factors. Prior to undertaking this routine, data for the above suites of water quality variables recorded in (i) the surface waters and (ii) both the surface and bottom waters, transformed where necessary (see above), were subjected to normalisation to place all variables (several of which were measured in different units) on the same measurement scale. The reference resemblance matrix employed in the BIOENV tests was the Bray-Curtis similarity matrix constructed from the pretreated fish assemblage data at each site in each period in (i) the nearshore waters (*i.e.* for those tests involving only the surface water quality data; see subsection 2.2.1.3.2) and (ii) the offshore waters (*i.e.* for those tests involving the surface and bottom water quality data; see subsection 2.2.1.4.2). The secondary matrices in the BIOENV tests comprised the pretreated water quality data recorded at each DoW monitoring site or fish sampling site in each period, for which Euclidean distance was considered an appropriate measure of resemblance. Note that, in order to achieve complementarity between samples in the reference (fish) and secondary (water quality) matrices (*i.e.* a requirement of the BIOENV procedure), the water quality data at each DoW monitoring site was matched, as closely as possible, to the nearest fish sampling site. In those cases where a particular water monitoring site lay the closest to more than one fish sampling site, the water quality data from that site was replicated for those fish sampling sites. The Spearman rank correlation coefficient (ρ_s) was used to match the complementary fish and water quality matrices, and the null hypothesis that there was no correlation between matrices was rejected if the significance level was ≤ 0.05. The relative extent of significant correlations was determined by the magnitude of ρ_s, *i.e.* values close to 0 indicate little correlation in rank order pattern between complementary matrices, while those close to +1 indicate a near perfect agreement.

In view of the fact that most of the above water quality variables were not recorded prior to 1994, the BIOENV tests for both the nearshore and offshore waters were carried out in two stages. Thus, one set of tests was undertaken for the fish assemblage data recorded in all available periods and employed only salinity and temperature in the secondary data matrix,

while the second set of tests were restricted to those fish assemblage data recorded after 1994 and employed the full suite of water quality variables in the secondary matrix.

Comparisons of the inter-period differences exhibited by the fish fauna *vs* the water quality variables selected by BIOENV were illustrated by, firstly, subjecting the Bray-Curtis similarity matrices constructed from the fish assemblage data to MDS ordination then, for each sample on the resultant plot, overlaying circles ("bubbles") of proportionate sizes that represented the magnitude of the selected water quality variable(s).

2.2.2 Development of a biotic index of estuarine health

2.2.2.1 Selection of metrics for constructing estuarine health indices

Two approaches were trialed in this study for selecting the most informative subset of fish assemblage metrics from an initial candidate list for inclusion in a multimetric index of ecosystem health for the Swan-Canning Estuary. The first approach sought to identify that metric subset that most strongly responded to spatial differences in habitat quality throughout the estuary, the latter of which was assessed using a novel and independent measure of habitat degradation (see subsection 2.2.2.1.3). The second approach focused on selecting metrics based on their sensitivity to changes in ecosystem condition between those periods in which fish were sampled in the Swan-Canning Estuary between the late 1970s and 2008/09 (see subsection 2.2.2.1.4).

2.2.2.1.1 Allocation of fish to ecological guilds

All fish species recorded in the Swan-Canning Estuary, both during the current study and in all previous studies of the fish fauna of this system (see Tables 2.2.1.1 and 2.2.1.2 and subsections 2.2.1.1.1 and 2.2.1.1.2), were first allocated to functional ecological guilds to enable the calculation of various candidate metrics (Table 2.2.2.1). This followed the rationale of Elliott *et al.* (2007) and was based on existing guild classification schemes (*e.g.* Potter and Hyndes 1999). Three categories of guilds were employed, namely (i) 'Habitat', which reflects the relative size and preferred position within the water column of each fish species, (ii) 'Estuarine Use', which reflects the proportion of their life cycle that each species spends in the estuary and their main activities in that environment, *i.e.* life history, and (iii) 'Feeding Mode', which reflects the diet of each species. With respect to the latter category, classification of feeding modes was limited to the trophic guild of adults, the life stage for which most data tend to be available (Noble *et al.* 2007). Guild allocations were made on the basis of information contained within FishBase (Froese and Pauly 2007), the Codes for Australian Aquatic Biota (CAAB; Rees *et al.* 1999) and published literature.

Table 2.2.2.1: List of fish species recorded in the Swan-Canning Estuary during the current and previous studies, and the functional guilds to which they were allocated. Abbreviations: P – large pelagic; D – demersal (species closely associated with substrate, rocks or weed); BP – benthopelagic; SP – small pelagic; SB – small benthic; MS – marine straggler; mm – marine migrant (including marine estuarine-opportunists); SA – semi-anadromous; ES – estuarine species; FM – freshwater migrant or straggler; PV – piscivore; ZB – zoobenthivore; ZP – zooplanktivore; DV – detritivore; OV – omnivore; HV – herbivore; OP – opportunist.

Species name	Common name	Habitat guild	Estuarine Use guild	Feeding Mode guild
Carcharinas leucas	Bull Shark	P	MS	PV
Myliobatis australis	Southern Eagle Ray	D	MS	ZB
Elops machnata	Giant Herring	BP	MS	PV
Hyperlophus vittatus	Sandy Sprat	SP	MM	ZP
Spratelloides robustus	Blue Sprat	SP	MM	ZP
Sardinops neopilchardus	Australian Pilchard	P	MS	ZP
Sardinella lemuru	Scaly Mackerel	P	MS	ZP
Nematalosa vlaminghi	Perth Herring	BP	SA	DV
Engraulis australis	Southern Anchovy	SP	ES	ZP
Galaxias occidentalis	Western Minnow	SB	FM	ZB
Carassius auratus	Goldfish	BP	FM	OV
Cnidoglanis macrocephalus	Estuarine Cobbler	D	MM	ZB
Tandanus bostocki	Freshwater Cobbler	D	FM	ZB
Hyporhamphus melanochir	Southern Sea Garfish	P	ES	HV
Hyporhamphus regularis	Western River Garfish	P	FM	HV
Gambusia holbrooki	Mosquito Fish	SP	FM	ZB
Atherinosoma elongata	Elongate Hardyhead	SP	ES	ZB
Leptatherina presbyteroides	Presbyter's Hardyhead	SP	MM	ZP
Atherinomorus vaigensis	Ogilby's Hardyhead	SP	MM	ZB
Craterocephalus mugiloides	Mugil's Hardyhead	SP	ES	ZB
Leptatherina wallacei	Wallace's Hardyhead	SP	ES	ZP
Cleidopus gloriamaris	Pineapplefish	D	MS	ZB
Stigmatophora nigra	Wide-Bodied Pipefish	D	MS	ZB
Vanacampus phillipi	Port Phillip Pipefish	D	MS	ZB
Hippocampus angustus	Western Australian Seahorse	D	MS	ZP
Phyllopteryx taeniolatus	Common Seadragon	D	MS	ZB
Stigmatophora argus	Spotted Pipefish	D	MS	ZP
Urocampus carinirostris	Hairy Pipefish	D	ES	ZP
Filicampus tigris	Tiger Pipefish	D	MS	ZP
Pugnaso curtirostris	Pugnose Pipefish	D	MS	ZP
Gymnapistes marmoratus	Devilfish	D	MS	ZB
Chelidonichthys kumu	Red Gurnard	D	MS	ZB
Platycephalus laevigatus	Rock Flathead	D	MS	PV
Platycephalus endrachtensis	Bar-Tailed Flathead	D	ES	PV
Leviprora inops	Long-Head Flathead	D	MS	PV
Platycephalus speculator	Southern Blue-Spotted Flathead	D	ES	PV
Pegasus lancifer	Sculptured Seamoth	D	MS	ZB
Amniataba caudavittata	Yellow-Tail Trumpeter	BP	ES	OP
Pelates octolineatus	Eight-Line Trumpeter	BP	MM	OV
Pelsartia humeralis	Sea Trumpeter	BP	MS	OV
Edelia vittata	Western Pygmy Perch	BP	FM	ZB
Apogon rueppelli	Gobbleguts	BP	ES	ZB
Siphamia cephalotes	Woods Siphonfish	BP	MS	ZB
Sillago bassensis	Southern School Whiting	D	MS	ZB
Sillago burrus	Trumpeter Whiting	D	MM	ZB
Sillaginodes punctata	King George Whiting	D	MM	ZB
Sillago schomburgkii	Yellow-Finned Whiting	D	MM	ZB
Sillago vittata	Western School Whiting	D	MM	ZB
Pomatomus saltatrix	Tailor	P	MM	PV

Trachurus novaezelandiae	Yellowtail Scad	P	MS	ZB
Pseudocaranx dentex	Silver Trevally	BP	MM	ZB
Pseudocaranx wrightii	Sand Trevally	BP	MM	ZB
Arripis georgianus	Australian Herring	P	MM	PV
Arripis esper	Southern Australian Salmon	P	MS	PV
Gerres subfasciatus	Roach	BP	MM	ZB
Pagrus auratus	Snapper	BP	MM	ZB
Acanthopagrus butcheri	Southern Black Bream	BP	ES	OP
Rhabdosargus sarba	Tarwhine	BP	MM	ZB
Argyrosomus japonicus	Mulloway	BP	MM	PV
Pampeneus spilurus	Black-Saddled Goatfish	D	MS	ZB
Enoplosus armatus	Old Wife	D	MS	ZB
Aldrichetta forsteri	Yellow-Eye Mullet	P	MM	OV
Mugil cephalus	Sea Mullet	P	MM	DV
Sphyraena obtusata	Striped Barracuda	P	MS	PV
Haletta semifasciata	Blue Weed Whiting	D	MS	OV
Siphonognathus radiatus	Long-Rayed Weed Whiting	D	MS	OV
Neoodax baltatus	Little Weed Whiting	D	MS	OV
Odax acroptilus	Rainbow Cale	D	MS	OV
Parapercis haackei	Wavy Grubfish	D	MS	ZB
Petroscirtes breviceps	Short-Head Sabre Blenny	SB	MS	OV
Omobranchus germaini	Germain's Blenny	SB	MS	ZB
Parablennius intermedius	Horned Blenny	D	MS	ZB
Istiblennius meleagris	Peacock Rockskipper	D	MS	HV
Cristiceps australis	Southern Crested Weedfish	D	MS	ZB
Pseudocalliurichthys goodladi	Longspine Stinkfish	D	MS	ZB
Eocallionymus papilio	Painted Stinkfish	D	MS	ZB
Nesogobius pulchellus	Sailfin Goby	SB	MS	ZB
Favonigobius lateralis	Long-Finned Goby	SB	MM	ZB
Afurcagobius suppositus	Southwestern Goby	SB	ES	ZB
Pseudogobius olorum	Blue-Spot / Swan River Goby	SB	ES	OV
Amoya bifrenatus	Bridled Goby	SB	ES	ZB
Callogobius mucosus	Sculptured Goby	SB	MS	ZB
Callogobius depressus	Flathead Goby	SB	MS	ZB
Papillogobius punctatus	Red-Spot Goby	SB	ES	ZB
Tridentiger trigonocephalus	Trident Goby	SB	MS	ZB
Pseudorhombus jenynsii	Small-Toothed Flounder	D	MM	ZB
Ammotretis rostratus	Longsnout Flounder	D	MM	ZB
Ammotretis elongate	Elongate Flounder	D	MM	ZB
Cynoglossus broadhursti	Southern Tongue Sole	D	MS	ZB
Acanthaluteres brownie	Spiny-Tailed Leatherjacket	D	MS	OV
Brachaluteres jacksonianus	Southern Pygmy Leatherjacket	D	MS	OV
Scobinichthys granulatus	Rough Leatherjacket	D	MS	OV
Meuschenia freycineti	Sixspine Leatherjacket	D	MM	OV
Monacanthus chinensis	Fanbellied Leatherjacket	D	MM	OV
Eubalichthys mosaicus	Mosaic Leatherjacket	D	MS	OV
Acanthaluteres vittiger	Toothbrush Leatherjacket	D	MS	OV
Acanthaluteres spilomelanurus	Bridled Leatherjacket	D	MM	OV
Torquigener pleurogramma	Banded Toadfish	BP	MM	OP
Contusus brevicaudus	Prickly Toadfish	BP	MS	OP
Polyspina piosae	Orange-Barred Puffer	BP	MS	OP
Diodon nichthemenus	Globefish	D	MS	ZB
Scorpis aequipinnis	Sea Sweep	P	MS	ZP
Neatypus obliquus	Footballer Sweep	P	MS	ZP

2.2.2.1.2 Candidate fish metrics

A list of candidate fish metrics was compiled from an extensive review of existing fish-based biotic indices for estuaries worldwide, and using expert knowledge of the fish fauna of the Swan-Canning Estuary. Despite a strong focus on functional guilds in multimetric indices, it has been suggested that metrics pertaining to individual ('sentinel') species may also be useful as potential metrics for assessing ecological integrity (Noble et al. 2007). These species are expected to exhibit predictable responses to ecosystem degradation and be useful in elucidating responses to specific stressors. The Blue-Spot or Swan River Goby, *Pseudogobius olorum*, represents one such species in the Swan Estuary. It was hypothesised that the abundance of *P. olorum* will increase in response to degradation of the estuarine environment, as this omnivorous species is tolerant of hypoxic conditions (H. Gill, Murdoch University, personal communication), is able to use atmospheric oxygen via aquatic surface respiration (Gee and Gee 1991) and, within the Swan Estuary, prefers silty substrates, to which it is well adapted (Gill and Potter 1993).

Where appropriate, two potential variants of each fish metric were tested, namely 'number of taxa' and 'proportion of total individuals', as recommended by Noble *et al.* (2007). For example, for the trophic generalist metric, both 'Number of trophic generalist taxa present' and 'Proportion of total individuals belonging to the trophic generalist category' were tested. An *a priori* hypothesis was then formulated for each candidate metric, reflecting its predicted response to increasing ecosystem degradation (Table 2.2.2.2). These hypotheses were subsequently used as a framework for testing the sensitivity of each candidate metric (*i.e.* and thus its usefulness for inclusion in the final index) by determining the extent to which metric values responded, in the manner predicted, to changes in ecosystem quality.

Table 2.2.2.2: Candidate metrics for potential inclusion in a biotic index of estuarine health, and their predicted responses to degradation of the estuarine environment. 'Trophic Specialist' comprises the feeding mode guilds Zooplanktivore, Zoobenthivore, Herbivore, Piscivore; 'Trophic Generalist' comprises the feeding mode guilds Omnivore, Opportunist; 'Benthic' comprises the habitat guilds Benthopelagic, Small Benthic, Demersal; 'Estuarine Spawner' comprises the habitat guilds Estuarine and Semi-Anadromous. Where appropriate, two variants of each metric were tested, namely 'number of taxa' and 'proportion of total individuals' (variants not shown for brevity).

Metric	Description	Predicted response to degradation (supporting references)
Species diversity / composition / abundance		
Species richness	Total number of species present	**Decrease** (Karr 1981, Karr *et al.* 1986)
Dominance	Number of species comprising 90% of total individuals	**Increase** *i.e.* no. of species decreases (Odum 1983; Harrison and Whitfield 2004)
Total density	Total number of individuals per net	**Decrease** (Karr 1981, Deegan *et al.* 1997)
Introduced	Contribution of alien/introduced species	**Increase** (Kennard *et al.* 2005)
Native	Contribution of native species	**Decrease** (Kennard *et al.* 2005)
Shannon diversity	Shannon Diversity Index	**Decrease** (Odum 1983)
Pielou's evenness	Pielou's Evenness Index	**Decrease** (Odum 1983)
Trophic structure		
Trophic Specialist	Contribution of trophic specialist species	**Decrease** (Hughes *et al.* 1998)
Carnivore	Contribution of carnivorous species	**Decrease** (Harris and Silveira 1999)
Piscivore	Contribution of piscivorous species	**Decrease** (Bilkovic and Roggero 2008)
Omnivore	Contribution of omnivorous species	**Increase** (Hughes *et al.* 1998)
Opportunist	Contribution of opportunist species	**Increase** (Hughes *et al.* 1998)

Trophic Generalist	Contribution of trophic generalist species	**Increase** (Hughes *et al.* 1998)
Detritivore	Contribution of detritivorous species	**Increase** (Pilati *et al.* 2009)
Feeding Guild Composition	The number of different trophic guilds present	**Decrease** (Coates *et al.* 2007)
Habitat / life history function		
Benthic	Contribution of benthic associated species	**Decrease** (Berkman and Rabeni 1987, Barbour *et al.* 1995
Estuarine Spawner	Contribution of estuarine spawning species	**Decrease** (Harrison and Whitfield 2004)
Estuarine Resident	Contribution of estuarine resident species	**Decrease** (Harrison and Whitfield 2004)
Sentinel species		
P. olorum	Contribution of *Pseudogobius olorum*	**Increase** (Gee and Gee 1991, Gill and Potter 1993)

2.2.2.1.3 Selection of metrics sensitive to spatial changes in habitat quality

<u>Physical habitat metrics</u>

The first approach to metric selection required an initial assessment of physical habitat quality throughout the Swan-Canning Estuary to facilitate identification of those fish metrics that most clearly responded to spatial differences in ecosystem degradation in this system. This aspect of the study focused on the nearshore waters of the estuary due to the relative ease with which their habitats could be assessed and their fish faunas sampled. From a review of the available literature and a consideration of the pressures affecting the Swan-Canning Estuary, six aspects (metrics) of physical habitat quality were selected to construct a habitat quality index for this system. The rationale for focusing on each of these habitat metrics is outlined below.

(i) INSTREAM COVER. This reflected the diversity and quantity of cover available for fish, *e.g.* boulders, coarse woody debris, seagrass, macroalgae and overhanging vegetation. Instream cover has been shown to be a major determinant of the diversity of fish communities due to the greater habitat complexity, food and/or shelter it provides (Koehn 1992, O'Connor 1992, Gippel *et al.* 1996, Ohio EPA 2006, Lester and Boulton 2008, Schneider and Winemiller 2008). This aspect of habitat quality also included the presence of small tributaries, backwaters and tidal pools, which might provide small fish with refugia from predators or suitable habitats in which to spawn (de Leeuw *et al.* 2007).

(ii) SUBSTRATE. The delivery of fine sediment to the Swan-Canning Estuary has increased over past decades due to the removal of natural vegetation cover from its catchment (Swan River Trust 1999). This has led to greater turbidity and nutrient loads within the estuary, which can adversely affect fish both directly, due to physiological effects (*e.g.* by clogging their gills; Waters 1995, Bunt *et al.* 2004), and indirectly, through effects on oxygen availability, habitat complexity and predator-prey relationships (Berkman and Rabeni 1987, Cyrus and Blaber 1987, Henley *et al.* 2000, Richardson and Jowett 2002). This physical habitat metric assessed substrate quality based on the assumption that coarse or complex sediments will exhibit lower rates of resuspension

and thus lead to higher quality habitats that support a wider range of fish species than those dominated by mud or silt (Parsons *et al.* 2002, Ohio EPA 2006).

(iii) BANK STABILITY. Erosion of shorelines and riverbanks has been highlighted as a major pressure on the ecological health of the Swan-Canning Estuary, leading to increased siltation and nutrient input, reduced habitat complexity and degraded riparian zones (Swan River Trust 1999). This physical habitat metric assessed the degree of shoreline erosion at a site. The presence of human attempts to reinforce the shoreline was also considered to be indicative of localised erosion pressures, as such intervention measures typically reduce habitat quality and negatively impact biological communities (Able *et al.* 1999, Bilkovic and Roggero 2008).

(iv) RIPARIAN ZONE WIDTH and (v) RIPARIAN ZONE LONGITUDINAL EXTENT / CANOPY COVER. A consistent feature of freshwater habitat assessment schemes is a focus on the extent and quality of riparian vegetation (Petersen 1992, Ladson *et al.* 1999, Parsons *et al.* 2002, Ohio EPA 2006). Such vegetation stabilizes shorelines and reduces erosion, moderates the input of nutrients and pollutants via runoff, mitigates the impacts of urban land use and provides allochthonous sources of detrital material and structural cover components (Steedman 1988, Swan River Trust 1999, Kennish 2002, Miltner *et al.* 2004). Several studies have demonstrated the importance of riparian buffer zones in maintaining the diversity of fish communities in streams and rivers (Lammert and Allan 1999, Meador and Goldstein 2003, Brooks *et al.* 2009), and this is also assumed to be the case for the Swan-Canning Estuary.

(vi) HUMAN STRESSORS OF HABITAT/RIPARIAN ZONE. These include the presence of structures within the water channel (*e.g.* bridges, jetties, boat moorings) and on adjacent shorelines (*e.g.* agriculture, roads, houses) which may impact estuarine habitat quality via effects on hydrology or increased pollutant loads (Swan River Trust 1999, Able *et al.* 1999, Paul and Meyer 2001, Kennish 2002, Foley *et al.* 2005, Uriarte and Borja 2009).

Site selection and field assessment

A site was considered to be an area of estuarine shoreline *ca* 50 m in length that extended into the nearshore waters to a depth of *ca* 1.5 m and also landward *ca* 30 m into the riparian zone. Sites at which the nearshore fish fauna could not be sampled by seine net (*e.g.* due to steep nearshore gradients, submerged hazards or a lack of access points) were not considered for assessment. A total of 136 sites across seven regions of the Swan-Canning Estuary were selected for habitat quality assessment (see Fig. 2.2.1.1 for regional classification). The number of sites varied among regions due to differences in the prevalence of areas suitable for sampling.

A rapid visual survey of the habitat quality at each site was carried out by assigning a score of 1-20 to each of the first five physical habitat metrics. The final metric, HUMAN STRESSORS OF HABITAT/RIPARIAN ZONE, was scored according to the presence/absence of a range of human stressors, which were summed to obtain a net human impact score (Fig. 2.2.2.1). Each site was also photographed and described for future reference. In addition, four water quality parameters were measured at three random locations within each site, which were later used

in analyses of factors potentially influencing spatial differences in fish metric values (see below). These included Secchi depth (m), which was measured using a Secchi disk, and water temperature (˚C), salinity (‰) and dissolved oxygen concentration (mg L^{-1}), which were all measured in the middle of the water column using a Yellow Springs Instrument 556 MPS water quality meter. The habitat assessment at each site was completed within 15 minutes, and all sites were assessed by the same individual to eliminate observer bias. Each site was assessed once only (during spring 2007), as the scores of the physical habitat metrics were not expected to change substantially across seasons (Parsons *et al.* 2002).

Estuarine Habitat Quality Assessment Field Sheet

Habitat Quality Index Score:

Site Number:_____ Region: CH / BA / CR / LS / MD / MU / US GPS Coord's: _____

Date:_____ Time of assessment:_____ Assessor's name:_____ Photo number(s): _____

Photo info: _____

Site description (key features): _____

Weather on assessment day: _____ Rain in last week? Y / N

Water level at time of assessment: _____Other info: _____

N.B. Assess all habitat metrics for a single-bank site of length 50m. All metrics have a maximum score of 20

SUBSTRATE

Assess dominant substrate / sediment type across depths representative of those sampled by beach seining, along a 50m walked transect of the site. Score within a category depends upon extent and quality of vegetation present (exclude algae). If scattered, unattached rocks of cobble size (up to 25cm in diameter) are present, add 2 points to score and tick here [ ].

Excellent					Good					Fair					Poor				
Gravel, coarse or firm sand prevalent. Root mats and/or submerged vegetation common					Soft sand dominant with possible silt surface layer. Some root mats and/or submerged vegetation present					Silt or mud dominant. Little submerged vegetation					All mud or silt bottom. No root mat or submerged vegetation. Score awarded depends on depth and softness of sediment				
20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1

INSTREAM COVER

Score habitat according to linear amount of 50m site offering functional in-stream cover to fish community. Cover types include large boulders, rocky outcrops, woody debris such as submerged logs and snags, tree roots and root wads, submerged or emergent aquatic macrophytes, banks overhanging water deeper than ≈30cm, small tributaries. Score within a category depends upon extent and quality of cover afforded.

Excellent					Good					Fair					Poor				
Extensive cover, present throughout site. Many cover types present along ≥75% of habitat					Moderate cover, 25-75% of site. May be much of a single cover type / moderate amounts of multiple cover types					Sparse cover, 5-25% of site. Usually present in one or more isolated patches					No cover / cover almost absent. <5% of site offers cover, with no large patches				
20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1

BANK STABILITY

Consider human attempts to reinforce, stabilize or protect banks (e.g. concrete structures, rock walls, fences, log groynes and barriers, rip-rap) as evidence of erosion / stress. Where replanting has occurred, score as if vegetation were natural, then subtract 3 points from final score and tick [ ].

Excellent					Good					Fair					Poor				
Banks stable, with no alteration by humans, water flow or animal grazing					Banks are stable, but with scattered / minor evidence of stress. <25% of bank is false, eroding or artificially reinforced					Moderate alteration along 25-50% of bank length, including false and eroding banks, or artificial reinforcement					Severe evidence of alteration to over 50% of bank, including false banks, erosion or artificial reinforcement				
20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1

RIPARIAN ZONE WIDTH

Estimate width of riparian (streamside) vegetation, averaged along length of assessed site. ONLY include trees, shrubs, reed beds and natural grassland. Agricultural fields, vineyards and maintained lawns / parks should not be included in the estimation.

Excellent					Good					Fair					Poor				
Riparian zone greater than 20m in width					Riparian zone of moderate width (5-20m)					Narrow zone of riparian vegetation <5m in width					No riparian zone exists				
20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1

RIPARIAN ZONE LONGITUDINAL EXTENT and CANOPY COVER

Estimate extent of riparian vegetation along 50m bank length. Do not include ground layer (i.e. grasses). Include only shrubs, trees and vegetation capable of offering a degree of shading or cover to the aquatic habitat. Score within a category depends upon extent and quality of cover afforded. If ground layer trailing vegetation overhangs water, add 1-3 points according to extent and tick here [ ]

Excellent					Good					Fair					Poor				
Riparian vegetation continuous or present along >75% of bank length					Riparian vegetation along 75-25% of bank length. May be regularly spaced or occur in clumps					Riparian vegetation along 5-25% of bank length, usually as a small number of scattered clumps					Riparian zone vegetation absent, or isolated occurrences along <5% of bank length				
20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1

HUMAN STRESSORS OF HABITAT / RIPARIAN ZONE

Tick box for each of the following pressures that apply within the 50m length of habitat or within an adjacent 50m wide riparian zone. Add 3 points to site total (to reflect assumed higher integrity of sites unaffected by local anthropogenic stressors), then subtract from, or add to, final index the number of points indicated in column 3.

Net human impact score:

Human Pressure	Tick	Points	Comments
Industry		- 2	
Construction		- 2	
Marina		- 2	
Bridge / jetty / boat ramp		- 2	
Agriculture (cropping)		- 2	
Agriculture (grazing)		- 2	
Yacht club / rowing club		- 1	
Artificial embayment		- 1	
Transport (paths, roads)		- 1	
Boat mooring(s)		- 1	
Discharge pipe / drain outlet		- 1	
Recreation (golf / park)		- 1	
Residential housing		- 1	

WATER QUALITY PARAMETERS

Measure each parameter at 3 locations within 50m site. *denotes variables should be measured in middle of water column

Secchi depth (m)	…………	…………	…………	AVERAGE = …………………
Temperature (°C) *	…………	…………	…………	AVERAGE = …………………
Salinity (ppt) *	…………	…………	…………	AVERAGE = …………………
Dissolved Oxygen (mg/l)* …………	…………	…………	AVERAGE = …………………

NOTES

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Figure 2.2.2.1: Field sheet employed in the rapid visual assessment of habitat quality at sites throughout the Swan-Canning Estuary.

Scores for all six physical habitat metrics were summed to produce an overall habitat quality index (HQI) score for each site. Sites were then allocated to one of four habitat quality categories (HQC), depending on whether their HQI scores were >79 (Excellent), 79-54 (Good), 53-31 (Fair) or <31 (Poor).

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Sampling of the nearshore fish community

The nearshore fish community was sampled during the day in spring 2007 at 71 of the 136 sites throughout the Swan-Canning Estuary that had been allocated to a particular HQC. The sites that were selected for fish sampling were those that maximised spatial coverage throughout the estuary and, where possible, ensured sufficient replication of each HQC within each region. Thus, where the number of sites belonging to a HQC in a region was less than or equal to four, all such replicate sites were sampled. When a HQC was represented by at least five sites in a region, four of those replicates were randomly selected.

Samples of the fish fauna at each selected site were collected using the 21.5 m seine net described in subsection 2.2.1.1.1. Fish samples were immediately placed in an ice slurry and taken to the laboratory for processing. All fish were identified to species and the total number of individuals and biomass ($\pm$ 0.1 g) of fish belonging to each species in each sample were recorded. The total length of each fish was measured to the nearest 1 mm, except when large numbers of individuals of any one species were encountered in a sample, in which case the lengths of a representative subsample of 50 individuals were measured.

Metric calculation and statistical analyses

Values for each of the candidate fish community metrics were calculated for all fish samples, and boxplots were used to visually examine the extent of the relationship between the fish metric values and HQCs across all sampling sites.

The following statistical analyses were then undertaken to more rigorously examine whether spatial differences in fish metric and community composition were related to differences in physical habitat and/or water quality. These analyses, and also those in all other subsequent subsections, were carried out using the PRIMER v6 software (Clarke and Gorley 2006) with the PERMANOVA+ add-on module (Anderson *et al.* 2008).

Prior to analysis, the data for the various fish metrics in each sample were subjected to Draftsman plots, or scatterplots between every pair of metrics, to visually assess the extent to which their distributions were notably skewed. This provided a basis for selecting the most appropriate transformation to ameliorate any such effect, and thus approximate the following test assumption of homogeneous sample dispersions among groups (see Table 2.2.2.3).

Table 2.2.2.3: Transformations applied to candidate fish metrics prior to analysis of metric responses to differences in habitat quality.

Metric	Transformation
Species richness	
Dominance	
Total density	$\log_e(x+1)$
Proportion introduced	$\log_e(x+1)$
Number of introduced species	$\log_e(x+1)$
Proportion native	$\log_e((100-x)+1)$
Shannon diversity	
Pielou's evenness	
Proportion of trophic specialists	

Number of trophic specialist species
Proportion of carnivores
Number of carnivorous species
Proportion of piscivores $\quad\quad\quad\quad\quad\quad\quad$ $\log_e(x+1)$
Number of piscivorous species $\quad\quad\quad\quad\quad$ $\log_e(x+1)$
Proportion of omnivores $\quad\quad\quad\quad\quad\quad\quad$ $\sqrt{\sqrt{x}}$
Number of omnivorous species
Proportion of trophic generalists
Number of opportunist species
Proportion of opportunists
Number of trophic generalist species
Proportion of detritivores $\quad\quad\quad\quad\quad\quad$ $\log_e(x+1)$
Number of detritivorous species $\quad\quad\quad\quad$ $\log_e(x+1)$
Proportion of benthic species
Number of benthic species
Feeding guild composition
Proportion of estuarine spawners
Number of estuarine spawning species
Proportion of estuarine residents
Number of estuarine resident species
Proportion of Pseudogobius olorum
Total number of Pseudogobius olorum

The transformed fish metric data were then normalised to place all metrics on a comparable measurement scale, and then used to construct a Euclidean distance matrix containing the resemblances between all pairs of samples. To ascertain whether the suite of fish metric responses differed significantly among HQCs, accounting for any confounding influence of differences in regions, the above Euclidean matrix was then subjected to a two-way crossed HQC x region PERMANOVA, with both factors being considered as fixed. The null hypothesis and method of interpretation for this test was the same as that described in subsection 2.2.1.3.2. The same Euclidean matrix was also subjected to MDS ordination to illustrate the extent to which fish metric composition differed among HQCs and regions of the estuary.

The above analyses were also carried out on the fish species abundance data recorded at each sampling site to help elucidate the cause of any significant differences in fish metric responses among HQCs and/or regions. Species abundances were $\log_e(x+1)$ transformed prior to analysis, which was considered appropriate after applying the transformation test described in subsection 2.2.1.3.2. A Bray-Curtis similarity matrix containing the resemblances between all pairs of sampling sites was then created from the transformed data and subjected to the same PERMANOVA and MDS routines described above.

Canonical correspondence analyses (CcorA) were then used to quantify the correlations between spatial differences in fish metric or community composition and those in (i) physical habitat quality and (ii) water quality. The results of these analyses were thus used to determine the relative influence of habitat quality *vs* water quality gradients on fish metric values and community composition. Normalisation was applied to (i) the scores for the habitat quality metrics and (ii) the averages for each of the water quality variables at each sampling site, to convert all variables in each of these data matrices to common measurement

scales. The normalised matrices were then each subjected to Principal Components Analysis (PCA) to identify the principle component axis (PC1) that best captured total variability in either physical habitat quality or water quality among the 71 sampling sites (Anderson *et al.* 2008). The Canonical Analysis of Principal Coordinates (CAP) routine was then employed to relate each of the above PC axes to (i) the Euclidean distance matrix created from the transformed and normalised fish metric data and (ii) the Bray-Curtis similarity matrix created from the transformed fish abundances. As employed here, CAP thus aimed to find the subset of m principal coordinate (PCO) axes through the multivariate fish (metric or abundance) data cloud that had the strongest correlation with physical habitat quality or water quality. To avoid over-parameterisation, the value of m chosen in each case was determined parsimoniously as that which best achieved the compromise of minimising the leave-one-out residual sum of squares, whilst maximising the squared coefficient (δ^2) in the canonical correlation.

2.2.2.1.4 Selection of metrics sensitive to temporal changes

Refinement of the candidate fish metric list
Prior to selecting those fish metrics that exhibited the most pronounced and consistent inter-period differences, and thus could be considered as the most sensitive to temporal shifts in ecosystem health, the following candidate metrics were eliminated from further consideration on the basis of their highly variable distributions, direct correlation with other metrics or lack of information.

  (i) *Pielou's evenness index*, which is not definable for samples in which no fish were caught.
  (ii) *Total fish density*, which often varied over several orders of magnitude between replicate samples. Moreover, several studies have found no correlation between ecosystem degradation and total fish abundance (*e.g.* Pont *et al.* 2007) or have found this metric to be highly variable (Hughes *et al.* 1998, Harris and Silveira 1999).
  (iii) *The contribution of introduced species* and its reciprocal *the contribution of native species*, which were largely inapplicable to the Swan-Canning Estuary. Although considered to be an important stressor of estuarine systems in other regions of Australia, the influence of introduced fish species in the Swan-Canning Estuary is limited largely to the occasional presence of the Mosquitofish, *Gambusia holbrooki*, which is found only in the upper reaches of this system and rarely accounts for >0.5% of the total catch throughout the estuary.
  (iv) Various trophic structure metrics, namely *the contribution of piscivores,* which are often few in number (one or two species per sample at most) and account for a small proportion of the total catch in the Swan-Canning Estuary (usually <2% of all individuals); *the contribution of carnivores*, which provided identical information to the *trophic specialist* metric due to the general absence of herbivorous fish in the Swan-Canning Estuary; *the contributions of omnivorous* and *opportunistic species*, as the data provided by these metrics is aggregated within the *trophic generalist* metric.

Elimination of the above metrics produced a refined list of candidate metrics to be tested for inclusion in the index of estuarine health (Table 2.2.2.4). Where appropriate, two potential variants of each metric were again tested, namely 'Number of species' and 'Proportion of total individuals' (see subsection 2.2.2.1.2).

Table 2.2.2.4: Refined list of candidate metrics for possible inclusion in a biotic index of estuarine health for the Swan-Canning Estuary.

Metric	Metric code	Metric description
Species diversity / composition / abundance		
Species richness	*No species*	Total number of species present
Dominance	*Dominance*	No. of species comprising 90% of total individuals
Shannon diversity	*Sh-div*	Shannon's diversity index
Trophic structure		
Proportion of trophic specialists	*Prop trop spec*	Trophic specialists as a proportion of total individuals
Number of trophic specialists	*No trop spec*	Number of trophic specialist species
Proportion of trophic generalists	*Prop trop gen*	Trophic generalists as a proportion of total individuals
Number of trophic generalists	*No trop gen*	Number of trophic generalist species
Proportion of detritivores	*Prop detr*	Detritivores as a proportion of total individuals
Number of detritivores	*No detr*	Number of detritivorous species
Feeding Guild Composition	*Feed guild comp*	Number of different trophic guilds present
Habitat / life history function		
Proportion of benthic species	*Prop benthic*	Benthic associated as a proportion of total individuals
Number of benthic species	*No benthic*	Number of benthic associated species
Proportion of estuarine spawners	*Prop est spawn*	Estuarine spawners as a proportion of total individuals
Number of estuarine spawning species	*No est spawn*	Number of estuarine spawning species
Proportion of estuarine residents	*Prop est res*	Estuarine residents as a proportion of total individuals
Number of estuarine resident species	*No est res*	Number of estuarine resident species
Sentinel species		
Proportion of *P. olorum*	*Prop P. olorum*	*P. olorum* as a proportion of total individuals
Total density of *P. olorum*	*Tot no P. olorum*	Total abundance (density) of *P. olorum*

Collation of data sets and calculation of metrics

The temporal approach to metric selection employed the various sets of fish species abundance data collected since the late 1970s throughout the nearshore and offshore waters of the Swan-Canning Estuary (see subsection 2.2.1.1, Tables 2.2.1.1-2.2.1.2 and Fig. 2.2.1.1). As marked seasonal and regional differences in fish community composition have been documented for this system (Loneragan *et al.* 1989, Loneragan and Potter 1990, Kanandjembo *et al.* 2001a, Hoeksema and Potter 2006), both of which would increase metric variability and potentially obscure their sensitivity to inter-period changes in ecosystem condition, the data used in these temporal analyses were those that had comparable sampling locations, intra-annual timing and sampling effort. Note that the spatial separation of replicate sites for these analyses was based on the regional rather than zone demarcations of the Swan-Canning Estuary adopted in subsection 2.2.1 (see Fig. 2.2.1.1 for both demarcations), as the former were originally employed by previous researchers and thus contain a balanced number of replicate sites. The data derived from fish samples collected using different net types

(subsections 2.2.1.1.1-2.2.1.1.2, Tables 2.2.1.1-2.2.1.2 and Fig. 2.2.1.1) were also analysed separately to completely overcome the sampling biases associated with each of those methods.

Values for each of the candidate metrics in the refined list were calculated for each historical and current fish sample. The resultant data were then subjected to the following analyses to identify the subset of metrics, in both the nearshore and offshore waters, that exhibited the most pronounced and consistent inter-period differences between the late 1970s and 2008/09.

Statistical analyses – nearshore data sets
The fish metric data derived from samples collected with the 21.5, 41.5 and 102.5-133 m seine nets (hereafter "21 m data set", "41 m data set" and "102-133 m data set", respectively) were used, in combination, to select the most appropriate metrics for incorporation into an index of health for the nearshore waters of the Swan-Canning Estuary. For each of these data sets, Draftsman plots were initially constructed between each pair of metrics to ascertain (i) whether the data distribution for any metric was notably skewed, and thus the most appropriate transformation to ameliorate any such effect and (ii) the extent to which pairs of metrics were highly correlated (*i.e.* $\rho_s \geq 0.95$) and thus the level of redundancy among metrics (Clarke and Warwick 2001). The metrics *Prop trop gen*, *No detr*, *No est res* and *Prop est res* were found to be highly correlated with other metrics in each nearshore data set, and were thus eliminated from further analyses. The remaining metrics were then subjected to the following transformations to address skewness in their distributions (Table 2.2.2.5).

Table 2.2.2.5: Transformations applied to metrics in the refined candidate list for each nearshore data set for the Swan-Canning Estuary. Metrics highlighted in grey were considered redundant and thus eliminated from subsequent analyses.

Metric	21 m data set	41 m data set	102-133 m data set
No species			
Dominance			
Sh-div			
Prop trop spec			
No trop spec		$\sqrt{x}$	$\sqrt{x}$
Prop trop gen			
No trop gen			$\sqrt{x}$
Prop detr	$\log_e(x+0.001)$	$\log_e(x+0.001)$	$\sqrt{x}$
No detr			
Feed guild comp			
Prop benthic			$\sqrt{(1-x)}$
No benthic			
Prop est spawn	$1-(\log_e(x+0.01))$	$\sqrt{(1-x)}$	$\sqrt{(1-x)}$
No est spawn	$\sqrt{x}$		
Prop est res			
No est res			
Prop P. olorum	$\sqrt{x}$	$\sqrt{\sqrt{x}}$	$\sqrt{\sqrt{x}}$
Tot no P. olorum	$\log_e(x+1)$	$\log_e(x+1)$	$\log_e(x+1)$

Secondly, as the fish metrics in each nearshore data set exhibited marked differences in their degree of variation within groups of replicate samples (even after transformation), the following weighting procedure was adopted to adjust each metric by its inherent variability.

Thus, for each metric in each data set, the transformed values were divided by their average standard deviation, which was calculated from the standard deviations of the various groups of region*season replicates (K. R. Clarke, Plymouth Marine Laboratory, pers. comm.). This pre-treatment step thus downweighted the influence of highly erratic, "noisy" metrics, whilst leaving the data for those metrics with more consistent values across replicate samples relatively unchanged.

In order to focus only on the inter-period differences in fish metric composition in each of the nearshore data sets, the confounding effects of regions and seasons were removed by moving all samples to a common centroid in Euclidean space (K. R. Clarke, Plymouth Marine Laboratory, pers. comm.). This was achieved for each pre-treated metric by calculating the mean of all samples in each region*season group, then subtracting the relevant region*season mean from each sample value. The resultant data thus simply comprised the residual values for each metric, and so lacked any influence of differences among regions and seasons. For each of the nearshore data sets, a Euclidean distance matrix containing all pairs of samples was then constructed from these metric residuals and used to create a 'model resemblance matrix', whereby samples from the same period had a distance of 0 and those from different periods had a distance of 1. This model resemblance matrix, in conjunction with the data matrix of metric residuals, was then used in the following two approaches to identify those metrics which exhibited the most consistent inter-period differences.

Firstly, a distance-based redundancy analysis (RDA) was performed using the distance-based linear modeling (DISTLM) routine. This approach sought to determine the subset of predictor variables (fish metrics) which best modeled the response data cloud (the 0-1 model matrix), and thus whose values were relatively constant within any period yet differed consistently between periods. The proportion of explained variation (r^2) was calculated for each model but, as the value of this selection criterion always increases with the number of predictor variables, it was not considered to be a good basis for selection of parsimonious metric subsets. The selection criterion employed in this analysis was thus a modified version of the information criterion (AIC) described by Akaike (1973), *i.e.* AIC_c, which was developed for situations in which the number of samples (N) relative to predictor variables (q) is small, *i.e. N / q* <40 (Burnham and Anderson 2002). The selection procedure used was the 'Best' procedure, which calculates AIC_c for all possible models (combinations of predictor variables) and identifies that with the lowest AIC_c value ($AIC_{c(min)}$) as the estimated 'best' of the candidate models.

It is important to note that competing models are also useful in estimating the uncertainty associated with any likely 'best' model (Burnham and Anderson 2002). Indeed, the latter workers suggest that models with AIC_c values within 2 units of $AIC_{c(min)}$ are also substantially supported by the evidence. AIC_c differences (Δ_i) can be calculated for a competing model (i) using the equation $\Delta_i = AIC_{c(i)} - AIC_{c(min)}$, and allow comparison and ranking of competing models. Thus, for each of the nearshore data sets, the subset of models with $\Delta_i \leq 2$ were identified and their relative log likelihoods were calculated as being equal to exp(-0.5*Δ_i). To better interpret the weight of evidence supporting each of these models, their

log likelihoods were then normalised to produce a set of positive Akaike weights (w_i) summing to 1 (Burnham and Anderson 2002). Finally, evidence ratios (w_1 / w_j, where model 1 is the estimated 'best' in the set) were calculated to examine the relative likelihood of each model compared to the estimated 'best' model. Burnham and Anderson (2002) have suggested that in cases where a number of models exhibit small evidence ratios, multi-model inference (MMI) should be employed to identify the relative importance of each of the variables (metrics) across all, or an appropriate subset, of the models. A weight of evidence approach was thus adopted for selecting those metrics that exhibited the most pronounced and consistent inter-period differences, based on their relative importance among the models in the $\Delta_i \leq 2$ subset. Only those metrics which occurred in >50% of the models in the $\Delta_i \leq 2$ subset were selected.

The above DISTLM approach fits a linear combination of the fish metrics to the 0-1 model matrix but, given that linear metric responses might not reasonably be assumed, a fully non-parametric (and thus not necessarily linear) multivariate approach was also used to identify that metric subset which best matched the inter-period model matrix. This approach employed the BIOENV or BVSTEP procedures, in which the reference (0-1 model) resemblance matrix and complementary set of explanatory data (fish metric residuals) were the same as those employed in the DISTLM routine. These procedures were thus used to search for that subset of fish metrics whose pattern of rank order of resemblances best matched that defined by the inter-period model matrix. The null hypothesis and method of interpretation for these tests were the same as those described in subsection 2.1.3.5. Note that BIOENV was used to search all possible metric combinations for the 21 and 41 m data sets, whilst the larger size of the 102-133 m data set necessitated the use of the BVSTEP routine, which searches only a subset of possible metric combinations. The forward selection/ backward elimination algorithm of BVSTEP was repeated multiple times, starting with different randomly selected subsets of one to six metrics (Clarke and Warwick 1998), in order to minimise the chances of not detecting the most suitable metric subset.

Given the range of different analyses outlined above, a weight of evidence approach was adopted for consolidating, into a single set, those fish metrics which were consistently identified as among the 'best' by the DISTLM and BIOENV/BVSTEP analyses of the 21, 41 and 102-133 m data sets. Thus, a metric was selected for inclusion in the nearshore index of estuarine health if it was identified by more than one of the six analyses.

Statistical analyses – offshore data set
The gill net data set was used to select metrics for incorporation into an ecosystem health index for the offshore waters of the Swan-Canning Estuary. Draftsman plots were constructed between each pair of fish metrics in the refined candidate list to determine the appropriate data transformation in each case and identify redundant metrics for elimination from subsequent analyses (Table 2.2.2.6). As for the nearshore data sets, the metrics *Prop trop gen*, *No detr*, *No est res* and *Prop est res* were eliminated from the offshore data set due to correlations of ≥0.95 with other metrics. In addition, the metrics *Prop P. olorum* and *Tot no*

P. olorum were also eliminated, as the small goby species *Pseudogobius olorum* is not captured by the gill nets employed to sample offshore waters.

Table 2.2.2.6: Transformations applied to fish metrics in the refined candidate list for the offshore data set for the Swan-Canning Estuary. Metrics highlighted in grey were considered redundant and thus eliminated from subsequent analyses.

Metric	Gill net data set
No species	
Dominance	
Sh-div	
Prop trop spec	$\sqrt{\sqrt{x}}$
No trop spec	
Prop trop gen	
No trop gen	
Prop detr	
No detr	
Feed guild comp	
Prop benthic	$\sqrt{(1-x)}$
No benthic	
Prop est spawn	$\sqrt{(1-x)}$
No est spawn	
Prop est res	
No est res	
Prop P. olorum	
Tot no P. olorum	

The data pre-treatment procedures and analyses adopted above for the nearshore data sets were also applied to the offshore data to identify that subset of metrics that best responded to inter-period changes in ecosystem health. However, given the small number of metrics identified by the DISTLM and BIOENV analyses of the gill net data set, and the fact that only two metrics were selected by both analyses (see subsection 2.3.2.2.2), a modified decision rule was applied in this case, whereby a metric was selected for inclusion in the offshore index if it was identified by either of the two analyses.

2.2.2.2 Establishing reference conditions for estuarine health indices

2.2.2.2.1 Reference data sets
All fish species abundance data collected throughout the nearshore and offshore waters of the Swan-Canning Estuary since 1976/77, which had been appropriately standardised to minimise the effects of net-induced and/or other sampling biases (see subsections 2.2.1.3.1 and 2.2.1.4.1), were employed in determining reference conditions for each of the selected nearshore and offshore fish metrics, respectively.

2.2.2.2.2 Establishing reference conditions and scoring metrics
Reference conditions for each nearshore or offshore metric were determined by identifying the "best available" value recorded during any of the fish faunal studies carried out between 1976/77 and 2008/09. Identification of these "best" values for each metric (*i.e.* whether they

were among the lowest or highest of all values ever recorded) depended on the *a priori* hypothesis of metric response to anthropogenic degradation of the ecosystem (Table 2.2.2.2).

Values for each of the selected fish metrics were calculated from the standardised nearshore or offshore data for each historical and current fish sample. To overcome the confounding influence of spatial and seasonal differences on reference conditions, reference values for each nearshore and offshore metric were established for each zone*season combination. Note that the Ecological Management Zones were employed here rather than regions (see subsection 2.2.1.1 and Fig. 2.2.1.1) in order to make the results as relevant as possible to the activities of local environmental management agencies.

The zone*season-specific reference conditions for each nearshore and offshore metric were then used to establish metric scores for each sample via continuous scaling, as outlined by Minns *et al.* (1994), Hughes *et al.* (1998) and Hering *et al.* (2006). Thus, for negative metrics (*i.e.* those that decrease with increasing ecosystem degradation), the upper threshold (95th percentile) of metric values represented the best available reference condition and was allocated a score of 10, with metric scores decreasing to zero as metric values approached the lower threshold (5th percentile). The opposite scaling was applied for positive metrics. Upper and lower thresholds were set using percentiles, rather than minima and maxima, to avoid the influence of extreme outliers (Gibson *et al.* 2000). Scores between these upper and lower thresholds were calculated by linear interpolation. Thus, for negative metrics, the metric value was divided by the observed range of reference values and then multiplied by 10 (Minns *et al.* 1994), *i.e.*

$$Metric\,score = \frac{(Observed\,metric\,value - Lower\,threshold)}{(Upper\,threshold - Lower\,threshold)} \times 10$$

For positive metrics, the quotient was subtracted from 1 before multiplying by 10 (Ganasan and Hughes 1998), *i.e.*

$$Metric\,score = \left(1 - \frac{(Observed\,metric\,value - Lower\,threshold)}{(Upper\,threshold - Lower\,threshold)}\right) \times 10$$

In cases where metric values exceeded the upper or lower reference value thresholds (*i.e.* outliers), a metric score of 10 was allocated. Moreover, when no fish were caught in a sample, all metrics received a score of zero.

2.2.2.3 Index calculation and validation

2.2.2.3.1 Index calculation
Index scores for both the nearshore and offshore health indices were calculated by summing the scores for their component metrics then adjusting the resultant value by the number of

metrics in the index. This produced a final index score that ranged from 0-100 (Ganasan and Hughes 1998). Index scores were calculated for each historical and current fish sample, and were then averaged to provide a measure of the health of the Swan-Canning Estuary in each of the periods in which fish were sampled between 1976/77 and 2008/09.

Index scores were then used to determine thresholds for establishing qualitative estuarine health status by subdividing the possible range of index scores into four equal classes (Table 2.2.2.7).

Table 2.2.2.7: Thresholds for qualitative classification of estuarine health status on the basis of index scores that ranged between 0 and 100.

Index score	Estuarine health status
≥ 75	Good
$\geq 50 < 75$	Fair
$\geq 25 < 50$	Poor
< 25	Very poor

2.2.2.3.2 Preliminary interpretation of index performance
The performance of each selected metric for the nearshore and offshore indices was examined by determining its relative contribution to the health index score across all samples. These analyses employed only those historical and current samples in which fish were caught to eliminate any bias caused by outlying zero scores. For each of these samples, scores for each metric were plotted against those for the health index, and Spearman's correlation test was used to determine if the Spearman correlation coefficient (ρ_s) differed significantly from zero at Bonferroni-corrected significance levels of $P=0.0045$ and $P=0.007$ for the nearshore and offshore scores, respectively.

2.2.2.3.3 Index validation
<u>Index sensitivity</u>
Given the absence of existing quantitative indicators of stressors and pressures affecting the Swan-Canning Estuary, which might otherwise have provided an independent means of testing the sensitivity of health index scores, an attempt was made to evaluate index sensitivity using water quality data (salinity, temperature and dissolved oxygen concentration) collected concurrently with fish sampling in the current study. Concerns have been raised over deteriorating water quality within the Swan-Canning Estuary (Swan River Trust 1999, 2000a) and reduced dissolved oxygen concentrations, in particular, have been identified as a major stressor affecting this system (Hamilton *et al.* 2001). Analyses were thus performed to determine whether the nearshore and offshore health indices for each fish sample responded to each of the three water quality parameters measured at the time of sample collection. In the case of the offshore index, an assessment was also made of the response of index scores to dissolved oxygen stratification of the water column, expressed in the form of a stratification index (*i.e.* the absolute difference between surface and bottom

dissolved oxygen concentrations measured on a site visit). The Spearman's correlation test with Bonferroni corrections, as described in subsection 2.2.2.3.2, was used to examine the correlation between the nearshore or offshore health index scores and data for each of the above water quality parameters.

Index variability

Cross-validation approaches were used to quantify the sources of variability in the nearshore and offshore health indices and thus assess their reliability. Data collected only during the current study were used for the following cross-validation analyses as, compared to the various historical studies, the data sets from this period were collected across all regions of the estuary in eight consecutive seasons, and were thus the most comprehensive and consistently recorded.

Index variability between replicate sites

To address the question of whether the variability of index scores within groups of replicate sites differed between regions and/or seasons, the standard deviations of the scores for each of those groups were calculated and compared. This analysis focused on the regions of the Swan-Canning Estuary rather than the Ecological Management Zones (Fig. 2.2.1.1), as the former provided a more balanced number of replicate sites and a finer spatial resolution, and was thus considered more conservative for investigating index variability among replicate samples. Note that, while the standard deviations of these replicate index scores could be compared among regions and seasons, small within-group sample sizes ($n=3$) precluded formal statistical testing of differences in dispersion among those groups (Anderson *et al.* 2008).

Relationships between inter-seasonal variability and ecological quality

To address whether inter-seasonal variation in index scores at a site was related to its ecological quality, the standard deviation of the index scores among seasons in each period at each site was plotted against the corresponding mean index score. Spearman's correlation test was used to determine if ρ_s, calculated between the standard deviations and the means of the scores, differed significantly from zero at $P=0.05$.

Index variability between consecutive periods

The extent of the variability in index scores between consecutive periods, and thus its effects on the consistency of health status classifications, was determined by plotting index scores from sites assessed in each season in 2007/08 against those from the same sites and seasons in 2008/09. Spearman's correlation test was used to determine if ρ_s, calculated between the scores from the first *vs* the second of the above periods, differed significantly from zero at $P=0.05$. It was assumed that, for the index to be reliable, index scores for each site in each season should be roughly similar between consecutive periods, *i.e.* in the absence of any documented major anthropogenic impacts on the system (Harris and Silveira 1999). Moreover, inter-annual variability in site scores should not often lead to a reclassification of their estuarine health status (Harrison and Whitfield 2006).

2.2.2.3.4 Random sampling variability

Bootstrap cross-validation was used to quantify the effects of random sampling variability on index scores, as described for the IBI by Fore *et al.* (1994) and Dolph *et al.* (2010). Bootstrapping is a resampling procedure which enables estimation of the accuracy of a statistic whose distribution is unknown (*e.g.* a multimetric index score determined from a single sample; Dixon 1993), and was employed to estimate the effect on index scores of changes in the fish faunal composition of a sample that might arise from random sampling variability (Dolph *et al.* 2010). One thousand bootstrap samples were created for each fish sample collected in the current study by randomly resampling from the original sample with replacement (Efron and Tibshirani 1993). An index score was calculated for each bootstrap sample, and these scores were then averaged for each site visit. The percentile method (Efron and Tibshirani 1993) was used to estimate a 95% confidence interval for these average index scores, and the lengths of these confidence intervals were determined from the difference between the upper and lower confidence limits (Dolph *et al.* 2010).

The results of the bootstrap resampling procedures were used to examine the precision and bias of index scores. The former was tested using simple linear regression to determine the relationship between confidence interval length and (i) total numbers of fish per sample and (ii) total numbers of species per sample, whilst the bias of the index was quantified for each site visit by subtracting the original index score from the mean bootstrapped score (Fore *et al.* 1994).

Finally, the effect of sampling variability on the consistency of health status classifications was also investigated by determining the proportion of samples for which the health status indicated by the mean bootstrapped score differed from that of the original index score.

2.3 Results

2.3.1 Changes in the characteristics of the fish fauna of the Swan-Canning Estuary between 1978 and 2009

2.3.1.1 Seine net comparison

For each of the five habitat guilds, when assuming a Poisson distribution for the counts of fish, the fitted model that produced the lowest values of the AIC was $c \sim O + N + offset$ $(log_e[A])$. In each case, however, the residual deviances greatly exceeded the residual degrees of freedom (with dispersion parameter φ in the range of 2.97 to 142.3), indicating that the counts for each of the habitat guilds exhibited high levels of overdispersion and were thus not well described by a Poisson distribution.

When a negative binomial distribution was assumed for the counts, the model structure that produced the lowest AIC value was again $c \sim O + N + offset (log_e[A])$ in the case of the small pelagic, demersal, pelagic and small benthic guilds, but was $c \sim O + offset (log_e[A])$ for the benthopelagic guild. The residual deviances were comparable to the residual degrees of freedom in each case (φ=0.37-1.73), indicating that the counts for each of the habitat guilds were reasonably well described by this distribution. As the best model for the counts of fish belonging to the benthopelagic guild did not include the parameter relating to net type, the null hypothesis was simply accepted in this case. For each of the other four habitat guilds, the best-fitted negative binomial model revealed statistically significant differences between the counts from the 41.5 and/or 133 m net and those from the 21.5 m net (Table 2.3.1.1).

Table 2.3.1.1: Parameter estimates, their associated standard errors (SE) and *z*-statistics for the effects of the 41.5 and 133 m seine nets relative to the 21.5 m seine, derived from negative binomial modelling of the counts of fish belonging to each of five habitat guilds; * denotes significant effect of net type on fish counts (*P*<0.001), NA denotes cases where the parameter of net type was absent from the best generalised linear model.

	Habitat guild				
	Small pelagic	Demersal	Benthopelagic	Pelagic	Small benthic
Residual deviance	134.83	6.144	128.73	29.115	131.23
Residual degrees of freedom	78	78	80	78	78
Dispersion parameter	1.73	0.98	1.61	0.37	1.68
41.5 m seine					
Estimate	-3.026	-0.097	NA	2.509	-1.042
SE	0.244	0.286	NA	0.592	0.207
z	-12.40*	-0.34	NA	4.24*	-5.04*
133 m seine					
Estimate	-2.432	-1.547	NA	-0.355	-3.348
SE	0.237	0.279	NA	2.76×10^6	0.211
z	-10.25*	5.55*	NA	-1.29×10^{-5}	-15.90*

Net equivalence factors were derived from each of the statistically significant net parameter coefficients, and were then used to standardise the counts for each species (*i.e.* on the basis of

their assigned guild) in each sample collected with the 41.5 or 133 m net to those that would have most likely been recorded in the 21.5 m net. Net equivalence factors for the 41.5 m net ranged from 0.03 for larger pelagic species to 8.73 for small pelagics and, for the 133 m net, from 0.19 for demersal species to 1.17 for small benthic species (Table 2.3.1.2). The 95% confidence intervals for some of these equivalence factors were large, reflecting the relatively large standard errors for the parameter estimates from which they were derived.

Table 2.3.1.2: Equivalence factors and associated upper and lower 95% confidence intervals (CI) derived for five fish habitat guilds, for standardising historical counts of fish obtained using the 41.5-133 m seine nets to equivalent counts per 21.5 m seine. Equivalence factors for the 102.5 m seine and half-net 133 m seine were derived from parameter estimates of the effect of the 133 m seine; NA denotes no significant effect of net type on fish counts identified from the best-fitting generalised linear model.

	Habitat guild				
	Small pelagic	Demersal	Benthopelagic	Pelagic	Small benthic
41.5 m seine					
Equivalence factor	8.73	NA	NA	0.03	1.20
Upper CI	14.22	NA	NA	0.11	1.81
Lower CI	5.36	NA	NA	0.01	0.79
133 m seine					
Equivalence factor	0.47	0.19	NA	NA	1.17
Upper CI	0.75	0.34	NA	NA	1.79
Lower CI	0.29	0.11	NA	NA	0.77
102.5 m seine					
Equivalence factor	0.79	0.33	NA	NA	1.98
Upper CI	1.27	0.57	NA	NA	3.01
Lower CI	0.49	0.19	NA	NA	1.30
133 m seine (half net)					
Equivalence factor	1.88	0.77	NA	NA	4.69
Upper CI	3.01	1.35	NA	NA	7.15
Lower CI	1.17	0.44	NA	NA	3.07

Figure 2.3.1.1 compares the biases of the 41.5 and 133 m nets relative to the 21.5 m net in terms of (a) total fish counts per 116 m^2 (*i.e.* the area swept by the 21.5 m seine) without any standardisation for net type and (b) total fish counts after standardisation of catch data to expected counts per 21.5 m seine. Without standardisation, estimates of total fish density in samples collected with the 41.5 and 133 m seines were far lower than those in samples obtained with the 21.5 m net. Following standardisation, the total fish densities in samples collected with the 41.5 m net were again underestimates relative to those for the 21.5 m net, whilst those predicted for samples collected with the 133 m net were overestimates. However, for both of these larger nets, the adjusted density estimates were far closer to those recorded using the 21.5 m seine, thus indicating that the standardisation of fish abundance data has greatly reduced the effects of the biases introduced by these different net types.
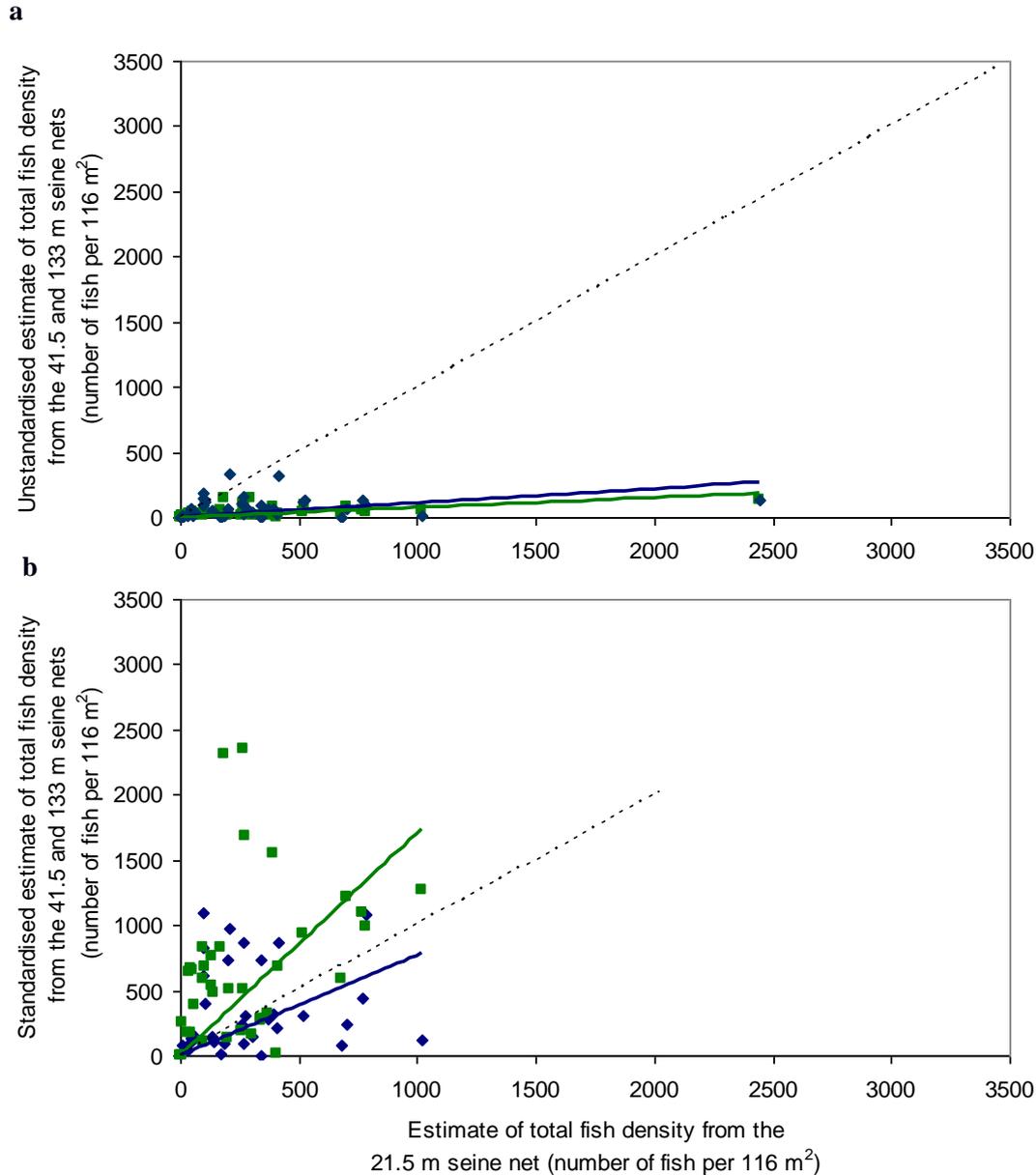
a

b

Estimate of total fish density from the
21.5 m seine net (number of fish per 116 m^2)

Figure 2.3.1.1: Plots of total fish densities obtained using the 41.5 m (blue) and 133 m (green) seine nets *vs* those obtained using the 21.5 m seine. Plot (a) shows unstandardised fish densities and plot (b) shows fish densities following standardisation of the counts of each species in the former two net types to expected counts per 21.5 m seine. The broken line illustrates a 1:1 relationship between density estimates from the different net types. N.B. plots presented exclude two obvious outlier samples.

2.3.1.2 Differences in the nearshore fish fauna among periods

2.3.1.2.1 Mean species densities
The mean density of each fish species caught in the nearshore waters of the Swan-Canning Estuary during each of the six studies carried out between 1978 and 2009 are provided in Table 2.3.1.3. Note that these species densities represent the number of fish per 100m^2, averaged across all samples collected in any given study, and that all data have been

Table 2.3.1.3: Mean density (Mean; *i.e.* number of fish 100m^{-2}), standard deviation (^{sd}), percentage contribution to the overall catch (%) and rank by density (R) of each fish species recorded in each study carried out in the nearshore waters of the Swan-Canning Estuary between 1978/79 and 2008/09. All data have been standardised for net type and spatio-temporal sampling intensity as per the methods described in subsection 2.2.1.3.1. Abundant species (*i.e.* those that contribute >5% to the catch) are highlighted in grey. The life-history category of each species (^{LH}) is also provided (*i.e.* ^{A}=semi-anadromous, ^{E}=estuarine, ^{EM}=estuarine and marine, ^{F}=freshwater, ^{O}=marine estuarine-opportunist, ^{S}=marine straggler). The total number of species and the total mean density of individuals are also given for each study.

Species [LH]	Common Name	Loneragan *et al.* 1989 (1978-81) Mean^{sd}	%	R	Kanandjembo *et al.* 2001a (1995-97) Mean^{sd}	%	R	Hoeksema and Potter 2006 (1999-01) Mean^{sd}	%	R	Valesini *et al.* 2005 (2003/04) M^{sd}	%	R	Valesini *et al.* 2009 (2005/06) Mean^{sd}	%	R	Current (2007-09) Mean^{sd}	%	R
Nematalosa vlaminghi ^{A}	Perth Herring	378.93$^{1365.34}$	44.89	1	47.22$^{147.62}$	3.64	6	4.55$^{17.77}$	1.85	6	1.31$^{4.82}$	0.11	18	0.92$^{2.83}$	0.40	15	17.05$^{70.24}$	3.65	8
Leptatherina presbyteroides ^{O}	Presbyter's Hardyhead	63.02$^{412.23}$	7.47	2	23.40$^{116.97}$	1.80	7				39.78$^{295.57}$	3.23	5	67.57$^{184.53}$	29.68	1	27.73$^{137.54}$	5.94	5
Leptatherina wallacei ^{E}	Wallace's Hardyhead	51.97$^{235.93}$	6.16	3	171.97$^{255.27}$	13.26	2	141.62$^{114.60}$	57.69	1	371.34$^{889.08}$	30.14	2	29.05$^{67.22}$	12.76	3	160.27$^{429.04}$	34.32	1
Favonigobius lateralis ^{EM}	Long-finned Goby	50.02$^{152.74}$	5.93	4				<0.01$^{0.03}$	<0.01	26	0.08$^{0.41}$	0.01	23	7.95$^{12.22}$	3.49	9	6.73$^{20.45}$	1.44	13
Atherinomorus ogilbyi ^{O}	Ogilby's Hardyhead	47.18$^{220.36}$	5.59	5	57.57$^{169.51}$	4.44	4	0.36$^{1.49}$	0.15	14	10.39$^{44.58}$	0.84	9	13.13$^{69.79}$	5.77	6	39.65$^{361.76}$	8.49	3
Pelates octolineatus ^{O}	Western Striped Grunter	34.66$^{200.63}$	4.11	6	3.30$^{16.91}$	0.25	15	1.34$^{4.00}$	0.54	10	2.49$^{7.12}$	0.20	14	2.24$^{7.02}$	0.98	13	15.80$^{145.24}$	3.38	10
Pseudogobius olorum ^{E}	Blue-spot Goby	34.64$^{140.78}$	4.10	7	7.89$^{15.14}$	0.61	10	54.88$^{64.58}$	22.35	2	6.40$^{11.05}$	0.52	11	8.32$^{34.70}$	3.65	8	18.15$^{81.89}$	3.89	6
Mugil cephalus ^{O}	Sea Mullet	34.51$^{52.17}$	4.09	8	0.59$^{0.69}$	0.05	20	2.24$^{5.99}$	0.91	8	0.02$^{0.12}$	<0.01	28	0.74$^{1.87}$	0.32	18	0.44$^{1.21}$	0.09	22
Amniataba caudavittata ^{E}	Yellowtail Grunter	26.72$^{203.86}$	3.17	9	11.78$^{40.70}$	0.91	8	3.84$^{9.62}$	1.56	7	11.45$^{30.50}$	0.93	8	3.53$^{7.20}$	1.55	12	10.91$^{47.19}$	2.34	12
Torquigener pleurogramma ^{O}	Banded Toadfish	24.29$^{89.23}$	2.88	10	1.90$^{6.30}$	0.15	17				21.01$^{48.97}$	1.71	6	31.36$^{43.81}$	13.78	2	30.95$^{76.75}$	6.63	4
Apogon rueppellii ^{EM}	Western Gobbleguts	22.66$^{65.90}$	2.68	11	1.64$^{6.70}$	0.13	18	0.01$^{0.10}$	<0.01	23	52.28$^{135.71}$	4.24	3	5.42$^{30.43}$	2.38	11	4.88$^{24.05}$	1.04	14
Aldrichetta forsteri ^{O}	Yellow-eye Mullet	19.10$^{53.60}$	2.26	12	0.35$^{0.61}$	0.03	23	1.06$^{4.94}$	0.43	11	0.06$^{0.22}$	<0.01	25	2.12$^{6.79}$	0.93	14	0.13$^{0.75}$	0.03	26
Engraulis australis ^{EM}	Southern Anchovy	14.66$^{68.17}$	1.74	13	755.80$^{6112.70}$	58.28	1	0.04$^{0.28}$	0.02	17	7.97$^{28.51}$	0.65	10	0.10$^{0.59}$	0.04	27	3.64$^{28.81}$	0.78	16
Papillogobius punctatus ^{E}	Red-spot Goby	8.66$^{24.85}$	1.03	14	8.02$^{21.41}$	0.62	9	0.53$^{1.74}$	0.22	13	5.74$^{12.78}$	0.47	13	6.03$^{12.42}$	2.65	10	16.62$^{35.96}$	3.56	9
Atherinosoma mugiloides ^{E}	Mugil's Hardyhead	7.13$^{19.52}$	0.84	15	51.36$^{169.62}$	3.96	5	0.16$^{0.62}$	0.07	16	619.70$^{3633.45}$	50.30	1	19.28$^{52.72}$	8.47	4	72.93$^{243.67}$	15.62	2
Hyperlophus vittatus ^{O}	Sandy Sprat	4.55$^{30.91}$	0.54	16	5.47$^{30.15}$	0.42	12							0.03$^{0.13}$	0.01	37	0.35$^{4.07}$	0.07	23
Atherinosoma elongata ^{E}	Elongate Hardyhead	4.09$^{16.81}$	0.49	17	131.11$^{287.97}$	10.11	3				49.62$^{325.85}$	4.03	4	0.68$^{3.34}$	0.30	19	11.76$^{65.16}$	2.52	11
Acanthopagrus butcheri ^{E}	Southern Black Bream	3.67$^{23.17}$	0.44	18	6.21$^{12.05}$	0.48	11	4.60$^{10.03}$	1.87	5	18.44$^{31.45}$	1.50	7	11.04$^{17.33}$	4.85	7	17.16$^{32.72}$	3.67	7
Pomatomus saltatrix ^{O}	Tailor	2.55$^{9.04}$	0.30	19	0.05$^{0.20}$	<0.01	26	0.03$^{0.17}$	0.01	20	0.02$^{0.12}$	<0.01	28				0.01$^{0.09}$	<0.01	38
Afurcagobius suppositus ^{E}	South-western Goby	2.24$^{18.71}$	0.27	20	2.02$^{3.42}$	0.16	16	19.49$^{28.27}$	7.94	3	2.34$^{7.52}$	0.19	15	0.88$^{2.26}$	0.39	16	4.35$^{14.59}$	0.93	15

		Loneragan *et al.* 1989 (1978-81)			Kanandjembo *et al.* 2001a (1995-97)			Hoeksema and Potter 2006 (1999-01)			Valesini *et al.* 2005 (2003/04)			Valesini *et al.* 2009 (2005/06)			Current (2007-09)		
Species [LH]	Common Name	Mean^{sd}	%	R	Mean^{sd}	%	R	Mean^{sd}	%	R	M^{sd}	%	R	Mean^{sd}	%	R	Mean^{sd}	%	R
Gerres subfasciatus [O]	Roach	1.97$^{10.59}$	0.23	21	0.72$^{2.90}$	0.06	19	2.22$^{5.04}$	0.90	9	1.08$^{2.78}$	0.09	19	0.25$^{0.78}$	0.11	23	0.99$^{5.06}$	0.21	20
Amoya bifrenatus [EM]	Bridled Goby	1.88$^{4.08}$	0.22	22	3.66$^{7.26}$	0.28	14	0.20$^{0.94}$	0.08	15	1.51$^{3.72}$	0.12	16	0.09$^{0.56}$	0.04	28	1.50$^{10.94}$	0.32	18
Gambusia affinis [F]	Eastern Gambusia	1.86$^{12.41}$	0.22	23	0.43$^{2.88}$	0.03	21	7.24$^{14.69}$	2.95	4	0.55$^{2.50}$	0.04	20	0.75$^{4.69}$	0.33	17	1.28$^{5.68}$	0.27	19
Spratelloides robustus [O]	Blue Sprat	1.10$^{9.56}$	0.13	24										13.77$^{85.35}$	6.05	5	1.78$^{16.26}$	0.38	17
Sillaginodes burrus [O]	Trumpeter Whiting	0.61$^{2.62}$	0.07	25	3.68$^{10.22}$	0.28	13	0.02$^{0.20}$	0.01	21	1.34$^{3.35}$	0.11	17	0.58$^{2.64}$	0.26	20	0.90$^{4.79}$	0.19	21
Galaxias occidentalis [F]	Western Minnow	0.26$^{1.49}$	0.03	26	0.10$^{0.40}$	0.01	25	0.95$^{1.79}$	0.39	12				0.05$^{0.31}$	0.02	32	0.06$^{0.50}$	0.01	30
Rhabdosargus sarba [O]	Tarwhine	0.16$^{0.75}$	0.02	27	0.12$^{0.53}$	0.01	24				0.26$^{1.00}$	0.02	21	0.04$^{0.25}$	0.02	34	0.06$^{0.44}$	0.01	32
Haletta semifasciata [S]	Blue Weed Whiting	0.15$^{0.88}$	0.02	28										0.32$^{1.13}$	0.14	22	0.18$^{0.82}$	0.04	24
Platycephalus endrachtensis [E]	Bar-tailed Flathead	0.11$^{0.21}$	0.01	29	0.02$^{0.14}$	<0.01	27	0.03$^{0.10}$	0.01	19	0.22$^{0.60}$	0.02	22	0.06$^{0.12}$	0.02	30	0.08$^{0.38}$	0.02	28
Trachurus novaezelandiae [S]	Yellowtail Scad	0.10$^{0.65}$	0.01	30															
Sardinella lemuru [S]	Scaly Mackerel	0.10$^{1.20}$	0.01	31															
Contusus brevicaudus [O]	Prickly Toadfish	0.06$^{0.25}$	0.01	32															
Sillago schomburgkii [O]	Yellow-finned Whiting	0.06$^{0.42}$	0.01	33	0.41$^{3.45}$	0.03	22	<0.01$^{0.03}$	<0.01	26	0.08$^{0.41}$	0.01	23	0.10$^{0.37}$	0.04	26	0.04$^{0.35}$	0.01	33
Urocampus carinirostris [EM]	Hairy Pipefish	0.05$^{0.14}$	0.01	34							0.06$^{0.36}$	<0.01	25	<0.01$^{0.03}$	<0.01	48	<0.01$^{0.06}$	<0.01	44
Cnidoglanis macrocephalus [EM]	Estuarine Cobbler	0.04$^{0.11}$	<0.01	35	0.02$^{0.14}$	<0.01	28										0.01$^{0.09}$	<0.01	38
Scorpis aequipinnis [S]	Sea Sweep	0.04$^{0.39}$	<0.01	36															
Acanthaluteres brownii [S]	Spiny-tail Leatherjacket	0.04$^{0.35}$	<0.01	37													<0.01$^{0.06}$	<0.01	44
Gymnapistes marmoratus [O]	Devilfish	0.04$^{0.17}$	<0.01	38	0.01$^{0.10}$	<0.01	30							0.20$^{0.51}$	0.09	24	0.06$^{0.35}$	0.01	31
Pseudorhombus jenynsii [O]	Small-toothed Flounder	0.03$^{0.17}$	<0.01	39							0.05$^{0.26}$	<0.01	27	0.04$^{0.11}$	0.02	33	0.03$^{0.17}$	0.01	34
Monacanthus chinensis [S]	Fanbelly Leatherjacket	0.03$^{0.28}$	<0.01	40										0.02$^{0.10}$	0.01	41			
Hyporhamphus regularis [E]	River Garfish	0.03$^{0.22}$	<0.01	41															
Carangid sp. (unid. juv.)		0.03$^{0.15}$	<0.01	42															
Scobinichthys granulatus [S]	Rough Leatherjacket	0.02$^{0.12}$	<0.01	43										0.01$^{0.05}$	0.01	42			
Stigmatophora argus [S]	Spotted Pipefish	0.02$^{0.09}$	<0.01	44										0.54$^{1.41}$	0.24	21	0.15$^{0.81}$	0.03	25

Species [LH]	Common Name	Loneragan et al. 1989 (1978-81)			Kanandjembo et al. 2001a (1995-97)			Hoeksema and Potter 2006 (1999-01)			Valesini et al. 2005 (2003/04)			Valesini et al. 2009 (2005/06)			Current (2007-09)		
		Mean^{sd}	%	R	Mean^{sd}	%	R	Mean^{sd}	%	R	M^{sd}	%	R	Mean^{sd}	%	R	Mean^{sd}	%	R
Meuschenia freycineti [S]	Six-spine Leatherjacket	$0.02^{0.15}$	<0.01	45										$0.05^{0.24}$	0.02	31			
Callogobius mucosus [S]	Sculptured Goby	$0.02^{0.15}$	<0.01	46															
Sillaginodes punctata [O]	King George Whiting	$0.01^{0.08}$	<0.01	47										$0.04^{0.14}$	0.02	36	$0.13^{0.93}$	0.03	27
Carassius auratus [F]	Goldfish	$0.01^{0.14}$	<0.01	48				$0.01^{0.07}$	<0.01	24									
Tridentiger trigonocephalus [S]	Trident Goby	$0.01^{0.11}$	<0.01	49															
Neoodax balteatus [S]	Little Weed Whiting	$0.01^{0.05}$	<0.01	50										$0.12^{0.38}$	0.05	25	$0.01^{0.09}$	<0.01	38
Edelia vittata [F]	Western Pygmy Perch	$<0.01^{0.04}$	<0.01	51				$0.01^{0.05}$	<0.01	24									
Hippocampus angustus [S]	Western Spiny Seahorse	$<0.01^{0.04}$	<0.01	52															
Parupeneus spilurus [S]	Blacksaddle Goatfish	$<0.01^{0.03}$	<0.01	53															
Enoplosus armatus [S]	Old Wife	$<0.01^{0.04}$	<0.01	54										$0.03^{0.16}$	0.01	38	$0.01^{0.14}$	<0.01	36
Sphyraeana obtusata [S]	Striped Seapike	$<0.01^{0.03}$	<0.01	55															
Eubalichthys mosaicus [S]	Mosaic Leatherjacket	$<0.01^{0.02}$	<0.01	56															
Arripis georgianus [O]	Australian Herring	$<0.01^{0.02}$	<0.01	57										$<0.01^{0.03}$	<0.01	48	$<0.01^{0.06}$	<0.01	49
Eocallionymus papilio [S]	Painted Stinkfish	$<0.01^{0.02}$	<0.01	57															
Hyporhamphus melanochir [EM]	Southern Garfish	$<0.01^{0.01}$	<0.01	59													$<0.01^{0.06}$	<0.01	44
Brachaluteres jacksonianus [S]	Sth[n] Pygmy Leatherjacket	$<0.01^{0.01}$	<0.01	59															
Platycephalus laevigatus [S]	Rock Flathead	$<0.01^{0.01}$	<0.01	61										$<0.01^{0.03}$	<0.01	48			
Leviprora inops [S]	Longhead Flathead	$<0.01^{0.01}$	<0.01	61															
Pelsartia humeralis [S]	Sea Trumpeter	$<0.01^{0.01}$	<0.01	61															
Cristiceps australis [S]	Southern Crested Weedfish	$<0.01^{0.01}$	<0.01	61										$0.01^{0.05}$	<0.01	44			
Diodon nicthemerus [S]	Globefish	$<0.01^{0.01}$	<0.01	61															
Siphamia cephalotes [S]	Wood's Siphonfish	$<0.01^{0.01}$	<0.01	66										$0.01^{0.04}$	<0.01	46			
Argyrosomus japonicus [O]	Mulloway	$<0.01^{0.01}$	<0.01	66															
Parablennius intermedius [S]	Horned Blenny				$0.02^{0.20}$	<0.01	28												

Species [LH]	Common Name	Loneragan et al. 1989 (1978-81)			Kanandjembo et al. 2001a (1995-97)			Hoeksema and Potter 2006 (1999-01)			Valesini et al. 2005 (2003/04)			Valesini et al. 2009 (2005/06)			Current (2007-09)		
		Mean^{sd}	%	R	Mean^{sd}	%	R	Mean^{sd}	%	R	M^{sd}	%	R	Mean^{sd}	%	R	Mean^{sd}	%	R
Elops machnata [S]	Australian Giant Herring				$0.01^{0.10}$	<0.01	30												
Sardinops neopilchardus [S]	Australian Sardine							$0.03^{0.28}$	0.01	18									
Atherinid sp. (unid. juv.)								$0.02^{0.11}$	0.01	22	$6.36^{23.15}$	0.52	12						
Sillago vittata [O]	Western School Whiting													$0.06^{0.37}$	0.03	29			
Ammotretis elongatus [O]	Elongate Flounder													$0.04^{0.13}$	0.02	35	$<0.01^{0.06}$	<0.01	43
Pugnaso curtirostris [S]	Pugnose Pipefish													$0.03^{0.13}$	0.01	38			
Acanthaluteres spilomelanurus [S]	Bridled Leatherjacket													$0.02^{0.08}$	0.01	40			
Arripis truttaceus [S]	Western Australian Salmon													$0.01^{0.09}$	0.01	42	$0.01^{0.13}$	<0.01	38
Monocanthid sp. (unid. juv.)														$0.01^{0.06}$	<0.01	45	$0.08^{0.55}$	0.02	29
Siphonognathus radiatus [S]	Long-ray Weed Whiting													$0.01^{0.04}$	<0.01	46	$0.01^{0.14}$	<0.01	36
Phyllopteryx taeniolatus [S]	Common Seadragon													$<0.01^{0.03}$	<0.01	48			
Filicampus tigris [S]	Tiger Pipefish													$<0.01^{0.03}$	<0.01	48			
Mullid sp. (unid. juv.)														$<0.01^{0.03}$	<0.01	48			
Acanthaluteres vittiger [S]	Toothbrush Leatherjacket													$<0.01^{0.03}$	<0.01	48			
Petroscirtes breviceps [S]	Shorthead Sabretooth Blenny																$0.02^{0.21}$	0.01	35
Omobranchus germaini [S]	Germain's Blenny																$0.01^{0.13}$	<0.01	38
Odax acroptilus [S]	Rainbow Cale																$<0.01^{0.06}$	<0.01	44
Gobiid sp. (unid. juv.)																	$<0.01^{0.06}$	<0.01	44
Number of species		**67**			**31**			**27**			**29**			**54**			**49**		
Total mean density		**844.11**			**1296.86**			**245.48**			**1231.94**			**227.68**			**466.96**		

standardised for differences in net type and spatio-temporal sampling intensity as per the methods described in subsection 2.2.1.3.1. However, despite these corrections, the comparability of the data among some studies is reduced by the fact that not all workers sampled all of the same zones of the estuary (see Table 2.2.1.1).

The most conspicuous difference was the marked reduction in the mean density of *Nematalosa vlaminghi* (Perth Herring) between the study carried out by Loneragan *et al.* (1989) in the late 1970s/early 1980s ($\approx$380 fish 100m^{-2}, comprising nearly 45% of the overall catch) and all other subsequent studies between 1995 and 2009 (0.92-47.22 fish 100m^{-2}, comprising 0.11-3.64% of the catch). This reduction was particularly marked in each of the three studies carried out between 1999 and 2006, the latter two of which examined all of the same zones as Loneragan *et al.* (1989) (Table 2.3.1.3). Other species that exhibited notable declines in mean density between the late 1970s/early 1980s and all other subsequent studies included *Favonigobius lateralis* (Long-finned Goby), *Pelates octolineatus* (Western Striped Grunter), *Mugil cephalus* (Sea Mullet), *Amniataba caudavittata* (Yellowtail Grunter) and *Aldrichetta forsteri* (Yellow-eye Mullet) (Table 2.3.1.3).

In contrast to the above, several other species exhibited marked increases in their mean density between the earliest study and all or most of the more recent studies. These included the estuarine atherinid *Leptatherina wallacei* (Wallace's Hardyhead), which was three to seven times less abundant in the late 1970s/early 1980s than in all other subsequent studies, except for that in 2005/06 by Valesini *et al.* (2009) (Table 2.3.1.3). However, despite these large differences in density, *L. wallacei* still ranked among the three most abundant species in all studies. Other species that have increased in prevalence include *Torquigener pleurogramma* (Banded Toadfish), *Atherinosoma mugiloides* (Mugil's Hardyhead) and *Acanthopagrus butcheri* (Southern Black Bream). Note that, as the first of these species is a marine-estuarine opportunist and thus largely tends to inhabit only the Lower Swan-Canning Estuary (LSCE), this trend in increased abundance from the earliest to more recent periods was derived only from comparing those studies in which that lower estuarine zone was sampled, namely Loneragan *et al.* (1989), Valesini *et al.* (2005, 2009) and the current study.

Several other species ranked among the most abundant in particular studies, but such findings were seemingly more a reflection of the particular estuary zones that were sampled and/or the pronounced schooling behaviour of those species rather than notable temporal trends. For example, *Leptatherina presbyteroides* (Presbyter's Hardyhead), a small, highly schooling marine-estuarine opportunist species, ranked in the top five species during the Loneragan *et al.* (1989), Valesini *et al.* (2005, 2009) and current studies, and had similarly high mean densities in the first and third of those cases. However, it was either not recorded or was recorded in substantially lower mean densities in the remaining studies, both of which did not sample the LSCE zone in which this species typically occurs (Tables 2.2.1.1 and 2.3.1.3). Similarly, the prominence of *Pseudogobius olorum* (Blue-spot Goby) and *Afurcagobius suppositus* (South-western Goby) in the Hoeksema and Potter (2006) study most likely reflects the restriction of sampling activity to the Middle Swan Estuary (MSE) and Upper Swan Estuary (USE) zones where these estuarine species are typically found. Lastly, the high

mean densities of *Engraulis australis* (Southern Anchovy) and *Atherinosoma elongata* (Elongate Hardyhead) in the Kanandjembo *et al.* (2001a) study and, to a lesser extent, of *Spratelloides robustus* (Blue Sprat) in the Valesini *et al.* (2009) study, most likely reflect chance intercepts of large schools of these small species on a relatively small number of occasions. This is supported by the high standard deviations associated with the mean densities of those species in those studies, particularly in the first of these cases.

The overall mean density of fish was notably higher in the studies carried out by Kanandjembo *et al.* (2001a) and Valesini *et al.* (2005) and the lowest, by far, in those undertaken by Hoeksema and Potter (2006) and Valesini *et al.* (2009). The high densities in the first two studies were due largely to the capture of large schools of *E. australis* and *A. mugiloides,* respectively, in a small number of replicate samples. The total number of species was notably higher in the study carried out by Loneragan *et al.* (1989), *i.e.* 67 species, followed by that recorded by Valesini *et al.* (2009) and in the current study, *i.e.* 54 and 49 species, respectively. However, many of these species were marine stragglers or marine estuarine-opportunists and reflect the fact that the above three studies included sampling in the LSCE, which was largely absent from the remaining three studies.

2.3.1.2.2 Mean overall density and taxonomic distinctness
Three-way period x season x zone PERMANOVA of the total fish densities recorded seasonally in each of the 12 periods sampled between 1978/79 and 2008/09 in the MSE, USE and Canning Estuary/Lower Canning River (CELCR) identified significant differences in this dependent variable among each of the three main effects and the interaction between periods and zones (P=0.001; Table 2.3.1.4a). The components of variation for each of these significant terms demonstrated that differences among seasons exerted the greatest influence on fish density, followed by those among the period x zone interaction and the period main effect. Differences among the above three zones, however, exerted a relatively small influence on fish densities.

A plot of the mean fish densities in each period and zone demonstrated that, in the MSE and USE, values were higher in 1978/79 and 1979/80 than in any other period, with exceptionally high densities being recorded in the former zone and period (Fig. 2.3.1.2a). This was also generally true for the CELCR, with the exception of 1996/97 and 2003/04. The lowest densities of fish were recorded in 2003/04 and 2005/06 in the MSE, 1981/82, 1995/96 and 2003/04 in the USE and in 2005/06 and 2008/09 in the CELCR. The significant period x zone interaction term was clearly the result of differences in the magnitude and pattern of inter-period differences among the three zones. For example, mean fish density differed markedly among zones in 1978/79 and 1981/82, whereas it differed very little among zones in 2005/06 and 2007/08 (Fig. 2.3.1.2a).

When the same three-way PERMANOVA design was used to test for differences in the quantitative average taxonomic distinctness of the fish assemblage (a diversity index), significant differences were detected among periods, zones and the interaction between these two main effects (P=0.001-0.002; Table 2.3.1.4b). As indicated by the associated

Table 2.3.1.4: Mean squares (MS), pseudo F-ratios, significance levels (*P*) and components of variation (COV) for zone x season x period PERMANOVAs on the data for nearshore fish (a) total density, (b) quantitative average taxonomic distinctness and (c) assemblage composition recorded seasonally throughout the CELCR, MSE and USE zones of the Swan-Canning Estuary in each period sampled between 1978/79 and 2008/09. df=degrees of freedom; E=exponential. Significant results involving period are highlighted in bold.

	df	**(a) Total density**				**(b) Quantitative Average Taxonomic Distinctness**				**(c) Assemblage composition**			
		MS	Pseudo-F	*P*	COV	MS	Pseudo-F	*P*	COV	MS	Pseudo-F	*P*	COV
Zone (Z)	2	11.564	6.8959	0.001	0.28897	19.042	9.1772	0.001	0.37855	24314	13.583	0.001	13.792
Season (S)	3	37.188	22.175	0.001	0.62739	3.8037	1.8331	0.155	0.13842	22286	12.450	0.001	15.073
Period (P)	11	**9.0958**	**5.4239**	**0.001**	**0.48072**	**9.1511**	**4.4102**	**0.001**	**0.46948**	**23077**	**12.892**	**0.001**	**25.750**
ZxS	6	1.0585	0.6312	0.685	-0.14455	0.3965	0.1911	0.975	-0.23812	3159.7	1.7652	0.002	6.8022
ZxP	20	**5.2385**	**3.1238**	**0.001**	**0.53941**	**7.2822**	**3.5096**	**0.002**	**0.65224**	**4271.7**	**2.3864**	**0.001**	**14.239**
SxP	33	1.6512	0.9846	0.486	-5.6711E-2	1.5821	0.7625	0.743	-0.24780	**3474.5**	**1.9411**	**0.001**	**14.487**
ZxSxP	60	1.7929	1.0691	0.327	0.19459	1.2986	0.6258	0.945	-0.50370	1798.7	1.0048	0.421	1.6843
Residual	336	1.6770			1.295	2.0750			1.44050	1790			42.309

Table 2.3.1.5: Mean squares (MS), pseudo F-ratios, significance levels (*P*) and components of variation (COV) for period x season PERMANOVAs on the data for nearshore fish (a) total density, (b) quantitative average taxonomic distinctness and (c) assemblage composition recorded seasonally throughout the LSCE zone of the Swan-Canning Estuary in each period sampled between 1978/79 and 2008/09. df=degrees of freedom; E=exponential. Significant results involving period are highlighted in bold.

|	df	**(a) Total density**				**(b) Quantitative Average Taxonomic Distinctness**				**(c) Assemblage composition**			
		MS	Pseudo-F	*P*	COV	MS	Pseudo-F	*P*	COV	MS	Pseudo-F	*P*	COV
Period (P)	7	**3.9877**	**3.288**	**0.003**	**0.4274**	2.0765	1.0468	0.389	7.821E-2	**11618**	**4.6208**	**0.001**	**24.481**
Season (S)	3	4.2331	3.4903	0.013	0.3342	1.2795	0.64502	0.573	-0.16136	6038.2	2.4017	0.001	11.416
PxS	21	0.4494	0.37052	0.996	-0.4484	2.0202	1.0185	0.407	9.818E-2	2245.1	0.89298	0.894	-8.418
Residual	92	1.2128			1.1013	1.9836			1.4084	2514.2			50.142
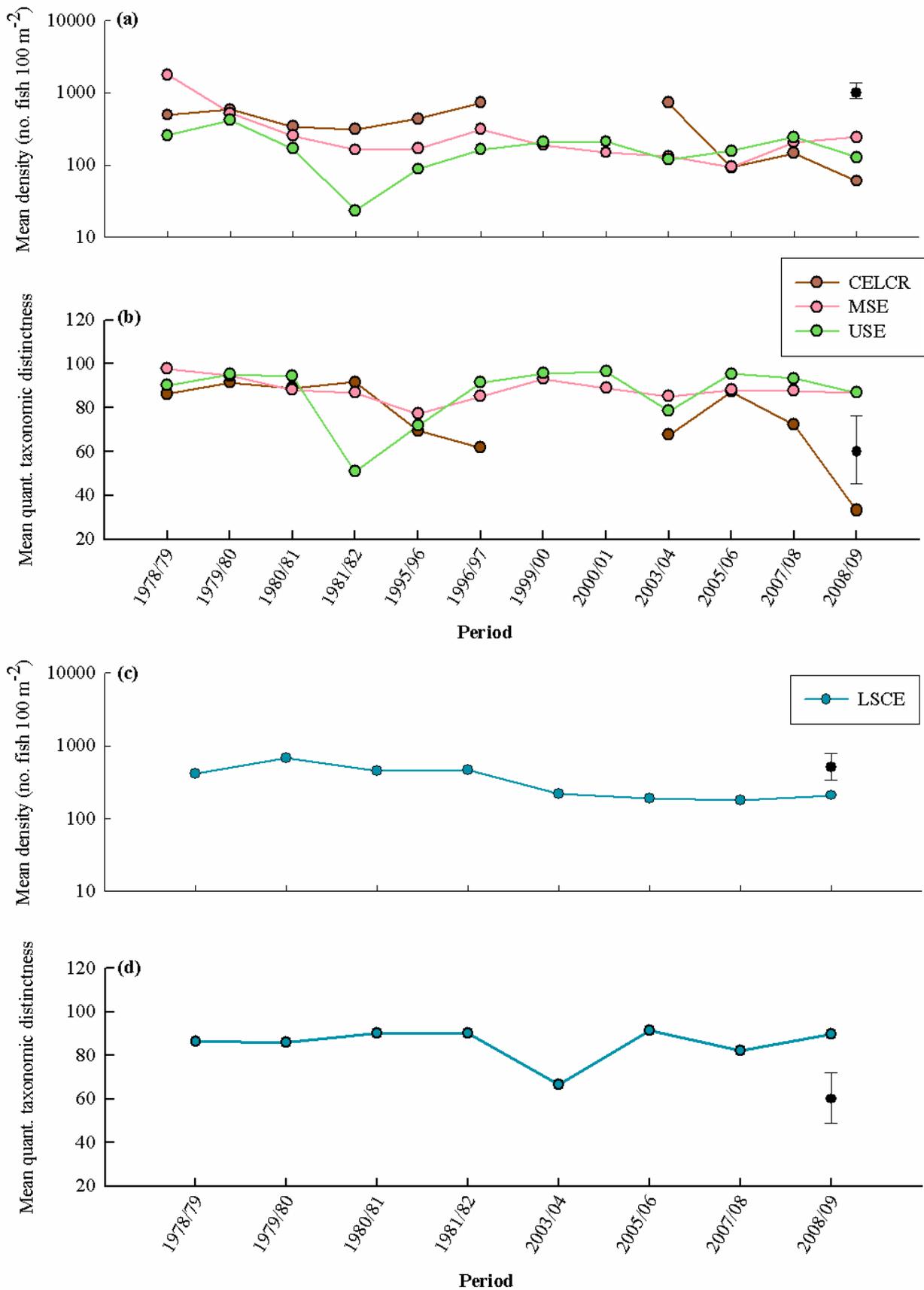
Figure 2.3.1.2: Mean (a) density of fish (number of individuals $100m^{-2}$) and (b) quantitative taxonomic distinctness of the fish fauna in the nearshore waters of the CELCR, MSE and USE and (c) density of fish and (d) quantitative taxonomic distinctness of the fish fauna in the nearshore waters of the LSCE in each period sampled between 1978/79 and 2008/09. For the sake of clarity, the average 95% confidence intervals have been presented for each of these plots.

components of variation, the period x zone interaction exerted the greatest influence on this diversity index, followed by the period main effect. A plot of the mean of this dependent variable in each period and zone demonstrated that among the most pronounced inter-period differences occurred in the CELCR, in which values in all periods since 1995/96 (with the exception of 2005/06) were notably lower than those in each period in the late 1970s/early 1980s. Moreover, values in 2008/09 were far lower than those for any other period in this zone (Fig. 2.3.1.2b). In contrast, species diversity in the MSE generally exhibited a very slight declining trend between 1978/79 and 2008/09, while that in the USE remained fairly similar over the above time frame, except for notably lower values in 1981/82 and, to a lesser extent, in 1995/96 and 2003/4 (Fig. 2.3.1.2b).

PERMANOVA of mean fish density and quantitative average taxonomic distinctness in the LSCE, which was undertaken separately from that for the above three zones due to the smaller number of periods in which this zone was sampled (see subsection 2.2.1.3.2), demonstrated that the former dependent variable differed significantly among periods and seasons (P=0.003-0.013, with periods exerting the greatest influence), while the latter did not exhibit any significant differences (Table 2.3.1.5a and 2.3.1.5b, respectively). A plot of the mean fish density in each of the eight periods sampled in the LSCE demonstrated that values recorded between 2003/04 and 2008/09 were all lower than those between 1978/79 and 1981/82 (Fig. 2.3.1.2c). In contrast, species diversity in this zone was virtually identical among all periods, except for during 2003/04 in which lower values were recorded (Fig. 2.3.1.2d).

2.3.1.2.3 Species composition of fish assemblages among periods
PERMANOVA demonstrated that the composition of the nearshore fish assemblages recorded seasonally throughout the MSE, USE and CELCR between 1978/79 and 2008/09 differed significantly among periods, zones and seasons and all two-way interactions between these main effects (Table 2.3.1.4c). The relative influence of period on fish composition was approximately twice that of any other significant term, as gauged by the magnitude of their associated components of variation. PERMANOVA of the fish assemblage data recorded in the LSCE identified significant differences among periods and seasons, and showed that the relative influence of the former factor was far greater than that of the latter (Table 2.3.1.5c).

To further explore the characteristics of the above inter-period differences, and to remove the confounding influence of the other significant factors, the data were separated on the basis of zone (including that for the LSCE) and, in each of these zones, a two-way crossed period x season ANOSIM test was carried out. The period component of these tests is shown in Table 2.3.1.6. These tests identified significant inter-period differences in each zone (P=0.001) and demonstrated that the overall extent of those differences ranged from moderately large in the USE and MSE (Global R=0.481-0.488) to moderately low in the LSCE (Global R=0.298).

In the USE and MSE, very large differences in fish faunal composition (*i.e.* R>0.700) were detected between almost all pairs of periods sampled during the late 1970s/early 1980s and

Table 2.3.1.6: R-statistic and/or significance level (*P*) values for global and pairwise comparisons in two-way crossed period x season ANOSIM tests of the nearshore fish faunal composition in the (a) Canning Estuary/Lower Canning River, (b) Upper Swan Estuary, (c) Middle Swan Estuary and (d) Lower Swan-Canning Estuary. Note that only the period component of these tests is shown. Insignificant pairwise comparisons are highlighted in grey.

(a) Canning Estuary/Lower Canning River; Global R=0.367, *P*=0.001

	1978/79	1979/80	1980/81	1981/82	1995/96	1996/97	2003/04	2005/06	2007/08
1979/80	0.019								
1980/81	0.037	-0.111							
1981/82	0.157	-0.028	-0.028						
1995/96	0.843	0.731	0.630	0.713					
1996/97	0.944	0.870	0.731	0.815	0.370				
2003/04	0.694	0.579	0.569	0.662	0.301	0.361			
2005/06	0.667	0.648	0.500	0.620	0.602	0.713	0.227		
2007/08	0.481	0.296	0.296	0.407	0.120	0.231	0.134	0	
2008/09	0.463	0.333	0.296	0.444	0.157	0.454	0.116	0.074	-0.139

(b) Upper Swan Estuary; Global R=0.481, *P*=0.001

	1978/79	1979/80	1980/81	1981/82	1999/00	2000/01	2003/04	2005/06	2007/08
1979/80	0.500								
1980/81	0.500	0.063							
1981/82	0.625	0.625	0.375						
1999/00	0.964	0.865	0.878	0.968					
2000/01	0.992	0.924	0.885	0.971	0.227				
2003/04	1.000	1.000	0.563	0.750	0.875	0.903			
2005/06	1.000	0.750	0.625	0.750	0.516	0.490	0.875		
2007/08	0.644	0.367	0.393	0.669	0.525	0.462	0.266	-0.005	
2008/09	0.633	0.364	0.244	0.529	0.413	0.318	0.115	-0.068	0.155

(c) Middle Swan Estuary; Global R=0.488, *P*=0.001

	1978/79	1979/80	1980/81	1981/82	1995/96	1996/97	1999/00	2000/01	2003/04	2005/06	2007/08
1979/80	0.313										
1980/81	0.125	0.250									
1981/82	0.500	0.188	0.063								
1995/96	0.641	0.391	0.541	0.509							
1996/97	0.564	0.500	0.586	0.536	0.34						
1999/00	0.938	0.938	0.875	0.938	0.336	0.345					
2000/01	1.000	1.000	1.000	0.938	0.550	0.332	0.563				
2003/04	0.875	0.818	0.854	0.901	0.719	0.669	0.826	0.771			
2005/06	0.809	0.691	0.686	0.686	0.533	0.556	0.686	0.555	0.505		
2007/08	0.667	0.536	0.573	0.615	0.516	0.563	0.701	0.458	0.475	0.183	
2008/09	0.549	0.396	0.510	0.555	0.518	0.516	0.628	0.365	0.370	0.196	0.038

(d) Lower Swan-Canning Estuary; Global R=0.298, *P*=0.001

	1978/79	1979/80	1980/81	1981/82	2003/04	2005/06	2007/08
1979/80	0.070						
1980/81	0.023	0.046					
1981/82	0.012	0.132	0.027				
2003/04	0.725	0.733	0.716	0.755			
2005/06	0.582	0.559	0.508	0.528	0.439		
2007/08	0.560	0.573	0.390	0.425	0.137	0.109	
2008/09	0.444	0.359	0.293	0.359	0.185	0.038	0.038

those sampled from 1999/00 to 2003/04. This was also often the case for 2005/06 *vs* each of the periods sampled between 1978/79 and 1981/82, particularly in the USE (Table 2.3.1.6b-c). These differences are illustrated by the MDS plots in Fig. 2.3.1.3b, c, which have been constructed from the fish assemblage data in each zone and season. Thus, samples from the earliest periods typically formed a discrete group on one side of the plot, while those from each of the more recent periods were located a relatively large distance away and also often formed pronounced and discrete groups. SIMPER demonstrated that, in both zones, these differences in fish composition were often driven by more abundant and consistent catches of *M. cephalus, N. vlaminghi* and *P. punctatus* in each of the periods from 1978/79 to 1981/82, and by a greater prevalence of *A. butcheri, P. olorum* and *A. suppositus* in 1999/00-2003/04 (Table 2.3.1.7b-c). Furthermore, in the MSE, *A. forsteri, A. rueppellii* and *A. georgiana* were also typically more prevalent in the earlier than later periods (Table 2.3.1.7c).

In the MSE, moderately large (*i.e.* R >0.500) pairwise differences were also detected between almost all periods sampled from 1978/79-1999/00 and those from 2007-09. However, this was the case for only a few of the corresponding pairwise comparisons in the USE, with the remainder typically exhibiting moderately small to small differences (Table 2.3.1.6b-c). Such findings were reflected by the considerable distances between and/or discrete groups formed by samples from these two sets of periods on the MDS plots for the MSE (particularly during autumn and summer; Fig. 2.3.1.3c), whereas samples from 2007-09 often lay between those from the late 1970/s/early 1980s and those from 1999/00 on the plots for the USE (Fig. 2.3.1.3b). Moreover, particularly in the USE, replicate samples from the most recent periods were often more dispersed than those from other periods. SIMPER showed that the above inter-period differences in the MSE were driven, in part, by a greater prevalence of *A. butcheri* and *P. punctatus* in 2007-09 than in each of the periods sampled from 1978/79 to 1999/00. More regular and abundant catches of *N. vlaminghi, M. cephalus, A. forsteri, A. rueppellii* and *A. georgiana* in the late 1970s/early 1980s also contributed to the ichthyofaunal differences between these periods and those from 2007-09 (Table 2.3.1.7c).

Moderately large to large compositional differences were also detected between several of the remaining pairs of periods in the MSE, such as those sampled from 1995-2001 *vs* those from 2003-06, with the frequent exception of those that were consecutive, *e.g.* 1995/96 *vs* 1996/97 (Table 2.3.1.6c, Fig. 2.3.1.3c). Differences in the fish faunas between the mid 1990s/early 2000s and early to mid 2000s in this zone were consistently due to greater and more regular catches of *A. butcheri* in the later than earlier periods. Several other species also variably contributed to these inter-period differences, as detailed in Table 2.3.1.7c.

The largest inter-period differences in the CELCR were detected between each of those periods sampled from 1978 to 1982 and those sampled from 1995 to 1997 (R=0.630-0.944). Moderately large pairwise differences were also detected between the former set of periods and those sampled from 2003 to 2006 (R=0.500-0.694; Table 2.3.1.6a). Such findings were clearly illustrated by the fact that, in each season, samples from the late 1970s/early 1980s

Table 2.3.1.7: Species that consistently typified (provided along the diagonal) and distinguished (provided in the sub-diagonal) the nearshore fish assemblages in each period sampled between 1978/79 and 2008/09 in the (a) Canning Estuary/Lower Canning River, (b) Upper Swan Estuary, (c) Middle Swan Estuary and (d) Lower Swan-Canning Estuary, as detected by two-way crossed period x season SIMPER. Note that only the period component of these tests is shown. The period in which each species was most abundant is given in superscript for each pairwise comparison. Insignificant pairwise comparisons (as detected by ANOSIM; Table 2.3.1.6) are highlighted in grey.

(a) Canning Estuary/Lower Canning River

	1978/79	1979/80	1980/81	1981/82	1995/96	1996/97	2003/04	2005/06	2007/08	2008/09
1978/79	M. cephalus P. olorum N. vlaminghi A. caudavittatus A. forsteri									
1979/80		M. cephalus N. vlaminghi								
1980/81			M. cephalus P. olorum A. georgiana L. wallacei							
1981/82				M. cephalus A. forsteri A. georgiana N. vlaminghi P. olorum						
1995/96	M. cephalus $^{78/79}$ A. elongata $^{95/96}$ L. wallacei $^{95/96}$ P. olorum $^{78/79}$ A. bifrenatus $^{95/96}$ A. forsteri $^{78/79}$	M. cephalus $^{79/80}$ N. vlaminghi $^{79/80}$ A. elongata $^{95/96}$ P. punctatus $^{95/96}$ L. wallacei $^{95/96}$ P. olorum $^{79/80}$	M. cephalus $^{80/81}$ A. elongata $^{95/96}$ P. olorum $^{80/81}$ L. wallacei $^{95/96}$ P. punctatus $^{95/96}$ A. bifrenatus $^{95/96}$	M. cephalus $^{81/82}$ A. elongata $^{95/96}$ L. wallacei $^{95/96}$ A. bifrenatus $^{95/96}$ A. georgiana $^{81/82}$	L. wallacei A. elongata P. punctatus					
1996/97	M. cephalus $^{78/79}$ A. elongata $^{96/97}$ L. wallacei $^{96/97}$ A. mugiloides $^{96/97}$ A. bifrenatus $^{96/97}$ L. presbyteroides $^{96/97}$ A. forsteri $^{78/79}$	M. cephalus $^{79/80}$ A. elongata $^{96/97}$ N. vlaminghi $^{79/80}$ L. wallacei $^{96/97}$ A. forsteri $^{79/80}$ A. mugiloides $^{96/97}$ A. bifrenatus $^{96/97}$ P. olorum $^{79/80}$ L. presbyteroides $^{96/97}$	M. cephalus $^{80/81}$ A. elongata $^{96/97}$ L. wallacei $^{96/97}$ A. butcheri $^{96/97}$ A. mugiloides $^{96/97}$ L. presbyteroides $^{96/97}$ N. vlaminghi $^{80/81}$	M. cephalus $^{81/82}$ A. elongata $^{96/97}$ L. wallacei $^{96/97}$ A. forsteri $^{81/82}$ A. butcheri $^{96/97}$ A. mugiloides $^{96/97}$ N. vlaminghi $^{81/82}$ A. bifrenatus $^{96/97}$ A. georgiana $^{81/82}$ L. presbyteroides $^{96/97}$	A. elongata $^{96/97}$ L. wallacei $^{96/97}$ A. mugiloides $^{96/97}$ P. olorum $^{95/96}$ L. presbyteroides $^{96/97}$ A. butcheri $^{96/97}$	A. elongata L. wallacei A. mugiloides L. presbyteroides				

	1978/79	1979/80	1980/81	1981/82	1995/96	1996/97	2003/04	2005/06	2007/08	2008/09
2003/04	M. cephalus [78/79] L. wallacei [03/04] A. forsteri [78/79]	M. cephalus [79/80] L. wallacei [03/04] N. vlaminghi [79/80] P. olorum [79/80] P. punctatus [79/80] A. forsteri [79/80]	M. cephalus [80/81] L. wallacei [03/04] A. butcheri [03/04]	M. cephalus [81/82] L. wallacei [03/04] A. georgiana [81/82] A. butcheri [03/04]	L. wallacei [03/04] A. elongata [95/96]	A. elongata [96/97] L. wallacei [03/04] A. butcheri [96/97] P. olorum [03/04] L. presbyteroides [96/97]	L. wallacei A. caudavittatus			
2005/06	M. cephalus [78/79] N. vlaminghi [78/79] A. butcheri [05/06] P. punctatus [05/06] T. pleurogramma [05/06] A. forsteri [78/79] L. wallacei [05/06]	N. vlaminghi [79/80] M. cephalus [79/80] A. butcheri [05/06] P. olorum [79/80] P. punctatus [05/06] A. forsteri [79/80] T. pleurogramma [05/06]	M. cephalus [80/81] P. olorum [80/81] A. butcheri [05/06] P. punctatus [80/81] T. pleurogramma [05/06] A. mugiloides [05/06]	M. cephalus [81/82] A. forsteri [81/82] A. butcheri [05/06] P. punctatus [05/06] A. georgiana [81/82] T. pleurogramma [05/06] A. caudavittatus [05/06] L. wallacei [05/06] A. mugiloides [05/06]	A. elongata [95/96] A. butcheri [05/06] L. wallacei [95/96] P. punctatus [95/96] A. bifrenatus [95/96] T. pleurogramma [05/06]	A. elongata [96/97] L. wallacei [96/97] A. butcheri [05/06] P. punctatus [05/06] A. mugiloides [96/97] P. olorum [96/97] T. pleurogramma [05/06] L. presbyteroides [96/97] A. caudavittatus [05/06] M. cephalus [05/06]		A. butcheri P. punctatus M. cephalus A. caudavittatus L. wallacei		
2007/08	M. cephalus [78/79] N. vlaminghi [78/79] P. punctatus [07/08] L. wallacei [07/08] A. forsteri [78/79]	M. cephalus [79/80] N. vlaminghi [79/80] P. punctatus [07/08] L. wallacei [07/08] A. mugiloides [07/08] P. olorum [79/80]	M. cephalus [80/81] P. olorum [80/81] P. punctatus [07/08] L. wallacei [07/08]	M. cephalus [81/82] P. punctatus [07/08] L. wallacei [07/08]		A. elongata [96/97] L. wallacei [96/97] A. mugiloides [96/97] L. presbyteroides [96/97]			L. wallacei P. punctatus A. butcheri P. olorum	
2008/09	M. cephalus [78/79] P. punctatus [08/09] A. forsteri [78/79]	M. cephalus [79/80] N. vlaminghi [79/80] P. punctatus [08/09] A. mugiloides [08/09]	M. cephalus [80/81] P. punctatus [08/09] L. wallacei [08/09] A. mugiloides [08/09]	M. cephalus [81/82] P. punctatus [08/09] A. butcheri [08/09]		A. elongata [96/97] L. wallacei [96/97] A. mugiloides [08/09] L. presbyteroides [96/97]				P. punctatus A. mugiloides L. wallacei

(b) Upper Swan Estuary

	1978/79	1979/80	1980/81	1981/82	1999/00	2000/01	2003/04	2005/06	2007/08	2008/09
1978/79	*M. cephalus* *P. endrachtensis* *N. vlaminghi* *A. butcheri*									
1979/80	*M. cephalus* [78/79] *L. wallacei* [79/80] *A. butcheri* [78/79] *P. olorum* [79/80] *P. punctatus* [79/80] *A. bifrenatus* [79/80] *G. affinis* [78/79]	*M. cephalus* *A. bifrenatus* *N. vlaminghi* *P. punctatus* *P. olorum* *L. wallacei*								
1980/81	*M. cephalus* [78/79] *P. endrachtensis* [78/79] *A. butcheri* [78/79] *P. punctatus* [80/81] *L. wallacei* [80/81]		*P. punctatus* *M. cephalus* *L. wallacei*							
1981/82	*M. cephalus* [78/79] *P. endrachtensis* [78/79] *A. butcheri* [78/79] *N. vlaminghi* [78/79] *P. punctatus* [78/79] *A. georgiana* [78/79]	*M. cephalus* [79/80] *L. wallacei* [79/80] *P. olorum* [79/80] *A. bifrenatus* [79/80] *P. punctatus* [79/80] *A. georgiana* [79/80] *A. forsteri* [81/82] *A. butcheri* [79/80]		*M. cephalus* *P. olorum*						
1999/00	*M. cephalus* [78/79] *P. olorum* [99/00] *N. vlaminghi* [78/79] *L. wallacei* [99/00] *A. suppositus* [99/00] *A. butcheri* [78/79] *P. punctatus* [78/79] *G. affinis* [99/00] *A. georgiana* [78/79]	*M. cephalus* [79/80] *L. wallacei* [79/80] *P. olorum* [99/00] *A. suppositus* [99/00] *N. vlaminghi* [79/80] *P. punctatus* [79/80] *A. bifrenatus* [79/80] *A. georgiana* [79/80]	*P. punctatus* [80/81] *A. suppositus* [99/00] *P. olorum* [99/00] *L. wallacei* [80/81] *G. affinis* [99/00] *M. cephalus* [80/81]	*A. suppositus* [99/00] *P. olorum* [99/00] *L. wallacei* [99/00] *G. occidentalis* [99/00] *G. affinis* [99/00] *M. cephalus* [81/82] *A. butcheri* [81/82]	*P. olorum* *A. suppositus* *L. wallacei*					
2000/01	*M. cephalus* [78/79] *L. wallacei* [00/01] *A. suppositus* [00/01] *N. vlaminghi* [78/79] *P. punctatus* [78/79] *A. georgiana* [78/79]	*M. cephalus* [79/80] *L. wallacei* [79/80] *A. suppositus* [00/01] *N. vlaminghi* [79/80] *A. bifrenatus* [79/80] *P. olorum* [00/01] *P. punctatus* [79/80] *A. butcheri* [00/01]	*P. punctatus* [80/81] *A. suppositus* [00/01] *L. wallacei* [00/01] *P. olorum* [00/01] *M. cephalus* [80/81] *A. butcheri* [00/01]	*A. suppositus* [00/01] *L. wallacei* [00/01] *P. olorum* [00/01] *G. occidentalis* [00/01] *A. butcheri* [00/01]	*P. olorum* [99/00] *A. suppositus* [00/01] *G. affinis* [99/00] *L. wallacei* [00/01] *A. butcheri* [00/01]	*L. wallacei* *A. suppositus* *P. olorum* *A. butcheri*				

	1978/79	1979/80	1980/81	1981/82	1999/00	2000/01	2003/04	2005/06	2007/08	2008/09
2003/04	*M. cephalus* [78/79] *N. vlaminghi* [78/79] *P. punctatus* [78/79] *A. suppositus* [03/04] *P. olorum* [03/04] *G. affinis* [78/79] *A. georgiana* [78/79]	*M. cephalus* [79/80] *L. wallacei* [79/80] *N. vlaminghi* [79/80] *P. punctatus* [79/80] *A. suppositus* [03/04] *A. bifrenatus* [79/80] *P. olorum* - *A. georgiana* [79/80]	*P. punctatus* [80/81] *L. wallacei* [80/81] *A. suppositus* [03/04] *P. olorum* [03/04] *M. cephalus* [80/81]	*P. punctatus* [03/04] *A. suppositus* [03/04] *P. olorum* [03/04] *L. wallacei* [03/04] *M. cephalus* [81/82] *A. butcheri* [03/04]	*A. suppositus* [99/00] *L. wallacei* [99/00] *P. olorum* [99/00] *G. affinis* [99/00]	*A. suppositus* [00/01] *L. wallacei* [00/01] *P. punctatus* [03/04] *G. occidentalis* [00/01] *A. butcheri* [03/04]	*A. suppositus* *P. olorum* *L. wallacei* *P. punctatus* *A. butcheri*			
2005/06	*M. cephalus* [78/79] *P. endrachtensis* [78/79] *N. vlaminghi* [78/79] *L. wallacei* [05/06] *P. olorum* [05/06] *P. punctatus* [78/79] *A. butcheri* [05/06]	*M. cephalus* [79/80] *L. wallacei* [79/80] *N. vlaminghi* [79/80] *A. butcheri* [05/06] *A. bifrenatus* [79/80] *P. punctatus* [79/80] *P. olorum* [05/06] *A. suppositus* [05/06] *A. georgiana* [79/80]	*P. punctatus* [80/81] *L. wallacei* [80/81] *A. butcheri* [05/06] *P. olorum* [05/06] *M. cephalus* [80/81]	*L. wallacei* [05/06] *P. olorum* [05/06] *A. butcheri* [05/06] *M. cephalus* [81/82] *N. vlaminghi* [81/82]	*A. suppositus* [99/00] *A. butcheri* [05/06] *P. olorum* [05/06] *G. affinis* [99/00] *M. cephalus* [05/06] *L. wallacei* [99/00]	*A. suppositus* [00/01] *L. wallacei* [05/06] *A. butcheri* [05/06] *M. cephalus* [05/06] *P. olorum* [05/06]	*L. wallacei* [05/06] *A. suppositus* [03/04] *A. butcheri* [05/06] *P. punctatus* [03/04] *M. cephalus* [05/06] *P. olorum* [05/06]	*A. butcheri* *L. wallacei* *P. olorum* *M. cephalus*		
2007/08	*M. cephalus* [78/79] *L. wallacei* [07/08] *A. butcheri* [07/08] *P. olorum* [07/08] *P. punctatus* [78/79] *A. georgiana* [78/79] *G. affinis* [78/79]	*M. cephalus* [79/80] *L. wallacei* [07/08] *A. butcheri* [07/08] *P. olorum* [07/08] *P. punctatus* [79/80] *A. bifrenatus* [79/80] *A. georgiana* [79/80] *A. caudavittatus* [79/80]	*L. wallacei* [07/08] *A. butcheri* [07/08] *P. punctatus* [80/81] *P. olorum* [07/08] *M. cephalus* [80/81]	*L. wallacei* [07/08] *A. butcheri* [07/08] *P. olorum* [07/08] *P. punctatus* [07/08] *M. cephalus* [81/82]	*P. olorum* [99/00] *A. butcheri* [07/08] *A. suppositus* [99/00] *L. wallacei* [07/08] *P. punctatus* [07/08]	*L. wallacei* [07/08] *A. suppositus* [00/01] *A. butcheri* [07/08] *P. olorum* -	*L. wallacei* [07/08] *A. butcheri* [07/08] *P. punctatus* [07/08] *P. olorum* [07/08] *A. suppositus* [03/04]		*A. butcheri* *L. wallacei* *P. punctatus* *A. suppositus*	
2008/09	*M. cephalus* [78/79] *N. vlaminghi* [78/79] *A. butcheri* [78/79] *L. wallacei* [08/09] *P. olorum* [08/09] *P. punctatus* [78/79] *A. georgiana* [78/79]	*M. cephalus* [79/80] *L. wallacei* [79/80] *N. vlaminghi* [79/80] *P. olorum* [08/09] *P. punctatus* [79/80] *A. bifrenatus* [79/80] *A. butcheri* [08/09] *A. georgiana* [79/80]	*P. punctatus* [80/81] *L. wallacei* [80/81] *P. olorum* [08/09] *M. cephalus* [80/81]	*P. olorum* [08/09] *L. wallacei* [08/09] *M. cephalus* [81/82] *N. vlaminghi* [81/82]	*A. suppositus* [99/00] *P. olorum* [99/00] *G. affinis* [99/00] *L. wallacei* [08/09] *A. butcheri* [08/09]	*A. suppositus* [00/01] *L. wallacei* [00/01] *P. olorum* [08/09] *G. occidentalis* [00/01] *P. punctatus* [08/09] *A. butcheri* [00/01]			*A. butcheri* [07/08] *L. wallacei* [07/08] *P. olorum* [08/09] *P. punctatus* [07/08]	*L. wallacei* *P. olorum* *P. punctatus*

(c) Middle Swan Estuary

|	1978/79	1979/80	1980/81	1981/82	1995/96	1996/97	1999/00	2000/01	2003/04	2005/06	2007/08	2008/09
1978/79	*N. vlaminghi* *M. cephalus* *A. caudavittatus* *P. punctatus* *A. forsteri* *G. subfasciatus* *A. butcheri* *A. rueppellii*											
1979/80		*N. vlaminghi* *P. punctatus* *M. cephalus* *A. forsteri* *A. georgiana* *A. caudavittatus* *A. butcheri* *A. ogilbyi* *A. rueppellii*										
1980/81			*M. cephalus* *P. punctatus* *A. caudavittatus* *A. rueppellii*									
1981/82	*N. vlaminghi* [78/79] *A. caudavittatus* [78/79] *A. forsteri* [78/79] *A. rueppellii* [78/79] *P. punctatus* [78/79] *A. bifrenatus* [81/82] *A. butcheri* [78/79] *P. octolineatus* [78/79]			*M. cephalus*								
1995/96	*N. vlaminghi* [78/79] *M. cephalus* [78/79] *A. caudavittatus* [78/79] *A. forsteri* [78/79] *P. punctatus* [78/79] *A. rueppellii* [78/79] *A. georgiana* [78/79] *G. subfasciatus* [78/79] *A. bifrenatus* [78/79] *A. butcheri* [78/79]	*N. vlaminghi* [79/80] *M. cephalus* [79/80] *P. punctatus* [79/80] *A. forsteri* [79/80] *A. bifrenatus* [79/80] *A. georgiana* [79/80]	*M. cephalus* [80/81] *P. punctatus* [80/81] *A. forsteri* [80/81] *A. rueppellii* [80/81] *L. wallacei* [95/96] *S. burrus* [80/81] *N. vlaminghi* [80/81] *A. butcheri* [95/96] *A. caudavittatus* [95/96]	*M. cephalus* [81/82] *A. bifrenatus* [81/82] *A. butcheri* [95/96] *N. vlaminghi* [81/82]	*A. suppositus* *L. wallacei* *M. cephalus*							

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	1978/79	1979/80	1980/81	1981/82	1995/96	1996/97	1999/00	2000/01	2003/04	2005/06	2007/08	2008/09
1996/97	*N. vlaminghi* [78/79] *M. cephalus* [78/79] *A. caudavittatus* [78/79] *A. forsteri* [78/79] *P. punctatus* [78/79] *A. rueppellii* [78/79] *A. butcheri* [96/97] *P. octolineatus* [78/79]	*P. punctatus* [79/80] *N. vlaminghi* [79/80] *M. cephalus* [79/80] *A. forsteri* [79/80] *A. forsteri* [79/80] *A. bifrenatus* [96/97] *A. georgiana* [79/80] *A. caudavittatus* [79/80] *A. ogilbyi* [79/80] *A. mugiloides* [79/80]	*M. cephalus* [80/81] *P. punctatus* [80/81] *A. forsteri* [80/81] *A. rueppellii* [80/81] *A. butcheri* [96/97] *N. vlaminghi* [80/81] *L. wallacei* [96/97] *A. caudavittatus* [80/81] *A. mugiloides* [80/81]	*M. cephalus* [81/82] *A. bifrenatus* - *A. butcheri* [96/97] *L. wallacei* [96/97]	*A. bifrenatus* [96/97] *A. elongata* [95/96] *L. wallacei* [95/96]	*L. wallacei* *S. burrus* *A. bifrenatus* *A. butcheri*						
1999/00	*N. vlaminghi* [78/79] *M. cephalus* [78/79] *A. caudavittatus* [78/79] *P. olorum* [99/00] *A. forsteri* [78/79] *A. rueppellii* [78/79] *P. punctatus* [78/79] *A. georgiana* [78/79] *A. suppositus* [99/00] *L. wallacei* [99/00] *G. subfasciatus* [78/79]	*P. olorum* [99/00] *N. vlaminghi* [79/80] *P. punctatus* [79/80] *M. cephalus* [79/80] *A. rueppellii* [79/80] *A. suppositus* [99/00] *A. forsteri* [79/80] *L. wallacei* [99/00] *A. georgiana* [79/80] *S. burrus* [79/80] *A. caudavittatus* [79/80] *A. mugiloides* [79/80] *A. ogilbyi* [79/80]	*P. olorum* [99/00] *M. cephalus* [80/81] *P. punctatus* [80/81] *A. suppositus* [99/00] *A. forsteri* [80/81] *A. rueppellii* [80/81] *S. burrus* [80/81] *L. wallacei* [99/00] *N. vlaminghi* [80/81] *A. bifrenatus* [99/00] *P. octolineatus* [80/81] *A. caudavittatus* [80/81]	*P. olorum* [99/00] *M. cephalus* [81/82] *A. suppositus* [99/00] *L. wallacei* [99/00] *N. vlaminghi* [81/82] *G. subfasciatus* [81/82] *A. rueppellii* [81/82] *A. bifrenatus* [81/82] *P. punctatus* [81/82] *A. butcheri* [99/00]	*P. olorum* [99/00] *A. suppositus* [99/00] *L. wallacei* [99/00] *A. bifrenatus* [99/00] *A. butcheri* [95/96]	*P. olorum* [99/00] *A. suppositus* [99/00] *A. bifrenatus* [96/97] *A. butcheri* [96/97] *L. wallacei* [99/00] *G. subfasciatus* [99/00]	*P. olorum* *A. suppositus* *L. wallacei* *A. caudavittatus*					
2000/01	*N. vlaminghi* [78/79] *M. cephalus* [78/79] *A. caudavittatus* [78/79] *P. olorum* [00/01] *G. subfasciatus* [00/01] *A. suppositus* [00/01] *A. forsteri* [78/79] *A. rueppellii* [78/79] *A. georgiana* [78/79] *P. punctatus* [78/79] *L. wallacei* [00/01] *A. bifrenatus* [78/79]	*N. vlaminghi* [79/80] *M. cephalus* [79/80] *P. punctatus* [79/80] *A. butcheri* [00/01] *P. olorum* [00/01] *A. rueppellii* [79/80] *A. suppositus* [00/01] *A. forsteri* [79/80] *A. georgiana* [79/80] *L. wallacei* [00/01] *A. bifrenatus* [79/80] *A. mugiloides* [79/80] *A. ogilbyi* [79/80]	*M. cephalus* [80/81] *P. olorum* [00/01] *A. butcheri* [00/01] *A. suppositus* [00/01] *P. punctatus* [80/81] *A. rueppellii* [80/81] *A. forsteri* [80/81] *A. georgiana* [80/81] *N. vlaminghi* [80/81] *S. burrus* [80/81] *L. wallacei* [00/01] *P. octolineatus* [80/81]	*M. cephalus* [81/82] *G. subfasciatus* [00/01] *A. suppositus* [00/01] *A. butcheri* [00/01] *P. olorum* [00/01] *L. wallacei* [00/01] *A. rueppellii* [81/82] *A. forsteri* [81/82]	*P. olorum* [00/01] *A. butcheri* [00/01] *A. suppositus* [00/01] *A. butcheri* [00/01] *P. olorum* [00/01] *L. wallacei* [00/01]	*G. subfasciatus* [00/01] *A. butcheri* [00/01] *A. suppositus* [00/01] *G. subfasciatus* [00/01]		*P. olorum* *G. subfasciatus* *A. butcheri* *A. suppositus* *L. wallacei*				
2003/04	*N. vlaminghi* [78/79] *M. cephalus* [78/79] *A. rueppellii* [78/79] *A. forsteri* [78/79] *A. butcheri* [03/04] *A. georgiana* [78/79] *P. punctatus* [78/79] *G. subfasciatus* [78/79] *A. bifrenatus* [78/79]	*N. vlaminghi* [79/80] *M. cephalus* [79/80] *A. rueppellii* [79/80] *P. punctatus* [79/80] *A. butcheri* [03/04] *A. forsteri* [79/80] *A. bifrenatus* [79/80] *A. georgiana* [79/80] *A. caudavittatus* - *A. ogilbyi* [79/80] *A. mugiloides* [79/80]	*M. cephalus* [80/81] *A. butcheri* [03/04] *A. rueppellii* [03/04] *A. forsteri* [80/81] *N. vlaminghi* [80/81] *A. georgiana* [80/81] *S. burrus* [80/81] *A. mugiloides* [80/81]	*M. cephalus* [81/82] *A. butcheri* [03/04] *N. vlaminghi* [81/82]	*P. punctatus* [03/04] *A. butcheri* [03/04]	*A. butcheri* [03/04] *A. bifrenatus* [96/97] *A. caudavittatus* [03/04]	*P. olorum* [99/00] *A. butcheri* [03/04] *A. suppositus* [99/00] *L. wallacei* [99/00] *A. bifrenatus* [99/00] *A. caudavittatus* [03/04]	*A. butcheri* [03/04] *P. olorum* [00/01] *P. punctatus* [03/04] *G. subfasciatus* [00/01] *A. suppositus* [00/01] *L. wallacei* [00/01]	*A. butcheri* *P. punctatus* *A. rueppellii*			

	1978/79	1979/80	1980/81	1981/82	1995/96	1996/97	1999/00	2000/01	2003/04	2005/06	2007/08	2008/09
2005/06	*N. vlaminghi* 78/79 *M. cephalus* 78/79 *A. caudavittatus* 78/79 *A. forsteri* 78/79 *A. rueppellii* 78/79 *A. georgiana* 78/79 *A. butcheri* 05/06 *P. punctatus* 78/79 *G. subfasciatus* 78/79 *A. bifrenatus* 78/79 *P. octolineatus* 78/79	*N. vlaminghi* 79/80 *M. cephalus* 79/80 *A. rueppellii* 79/80 *P. punctatus* 79/80 *A. butcheri* 05/06 *A. forsteri* 79/80 *T. pleurogramma* 05/06 *A. georgiana* 79/80 *A. bifrenatus* 79/80 *A. mugiloides* 79/80 *A. caudavittatus* 79/80 *A. ogilbyi* 79/80	*M. cephalus* 80/81 *G. subfasciatus* 80/81 *A. butcheri* 05/06 *A. rueppellii* 80/81 *A. forsteri* 80/81 *P. punctatus* 80/81 *A. georgiana* 80/81 *T. pleurogramma* 05/06 *N. vlaminghi* 80/81 *S. burrus* 80/81 *P. octolineatus* 80/81 *A. mugiloides* 05/06	*M. cephalus* 81/82 *A. butcheri* 05/06 *P. punctatus* 05/06 *G. subfasciatus* 81/82 *T. pleurogramma* 05/06 *N. vlaminghi* 81/82	*A. butcheri* 05/06 *P. punctatus* 05/06	*P. punctatus* 05/06 *A. butcheri* 05/06 *T. pleurogramma* 05/06	*P. olorum* 99/00 *A. butcheri* 05/06 *A. suppositus* 99/00 *P. punctatus* 05/06 *T. pleurogramma* 05/06 *L. wallacei* 99/00 *A. bifrenatus* 99/00 *A. caudavittatus* 05/06	*P. olorum* 00/01 *A. butcheri* 05/06 *A. suppositus* 00/01 *P. punctatus* 05/06 *T. pleurogramma* 05/06 *L. wallacei* 00/01	*P. punctatus* 05/06 *A. butcheri* 03/04	*A. butcheri* *P. punctatus* *T. pleurogramma*		
2007/08	*N. vlaminghi* 78/79 *M. cephalus* 78/79 *A. caudavittatus* 78/79 *P. punctatus* 07/08 *A. forsteri* 78/79 *A. rueppellii* 78/79 *A. georgiana* 78/79 *G. subfasciatus* 78/79 *A. bifrenatus* 78/79 *P. octolineatus* 78/79 *P. olorum* 78/79	*N. vlaminghi* 79/80 *M. cephalus* 79/80 *A. butcheri* 07/08 *A. rueppellii* 79/80 *P. punctatus* 07/08 *A. forsteri* 79/80 *A. bifrenatus* 79/80 *A. georgiana* 79/80 *P. olorum* 79/80 *A. caudavittatus* 79/80 *A. mugiloides* 07/08 *A. ogilbyi* 79/80	*M. cephalus* 80/81 *A. butcheri* 07/08 *P. punctatus* 07/08 *A. forsteri* 80/81 *A. rueppellii* 80/81 *S. burrus* 80/81 *A. georgiana* 80/81 *N. vlaminghi* 80/81 *A. caudavittatus* 80/81 *A. mugiloides* 07/08	*M. cephalus* 81/82 *A. butcheri* 07/08 *P. punctatus* 07/08	*A. butcheri* 07/08 *P. punctatus* 07/08 *L. wallacei* 95/96	*P. punctatus* 07/08 *A. butcheri* 07/08	*P. olorum* 99/00 *P. punctatus* 07/08 *A. butcheri* 07/08 *L. wallacei* 99/00 *A. suppositus* 99/00 *A. caudavittatus* 99/00	*P. olorum* 00/01 *P. punctatus* 07/08 *A. butcheri* 07/08 *G. subfasciatus* 00/01 *A. suppositus* 00/01	*A. butcheri* 03/04 *P. punctatus* 07/08	*P. punctatus* 07/08 *A. butcheri* 07/08 *T. pleurogramma* 05/06	*A. butcheri* *P. punctatus*	
2008/09	*N. vlaminghi* 78/79 *M. cephalus* 78/79 *A. caudavittatus* 78/79 *A. butcheri* 08/09 *A. forsteri* 78/79 *A. rueppellii* 78/79 *A. georgiana* 78/79 *G. subfasciatus* 78/79 *P. octolineatus* 78/79	*N. vlaminghi* 79/80 *A. butcheri* 08/09 *M. cephalus* 79/80 *A. rueppellii* 79/80 *P. punctatus* 08/09 *A. forsteri* 79/80 *A. bifrenatus* 79/80 *A. georgiana* 79/80 *A. mugiloides* 08/09 *S. burrus* 79/80	*M. cephalus* 80/81 *A. butcheri* 08/09 *P. punctatus* 08/09 *A. forsteri* 80/81 *A. rueppellii* 80/81 *A. georgiana* 80/81 *S. burrus* 80/81 *N. vlaminghi* 80/81 *A. mugiloides* 08/09	*M. cephalus* 81/82 *P. punctatus* 08/09 *A. butcheri* 08/09	*P. punctatus* 08/09 *A. butcheri* 08/09	*P. punctatus* 08/09 *A. butcheri* 08/09	*P. olorum* 99/00 *P. punctatus* 08/09 *A. butcheri* 08/09 *A. suppositus* 99/00 *L. wallacei* 99/00 *A. bifrenatus* 99/00	*P. punctatus* 08/09 *P. olorum* 00/01 *A. butcheri* 08/09 *A. suppositus* 00/01 *G. subfasciatus* 00/01 *L. wallacei* 00/01	*P. punctatus* 08/09 *A. butcheri* 08/09	*P. punctatus* 08/09 *A. butcheri* 08/09 *T. pleurogramma* - *G. subfasciatus* 08/09		*P. punctatus* *A. butcheri*

(d) Lower Swan-Canning Estuary

	1978/79	1979/80	1980/81	1981/82	2003/04	2005/06	2007/08	2008/09
1978/79	*M. cephalus* *F. lateralis* *A. forsteri* *T. pleurogramma* *A. rueppellii*							
1979/80		*F. lateralis* *M. cephalus* *A. rueppellii* *P. olorum*						
1980/81			*F. lateralis* *M. cephalus* *A. forsteri* *T. pleurogramma*					
1981/82		*A. forsteri* [81/82] *F. lateralis* [81/82] *M. cephalus* [79/80] *T. pleurogramma* [81/82] *L. presbyteroides* [81/82] *P. octolineatus* [81/82] *A. bifrenatus* [79/80] *P. olorum* [79/80]		*F. lateralis* *M. cephalus* *A. forsteri* *T. pleurogramma* *P. octolineatus* *A. rueppellii*				
2003/04	*M. cephalus* [78/79] *F. lateralis* [78/79] *A. forsteri* [78/79] *T. pleurogramma* [03/04] *P. punctatus* [03/04] *A. rueppellii* [03/04] *S. burrus* [03/04]	*F. lateralis* [79/80] *T. pleurogramma* [03/04] *P. punctatus* [03/04] *A. elongata* [03/04] *A. bifrenatus* [79/80]	*A. forsteri* [80/81] *M. cephalus* [80/81] *F. lateralis* [80/81] *T. pleurogramma* [80/81] *P. punctatus* [03/04] *A. rueppellii* [03/04] *S. burrus* [03/04]	*F. lateralis* [81/82] *A. forsteri* [81/82] *M. cephalus* [81/82] *T. pleurogramma* [81/82] *P. punctatus* [03/04] *A. rueppellii* [03/04] *P. octolineatus* [81/82] *P. olorum* [81/82]	*T. pleurogramma* *A. rueppellii* *P. punctatus*			
2005/06	*M. cephalus* [78/79] *A. forsteri* [78/79] *T. pleurogramma* [05/06] *G. marmoratus* [05/06] *L. presbyteroides* [05/06] *S. argus* [05/06] *A. rueppellii* [78/79]	*F. lateralis* [79/80] *T. pleurogramma* [05/06] *L. presbyteroides* [79/80] *G. marmoratus* [05/06] *S. argus* [05/06] *P. olorum* [79/80]	*A. forsteri* [80/81] *M. cephalus* [80/81] *T. pleurogramma* [80/81] *S. argus* [05/06] *A. rueppellii* [80/81]	*A. forsteri* [81/82] *M. cephalus* [81/82] *T. pleurogramma* [81/82] *L. presbyteroides* [81/82] *P. octolineatus* [81/82] *S. argus* [05/06] *A. rueppellii* [81/82]	*T. pleurogramma* [03/04] *A. rueppellii* [03/04] *P. punctatus* [03/04] *F. lateralis* [05/06] *L. presbyteroides* [05/06] *S. argus* [05/06]	*T. pleurogramma* *F. lateralis* *L. presbyteroides*		
2007/08	*M. cephalus* [78/79] *A. forsteri* [78/79] *T. pleurogramma* [07/08] *A. rueppellii* [78/79] *P. olorum* [78/79]	*F. lateralis* [79/80] *T. pleurogramma* [07/08] *P. olorum* [79/80]	*A. forsteri* [80/81] *M. cephalus* [80/81] *T. pleurogramma* [80/81] *F. lateralis* [80/81] *A. rueppellii* [80/81]	*F. lateralis* [81/82] *A. forsteri* [81/82] *M. cephalus* [81/82] *T. pleurogramma* [81/82] *P. octolineatus* [81/82] *A. rueppellii* [81/82]		*T. pleurogramma* [07/08] *S. argus* [05/06] *L. presbyteroides* [05/06] *F. lateralis* [05/06]	*T. pleurogramma* *F. lateralis*	
2008/09	*M. cephalus* [78/79] *A. forsteri* [78/79] *T. pleurogramma* [08/09] *A. rueppellii* [78/79] *P. olorum* [78/79]	*F. lateralis* [79/80] *L. presbyteroides* [79/80] *T. pleurogramma* [08/09] *P. olorum* [79/80]	*A. forsteri* [80/81] *M. cephalus* [80/81] *T. pleurogramma* [80/81] *F. lateralis* [80/81] *A. rueppellii* [80/81]	*A. forsteri* [81/82] *M. cephalus* [81/82] *T. pleurogramma* [81/82] *P. olorum* [81/82]	*T. pleurogramma* [03/04] *A. rueppellii* [03/04] *F. lateralis* [08/09] *P. punctatus* [03/04]			*T. pleurogramma* *F. lateralis*

(a) Canning Estuary/Lower Canning River
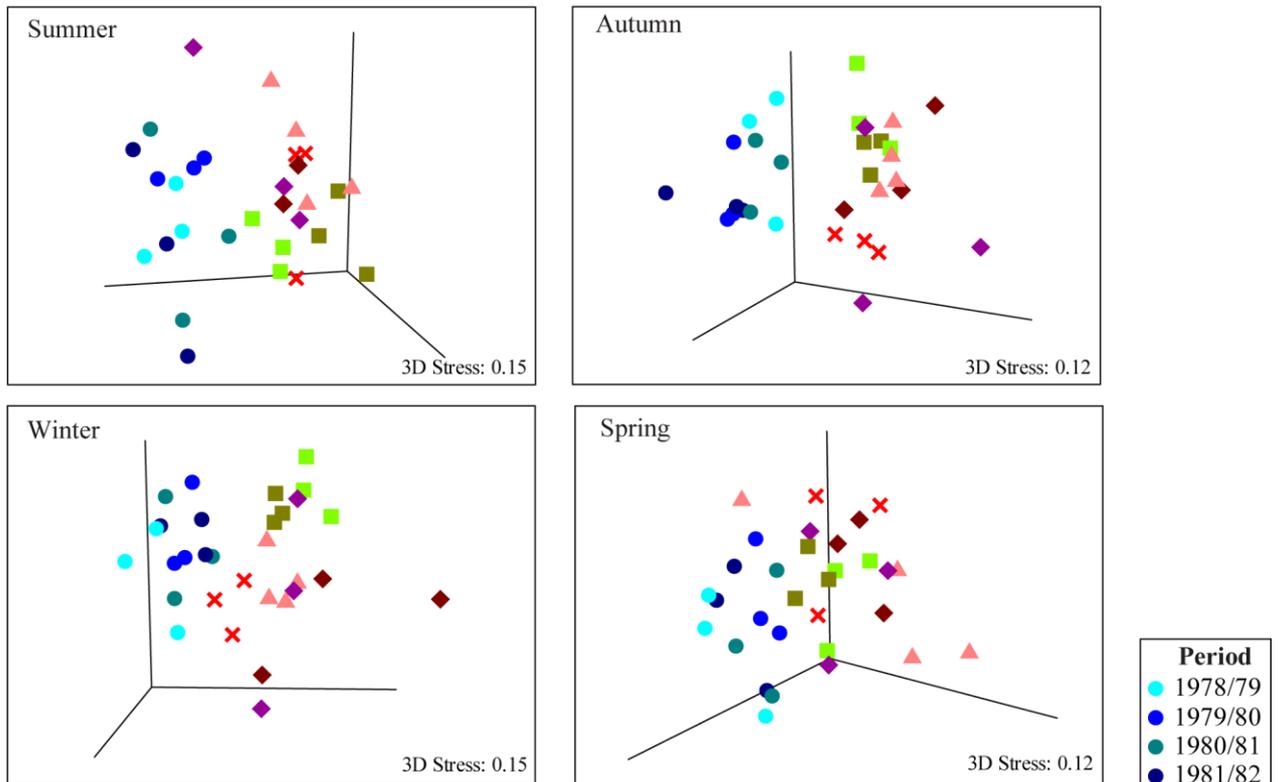
(b) Upper Swan Estuary

Figure 2.3.1.3: MDS ordination plots constructed from the nearshore fish species abundance data recorded during each period in each season in the (a) Canning Estuary/Lower Canning River and (b) Upper Swan Estuary.

(c) Middle Swan Estuary
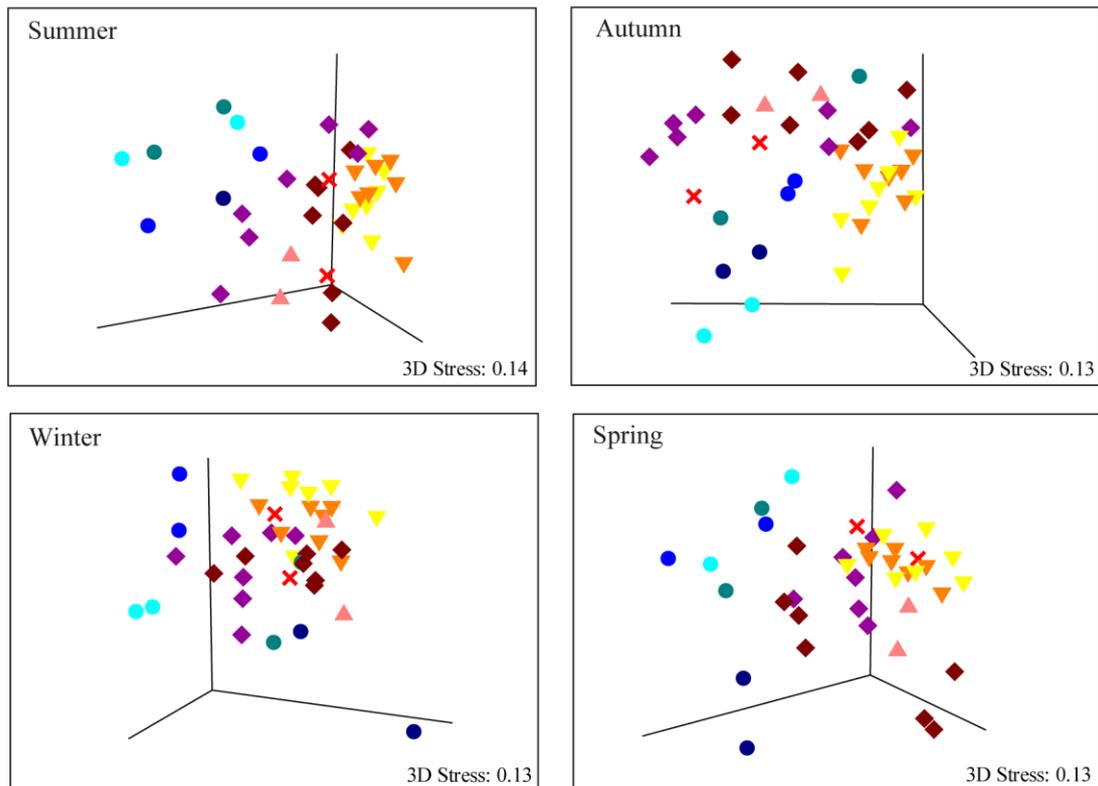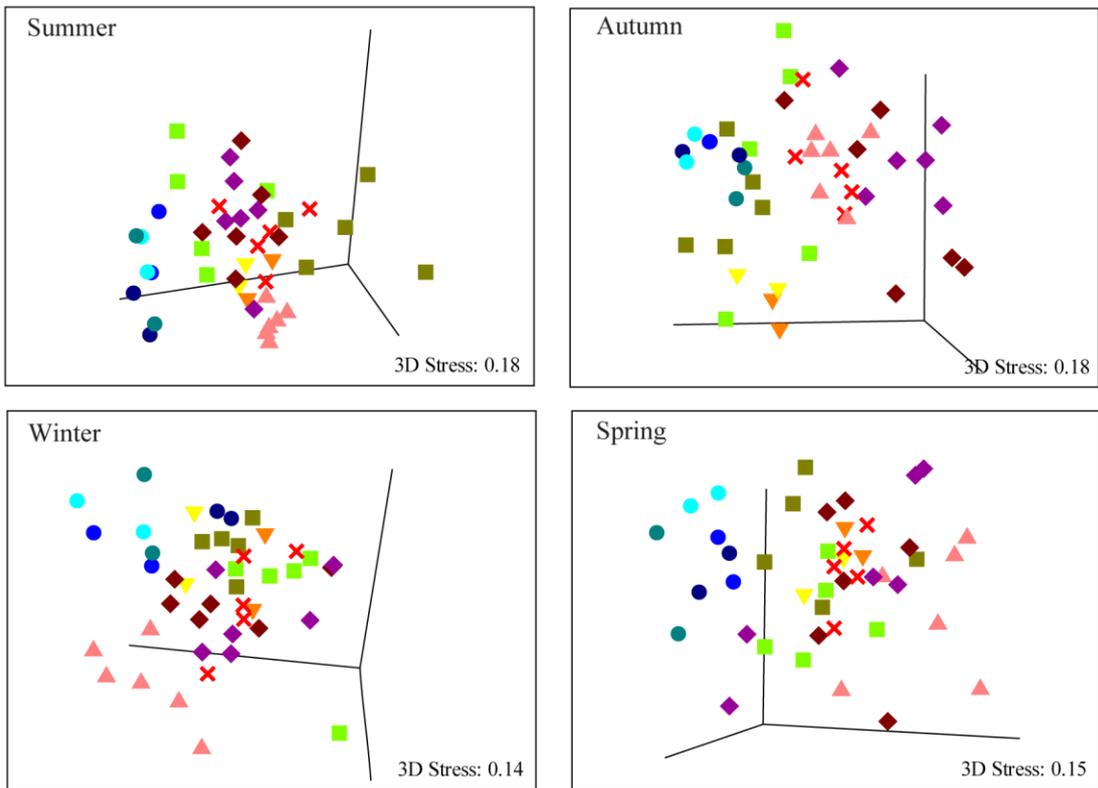

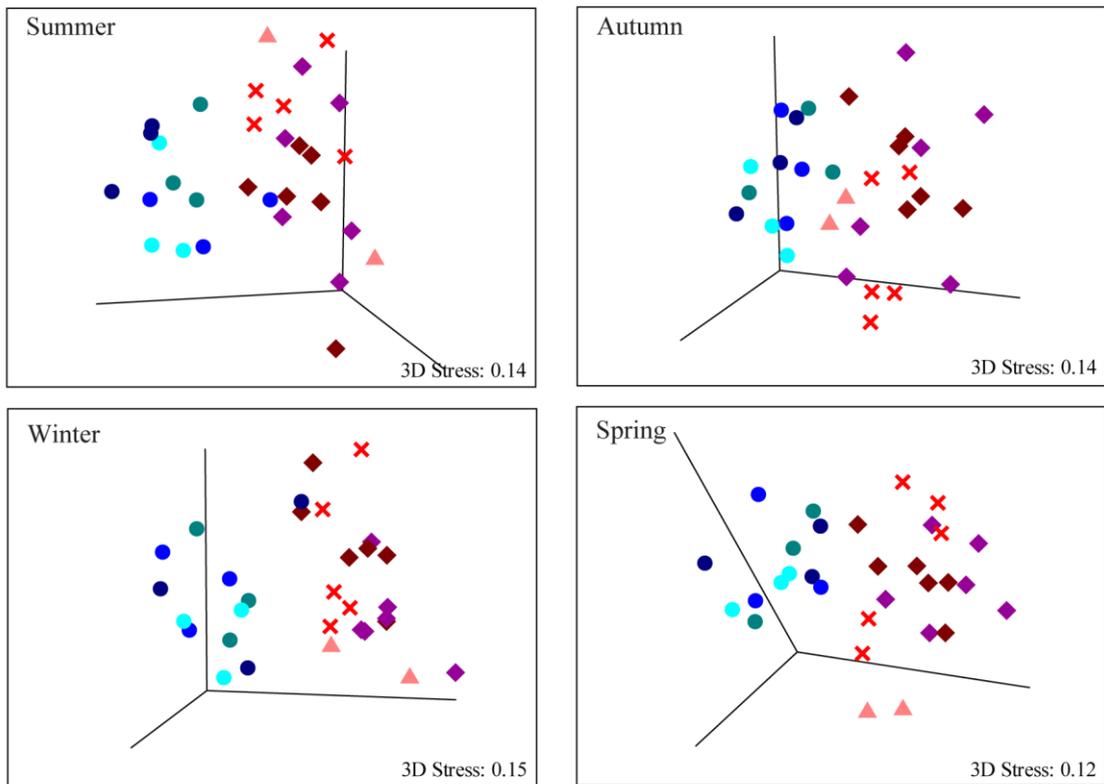**(d) Lower Swan-Canning Estuary**

Figure 2.3.1.3 (cont.):MDS ordination plots constructed from the nearshore fish species abundance data recorded during each period in each season in the (c) Middle Swan Estuary and (d) Lower Swan-Canning Estuary.

formed a distinct group on one side of the MDS plots shown in Fig. 2.3.1.3a, while those from 1995 to 2006 often formed relatively tight and discrete groups that lay towards the opposite side of the plots. SIMPER demonstrated that these differences were often attributable to comparatively greater catches of *M. cephalus* and, to a lesser extent, of *N. vlaminghi* and *A. forsteri* in the earlier periods, and also to a greater prevalence of *L. wallacei* and *A. butcheri* in the later periods. *Atherinosoma elongata* was also consistently more abundant in 1995-97 than 1978-82, as were *A. mugiloides* and *L. presbyteroides* in 1996/97 and *T. pleurogramma* in 2005/06 (Table 2.3.1.7a). Many of the pairs of periods sampled between 1978/79 and 1981/82 in the CELCR did not differ significantly from each other, which was also the case for those sampled between 2003/04 and 2008/09. This was reflected by the high degree of intermingling of samples from each of those sets of periods on the MDS plots shown in Fig. 2.3.1.3a.

In the LSCE, large differences in nearshore fish composition were detected between each of the periods sampled in the late 1970s/early 1980s and 2003/04 (R=0.716-0.755), while moderately large differences occurred between the former periods and 2005/06 and, to a lesser extent, 2007/08 (Table 2.3.1.6d). However, like the CELCR, many pairs of periods sampled between 1978/79 and 1981/82 did not differ significantly from each other, as was also the case for several pairs of periods sampled between 2003/04 and 2008/09. These results were reflected by the seasonal MDS plots shown for this zone in Fig. 2.3.1.3d, in which samples from the late 1970s/early 1980s formed a discrete group on one side of the plots, while those from 2003/04 and 2005/06 typically each formed groups that lay towards the opposite side of the plots. Samples collected in 2007-09 also tended to occupy the opposite side of the plots to those collected in the earliest periods, but, as for some of the other zones, they were often more dispersed than those in other periods, particularly in summer and autumn (Fig. 2.3.1.3d). SIMPER showed that the above inter-period differences were driven, in part, by consistently greater catches of *M. cephalus* and *A. forsteri* during 1978/79-1981/82 than in 2003/04-2007/08. They were also commonly due to greater catches of *T. pleurogramma* in each of the more recent periods than in 1978/79-1979/80, while the opposite was true for 1980/81-1981/82 (Table 2.3.1.7d). A range of other species also variably contributed to the above compositional differences, such as the frequently greater prevalence of *F. lateralis* and *A. rueppellii* in the late 1970s/early 1980s than in the later periods, except 2003/4 for the latter species, when the opposite was true (Table 2.3.1.7d).

2.3.1.3 Differences in the offshore fish fauna among periods

2.3.1.3.1 Mean species catch-rates
The mean catch-rates of each fish species caught in the offshore waters of the Swan-Canning Estuary during each of the four studies carried out between 1993 and 2009 are provided in Table 2.3.1.8. The total mean catch-rate declined progressively from the earliest to the latest of those studies, such that values in 2007/09 were about 1.5 times less than those in 1993/94. The total number of species, however, which ranged from 11 to 16, was identical for the earliest and latest studies (*i.e.* 14).

Table 2.3.1.8: Mean catch rate (Mean; *i.e.* number of fish 1h^{-1}), standard deviation (^{sd}), percentage contribution to the overall catch (%) and rank by density (R) of each fish species recorded in each study carried out in the offshore waters of the Swan-Canning Estuary between 1993/94 and 2008/09. All data have been standardised for spatio-temporal sampling intensity as per the methods described in subsection 2.2.1.4.1. Abundant species (*i.e.* those that contribute >5% to the catch) are highlighted in grey. The life-history category of each species (^{LH}) is also provided (*i.e.* ^{A}=semi-anadromous, ^{E}=estuarine, ^{EM}=estuarine and marine, ^{F}=freshwater, ^{O}=marine estuarine-opportunist, ^{S}=marine straggler). The total number of species and the total mean catch rate of individuals are also given for each study.

Species	Common name	Sarre (unpubl.) (1993/94) Mean^{sd}	%	R	Kanandjembo *et al.* 2001a (1995-97) Mean^{sd}	%	R	Valesini *et al.* 2005 (2003/04) Mean^{sd}	%	R	Current (2007-09) Mean^{sd}	%	R
Nematalosa vlaminghi ^{A}	Perth Herring	$9.94^{11.80}$	57.70	1	$7.21^{8.57}$	45.95	1	$6.44^{8.86}$	41.81	1	$5.49^{9.20}$	49.91	1
Acanthopagrus butcheri ^{E}	Southern Black Bream	$3.28^{3.49}$	19.04	2	$2.13^{6.70}$	13.55	3	$3.00^{5.23}$	19.49	3	$0.47^{0.82}$	4.26	3
Amniataba caudavittata ^{E}	Yellowtail Grunter	$2.24^{3.02}$	13.01	3	$4.02^{7.78}$	25.63	2	$3.71^{6.33}$	24.09	2	$4.14^{9.93}$	37.59	2
Mugil cephalus ^{O}	Sea Mullet	$0.64^{0.88}$	3.74	4	$1.19^{3.12}$	7.57	4	$1.52^{4.81}$	9.88	4	$0.30^{1.20}$	2.75	4
Platycephalus endrachtensis ^{E}	Bar-tailed Flathead	$0.30^{0.42}$	1.75	5	$0.23^{0.52}$	1.46	5	$0.15^{0.36}$	0.95	6	$0.13^{0.33}$	1.14	6
Argyrosomus japonicus ^{O}	Mulloway	$0.25^{0.34}$	1.48	6	$0.23^{0.66}$	1.46	6				$0.02^{0.14}$	0.19	12
Pelates octolineatus ^{O}	West^{n} Striped Grunter	$0.22^{0.43}$	1.29	7	$0.13^{0.44}$	0.80	9	$0.15^{0.62}$	0.95	7			
Cnidoglanis macrocephalus ^{EM}	Estuarine Cobbler	$0.11^{0.21}$	0.65	8	$0.08^{0.28}$	0.53	10				$0.02^{0.14}$	0.19	11
Gerres subfasciatus ^{O}	Roach	$0.10^{0.23}$	0.56	9	$0.02^{0.14}$	0.13	16	$0.10^{0.42}$	0.68	8	$0.01^{0.10}$	0.09	13
Aldrichetta forsteri ^{O}	Yellow-eye Mullet	$0.07^{0.17}$	0.40	10	$0.04^{0.20}$	0.27	12	$0.04^{0.20}$	0.27	11			
Elops machnata ^{S}	Australian Giant Herring	$0.03^{0.09}$	0.16	11	$0.15^{0.41}$	0.93	8				$0.01^{0.10}$	0.09	14
Tandanus bostocki ^{F}	Freshwater Cobbler	$0.02^{0.11}$	0.11	12									
Pomatomus saltatrix ^{O}	Tailor	$0.01^{0.06}$	0.05	13	$0.17^{0.48}$	1.06	7				$0.09^{0.33}$	0.85	7
Siphamia cephalotes ^{S}	Wood's Siphonfish	$0.01^{0.06}$	0.05	14									
Engraulis australis ^{EM}	Southern Anchovy				$0.04^{0.29}$	0.27	11	$0.21^{0.41}$	1.35	5	$0.23^{0.55}$	2.08	5
Rhabdosargus sarba ^{O}	Tarwhine				$0.02^{0.14}$	0.13	13	$0.04^{0.20}$	0.27	9			
Torquigener pleurogramma ^{O}	Banded Toadfish				$0.02^{0.14}$	0.13	13	$0.04^{0.20}$	0.27	9	$0.04^{0.20}$	0.38	8
Callogobius depressus ^{S}	Flathead Goby				$0.02^{0.14}$	0.13	15						
Arripis georgianus ^{O}	Australian Herring										$0.03^{0.17}$	0.28	9
Carcharhinus leucas ^{S}	Bull Shark										$0.02^{0.14}$	0.19	10
Total mean catch rate		**17.22**			**15.69**			**15.40**			**11.00**		
Number of species		**14**			**16**			**11**			**14**		

Nematalosa vlaminghi ranked first and *A. butcheri* and *A. caudavittata* ranked either second or third in terms of mean abundance in all offshore studies. However, their mean catch-rates and contributions to the overall catch varied considerably (Table 2.3.1.8). Thus, whereas an average of *ca* 10 fish/h^{-1} of the first of these species was recorded in 1993/94 (contributing nearly 60% to the overall catch), progressively lower averages were recorded in each successive study (representing *ca* 42-50% of the overall catches), such that only about 5 fish/h^{-1} were recorded in 2007-09. Furthermore, the mean catch-rate of *A. butcheri* in 2007-09 was markedly lower than in any other study (*ca* 0.5 fish/h^{-1}), but particularly compared to that in 1993/94 (*ca* 3.2 fish/h^{-1}). In contrast, the opposite was true for *A. caudavittata*, with higher mean catch-rates and contributions to the overall catch in all studies since 1995 than that in 1993/4 (Table 2.3.1.8).

2.3.1.3.2. Mean overall number of species, catch-rates and taxonomic distinctness
Four-way PERMANOVA of the mean catch-rate, number of species and taxonomic distinctness in offshore fish samples collected seasonally from a common suite of sites in the MSE and USE during 1993/94, 2003/04 and 2007-09 demonstrated that each of these dependent variables differed significantly among periods and seasons and the interaction between these two main effects (Table 2.3.1.9a-c). Mean catch-rate also differed significantly among sites within zones and all remaining interaction terms except zone x period, while mean taxonomic distinctness also exhibited a significant period x site(zone) interaction.

Plots of the mean catch-rate in each period, constructed separately for each season and zone to overcome the influence of those confounding factors, showed that by far the most pronounced inter-period shifts occurred in the USE in summer, with values declining progressively from *ca* 38 fish h^{-1} in 1993/94 to *ca* 8 fish h^{-1} in 2008/09 (Fig. 2.3.1.4b). Although less marked, mean catch-rate also declined over the above periods in autumn and winter in the USE and in all seasons except autumn in the MSE (except for a slight increase from 2007/08 to 2008/09 in some cases). During spring in the USE, however, catch-rates increased from similar values in 1993/94 and 2003/04 to their highest value in 2008/09, while those for autumn in the MSE showed no consistent inter-period trends (Fig. 2.3.1.4a, b).

The mean number of species also generally declined progressively from 1993/94 to 2008/09, again with the exception of a slight increase from 2007/08 to 2008/09 in some seasons (Fig. 2.3.1.4c). The most pronounced declines occurred in autumn, with values falling steadily from a maxima of *ca* 7 in 1993/94 to a minima of *ca* 2 in 2007/08, while the least pronounced were recorded in winter (*i.e. ca* 4 and 1 species in 1993/94 and 2008/09, respectively) and spring (*i.e. ca* 5 and 2 species in 1993/94 and 2007/08, respectively; Fig. 2.3.1.4c).

Trends in the mean average quantitative taxonomic distinctness of the fish assemblage among periods paralleled, to a large extent, those observed for both of the above dependent variables. However, rather than declining progressively from the earliest to most recent period, the mean values of this diversity index were almost identical in 1993/94 and 2003/04

Table 2.3.1.9: Mean squares (MS), pseudo F-ratios, significance levels (*P*) and components of variation (COV) for zone x season x period x site[zone] PERMANOVAs on the data for offshore fish (a) total catch-rate, (b) number of species, (c) quantitative average taxonomic distinctness and (d) assemblage composition recorded seasonally throughout the MSE and USE zones of the Swan-Canning Estuary in each period sampled between 1993/94 and 2008/09 (excluding 1995/96 and 1996/97). df=degrees of freedom; E=exponential. Significant results involving period are highlighted in bold.

		(a) Total catch-rate				(b) Number of species			
	df	MS	Pseudo-F	*P*	COV	MS	Pseudo-F	*P*	COV
Zone (Z)	1	1.3665	0.8962	0.361	-4.7165E-2	5.7781	5.4132	0.075	0.25738
Season (S)	3	13.394	16.119	0.001	0.59443	26.459	23.355	0.001	0.84397
Period (P)	3	**5.6641**	**6.7531**	**0.002**	**0.36839**	**75.251**	**44.906**	**0.001**	**1.43850**
Site (si) [Zone]	7	1.5247	3.8157	0.002	0.26518	1.0674	0.7027	0.679	-0.16800
ZxS	3	3.9005	4.6940	0.011	0.41552	1.1346	1.0015	0.420	9.8349E-3
ZxP	3	0.1455	0.1734	0.914	-0.19747	1.4087	0.8406	0.480	-0.12257
SxP	9	**1.3397**	**3.3527**	**0.001**	**0.32521**	**4.6201**	**3.0415**	**0.008**	**0.59066**
Sxsi[Z]	21	0.8310	2.0795	0.014	0.32839	1.1329	0.7458	0.758	-0.31069
Pxsi(Z)	21	**0.8387**	**2.0990**	**0.009**	**0.33134**	1.6757	1.1032	0.363	0.19796
ZxSxP	9	**1.0557**	**2.6418**	**0.018**	**0.38421**	2.9627	1.9504	0.075	0.56994
Residual	63	0.3996			0.63213	1.5190			1.23250

		(c) Quantitative Average Taxonomic Distinctness				(d) Assemblage composition			
	df	MS	Pseudo-F	*P*	COV	MS	Pseudo-F	*P*	COV
Zone (Z)	1	2.381E-2	2.029E-3	0.964	-0.4058	7495.4	5.5646	0.012	9.2985
Season (S)	3	62.910	11.893	0.001	1.2730	9386.1	12.480	0.001	15.583
Period (P)	3	**76.455**	**6.3716**	**0.006**	**1.3464**	**9992.3**	**13.271**	**0.001**	**16.120**
Site (si) [Zone]	7	11.736	2.0427	0.070	0.6119	1347.0	2.2662	0.001	6.8584
ZxS	3	5.4816	1.0363	0.401	0.1039	2812.6	3.7398	0.001	10.766
ZxP	3	2.2620	0.1885	0.905	-0.7401	1146.6	1.5229	0.119	4.7059
SxP	9	**19.062**	**3.3177**	**0.007**	**1.2240**	**1627.3**	**2.7378**	**0.001**	**10.780**
Sxsi[Z]	21	5.2897	0.9207	0.538	-0.3376	752.07	1.2653	0.072	6.2787
Pxsi(Z)	21	**11.999**	**2.0885**	**0.009**	**1.2504**	752.93	1.2667	0.062	6.2958
ZxSxP	9	8.5428	1.4869	0.171	0.7933	**897.68**	**1.5103**	**0.026**	**8.2609**
Residual	63	5.7455			2.3970	594.38			24.380
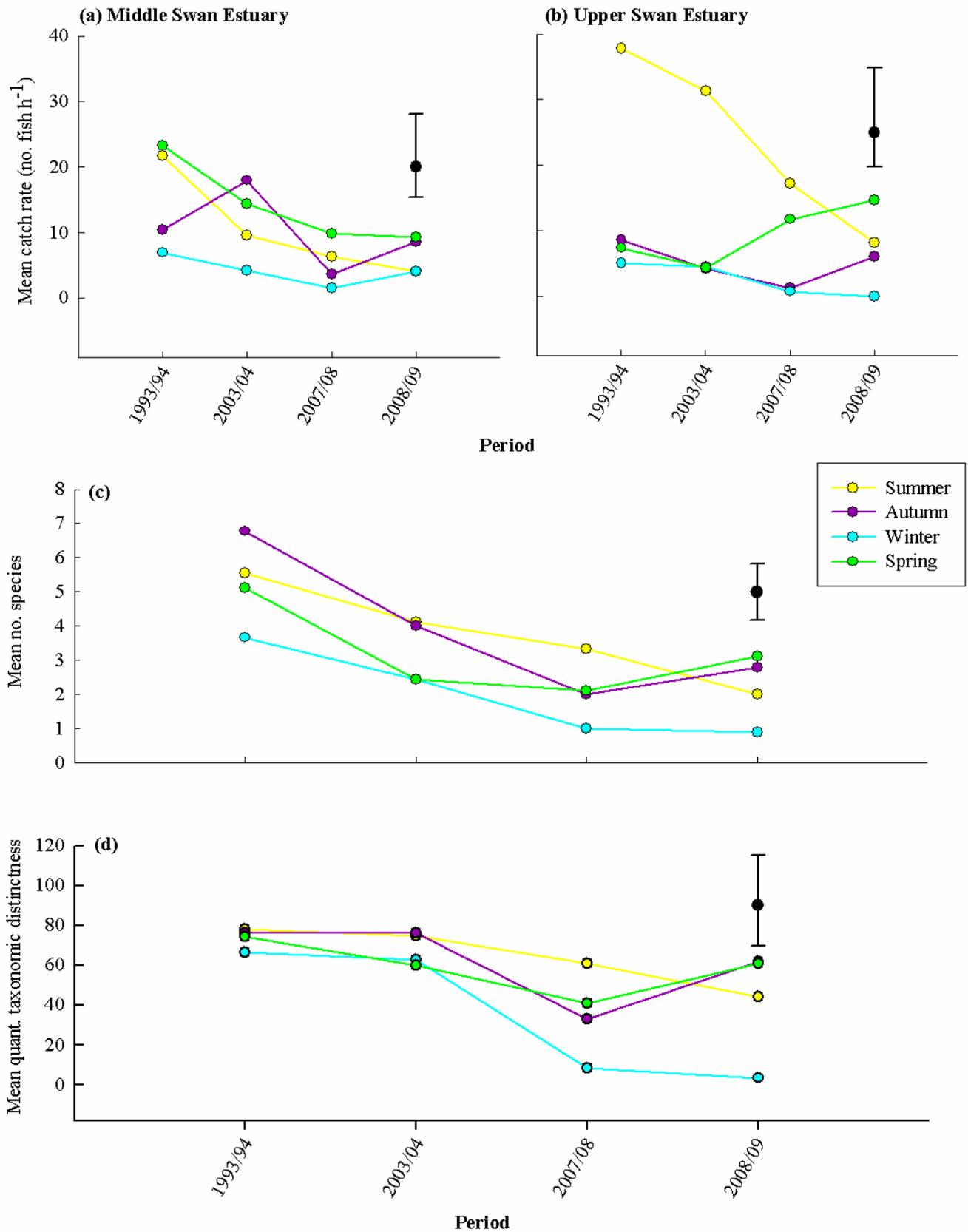
Figure 2.3.1.4: Mean catch-rate (fish h^{-1}) of fish in the offshore waters of (a) the MSE and (b) the USE during each season in each period sampled between 1993/94 and 2008/09. Mean (c) number of species and (d) quantitative taxonomic distinctness of the fish fauna in the offshore waters during each season in each period sampled between 1993/94 and 2008/09. For the sake of clarity, the average 95% confidence intervals have been presented for each of these plots.

in most seasons, then underwent a marked decline in 2007/08 and, in summer and winter, further declines in 2008/09 (Fig. 2.3.1.4d). Although values increased slightly from 2007/08 to 2008/09 in autumn and spring, they were still less than those recorded in the same seasons in 1993/94 (Fig. 2.3.1.4d).

2.3.1.3.3 Species composition of fish assemblages among periods
Four-way PERMANOVA, containing period, season, zone and site nested within zone as factors, demonstrated that the composition of the offshore fish fauna at the common suite of sites sampled seasonally in the MSE and USE during 1993/94, 2003/04 and 2007-09 differed significantly among all main effects, the zone x season and season x period two-way interactions and the interaction between the first three of the above main effects (Table 2.3.1.9d). The components of variation associated with each of these significant terms demonstrated that both the period and season main effects had the greatest influence on offshore fish composition, followed by both of the above two-way interactions.

To examine the significant inter-period differences in offshore fish composition in more detail, and to remove the confounding influence of the remaining factors, the data were separated on the basis of zone and, in each zone, a two-way crossed period x season ANOSIM test was carried out. The period component of these tests is shown in Table 2.3.1.10. Note that two separate ANOSIM tests were carried out for the USE, the first of which employed the data described above, and the second of which employed the data at the seven common sites sampled in that zone in 2003/04 and the current study (see Table 2.2.1.2). Furthermore, the ANOSIM test for the MSE also included data collected in 1995-97, and thus incorporated data from all studies undertaken in that zone between 1993/94 and 2008/09 (see Table 2.2.1.2).

Significant inter-period differences were detected for both ANOSIM tests in the USE and in the MSE (P=0.001), but their overall extent was moderate to moderately low in each case (*i.e.* Global R= 0.394-0.261; Table 2.3.1.10). The greatest differences occurred in the USE, and particularly for that test including all periods sampled between 1993/94 and 2008/09. For both tests in this zone, the most pronounced differences in offshore fish composition typically occurred between those pairs of periods that were the most temporally disparate, *i.e.* 1993/94 and/or 2003/04 *vs* 2007/08 and/or 2008/09 (Table 2.3.1.10a, b). This was clearly reflected by the relative positions of samples from those periods on the MDS plots shown in Fig. 2.3.1.5a, b, especially in autumn and winter. Moreover, and particularly for the first of the above USE data sets, the replicate samples collected in 2007-09 were generally far more dispersed than those from earlier periods. SIMPER demonstrated that, for both tests in the USE, the most pronounced inter-period differences were driven largely by the greater and more consistent catches of *A. butcheri, M. cephalus* and *N. vlaminghi* in the earlier than later periods (Table 2.3.1.11a, b).

In the MSE, moderately large to large differences in fish faunal composition occurred between 2007/08 and both 1993/94 and 1996/97 (R=0.492-0.632), with the remainder of the pairwise differences being moderately low to low (Table 2.3.1.11c). The MDS plots

Table 2.3.1.10: R-statistic and/or significance level (*P*) values for global and pairwise comparisons in two-way crossed period x season ANOSIM tests of the offshore fish faunal composition in (a) the Upper Swan Estuary *(i)* (*i.e.* common sites sampled in 1993/94, 2003/04 and 2007-09), (b) the Upper Swan Estuary *(ii)* (*i.e.* all common sites sampled in 2003/04 and 2007-09) and (c) the Middle Swan Estuary. Note that only the period component of these tests is shown. Insignificant pairwise comparisons are highlighted in grey.

(a) Upper Swan Estuary *(i)*; Global R=0.394, *P*=0.001

	1993/94	2003/04	2007/08
2003/04	0.370		
2007/08	0.573	0.336	
2008/09	0.523	0.477	0.143

(b) Upper Swan Estuary *(ii)*; Global R=0.293, *P*=0.001

	2003/04	2007/08
2007/08	0.281	
2008/09	0.438	0.179

(c) Middle Swan Estuary; Global R=0.261, *P*=0.001

	1993/94	1995/96	1996/97	2003/04	2007/08
1995/96	0.253				
1996/97	0.258	0.157			
2003/04	0.315	0.188	0.161		
2007/08	0.632	0.353	0.492	0.278	
2008/09	0.379	0.256	0.251	0.275	0.007

Table 2.3.1.11: Species that consistently typified (provided along the diagonal) and distinguished (provided in the sub-diagonal) the offshore fish assemblages in each period sampled between 1993/94 and 2008/09 in (a) the Upper Swan Estuary *(i)* (*i.e.* all common sites sampled in 1993/94, 2003/04 and 2007-09), (b) the Upper Swan Estuary *(ii)* (*i.e.* all common sites sampled in 2003/04 and 2007-09) and (c) the Middle Swan Estuary, as detected by two-way crossed period x season SIMPER. Note that only the period component of these tests is shown. The period in which each species was most abundant is given in superscript for each pairwise comparison. Insignificant pairwise comparisons (as detected by ANOSIM; Table 2.3.1.10) are highlighted in grey.

(a) Upper Swan Estuary *(i)*

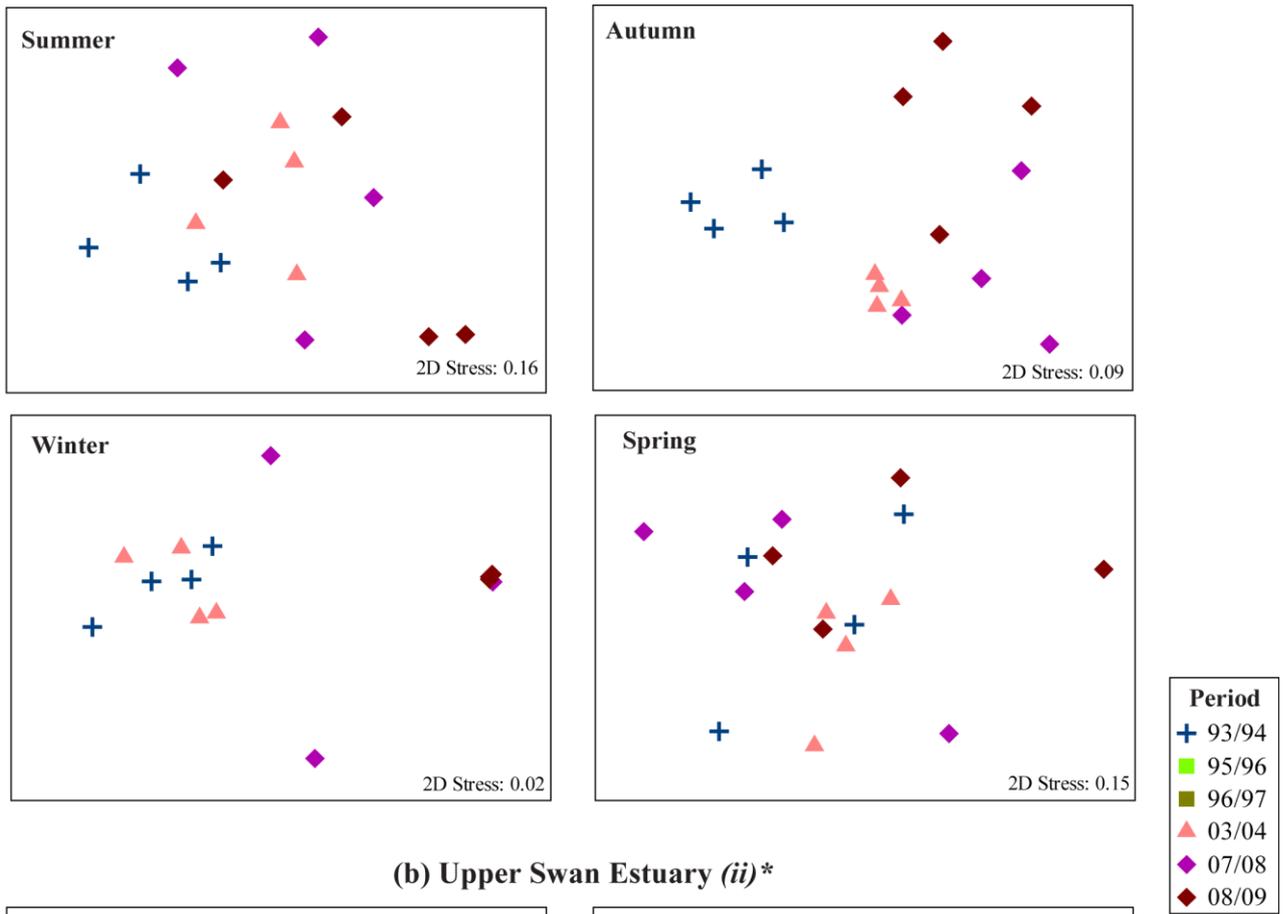|	1993/94	2003/04	2007/08	2008/09
1993/94	A. butcheri A. caudavittatus N. vlaminghi M. cephalus			
2003/04	A. caudavittatus $^{93/94}$ A. butcheri $^{93/94}$ N. vlaminghi $^{93/94}$ M. cephalus $^{93/94}$ A. japonicus $^{93/94}$ P. endrachtensis $^{93/94}$	A. butcheri N. vlaminghi A. caudavittatus M. cephalus		
2007/08	A. butcheri $^{93/94}$ A. caudavittatus $^{07/08}$ M. cephalus $^{93/94}$ N. vlaminghi $^{93/94}$	A. butcheri $^{03/04}$ A. caudavittatus $^{07/08}$ N. vlaminghi $^{03/04}$ M. cephalus $^{03/04}$	A. caudavittatus N. vlaminghi	
2008/09	A. butcheri $^{93/94}$ M. cephalus $^{93/94}$ N. vlaminghi $^{93/94}$ A. caudavittatus $^{93/94}$	A. butcheri $^{03/04}$ M. cephalus $^{03/04}$ A. caudavittatus $^{03/04}$ N. vlaminghi $^{03/04}$ E. australis $^{08/09}$		A. caudavittatus N. vlaminghi A. butcheri E. australis

(b) Upper Swan Estuary *(ii)*

	2003/04	2007/08	2008/09
2003/04	A. butcheri N. vlaminghi A. caudavittatus M. cephalus		

	2003/04	2007/08	2008/09
2007/08	*A. butcheri* [03/04] *A. caudavittatus* [07/08] *M. cephalus* [03/04] *N. vlaminghi* [03/04]	*A. caudavittatus* *N. vlaminghi* *A. butcheri*	
2008/09	*A. butcheri* [03/04] *M. cephalus* [03/04] *N. vlaminghi* [03/04] *A. caudavittatus* [03/04]	*A. caudavittatus* [07/08] *N. vlaminghi* [08/09] *A. butcheri* [07/08]	*A. caudavittatus* *N. vlaminghi* *A. butcheri*

(c) Middle Swan Estuary

	1993/94	1995/6	1996/7	2003/04	2007/08	2008/09
1993/94	*N. vlaminghi* *A. butcheri* *P. endrachtensis* *A. caudavittatus*					
1995/96	*A. butcheri* [93/94] *N. vlaminghi* [93/94] *M. cephalus* [95/96] *A. caudavittatus* [95/96] *P. endrachtensis* [93/94]	*N. vlaminghi* *A. caudavittatus* *M. cephalus*				
1996/97	*A. butcheri* [93/94] *N. vlaminghi* [93/94] *M. cephalus* [93/94] *P. endrachtensis* [93/94] *A. caudavittatus* -	*A. caudavittatus* [95/96] *N. vlaminghi* [95/96] *A. butcheri* [95/96] *M. cephalus* [95/96]	*N. vlaminghi* *A. butcheri* *M. cephalus* *A. caudavittatus*			
2003/04	*A. butcheri* [93/94] *N. vlaminghi* [93/94] *A. caudavittatus* [03/04] *P. endrachtensis* [93/94]	*A. butcheri* [95/96] *A. caudavittatus* [95/96] *N. vlaminghi* [95/96] *M. cephalus* [95/96]	*N. vlaminghi* [03/04] *A. caudavittatus* [03/04] *A. butcheri* [03/04] *P. endrachtensis* [03/04]	*N. vlaminghi* *A. butcheri* *A. caudavittatus*		
2007/08	*A. butcheri* [93/94] *N. vlaminghi* [93/94] *P. endrachtensis* [93/94] *A. caudavittatus* [93/94]	*A. caudavittatus* [95/96] *N. vlaminghi* [95/96] *M. cephalus* [95/96] *A. butcheri* [95/96]	*A. butcheri* [96/97] *N. vlaminghi* [96/97] *M. cephalus* [96/97]	*A. butcheri* [03/04] *N. vlaminghi* [03/04] *A. caudavittatus* [03/04]	*N. vlaminghi* *E. australis*	
2008/09	*A. butcheri* [93/94] *N. vlaminghi* [93/94] *P. endrachtensis* [93/94] *A. caudavittatus* [93/94]	*A. caudavittatus* [95/96] *N. vlaminghi* [95/96] *M. cephalus* [95/96] *A. butcheri* [95/96]	*N. vlaminghi* [08/09] *A. butcheri* [96/97] *M. cephalus* [96/97]	*N. vlaminghi* [08/09] *A. butcheri* [03/04] *A. caudavittatus* [03/04]		*N. vlaminghi*

Figure 2.3.1.5: MDS ordination plots constructed from the offshore fish species abundance data recorded during each period in each season at all common sites sampled in the Upper Swan Estuary in (a) 1993/94, 2003/04 and 2007-09 *(i)* and (b) 2003/04 and 2007-09 *(ii)*.
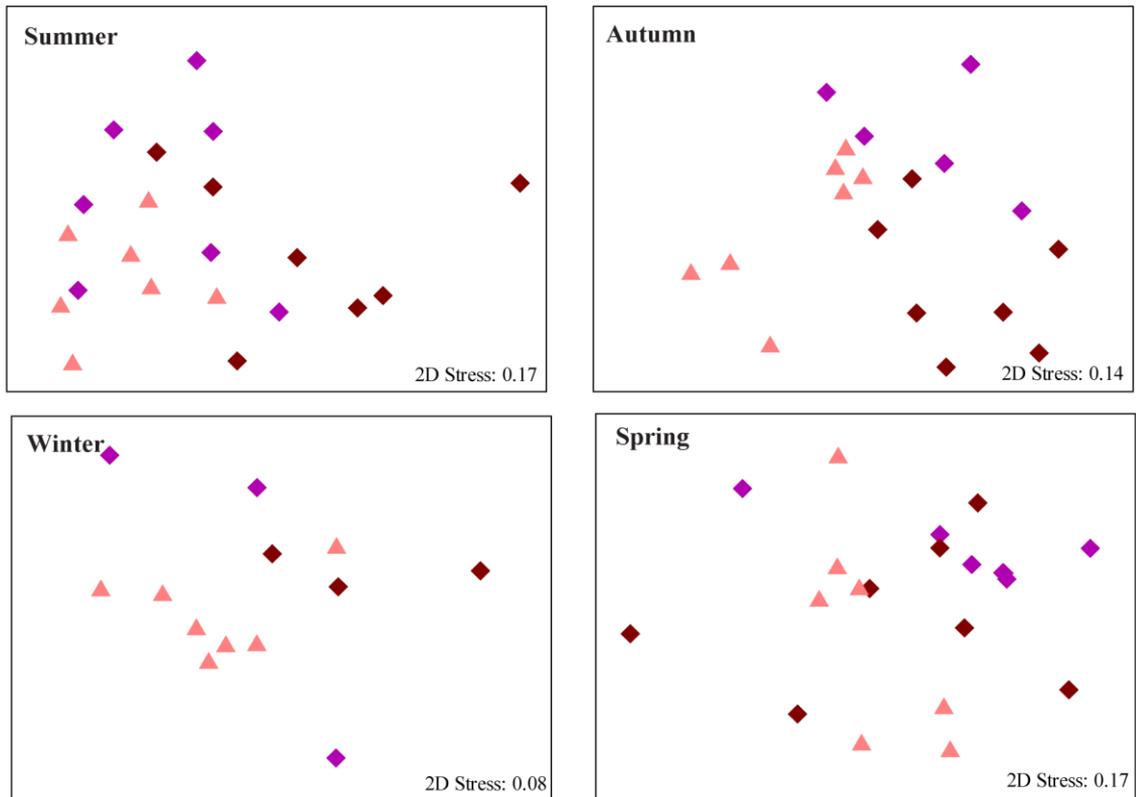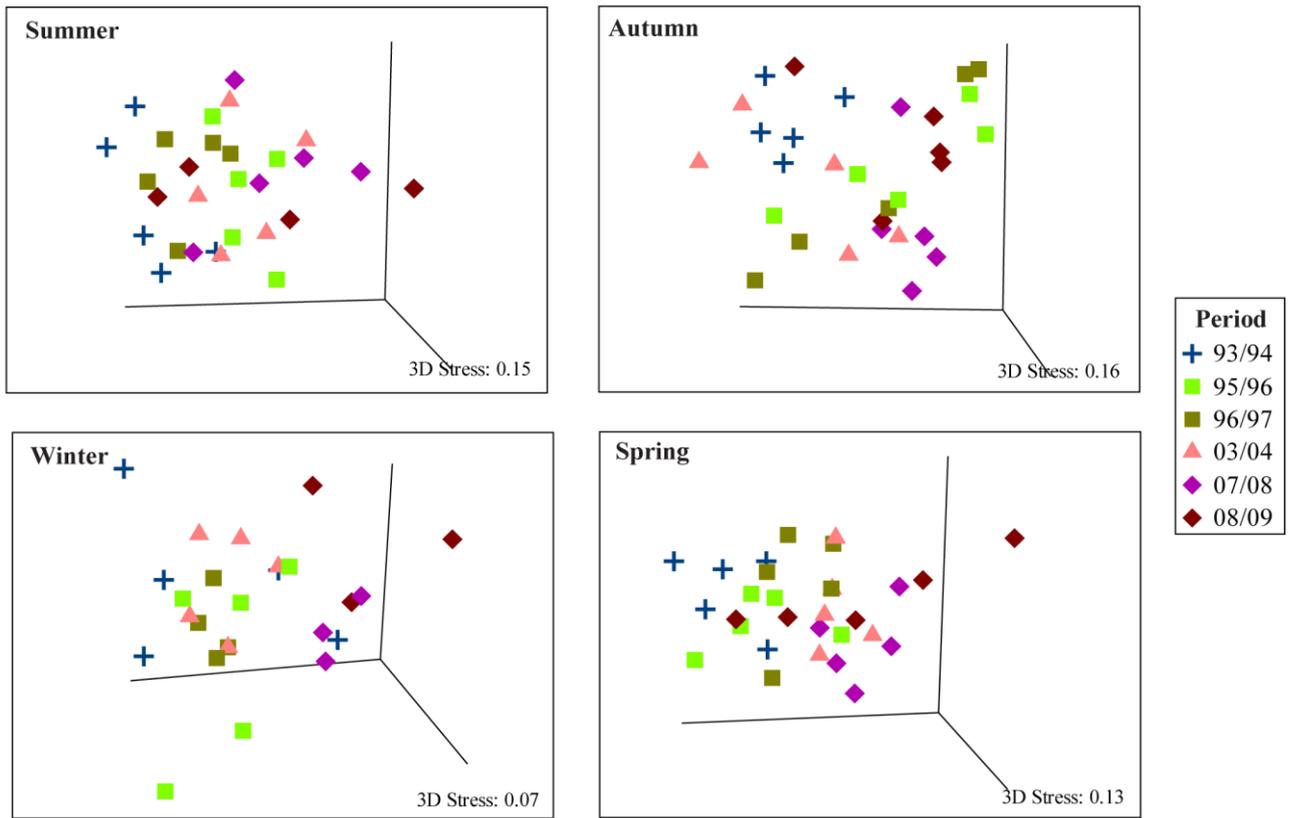
(c) Middle Swan Estuary

Figure 2.3.1.5 (cont.): MDS ordination plots constructed from the offshore fish species abundance data recorded during each period in each season in the Middle Swan Estuary.

constructed from the data recorded in this zone showed that samples from 2007/08 and 1993/94 tended to occupy opposite sides of the plot, with varying degrees of within-group dispersion in the different seasons (Fig. 2.3.1.5c). Moreover, samples from 1996/97 were generally located alongside those from 1993/94, and again exhibited varying degrees of dispersion, with those in autumn being the most dispersed (Fig. 2.3.1.5c). SIMPER showed that the above inter-period differences were due, in part, to consistently lower catches of *A. butcheri* and *N. vlaminghi* in 2007/08 than in both of the earlier periods (Table 2.3.1.11c).

2.3.1.4 Relationships between fish faunal composition and water quality in nearshore and offshore waters

2.3.1.4.1 Nearshore (surface) waters
The surface water concentrations of chlorophyll *a*, total N, total P and dissolved oxygen, recorded by the DoW at their regular monitoring sites in the MSE, USE and CELCR during those periods in which the nearshore fish were sampled between 1995/96 and 2008/09, were each shown by PERMANOVA to differ significantly among periods and the season x period interaction ($P=0.001$). All other interaction terms involving period were also significant in the case of dissolved oxygen concentration (Table 2.3.1.12a-d). Furthermore, PERMANOVA of the surface salinity and temperature data recorded in the above three zones by the DoW or Loneragan *et al.* (1989) in each fish sampling period since the late 1970s also detected significant inter-period differences in each case ($P=0.001$). Surface water temperature also differed significantly among the period x zone and zone x season x period interactions (Table 2.3.1.12e-f). However, for each of the above surface water quality variables, the relative influence of the significant period main effect and/or interactions was less than that of the significant season and/or zone main effects (Table 2.3.1.12a-f).

Plots of the means of each of the above surface water quality variables in each fish sampling period (within each season and/or zone where necessary) demonstrated that, in the case of chlorophyll *a*, the main causes of the significant inter-period differences were the notably higher concentrations in 1996/97, 1999/00, 2000/01 and 2005/06 than in the remaining periods during summer and/or autumn. Moreover, the lowest chlorophyll *a* concentrations, or those close to the lowest, were recorded in 2003/04 in every season (Fig. 2.3.1.6a).

The highest concentrations of both total N and P were recorded in 1996/97 in all seasons except summer with respect to the former variable (Fig. 2.3.1.6b, c). The concentration of total P was also higher in 1995/96 than in all periods between 1999/00 and 2008/09 in each season except autumn, and the same was true for total N in winter. Concentrations of each of these nutrients were generally similar between 1999/00 and 2008/09, with a few minor exceptions, *e.g.* the comparatively low concentrations of total N in most seasons in 2000/01 and the relatively high concentrations of total P in autumn 1999/00 (Fig. 2.3.1.6b, c).

Table 2.3.1.12: Mean squares (MS), pseudo F-ratios, significance levels (*P*) and components of variation (COV) for zone x season x period PERMANOVAs of the surface water concentrations of (a) chlorophyll *a*, (b) total nitrogen, (c) total phosphorous and (d) dissolved oxygen recorded seasonally by the DoW at their water quality monitoring sites throughout the CELCR, MSE and USE zones of the Swan-Canning Estuary in each nearshore fish sampling period between 1995/96 and 2008/09, and of (e) salinity and (f) temperature recorded seasonally by Loneragan *et al.* (1989) or the DoW throughout the same estuary zones in each nearshore fish sampling period between 1978/79 and 2008/09. df=degrees of freedom; E=exponential. Significant results involving period are highlighted in bold.

	df	**(a) Chlorophyll *a* concentration** MS	Pseudo-F	*P*	COV	**(b) Total nitrogen concentration** MS	Pseudo-F	*P*	COV
Zone (Z)	2	9.8898	17.338	0.001	0.33751	0.12395	50.260	0.001	3.853E-2
Season (S)	3	22.629	39.671	0.001	0.60505	9.055E-2	36.717	0.001	3.823E-2
Period (P)	7	**4.8055**	**8.4245**	**0.001**	**0.35754**	**3.480E-2**	**14.113**	**0.001**	**3.124E-2**
ZxS	6	3.966	6.9528	0.001	0.40738	1.348E-2	5.4673	0.001	2.321E-2
ZxP	10	0.6453	1.1313	0.332	7.3868E-2	1.297E-3	0.5260	0.864	-9.228E-3
SxP	21	**2.0316**	**3.5616**	**0.001**	**0.41983**	**1.128E-2**	**4.5726**	**0.001**	**3.260E-2**
ZxSxP	30	0.7188	1.2601	0.187	0.20781	2.649E-3	1.0741	0.381	7.292E-3
Residual	199	0.5704			0.75526	2.466E-3			4.966E-2

	df	**(c) Total phosphorous concentration** MS	Pseudo-F	*P*	COV	**(d) Dissolved oxygen concentration** MS	Pseudo-F	*P*	COV
Zone (Z)	2	3.731E-2	33.852	0.001	2.1037E-2	17.920	81.177	0.001	0.46514
Season (S)	3	5.173E-2	46.939	0.001	2.8987E-2	39.491	178.89	0.001	0.80730
Period (P)	7	**1.276E-2**	**11.576**	**0.001**	**1.8757E-2**	**1.2536**	**5.6785**	**0.001**	**0.17657**
ZxS	6	4.060E-3	3.6836	0.001	1.2023E-2	1.1799	5.3448	0.001	0.21652
ZxP	10	1.198E-3	1.0868	0.402	2.6401E-3	**1.7055**	**7.7255**	**0.001**	**0.32888**
SxP	21	**2.867E-3**	**2.6016**	**0.001**	**1.4591E-2**	**0.5816**	**2.6345**	**0.001**	**0.20862**
ZxSxP	30	9.363E-4	0.8496	0.692	-6.9475E-3	**0.5000**	**2.2651**	**0.001**	**0.28513**
Residual	199	1.102E-3			3.3198E-2	0.2208			0.46985

	df	**(e) Salinity** MS	Pseudo-F	*P*	COV	**(f) Temperature** MS	Pseudo-F	*P*	COV
Zone (Z)	2	2103.2	52.908	0.001	4.2653	11.086	22.494	0.001	0.30561
Season (S)	3	5455.1	137.23	0.001	7.8041	2071.9	4203.9	0.001	4.82660
Period (P)	11	**234.23**	**5.8922**	**0.001**	**2.4959**	**6.3379**	**12.860**	**0.001**	**0.43270**
ZxS	6	128.07	3.2219	0.007	1.7648	3.6683	7.4432	0.001	0.33463
ZxP	18	38.523	0.9691	0.501	-0.3222	**2.8670**	**5.8173**	**0.001**	**0.44779**
SxP	33	51.494	1.2954	0.151	1.2261	**1.8795**	**3.8136**	**0.001**	**0.42136**
ZxSxP	54	10.774	0.2710	1.000	-3.1277	**1.1744**	**2.3828**	**0.001**	**0.47966**
Residual	263	39.752			6.3049	0.4928			0.70202
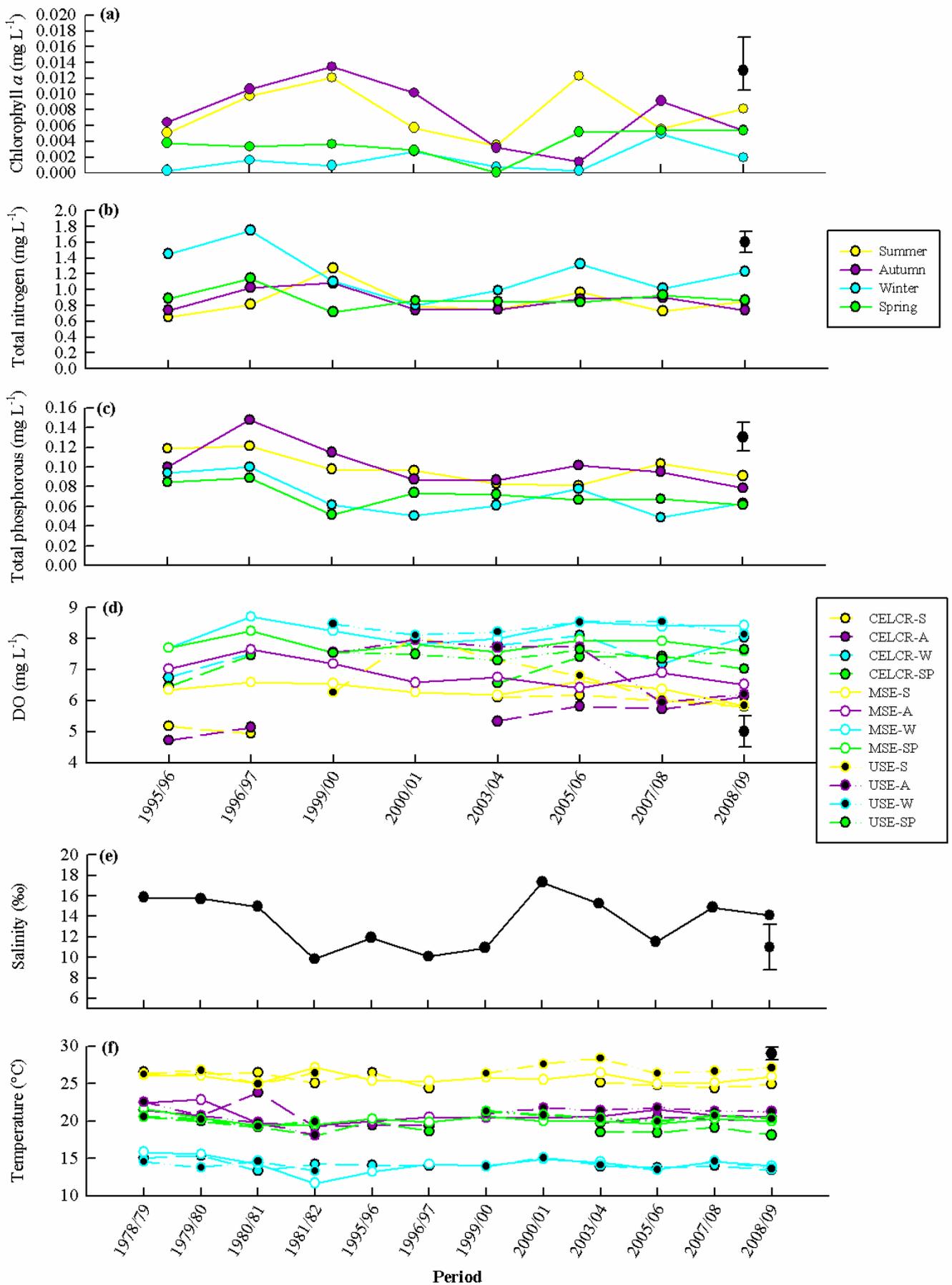
Figure 2.3.1.6: Mean (a) chlorophyll *a*, (b) total nitrogen, (c) total phosphorous, (d) dissolved oxygen (DO) concentration, (e) salinity and (f) temperature in the surface waters of the CELCR, MSE and USE during each season in each period in which nearshore fish were sampled between 1995/96 and 2008/09 (a-d) or 1978/79 and 2008/09 (e-f). For the sake of clarity, the average 95% confidence intervals have been presented for each of these plots.

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Differences among periods in surface dissolved oxygen concentration varied considerably in their nature and extent between the different estuary zones and seasons (Fig. 2.3.1.6d). Some of the most pronounced inter-period differences were recorded in the CELCR during summer and, to a lesser extent, autumn, in which values in 1995-97 were notably lower than those in any other period in this zone and time of year. Moreover, the dissolved oxygen concentrations in the CECLR during winter and spring in 1995/96 were also lower than those in each of the subsequent periods. However, in contrast to the CELCR, the mean values in the MSE during 1996/97 were slightly higher than those in each of the remaining periods (in all seasons except summer). Lastly, dissolved oxygen concentrations in the USE exhibited marked inter-period variability during summer and autumn, with notable reductions in values since 2000/01 in the former season, and considerably lower values in 2007/08 and 2008/09 during the latter season (Fig. 2.3.1.6d).

Mean surface salinity exhibited considerable variability among periods (Fig. 2.3.1.6e). Thus, whereas values of *ca* 15-16‰ were recorded between 1978/79 and 1980/81, they declined to *ca* 10‰ in 1981/82 and remained between this value and 12‰ until 1996/97, after which they increased to their maxima of 17.4‰ in 1999/00. Relatively high mean salinities of *ca* 14-15‰ were recorded in all subsequent periods, with the exception of 2003/04 (11.5‰; Fig. 2.3.1.6e).

General inter-period trends in surface water temperature were difficult to discern, due both to the significant interactions among all main effects and the fact that period differences were relatively small compared to the pronounced influence of season on this water quality variable (Fig. 2.3.1.6f). Some of the more notable inter-period trends in temperature were detected in the USE during summer and autumn, in which values in periods after and including 1999/00 and/or 2000/01 were often 1-2°C higher than those in several periods during the late 1970s/early 1980s (Fig. 2.3.1.6f).

Two-way period x season PERMANOVA of each of the above water quality variables in the LSCE also detected significant period and period x season differences in all cases (P=0.001-0.044), except for chlorophyll *a* concentration, for which neither was significant, and salinity, for which the interaction term was not significant (Table 2.3.1.13). The relative influence of seasonal differences was again substantially greater than that of period or the interaction term for each water quality variable except total P concentration, for which the relative importance of inter-period differences was notably greater than that of any other term (Table 2.3.1.13).

The plot of the mean total N concentration in the LSCE clearly demonstrated that the main cause of the significant period x season interaction was that while there were marked inter-period differences in this variable during winter, very little variability occurred among periods in the remaining seasons (Fig. 2.3.1.7b). Thus, in that former season, considerably greater mean concentrations were recorded in 2005/06 (0.74 mg L^{-1}), followed by 2008/09 (0.60 mg L^{-1}), than in 2007/08 and particularly 2003/04 (0.36-0.45 mg L^{-1}). However, the mean values in this region were typically far lower than in the other three zones of the estuary (*cf* Fig. 2.3.1.6b and 2.3.1.7b).

Table 2.3.1.13: Mean squares (MS), pseudo F-ratios, significance levels (*P*) and components of variation (COV) for period x season PERMANOVAs of the surface water concentrations of (a) chlorophyll *a*, (b) total nitrogen, (c) total phosphorous and (d) dissolved oxygen recorded seasonally by the DoW at their water quality monitoring sites throughout the LSCE zone of the Swan-Canning Estuary in each nearshore fish sampling period between 1995/96 and 2008/09, and of (e) salinity and (f) temperature recorded seasonally by Loneragan *et al.* (1989) or the DoW in the same estuary zone in each nearshore fish sampling period between 1978/79 and 2008/09. df=degrees of freedom; E=exponential. Significant results involving period are highlighted in bold.

		(a) Chlorophyll *a* concentration				**(b) Total nitrogen concentration**			
	df	MS	Pseudo-F	*P*	COV	MS	Pseudo-F	*P*	COV
Period (P)	3	0.4834	2.2011	0.123	0.18158	**4.213E-3**	**8.8321**	**0.002**	**2.161E-2**
Season (S)	3	1.0529	4.7942	0.012	0.32274	4.033E-2	84.547	0.001	7.058E-2
PxS	9	0.2377	1.0825	0.444	9.519E-2	**2.678E-3**	**5.6145**	**0.001**	**3.317E-2**
Residual	16	0.2196			0.46863	4.770E-4			2.184E-2

		(c) Total phosphorous concentration				**(d) Dissolved oxygen concentration**			
	df	MS	Pseudo-F	*P*	COV	MS	Pseudo-F	*P*	COV
Period (P)	3	**5.755E-3**	**21.172**	**0.001**	**2.618E-2**	**0.7210**	**26.852**	**0.001**	**0.2946**
Season (S)	3	1.299E-3	4.7793	0.016	1.1332E-2	9.0129	335.66	0.001	1.0598
PxS	9	**9.859E-4**	**3.6269**	**0.008**	**1.8895E-2**	**0.37131**	**13.828**	**0.001**	**0.4150**
Residual	16	2.718E-4			1.6487E-2	2.685E-2			0.1639

		(e) Salinity				**(f) Temperature**			
	df	MS	Pseudo-F	*P*	COV	MS	Pseudo-F	*P*	COV
Period (P)	7	**30.153**	**2.0712**	**0.044**	**1.2524**	**5.8793**	**7.8505**	**0.001**	**0.7183**
Season (S)	3	834.41	57.316	0.001	6.5346	274.42	366.42	0.001	3.7754
PxS	21	18.041	1.2393	0.257	1.1837	**2.8547**	**3.8118**	**0.001**	**0.9204**
Residual	48	14.558			3.8155	0.7489			0.8654

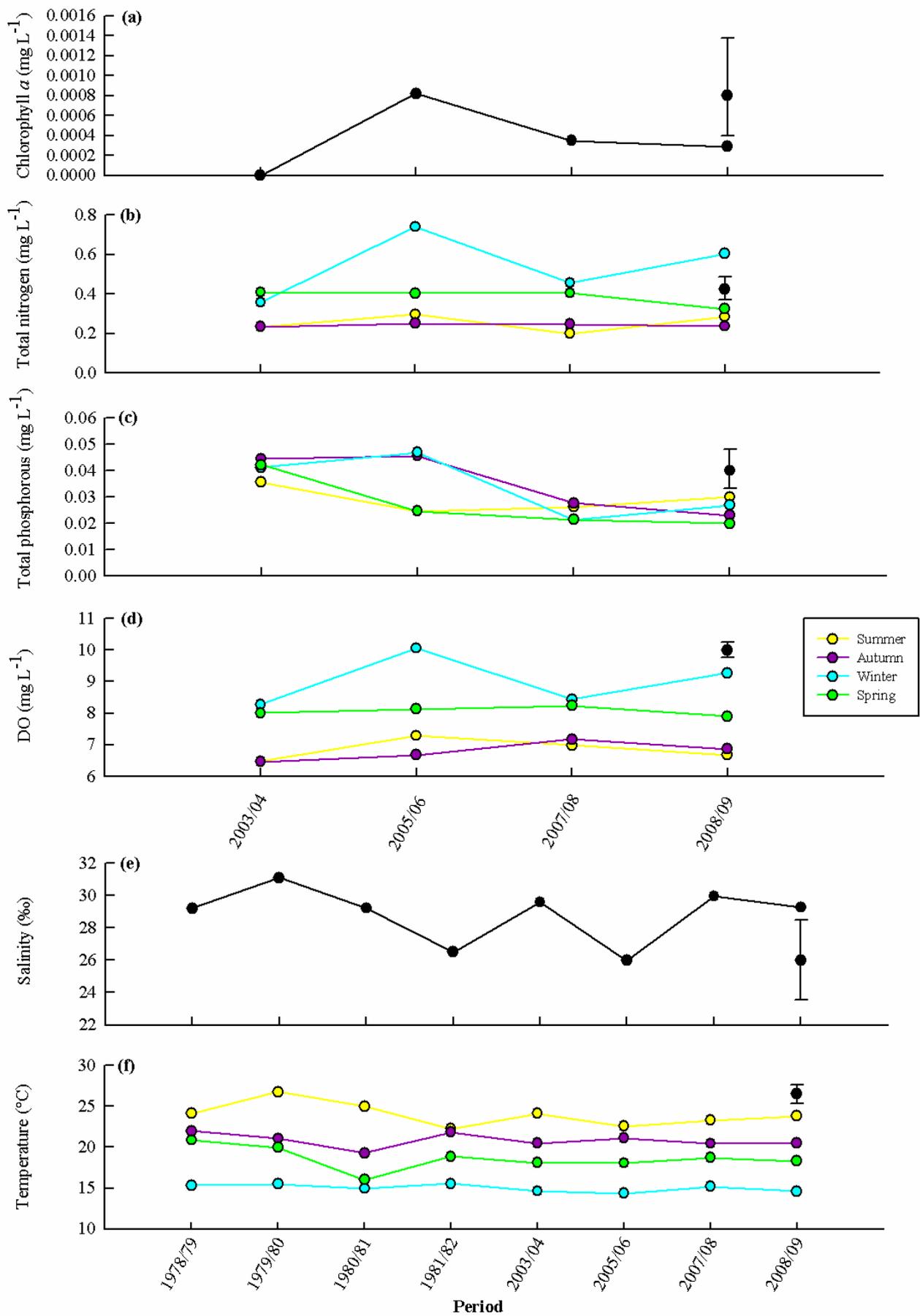**Figure 2.3.1.7:** Mean (a) chlorophyll *a*, (b) total nitrogen, (c) total phosphorous, (d) dissolved oxygen (DO) concentration, (e) salinity and (f) temperature in the surface waters of the LSCE during each season in each period in which nearshore fish were sampled between 2003/04 and 2008/09 (a-d) or 1978/79 and 2008/09 (e-f). For the sake of clarity, the average 95% confidence intervals have been presented for each of these plots.

The significant inter-period differences in total P concentration were mainly attributable to the fact that, in all seasons, values in 2003/04 were greater than those in 2007/08 and 2008/09 and that, in autumn and winter, those in 2005/06 were markedly greater than in the two most recent periods (Fig. 2.3.1.7c). As for total N, the concentrations of total P in the LSCE were generally far lower than in the other three zones located further upstream (*cf* Fig. 2.3.1.6c and 2.3.1.7c).

The inter-period trends in dissolved oxygen concentration in the LSCE were very similar to those of total N concentration (*cf* Fig. 2.3.1.7d and 2.3.1.7b). Thus, in winter, notably higher mean values were recorded in 2005/06 (*ca* 10 mg L^{-1}) than in 2003/04 and 2007/08 (8.3-8.4 mg L^{-1}), but there was comparatively little inter-period variation in the remaining seasons (Fig. 2.3.1.7d).

As for the other zones, mean salinity in the LSCE varied considerably among sampling periods. Notably lower values were recorded in 1981/82 and 2005/06 (*ca* 26‰) than in each of the other sampling periods (*ca* 29-31‰), while the greatest values were recorded in 1979/80 (Fig. 2.3.1.7e).

Surface water temperature in the LSCE exhibited the greatest inter-period variability between 1978 and 1981, then remained relatively constant in most subsequent periods. During summer, the greatest values were recorded in 1979/80 followed by 1980/81 (*i.e.* 25-26.8 °C, compared with 22.3-24.1°C in each of the remaining periods), while in spring and to a lesser extent autumn, notably lower mean values were recorded in 1980/81 than in any other period, *i.e.* 16 *vs* 18.1-20.8°C and 19.3 *vs* 20.4-21.8°C, respectively (Fig. 2.3.1.7f).

BIOENV was then employed to ascertain whether the pattern of inter-period differences exhibited by any particular subset of the above water quality variables was significantly correlated with that displayed by the nearshore fish fauna and, if so, which subset provided the best match. These BIOENV tests, which were carried out separately for each zone and season, demonstrated that for those periods between 1995/96 and 2008/09 (*i.e.* during which all of the above water quality variables were measured), the complementary fish and water quality matrices were significantly matched in all seasons in the MSE, USE and CELCR (except for winter in the USE) and only during spring in the LSCE (*P*=0.01-0.04; Table 2.3.1.14a). The extent of those significant matches was moderate during summer and autumn in the CELCR ($\rho_{s=}$0.543-0.556) and moderately low to low in the remaining cases ($\rho_{s=}$0.237–0.388). The particular subsets of surface water quality variables that were responsible for providing those matches are given in Table 2.3.1.14a.

The relationships between the inter-period differences in the nearshore fish assemblages and those of the water quality parameter(s) selected by BIOENV are illustrated, for each season and zone, by the MDS and associated bubble plots shown in Fig. 2.3.1.8. Note that plots for only those correlations which displayed relatively clear patterns are presented in that figure. Thus, in the CELCR in both summer and spring, the relative distinctness of the fish fauna in 2003/04 (*i.e.* whose samples were typically located to one side of the plots) was paralleled by

**Table 2.3.1.14:** Significance levels (P), Spearman rank correlation values (ρ_s) and (for significant findings) the subset of water quality variables derived from BIOENV tests between complementary nearshore fish assemblage data and surface water measurements of **(a)** chlorophyll *a* (chl. *a*), total nitrogen (N), total phosphorous (P) and dissolved oxygen concentration (DO) and salinity (sal) and temperature (temp) recorded in each fish sampling period between 1995/96 and 2008/09 and **(b)** salinity and temperature recorded in each fish sampling period between 1978/79 and 2008/09, performed separately for each season and estuary zone.

		(a) All periods ≥ 1995/96 (all surface water quality variables)			(b) All periods ≥ 1978/79 (surface salinity & temperature only)		
		P	ρ_s	selected subset	P	ρ_s	selected subset
LSCE	Summer	0.16	0.163		0.46	0.085	
	Autumn	0.05	0.243	N	0.60	0.082	
	Winter	0.13	0.193		0.27	0.227	
	Spring	0.01	0.320	P, temp	0.84	-0.075	
MSE	Summer	0.03	0.237	P, sal	0.39	0.133	
	Autumn	0.02	0.239	DO, P	0.62	0.046	
	Winter	0.01	0.326	DO, P, temp	0.69	-0.001	
	Spring	0.01	0.340	Sal, temp	0.07	0.269	
USE	Summer	0.02	0.293	Chl. *a*, sal	0.16	0.340	
	Autumn	0.03	0.243	DO, P	0.03	0.601	Temp
	Winter	0.10	0.210		0.80	-0.066	
	Spring	0.04	0.248	Sal	0.78	-0.019	
CELCR	Summer	0.01	0.556	Chl. *a*, N, DO, sal	0.24	0.198	
	Autumn	0.01	0.543	DO, sal, temp	0.48	0.071	
	Winter	0.01	0.388	Temp	0.67	0.019	
	Spring	0.03	0.384	Chl. *a*, DO, sal	0.67	0.037	

Canning Estuary/Lower Canning River: Summer
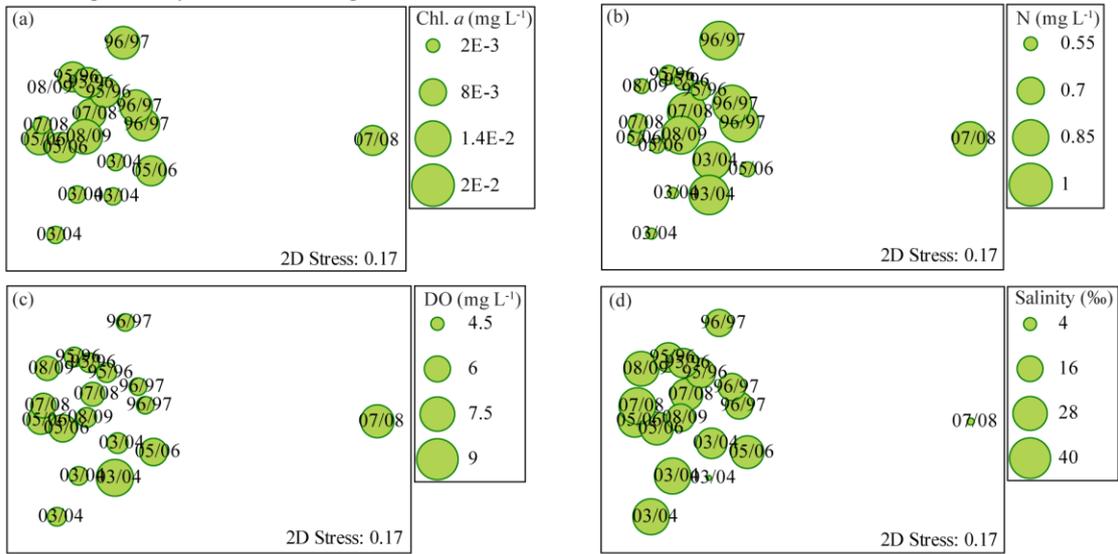
Canning Estuary/Lower Canning River: Autumn
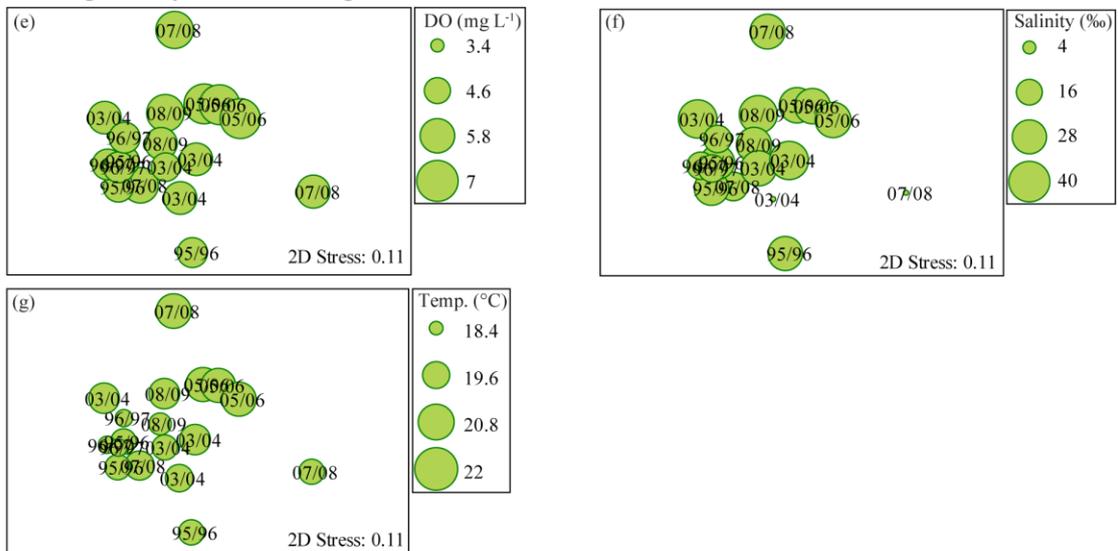
Canning Estuary/Lower Canning River: Spring
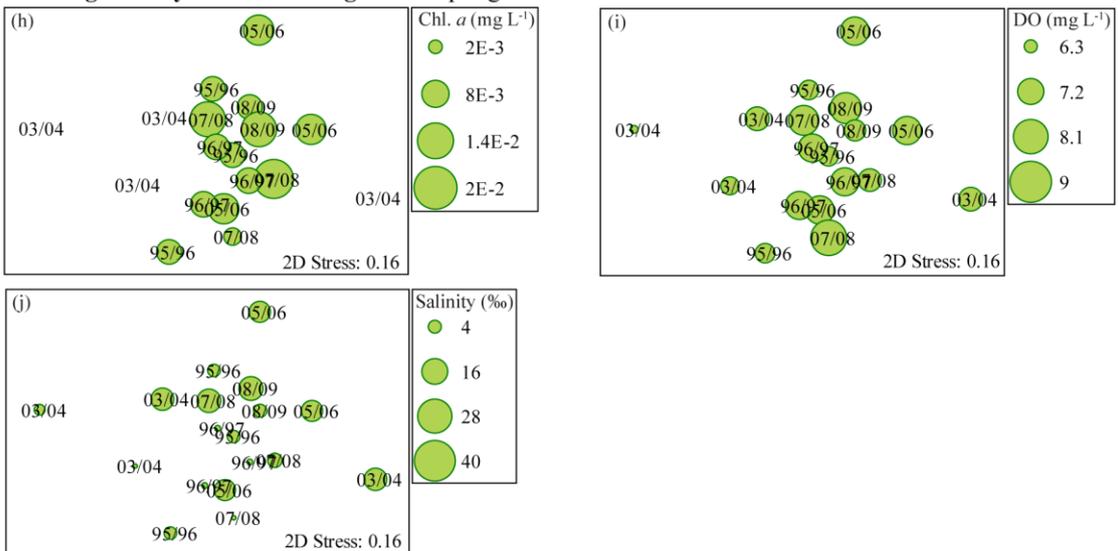
Figure 2.3.1.8: MDS ordination plots constructed from the nearshore fish species abundance data recorded during each period between 1995/96 and 2008/09 in the Canning Estuary/Lower Canning River in summer (a-d), autumn (e-g) and spring (h-j) with the magnitude of each surface water quality variable selected by BIOENV overlain as circles of proportionate sizes.

Middle Swan Estuary: Winter
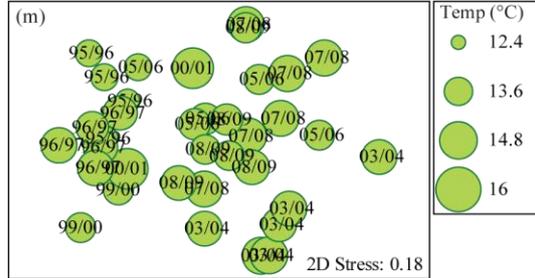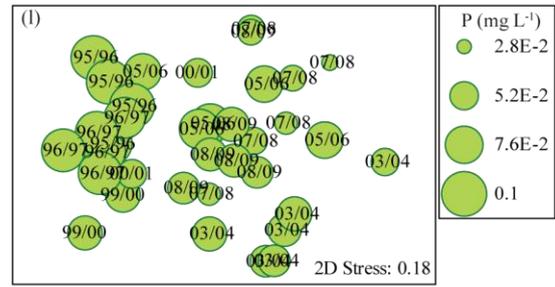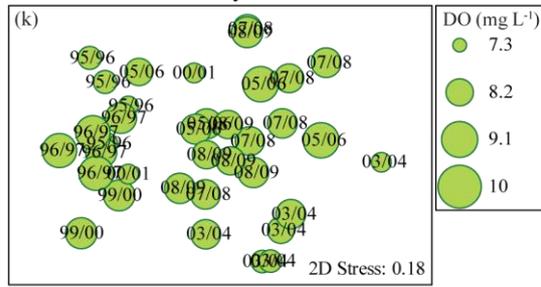
Middle Swan Estuary: Spring
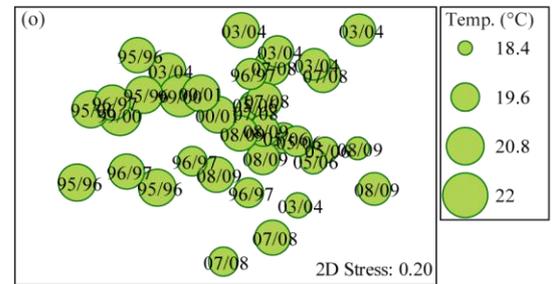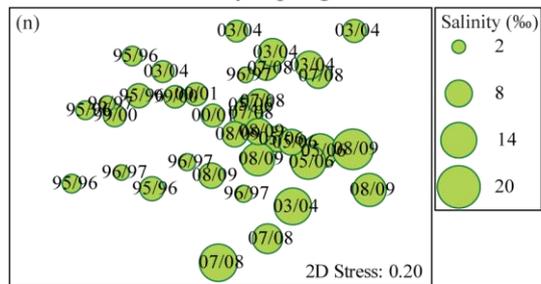
Upper Swan Estuary: Autumn
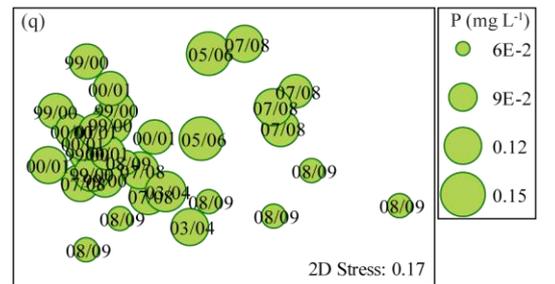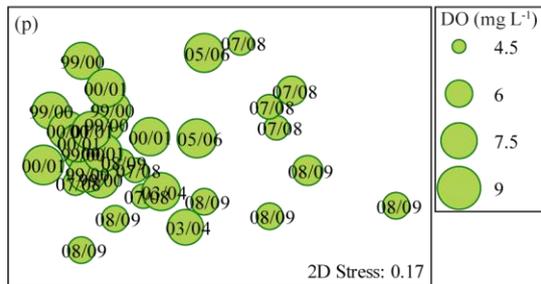
Upper Swan Estuary: Spring
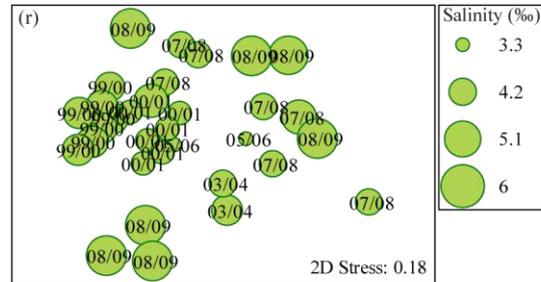
Figure 2.3.1.8 (cont.): MDS ordination plots constructed from the nearshore fish species abundance data recorded during each period between 1995/96 and 2008/09 in the Middle Swan Estuary in winter (k-m) and spring (n-o) and in the Upper Swan Estuary in autumn (p-q) and spring (r) with the magnitude of each surface water quality variable selected by BIOENV overlain as circles of proportionate sizes.

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notably lower concentrations of (or no) chlorophyll *a* (Fig. 2.3.1.8a, h). Moreover, the comparative distinctiveness of the fish fauna in 1995/96, 1996/97 and, in several cases, 2003/04, during summer, autumn and spring, typically corresponded with lower concentrations of dissolved oxygen than in later periods (Fig. 2.3.1.8c, e, i). During autumn in the CELCR, the largely gradational change in fish composition from 1995/96-2003/04 to 2005/06-2008/09 was typically accompanied by increases in water temperature (Fig. 2.3.1.8g), while in summer and spring, the relative difference in the fish faunas of earlier *vs* later periods generally corresponded with increases in salinity (Fig. 2.3.1.8d, j).

In the MSE, the gradual shift in fish assemblage composition from earlier periods (*i.e.* 1995/96–2000/01) to later periods (*i.e.* 2003/04–2008/09) during winter and spring was generally paralleled by increasing temperature and declining total P concentrations in the former season, and increasing salinity and declining temperature in the latter season (Fig. 2.3.1.8l, m, n, o). Lastly, the significant correlations between the fish and water quality data in the USE during autumn reflected the fact that the gradational change in fish composition from the earlier to later sampling periods was often mirrored by declining concentrations of dissolved oxygen and, to a lesser extent, of total P (Fig. 2.3.1.8p, q). In spring, however, the relative distinctiveness of fish samples in 2008/09 was correlated with higher salinities than those recorded in all other periods (Fig. 2.3.1.8r).

When BIOENV was used to correlate those complementary nearshore fish and surface water quality matrices containing data recorded from 1978/79 to 2008/09 (*i.e.* during which salinity and water temperature were the only water quality variables measured), a significant match was detected only for the USE during autumn (p=0.03, ρ_s=0.601) when temperature was the only water quality variable employed (Table 2.3.1.14b). The plot of this data showed that the distinctness of the fish assemblages in the late 1970s/early 1980s was reflected by considerable variability in water temperature (plot not shown).

2.3.1.4.2 Offshore (surface and bottom) waters
The results of the four-way period x depth x season x site PERMANOVAs on the surface and bottom water concentrations of total N, total P and dissolved oxygen, which were recorded by the DoW at their regular monitoring sites during those periods in which the offshore fish were sampled between 1995/96 and 2008/09, are presented for the MSE and USE in Tables 2.3.1.15 and 2.3.1.16, respectively.

In the MSE, each of the above water quality variables differed significantly among periods and the period x season interaction and, in the case of total N and dissolved oxygen concentration, also among the depth x period x season interaction. A significant depth x period interaction was also detected for total N (*P*=0.001–0.047; Table 2.3.1.15). The components of variation for each of the significant terms in the above tests demonstrated that, for total N and particularly P, the relative influences of the period main effect and period x season interaction were comparatively strong, ranking second and third (respectively) behind the site main effect for the latter variable, and third and fourth (respectively) behind the site and season main effects for the former variable. In the case of dissolved oxygen, however, the

Table 2.3.1.15: Mean squares (MS), pseudo F-ratios, significance levels (*P*) and components of variation (COV) for depth x period x season x site PERMANOVAs on the surface and bottom water concentrations of (a) total nitrogen, (b) total phosphorous and (c) dissolved oxygen recorded seasonally by the DoW at their water quality monitoring sites in the MSE zone of the Swan-Canning Estuary in each offshore fish sampling period between 1995/96 and 2008/09. df=degrees of freedom; E=exponential. Significant results involving period are highlighted in bold.

		(a) Total nitrogen concentration				(b) Total phosphorous concentration				(c) Dissolved oxygen concentration			
	df	MS	Pseudo-F	*P*	COV	MS	Pseudo-F	*P*	COV	MS	Pseudo-F	*P*	COV
Depth (D)	1	1.7194	27.36	0.024	0.1439	0.3562	3.6769	0.128	5.694E-2	367.97	444.89	0.026	2.1423
Period (P)	4	**1.0644**	**83.856**	**0.001**	**0.1813**	**1.4640**	**20.331**	**0.001**	**0.2086**	**2.9635**	**3.6258**	**0.047**	**0.2590**
Season (S)	3	3.5130	174.05	0.001	0.2955	0.6109	5.0272	0.028	0.1106	19.300	8.7342	0.009	0.6536
Site (si)	3	2.9040	375.48	0.001	0.2691	2.2065	206.77	0.001	0.2343	2.8249	6.6360	0.002	0.2449
DxP	4	**3.290E-2**	**6.5208**	**0.003**	**4.172E-2**	1.879E-2	1.1273	0.389	1.151E-2	0.8251	1.4987	0.244	0.1310
DxS	3	7.923E-2	1.6544	0.268	3.958E-2	0.2138	10.805	0.005	9.850E-2	7.7301	2.7706	0.091	0.4970
Dxsi	3	6.284E-2	8.1257	0.001	5.249E-2	9.688E-2	9.0782	0.001	6.565E-2	0.8271	1.9429	0.130	0.1417
PxS	12	**0.2350**	**14.139**	**0.001**	**0.16524**	**0.1989**	**9.9192**	**0.001**	**0.1495**	**1.8134**	**5.4941**	**0.001**	**0.4306**
Pxsi	12	1.269E-2	1.6412	0.114	2.490E-2	**7.201E-2**	**6.7477**	**0.001**	**8.756E-2**	**0.8173**	**1.9200**	**0.048**	**0.2213**
Sxsi	9	2.018E-2	2.6098	0.017	3.528E-2	0.1215	11.388	0.001	0.1053	2.2097	5.1907	0.001	0.4224
DxPxS	12	**5.149E-2**	**6.6571**	**0.001**	**0.10458**	2.169E-2	2.0329	0.059	5.249E-2	**1.8653**	**4.3818**	**0.001**	**0.5999**
DxPxsi	12	5.046E-3	0.6524	0.773	-2.592E-2	1.666E-2	1.5617	0.146	3.871E-2	0.5506	1.2933	0.267	0.1767
DxSxsi	9	4.789E-2	6.1920	0.001	8.961E-2	1.979E-2	1.8545	0.095	4.270E-2	2.7901	6.5541	0.001	0.6876
PxSxsi	36	**1.662E-2**	**2.1495**	**0.012**	**6.667E-2**	**2.005E-2**	**1.8790**	**0.030**	**6.849E-2**	0.3301	0.7753	0.790	-0.2187
Residual	36	7.734E-3			8.794E-2	1.067E-2			0.1033	0.4257			0.6525

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Table 2.3.1.16: Mean squares (MS), pseudo F-ratios, significance levels (*P*) and components of variation (COV) for depth x period x season x site PERMANOVAs on the surface and bottom water concentrations of (a) total nitrogen, (b) total phosphorous and (c) dissolved oxygen recorded seasonally by the DoW at their water quality monitoring sites in the USE zone of the Swan-Canning Estuary in each offshore fish sampling period between 2003/04 and 2008/09. df=degrees of freedom; E=exponential. Significant results involving period are highlighted in bold.

	df	**(a) Total nitrogen concentration**				**(b) Total phosphorous concentration**				**(c) Dissolved oxygen concentration**			
		MS	Pseudo-F	*P*	COV	MS	Pseudo-F	*P*	COV	MS	Pseudo-F	*P*	COV
Depth (D)	1	0.2095	31.917	0.085	7.5082E-2	0.5747	205.67	0.120	0.1260	164.66	230.82	0.087	2.1340
Period (P)	2	3.127E-2	9.0956	0.051	3.4052E-2	6.420E-2	5.9041	0.075	4.714E-2	0.9022	1.7246	0.317	0.1257
Season (S)	3	0.1991	10.468	0.018	0.10002	1.6234	43.238	0.005	0.2968	16.705	24.296	0.006	0.9433
Site (si)	2	7.231E-2	23.227	0.001	5.3696E-2	2.958E-2	1.6615	0.229	2.215E-2	4.874E-2	0.2374	0.795	-8.071E-2
DxP	2	4.037E-3	0.7633	0.510	-1.0213E-2	6.106E-2	2.7443	0.161	5.687E-2	1.0583	4.2048	0.109	0.2593
DxS	3	7.438E-2	37.129	0.001	8.9677E-2	9.072E-2	7.3543	0.028	9.332E-2	1.1663	1.5702	0.272	0.2169
Dxsi	2	6.564E-3	2.1085	0.175	1.6958E-2	2.794E-3	0.1569	0.855	-3.537E-2	0.7134	3.4796	0.082	0.2058
PxS	6	**8.215E-2**	**31.010**	**0.001**	**0.11511**	**0.1626**	**12.522**	**0.001**	**0.1579**	**2.6345**	**13.349**	**0.001**	**0.6373**
Pxsi	4	3.437E-3	1.1042	0.382	6.367E-3	1.087E-2	0.6107	0.668	-2.944E-2	0.5231	2.5517	0.089	0.1994
Sxsi	6	1.902E-2	6.1094	0.005	5.1489E-2	3.754E-2	2.1086	0.107	5.736E-2	0.6876	3.3538	0.031	0.2836
DxPxS	6	**1.170E-2**	**3.7574**	**0.024**	**5.3493E-2**	1.906E-2	1.0705	0.442	2.0451E-2	**2.1611**	**10.541**	**0.003**	**0.8075**
DxPxsi	4	5.289E-3	1.6988	0.219	2.3322E-2	2.225E-2	1.2495	0.343	3.333E-2	0.2517	1.2276	0.348	0.1080
DxSxsi	6	2.003E-3	0.6435	0.708	-1.9235E-2	1.233E-2	0.6928	0.682	-4.270E-2	0.7427	3.6228	0.022	0.4234
PxSxsi	12	2.649E-3	0.8509	0.587	-1.5234E-2	1.298E-2	0.7291	0.700	-4.911E-2	0.1974	0.9626	0.516	-6.188E-2
Residual	12	3.113E-3			5.5796E-2	1.781E-2			0.1334	0.2050			0.4528

relative influence of the depth main effect was far more important than that of any other term. Even so, the significant three-way and two-way interactions involving period, and the period main effect, ranked fourth, sixth and seventh, respectively, in terms of relative importance (Table 2.3.1.15).

The plot of the mean total N concentration in each period, season and depth in the MSE showed that by far the most obvious inter-period differences were those in both the surface and bottom waters during winter, in which values in 1996/97, followed by those in 1995/96, were much greater than in any other period (Fig. 2.3.1.9a). The same was also true, but to a lesser extent, during 1996/97 in the surface waters in both autumn and spring, and in the deeper waters in the latter season. Relatively little inter-period variation in total N concentration was detected for the remaining combinations of season and water depth (Fig. 2.3.1.9a).

The mean total P concentration also exhibited obvious peaks in 1996/97, which was true for all seasons except winter, in which the values in that period were second to those recorded in 1995/96 (Fig. 2.3.1.9b).

Mean dissolved oxygen concentration underwent little inter-period variation in the surface waters of the MSE in each season, but exhibited relatively pronounced differences among periods in the deeper waters, particularly during winter and autumn (Fig. 2.3.1.9c). Thus, during the former season, considerably lower concentrations were recorded in 2003/04 than in all other periods and especially 1996/97, while in the latter season, notably lower concentrations were present in 1996/97 than in most other periods (Fig. 2.3.1.9c).

In the USE, significant period x season differences were detected for total N, total P and dissolved oxygen concentrations, and a significant depth x period x season interaction was detected for the first and last of these variables (Table 2.3.1.16). The period x season interaction exerted the greatest influence above all other terms on the concentration of total N, and was the second most important influence (behind season) on the concentration of total P. In the case of dissolved oxygen, the above three-way and two-way interactions ranked third and fourth, respectively, in terms of their relative influence on this water quality variable (Table 2.3.1.16).

The mean total N concentration in the USE underwent notable inter-period differences during most seasons in both the surface and bottom waters, but there was considerable inconsistency in those trends (Fig. 2.3.1.9d). For example, whereas values increased progressively from 2003/04 to 2008/09 in the surface and bottom waters in winter and in the bottom waters in spring, they increased considerably from 2003/04 to 2007/08 then declined sharply in 2008/09 in the surface waters in autumn. The opposite trend occurred in the surface waters in summer (Fig. 2.3.1.9d).

The most obvious inter-period differences in mean total P concentration in the USE occurred in autumn, during which values declined markedly between 2007/08 and 2008/09. A slight
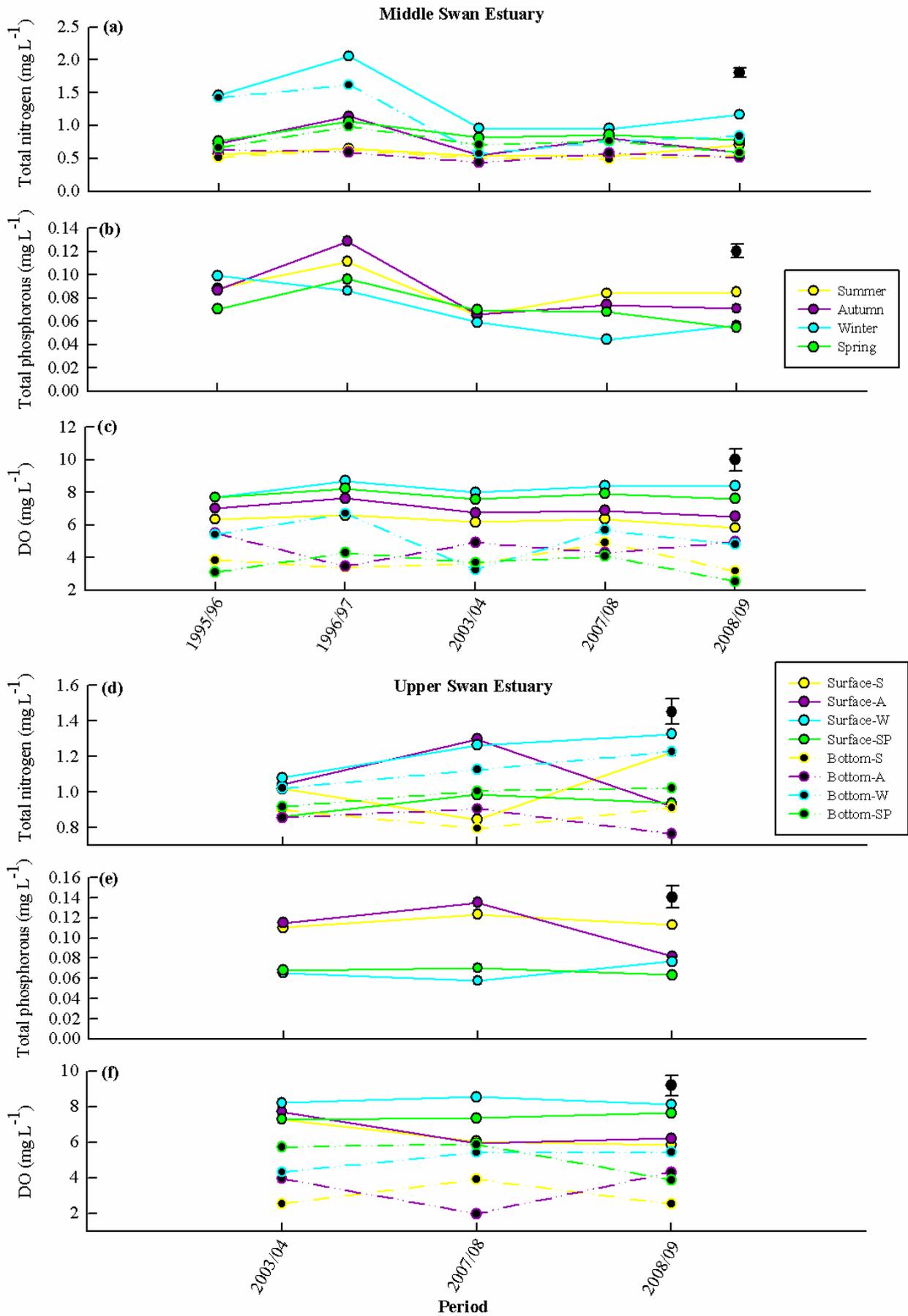
Figure 2.3.1.9: Mean (a) total nitrogen, (b) total phosphorous and (c) dissolved oxygen (DO) concentration in the surface and bottom waters of the MSE and the same three parameters in the same waters of the USE (d-f) during each season in each period in which offshore fish were sampled between 1995/96 and 2008/09 (a-c) or 2003/04 and 2008/09 (d-f). For the sake of clarity, the average 95% confidence intervals have been presented for each of these plots.

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increase was detected between these two periods in winter, whereas concentrations remained relatively stable among periods in the other two seasons (Fig. 2.3.1.9e).

The mean dissolved oxygen concentration in the USE displayed considerable inter-period variability in all seasons and both water depths, except for in the surface waters during winter and spring (Fig. 2.3.1.9f). However, like total N concentration, those inter-period differences were highly variable. For example, while surface water values in summer and autumn declined between 2003/04 and 2007/08 then remained virtually stable, they increased between those two periods in the bottom waters in summer, then subsequently declined (Fig. 2.3.1.9f).

When PERMANOVA was used to test whether salinity and temperature differed significantly among all offshore fish sampling periods since 1993/94 (but excluding 1995/96 and 1996/7, during which no sampling of the offshore fish in the USE was undertaken), significant period, zone x period and period x season differences were detected in both cases (Table 2.3.1.17). Salinity also differed significantly among the depth x period interaction, and temperature also exhibited a significant depth x period x season interaction. The relative influences of period and the above significant interactions were substantially lower than that of season for both of these water quality variables. However, they ranked relatively highly among the remaining terms in the PERMANOVA model (see components of variation values in Table 2.3.1.17).

The plot of mean salinity showed that the most pronounced inter-period difference in many of the zone, season and depth combinations was the increase in values between 1993/94 and 2003/04 (Fig. 2.3.1.10a, b). Although not shown on that figure, this increasing trend was also consistent, in most seasons and both depths, across 1995/96 and 1996/97 in the MSE. In several cases, this increase was marked, *e.g.* from *ca* 6 to 24‰ in the bottom waters of the MSE in winter, *ca* 6 to 16‰ in the same zone and depth in spring and *ca* 20 to 29 in the bottom waters of the USE in autumn (Fig. 2.3.1.10a, b). Mean salinities then remained relatively stable in subsequent periods in the majority of the different zone, season and depth combinations. In other cases, however, salinity continued to fluctuate among the remaining periods, *e.g.* during autumn in the surface waters of both zones. In contrast, little inter-period variation in mean salinity was detected in the surface waters of both zones in spring (Fig. 2.3.1.10a, b).

The greatest differences in mean temperature among periods in the MSE occurred in the bottom waters in spring, in which values declined from 21.4°C in 1993/94 to 18.8°C in 2003/04, and in the surface waters in winter, where values fell from *ca* 16°C in the earliest period to 13.9°C in the latest period (Fig. 2.3.1.10c, d). In the USE, however, the greatest inter-period differences were recorded in summer in both the surface and bottom waters, in which values alternately increased and decreased between consecutive periods, peaking in 2003/04 (Fig. 2.3.1.10c, d).

BIOENV was then used to test whether the inter-period patterns in offshore fish assemblages between 1995/96 and 2008/09 were significantly matched with those of any particular subset

Table 2.3.1.17: Mean squares (MS), pseudo F-ratios, significance levels (*P*) and components of variation (COV) for depth x zone x period x season PERMANOVAs on the surface and bottom water values of (a) salinity and (b) temperature recorded seasonally by Sarre (unpubl.) or the DoW in the MSE and USE zones of the Swan-Canning Estuary in each offshore fish sampling period between 1993/94 and 2008/09 (excluding 1995-97). df=degrees of freedom; E=exponential. Significant results involving period are highlighted in bold.

		(a) Salinity				(b) Temperature			
	df	MS	Pseudo-F	*P*	COV	MS	Pseudo-F	*P*	COV
Depth (D)	1	1123	92.737	0.001	3.0897	0.2698	0.8358	0.373	-2.134E-2
Zone (Z)	1	3145.9	259.79	0.001	5.1895	4.3549	13.493	0.001	0.1861
Period (P)	3	**766.09**	**63.265**	**0.001**	**3.5858**	**11.407**	**35.340**	**0.001**	**0.4348**
Season (S)	3	3953.4	326.48	0.001	8.2305	1328.2	4115	0.001	4.7773
DxZ	1	34.648	2.8613	0.082	0.62241	0.6796	2.1056	0.154	7.832E-2
DxP	3	**96.521**	**7.9709**	**0.001**	**1.6967**	0.2270	0.7033	0.545	-5.715E-2
DxS	3	43.479	3.5906	0.025	1.0384	3.3483	10.374	0.001	0.3225
ZxP	3	**48.762**	**4.0268**	**0.007**	**1.1181**	**2.7120**	**8.4024**	**0.001**	**0.2855**
ZxS	3	56.306	4.6498	0.004	1.2326	8.6794	26.891	0.001	0.5360
PxS	9	**78.294**	**6.4657**	**0.001**	**2.1248**	**2.3721**	**7.3492**	**0.001**	**0.3739**
DxZxP	3	5.0118	0.4139	0.752	-0.69579	0.1683	0.5214	0.667	-0.1026
DxZxS	3	35.214	2.9080	0.035	1.2603	9.076E-2	0.2812	0.847	-0.1263
DxPxS	9	17.287	1.4276	0.201	0.84047	**0.7593**	**2.3524**	**0.018**	**0.2440**
ZxPxS	9	12.079	0.9975	0.434	-6.4529E-2	0.2350	0.7281	0.680	-0.1094
DxZxPxS	9	2.5973	0.2145	0.992	-1.611	0.1373	0.4253	0.910	-0.2250
Residual	176	12.109			3.4798	0.3228			0.5681

Table 2.3.1.18: Significance levels (*P*), Spearman rank correlation values (ρ_s) and (for significant findings) the subset of water quality variables derived from BIOENV tests between complementary offshore fish assemblage data and surface (s) / bottom (b) measurements of **(a)** total nitrogen (N), total phosphorous (P) and dissolved oxygen concentration (DO) and salinity (sal) and temperature (temp) recorded in each fish sampling period between 1995/96 and 2008/09 and **(b)** salinity and temperature recorded in each fish sampling period between 1993/94 and 2008/09, performed separately for each season in the MSE and USE.

		(a) All periods ≥ 1995/96 (all surface & bottom water quality variables)			(b) All periods ≥ 1993/94 (surface & bottom salinity & temperature only)		
		P	ρ_s	selected subset	*P*	ρ_s	selected subset
MSE	Summer	0.52	0.159		0.68	0.059	
	Autumn	0.16	0.177		0.25	0.092	
	Winter	0.04	0.246	s-P, s-temp, b-temp	0.17	0.116	
	Spring	0.10	0.290		0.01	0.266	s-sal, b-sal, b-temp
USE	Summer	0.04	0.359	s-sal, b-sal	0.35	0.14	
	Autumn	0.05	0.296	s-P, s-sal, b-N, b-DO	0.36	0.155	
	Winter	0.04	0.260	s-N	0.15	0.172	
	Spring	0.02	0.483	s-temp, b-DO, b-temp	0.29	0.226	
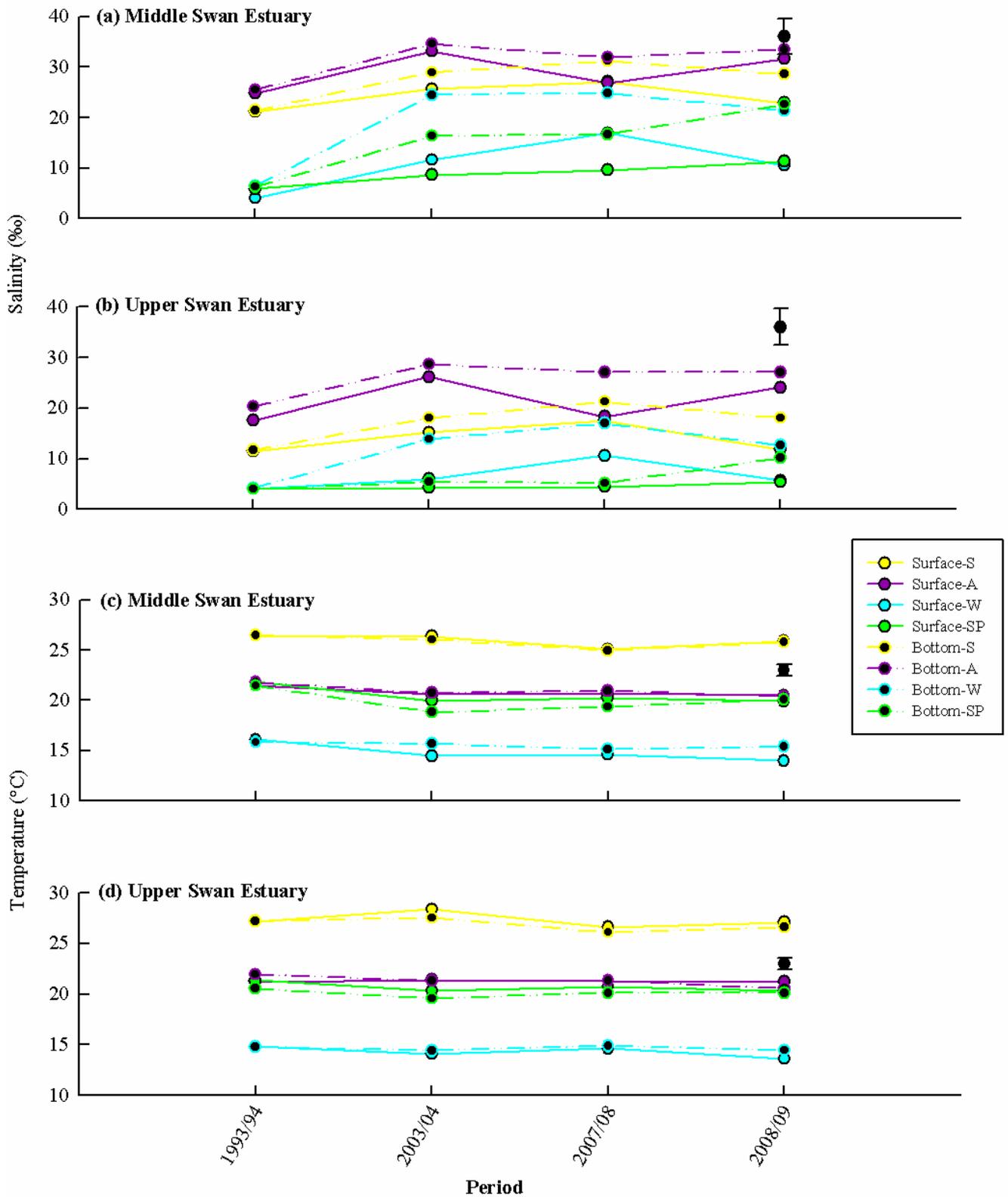
Figure 2.3.1.10: Mean salinity in (a) the MSE and (b) the USE and mean temperature in (c) the MSE and (d) the USE in the surface and bottom waters during each season in each period in which offshore fish were sampled between 1993/94 and 2008/09 (except 1995/96 and 1996/97). For the sake of clarity, the average 95% confidence intervals have been presented for each of these plots.

Middle Swan Estuary: Winter
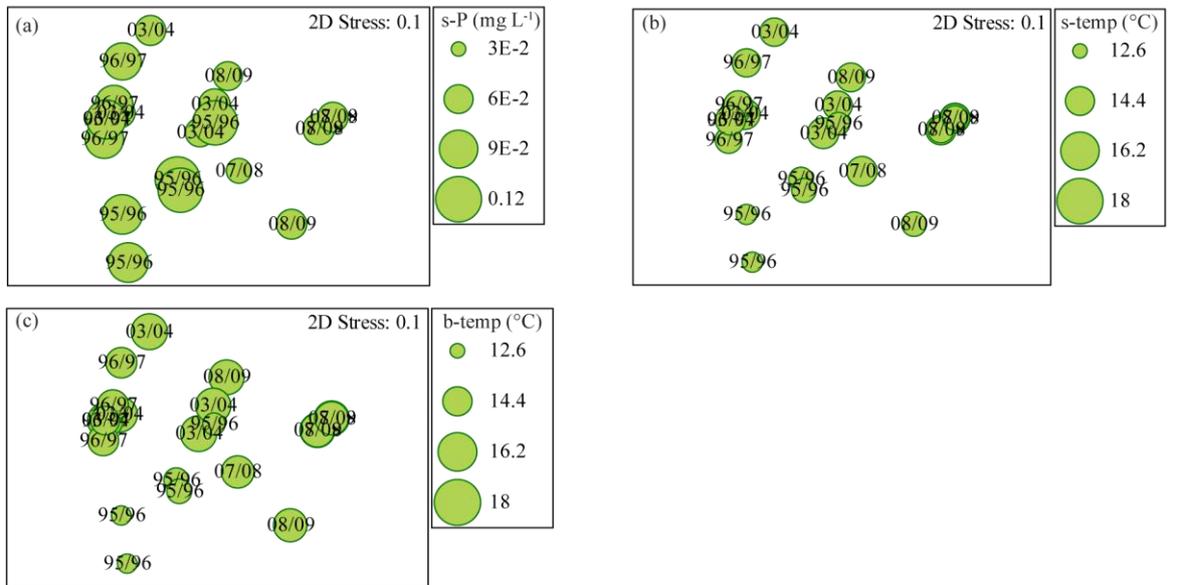
Upper Swan Estuary: Summer
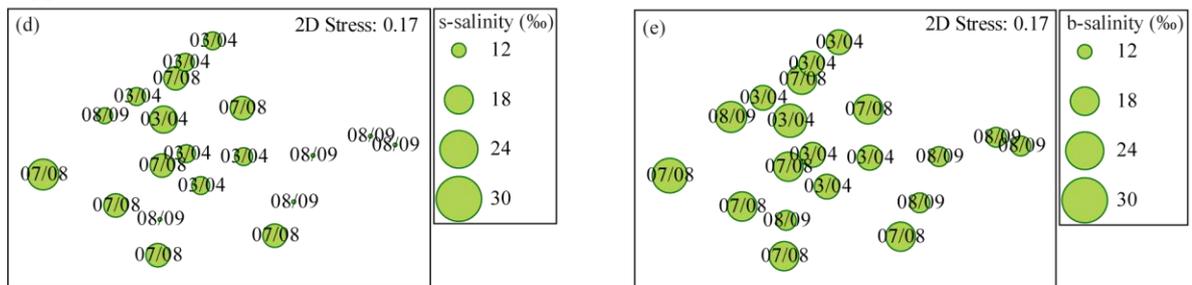
Upper Swan Estuary: Autumn
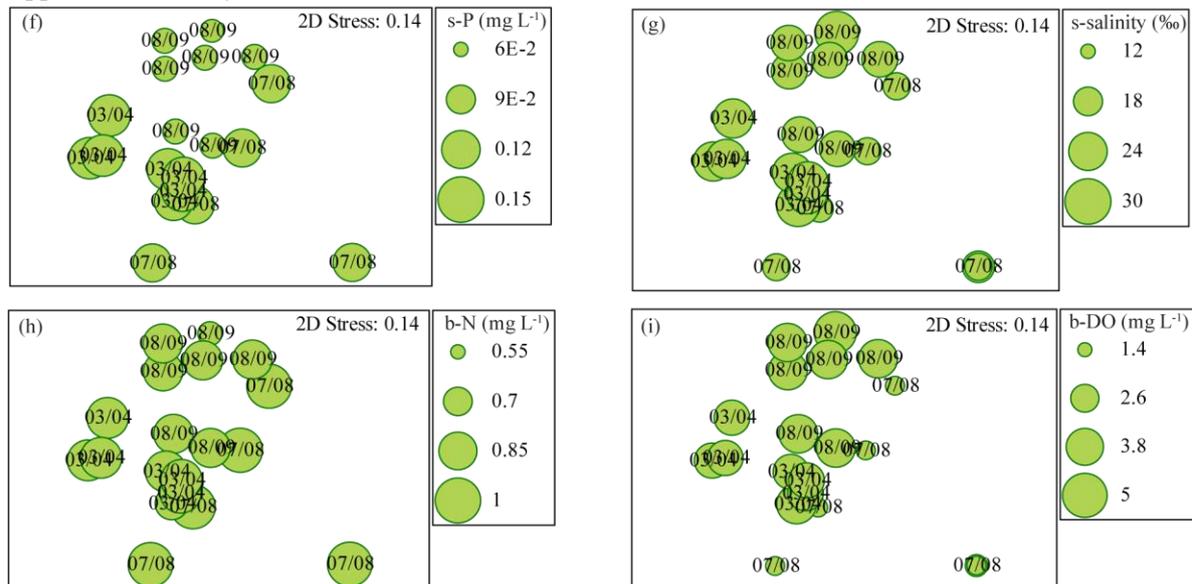
Figure 2.3.1.11: MDS ordination plots constructed from the offshore fish species abundance data recorded during each period between 1995/96 and 2008/09 in the Middle Swan Estuary in winter (a-c) and in the Upper Swan Estuary in summer (d-e) and autumn (f-i) with the magnitude of each surface (s) or bottom (b) water quality variable selected by BIOENV overlain as circles of proportionate sizes.

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Upper Swan Estuary: Winter
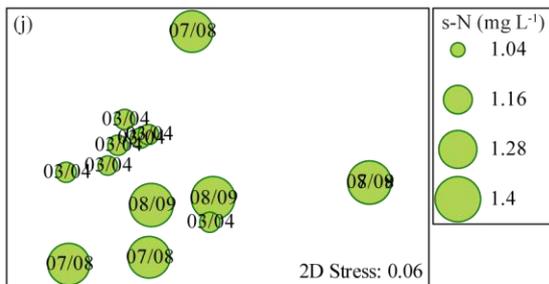
Upper Swan Estuary: Spring
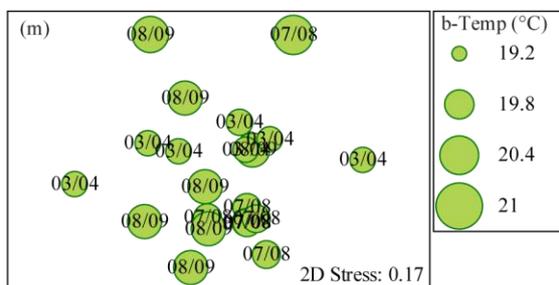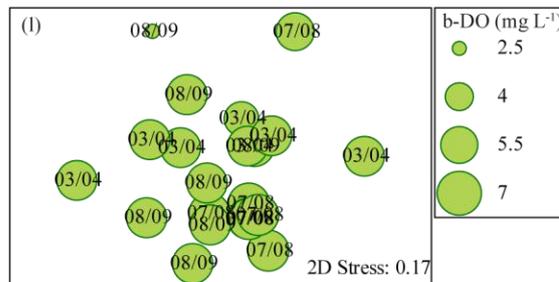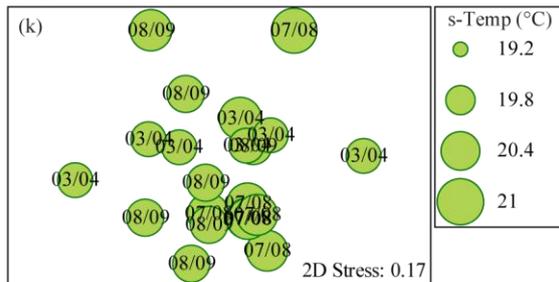
Middle Swan Estuary: Spring (1993/94-2008/09)
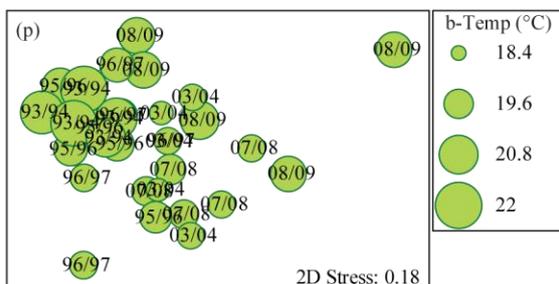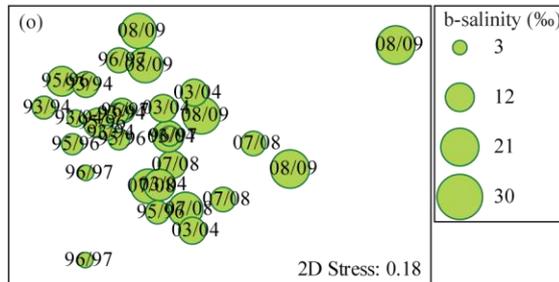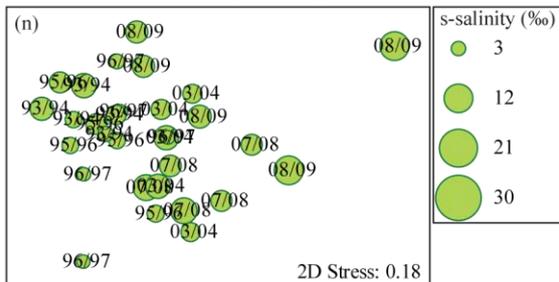
Figure 2.3.1.11 (cont.): MDS ordination plots constructed from the offshore fish species abundance data recorded during each period between 2003/04 and 2008/09 in the Upper Swan Estuary in winter (q) and spring (k-m) and in the Middle Swan Estuary in each period between 1993/94 and 2008/09 in spring (n-p) with the magnitude of each surface (s) or bottom (b) water quality variable selected by BIOENV overlain as circles of proportionate sizes.

of the above surface and bottom water quality variables. These tests detected significant correlations in each season in the USE, but only during winter in the MSE (P=0.02-0.05). The extent of those significant correlations was moderate to low (ρ_s =0.246-0.483), and the subsets of water quality variables selected in each case are provided in Table 2.3.1.18a.

The MDS plots of the offshore fish assemblage data, overlain with the values for each of the water quality variables selected by BIOENV, are shown for each of the above zone and season combinations in Fig. 2.3.1.11. Thus, in the MSE in winter, the gradational shift in offshore fish composition between 1995/96 and 2008/09 was paralleled by decreasing concentrations of total P in the surface waters, and the relative distinctness of the fish fauna in the former period was accompanied by lower surface and bottom water temperatures (Fig. 2.3.1.11a-c). In the USE during summer, the comparative distinctness of the fish composition in 2008/09 was associated with notably lower surface and bottom salinities (Fig. 2.3.1.11d, e), while in autumn, it was associated with lower concentrations of both surface total P and bottom total N and higher levels of bottom dissolved oxygen (Fig. 2.3.1.11f, h, i). During winter, the notable difference in the composition of the offshore fish fauna between 2003/04 and 2007-09 was mirrored by lower concentrations of surface total N in the former period, while in spring, samples from particular sites in 2007/08 and/or 2008/09 with relatively distinct fish compositions also had higher surface and bottom temperatures and lower dissolved oxygen concentrations in the bottom waters (Fig. 2.3.1.11k-m).

When BIOENV was used to correlate the inter-period patterns in offshore fish composition between 1993/94 and 2008/09 with those of surface and bottom salinity and temperature, significant results were obtained only for the MSE in spring (Table 2.3.1.18b). The MDS plot of this fish data with the selected water quality variables overlaid showed that the gradational shift in fish composition from the earliest to the latest period was matched by increasing surface and bottom salinity and generally decreasing bottom temperature (Fig. 2.3.1.11n-p).

2.3.2 Development of a biotic index of estuarine health

2.3.2.1 Selection of metrics sensitive to spatial changes in habitat quality
Of the 136 sites examined for habitat quality throughout the Swan-Canning Estuary, 18, 65 and 46 sites were deemed to be of Poor, Fair and Good quality, respectively, whilst only seven were allocated a Habitat Quality Category (HQC) of Excellent (Table 2.3.2.1). For the 71 of those 136 sites at which the fish community was subsequently sampled, boxplots were employed to ascertain whether each of the fish metrics measured in those samples responded to differing habitat quality as hypothesised (Table 2.2.2.2, Fig. 2.3.2.1). Visual examination of those boxplots revealed no apparent relationship between HQCs and fish metric values in all cases, as shown by the large degree of overlap in the inter-quartile ranges across all four HQCs.

Table 2.3.2.1: Total number of sites allocated to each of the four Habitat Quality Categories (HQCs) in each of the seven regions of the Swan-Canning Estuary. Numbers of sites at which the fish community was sampled are given in parentheses. See Fig. 2.2.1.1 for the location of each region.

HQC	Channel	Basin	Canning River	Lower Swan	Middle Downstream Swan	Middle Upstream Swan	Upper Swan	Total
Excellent	2 (2)	2 (2)	1 (1)	1 (1)	0 (0)	0 (0)	1 (1)	**7 (7)**
Good	7 (4)	18 (4)	5 (4)	6 (4)	5 (4)	3 (3)	2 (2)	**46 (25)**
Fair	12 (4)	24 (4)	10 (4)	4 (4)	7 (4)	5 (4)	3 (3)	**65 (27)**
Poor	2 (2)	6 (4)	8 (4)	1 (1)	1 (1)	0 (0)	0 (0)	**18 (12)**
Total	**23 (12)**	**50 (14)**	**24 (13)**	**12 (10)**	**13 (9)**	**8 (7)**	**6 (6)**	**136 (71)**
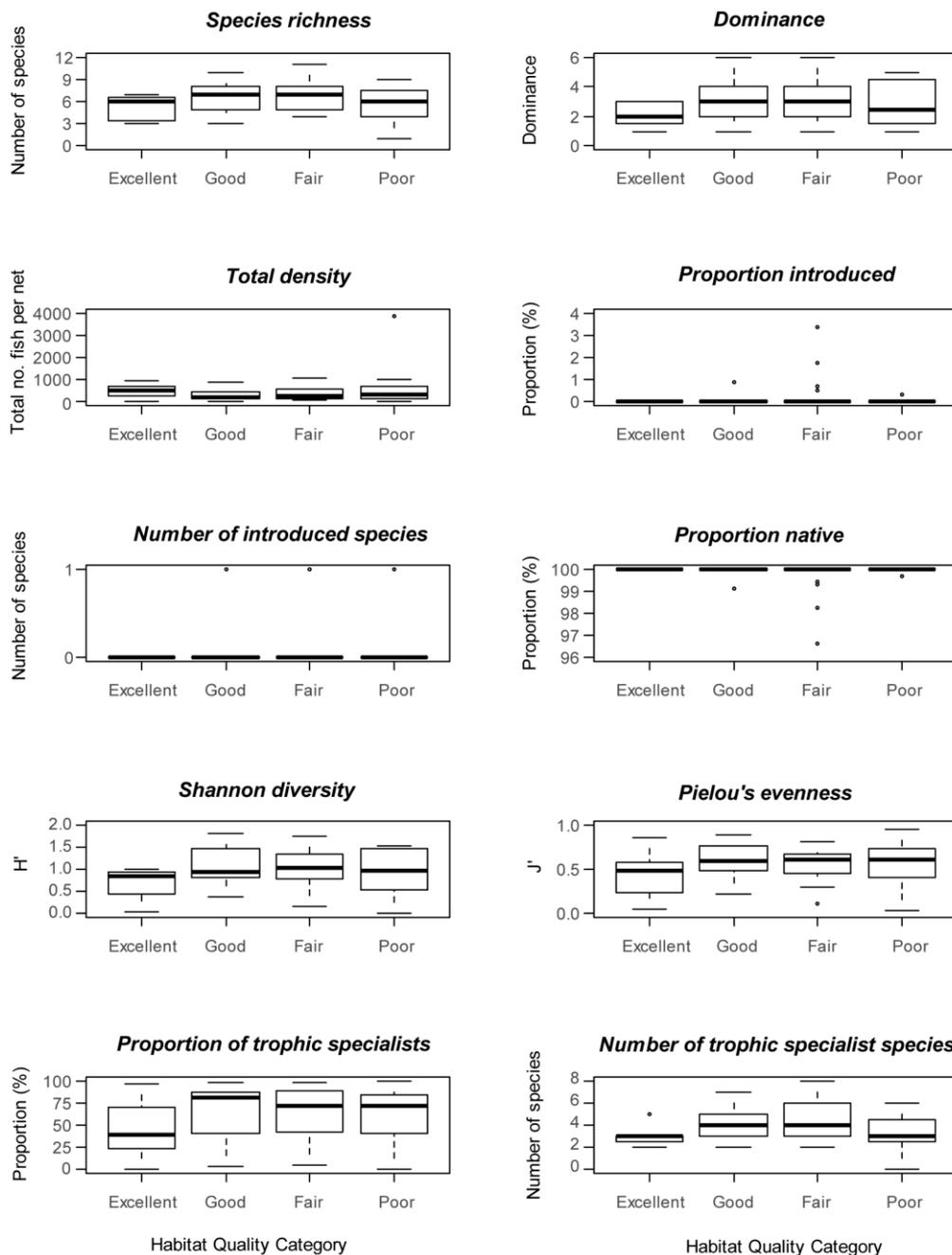
Figure 2.3.2.1: Boxplots of candidate fish metric responses across Habitat Quality Categories.

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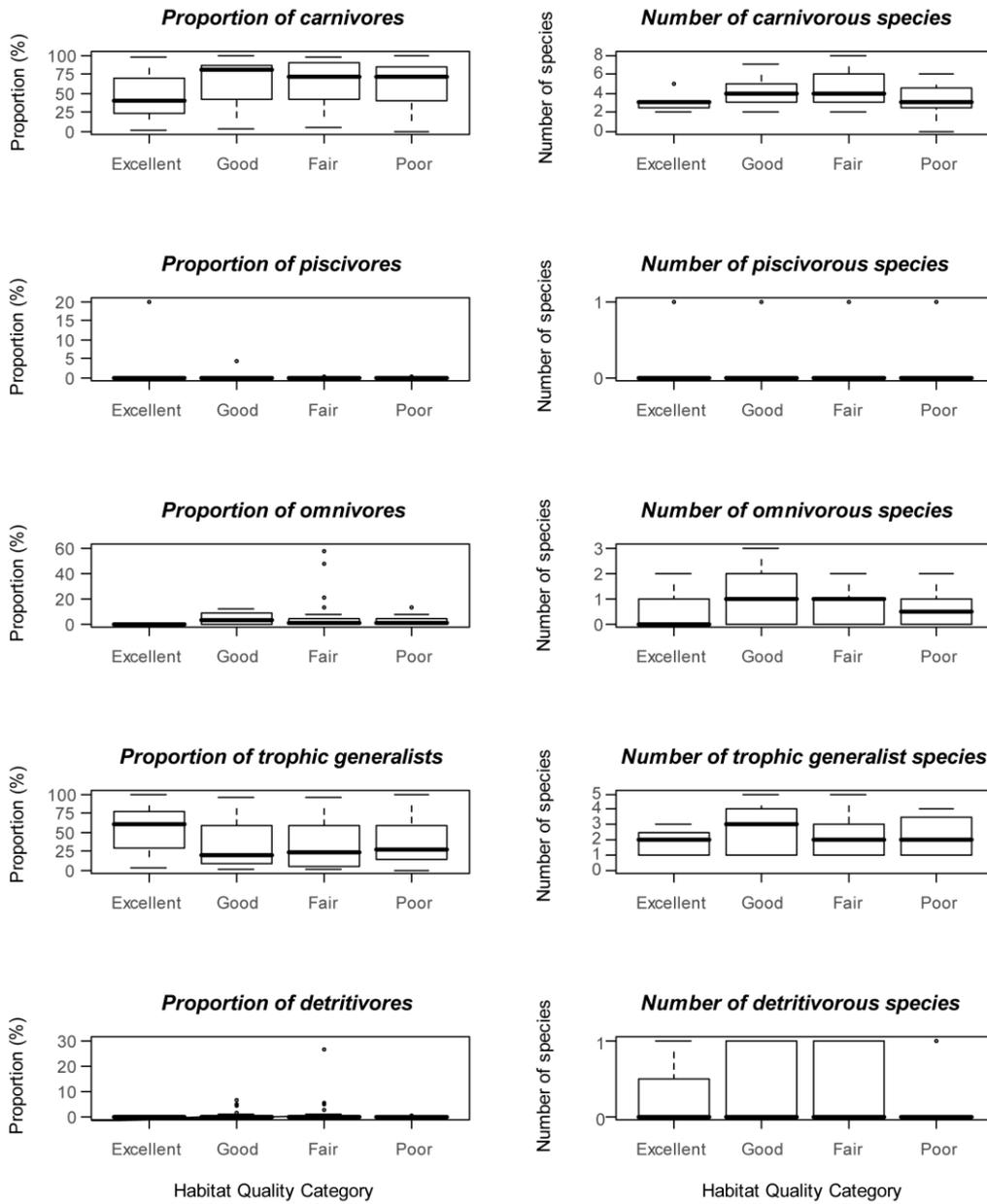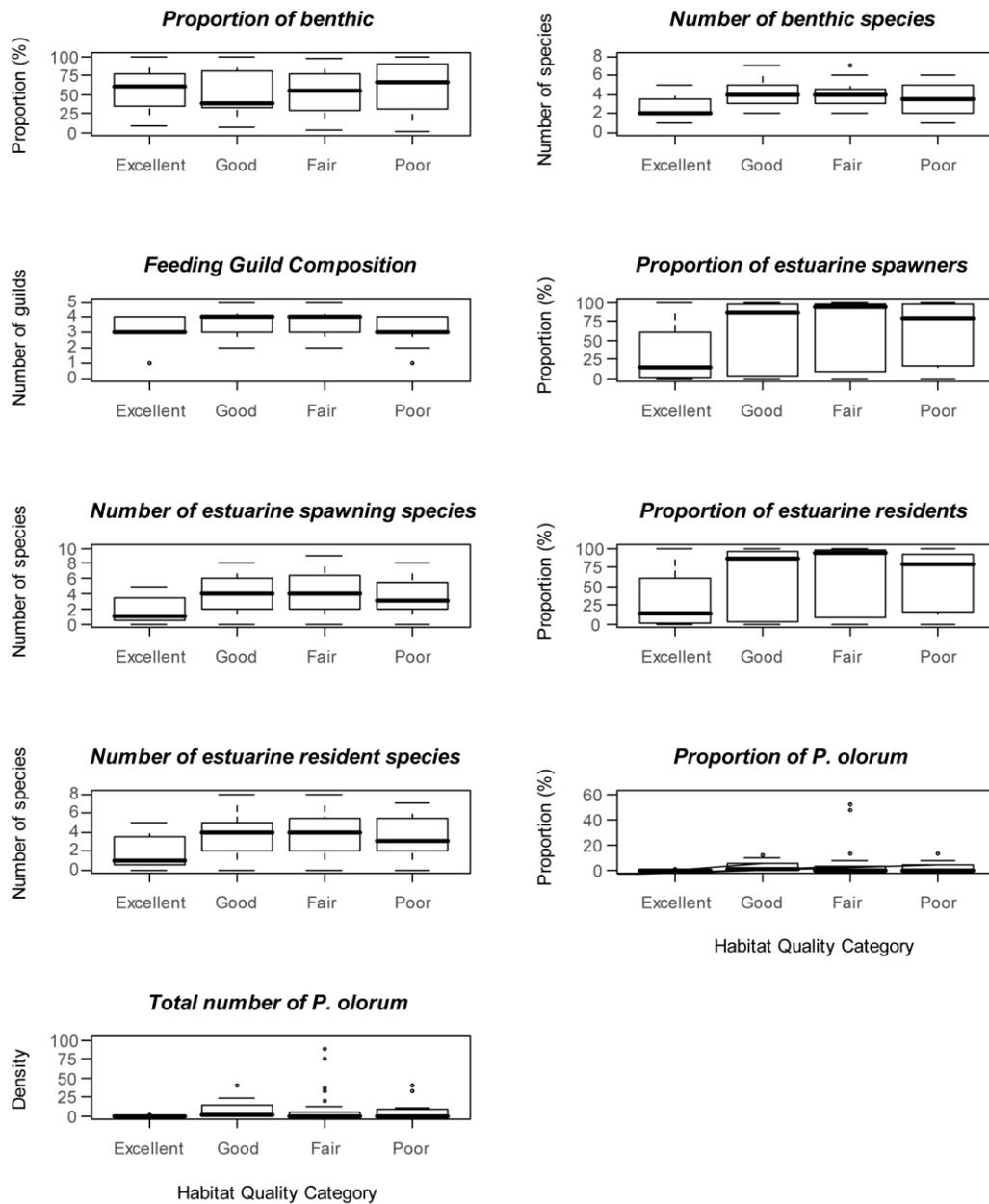
Figure 2.3.2.1: (cont'd).

Figure 2.3.2.1: (cont'd).

Similarly, MDS ordination of the data for the suite of fish metrics recorded at each site did not reveal any obvious overall differences among the four HQCs to which those sites were assigned (Figure 2.3.2.2a). The HQC x region PERMANOVA performed on these data confirmed no significant difference in metric values between HQCs, either as a main effect or after the potentially confounding influence of regional differences had been removed, *i.e.* the HQC x region interaction (Table 2.3.2.2). However, when the sites on the same MDS plot were coded according to region of the estuary, a relatively pronounced gradation was

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detected, with samples from the lower regions of the estuary (*i.e.* entrance channel and basin) being located on one side of the plot, while those from the upper estuary (*i.e.* regions of the Swan River) were located on the opposite side (Figure 2.3.2.2b). These differences in fish metric composition among regions were confirmed as significant by PERMANOVA (*P*=0.0001; Table 2.3.2.2).
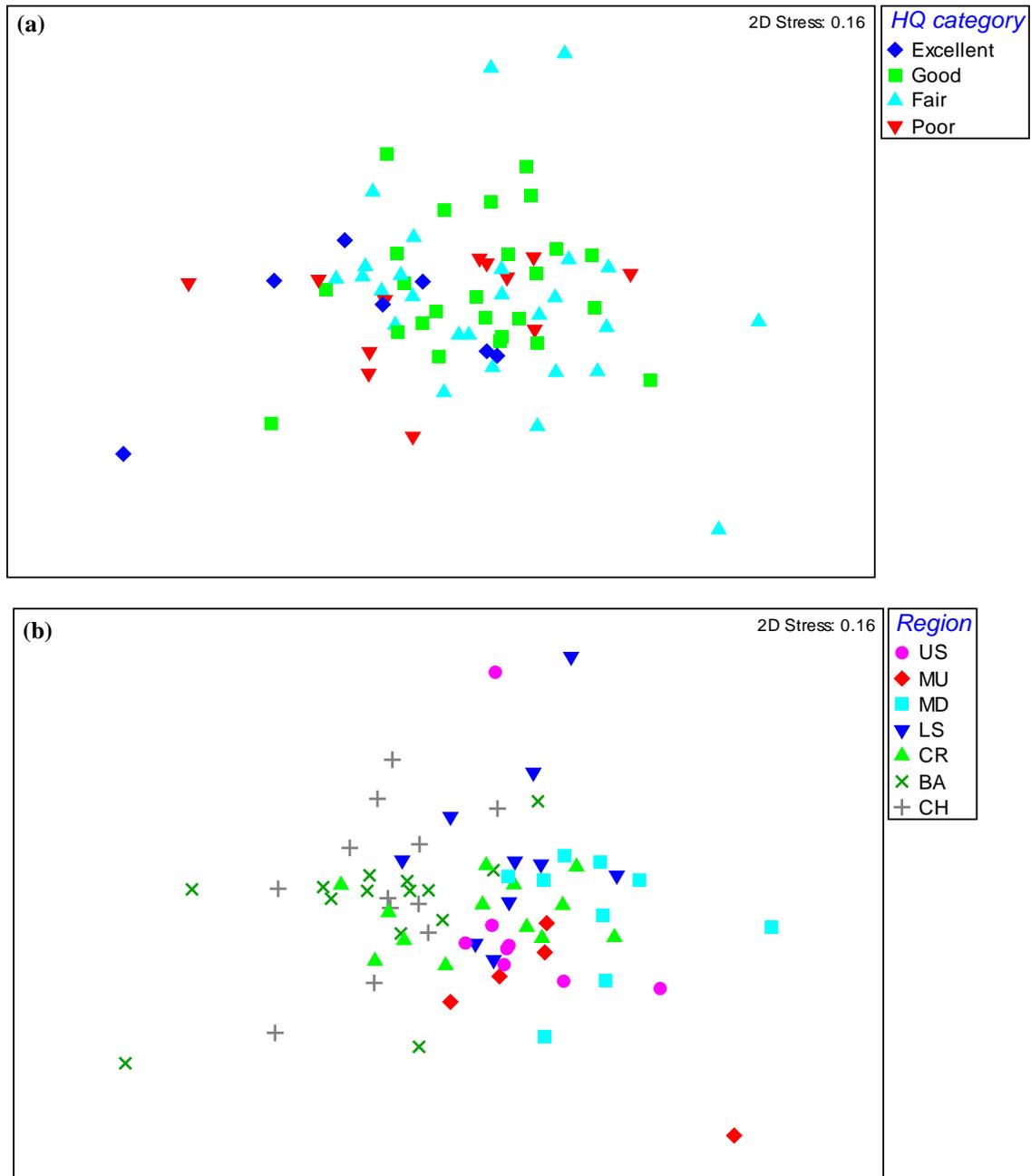
Figure 2.3.2.2: Non-metric multidimensional scaling ordination plots of the pre-treated fish metric composition data recorded from 71 sites throughout the Swan-Canning Estuary. Samples are coded by (a) Habitat Quality Category and (b) region of the estuary (US=Upper Swan River, MU=Middle Upstream Swan River, MD=Middle Downstream Swan River, LS=Lower Swan River, CR=Canning River, BA=Basin, CH=Channel).

Table 2.3.2.2: Mean squares (MS), pseudo-F ratios (Pseudo-F), significance levels (*P*) and components of variation (COV) for a region x Habitat Quality Category (HQC) PERMANOVA on the pre-treated fish metric composition data recorded throughout the Swan-Canning Estuary (df=degrees of freedom, * denotes rejection of the null-hypothesis at $P \leq 0.05$).

Source	df	MS	Pseudo-F	*P*	COV
Region	6	74.48	3.238	* 0.0001	2.627
HQC	3	18.52	0.805	0.707	-0.577
Region x HQC	14	19.68	0.856	0.808	-1.111
Residual	47	23.00			4.796
Total	70				

When the same analyses were performed on the fish species abundance data, examination of the MDS ordination plot again revealed no apparent differences among HQCs (Fig. 2.3.2.3a), but pronounced overall differences among regions of the estuary, with sites from the upstream reaches again forming a group to one side of the plot, while those from the basin and entrance channel formed a group on the opposite side of the plot (Fig. 2.3.2.3b).
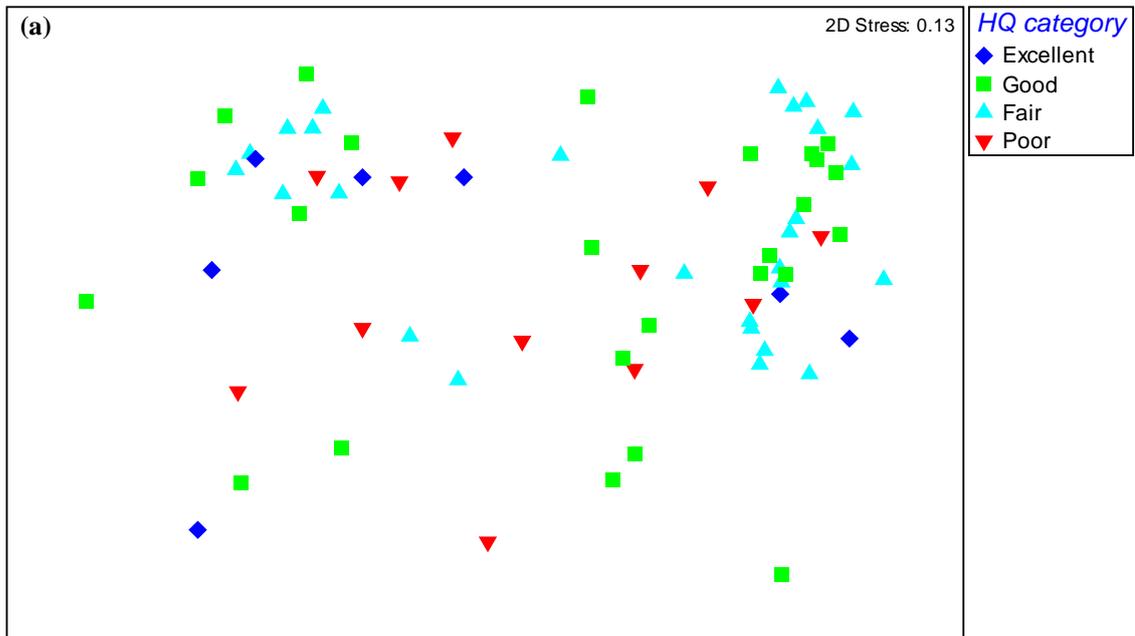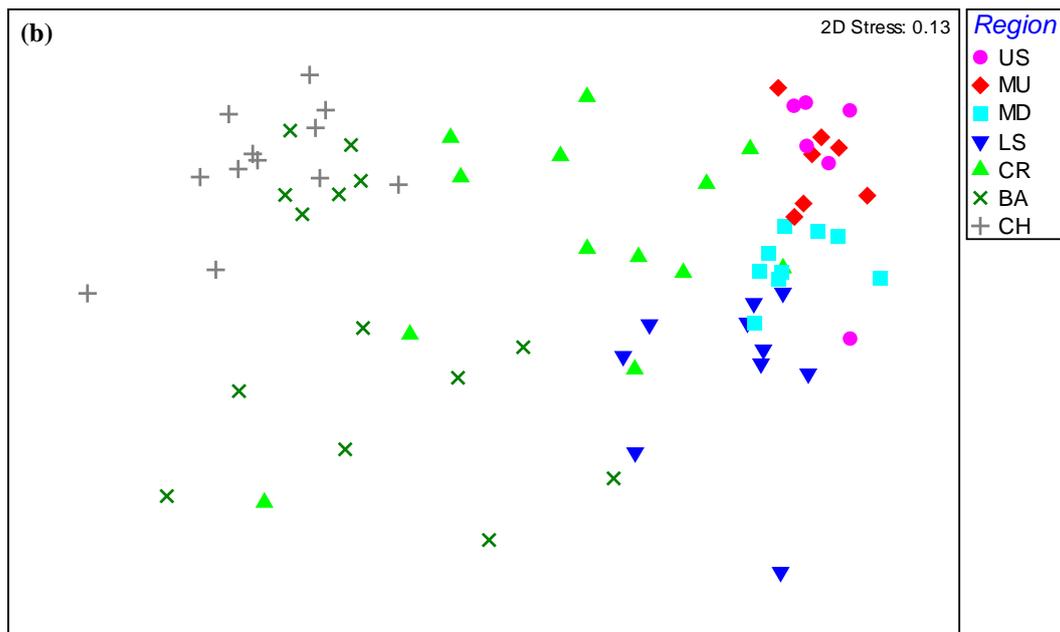
Figure 2.3.2.3: Non-metric multidimensional scaling ordination plots of the pre-treated fish abundance data recorded from 71 sites throughout the Swan-Canning Estuary. Samples are coded by (a) Habitat Quality Category and (b) region of the estuary (US=Upper Swan River, MU=Middle-Upstream Swan River, MD=Middle-Downstream Swan River, LS=Lower Swan River, CR=Canning River, BA=Basin, CH=Channel).

As was also the case for the fish metric data, PERMANOVA confirmed that no significant differences in fish community composition occurred between HQCs, either as a main effect or as part of the HQC x region interaction, but a significant difference was identified between regions of the estuary (P=0.0001; Table 2.3.2.3). Such findings indicate that both fish metric and community composition were influenced by regional differences in some other factor than habitat quality.

Table 2.3.2.3: Mean squares (MS), pseudo-F ratios (Pseudo-F), significance levels (P) and components of variation (COV) for a region x Habitat Quality Category (HQC) PERMANOVA on the pre-treated fish species abundance data recorded throughout the Swan-Canning Estuary (df=degrees of freedom, * denotes rejection of the null-hypothesis at $P \leq 0.05$).

Source	df	MS	Pseudo-F	P	COV
Region	6	13424	9.835	* 0.0001	39.054
HQC	3	790.6	0.579	0.938	-6.348
Region x HQC	14	1077.3	0.789	0.914	-10.232
Residual	47	1364.9			36.944
Total	70				

The relative influence of regional differences in one such alternative factor, namely water quality, on both fish metric and community composition was then further elucidated using a combination of PCA and CCorA ordination analyses. Thus, initial PCA ordination of the 71 fish sampling sites for (a) the scores of the six physical habitat quality metrics (Table 2.3.2.4, Fig. 2.3.2.4) and (b) the four water quality variables (Table 2.3.2.4, Fig. 2.3.2.5) demonstrated that, in the first of these cases, the first principal component axis (subsequently

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denoted as PC1a) explained over 41% of the total variability among sites. The greatest contributions to PC1a were made by riparian width and riparian zone longitudinal extent/canopy cover, whose scores decreased from left (Excellent sites) to right (Poor sites) along that axis. This principal component was thus considered to be a reasonable proxy 'variable' for describing the maximum spatial differences in physical habitat quality throughout the estuary. In the second of the above cases, the first PC axis (subsequently denoted as PC1b) explained approximately 80% of the variability among sites, with the greatest contributions being made by salinity and water temperature, which decreased and increased, respectively, from left to right along PC1b (*i.e.* from the downstream to upstream regions of the estuary). This axis was thus considered to be a good proxy variable for capturing the overall differences in water quality throughout the estuary.
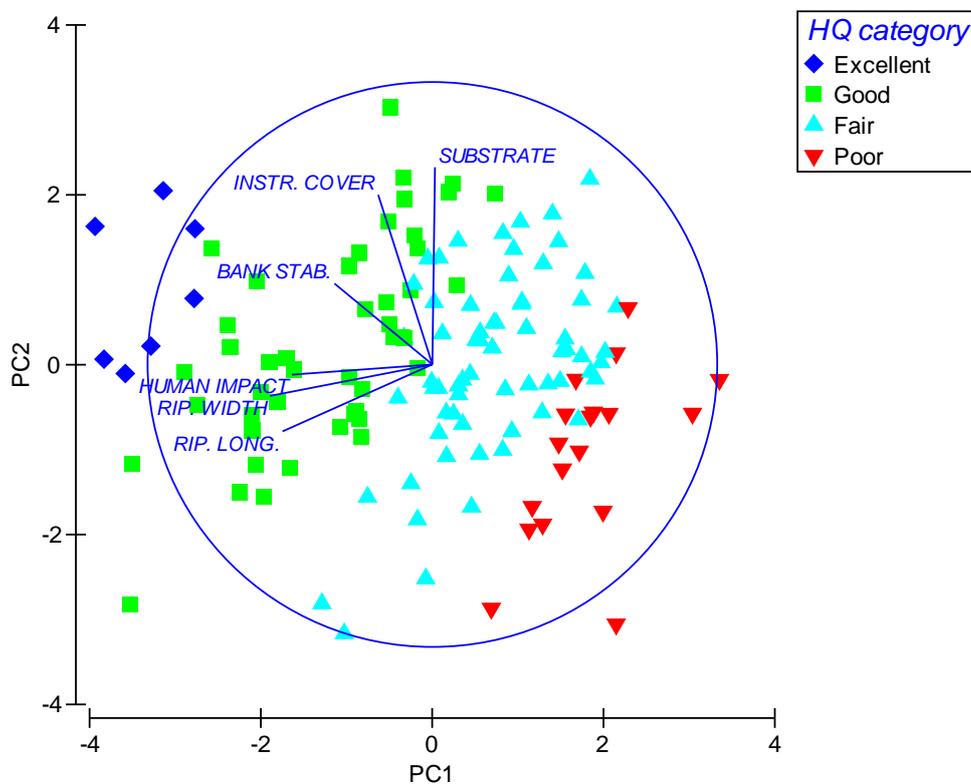
Figure 2.3.2.4: PCA ordination of normalised scores for the various physical habitat quality metrics measured at the 71 sites fished throughout the Swan-Canning Estuary. Samples are coded by habitat quality (HQ) category. Vector overlays denote the direction and magnitude of the contributions to the PC axes of each of the habitat metrics: Substrate, Instream Cover (Instr. Cover), Bank Stability (Bank Stab.), Riparian Zone Width (Rip. Width), Riparian Zone Length and Canopy Cover (Rip. Long.) and Human Stressors of Habitat/Riparian Zone (Human Impact).
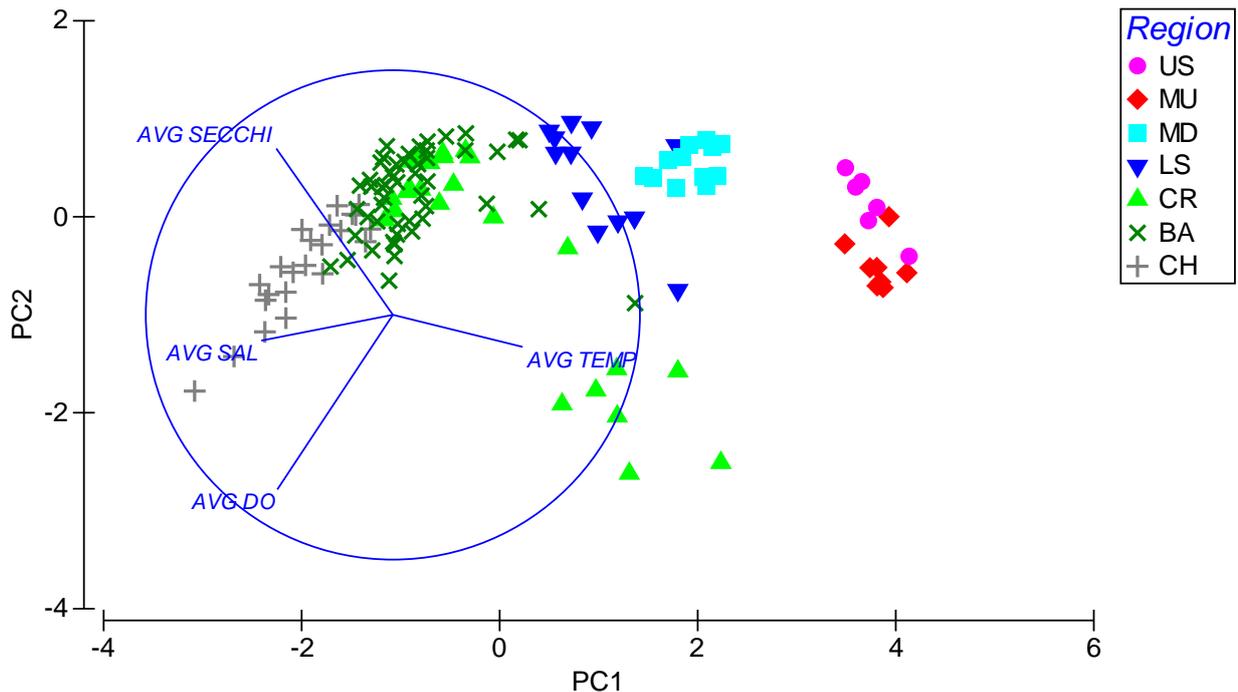
Figure 2.3.2.5: PCA ordination of normalised scores for the various water quality variables measured at 71 sites fished throughout the Swan-Canning Estuary. Samples are coded by region of the estuary (US=Upper Swan River, MU=Middle-Upstream Swan River, MD=Middle-Downstream Swan River, LS=Lower Swan River, CR=Canning River, BA=Basin, CH=Channel). Vector overlays denote the direction and magnitude of the contributions to the PC axes of each of the water quality variables: Secchi depth (Avg Secchi), Temperature (Avg Temp), Salinity (Avg Sal) and Dissolved oxygen (Avg DO).

Table 2.3.2.4: Eigenvectors from PCAs of the normalised scores for (a) physical habitat metrics and (b) water quality variables recorded at the 71 sites fished throughout the Swan-Canning Estuary. Eigenvectors highlighted in bold denote those variables that contributed most strongly to the first PC axis in each of the two analyses.

Variable	PC1a	PC1b
(a) Physical habitat quality metrics		
Substrate	0.009	
Instream cover	-0.190	
Bank stability	-0.344	
Riparian width	**-0.570**	
Riparian zone longitudinal extent / canopy cover	**-0.526**	
Human stressors of habitat / riparian zone	-0.493	
(b) Water quality variables		
Secchi depth		-0.472
Water temperature		**0.525**
Salinity		**-0.532**
Dissolved oxygen		-0.467

The above PC1 axes were then each employed in a canonical correlation analysis (CCorA) to investigate whether the habitat quality or water quality gradients they defined were correlated with spatial differences in fish metric or community composition. These ordination analyses, which were carried out using the CAP routine, revealed very little correlation between the

gradient of physical habitat quality (represented by PC1a) and fish metric or community composition at the 71 sampling sites (Figs 2.3.2.6 and 2.3.2.7), as evidenced by the weak squared canonical correlation coefficients (δ^2) of 0.027 and 0.015, respectively. The choices of m=4 and m=2 PCO axes, respectively, for these analyses encapsulated 64 and 63% of the variability in the respective fish metric and community resemblance matrices, and were deemed reasonable in each case. Thus, any further increase in m would have increased the leave-one-out residual sum of squares without appreciably increasing the squared canonical correlation coefficient.
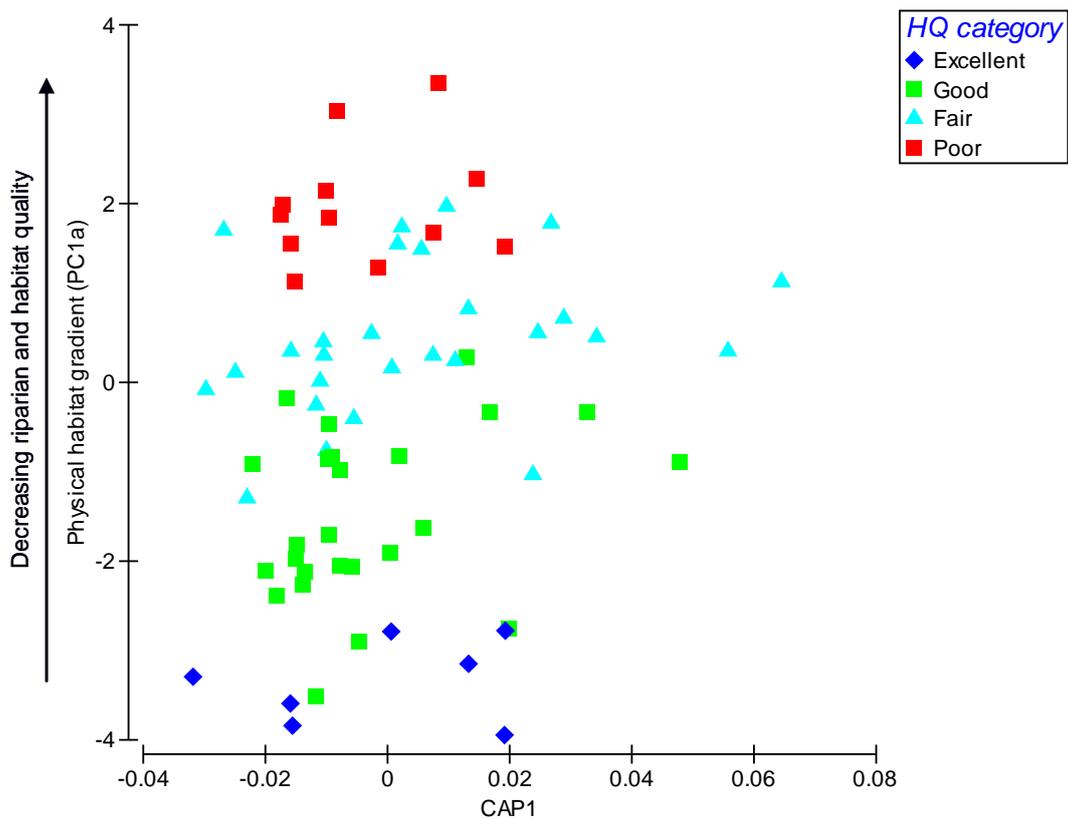
Figure 2.3.2.6: Canonical Analysis of Principal Coordinates (CAP) ordination, relating the fish metric composition (CAP1) to the physical habitat quality gradient (PC1a) across the 71 sites fished throughout the Swan-Canning Estuary. Proportion of variation in the data cloud explained by the first 4 (*m*) PCO axes=64%.
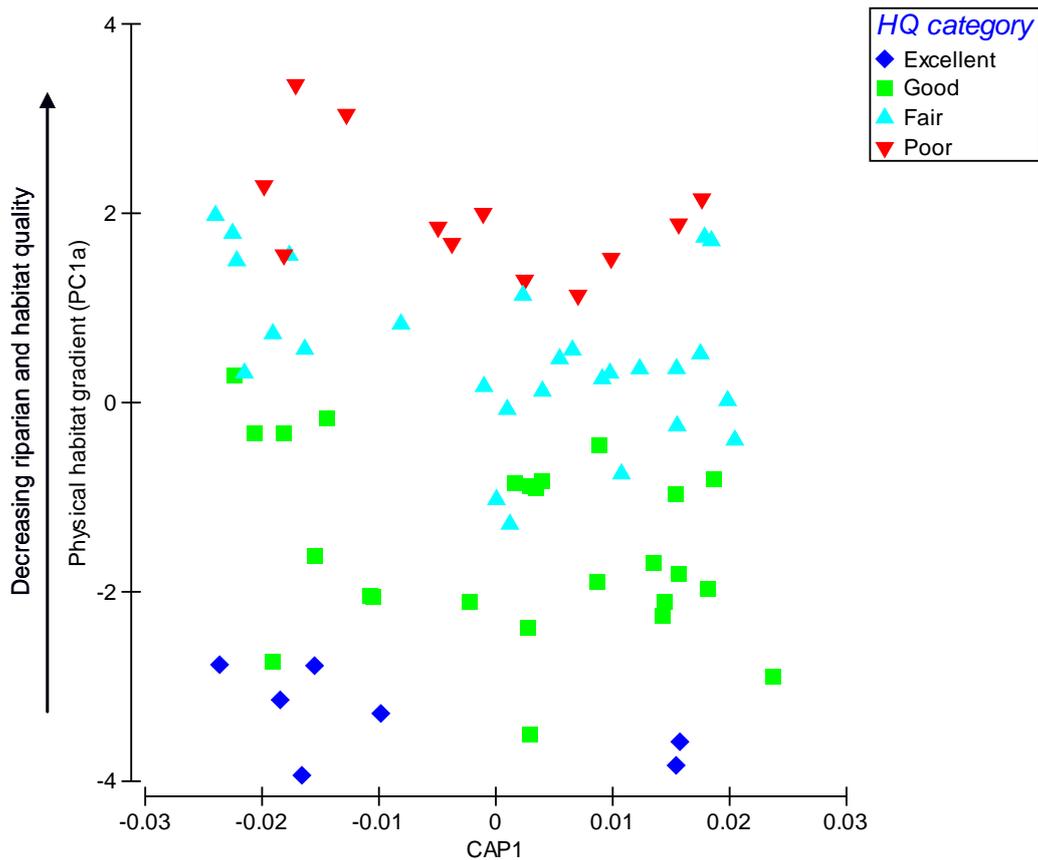
Figure 2.3.2.7: Canonical Analysis of Principal Coordinates (CAP) ordination, relating the fish community composition (CAP1) to the physical habitat quality gradient (PC1a) across the 71 sites fished throughout the Swan-Canning Estuary. Proportion of variation in the data cloud explained by the first 2 (*m*) PCO axes=63%.

In contrast, strong correlations with the gradient of water quality (represented by PC1b) were demonstrated for both fish metric and community composition, as evidenced by squared canonical correlation coefficients of 0.760 and 0.816, respectively (Figs 2.3.2.8 and 2.3.2.9). The choices of *m*=6 and *m*=2 PCO axes, respectively, for these analyses encapsulated 79 and 63% of the variability in the respective resemblance matrices. These values of m were considered reasonable in each case, as further increases would have increased the leave-one-out residual sum of squares without appreciably increasing the squared canonical correlation coefficient, whilst further reductions in m would have both increased the residual sum of squares and decreased the value of δ^2.
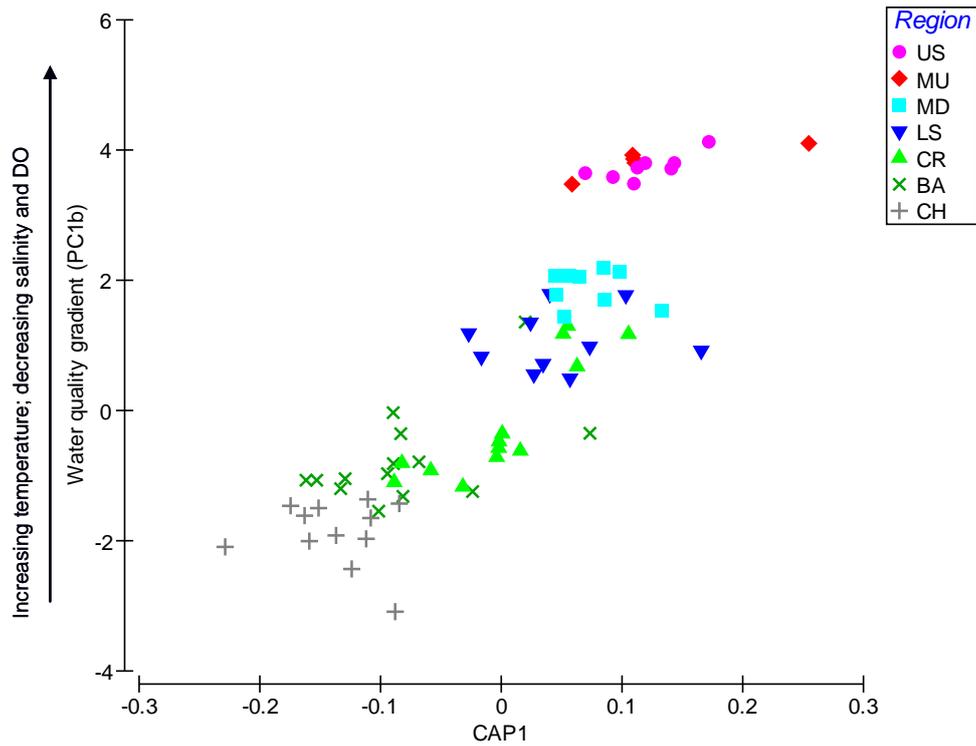
Figure 2.3.2.8: Canonical Analysis of Principal Coordinates (CAP) ordination, relating the fish metric composition (CAP1) to the water quality gradient (PC1b) across the 71 sites fished throughout the Swan-Canning Estuary. Proportion of variation in the data cloud explained by the first 6 (*m*) PCO axes=79%.
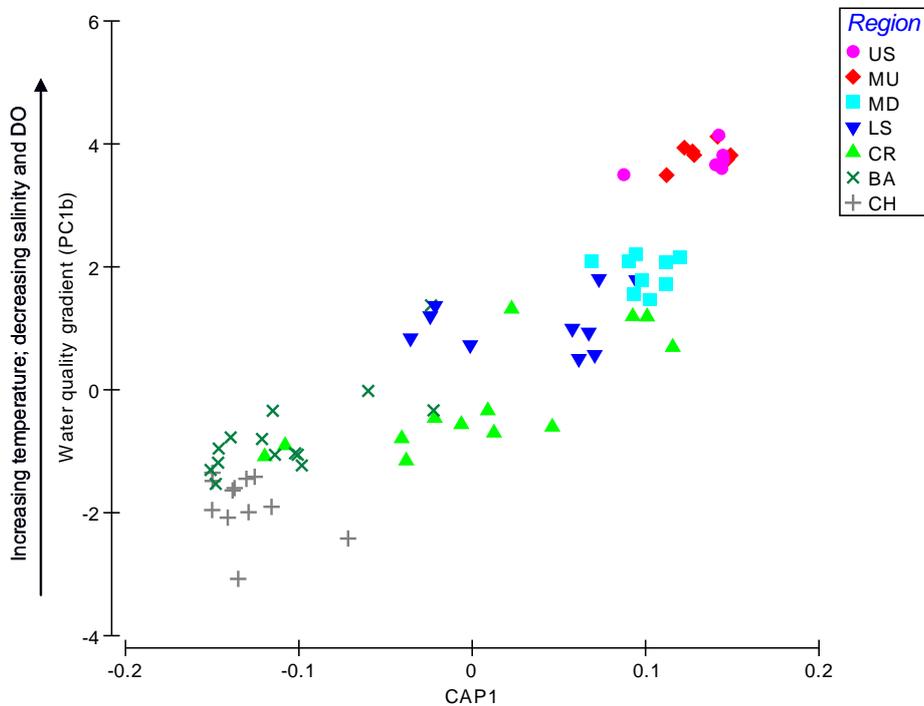
Figure 2.3.2.9: Canonical Analysis of Principal Coordinates (CAP) ordination, relating the fish community composition (CAP1) to the water quality gradient (PC1b) across the 71 sites fished throughout the Swan-Canning Estuary. Proportion of variation in the data cloud explained by the first 2 (*m*) PCO axes=63%.

2.3.2.2 Selection of metrics sensitive to temporal changes

2.3.2.2.1 Nearshore data sets

The DISTLM analysis of the fish metric data derived from the 21 m data set identified a combination of eight metrics (*i.e. No species, Dominance, Prop trop spec, No trop spec, Prop trop gen, Prop est spawn, Prop P. olorum, Tot no P. olorum*) as the estimated 'best' model, which was denoted as $AIC_{c(min)}$. However, a set of 20 models with r^2 values ranging between 0.194 and 0.216 were also identified as being within 2 units of $AIC_{c(min)}$ (*i.e.* $\Delta_i \leq 2$), and were thus considered to be substantially supported by the evidence (Appendix 2.5.1). The Akaike weights for each of these models revealed that none had a high probability of being the single best, and the evidence ratios showed that the estimated best model was only 2.7 times more likely to be the best model compared to the 20th best model. Such small evidence ratios highlight considerable uncertainty surrounding the identity of the best model, and also a degree of redundancy among the variables within the model set, thus indicating that it was appropriate to adopt a multi-model inference (MMI) strategy, based on a weight of evidence approach. Metrics were thus selected according to their relative importance among the models in the $\Delta_i \leq 2$ subset and, specifically, whether they occurred at a relative frequency of >50% among those models. The metrics that satisfied these criteria are listed in Table 2.3.2.5.

Table 2.3.2.5: Fish metrics selected (highlighted) by distance-based redundancy analysis of the 21 m seine net data.

Metric	Relative frequency among subset (%)
No species	65
Dominance	45
Sh-div	25
Prop trop spec	100
No trop spec	100
No trop gen	85
Prop detr	65
Feed guild comp	5
Prop benthic	15
No benthic	5
Prop est spawn	100
No est spawn	85
Prop P. olorum	100
Tot no P. olorum	100

Similarly, the results of the DISTLM analysis of the fish metric data calculated from the 41 m data set (Appendix 2.5.2) showed that a model containing *Prop trop spec, No trop spec, Prop detr, No benthic, Prop est spawn, No est spawn* and *Prop P. olorum* provided the estimated 'best' combination of metrics ($AIC_{c(min)}$), although a set of 66 models with r^2 values ranging from 0.237 to 0.329 were also identified as having substantial support from the evidence (*i.e.* $\Delta_i \leq 2$). Akaike weights again revealed that none of these fish metric combinations had a high probability of being the single best model, and the evidence ratios showed that the estimated best model was only 2.7 times more likely than the 66th model to be the best. Therefore, MMI was again shown to be appropriate in selecting those metrics which occurred at a relative frequency of >50% among those models in the $\Delta_i \leq 2$ subset (Table 2.3.2.6).

Table 2.3.2.6: Fish metrics selected (highlighted) by distance-based redundancy analysis of the 41 m seine net data.

Metric	Relative frequency among subset (%)
No species	58
Dominance	3
Sh-div	6
Prop trop spec	91
No trop spec	100
No trop gen	27
Prop detr	71
Feed guild comp	5
Prop benthic	56
No benthic	86
Prop est spawn	53
No est spawn	59
Prop P. olorum	73
Tot no P. olorum	5

The DISTLM analysis carried out on the fish metric data calculated from the 102-133 m data set identified a model containing nine metrics (*No species, Dominance, Prop trop spec, No trop spec, Prop detr, Prop benthic, No benthic, Feed guild comp, No est spawn*) to be the estimated 'best' combination (AIC$_{c(min)}$), although a set of 51 models with r^2 values ranging from 0.133 to 0.145 were also identified as having substantial support from the evidence. Akaike weights again demonstrated that none of these fish metric combinations had a high probability of being the single best model, and the evidence ratios showed that the estimated best model was only 2.7 times more likely than the 51st model to be the best (Appendix 2.5.3). Therefore, MMI was again shown to be appropriate in selecting those metrics which occurred at a relative frequency of >50% among those models in the $\Delta_i \leq 2$ subset (Table 2.3.2.7).

Table 2.3.2.7: Fish metrics selected (highlighted) by distance-based redundancy analysis of the 102-133 m seine net data.

Metric	Relative frequency among subset (%)
No species	100
Dominance	63
Sh-div	39
Prop trop spec	57
No trop spec	100
No trop gen	29
Prop detr	100
Feed guild comp	100
Prop benthic	86
No benthic	100
Prop est spawn	39
No est spawn	100
Prop P. olorum	20
Tot no P. olorum	12

BIOENV determined that, for the 21 m data set, the metrics *No trop spec, Prop detr, Prop P. olorum* and *Tot no P. olorum* best matched the pattern of inter-period differences in the

model matrix (ρ_s=0.128, P=0.01), while for the 41 m data set, *No trop gen, Prop detr, Prop benthic* and *Prop est spawn* were most highly correlated with the model matrix (ρ_s=0.176, P=0.01). For the 102-133 m data set, the BVSTEP procedure consistently identified the subset of metrics *Prop trop spec, No benthic* and *No est spawn* as being the best matched to the inter-period model matrix (ρ_s=0.071, P=0.001). Although each of the above correlations were significant, their extents were low in all cases, thus indicating a weak match between the inter-period differences exhibited by the selected subsets of fish metrics and those defined by the model matrix.

Neither DISTLM nor BIOENV/BVSTEP alone could thus be considered to have selected a definitive, best set of fish metrics for the nearshore waters of the Swan-Canning Estuary. Consideration of the combined outputs of these analyses via a weight of evidence approach was thus deemed appropriate for identifying the most reliable, informative metric subset. The set of 11 metrics selected for inclusion in a nearshore index of estuarine health (*i.e.* those selected by more than one of the six analyses) are shown in Table 2.3.2.8.

Table 2.3.2.8: Summary of the fish metrics selected by the DISTLM and BIOENV/BVSTEP analyses of each of the nearshore data sets (light highlight), including those metrics selected by multiple analyses and thus chosen for incorporation into a nearshore index of estuarine health for the Swan-Canning Estuary (dark highlight).

Metric	21 m data set		41 m data set		102-133 m data set		Selected
	DISTLM	BIOENV	DISTLM	BIOENV	DISTLM	BVSTEP	
No species	▓		▓		▓		██
Dominance					▓		
Sh-div							
Prop trop spec	▓	▓	▓		▓	▓	██
No trop spec	▓	▓			▓		██
No trop gen	▓			▓	▓		██
Prop detr	▓	▓		▓	▓	▓	██
Feed guild comp					▓		
Prop benthic			▓	▓		▓	██
No benthic			▓		▓	▓	██
Prop est spawn	▓		▓				██
No est spawn	▓	▓		▓	▓	▓	██
Prop P. olorum	▓	▓	▓				██
Tot no P. olorum	▓		▓				██

2.3.2.2.2 Offshore data set

The estimated 'best' model (AIC$_{c(min)}$) as identified by DISTLM of the offshore fish data set contained five fish metrics (*i.e. No species, No trop spec, No trop gen, Prop benthic, Prop est spawn*), although a set of 66 models with r^2 values ranging between 0.098 and 0.329 were again identified as having substantial support from the evidence (Appendix 2.5.4). As for the nearshore data sets, Akaike weights demonstrated that none of these models had a high probability of being the single best, and the estimated best model was again shown by evidence ratios to be only 2.7 times more likely to be the best than the model ranked 66th. Selection of those metrics occurring at a relative frequency of >50% among the models in the $\Delta_i \leq 2$ subset generated the set of metrics highlighted in Table 2.3.2.9.

Table 2.3.2.9: Set of fish metrics selected (highlighted) by distance-based redundancy analysis of the gill net data.

Metric	Relative frequency among subset (%)
No species	80
Dominance	24
Sh-div	39
Prop trop spec	12
No trop spec	88
No trop gen	42
Prop detr	39
Feed guild comp	44
Prop benthic	100
No benthic	18
Prop est spawn	100
No est spawn	21

The BIOENV routine identified a set of five metrics from the offshore data set (*Sh-div, No trop spec, No trop gen, Prop detr* and *Prop benthic*) as being the best matched to the inter-period trends in the model matrix (ρ_s=0.068, *P*=0.07). Although the extent of this correlation was weak, it was close to statistical significance at *P*=0.05, and was thus accepted for further consideration as part of the broader, evidence-based approach. As only two metrics were selected by both the DISTLM and BIOENV analyses of this data set, the modified decision rule to select a metric for inclusion in an offshore index of estuarine health if it was identified by either of the two analyses, subsequently generated a set of seven metrics (Table 2.3.2.10).

Table 2.3.2.10: Fish metrics selected by the DISTLM or BIOENV analyses of the offshore data set (light highlight) and thus chosen for incorporation into an offshore index of estuarine health for the Swan-Canning Estuary (dark highlight).

Metric	Gill net data set		Selected
	DISTLM	BIOENV	
No species	▨		▉
Dominance			
Sh-div		▨	▉
Prop trop spec			
No trop spec	▨		▉
No trop gen		▨	▉
Prop detr		▨	▉
Feed guild comp			
Prop benthic	▨	▨	▉
No benthic			
Prop est spawn	▨		▉
No est spawn			

2.3.2.3 Establishing reference conditions and scoring metrics

The reference conditions for each selected nearshore metric, as determined from the "best available" metric values derived from the standardised seine net data collected between 1976 and 2009, are presented for each zone*season combination in Table 2.3.2.11. For several of these metrics, there were clear differences in reference condition values both between different zones in a given season, and between seasons within a zone. For example, the

reference condition for the metric *No species* varied from as few as five species in the USE in winter to as many as 14 species in the MSE or CELCR in summer or autumn.

Table 2.3.2.11: Reference conditions for each of the selected nearshore fish metrics, determined from standardised historical and current seine net data collected from each zone of the Swan-Canning Estuary (Lower Swan-Canning Estuary [LSCE], Canning Estuary/Lower Canning River [CELCR], Middle Swan Estuary [MSE] and Upper Swan Estuary [USE]) in each season; *n*=number of samples per zone*season combination. Metric abbreviations and the predicted responses of metrics to degradation, *i.e.* positive (+) or negative (-), are described in subsection 2.2.2.1.2.

Zone*season	n	No species (-)	Prop trop spec (-)	No trop spec (-)	No trop gen (+)	Prop detr (+)	Prop benthic (-)	No benthic (-)	Prop est spawn (-)	No est spawn (-)	Prop P. olorum (+)	Tot no P. olorum (+)
LSCE*summer	174	11	0.99	8	1	0	1.0	9	0.96	5	0	0
LSCE*autumn	156	13	0.99	8	1	0	1.0	9	0.83	5	0	0
LSCE*winter	173	8	1.0	6	0	0	1.0	6	0.79	4	0	0
LSCE*spring	179	11	0.98	7	1	0	1.0	8	0.76	5	0	0
CELCR*summer	66	14	0.99	9	1	0	1.0	9	1.0	9	0	0
CELCR*autumn	68	13	0.99	8	0	0	1.0	6	1.0	7	0	0
CELCR*winter	79	10	0.99	5	0	0	1.0	5	1.0	6	0	0
CELCR*spring	84	12	0.98	8	1	0	1.0	7	1.0	8	0	0
MSE*summer	119	14	0.96	8	1	0	1.0	9	1.0	9	0	0
MSE*autumn	123	14	1.0	9	0	0	1.0	9	1.0	8	0	0
MSE*winter	115	10	0.98	6	0	0	1.0	7	1.0	6	0	0
MSE*spring	144	13	0.93	8	1	0	1.0	9	1.0	8	0	0
USE*summer	108	10	0.98	6	1	0	0.98	7	1.0	8	0	0
USE*autumn	111	9	1.0	5	0	0	1.0	6	1.0	7	0	0
USE*winter	99	5	0.99	3	0	0	0.95	3	1.0	4	0	0
USE*spring	132	9	0.98	5	1	0	1.0	6	1.0	7	0	0

Similarly, there were clear differences in reference condition values for several of the selected offshore metrics, both between zones in each season and *vice versa* (Table 2.3.2.12).

Table 2.3.2.12: Reference conditions for each of the selected offshore fish metrics, determined from historical and current gill net data collected from each zone of the Swan-Canning Estuary (Lower Swan-Canning Estuary [LSCE], Canning Estuary/Lower Canning River [CELCR], Middle Swan Estuary [MSE] and Upper Swan Estuary [USE]) in each season; *n*=number of samples per zone*season combination. Metric abbreviations and the predicted responses of metrics to degradation, *i.e.* positive (+) or negative (-), are described in subsection 2.2.2.1.2.

Zone*season	*n*	No species (-)	Sh-div (-)	No trop spec (-)	No trop gen (+)	Prop detr (+)	Prop benthic (-)	Prop est spawn (-)
LSCE*summer	11	6	1.51	4	0	0	1.0	1.0
LSCE*autumn	12	6	1.63	4	0	0	1.0	0.92
LSCE*winter	12	8	1.87	5	0	0	1.0	0.41
LSCE*spring	8	5	1.47	5	0	0	1.0	1.0
CELCR*summer	10	7	1.71	4	0	0.20	1.0	0.83
CELCR*autumn	8	8	1.69	4	0	0.36	1.0	0.72
CELCR*winter	10	4	1.36	3	0	0	1.0	1.0
CELCR*spring	8	9	1.71	4	0	0	0.96	1.0
MSE*summer	37	6	1.67	2	0	0.09	1.0	1.0
MSE*autumn	45	6	1.44	3	0	0.16	1.0	1.0
MSE*winter	42	5	1.44	2	0	0	1.0	1.0
MSE*spring	42	5	1.29	2	0	0.20	1.0	1.0
USE*summer	35	5	1.18	2	1	0	1.0	1.0
USE*autumn	39	5	1.55	3	0	0	1.0	1.0
USE*winter	39	4	1.18	1	0	0	1.0	1.0
USE*spring	37	4	1.27	1	1	0	1.0	1.0

Metric values for each historical and current nearshore and offshore fish sample were then scored on a scale of 0-10 according to the extent of their deviation from the relevant zone*season reference condition, thus enabling the subsequent calculation of final index values.

2.3.2.4 Preliminary interpretation of index performance

Examination of the changes in mean nearshore index scores between the late 1970s and late 2000s indicates that the health of the nearshore waters of the Swan-Canning Estuary has undergone a moderate degree of variation, but that the health status has remained as fair throughout this time (Fig. 2.3.2.10). However, it is important to note that reliable interpretation of longer-term trends in these mean index scores is impeded by differences among studies in the location, timing and intensity of sampling, as well as by the inability to standardise values of species richness among samples collected using different net types (subsection 2.2.1.3.1). Changes in nearshore index scores since the mid-1990s, and particularly those from 2005/06 to 2008/09, may, however, be interpreted more reliably, due

to greater standardisation of the sampling methodology across that period. Although the lack of sampling in consecutive years between 1995/96 and 2003/04 reduces the ability to discern index trends over that time, there is evidence to suggest that the health of the nearshore waters of the estuary has increased in more recent years, from a mean health index score of *ca* 58 in 2005/06 to 64 in 2008/09.
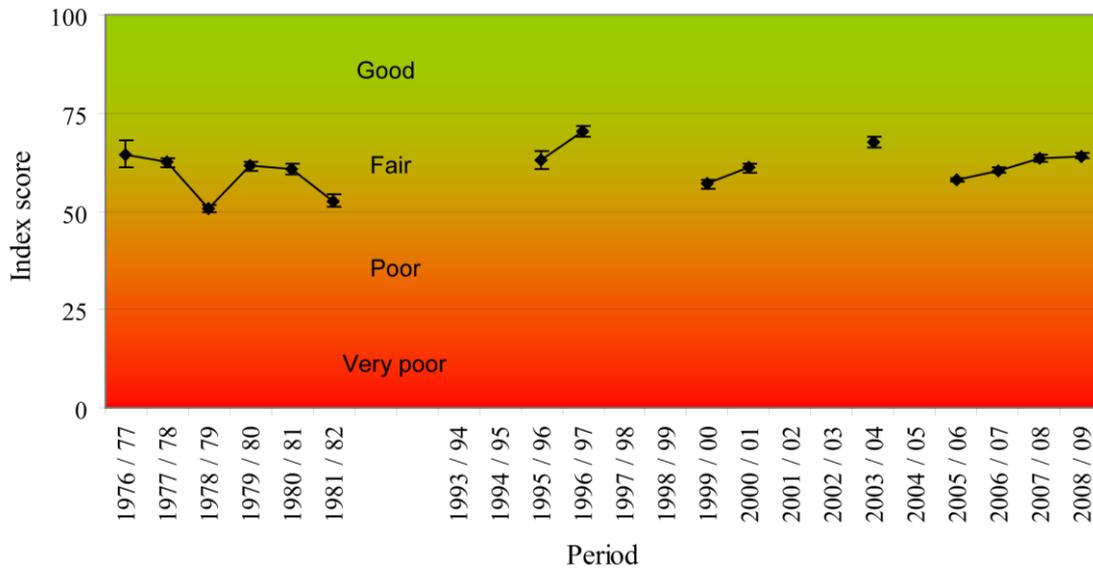
Figure 2.3.2.10: Mean (±SE) nearshore health index scores across all sites sampled throughout the Swan-Canning Estuary, 1976-2009.

In contrast, the longer-term changes in the health of the offshore waters of the Swan-Canning Estuary may be interpreted reliably, due to the greater consistency of sampling methodologies among all historical and current fish community studies of those waters. The mean offshore index score has decreased consistently from 56.5 in the late 1970s to 47 in 2008/09, resulting in the health status of these waters being classified as poor during the most recent study period for the first time in three decades (Fig. 2.3.2.11).
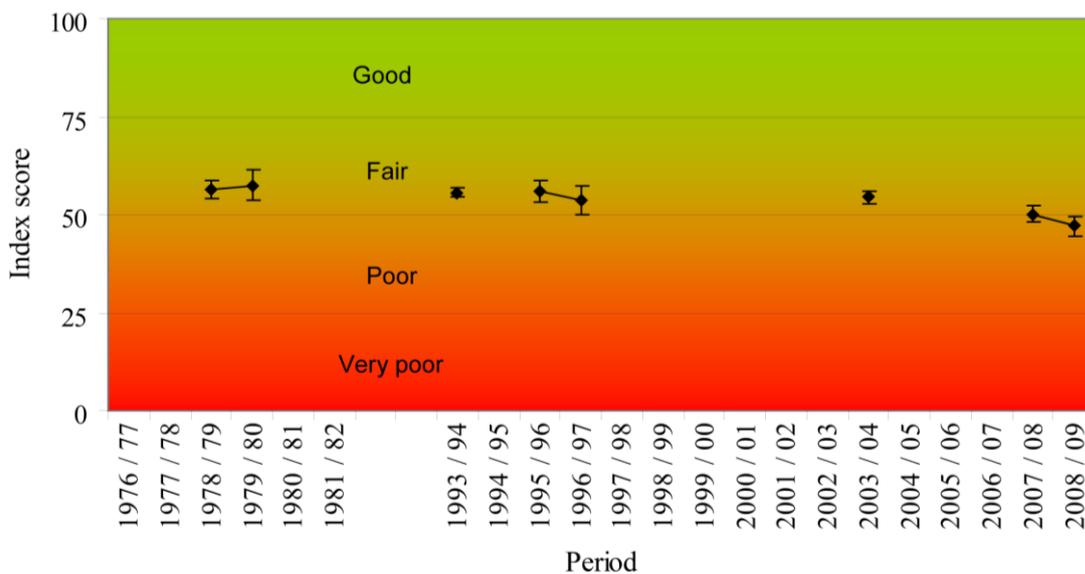
Figure 2.3.2.11: Mean (± SE) offshore health index scores across all sites sampled throughout the Swan-Canning Estuary, 1978-2009.

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Scores for eight of the selected nearshore metrics were significantly correlated with those for the nearshore health index, following Bonferroni correction ($P<0.0045$; Table 2.3.2.13). Four of the metrics were positively and highly correlated with the health index scores ($\rho_s >0.5$; *No species, No trop spec, No benthic, No est spawn*) and two were reasonably well correlated in a positive direction ($0.2< \rho_s< 0.5$) (Table 2.3.2.13, Fig. 2.3.2.12). The remaining metrics formed either weak positive or weak negative correlations with index scores ($\rho_s<0.2$).
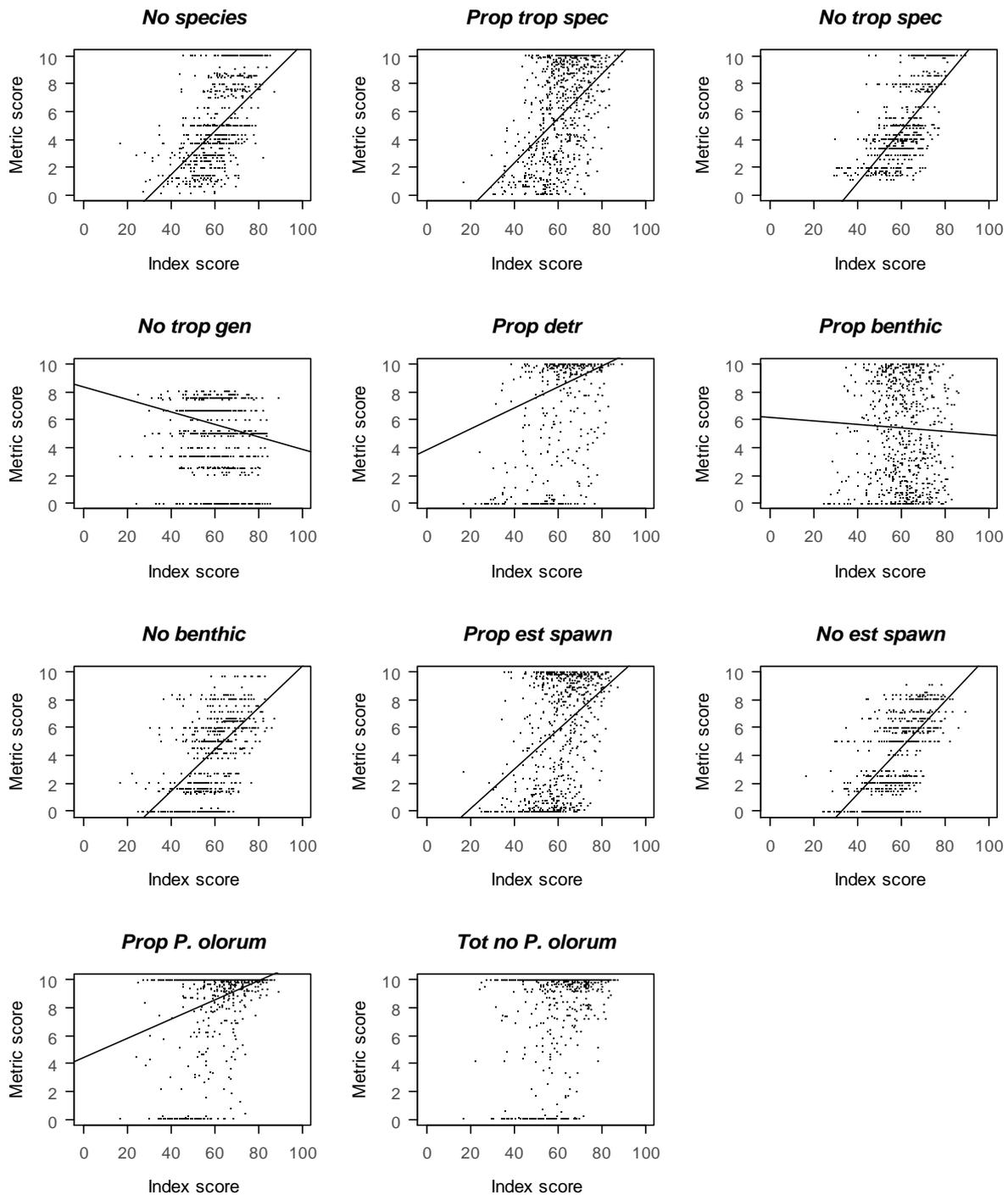
Figure 2.3.2.12: Scores for the 11 selected nearshore fish metrics *vs* the nearshore health index scores in each (non-zero) fish sample collected throughout the Swan-Canning Estuary, 1976-2009. Lines on plots indicate statistically significant correlations ($P <0.05$).

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Table 2.3.2.13: Spearman correlation coefficients (ρ_s) and associated *P*-values for correlations between the scores for each of the selected nearshore fish metrics and those for the nearshore health index; * denotes rejection of the null-hypothesis of $\rho_s=0$ at *P* <0.0045 (following Bonferroni correction for multiple tests).

Metric	ρ_s	P
No species	0.612	<0.001*
Prop trop spec	0.483	<0.001*
No trop spec	0.727	<0.001*
No trop gen	-0.176	<0.001*
Prop detr	0.047	0.041
Prop benthic	-0.088	<0.001*
No benthic	0.562	<0.001*
Prop est spawn	0.351	<0.001*
No est spawn	0.643	<0.001*
Prop P. olorum	0.048	0.034
Tot no P. olorum	0.011	0.639

Scores for five of the seven selected offshore metrics (*No species*, *Sh-div*, *No trop spec*, *Prop detr*, *Prop benthic*) were significantly and positively correlated with those for the offshore health index. The first three of these metrics showed strong correlations (ρ_s >0.5), the fourth was reasonably well correlated (0.3< ρ_s<0.5), while the fifth exhibited a weak correlation (Table 2.3.2.14, Fig. 2.3.2.13). Although not significant, scores for the metric *No trop gen* were again weakly and negatively correlated with the offshore index scores.
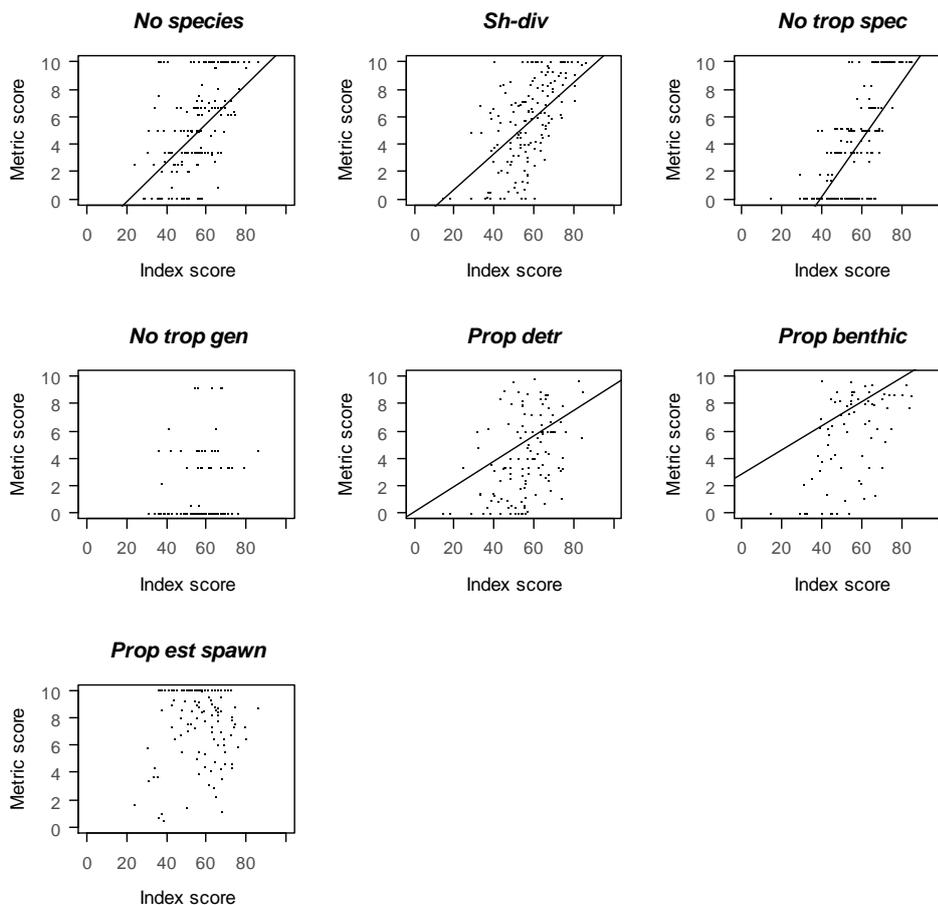
Figure 2.3.2.13: Scores for the seven selected offshore fish metrics *vs* the offshore health index scores in each (non-zero) fish sample collected throughout the Swan-Canning Estuary, 1978-2009. Lines on plots indicate statistically significant correlations (*P* <0.05).

Table 2.3.2.14: Spearman correlation coefficients (ρ_s) and associated *P*-values for correlations between the scores for each of the selected offshore fish metrics and those for the offshore health index; * denotes rejection of the null-hypothesis of ρ_s=0 at *P* <0.007 (following Bonferroni correction for multiple tests).

Metric	ρ_s	P
No species	0.572	<0.001*
Sh-div	0.516	<0.001*
No trop spec	0.673	<0.001*
No trop gen	-0.055	0.284
Prop detr	0.324	<0.001*
Prop benthic	0.188	<0.001*
Prop est spawn	0.044	0.639

2.3.2.5 Index validation

2.3.2.5.1 Index sensitivity
An analysis of all nearshore and offshore fish samples collected seasonally between 2007 and 2009 failed to identify any significant correlation between their health index scores and dissolved oxygen concentration or salinity recorded concurrently with fish collection (Table 2.3.2.15). However, offshore index scores showed a weak positive correlation with both surface and bottom water temperature ($P \leq 0.001$).

Table 2.3.2.15: Spearman correlation coefficients (ρ_s) and associated *P*-values for correlations between (a) nearshore and (b) offshore health index scores and water quality parameters measured concurrently with sampling of the fish community during 2007-2009. * denotes rejection of the null-hypothesis of ρ_s=0 at *P* <0.017 and *P* <0.007 for the nearshore and offshore tests, respectively (following Bonferroni correction for multiple tests).

Water quality parameter	ρ_s	P
(a) Nearshore		
Dissolved oxygen (mg L^{-1})	0.096	0.155
Salinity (‰)	-0.004	0.954
Temperature (°C)	0.084	0.216
(b) Offshore		
Bottom dissolved oxygen (mg L^{-1})	-0.167	0.165
Surface dissolved oxygen (mg L^{-1})	-0.044	0.606
Stratification index	-0.015	0.858
Bottom salinity (‰)	-0.053	0.542
Surface salinity (‰)	-0.012	0.890
Bottom temperature (°C)	0.297	<0.001*
Surface temperature (°C)	0.276	0.001*

2.3.2.5.2 Index variability

Index variability between replicate sites
In both years of the current study, between-site variability of the nearshore index within any given season was, on average, lower in the more upstream regions of the Swan-Canning

Estuary (*i.e.* Middle Downstream to Upper Swan River) than in those regions nearer the mouth of the system (*i.e.* Channel, Basin and Canning River) (Fig. 2.3.2.14). A similar pattern was also observed in the degree to which the standard deviations of nearshore index scores varied among seasons, with those in the upstream regions often being considerably less pronounced than in regions further downstream, most notably in 2008/09. Across all regions, the seasons with the lowest variability of index scores (*i.e.* those with the most points below the average standard deviation) were summer and autumn in 2007/08 and summer and winter in the following year (Fig. 2.3.2.14).
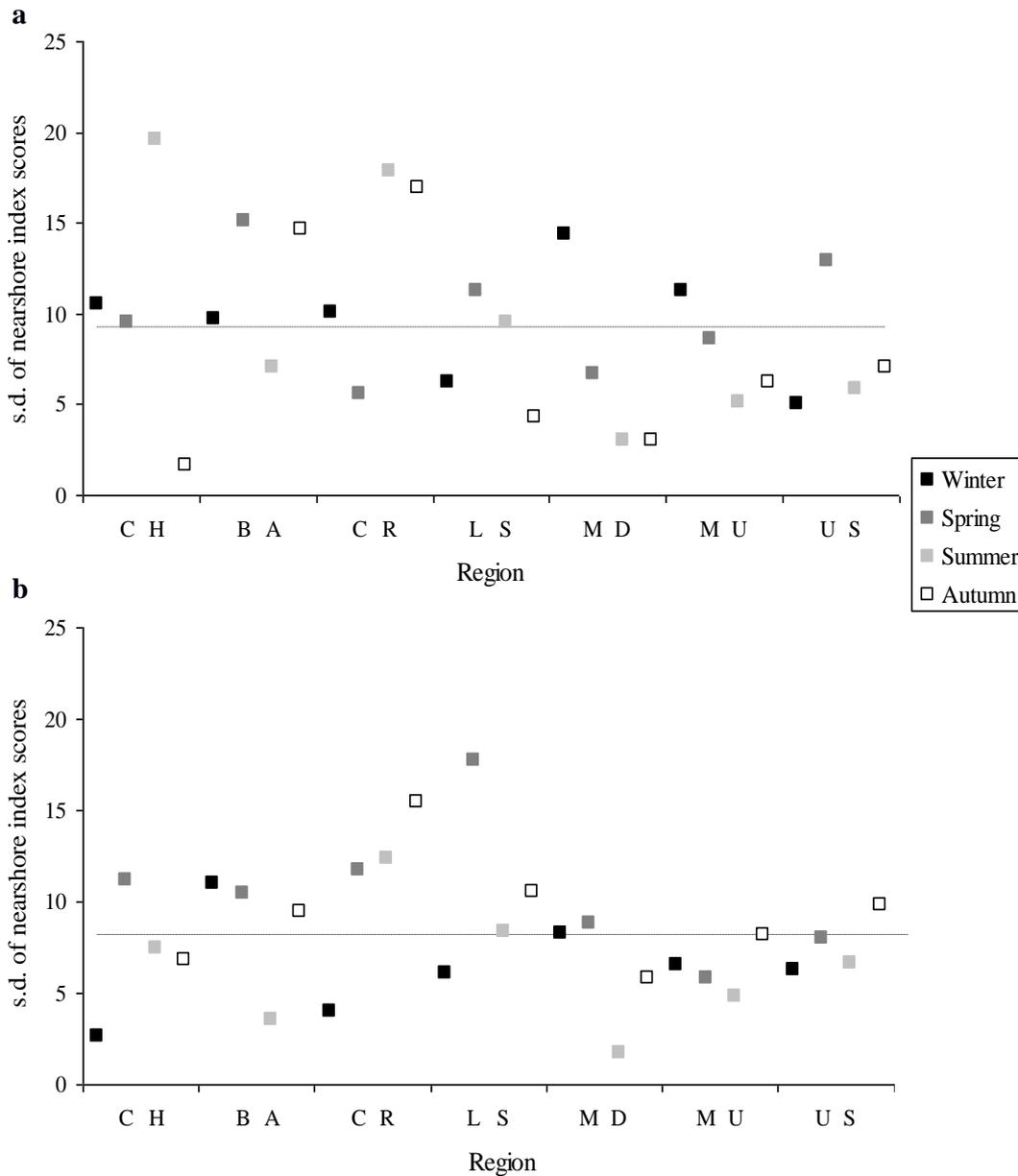
Figure 2.3.2.14: Plots of the standard deviation (s.d.) in nearshore health index scores among the three sites within each region of the Swan-Canning Estuary in each season during (a) 2007/08 and (b) 2008/09. See Fig. 2.2.1.1 for region codes. Dashed lines represent the average inter-site variability for each year, across all regions and seasons.

Variability of index scores among replicate sites was generally greater for the offshore index than its nearshore equivalent (Fig. 2.3.2.15). Also, and unlike the nearshore index, between-

site variability of offshore index scores generally decreased in a downstream direction during autumn and particularly winter in 2007/08 (Fig. 2.3.2.15a), while the same was often true in winter 2008/09 (Fig. 2.3.2.15b). The variability of offshore index scores was lowest, on average, in spring and summer in 2007/08 and in autumn during 2008/09.
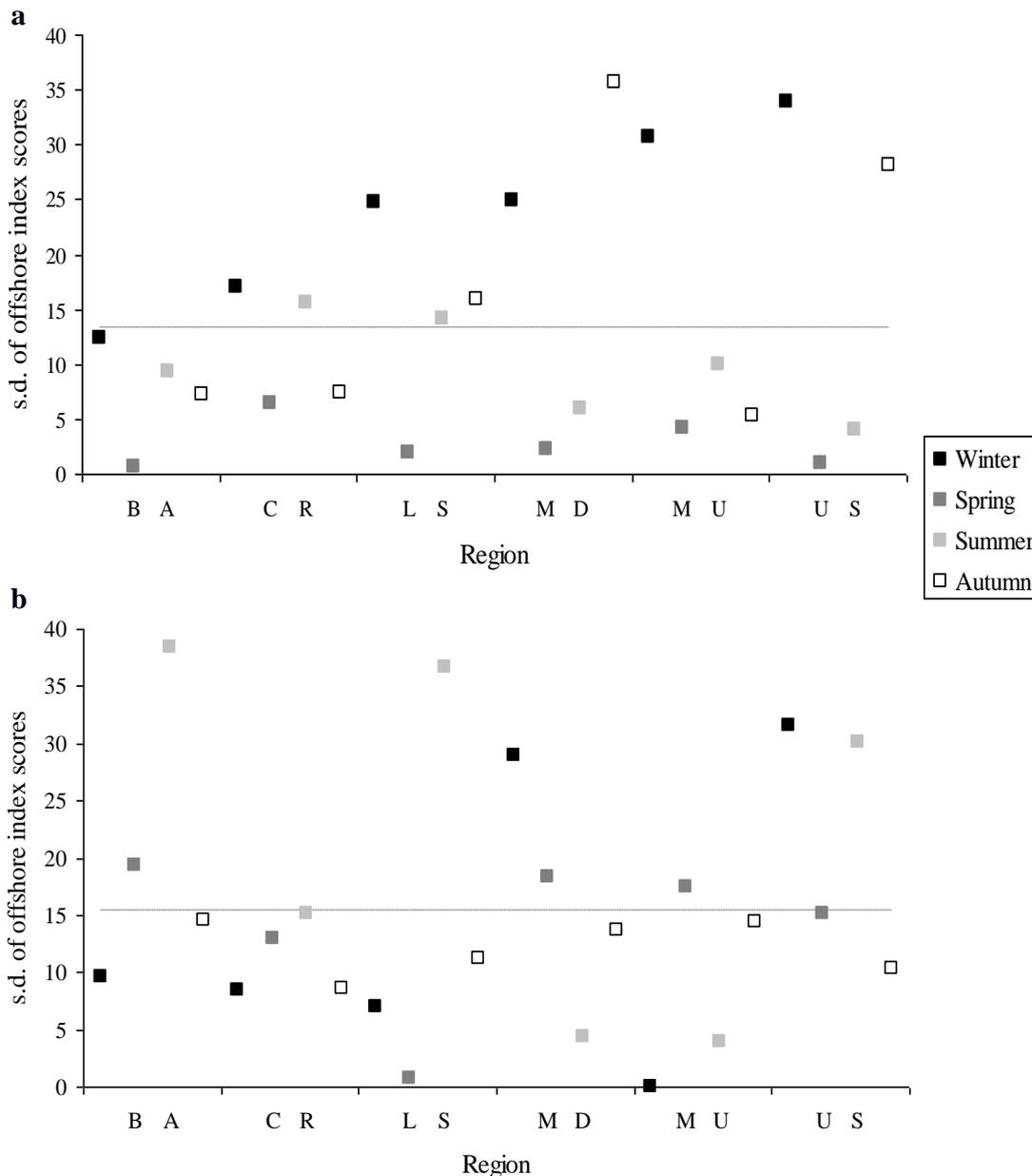
Figure 2.3.2.15: Plots of the standard deviation (s.d.) in offshore health index scores among the three sites within each region of the Swan-Canning Estuary, in each season during (a) 2007/08 and (b) 2008/09. See Fig. 2.2.1.1 for region codes. Dashed lines represent average inter-site variability for each year, across all regions and seasons.

Relationships between inter-seasonal variability and ecological quality
The standard deviations of index scores among seasons at each nearshore site sampled between 2007 and 2009 exhibited a weak, negative correlation with the means of those scores, which was close to being statistically significant (ρ_s=-0.246, P=0.056). Thus, inter-seasonal variation in nearshore index scores at the various sites in any given period was largely unrelated to the ecological quality of those sites. Moreover, there was no evidence to

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suggest that seasonal variability in index scores at a site was related to the zone of the estuary in which the site was located (Fig. 2.3.2.16).
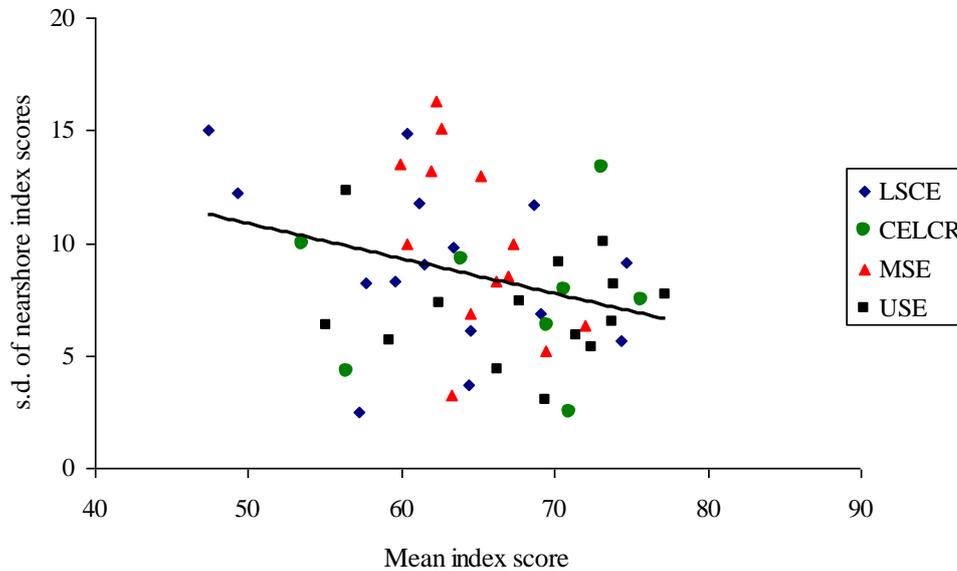
Figure 2.3.2.16: Mean *vs* standard deviation (s.d.) of nearshore health index scores among seasons at each of the sites assessed in 2007-2009. Sites are colour-coded for zone of the estuary (see Fig. 2.2.1.1 for zone codes). Solid lines are simple linear regressions.

In contrast, a significant and moderate negative correlation was observed between the inter-seasonal variation in index scores and the averages of those scores at each offshore site in 2007-09 (ρ_s=-0.553, P<0.001). These results thus demonstrated that inter-seasonal variation in offshore index scores was inversely related to site quality. Moreover, sites in the USE were often of lower ecological quality and, in accordance with the above significant relationship, experienced greater seasonal variability of index scores than sites from other zones (Fig. 2.3.2.17).
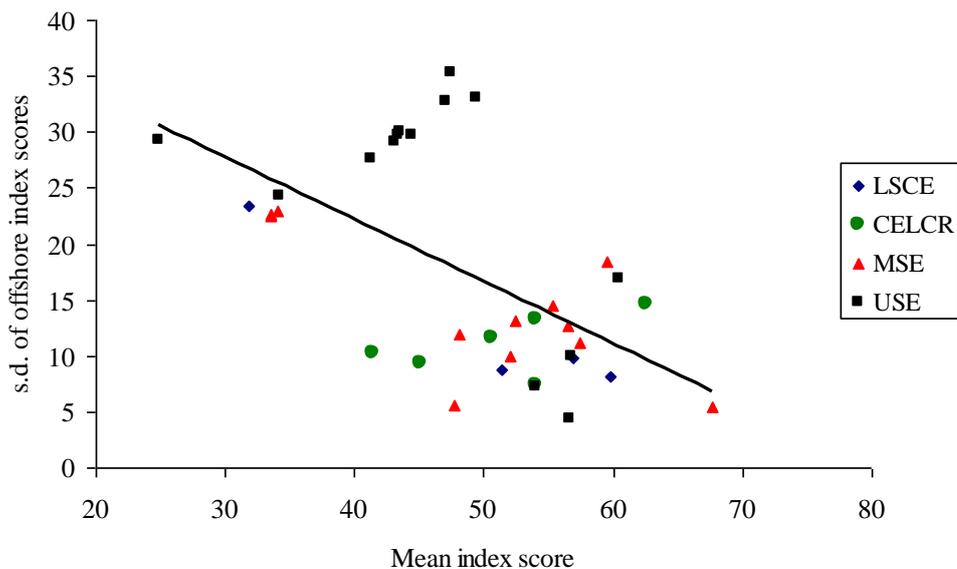
Figure 2.3.2.17: Mean *vs* standard deviation (s.d.) of offshore health index scores among seasons at each of the sites assessed in 2007-2009. Sites are colour-coded for zone of the estuary (see Fig. 2.2.1.1 for zone codes). Solid lines are simple linear regressions.

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Index variability between consecutive periods

Nearshore index scores recorded at each site in each season of 2007/08 were significantly, yet weakly, positively correlated with those for the corresponding samples in 2008/09 (ρ_s=0.211, P=0.027). The small extent of this correlation suggests that there were often considerable differences in index scores between the two periods. Nonetheless, this inter-period variability had a relatively minor impact on the consistency of health status classifications, as most nearshore sites were assessed as good/fair in both periods (upper right quadrant of Fig. 2.3.2.18). The health status of several sites improved from poor in 2007/08 to fair in 2008/09 (upper left quadrant), and only a small number of sites changed from good or fair in the first of these periods to poor in the second period (lower right quadrant). Zero catches were obtained on only four occasions, all during 2008/09.
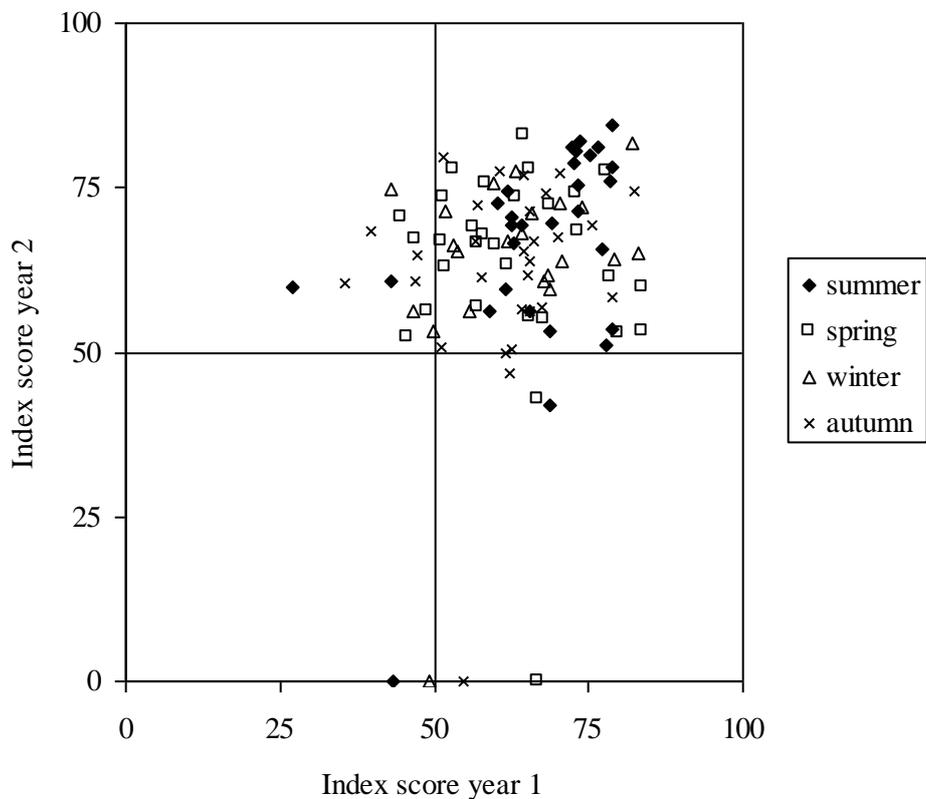
Figure 2.3.2.18: Comparison of nearshore health index scores at each site in year 1 (2007/08) *vs* year 2 (2008/09) of the current study. Line at index score of 50 indicates the threshold between fair and poor health status.

Index scores from offshore sites also exhibited a weak, positive correlation between the two consecutive periods, although this was not significant (ρ_s=0.224, P=0.059). Inter-period variability in offshore index scores had a greater impact on the consistency of health status classifications than in the case of the nearshore index. Thus, while the majority of sites were assessed as either good or fair in both periods or, alternatively, poor or very poor in both periods, the health status of a number of sites changed markedly between 2007/08 and 2008/09 (upper left and lower right quadrants of Fig. 2.3.2.19). This was due, in part, to zero catches being more common among offshore samples.
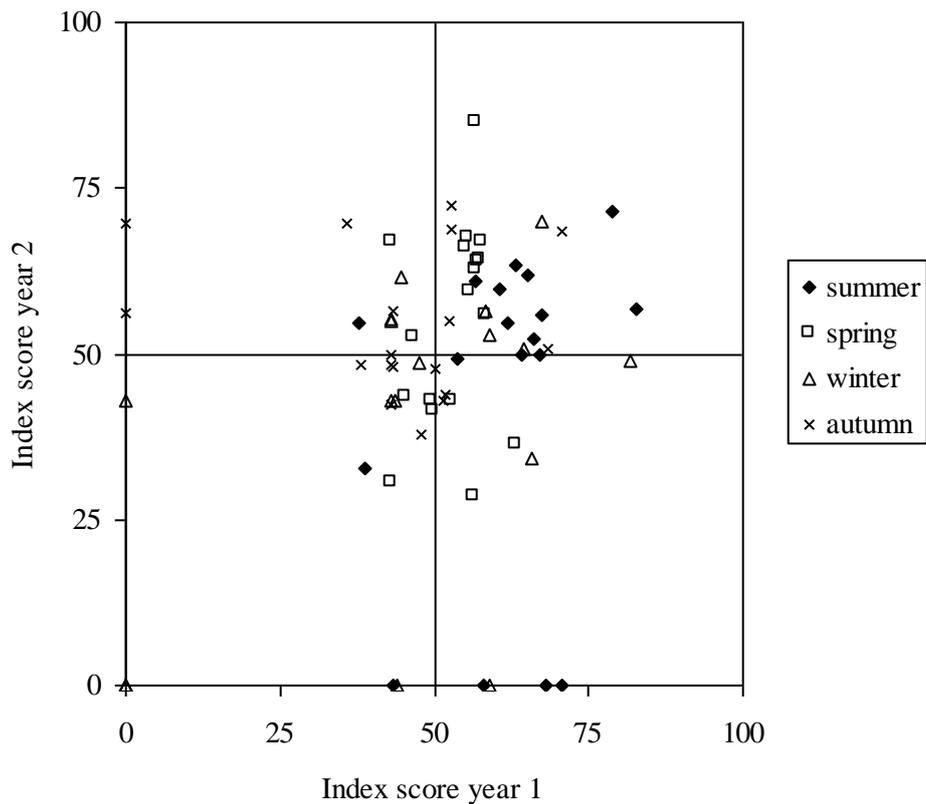
Figure 2.3.2.19: Comparison of offshore health index scores at each site in year 1 (2007/08) *vs* year 2 (2008/09) of the current study. Line at index score of 50 indicates the threshold between fair and poor health status.

Random sampling variability

The length of the 95% confidence intervals (CI) around bootstrapped mean nearshore index scores ranged from zero to approximately 27 points, with a mean of seven points. Although the evidence was not strong, there was a suggestion that CI length decreased with increases in the total number of fish (Fig. 2.3.2.20a) and increased with species richness in the original sample (Fig. 2.3.2.20b).

The bias of original nearshore index scores ranged from one point (underestimation) to approximately -7 points (overestimation), with a mean negative bias of one to two points. Original index scores thus consistently overestimated estuarine health, most notably among higher quality sites (Fig. 2.3.2.21). However, for only 16 out of 233 site visits (approximately 7%) did the difference between the mean bootstrap score and the original index score represent a change in health status classification. In most of these cases, the original index score indicated a higher health status than did the bootstrap score (fair *vs* poor or good *vs* fair, respectively).
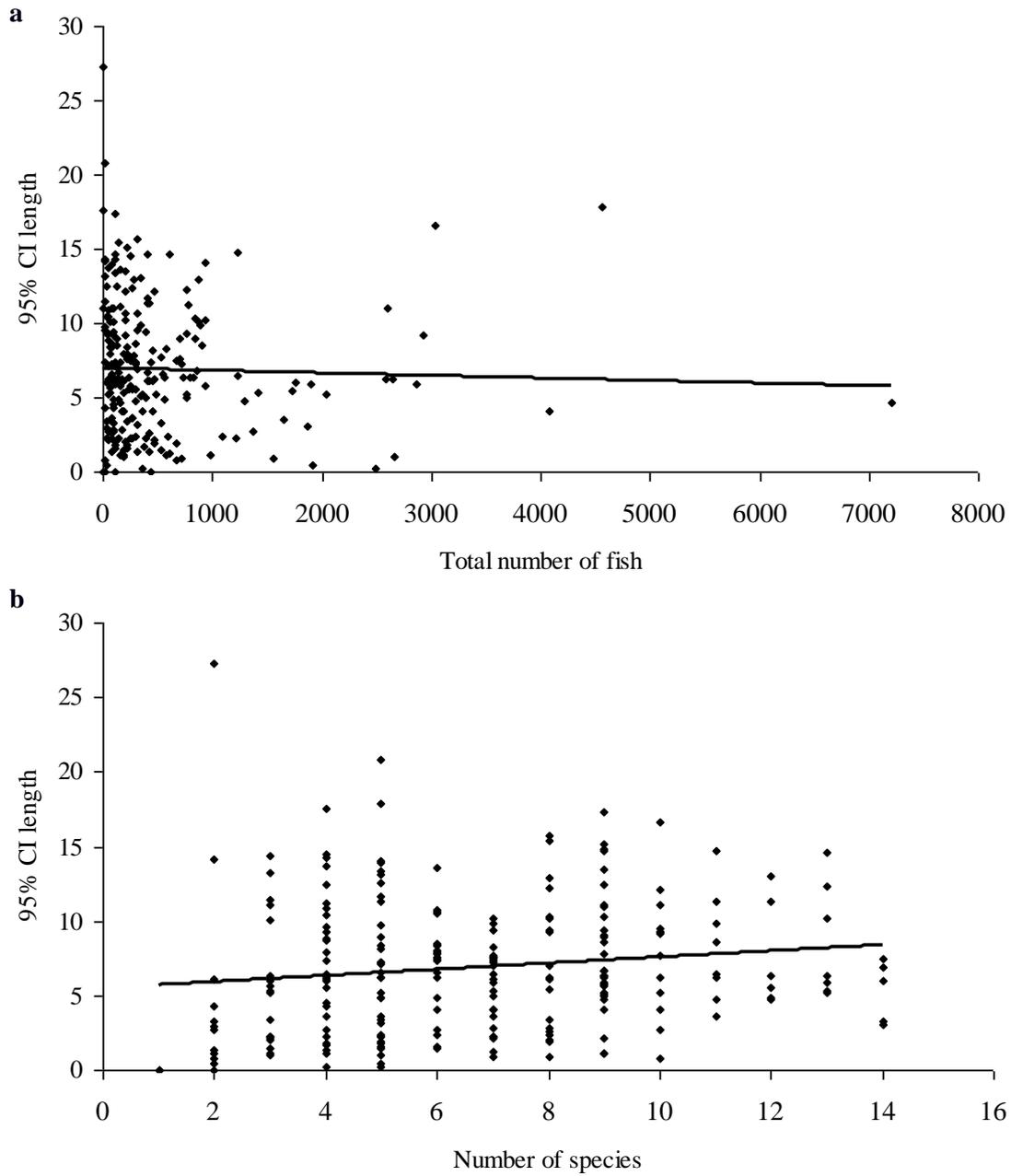
Figure 2.3.2.20: Confidence interval (CI) length for the nearshore health index as a function of (a) total number of fish and (b) total number of species in the original sample. Solid lines are simple linear regressions.
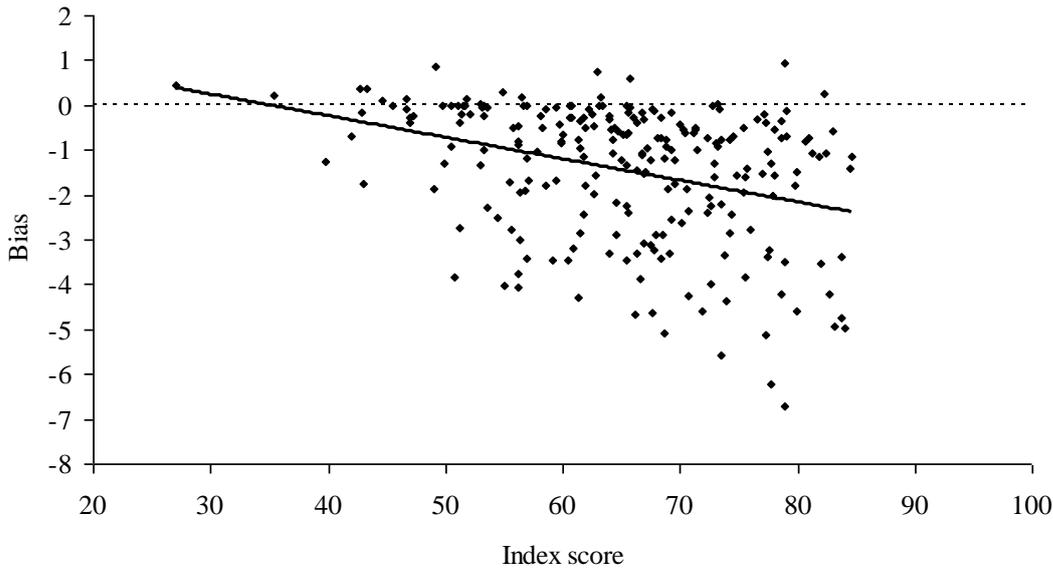
Figure 2.3.2.21: Bias (mean bootstrap index score minus original index score) of the nearshore index scores from site visits throughout the Swan-Canning Estuary in 2007-09. Dashed line represents zero bias expected if bootstrap index scores matched original index scores. Solid line is the simple linear regression of bias as a function of original index score.

In the case of the offshore index, the length of the 95% CIs around bootstrapped mean index scores ranged from zero to 40 points, with a mean of approximately 14 points. Again, CI length was not strongly related to the total number of fish in the original sample (Fig. 2.3.2.22a), but it clearly increased with increasing species richness (Fig. 2.3.2.22b). The latter finding thus demonstrates that offshore samples containing greater numbers of species are likely to exhibit greater differences in index scores due to random sampling variability.

The bias of original offshore index scores ranged from a 12 point underestimation to an overestimation of approximately -30 points, with a mean bias of *ca* -4 points. Original index scores of <45 thus represented probable underestimates of estuarine health, but those at the higher end of the index scale tended to overestimate health (Fig. 2.3.2.23). The difference between the mean bootstrap score and the original index score represented a change in health status classification for 31 out of 119 site visits (*i.e.* 26%), of which two-thirds were overestimates, *i.e.* the original index score indicated a higher health status than did the bootstrap score.
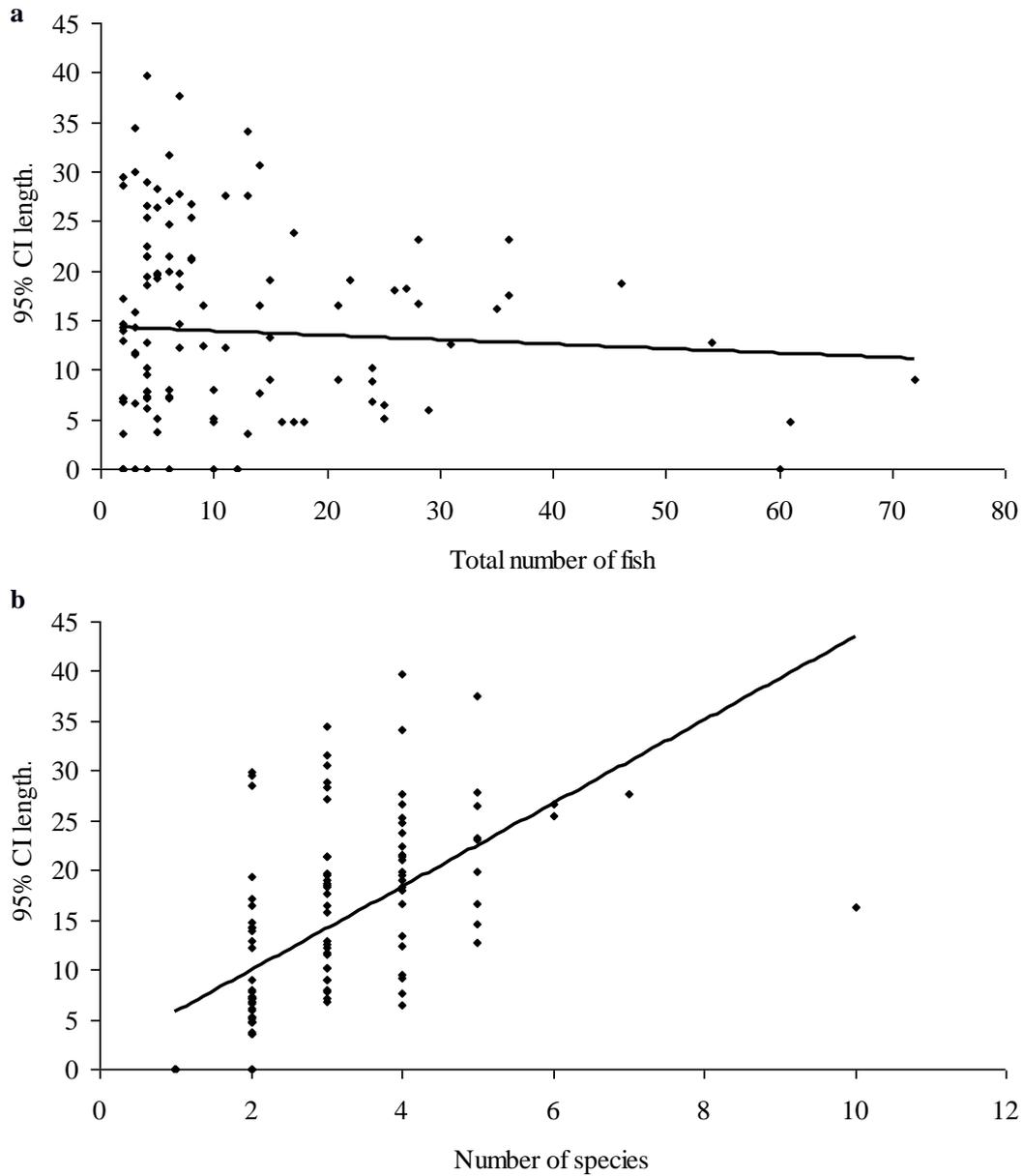
Figure 2.3.2.22: Confidence interval (CI) length for the offshore health index as a function of (a) total number of fish and (b) total number of species in the original sample. Solid lines are simple linear regressions.
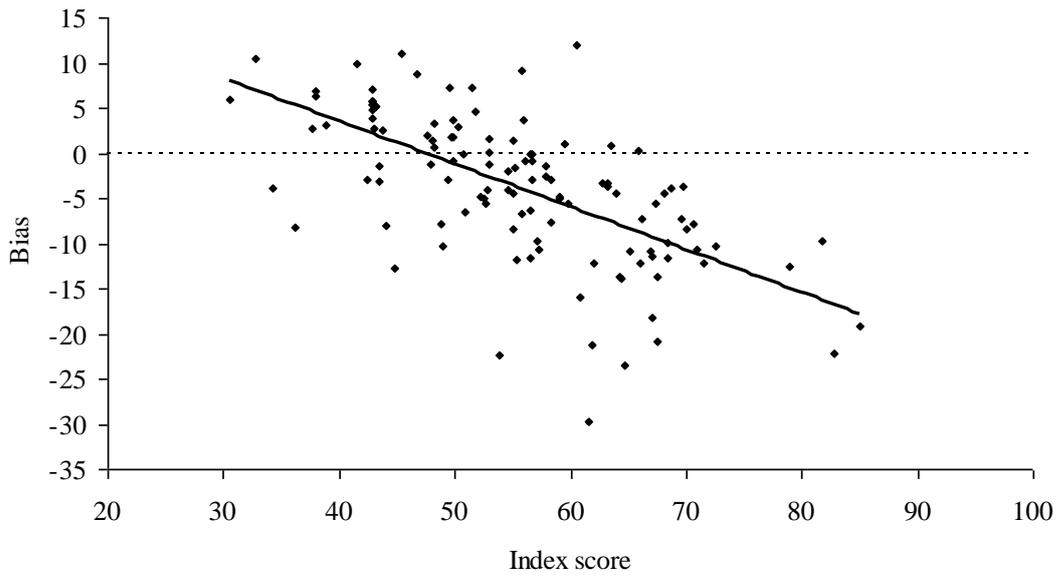
Figure 2.3.2.23: Bias (mean bootstrap index score minus original index score) of the offshore index scores from site visits throughout the Swan-Canning Estuary in 2007-09. Dashed line represents zero bias expected if bootstrap index scores matched original index scores. Solid line is the simple linear regression of bias as a function of original index score.

2.4 Discussion

2.4.1 Changes in the characteristics of the fish fauna of the Swan-Canning Estuary since the late 1970s

2.4.1.1 Seine net comparison study and data standardisation for seine net type

Numerous studies have attempted to quantify the specific selectivities (efficiencies) of different seine nets for a range of fish species. Such studies have commonly involved either repeated sampling of a known community contained within block nets and/or mark-recapture techniques to quantify the proportion of individuals of each species caught and retained by each net type (Weinstein and Davis 1980, Lyons 1986, Parsley *et al.* 1989, Pierce *et al.* 1990, Allen *et al.* 1992, Bayley and Herendeen 2000, Steele *et al.* 2006). In contrast, the method implemented in the current study sought not to determine the absolute bias of each main seine net type for each species, but rather the relative biases of each of the larger nets (*i.e.* the 133 and 41.5 m nets) compared to the small net (21.5 m) for each fish guild. Maki *et al.* (2006) independently developed a similar approach for adjusting gill net catch data to account for the effects of differences in fishing gear characteristics, and concluded that historical reference points derived from unadjusted *vs* adjusted catch data differed substantially. Such findings highlight the importance of adopting appropriate standardisation methods prior to comparative analyses of fish abundance or catch-rate data collected using different net types.

The equivalence factors derived for each habitat guild in the current study (*i.e.* small pelagic, small benthic, benthopelagic, pelagic and demersal fish) are interpretable in terms of the relative abilities of each of the three main net types to capture and retain fish belonging to those guilds, accounting for differences in the areas swept by each net. For example, despite the far greater area encircled by the 41.5 and particularly the 133 m net, the equivalence factors for standardising counts of small pelagic fish obtained with these two net types reflect the fact that many representatives of this guild (*e.g.* those belonging to the Atherinidae) were able to pass through the larger meshes of those nets, yet were retained by the smaller mesh of the 21.5 m net. Similarly, the equivalence factors for small benthic fish reflect a greater tendency for the lead line of the 21.5 m net to maintain contact with the substrate compared to those of the larger nets.

Although the design of the net comparison study aimed to incorporate the spatial and temporal variability of fish communities as much as possible, the standard errors of the parameter estimates for the effects of net type were occasionally large, leading to wide confidence intervals for several of the equivalence factors. However, residual deviances were comparable to the residual degrees of freedom for the models which assumed a negative binomial distribution of the fish counts, thus indicating that this distribution was satisfactory for modelling the data and that the adjusted fish abundance estimates were as robust as possible.

As the data distribution employed in the current standardisation procedure was shown to be appropriate, the wide confidence intervals associated with some of the equivalence factors are more likely to be due to the inherent variability in the efficiency of any given seine net between sampling occasions (*i.e.* different sites and times). Such variability may result from factors such as substrate topography, the presence of snags and submerged vegetation, blocking and rolling of nets due to accumulation of weed and spatio-temporal differences in fish behaviour and distribution (Kjelson and Colby 1977, Weinstein and Davis 1980, Parsley *et al.* 1989, Pierce *et al.* 1990, Allen *et al.* 1992, Rozas and Minello 1997, Macbeth *et al.* 2005, Steele *et al.* 2006). However, the degree to which the reliability of the standardised data is affected by the uncertainty associated with each equivalence factor is an area requiring further investigation in the future.

Lastly, there remains an insurmountable problem associated with the current standardisation procedure, in that while it is possible to model and adjust for the effects of different net types on fish abundances, it is not possible to adjust for those on the numbers and identity of species captured (or not captured). That is, it is impossible to standardise the abundance of a species which was never captured in a given sample. Moreover, there is no way of knowing whether the failure of a given net to capture a particular species was due to the characteristics of the net precluding its capture (*i.e.* "false" zeros, *sensu* Martin *et al.* 2005) or to that species not having been present for capture ("true" zeros). The number and identity of species present in each sample thus remains invariant, irrespective of the net type used to collect it.

Given the above, some degree of caution must be exercised when interpreting changes in the composition of the nearshore fish fauna (subsection 2.4.1.2) and trends in the nearshore index of estuarine health (subsection 2.4.2) among periods in which different net types were used.

2.4.1.2 Differences among periods in the nearshore fish assemblages

The results of this study provide strong indications that the composition of the fish assemblages in the nearshore waters of the Swan-Canning Estuary has changed markedly between 1978/79 and 2008/09, with the largest of those changes typically occurring between 1978/79-1981/82 and all or several of the later sampling periods. Indeed, PERMANOVA demonstrated that the relative influence of differences among periods on nearshore fish composition was about twice that of differences among seasons or zones of the estuary. Moreover, ANOSIM detected very large differences and, in some cases, complete disparity (*i.e.* R=1), between the fish compositions in the late 1970s/early 1980s *vs* those in other periods. However, as noted above in subsection 2.4.1.1, these observed differences must be tempered by the uncertainties associated with the procedure used to standardise fish species abundances among samples collected with divergent net types, and the fact that the late 1970s/early 1980s was the only period in which the large 133 m seine (*i.e.* the most divergent from the 21.5 and 41.5 m seines) was used. Moreover, despite attempts to maximise the comparability of the various nearshore fish data sets wherever possible, the reliability of some inter-period comparisons is also weakened by the fact that the same zones of the

estuary were not sampled during all studies, and that the spatial and temporal intensity of sampling differed among studies.

The pronounced changes in the nearshore fish fauna of the CELCR, MSE and USE zones of the Swan-Canning Estuary between the late 1970s/early 1980s and most or all of the subsequent periods were commonly attributable to the far more consistent and abundant catches of Perth Herring (*Nematalosa vlaminghi*) in the former set of periods. The significant and marked decline in the prevalence of this species is clearly reflected by the fact that it represented nearly 45% of fish collected during 1978/79-1981/82, compared with less than 4% in all other subsequent periods. Other species that have also declined notably in the above zones include Sea Mullet (*Mugil cephalus*) and Yellow-eye Mullet (*Aldrichetta forsteri*). These two species were also more prevalent in the nearshore waters of the LSCE in 1978/79-1981/82 than in most of the following periods in which fish were sampled in that zone.

The marked decline in the abundance of *N. vlaminghi* in the nearshore waters since the late 1970s is paralleled by a pronounced drop in the catch per unit effort (CPUE) of this species by commercial fishers in the Swan-Canning Estuary since the mid 1980s (Smith 2006). Chubb *et al.* (1984) also reported declines in the CPUE of Perth Herring in the estuary between 1977 and 1983, and attributed these findings mainly to the impact of very large catches of this semi-anadromous species by commercial fishers in the local coastal waters from 1976 to 1979, which were considered to be fish that had migrated out of the estuary following spawning in its upper reaches. The CPUE of *N. vlaminghi* in the Swan-Canning Estuary increased in 1984 then underwent a progressive decline, which was particularly marked from the early 1990s (Smith 2006). Although a reduction in the demand for Perth Herring may have influenced these more recent trends in CPUE, our fishery-independent data also strongly indicate that the density of this species is now far lower than in the late 1970s/early 1980s.

Since little has been published about the environmental conditions that *N. vlaminghi* is able to tolerate and/or prefers to inhabit, it is difficult to nominate particular physico-chemical characteristics that may be related to the pronounced reductions in the abundance of this species in the Swan-Canning Estuary. However, since *N. vlaminghi* migrates into the upper reaches of the estuary in late spring to mid-summer to spawn (Chubb and Potter 1984), it is likely that either its reproductive success and/or survival of its eggs or larvae (which remain in those reaches due to low river flow at that time of year) is enhanced by the reduced salinities in that part of the system. Although not reflected by the plot of mean surface salinity shown in subsection 2.3.1.4.1, which was averaged over all seasons and zones in accordance with the results of the PERMANOVA test, examination of the data for this water quality variable in each season and zone showed that values in the USE during summer and autumn from 2000/01 to 2008/09 were higher than those in the late 1970s/early 1980s (data not shown). Such increases in salinity, which also occurred to far greater extents in the bottom waters of the USE and MSE (discussed further in subsections 2.4.1.3 and 2.4.1.4), are consistent with the reductions in rainfall and hence river flow in south-western Australia since at least the mid 1970s, which has been attributed to the effects of climate change

(CSIRO 2009). Moreover, dissolved oxygen concentrations in the surface waters of the USE during summer and, to a lesser extent, autumn, have undergone notable reductions since 2000/01. The above changes in the water quality of the middle to upper estuary, combined with the apparent increase of large phytoplankton blooms and/or fish kills in these reaches at those times of year when *N. vlaminghi* spawns, may all have contributed to the pronounced declines in the abundance of this species in the Swan-Canning Estuary. Commercial fishing pressure, which was particularly high in the 1960s and 1970s (Smith 2006), may have also played a role in the reduction of this species. The proposed influence of such factors is also compounded by the relatively slow growth rate of *N. vlaminghi* (Chubb and Potter 1986), and thus its reduced ability to recover from periods of poor reproduction and/or recruitment, and the fact that the stock of this species in the Swan-Canning Estuary is reliant on self-replenishment (Smith 2006). Lastly, given that *N. vlaminghi* spends part of its life in marine areas, the causes of its decline may also be related to conditions in those waters.

It is possible that the lower catches of *M. cephalus* in more recent than earlier periods are also related, at least in part, to the rise in salinities in the Swan-Canning Estuary since the late 1970s. Several studies have indicated that the juveniles of this mullet species, which recruit into the estuary from marine waters during winter to spring and then move rapidly into the upper estuary, have a marked preference for fresh and oligohaline waters, *i.e.* 0-5‰ (*e.g.* Thomson 1955, Chubb *et al.* 1981, Nordlie *et al.* 1982, Cardona 2000). While other studies have shown that juvenile *M. cephalus* are more prevalent in mesohaline waters, *i.e.* 5.1-18.0‰, (*e.g.* De Silva and Perera 1976, McDonough and Wenner 2003), such findings still suggest that this species exhibits some preference for reduced salinities. Furthermore, Chubb *et al.* (1981) suggested that new recruits of *M. cephalus* in coastal waters respond to stimuli emanating from estuaries, presumably as a cue to enter these systems and commence their migration upstream. It is thus relevant that the mean annual rainfall in south-western Australia has declined by 10-15% since 1975 (CSIRO and Australian Bureau of Meteorology 2008) and that, in the Swan Coastal Basin, the fraction of rainfall that becomes runoff has declined by 30-40% since the late 1990s (CSIRO 2009). Furthermore, given that *M. cephalus* is targeted by commercial fishers in the Swan-Canning Estuary, the influence of fishing pressure cannot be ruled out. The commercial CPUE of this species has declined steadily since the mid 1970s, which may reflect a decrease in market demand and/or its abundance. However, given that the population of *M. cephalus* in the Swan-Canning Estuary is part of a stock that is widespread throughout south-western Australia, the impact of commercial harvesting on this species in the estuary is likely to be less than that for the comparatively localised stock of *N. vlaminghi* (Smith 2006). Lastly, as *M. cephalus* spawns at sea, particular local marine conditions may have also contributed to the decline of this species in the Swan-Canning Estuary.

In contrast to the above species, several others have exhibited considerable increases in abundance and consistency of occurrence in the nearshore waters of the Swan-Canning Estuary since the late 1970s/early 1980s. In the CELCR, MSE and USE, these commonly included the Southern Black Bream (*Acanthopagrus butcheri*), which has become more prevalent particularly since 2003/04. Such findings parallel the overall increase in the

commercial CPUE of this species in the Swan-Canning Estuary since the late 1980s (Smith 2006). This extremely hardy estuarine species is able to tolerate a wide range of environmental conditions. Thus, although it exhibits signs of osmotic stress at *ca* 60‰, it can tolerate salinities between *ca* 0 and 75‰ (Partridge and Jenkins 2002, Hoeksema *et al.* 2006) and has been shown to spawn in waters that range in salinity from 3.5 to 45‰ and temperatures from 17.5 to 28.5°C (Sarre and Potter 1999, 2000). Moreover, under normoxic conditions, its larvae hatch at similar rates under a wide range of salinities (15-35‰) and water temperatures (16-20°C; Hassell *et al.* 2008). However, the hatch rates and embryo survival of *A. butcheri* are compromised by hypoxic conditions (Hassell *et al.* 2008). This larger and fast-swimming species is also a highly opportunistic omnivore, and thus feeds on a wide range of food types (Sarre *et al.* 2000). The greater abundance of *A. butcheri* in the nearshore waters in more recent than earlier periods may thus be attributable to its capacity to reproduce, survive and flourish in a wide range of environmental conditions.

In the nearshore waters of the LSCE, the greater prevalence of the Banded Toadfish (*Torquigener pleurogramma)* in each of the periods between 2003/04 and 2008/09 than in 1978/79 and 1979/80 may at least partly reflect the preference of this marine-estuarine opportunist for higher salinities. Although salinities in the LSCE did not show any obvious increasing trends from earlier to later periods on the plot provided in subsection 2.3.1.4.1, examination of the inter-period trends in this water quality variable during each season in this zone showed that, particularly during winter, values in some of the more recent periods were considerably higher than those in earlier periods. These findings, together with the documented reductions in rainfall, runoff and later onset of winter rains associated with climate change (CSIRO 2009), suggest that the conditions in the LSCE are more saline for longer periods during the year, and thus are more suitable for marine species such as *T. pleurogramma*. However, the abundances of this species are also known to exhibit large inter-annual variability (Potter *et al.*1988), which was reflected by the fact that its numbers in 1980/81 and 1981/82 were substantially higher than in 1978/79 and 1979/80 and in each of the periods sampled since 2003/04. Given that this species spawns in the nearby coastal waters in summer and that its juveniles do not start entering the estuary until winter (Potter *et al.* 1988), the reasons for such inter-annual variability in its abundance may also be related to conditions in the local marine waters.

The far higher total number of species in the nearshore waters in the late 1970s/early 1980s was due mainly to the capture of marine species, which were generally recorded in low numbers. Such findings at least partly reflect artefacts of the sampling regime adopted by Lonergan *et al.* (1989), namely that it (i) like some of the other studies, comprised sites in the LSCE where these species mainly occur, (ii) was undertaken more regularly than those in other studies, *i.e.* two weekly to bimonthly *vs* seasonally and (iii) was the only one that used the large 133 m seine net, which would have positively biased the total number of species caught. As recognised in subsection 2.4.1.1, the latter gear-induced biases could not be corrected with the data standardisation method employed in this study, nor by any other such technique. As a consequence, differences in the mean number of species could not be reliably tested among periods in the nearshore waters. These limitations were able to be overcome for

species diversity, however, by employing average quantitative taxonomic distinctness as the measure. Thus, this diversity index, which accounts for the degree of taxonomic separation of any two individuals from different species throughout the hierarchical levels of the Linnaean tree, is not affected by sampling effort (Clarke and Warwick 1998). The most marked inter-period differences in this diversity index were found in the CELCR, in which values in most periods since 1995/96 were considerably lower than those in the late 1970s/early 1980s. Such findings were due mainly to the faunas in more recent periods being largely dominated by atherinid species from the same genus and/or family (*i.e. Lepthatherina wallacei, L. presbyteroides, Atherinosoma mugiloides* and *A. elongata*), whereas those in the earlier periods comprised species from diverse orders and/or families that were almost never recorded in later periods, *e.g. Galaxias occidentalis* (Osmeriformes, Galaxiidae), *Cnidoglanis macrocephalus* (Siluriformes, Plotsidae), *Urocampus carinirostris* (Syngnathiformes, Syngnathidae), *Pomatomus saltatrix* (Perciformes, Pomatomidae) and *Trachurus novaezelandiae* (Perciformes, Carangidae), or were recorded in far lower numbers, *e.g. N. vlaminghi, M. cephalus* and *Pseudogobius olorum.*

Lastly, in some zones and seasons, the composition of the nearshore fish assemblages was more variable among replicate samples in the most recent than earlier periods, as indicated by their greater degree of dispersion on the MDS plots shown in subsection 2.3.1.2.3. Such results are typically more reflective of stressed faunal assemblages (*e.g.* Wildsmith *et al.* 2009), and thus are potentially indicative of localised declines in the ecosystem health of the Swan-Canning Estuary.

2.4.1.3 Differences among periods in the offshore fish assemblages

The total and mean catch-rate of fish in the deeper offshore waters of the MSE and USE zones of the Swan-Canning Estuary have undergone a significant and pronounced decline since the early to mid-1990s. By far the most pronounced inter-period shifts in mean catch-rate occurred in the USE in summer, with values declining progressively from *ca* 38 fish h^{-1} in 1993/94 to *ca* 8 fish h^{-1} in 2008/09. Moreover, while the total number of species has remained relatively similar among periods, the mean number of species in both zones has fallen from the earlier to later periods, particularly during autumn, *i.e. ca* 7 to 2 species in 1993/94 and 2007/08, respectively. It is pertinent that the most pronounced reductions in mean catch-rate and number of species have occurred at those times of year in which water quality conditions in the bottom and/or surface waters of the upper estuary are typically the least favourable, *e.g.* reduced dissolved oxygen levels and greatest tendency for phytoplankton blooms to occur. Inter-period trends in species diversity, as reflected by the quantitative average taxonomic distinctness index, were similar to those of mean catch-rate and number of species, but exhibited the most pronounced decline between 2003/04 and 2007-09.

The reductions in total and mean catch-rate from 1993/94 to 2007-09, and also the notable inter-period differences in offshore fish composition, were due largely to the significantly lower catches of *N. vlaminghi* and *A. butcheri* in the later than earlier periods. The inter-

period trends in the first of these species parallel those found in the nearshore waters (see subsection 2.4.1.2), but those in the second species oppose those recorded in the nearshore waters. There may be several reasons for this latter result. Firstly, it is possible that, given the notably lower levels of dissolved oxygen in the bottom than surface waters in the MSE and USE, individuals of this species are spending more time on the shallower banks than in deeper waters. Such onshore movements may also reflect a greater availability of their preferred food source, benthic macroinvertebrates, in the shallows, due possibly to the negative effects on those invertebrate fauna of reduced oxygen levels in the deeper waters. Changes in the composition of the benthic macroinvertebrate fauna in the shallow and deeper waters of the MSE and USE between the mid-1990s and the present is the subject of a current PhD project being undertaken in the Centre for Fish and Fisheries Research at Murdoch University (A. Buckland), and the findings of this work will be crucial for interpreting changes such as those described above for *A. butcheri*. Lastly, it is also possible that the decline in the prevalence of *A. butcheri* in the deeper waters reflects differences in the intra-seasonal timing of sampling by Sarre (unpubl.) in 1993/94 *vs* that in each of the subsequent studies. Thus, the former worker targeted particular moon phases on some sampling occasions to improve catchability of this fish species, whereas that was not the case in each of the other studies. However, while the greatest catch-rates of *A. butcheri* were recorded in 1993/94 (3.28 fish h^{-1}), the values recorded between 1995/96 and 2003/04 by Kanandjembo *et al.* (2001a) and Valesini *et al.* (2005), *i.e.* 2.13-3 fish h^{-1}, were still far greater than that in 2007-09 (*i.e. ca* 0.5 fish h^{-1}). Such results thus indicate that the reductions in the abundance of *A. butcheri* in the offshore waters from the earlier to later periods are not simply the result of this difference in sampling technique.

The reduction in the species diversity of the offshore fish fauna since the early 1990s, and probably also that of the mean number of species, was due mainly to the lower rate of capture, or lack of capture, of species such as Mulloway (*Argyrosomus japonicas*), Estuarine Cobbler (*C. macrocephalus*), Freshwater Cobbler (*Tandanus bostocki*) and Western Striped Grunter (*P. octolineatus*), several of which comprise families and/or orders that were not represented by other species in the offshore fish assemblage. The decline in the prevalence of these species may be attributable to reductions in habitat quality (*e.g.* nesting burrows in the case of *C. macrocephalus* and increased salinities in the case of the freshwater species *T. bostocki*) and/or, for the first two species, commercial and/or recreational fishing pressure. Moreover, given that both *A. japonicas* and *P. octolineatus* spend part of their life cycle in marine waters, the possibility of the influence of environmental conditions and/or fishing pressure in those waters cannot be excluded.

In contrast to the above, the catch-rate of Yellowtail Grunter (*Amniataba caudavittata*) in the offshore waters of the MSE and USE increased from the early 1990s to the late 2000s. Given that this estuarine species is known to prefer more saline waters (Wise *et al.* 1994), and that its gonadal development is thought to be stimulated by rising salinities during spring (Potter *et al.* 1994), such findings may be at least partly related to the marked increase in the salinities of the above zones, particularly in the bottom waters.

2.4.1.4 Relationships between fish faunal composition and water quality in nearshore and offshore waters

For both the nearshore and offshore waters, the relationships between the inter-period differences in fish faunal composition and those exhibited by each combination of the various water quality variables were examined to determine whether they were significantly correlated and, if so, which variables provided the best match. These water quality variables were thus regarded as those that best "explained" the observed differences among periods in fish faunal composition. However, it should be noted that these analyses were compromised by the fact that not all of the water quality variables were recorded consistently (i) across the range of fish sampling periods and (ii) in both the surface and bottom waters.

The above inconsistencies in water quality records had three main consequences. Firstly, the greatest inter-period differences in fish composition were typically recorded for the late 1970s/early 1980s *vs* subsequent periods in the nearshore waters, and for 1993/94 *vs* 2007-09 in the offshore waters (see subsections 2.4.1.2 and 2.4.1.3). However, as the current Swan-Canning Estuary water quality monitoring program undertaken by the Department of Water (DoW) did not commence until 1994, the correlations between the fish data and the full suite of relevant water quality variables could only be carried out for those fish sampling periods between 1995/96 and 2008/09. Such restrictions thus meant that the strongest inter-period trends in fish composition could not be assessed with respect to their potential relationships with water quality. The only water quality variables for which data was available across all fish sampling periods were salinity and water temperature, which were recorded by all researchers during fish sampling. However, the combination of that research data collected prior to 1994 with the measurements collected by DoW in subsequent periods potentially introduced further problems regarding the consistency of water quality recording. Thus, it is possible that the presented trends in salinity and temperature across the full range of nearshore and offshore fish sampling periods, and hence the results of the BIOENV tests, are skewed by the above differences in water quality measurement. The third main issue was that some of the relevant water quality variables recorded by DoW were not measured consistently for all sites, seasons and periods, and thus had to be excluded from analysis. These included turbidity and chlorophyll *a* concentration in either or both of the surface and bottom waters. Given the potentially large impacts of both of these variables on fish distribution, the loss of such data was a considerable impediment.

Significant correlations between the inter-period differences in fish composition and those of particular combinations of water quality variables, which ranged from moderate to low in their extent, were detected in most zones and seasons in both the nearshore and offshore waters when the data recorded between 1995/96 and 2008/09 was employed. However, significant results were detected in very few cases for those tests that employed the fish data recorded in all sampling periods and only the complementary salinity and water temperature data. The latter findings may be indicative of the very restricted suite of water quality variables that were available for use in those tests. In the former tests, however, while there was considerable variability in the particular water quality variables selected in each case, there were also a number of common patterns. Thus, distinctive or gradational shifts in the

composition of the nearshore or offshore fish fauna from earlier to later periods were often correlated with one or more of the following trends in surface and/or bottom water quality.

(i) Decreasing concentrations of total P and, to a lesser extent, of total N.

(ii) Decreasing concentrations of dissolved oxygen.

(iii) Increasing salinity.

(iv) Increasing water temperature.

Each of the trends in the above water quality variables are congruent with the recognised effects of climate change in south-western Australia, namely reduced rainfall and warmer temperatures (CSIRO 2009). It is also recognised that a range of other factors may have contributed to these trends, such as improvements in the effects of management actions to reduce nutrient loads entering the Swan-Canning Estuary (Swan River Trust 2009). Irrespectively, the results of this study demonstrate that the above environmental changes are significantly correlated, either directly or indirectly, with the observed changes in the composition of the fish faunas in this system over at least the last 15 years.

2.4.2 Development of a biotic index of estuarine health

2.4.2.1 Metric selection

2.4.2.1.1 Selection of metrics sensitive to spatial changes in habitat quality

This approach failed to confirm hypothesised responses of fish metrics to physical habitat degradation, and was thus unsuccessful in selecting a metric subset that was sensitive to spatial changes in habitat quality throughout the Swan-Canning Estuary. Several explanations may be offered to account for these findings. Firstly, it is possible that either (i) the current habitat quality assessment scheme failed to adequately measure those aspects of the physical habitat that are important in structuring fish communities or (ii) the candidate fish metrics considered do not in fact respond to differences in physical habitat degradation within the Swan-Canning Estuary. However, the habitat quality assessment scheme developed in this study incorporates measures of physical habitat quality with well-documented roles in structuring riverine and lacustrine fish communities worldwide (see subsection 2.2.2.1.3) and employed established and widely-used visual survey methods. Moreover, a large number of independent estuarine studies have successfully identified changes in the fish metrics employed in the current scheme in response to habitat degradation (*e.g.* Deegan *et al.* 1997, Hughes *et al.* 2002, Bilkovic *et al.* 2005, Harrison and Whitfield 2006).

Alternatively, the failure of the current study to identify fish metrics sensitive to spatial differences in habitat quality might have been due to the confounding effects on fish community structure of factors other than localised habitat quality. This is suggested by the fact that whilst PERMANOVA identified no significant differences in fish metric, or indeed, fish community composition among habitat quality categories (HQCs), significant differences in both of these attributes were detected among regions of the estuary. The results of the canonical correlation analyses also demonstrate that water quality gradients, and particularly

those of salinity and water temperature, have a marked influence on fish community composition throughout the Swan-Canning Estuary, and thus might be expected to obscure possible responses of fish community metrics to habitat quality. Other authors, including Bilkovic and Roggero (2008), have similarly highlighted the confounding effects of salinity gradients on fish community responses to environmental degradation. However, whilst regionality was identified in the current study as having a pronounced influence on fish community structure over the whole of the Swan-Canning Estuary, significant differences in fish metric and community composition between HQCs were not even observed within individual regions of the estuary. Thus, it appears that fish metric and community composition did not respond to local differences in physical habitat quality within the Swan-Canning Estuary, even among groups of sites with similar water quality conditions.

A third possible explanation for the observed lack of metric responses is that habitat quality acts to structure fish communities at a different scale to that which has been assessed in the current study. Several authors have demonstrated that in some cases, fish community metrics may not respond clearly to habitat quality at local spatial scales, but better reflect the complex suite of stressors acting over larger areas (Bilkovic and Roggero 2008, Brooks *et al.* 2009, Infante *et al.* 2009, Yates and Bailey 2010). Further work is thus needed to determine the scales at which habitat quality, catchment land use and water quality characteristics act to influence the structure and function of fish communities, both within and among estuaries across south-western Australia. Nonetheless, it is pertinent to note that the habitat assessment scheme developed here has potential utility as an independent tool for assessing any future habitat degradation in the Swan-Canning Estuary. Moreover, it might also be useful for testing and validating other prospective biotic indices, particularly those based on taxa such as benthic macroinvertebrates which may respond more sensitively than fish to local scale determinants (Plafkin *et al.* 1989, Yates and Bailey 2010).

2.4.2.1.2 Selection of metrics sensitive to temporal changes
This alternative approach aimed to select that subset of fish metrics that most consistently exhibited inter-period changes at the ecosystem level, and thus were the most sensitive to temporal changes in ecosystem condition. Managers of the Swan-Canning Estuary are currently faced with considerable uncertainty over the broad health status of the system due to the lack of reliable tools for quantifying and monitoring such trends. Unlike many estuarine systems throughout Europe, the United States and South Africa, there currently exists no independent and easily interpreted measure of how the ecological condition of the Swan-Canning Estuary, or any estuary in south-western Australia, has changed over time, and against which the sensitivity of candidate fish metrics for a biotic index of ecosystem health might be assessed. Existing indicators for this system focus on various aspects of water quality, including salinity, temperature, total suspended solids, the concentrations of chlorophyll *a* and several key nutrients and counts of various phytoplankton groups. However, they provide little or no information on the ecological status of the estuarine biota, and exhibit trends which are often inconsistent, contrary and difficult to interpret (*e.g.* see Henderson and Kuhnert 2006, Kuhnert and Henderson 2006). At present, it is thus difficult to quantify how the ecological status of the Swan-Canning Estuary, and the magnitude of the

various stressors impacting on it, has changed over recent decades. The second approach to metric selection adopted in this study therefore rested on the overarching and highly simplified assumption that the ecological condition of the Swan-Canning Estuary has varied over time (*i.e.* non-directional change, in which each period studied is equally different from every other period) in response to changes in the suite of stressors acting upon the system (see subsection 2.2.2.1.4).

In line with the recommendations of Roset *et al.* (2007), a large number of candidate metrics, encompassing a wide range of fish community attributes, were proposed in the current study as potential components of a multimetric health index. Furthermore, the prior exclusion of erratically variable and/or highly correlated metrics increased the reliability and reduced redundancy, respectively, within the resultant candidate metric set (Hering *et al.* 2006). Finally, selection from among the remaining candidate metrics was carried out via rigorous statistical testing of metric sensitivity to inter-period changes in the condition of the Swan-Canning Estuary. The novel statistical approach adopted here, which employed a combination of multivariate analyses (DISTLM and BIOENV/BVSTEP) and information-theoretic multi-model inference (MMI) techniques, allowed metrics to be selected objectively according to the weight of evidence from multiple analyses of numerous data sets, each of which was collected over differing time frames using divergent sampling techniques.

Despite prior elimination of highly correlated metrics to reduce redundancy among the candidate metric set, the results of the distance-based linear modelling analyses highlighted considerable redundancy among the remaining candidate metrics, and indicated substantial uncertainty regarding the particular metric subset that best responded to inter-period differences. Moreover, the consistently low r^2 and ρ_s values from the DISTLM and BIOENV/BVSTEP analyses, respectively, revealed that no single combination of metrics (model) explained a large proportion of the inter-period patterns in the reference resemblance matrix. Thus, for each of the nearshore and offshore fish data sets, acceptance of a single 'best' model was deemed inappropriate and weight of evidence-based MMI techniques were applied. Although the selection of variables via exhaustive testing of all possible models has been labeled as 'data dredging' and warned against (*e.g.* see Burnham and Anderson 2002), the aim in the present case was not to determine significant explanatory variables and thus fit parameters to model causative relationships, but rather to identify the most useful signals from which to construct a working model of an estuarine health index, and which will subsequently be validated using larger data sets. The weight of evidence approach adopted in this study, *i.e.* selection of those metrics which appeared most consistently among the likely 'best' sets of models from multiple analyses, thus accounts for model uncertainty and is compatible with the ideological demands of constructing a multimetric index that integrates information from a range of fish community attributes.

Multimetric biotic indices derived using an objective, statistical approach to metric selection are widely regarded as being more robust than those in which metric selection is based on expert judgment alone (Hering *et al.* 2006, Roset *et al.* 2007). The multifaceted statistical approach employed in the current study has succeeded in objectively selecting that

combination of fish metrics which is best able to reflect inter-period changes in the environmental condition of the Swan-Canning Estuary. Moreover, by employing both linear modelling and non-parametric techniques, this approach has minimised bias attributable to any given statistical method.

A potential weakness of the current approach is that it has not been possible to demonstrate *a priori* the sensitivity of metrics to human disturbance, *i.e.* to establish empirical relationships between fish metrics and human impacts on environmental quality. This is attributable largely to a lack of independent data on human pressures at appropriate scales, and also to the focus of the current study being restricted to a single estuary. Consequently, *a posteriori* tests of sensitivity, redundancy and consistency were essential to demonstrate the ecological relevance and robustness of the selected metrics and resultant indices.

2.4.2.2 Reference conditions and scoring

2.4.2.2.1 Biases affecting reference data sets

Previous and current studies of the abundances of fish species from offshore waters of the Swan-Canning Estuary have employed relatively consistent sampling methods and effort, such that the collective data sets were largely free from sampling bias and thus required little standardisation prior to establishing reference conditions. A multimetric index based on the reference conditions and associated scores derived from these data may thus be interpreted without concerns over the influence of methodological biases on its reliability.

In contrast, different seine nets were employed to sample nearshore fish communities in each of the historical and current studies comprising the collective nearshore data set. The advantages of the shortest (21.5 m) of these nets over its larger counterparts (41.5, 102.5 and 133 m), including the greater speed and ease of deployment and the greater number of habitats in which it can be used, suggest that it should become the standard method for future monitoring of the nearshore fish community in the Swan-Canning Estuary. Thus, the current study sought to derive equivalence factors for standardising fish counts in samples collected with one of the larger nets to those expected per 21.5 m net, so as to obtain a comparable set of fish community data spanning three decades. The findings of that net comparability experiment and the subsequent data standardisation process are discussed above in subsection 2.4.1.1. However, despite that standardisaton process, some elements of the effects of gear type could not be accounted for, and the nearshore reference conditions and multimetric index derived from these data thus remain affected to some degree (subsections 2.4.1.1 and 2.4.2.3.2).

2.4.2.2.2 Reference conditions and scoring thresholds

For each of the selected nearshore and offshore fish metrics, appropriate reference conditions were defined statistically for each zone*season combination to eliminate the potential for spatial and temporal biases to impact their reliability (Karr 1999, Kennard *et al.* 2006, Coates *et al.* 2007). Whereas several authors have reported that fish-based multimetric indices

developed for riverine systems are not unduly affected by within-year variability in fish community composition (Karr *et al.* 1986, Pyron *et al.* 2008, Qadir and Malik 2009), the effects on fish faunas of highly seasonal freshwater flows and strong physico-chemical gradients in estuaries potentially impacts the reliability of such indicators for these ecosystems (Lobry *et al.* 2006, Chainho *et al.* 2007, Pérez-Ruzafa *et al.* 2007, Bilkovic and Roggero 2008, Rashleigh *et al.* 2009). Thus, as in the present case, natural spatio-temporal variability of biotic assemblages should be accounted for when establishing reference conditions (Chainho *et al.* 2007, Coates *et al.* 2007, Roset *et al.* 2007, Mazor *et al.* 2009).

It is widely recommended that appropriate reference conditions for ecological integrity metrics be established from a population of minimally-impaired reference sites that are (i) located across multiple systems subject to differing levels of human stress and (ii) identified using independent measures of environmental quality (Hughes 1995, Gibson *et al.* 2000, USEPA 2006). However, the present study has attempted to develop a multimetric index for a single estuarine system, without access to an established, independent means of identifying minimally-impacted sites or gradients of anthropogenic disturbance. Consequently, zone*season-specific reference conditions were defined for each of the selected nearshore and offshore metrics from the "best" fraction of metric values observed in the Swan-Canning Estuary between the late 1970s and 2009 (Gibson *et al.* 2000, Blocksom 2003). This approach thus enables the future health of the system and the success of its management to be measured in terms of deviation from this "best available" reference state.

Continuous methods for scoring metrics in relation to their degree of deviation from the reference condition, such as that employed in the current study, are considered objective and avoid gaps in possible scores. Unlike discrete or stepped scoring methods, all of the information provided by the metric is thus retained, thereby increasing its sensitivity and precision (Gibson *et al.* 2000, Roset *et al.* 2007). Such conclusions were drawn by Blocksom (2003) in her assessment of the effects of different scoring methods on the performance of a benthic macroinvertebrate index of stream health, and by Dolph *et al.* (2010), who showed that a fish-based IBI calculated using a continuous scoring method was less biased than one which employed discrete scoring.

2.4.2.3 Index calculation and validation

2.4.2.3.1 Index calculation
Calculation of multimetric index scores provides a means of quantifying estuarine health. However, for the purposes of management and reporting, it is also often useful to identify qualitative categories or classes on the basis of these scores, which correspond with varying degrees of ecosystem health. Whereas numerous schemes have sought to establish more or fewer classes, thresholds for establishing qualitative ecosystem condition in the present case were determined by subdividing the possible range of index scores (0-100) into four equal classes, representing very poor to good health status. It was considered that more classes than this would make decisions regarding management actions more problematic (Ganasan and

Hughes 1998, Qadir and Malik 2009), whilst fewer classes might allow the health of an estuary to decline markedly before a health status threshold is crossed and management actions are invoked.

2.4.2.3.2 Preliminary interpretation of index performance
Average health index scores for the nearshore waters of the Swan-Canning Estuary have undergone a moderate degree of variation from 1976 to 2009, although the resulting health status has remained as fair throughout that time. This suggests that the above health classification is likely to be robust to natural variability over longer time scales. More detailed examination of the trends in nearshore index values should, however, be undertaken with caution at this stage, as the lack of methodological consistency between the various nearshore fish community studies presents some problems for index interpretation. As discussed in subsection 2.4.1.1, whilst the equivalence factors derived for standardising data across all historical and current nearshore fish samples appear to provide a satisfactory means of adjusting fish densities to account for differences in net bias, they do not enable the adjustment of species richness. Consequently, those fish metrics based on numbers of species in a sample will remain subject to the bias associated with the net used to obtain the sample. As the 102.5-133 m seine nets were employed exclusively from 1976 to 1982, whereas sampling in subsequent periods employed the more comparable 41.5 and 21.5 m seines, inter-period changes in nearshore index scores can be interpreted with greater confidence among the more contemporary studies, *i.e.* those from the mid-1990s onwards. The comparability of those latter studies is also improved by the greater consistency in the location, timing and intensity of sampling, compared to that of the earliest survey periods.

In contrast, inter-period changes in the health of the offshore waters of the Swan-Canning Estuary may be interpreted more reliably, due to the largely consistent sampling methodology employed among all studies of these waters. The fact that mean offshore index scores have decreased over the last three decades, and that their health status is now classed as poor as opposed to fair in all previous periods, indicates that the ecological health of the deeper waters of the estuary has declined over this time. However, trends in the nearshore index since the mid 2000s indicate the opposite, and it is suggested that this may reflect a movement of the fish community inhabiting deeper waters toward nearshore habitats. This is reinforced by the inter-period trends in mean catch-rate, number of species and species diversity in the offshore waters, and also by those in the prevalence of *A. butcheri* in the nearshore *vs* the offshore waters of the estuary (subsections 2.4.1.2 and 2.4.1.3).

It must be emphasised that the above broad conclusions are based on changes in mean index scores, and that plots of such means mask the complexity of index differences between zones and seasons. They are also potentially biased by differences between studies in the spatio-temporal collection of samples. Before any detailed examination or statistical testing of trends in index scores can proceed, it is thus essential that a thorough evaluation of index performance, sensitivity and variability be performed, and that a consistent sampling regime is implemented to enable genuine trends in ecological health to be reliably distinguished.

The performance of both the nearshore and offshore indices was examined with respect to the relative contributions made to the final index scores by each of the component metrics (Angermeier and Karr 1986). Such analyses may aid in the interpretation of the ecological stressors to which index scores are primarily responding, and allow for the possible refinement of the index via the elimination of metrics which contribute little to index scores. For both the nearshore and offshore waters, the majority of metrics were positively correlated with index scores, with the metric *No trop spec* contributing strongly to each. In contrast, scores for the metric *No trop gen* exhibited a weak negative correlation with those for both indices. Some authors have eliminated those metrics which were found to lack correlation or to be negatively correlated with index scores (Hughes *et al.* 1998, Harris and Silveira 1999). However, it can be reasoned that as multimetric indices aim to integrate the complex effects of ecosystem degradation on different aspects of the structure and function of biotic communities, they should not consist solely of metrics which respond in an identical, linear manner. For example, Karr *et al.* (1987) emphasised that the relative contribution of metrics to IBI scores varied over large spatial scales, and argued against the automatic exclusion of apparently 'non-significant' metrics. With regard to the current indices, their component metrics therefore remained unaltered.

2.4.2.3.3 Index validation

A key requirement in developing any biotic index is that its sensitivity and reliability are validated to ensure that it has sufficient precision to detect changes in ecological health against a background of natural ecosystem variability (Fausch *et al.* 1990, Jackson *et al.* 2000, Pérez-Ruzafa *et al.* 2007). Attempts were therefore made to validate the sensitivity and robustness to natural variability of the nearshore and offshore indices developed during the present study, the outcomes of which varied in their degree of success.

Index sensitivity

Ideally, the ecological sensitivity of an index should be demonstrated via *a posteriori* testing of its response to ecosystem degradation. Most commonly, this is achieved by determining index sensitivity to human pressures and stressors using independently-derived, existing indices (*e.g.* Bilkovic *et al.* 2005, Romero *et al.* 2007). However, such indicators are not currently available for the Swan-Canning Estuary, with the exception of simple physico-chemical measures such as water quality parameters. However, besides exhibiting trends which are often highly variable and difficult to interpret, measurement of these water quality parameters in the Swan-Canning Estuary has been undertaken at spatial and temporal scales which are inconsistent with those at which fish sampling has been performed. Thus, the sites at which water quality is monitored by the DoW (Fig. 2.2.1.3) are often at broader spatial scales to those at which fish have been collected, and regular monitoring of these physico-chemical parameters did not commence until 1994. Such factors reduce the utility of these water quality data for accurately validating index sensitivity.

In an attempt to partly overcome the above restrictions, index sensitivity was evaluated using water quality data collected concurrently with fish sampling during the current study. Neither the nearshore nor offshore index scores from 2007 to 2009 were found to reflect changes in

the concentration of dissolved oxygen, a water quality parameter which is considered to be an important stressor within this system (Douglas *et al.* 1997, Hamilton *et al.* 2001). Such results highlight (i) the difficulties of determining the stressors to which the current indices are responding and the scales over which those stressors act, (ii) constraints imposed by the scope of the current study being limited to a single system and, for the purposes of these sensitivity analyses, to data collected only over two years and (iii) the lack of independent indicators of stressors and pressures measured at comparable spatial and temporal scales. However, it is important to note that although the sensitivity of the indices has not been quantified, the consistent decrease observed in offshore health index scores over the last three decades suggests that this index is capable of detecting the widely-perceived, long-term decline in the condition of the offshore waters of the Swan-Canning Estuary (Swan River Trust 1999, 2003, Valesini *et al.* 2005).

Karr *et al.* (1987) suggested that while testing index sensitivity via the preferred method of experimental manipulation is not feasible, the opportunistic evaluation of index responses to major anthropogenic perturbations, such as channelization or effluent spills (*e.g.* Harrison and Whitfield 2004), represents the best practical alternative to this theoretical ideal. In the present case, it is therefore suggested that the most promising approach for demonstrating the sensitivity of the nearshore and offshore health indices will be to evaluate their responses to one of the large fish kills which periodically affect the Swan-Canning Estuary, using a Before-After-Control-Impact approach. To do so effectively will require sampling of the fish community throughout the estuary over shorter than seasonal timescales, to enable the effect of such a perturbation to be discerned against the natural spatial and temporal variability of index scores.

Index variability

Index variability between sites

Differences in the variability of index scores among replicate sites were identified between both regions and seasons in each year of the current study. Moreover, this index variability was generally greater in the offshore than nearshore waters. Within any given season, nearshore index scores were less spatially variable in the more upstream regions of the Swan-Canning Estuary, which is possibly explained by the reduced habitat heterogeneity of the shallows in those regions compared to those nearer the mouth of the system. However, the opposite was true for offshore index scores, particularly during winter, which largely reflected a greater prevalence of zero catches. It should be noted that the measure of index variability employed was strongly affected by zero catches, as the standard deviations of index scores in each region and season were calculated from only three replicate site visits. If more sites were sampled within each region and/or if sites were sampled more regularly, it would be possible to determine whether such zero catches are more likely to be anomalous (*i.e.* false zeros; Cunningham and Lindenmayer 2005) or reflective of a genuine tendency across the region or season towards low index scores in a given period (true zeros). Further work is thus needed to quantify the effects of sampling intensity within a region and season on the precision of the health indices, and thus to determine the optimum spatio-temporal

level of sampling required for a robust future monitoring regime in the Swan-Canning Estuary.

The between-site variability of the nearshore and offshore indices was most consistently low in summer and autumn, suggesting that the optimum sampling period for applying these indices in the Swan-Canning Estuary is from December to May. However, as discussed below, the variability of index scores within seasons must also be examined before an optimum sampling period can be definitively identified (Yoder and Rankin 1995).

Index variability within seasons

A snapshot approach to bioassessment, which does not encompass repeated testing within seasons, may lead to erroneous conclusions about ecosystem health (Mazor *et al.* 2009). Thus, for data collected from a large number of sites during a single reporting period or season, it is essential to determine variability within that season (Stewart and Loar 1994, Kurtz *et al.* 2001). The design of the current study did not encompass repeated sampling within seasons, and thus intra-seasonal variability of index scores remains unquantified. There is thus a clear requirement that further sampling be performed to address this aspect of index variability, and to determine the appropriate timing and intensity of sampling within any proposed monitoring period.

Relationships between inter-seasonal variability and ecological quality

No evidence was observed of a relationship between the ecological quality of nearshore sites and the inter-seasonal variability of their index scores, or of differences in inter-seasonal index variability among zones. Such findings parallel those of Pyron *et al.* (2008). In contrast, the variability of index scores among seasons at offshore sites was inversely related to the quality of those sites, and most sites of poorer quality were located in the upper estuary. These latter findings parallel those of numerous other workers (*e.g.* Karr *et al.* 1987, Steedman 1988, Fore *et al.* 1994, Deegan *et al.* 1997, Bilkovic *et al.* 2005, Brooks *et al.* 2009). Variability in index scores has therefore been proposed as a signal of ecological degradation, with impacted sites thought to be less resilient to natural temporal changes in abiotic factors (Fore *et al.* 1994, Simon 1999, Paller 2002). The far greater inter-seasonal index variability among poorer quality offshore than nearshore sites may thus be further evidence that the deeper, offshore waters of the Swan-Canning Estuary are in poorer health than the nearshore waters of this system. In particular, the low and highly variable index scores for most of the offshore sites in the upper reaches of the system support the contention that this zone is the most severely impacted (Swan River Trust 1999, 2003). However, these findings are probably also related to the more pronounced seasonal differences in water quality conditions in this zone, which are primarily associated with the extent of river flow.

Index variability between consecutive years

Inter-annual changes in index scores between the two consecutive years of the current study were relatively large, and were notably higher than those reported by Harris and Silveira (1999) for an IBI applied to rivers in New South Wales. The weak positive correlations between 2007/08 and 2008/09 for both the nearshore and offshore indices, and particularly

the latter, highlight the fact that the scores for numerous sites varied considerably between these years, which was sometimes due to a failure to capture any fish on certain sampling occasions. Several workers have suggested that such index variability may be indicative of ecological disturbance (Fore *et al.* 1994, Deegan *et al.* 1997, Hughes *et al.* 1998, 2002, Paller 2002), and thus the current findings may be further evidence of declining health status in the deeper waters of the Swan-Canning Estuary. Nonetheless, it is important to note that, although the inter-annual variability of index scores was relatively high, health status classifications were fairly robust to these changes.

Random sampling variability

Fewer than 25% of nearshore index scores varied by more than 10 points as a result of random sampling error. Thus, the precision of the current nearshore index is comparable to that reported for an IBI from Ohio streams (Fore *et al.* 1994), and is higher than that of a fish-based IBI applied to Minnesotan river basins, for which almost 25% of scores varied by 15 or more points (Dolph *et al.* 2010). The lengths of the confidence intervals for the current nearshore index were not strongly related to either the total number of fish or species in the original sample, although index precision tended to be lowest for those samples with fewer than several hundred fish, as reported in other studies (Fore *et al.* 1994, Dolph *et al.* 2010). The precision of offshore index scores was lower than that of the nearshore index, with the most variable score having a range of 40 points, although this precision was comparable to that documented by Dolph *et al.* (2010) for the IBI. The length of the confidence intervals for the offshore index was significantly related to species richness of the original sample, reflecting the fact that several of the species in samples with high species richness were found in low numbers, and were commonly "lost" during resampling.

Bootstrap scores tended to be higher than the original scores for the nearshore index, indicating that it consistently overestimated the health of sites, and most notably for those with higher index scores, as also noted by Dolph *et al.* (2010). However, the mean bias of the nearshore index across all sites was only one or two points, and the difference between the mean bootstrap score and the original index score represented a change in health status classification on only 7% of occasions. Such findings suggest that the method developed for classifying the health of nearshore sites in the present study is robust to the effects of random sampling variability. Nonetheless, as this index tends to overestimate health status, those nearshore sites with scores that are only one or two points above a class threshold should perhaps be allocated the health status of the lower of the two classes. In contrast, the bias of the offshore index resulted in original scores exceeding mean bootstrap scores by 20 or more points in some cases, and indicated the potential for a change in health status classification for 26% of site visits during 2007-09. Given also that the bias of the offshore index is inconsistent, confidence limits around health status thresholds may be appropriate to account for the observed lack of index precision, as have been established for the IBI in some jurisdictions (Gibson *et al.* 2000, Wan *et al.* 2010).

2.4.2.4 Synthesis and future work

This Chapter has included a detailed technical description of the development and initial validation of multimetric health indices for the nearshore and offshore waters of the Swan-Canning Estuary. It is important to note that any future implementation of these indices would not require the technical stages of index development and validation detailed in the current study ("Index Development"; Fig. 2.3.2.24) to be performed again.
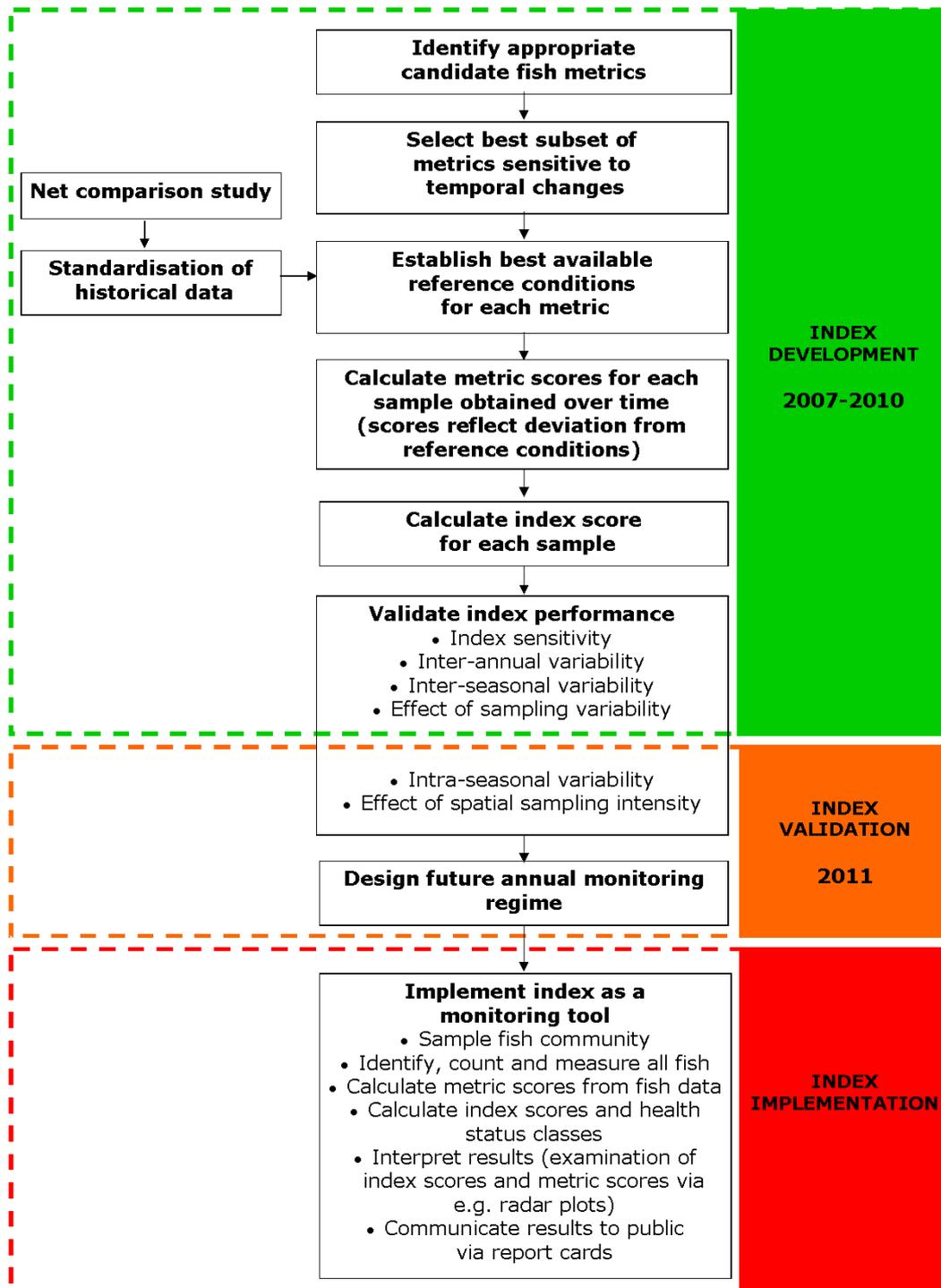
Figure 2.3.2.24: Flowchart of stages in the development, validation and potential implementation of the nearshore and offshore estuarine health indices.

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The current evaluations of the nearshore and offshore health indices developed for the Swan-Canning Estuary have demonstrated their capability for tracking long-term changes in the perceived health of this system. However, their sensitivity to specific stressors affecting the estuary remains unquantified. There are also unanswered questions concerning aspects of the spatial and temporal variability of index values, which might hamper the interpretation of trends in ecosystem health. These issues affect the design of any future fish monitoring regime for the Swan-Canning Estuary, and must be addressed to ensure that the indices provide a sensitive and reliable tool for ecological assessment. A further one-year study has been proposed, and is currently underway ("Index Validation"; Fig. 2.3.2.24), incorporating sampling of sufficient spatial and temporal intensity to address the following aims.

(1) Quantify the effects of spatial sampling intensity on the precision of health status classifications by establishing the relationship between the number of sites sampled per region and the resulting variability of index scores. Although the current study included some assessment of intra-regional index variability, the small number of replicate sites did not facilitate robust statistical testing among pairs of regions. Further work in this area will thus enable determination of the optimum spatial intensity of sampling required for future annual monitoring.

(2) Examine intra-seasonal variability of the health indices by comparing index values for sites sampled repeatedly within the same season, *i.e.* in each month. This will help to ascertain the optimum timing and length of the sampling period required for future annual monitoring.

(3) Quantify index responses to any major environmental perturbations (*e.g.* large phytoplankton blooms, fish kills) which might occur during this period, thus further validating the sensitivity of the indices.

In light of the findings of this extended research, a suitable monitoring regime and sampling protocol will be defined to enable the health of the Swan-Canning Estuary to be quantified into the future using the current fish-based indices ("Index Implementation"; Fig. 2.3.2.24). Subsequent implementation of these indices would require only a conceptual understanding of the rationale behind each of the development stages, and could then be carried out by anyone supplied with basic training and the spreadsheet-based tools developed in the current project to calculate metric and index values.

The annual implementation of this monitoring program will be essential for reliable interpretation of estuarine health trends, and also for reasonably construing increases in index variability as an additional signal of reduced resilience to ecological stress (Costanza and Mageau 1999, Pyron *et al.* 2008). Moreover, to maximise the utility of the current indices as an ongoing monitoring tool, several technical considerations will need to be emphasised when designing such a monitoring program. These include (i) the consistent implementation of standardised methodologies with regard to sampling location, timing, intensity and gear type, (ii) undertaking sampling at appropriate spatio-temporal scales to achieve a balance

between maximising index reliability and the cost-effectiveness of the monitoring program and (iii) quality control in terms of operating procedures and data management.

It must also be emphasised that the lack of suitable indicators of stressors and pressures represents a serious impediment to the future understanding of estuarine health, and must be addressed if we are to understand the complex ways in which the Swan-Canning Estuary responds to natural and anthropogenic influences, and thus the management actions required to improve or maintain it. This will only be possible if the indices developed in this study form part of a wider assessment and monitoring framework that employs multiple indicators. Such a framework would be characterised by the following.

(1) Be implemented via a cooperative approach involving government agencies, academic institutions, stakeholder groups and local councils (de Jonge 2007, EHMP 2007, Hartig *et al.* 2009).

(2) Focus on multiple scales of assessment, incorporating relevant attributes measured at landscape or catchment scales, regional scales within an estuary and localised, site-specific scales (Brooks *et al.* 2009, Infante *et al.* 2009, Nestlerode *et al.* 2009).

(3) Represent a bottom-up approach which focuses primarily on the relevant stressors and pressures affecting the estuary (Scheltinga and Moss 2007) and assesses their impacts on estuarine condition and local human populations.

(4) Employ multiple indicators at each level of the DPSIR model (Whitfield and Elliott 2002), including those which measure pressures or stressors (Walker *et al.* 2006, Degerman *et al.* 2007), the status or condition of various ecosystem components (Jackson *et al.* 2000, Griffith *et al.* 2005, Marchant *et al.* 2006, Scanes *et al.* 2007), processes and functions (Fairweather 1999, Rakocinski and Zapfe 2005, Udy *et al.* 2006, Horwitz 2007, Rowe *et al.* 2009) and the provision of ecosystem services to human populations (Worm *et al.* 2006). The advantages of using a broad suite of indicators to assess ecological condition (Dale and Beyeler 2001, Scanes *et al.* 2007, Puente and Diaz 2008), or of integrating multiple biotic and physical indicators into one (Ferreira 2000, Jordan and Vaas 2000, Kiddon *et al.* 2003, Bilkovic *et al.* 2005, Griffith *et al.* 2005), are widely acknowledged.

These issues must be addressed to develop an effective estuarine monitoring program whose focus extends beyond water quality and considers the requirements of the biological and human communities that live in and around the Swan-Canning Estuary.

2.5 Appended Tables

Appendix 2.5.1: Selection criterion (AIC$_c$) and associated measures of the evidence in favour of each of the subsets of models (fish metric combinations) identified as being substantially supported by the evidence ($\Delta_i \leq 2$) from distance-based linear modelling of the 21 m data set. The estimated 'best' model (AIC$_{c(min)}$) is italicised.

AIC$_c$	Number of metrics	Metrics selected *	AIC$_c$ difference (Δ_i)	log likelihood	Akaike weight (w_i)	Evidence ratio
-338.28	*8*	*1,2,4,5,6,11,13,14*	*0*	*1.00*	*0.09*	*1.00*
-338.01	7	1,4,5,6,11,13,14	0.27	0.87	0.08	1.14
-337.71	8	1,3,4,5,6,11,13,14	0.57	0.75	0.07	1.33
-337.44	9	1,2,4,5,6,11,12,13,14	0.84	0.66	0.06	1.52
-337.38	7	4,5,7,11,12,13,14	0.90	0.64	0.06	1.57
-337.32	7	4,5,6,7,11,13,14	0.96	0.62	0.06	1.62
-337.29	8	2,4,5,6,7,11,13,14	0.99	0.61	0.06	1.64
-337.10	9	1,3,4,5,6,11,12,13,14	1.18	0.55	0.05	1.80
-337.00	8	1,4,5,6,11,12,13,14	1.28	0.53	0.05	1.90
-336.97	8	3,45,6,7,11,13,14	1.31	0.52	0.05	1.93
-336.76	9	1,2,4,5,6,9,11,13,14	1.52	0.47	0.04	2.14
-336.69	8	3,4,5,7,11,12,13,14	1.59	0.45	0.04	2.21
-336.59	8	1,4,5,6,9,11,13,14	1.69	0.43	0.04	2.33
-336.57	8	2,4,5,7,11,12,13,14	1.71	0.43	0.04	2.35
-336.37	9	1,2,4,5,6,7,11,13,14	1.91	0.38	0.04	2.60
-336.36	8	1,4,5,6,7,11,13,14	1.92	0.38	0.04	2.61
-336.35	9	1,2,4,5,6,10,11,13,14	1.93	0.38	0.04	2.62
-336.30	9	2,4,5,6,7,11,12,13,14	1.98	0.37	0.03	2.69
-336.29	9	1,2,4,5,6,8,11,13,14	1.99	0.37	0.03	2.70
-336.28	9	1,3,4,5,6,9,11,13,14	2.00	0.37	0.03	2.72

* **Metric Numbers:** 1. *No species*, 2. *Dominance*, 3. *Sh-div*, 4. *Prop trop spec*, 5. *No trop spec*, 6. *No trop gen*, 7. *Prop detr*, 8. *Prop benthic*, 9. *No benthic*, 10. *Feed guild comp*, 11. *Prop est spawn*, 12. *No est spawn*, 13. *Prop P. olorum*, 14. *Tot no P. olorum*.

Appendix 2.5.2: Selection criterion (AIC$_c$) and associated measures of the evidence in favour of each of the subsets of models (fish metric combinations) identified as being substantially supported by the evidence ($\Delta_i \leq 2$) from distance-based linear modelling of the 41 m data set. The estimated 'best' model (AIC$_{c(min)}$) is italicised.

AIC$_c$	Number of metrics	Metrics selected *	AIC$_c$ difference (Δ_i)	Log likelihood	Akaike weight (w_i)	Evidence ratio
-111.54	*7*	*4,5,7,9,11,12,13*	*0*	*1.00*	*0.03*	*1.00*
-111.48	7	4,5,7,8,9,12,13	0.06	0.97	0.03	1.03
-111.35	8	4,5,7,8,9,11,12,13	0.19	0.91	0.03	1.10
-111.19	6	4,5,7,8,12,13	0.35	0.84	0.02	1.19
-111.09	6	1,4,5,7,9,11	0.45	0.80	0.02	1.25
-111.04	6	1,4,5,6,9,11	0.50	0.78	0.02	1.28
-110.86	7	4,5,7,8,11,12,13	0.68	0.71	0.02	1.40
-110.72	5	1,4,5,9,11	0.82	0.66	0.02	1.51
-110.71	7	1,4,5,7,9,11,13	0.83	0.66	0.02	1.51
-110.68	7	4,5,6,7,8,12,13	0.86	0.65	0.02	1.54
-110.66	8	1,4,5,7,8,9,12,13	0.88	0.64	0.02	1.55
-110.62	7	1,4,5,6,9,11,13	0.92	0.63	0.02	1.58
-110.56	8	1,4,5,6,8,9,12,13	0.98	0.61	0.02	1.63
-110.44	6	4,5,7,9,11,12	1.10	0.58	0.02	1.73

-110.40	6	5,7,8,9,11,12,13	1.14	0.57	0.02	1.77
-110.35	6	5,7,8,9,12,13	1.19	0.55	0.02	1.81
-110.34	5	1,5,7,9,11	1.20	0.55	0.02	1.82
-110.32	5	5,7,8,12,13	1.22	0.54	0.02	1.84
-110.29	8	4,5,6,7,8,11,12,13	1.25	0.54	0.02	1.87
-110.28	7	1,4,5,8,9,12,13	1.26	0.53	0.02	1.88
-110.27	6	1,4,5,9,11,13	1.27	0.53	0.02	1.89
-110.20	6	4,5,7,9,12,13	1.34	0.51	0.02	1.95
-110.19	7	1,4,5,7,9,12,13	1.35	0.51	0.02	1.96
-110.16	5	1,4,5,6,9	1.38	0.50	0.01	1.99
-110.14	7	1,4,5,7,8,9,11	1.40	0.50	0.01	2.01
-110.12	8	1,4,5,7,9,11,12,13	1.42	0.49	0.01	2.03
-110.12	6	1,4,5,6,8,9	1.42	0.49	0.01	2.03
-110.12	5	1,4,5,7,9	1.42	0.49	0.01	2.03
-110.11	7	1,4,5,6,9,12,13	1.43	0.49	0.01	2.04
-110.10	7	1,4,5,6,8,9,11	1.44	0.49	0.01	2.05
-110.10	6	1,4,5,7,8,9	1.44	0.49	0.01	2.05
-110.09	7	1,4,5,6,8,9,13	1.45	0.48	0.01	2.06
-110.05	6	1,4,5,9,12,13	1.49	0.47	0.01	2.11
-109.99	7	1,4,5,9,11,12,13	1.55	0.46	0.01	2.17
-109.97	6	1,5,7,9,11,13	1.57	0.46	0.01	2.19
-109.96	8	1,4,5,6,8,9,11,13	1.58	0.45	0.01	2.20
-109.96	8	3,4,5,7,9,11,12,13	1.58	0.45	0.01	2.20
-109.96	8	1,4,5,7,8,9,11,13	1.58	0.45	0.01	2.20
-109.94	8	1,4,5,6,9,11,12,13	1.60	0.45	0.01	2.23
-109.92	9	1,4,5,7,8,9,11,12,13	1.62	0.44	0.01	2.25
-109.90	8	2,4,5,7,8,9,12,13	1.64	0.44	0.01	2.27
-109.89	8	4,5,7,8,9,12,13,14	1.65	0.44	0.01	2.28
-109.86	8	3,4,5,7,8,9,12,13	1.68	0.43	0.01	2.32
-109.85	7	1,4,5,7,8,9,13	1.69	0.43	0.01	2.33
-109.80	7	1,4,5,6,7,9,11	1.74	0.42	0.01	2.39
-109.80	9	1,4,5,6,8,9,11,12,13	1.74	0.42	0.01	2.39
-109.78	6	1,4,5,6,9,13	1.76	0.41	0.01	2.41
-109.75	8	4,5,6,7,8,9,12,13	1.79	0.41	0.01	2.45
-109.73	9	4,5,7,8,9,11,12,13,14	1.81	0.40	0.01	2.47
-109.73	7	5,7,8,9,11,12,13	1.81	0.40	0.01	2.47
-109.68	8	4,5,7,9,10,11,12,13	1.86	0.39	0.01	2.53
-109.65	6	4,5,6,7,8,13	1.89	0.39	0.01	2.57
-109.64	7	1,4,5,7,9,10,11	1.90	0.39	0.01	2.59
-109.64	7	4,5,7,8,12,13,14	1.90	0.39	0.01	2.59
-109.62	9	3,4,5,7,8,9,11,12,13	1.92	0.38	0.01	2.61
-109.61	7	2,4,5,7,8,12,13	1.93	0.38	0.01	2.62
-109.61	6	4,5,7,8,9,12	1.93	0.38	0.01	2.62
-109.60	6	1,4,5,7,9,13	1.94	0.38	0.01	2.64
-109.60	6	1,4,5,8,9,11	1.94	0.38	0.01	2.64
-109.59	7	1,3,4,5,7,9,11	1.95	0.38	0.01	2.65
-109.59	8	1,4,5,8,9,11,12,13	1.95	0.38	0.01	2.65
-109.59	7	1,4,5,7,9,11,12	1.95	0.38	0.01	2.65
-109.58	8	4,5,7,8,9,10,12,13	1.96	0.38	0.01	2.66
-109.58	9	4,5,6,7,8,9,11,12,13	1.96	0.38	0.01	2.66
-109.54	5	4,5,7,9,11	2.00	0.37	0.01	2.72
-109.54	7	1,4,5,7,8,12,13	2.00	0.37	0.01	2.72

* **Metric Numbers:** 1. *No species*, 2. *Dominance*, 3. *Sh-div*, 4. *Prop trop spec*, 5. *No trop spec*, 6. *No trop gen*, 7. *Prop detr*, 8. *Prop benthic*, 9. *No benthic*, 10. *Feed guild comp*, 11. *Prop est spawn*, 12. *No est spawn*, 13. *Prop P. olorum*, 14. *Tot no P. olorum.*

Appendix 2.5.3: Selection criterion (AIC$_c$) and associated measures of the evidence in favour of each of the subsets of models (fish metric combinations) identified as being substantially supported by the evidence ($\Delta_i \leq 2$) from distance-based linear modelling of the 102-133 m data set. The estimated 'best' model (AIC$_{c(min)}$) is italicised.

AIC$_c$	Number of metrics	Metrics selected *	AIC$_c$ difference (Δ_i)	log likelihood	Akaike weight (w_i)	Evidence ratio
-638.51	*9*	*1,2,4,5,7,8,9,10,12*	*0*	*1.00*	*0.04*	*1.00*
-638.23	8	1,4,5,7,8,9,10,12	0.28	0.87	0.03	1.15
-638.11	10	1,2,3,4,5,7,8,9,10,12	0.40	0.82	0.03	1.22
-637.94	9	1,2,5,7,8,9,10,11,12	0.57	0.75	0.03	1.33
-637.82	8	1,2,5,7,8,9,10,12	0.69	0.71	0.03	1.41
-637.75	10	1,2,4,5,7,8,9,10,12,13	0.76	0.68	0.03	1.46
-637.72	10	1,2,4,5,6,7,8,9,10,12	0.79	0.67	0.03	1.48
-637.70	9	1,2,5,6,7,8,9,10,12	0.81	0.67	0.03	1.50
-637.66	9	1,3,4,5,7,8,9,10,12	0.85	0.65	0.03	1.53
-637.58	10	1,2,4,5,7,8,9,10,11,12	0.93	0.63	0.02	1.59
-637.48	9	1,4,5,6,7,8,9,10,12	1.03	0.60	0.02	1.67
-637.42	10	1,2,5,6,7,8,9,10,11,12	1.09	0.58	0.02	1.72
-637.36	11	1,2,3,4,5,7,8,9,10,12,13	1.15	0.56	0.02	1.78
-637.29	10	1,2,4,5,7,8,9,10,12,14	1.22	0.54	0.02	1.84
-637.27	9	1,2,4,5,7,9,10,11,12	1.24	0.54	0.02	1.86
-637.22	9	1,2,3,5,7,8,9,10,12	1.29	0.52	0.02	1.91
-637.19	9	1,2,5,7,8,9,10,12,13	1.32	0.52	0.02	1.93
-637.18	10	1,2,3,5,7,8,9,10,11,12	1.33	0.51	0.02	1.94
-637.16	8	1,5,6,7,8,9,10,12	1.35	0.51	0.02	1.96
-637.16	11	1,2,3,4,5,6,7,8,9,10,12	1.35	0.51	0.02	1.96
-637.14	7	1,5,7,8,9,10,12	1.37	0.50	0.02	1.98
-637.12	8	1,2,4,5,7,9,10,12	1.39	0.50	0.02	2.00
-637.06	10	1,2,5,7,8,9,10,11,12,13	1.45	0.48	0.02	2.06
-637.03	9	1,4,5,7,8,9,10,12,14	1.48	0.48	0.02	2.10
-637.01	10	1,3,4,5,7,8,9,10,12,13	1.50	0.47	0.02	2.12
-637.01	11	1,2,3,4,5,7,8,9,10,11,12	1.50	0.47	0.02	2.12
-636.99	10	1,3,4,5,6,7,8,9,10,12	1.52	0.47	0.02	2.14
-636.93	10	1,2,3,5,6,7,8,9,10,12	1.58	0.45	0.02	2.20
-636.93	9	1,4,5,7,8,9,10,11,12	1.58	0.45	0.02	2.20
-636.92	11	1,2,3,4,5,7,8,9,10,12,14	1.59	0.45	0.02	2.21
-636.92	9	1,4,5,7,8,9,10,12,13	1.59	0.45	0.02	2.21
-636.90	9	1,3,5,6,7,8,9,10,12	1.61	0.45	0.02	2.24
-636.78	9	1,2,5,7,8,9,10,12,14	1.73	0.42	0.02	2.38
-636.77	8	1,3,5,7,8,9,10,12	1.74	0.42	0.02	2.39
-636.77	11	1,2,4,5,6,7,8,9,10,12,13	1.74	0.42	0.02	2.39
-636.75	10	1,2,5,6,7,8,9,10,12,13	1.76	0.41	0.02	2.41
-636.74	9	1,3,5,7,8,9,10,11,12	1.77	0.41	0.02	2.42
-636.71	10	1,2,3,4,5,7,9,10,11,12	1.80	0.41	0.02	2.46
-636.71	10	1,2,5,7,8,9,10,11,12,14	1.80	0.41	0.02	2.46
-636.70	8	1,2,5,7,9,10,11,12	1.81	0.40	0.02	2.47
-636.67	11	1,2,4,5,7,8,9,10,11,12,13	1.84	0.40	0.02	2.51
-636.66	11	1,2,4,5,6,7,8,9,10,11,12	1.85	0.40	0.02	2.52
-636.65	9	1,2,3,4,5,7,9,10,12	1.86	0.39	0.02	2.53
-636.64	10	1,3,4,5,7,8,9,10,12,14	1.87	0.39	0.02	2.55
-636.64	8	1,4,5,7,9,10,11,12	1.87	0.39	0.02	2.55
-636.60	11	1,2,3,5,6,7,8,9,10,11,12	1.91	0.38	0.01	2.60
-636.60	8	1,5,7,8,9,10,11,12	1.91	0.38	0.01	2.60

-636.60	10	1,2,3,5,7,8,9,10,12,13	1.91	0.38	0.01	2.60
-636.56	10	1,3,4,5,7,8,9,10,11,12	1.95	0.38	0.01	2.65
-636.55	9	1,2,5,6,7,9, 10,11,12	1.96	0.38	0.01	2.66
-636.54	10	1,3,5,6,7,8,9,10,11,12	1.97	0.37	0.01	2.68

* **Metric Numbers:** 1. *No species,* 2. *Dominance,* 3. *Sh-div,* 4. *Prop trop spec,* 5. *No trop spec,* 6. *No trop gen,* 7. *Prop detr,* 8. *Prop benthic,* 9. *No benthic,* 10. *Feed guild comp,* 11. *Prop est spawn,* 12. *No est spawn,* 13. *Prop P. olorum,* 14. *Tot no P. olorum.*

Appendix 2.5.4: Selection criterion (AIC_c) and associated measures of the evidence in favour of each of the subset of models (fish metric combinations) identified as being substantially supported by evidence ($\Delta_i \leq 2$) from distance-based linear modelling of the gill net data set. The estimated 'best' model, termed $AIC_{c(min)}$, is italicised.

AIC_c	Number of metrics	Metrics selected *	AIC_c difference (Δ_i)	log likelihood	Akaike weight (w_i)	Evidence ratio
-240.16	*5*	*1,5,6,8,11*	*0*	*1.00*	*0.03*	*1.00*
-239.97	6	1,5,7,8,10,11	0.19	0.91	0.03	1.10
-239.93	5	1,5,8,10,11	0.23	0.89	0.03	1.12
-239.85	6	1,5,6,8,10,11	0.31	0.86	0.03	1.17
-239.78	4	6,7,8,11	0.38	0.83	0.02	1.21
-239.58	5	1,5,7,8,11	0.58	0.75	0.02	1.34
-239.50	4	1,5,8,11	0.66	0.72	0.02	1.39
-239.49	7	1,2,3,5,6,8,11	0.67	0.72	0.02	1.40
-239.38	6	1,3,5,6,8,11	0.78	0.68	0.02	1.48
-239.30	3	6,8,11	0.86	0.65	0.02	1.54
-239.24	6	1,5,6,7,8,11	0.92	0.63	0.02	1.58
-239.17	5	1,3,5,8,11	0.99	0.61	0.02	1.64
-239.12	6	1,3,5,8,10,11	1.04	0.59	0.02	1.68
-239.11	6	1,2,3,5,8,11	1.05	0.59	0.02	1.69
-239.10	6	1,5,8,9,10,11	1.06	0.59	0.02	1.70
-239.10	7	1,2,3,5,8,10,11	1.06	0.59	0.02	1.70
-239.08	7	1,5,7,8,9,10,11	1.08	0.58	0.02	1.72
-238.97	6	1,5,6,8,9,11	1.19	0.55	0.02	1.81
-238.95	8	1,2,3,5,6,8,10,11	1.21	0.55	0.02	1.83
-238.94	7	1,5,6,7,8,10,11	1.22	0.54	0.02	1.84
-238.91	5	1,5,8,9,11	1.25	0.54	0.02	1.87
-238.91	6	1,5,7,8,9,11	1.25	0.54	0.02	1.87
-238.90	7	1,5,7,8,10,11,12	1.26	0.53	0.02	1.88
-238.88	6	1,5,6,8,11,12	1.28	0.53	0.02	1.90
-238.86	8	1,2,3,5,6,8,11,12	1.30	0.52	0.02	1.92
-238.83	7	1,3,5,6,8,10,11	1.33	0.51	0.02	1.94
-238.80	6	1,5,8,10,11,12	1.36	0.51	0.02	1.97
-238.71	6	5,7,8,9,10,11	1.45	0.48	0.01	2.06
-238.67	7	1,4,5,7,8,10,11	1.49	0.47	0.01	2.11
-238.66	5	5,8,9,10,11	1.50	0.47	0.01	2.12
-238.65	7	1,5,6,8,9,10,11	1.51	0.47	0.01	2.13
-238.63	6	1,5,7,8,11,12	1.53	0.47	0.01	2.15
-238.61	6	5,7,8,10,11,12	1.55	0.46	0.01	2.17
-238.57	8	1,2,3,5,6,8,9,11	1.59	0.45	0.01	2.21
-238.55	6	1,3,5,7,8,11	1.61	0.45	0.01	2.24
-238.55	7	1,5,6,8,10,11,12	1.61	0.45	0.01	2.24
-238.54	5	1,5,8,11,12	1.62	0.44	0.01	2.25
-238.51	7	1,3,5,7,8,10,11	1.65	0.44	0.01	2.28
-238.50	6	1,3,4,5,8,11	1.66	0.44	0.01	2.29

-238.49	6	1,4,5,7,8,11	1.67	0.43	0.01	2.30
-238.47	6	1,4,5,8,10,11	1.69	0.43	0.01	2.33
-238.43	5	2,6,7,8,11	1.73	0.42	0.01	2.38
-238.42	6	1,4,5,6,8,11	1.74	0.42	0.01	2.39
-238.42	7	1,2,3,4,5,8,11	1.74	0.42	0.01	2.39
-238.42	4	5,8,10,11	1.74	0.42	0.01	2.39
-238.42	5	3,6,7,8,11	1.74	0.42	0.01	2.39
-238.41	6	1,2,5,6,8,11	1.75	0.42	0.01	2.40
-238.41	7	1,3,5,6,8,11,12	1.75	0.42	0.01	2.40
-238.38	5	5,8,10,11,12	1.78	0.41	0.01	2.44
-238.35	5	6,7,8,11,12	1.81	0.40	0.01	2.47
-238.32	7	1,3,5,6,8,9,11	1.84	0.40	0.01	2.51
-238.32	6	1,3,5,8,9,11	1.84	0.40	0.01	2.51
-238.31	9	1,2,3,5,6,8,10,11,12	1.85	0.40	0.01	2.52
-238.27	5	5,7,8,10,11	1.89	0.39	0.01	2.57
-238.26	7	1,2,3,5,8,9,11	1.90	0.39	0.01	2.59
-238.24	7	1,2,3,8,11,12	1.92	0.38	0.01	2.61
-238.24	7	1,2,5,7,8,10,11	1.92	0.38	0.01	2.61
-238.24	5	1,6,7,8,11	1.92	0.38	0.01	2.61
-238.23	5	1,4,5,8,11	1.93	0.38	0.01	2.62
-238.22	7	1,2,3,5,7,8,11	1.94	0.38	0.01	2.64
-238.22	8	1,2,3,5,7,8,10,11	1.94	0.38	0.01	2.64
-238.21	5	4,6,7,8,11	1.95	0.38	0.01	2.65
-238.21	5	6,7,8,10,11	1.95	0.38	0.01	2.65
-238.19	8	1,2,3,5,8,10,11,12	1.97	0.37	0.01	2.68
-238.19	7	1,3,5,6,7,8,11	1.97	0.37	0.01	2.68
-238.18	7	1,3,4,5,8,10,11	1.98	0.37	0.01	2.69

* Metric Numbers: 1. *No species,* 2. *Dominance,* 3. *Sh-div,* 4. *Prop trop spec,* 5. *No trop spec,* 6. *No trop gen,* 7. *Prop detr,* 8. *Prop benthic,* 9. *No benthic,* 10. *Feed guild comp,* 11. *Prop est spawn,* 12. *No est spawn.*

Chapter 3. Employing estimates of biomass production to assess changes in the dynamics of the Black Bream *Acanthopagrus butcheri* population in the Swan-Canning Estuary

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Executive summary

The Black Bream *Acanthopagrus butcheri* is one of Australia's most important fish species, which is particularly true for the Perth metropolitan region. In recent years, the public have become increasingly aware that the Swan-Canning Estuary, which flows through this area, is experiencing environmental problems that could be having a marked detrimental impact on its wildlife, including fish populations. Toxic algal blooms and poor water quality conditions have been associated with several fish kills and thus present a clear threat to Black Bream. Of further concern is a lack of information to determine whether Black Bream in this estuary is being fished at sustainable levels.

Until relatively recently, Black Bream in the Swan-Canning Estuary was fished commercially by gillnetting. The available catch per unit effort (CPUE) data for that fishery between about 1988 and 1997 (when Black Bream was caught by at least 5 commercial fishers) indicate that the abundance of this species had increased during that period, but those data may not precisely reflect the true abundance of this population and this type of data is no longer able to be collected. Previous studies of Black Bream in the Swan-Canning Estuary in the 1990s showed that, at that time, most individuals were young, suggesting that the population had experienced high mortality. Thus, more information is needed to provide a clear picture of the current status of Black Bream in the Swan-Canning Estuary.

Complicating any assessment of the health of a Black Bream population is that a number of its biological attributes, *e.g.* its pattern of growth, size and age at maturity and annual recruitment levels, are all highly variable. This means that many traditional approaches for assessing stock status are inappropriate for Black Bream as they assume that the population is at equilibrium under process such as growth, recruitment and mortality.

One useful approach for understanding the dynamics of a fish population that does not require CPUE data or equilibrium assumptions is to measure productivity, typically defined as the amount of tissue elaborated per unit time per unit area, including what is formed by individuals that do not survive. In this study, we first focused on determining the extent to which the biology of Black Bream in the Swan-Canning Estuary has changed since the mid 1990s. We then used the available biological and abundance data for this population of Black Bream from this and previous studies to produce estimates of annual biomass production to track changes in the dynamics of this population over time.

The results show that the growth performance of Black Bream has declined markedly, with fish, on average, now taking 6 years to reach the minimum legal length for retention (MLL) of 250 mm, compared with only 2.7 years in 1993-95. At 250 mm, Black Bream are now about 20 g lighter than in 1993-95 (= 9% reduction), suggesting that, on average, individuals are in poorer condition. There has also been a marked decline in the size at maturity, with

females, for example, typically maturing at 196 mm in 1993-95 compared with only 168 mm in 2007-08.

Estimates of density produced from research seine netting data in 1993-95, 2003-04 and 2007-08 strongly indicate that the abundance of Black Bream in nearshore waters of the Swan-Canning Estuary increased greatly between those first two periods and is currently at a level similar to that recorded in 2003-04. The concomitant decline growth performance and increased abundance of Black Bream provides strong support for the hypothesis that the growth of this species is strongly influenced by density-dependent factors.

When calculated on an individual fish basis, annual biomass production declined progressively over the three study periods, reflecting a progressive decline in growth performance of Black Bream. When calculated on a per unit area basis, annual biomass production was markedly higher in 2003-04 than in 1993-94, but was only slightly higher in 2007-08 than in the earliest period. It is concluded that the initial increase in biomass production per unit area was due to the increase in Black Bream abundance and that the lower annual biomass production per unit area in 2007-08 than in 2003-04 reflects the poorer growth performance of Black Bream in 2007-08 (and similar fish densities recorded for those two periods).

Although the results strongly indicate that the abundance of Black Bream has increased, at least in nearshore waters, the new age composition data confirm that the vast majority of fish are young. This latter finding suggests that Black Bream in this estuary is currently, or has recently experienced high mortality. However, as Black Bream are far more abundant than in the past and individuals can now potentially spawn for several years before they are legally able to be caught and retained, it would appear that recreational fishing pressure currently presents a low risk to the sustainability of the population in the Swan-Canning Estuary. The relatively poor growth performance of Black Bream in more recent years has implications for the quality of recreational fishing, as fewer fish are now of a size where they can be legally caught and retained by fishers.

Although the question as to the main source of the high mortality of Black Bream still remains, it may be relevant that age composition data for 2007-08 show a marked change in the abundance of fish hatched before and after 2003, at which time there was a major fish kill event in the estuary. Determining the extent to which environmental events, such as fish kills, influence Black Bream abundance in the Swan-Canning Estuary is likely to require continuous monitoring of this population.

3.1 Introduction

3.1.1 Background

The Black Bream *Acanthopagrus butcheri* (family Sparidae) is a medium-sized fish species (maximum length and weight of 533 mm and 3,450 g, respectively) (Hutchins and Thompson 2004), which completes its entire lifecycle within its natal estuary. It is also an iconic recreational finfish species (Norris *et al*. 2002) endemic to estuaries of southern Australia (Rowland 1984, Loneragan *et al*. 1989, Potter *et al*. 1990). Historically, Black Bream was also an important commercial species in this region (Lenanton and Potter 1987, Kailola *et al*. 1993), but this is less so today due to a combination of government "buy-backs" of commercial fishing licences, *e.g.* Swan-Canning Estuary and in some estuaries, greatly-reduced abundances of fish, *e.g.* Culham Inlet (Norris *et al.* 2002).

Biological studies have shown that a range of aspects of the biology of this species, including its pattern of growth, the length and age at maturity, and diet, differ markedly among estuaries (*e.g.* Hobday and Moran 1983, Coutin *et al*. 1997, Morrison *et al*. 1998, Sarre and Potter 2000, Hoeksema *et al*. 2006). For example, Sarre and Potter (2000) showed that Black Bream grows very differently in the Swan River and Moore River estuaries which are only *ca* 85 km apart. On the basis of von Bertalanffy growth curves for Black Bream in those estuaries this species, at 3 and 6 years of age, individuals attained 266 and 368 mm, respectively, in the Swan-Canning Estuary, compared with only 146 and 232 mm, respectively, in the Moore River Estuary. The lengths at which 50% of *A. butcheri* in those estuaries attained maturity, *i.e.* the L_{50}s as determined from logistic regression analysis, also differed substantially (*e.g.,* for females, Swan-Canning Estuary, $L_{50} = 218$ mm *vs* Moore River Estuary, $L_{50} = 157$ mm) (Sarre and Potter 2000).

Although the populations of *A. butcheri* in different south-western Australian estuaries have been shown to be genetically distinct (Chaplin *et al*. 1998), it has been demonstrated that the very different growth patterns of this species among estuaries does not have a strong genetic basis (Partridge *et al*. 2004). This suggests that the growth of *A. butcheri* is plastic and thus also that its growth in a given location could potentially change over time. Differences in fish density and in dietary intake have been proposed as factors likely to be important factor in influencing the growth performance of Black Bream (*e.g.* Sarre and Potter 2000, Hoeksema *et al.* 2006). As a study by Hoeksema *et al.* (2006) on a low density population Black Bream in Hamersley Inlet showed that individuals grew particularly rapidly despite having a largely vegetative diet, this suggests that fish density is particularly important for regulating the growth performance of Black Bream.

In the Swan-Canning Estuary, an increase in CPUE of Black Bream between about 1988 and 1997 by commercial gillnetting indicated that the abundance of this population had increased over this period (Smith 2006). It may be relevant that after 1988, there was a marked decline in the number of commercial fishers operating in the estuary (Smith 2006). Although the

above data indicate that the abundance of *A. butcheri* had increased in the 1990s, age composition data collected for Black Bream by Hoeksema and Smith in 2003-04 (unpubl. data) showed that most fish at that time were young (< 5 years old), suggesting that the population of Black Bream had experienced a high level of mortality. There is thus clearly a need for a robust assessment of the current state of the stock of Black Bream in the Swan-Canning Estuary.

Although there are past commercial CPUE data for *A. butcheri* in the Swan-Canning Estuary, it is recognized that those data may not have precisely reflected the true abundance of Black Bream in the estuary (Smith 2006) and, with the cessation of commercial fishing, such data will not be available for future years. As biological data exist for Black Bream in the Swan-Canning Estuary, some attention has been given to the use of per-recruit analyses for assessing this population (Ayvazian and Nowara 2000). Although per-recruit analyses are widely used to assess the status of fish stocks when there are limited or no reliable CPUE data, these and many other stock assessment methods assume that the fish stock is at equilibrium under processes such as recruitment and mortality, assumptions which are demonstrably invalid for many fish species (*e.g.* Hilborn and Walters 1992). Such assumptions would be implausible for a species like Black Bream which, for example, is known to exhibit high levels of inter-annual variability in recruitment (*e.g.* Hobday and Moran 1983, Sarre and Potter 2000). Furthermore, it would also be inappropriate to use per recruit analyses to assess the how the state of a fish stock had changed over time when growth is temporally variable because variability in growth, for example, will influence the relationship between fishing mortality and spawning biomass per recruit.

Another alternative approach that could be taken to assess the state of the Black Bream stock, if age composition data are available, is to use mortality-based reference points. However, as Black Bream exhibits high inter-annual variability in recruitment (Hobday and Moran 1983, Sarre and Potter 2000), any estimates of mortality derived using catch curve analyses are likely to be imprecise. Furthermore, such analysis would still require that the assumptions of constant recruitment and total mortality are satisfied if it was to produce reliable estimates. Although methods do exist to estimate total mortality from age composition data which allow for recruitment variability, the presence of only a small number of age classes of Black Bream in the Swan River means that there is likely to be little information content in the data to yield reliable estimates of the value of this parameter.

Given the problems with applying each of the above-mentioned stock approaches to Black Bream in the Swan-Canning Estuary, how then can the state of the stock be assessed? One approach is to assess production rate. Production is defined as the "amount of tissue elaborated per unit time per unit area, regardless of fate" (Clarke 1946; see Chapman, 1978). Key formulations for estimating production were provided by Ricker (1946) and Allen (1950, 1971). Production can be measured in terms of wet weight (by far the most common), dry weight, nitrogen content and energy content (Chapman 1978). According to Waters (1982), estimates of production provide one of the most useful bases for assessing the dynamic state

of a population and can be very valuable in helping establish upper limits to annual harvests (Ricker 1975, Downing *et al.* 1990; see also Mertz and Myers, 1998).

3.1.2 Aims and objectives

In this study, a range of biological and abundance data for Black Bream in the Swan-Canning Estuary, collected during 1993-95 (Sarre 1999), 2003-04 (Hoeksema and Smith, unpubl. data), and 2007-09 (current study) have been collated. Comparisons of size and age compositions, growth, length-weight relationships and the lengths and ages at which Black Bream attain maturity have been compared to assess the extent to which the biology of this species has changed over the last ~15 years and, if the changes are marked, to assess which factors are likely to have contributed most to those changes. These data have also been used to calculate the level of annual biomass production of a Black Bream population in the Swan-Canning Estuary as a reliable measure that can be used in the future to inform stock assessments for this species.

3.2 Materials and Methods

3.2.1 Sampling regime

During this study, *Acanthopagrus butcheri* was collected seasonally from the nearshore, shallow waters (<2 m deep) and offshore deeper waters (>2 m deep) of the middle and upper regions of the Swan-Canning Estuary (*i.e.* Swan and Canning rivers and Swan-Canning basin) using a combination of seining and gillnetting (Fig. 3.1). Four sites in the basin and twelve sites in Swan and Canning rivers were sampled during the day using a 21.5 m seine net. This net was also used to sample the middle downstream (MD), middle upstream (MU) and upper Swan River (US) at night, both in this study and during 2003-04 (Hoeksema and Smith, unpubl. data) (Fig. 3.1). The 21.5 m seine net, which consisted of a 1.5 m wide bunt of 3 mm mesh and two 10 m long wings (each comprising 4 m of 3 mm mesh and 6 m of 9 mm mesh) and swept an area of 116 m^2, was laid parallel to the bank and then hauled onto the shore.

Four nearshore sites in the basin and twelve sites in the Swan and Canning rivers were sampled in this study during the day using a 41.5 m seine net. This net was also used to sample the lower Swan (LS), MD, Canning River and the basin during 2003-04 (Hoeksema, unpubl. data) and the LS and MD regions during 1993-95 (Sarre 1999). The 41.5 m seine net, which consisted of two 20 m long wings made of 25 mm mesh and a 1.5 m cod end made of 9 mm mesh, swept an area of 274 m^2. This net was deployed from a small boat in a semi-circle from the bank and likewise hauled on to the shore.

Offshore waters (>2 m) were sampled in this study using composite sunken gillnets at three sites in the basin and twelve sites in the Swan and Canning rivers. These nets were also used to sample similar sites throughout the entire estuary during 2003-04 and in the LS, MD and MU during 1993-95 (see Fig.3.1). These gillnets consisted of a combination of between six and eight panels that were each 20 m long and 2 m high and ranged in mesh size from 35-127 mm, and were set parallel to the shore in water depths of 2 to 6 m, just after sunset and retrieved three hours later.

In 1993-95, further samples of Black Bream were collected by rod and line fishing. Such sampling was not repeated in later studies. Estimates of Black Bream density and biomass production per unit area have been compared for the three study periods using data collected by the 41 m seine net at the same six sites of the middle downstream and lower regions of the Swan River, *i.e.* the only sites that were sampled in all of the three study periods in each season using this method.
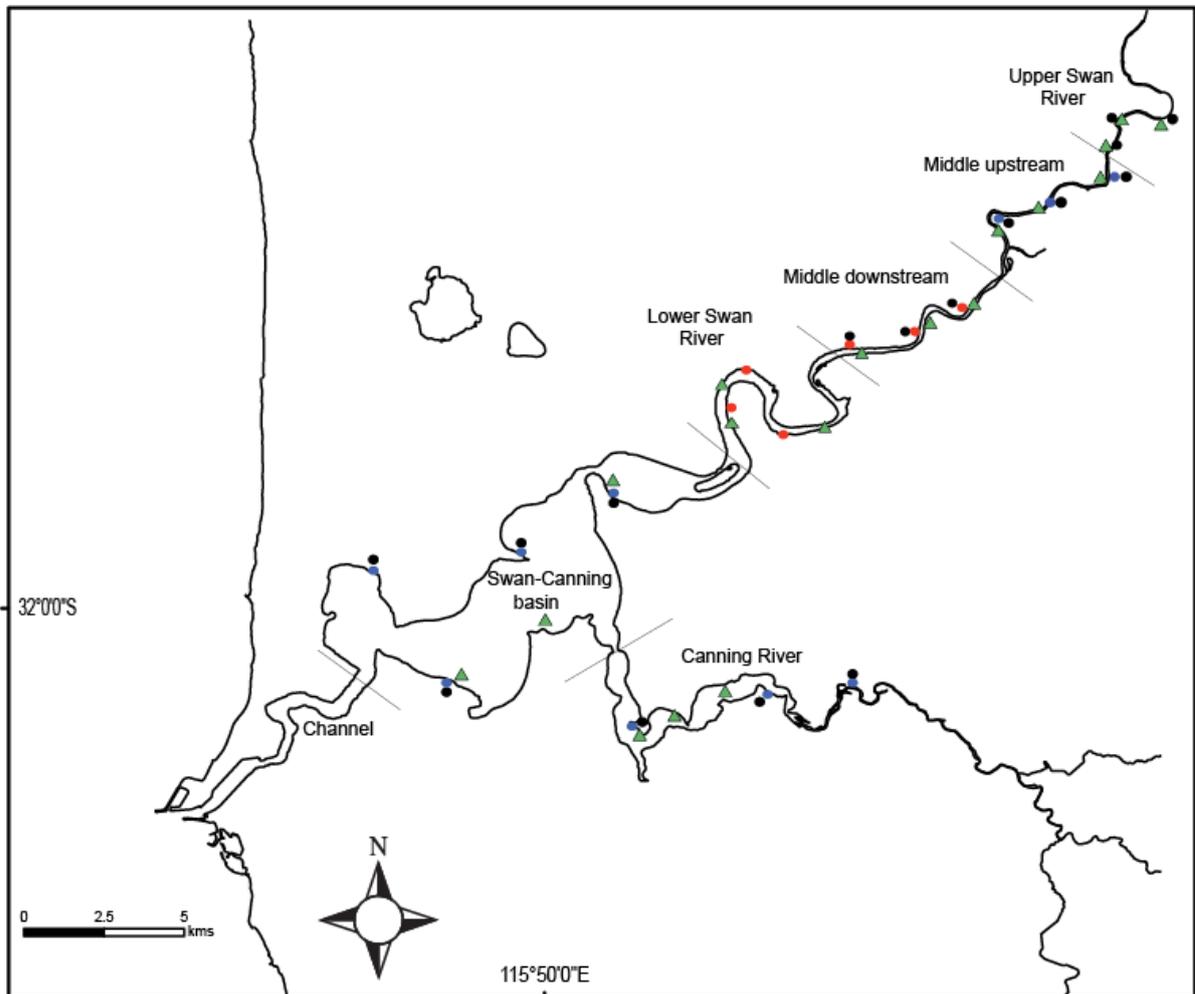
Figure 3.1: Map of the Swan-Canning Estuary in south-western Australia showing sites at which Black Bream was collected using multifilament gillnets (green triangles), a 21.5 m seine net (black circles) and a 41.5 m seine net (blue circles). Red circles show the locations of sites where 41.5 m seine net data was used to compare estimates of Black Bream density during this study those in 1993-95 (Sarre 1999) and 2003-04 (Hoeksema and Smith, unpubl. data). Grey lines denote boundaries of different sections of the estuary.

3.2.2 Biological analyses

Acanthopagrus butcheri was measured to the nearest 1 mm (total length), weighed to the nearest 0.1 g and sexed (on the basis of a macroscopic examination of their gonads). The gonads were then removed, weighed to the nearest 0.01 g and assigned to one of eight gonadal maturity stages, according to the staging scheme of Laevastu (1965), *i.e.* I and II = virgin/immature, III = developing, IV = maturing, V and VI = mature/spawning, VII = spent and VIII = recovering. The sagittal otoliths of each *A. butcheri* were removed, cleaned and stored. All otoliths were initially examined whole using a dissecting microscope under reflected light. If the number of opaque zones exceeded 6, the otolith was sectioned. For sectioning, otoliths were embedded in clear epoxy resin, sectioned transversely (*ca* 400 μm) through their primordia and mounted on glass microscope slides using DePX mounting adhesive.

As Sarre and Potter (2000) provided conclusive evidence, using marginal increment analysis, that the opaque zones in the otoliths of *A. butcheri* in the Swan-Canning Estuary are formed annually, validation of the use of annuli in otoliths for ageing individuals was not repeated for fish caught in 2003-04 and 2007-09. To assess the level of precision for the ageing of Black Bream during 2007-09, the numbers of opaque zones in a subsample of 200 otoliths for fish of a wide size range (and using whole and sectioned otoliths) were counted independently by two readers, *i.e.*, A. Hesp and A. Cottingham, and compared using the coefficient of variation (CV) (Chang 1982, Campana 2001). The equation is as follows.

$$CV_j = 100\% \left\{ \sqrt{\sum_{i=1}^{R} \frac{(X_{ij} - X_j)^2}{R-1}} / X_j \right\},$$

where CV_j is the age precision estimate for the *j*th fish, X_{ij} is the *i*th age determination of the *j*th fish, X_j is the mean age estimate of the *j*th fish, and R is the number of times each fish is aged. The overall *CV* for was taken as the average of the *CV*s for all 200 fish. This analysis produced a value of 0.2% for the CV for Black Bream, indicating a high degree of precision for the ageing of Black Bream, given that a value for the CV of approximately 5% is considered acceptable for medium to long-lived species (Campana 2001).

The growth of *A. butcheri* was described by fitting von Bertalanffy growth curves to the lengths at age of individuals for each time period. The von Bertalanffy growth equation is: $\hat{L}_t = L_\infty (1 - e^{-k(t-t_0)})$, where $\hat{L}_t$ is the estimated length (mm) at age t (years), L_∞ is the asymptotic length (mm), k is the growth coefficient (year^{-1}) and t_0 is the hypothetical age (years) at which the fish would have zero length. Point estimates and 95% confidence limits for the growth parameters were estimated by fitting the von Bertalanffy growth equation using the non-linear regression procedure in the Statistical Package for the Social Sciences (version 15.0, SPSS Inc.).

Separate length-weight relationships were determined for female and male *A. butcheri* collected during 1993-95, 2003-04 and 2007-09, using the equation $\ln W = b\ln TL - \ln a$, where *ln* refers to the natural logarithm, *W* and *TL* are the body weights (g) and total lengths (mm) of fish, respectively, and *a* and *b* are constants. The length-weight relationships were determined in each period using a sample of 30 fish caught using in spring, summer and autumn using the 41.5 m seine net in the lower and middle downstream regions of the Swan River. Note that samples from winter were not used for determining length-weight relationships because few or no fish were collected by seine netting during this season in the earlier studies. As recommended by Froese (2006), the fish in each season from which the subsamples were taken were divided into three size categories (*i.e.* <140, 160-190, >210 mm) and an equal number of fish (*i.e.* 10 individuals) were taken from each of those size categories so that the data were weighted equally for each size category. Analysis of covariance (ANCOVA), in SPSS, was used to test first, whether the length–weight relationships in each period differed between the sexes. As there were no sex-based differences in those relationships (*P* >0.05), the data for females and males were pooled. ANCOVA was then used to determine whether the length-weight relationships for *A. butcheri* differed among the different study periods and thus, whether separate equations needed to be used for each study period when estimating annual biomass production (see below).

3.2.3 Maturation

Logistic regression analysis was used to estimate separately the lengths at which 50% of *A. butcheri* were "mature" (*i.e.* fish possessed gonads at stages V-VIII during the peak period of spawning in November and December) in the two study periods for which there were reproductive data, *i.e.* 1993-95 and 2007-09. The logistic equation is as follows.

$$P = 1/\{1 + \exp(-\mathrm{ln}(19)(L - L_{50})/(L_{95} - L_{50}))\}$$

where *P* is the probability of *A. butcheri* possessing mature gonads, *ln* is natural logarithm, and L_{50} and L_{95} are the lengths at which 50 and 95% of the population are mature, respectively. The data were randomly resampled, *i.e.* bootstrapped, to create 200 sets of estimates for the parameters of the logistic equation and of the probabilities of maturity for a range of specified lengths. The 95% confidence limits of the maturity parameters and probabilities of maturity at each specified length were taken as the 0.025 and 0.975 percentiles of the 200 bootstrap estimates. A likelihood ratio test (Cerrato 1990) demonstrated that, for corresponding sexes, the L_{50}s at maturity differed between the study periods (*P* < 0.001).

Using ANCOVA, the mean monthly gonad weights (standardized for a common fish length) for females and males of *A. butcheri* ($\geq L_{50}$ at maturity for each sex) were determined for 1993-95 and 2007-09 to assess whether Black Bream spawned at the same time of year in those two study periods.

3.2.4 Estimating biomass production

Indices of biomass production for Black Bream in the Swan-Canning Estuary were determined for each of the 1993-95, 2003-04, 2007-08 and 2008-09 time periods (see below for methods of calculation) and compared. The biomass production calculations for all four time periods were based on seine net sample data for Black Bream collected at the same six sampling sites and seasons during the three studies. Biomass production estimates, calculated using 41.5 m seine net data, were based on a total of 18 Black Bream seine net samples for each time period (*i.e.* 6 samples collected in each of summer, autumn and spring).

For each of the time periods, the biomass production was estimated as follows:

(1) Estimation of the weights of each fish in the samples, using their recorded lengths and the length-weight relationship specific to the study period during which the fish was caught, and correcting for bias (associated with back transformation of the log-transformed data) (see Beauchamp and Olson 1973),

i.e. $Est_{corr} = Est_{uncorr} \exp(ms/2)$, where Est_{corr} and Est_{uncorr} are the corrected and uncorrected estimates of fish weight, respectively, and ms is the mean square value.

(2) Summation of the weights of all fish in the samples.

(3) Estimation of the length that each fish would have attained, if it had survived a further year after its date of capture, based on the von Bertalanffy growth curve specific to each study period and by adding one year to the estimated age of that fish.

(4) Estimation of the weight that each fish would have attained, if it had survived a further year, using the length-weight relationship for the relevant study period and then summation of the weights for each period.

(5) Calculation of the difference in total fish "sample" weights between the date of capture and one year after that date. Note that, as this analysis does not take into account the mortality of fish during the one year period, it is an index of potential annual biomass production rather than of actual biomass production. A. Cottingham is currently working on developing an instantaneous measure of biomass production for his PhD studies, a measure which removes any confounding influence of mortality on the estimates of the indices of biomass production.

(6) Division of the difference in sample weights calculated in 5) by the total number of fish. This provides an estimate of mean per capita annul biomass production.

(7) Use of resampling techniques to account for sources of uncertainty in growth and abundance of Black Bream in the biomass production calculations. For each study and sampling method, resampling was used to produce 200 estimates of Black Bream density (fish 100 m^{-2}), biomass production (kg 100 m^{-2} year^{-1}) and per capita biomass production (kg fish^{-1} year^{-1}).

The distributions of the abundances of Black Bream in seine net samples were always highly skewed and often contained zero values. Therefore, the analyses used for calculating Black Bream biomass production assumed the abundances of this species in seine net catches were delta log-normally distributed (Pennington 1996). WinBUGS software (Bayesian Inference using Gibbs Sampling, for Windows, version 1.4.1) was used resample the Black Bream

41.5 m seine net abundance data to generate expected distributions for the numbers of zero and non-zero catches of this species in samples, employing 1,000,000 iterations, with a lag of 1000 and a thinning interval of 250. The means Sampler lag-autocorrelation plots produced by WinBUGS were examined to assess whether convergence was likely to have been achieved.

WinBUGS was also used to produce estimates of the mean and standard deviation for von Bertalanffy growth parameters employing length-at-age data for each study period, using the same number of iterations, lag and thinning interval, as described for the above WinBUGS analysis. The priors for the growth parameters were specified as being normally distributed and as: $L_\infty = 250$ mm, tau $= 0.0001$; $k = 0.2$, tau $= 0.001$; $t_0 = 0$, 0.001. The error term for the von Bertalanffy growth model was specified as having a gamma distribution, with a mean value of 0.001, tau $= 0.001$. The mean and standard deviation values for the growth parameters were copied to Excel and used to generate multiple estimates of length at age based on parametric resampling, *i.e.* using the NORMSINV() and RAND() functions within Excel.

3.3 Results

3.3.1 Growth and body condition

The maximum length, weight and age recorded for *A. butcheri* during 1993-95 (480 mm, 2.20 kg and 21 years, respectively) were all considerably greater than the corresponding values recorded during 2007-09, *i.e.* 390 mm, 1.18 kg and 15 years, respectively (Table 3.1). The separate von Bertalanffy growth curves for each study period demonstrate that Black Bream now grow less rapidly than in the two former study periods. For example, in 1993-95, by ages 2, 4 and 6 years, Black Bream had, on average, attained lengths of 202, 304 and 360 mm, respectively, compared with 187, 249 and 274 mm, respectively, during 2003-04, and only 141, 204 and 249 mm, respectively, in 2007-09. The greater estimate for L_∞ for *A. butcheri* in 1993-95 (428 mm) than in 2007-09 (358 mm), during which periods relatively old fish were caught (> 15 years), demonstrates that the oldest Black Bream in the estuary are now typically far smaller in size (Table 3.1 and Fig. 3.2). On the basis of the von Bertalanffy growth curves, the age at which *A. butcheri* typically attained the minimum legal length for retention of 250 mm during 1993-95 (*ca* 3 years old) was less than that for 2003-04 (*ca* 4 years old) and even more so for 2007-09 (*ca* 6 years old) (Table 3.1).

Table 3.1: Parameters describing the growth of *Acanthopagrus butcheri* in the Swan-Canning Estuary collected during 1993-1995 (Sarre 1999), 2003-04 (Hoeksema and Smith, unpubl. data) and 2007-09 (current study). n refers to the number of fish caught in each period, L_{max}, W_{max} and A_{max} to the maximum total length (mm), weight (g) and age (years), respectively, and A_{MLL} to the average age (years) at which fish attain the minimum legal length of 250 mm (as determined from the von Bertalanffy growth curve for each of the three study periods). The von Bertalanffy growth parameters and their confidence limits (95% CLs) are also provided. L_∞ refers to the asymptotic length (mm), k to the growth coefficient (years^{-1}) and t_0 to the hypothetical age (years) at which a fish have zero length. r^2 refers to the coefficient of determination for the growth curve.

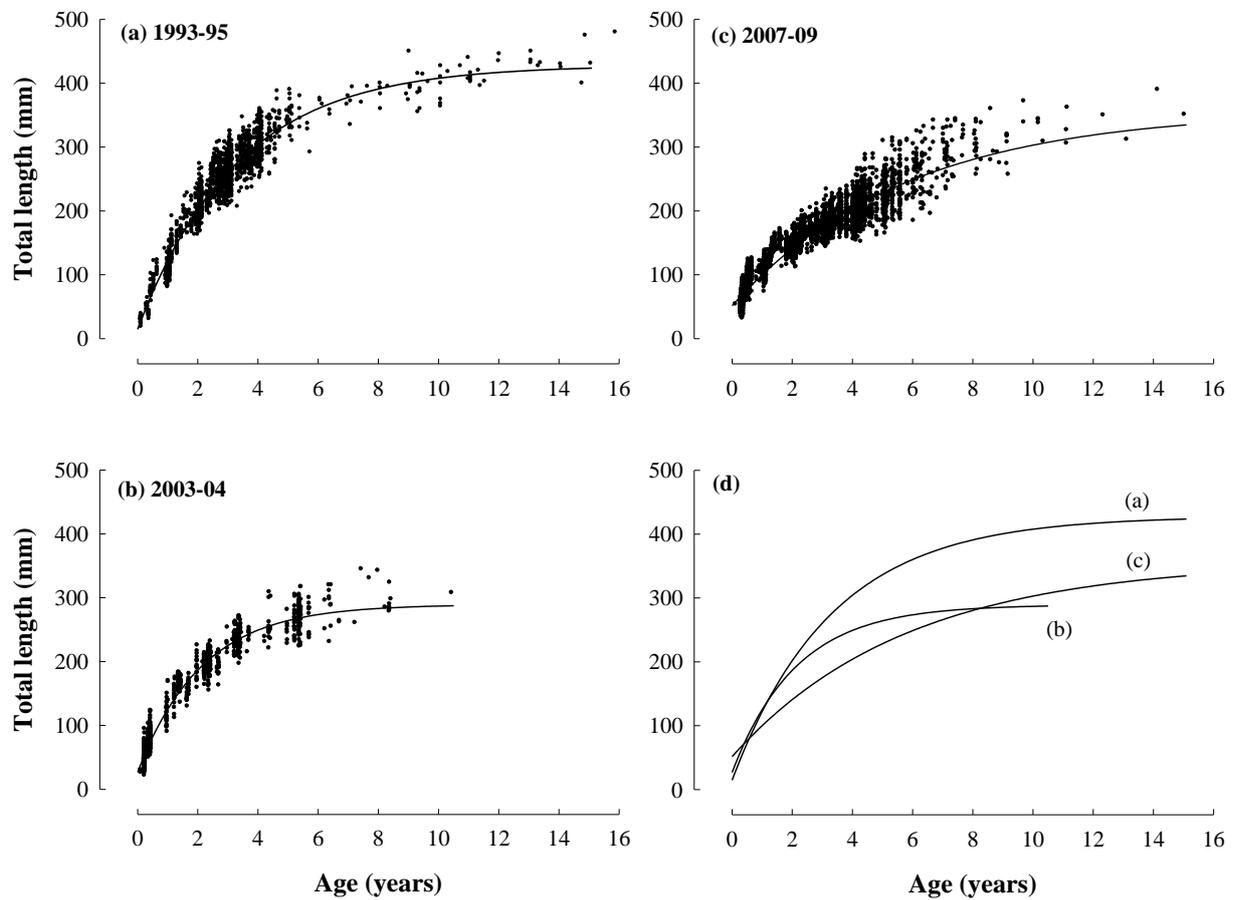| Year	95%CLs	n	L_{max}	W_{max}	A_{max}	A_{MLL}	L_∞	k	t_0	r^2
							von Bertalanffy parameters			
1993-95		1627	480	2196	21	2.7	428	0.30	-0.11	0.93
	(lower)						420	0.29	-0.14	
	(upper)						437	0.32	-0.08	
2003-04		747	n/a	n/a	n/a	4.0	288	0.47	-0.21	0.97
	(lower)						282	0.45	-0.22	
	(upper)						295	0.50	-0.19	
2007-09		4463	390	1180	15	6.0	358	0.17	-0.90	0.91
	(lower)						336	0.16	-0.96	
	(upper)						373	0.19	-0.84	

Figure 3.2: von Bertalanffy growth curves fitted to the lengths at age for *Acanthopagrus butcheri* in the Swan-Canning Estuary collected during a) 1993-95 (Sarre 1999), b) 2003-04 (Hoeksema and Smith, unpubl. data), and c) 2007-09 (current study), and d) a comparison of the growth curves for the three time periods.

ANCOVA demonstrated that the length-weight relationships for *A. butcheri* differed between 1993-95 and 2003-04 and between 1993-95 and 2007-08 (both <0.001) (Table 3.2). The values for the estimated marginal means and their 95% confidence limits (at a standardized length of 172 mm) demonstrated that, at each length, the mean weight of Black Bream in 1993-95 was greater than in either 2003-04 or 2007-09 (Table 3.2). The length-weight relationships for the three study periods were:

$$1993\text{-}95: \quad \ln W = 3.145 \ln TL - 11.703 \quad (r^2 = 0.99)$$
$$2003\text{-}04: \quad \ln W = 3.089 \ln TL - 11.497 \quad (r^2 = 0.99)$$
$$2007\text{-}09: \quad \ln W = 3.139 \ln TL - 11.737 \quad (r^2 = 0.99)$$

On the basis of the length-weight relationships, *A. butcheri* at the minimum legal length for retention (250 mm) weighed 291, 262 and 271 g in 1993-95, 2003-04 and 2007-09, respectively. Thus, on average, in 1993-95, Black Bream with a length of 250 mm, *i.e.* the current minimum legal length for this species in south-western Australia, were 11% heavier than in 2003-04 and 7% heavier than in 2007-09.

Table 3.2: Estimated mean wet body weights (g) and their 95% confidence limits (standardised for fish length of 172 mm) for *Acanthopagrus butcheri* caught in the Swan-Canning Estuary in 1993-95 (Sarre 1999), 2003-04 (Hoeksema and Smith, unpubl. data) and 2007-09 (current study).

Period	Mean weight (g)	95% confidence limit	
		Lower	Upper
1993-95	89.2	88.0	90.3
2003-04	82.2	81.1	83.2
2007-09	83.3	82.3	84.4

3.3.2 Year class strengths and age and length compositions

In all three study periods, most fish were less than six years old (Fig. 3.3). The data also show that Black Bream in the Swan-Canning Estuary exhibit considerable variability in annual recruitment strength. Thus, for example, while the 2006 year class was poorly represented in both 2007-08 and 2008-09, the 2003 year class was well represented in 2003-04, 2007-08 and 2008-09. The most recent (2008) year class appears to be particularly well represented. Note that, as different combinations of sampling methods were used to collect Black Bream in the different studies, the age compositions for Black Bream are not directly comparable. As in Fig. 3.3, the seasonal length-frequency histograms for the different year classes of Black Bream caught between the winter of 2007 and autumn of 2009 highlight the exceptional recruitment of *A. butcheri* in 2008 (Fig. 3.4). The majority of fish above the minimum legal length for capture of 250 mm in this period belong to the 2003 year class, and the remaining fish above this length are typically older than the 2003 year class (Fig. 3.4).
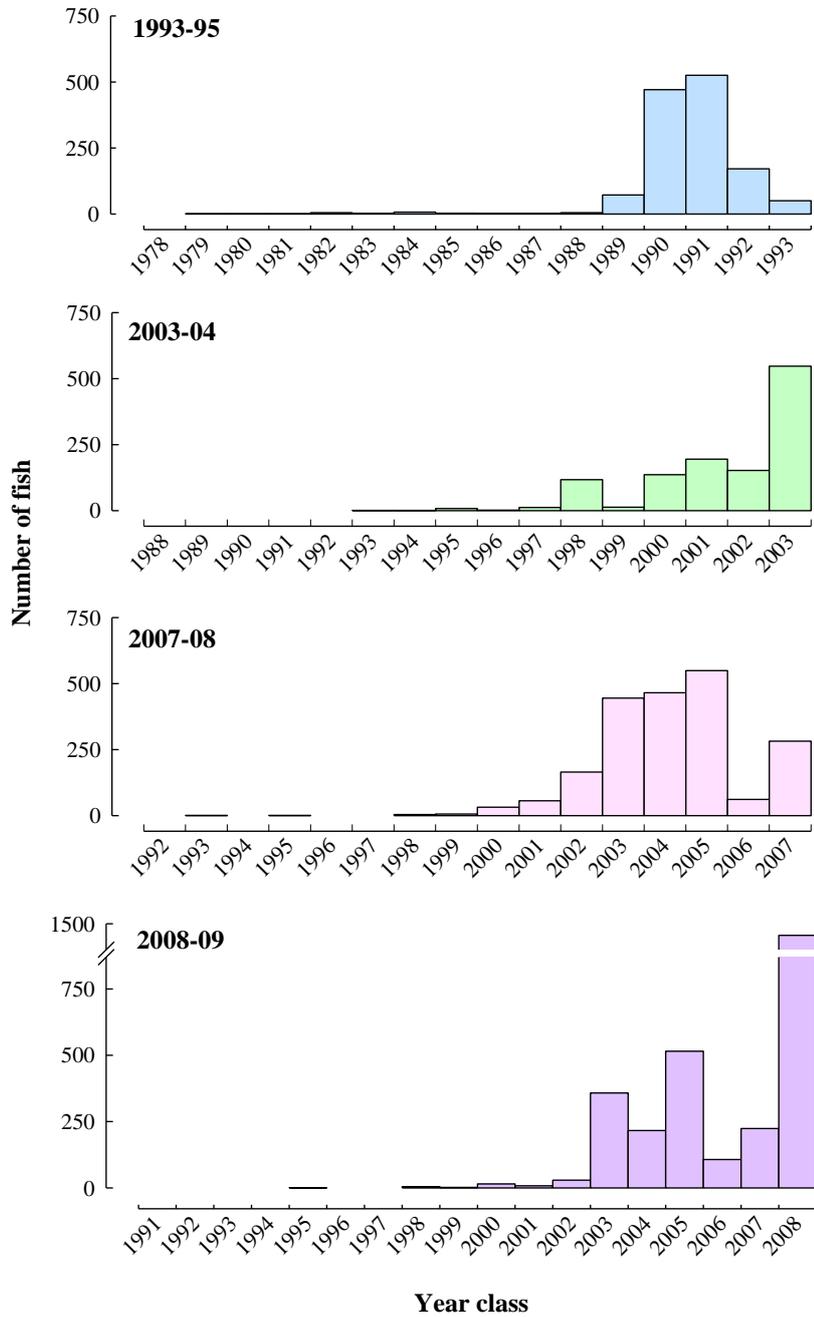
Figure 3.3: Number of *Acanthopagrus butcheri* in different year classes collected during 1993-95 (Sarre 1999), 2003-04 (Hoeksema and Smith, unpubl. data) and 2007-09 (current study) in the Swan-Canning Estuary.

Although the majority of *A. butcheri* caught by gillnetting ($n = 1709$) and rod and line fishing ($n = 184$) during 1993-95 were relatively large (modal length class 260-279 mm and 280-299 mm, respectively), fish caught by this method ranged widely in length, *i.e.* 100-479 mm (Fig. 3.5). In that period, the *A. butcheri* collected using the 41.5 m seine net ($n = 410$) were typically smaller than those caught by gillnetting (modal length class = 100-119 mm), although some fish caught by the former method were still relatively large (max length = 427 mm). The modal length class of Black Bream caught by gillnetting in 2003-04 was substantially less (220-239 mm) than in 1993-95. Furthermore, the size ranges of fish caught by gillnetting decreased from 100-479 mm in 1993-95, to 100-379 mm in 2003-04, and then to 100-329 mm in 2007-08 and 2008-09. In 2003-04, 2007-08 and 2008-09, the majority of fish had been collected using the 41.5 m seine net ($n = 59\%$, 72% and 65%, respectively). The size range of *A. butcheri* caught using the 41.5 m seine was similar in all time periods, *i.e.* 20-429 mm, and with the exception of 1993-95, encompassed all size classes that were caught by gillnet fishing (Fig. 3.5).

3.3.3 Reproduction

The mean monthly gonad weights (standardised for a total fish length of 238 mm) for female *A. butcheri* $> L_{50}$ caught in 2007-08 remained between 2.1 and 5.0 g in January to July and then increased to between 5.2 and 6.8 g through August to December (Fig. 3.6). The mean monthly gonad weights recorded for female Black Bream in the present study exhibited similar trends to those for 1993-95 (Sarre 1999), except that in the former period, they peaked at a much higher level (13.3 g) in the month of October. The trends exhibited by the mean monthly gonad weights for male *A. butcheri* in 1993-95 were virtually the same as those for females. Likewise, in 2007-08, the trends for males were similar to those for females, except that in November and December, the mean testis weights were greater than for ovaries (9.1 and 7.7 g, respectively) (Fig. 3.6).

Between March and June of 2007-08, the vast majority ($> 84\%$) of females possessed ovaries at stages I and II (Fig. 3.7). Fish with ovaries at stages III and IV during 2007-08 were first caught in May and were most prevalent in July and August (13-54%, collectively). Black Bream with ovaries at stages V and VI were caught in July and between October and January, with most being taken in October (22%) and November (64%). The vast majority of female Black Bream with ovaries at stages VII or VIII were found between November and January. The trends for male Black Bream in 2007-08 were very similar to those exhibited by females (Fig. 3.7).

During November and December of 2007, all female Black Bream < 140 mm were immature (*i.e.* possessed gonads at stages I-IV) (Fig. 3.8). Fifteen and 50% of females belonging to the 140-159 and 160-179 mm length classes, respectively, and all female *A. butcheri* >200 mm, were mature (*i.e.* possessed gonads at stages V-VIII). Similar trends were exhibited by males, with 20 and 22% of the individuals of this sex in the 120-139 and 140-159 mm length classes, respectively, being mature, and almost all males ≥ 160 mm being mature (Fig. 3.8). The
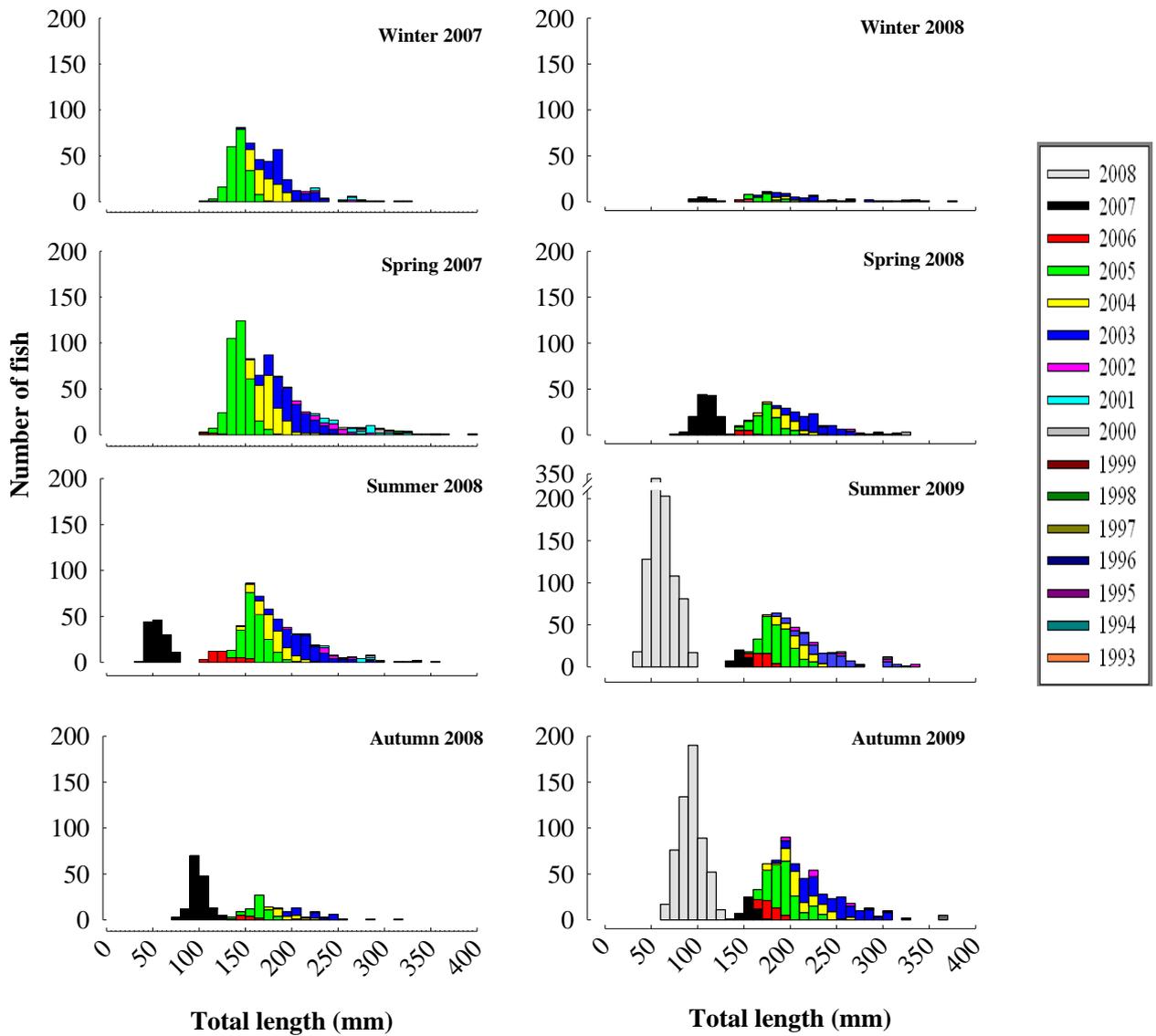
Figure 3.4: Length-frequency histograms for different year classes of *Acanthopagrus butcheri* sequential in 10 mm length classes collected from the Swan River Estuary in each season between winter 2007 and autumn 2009.
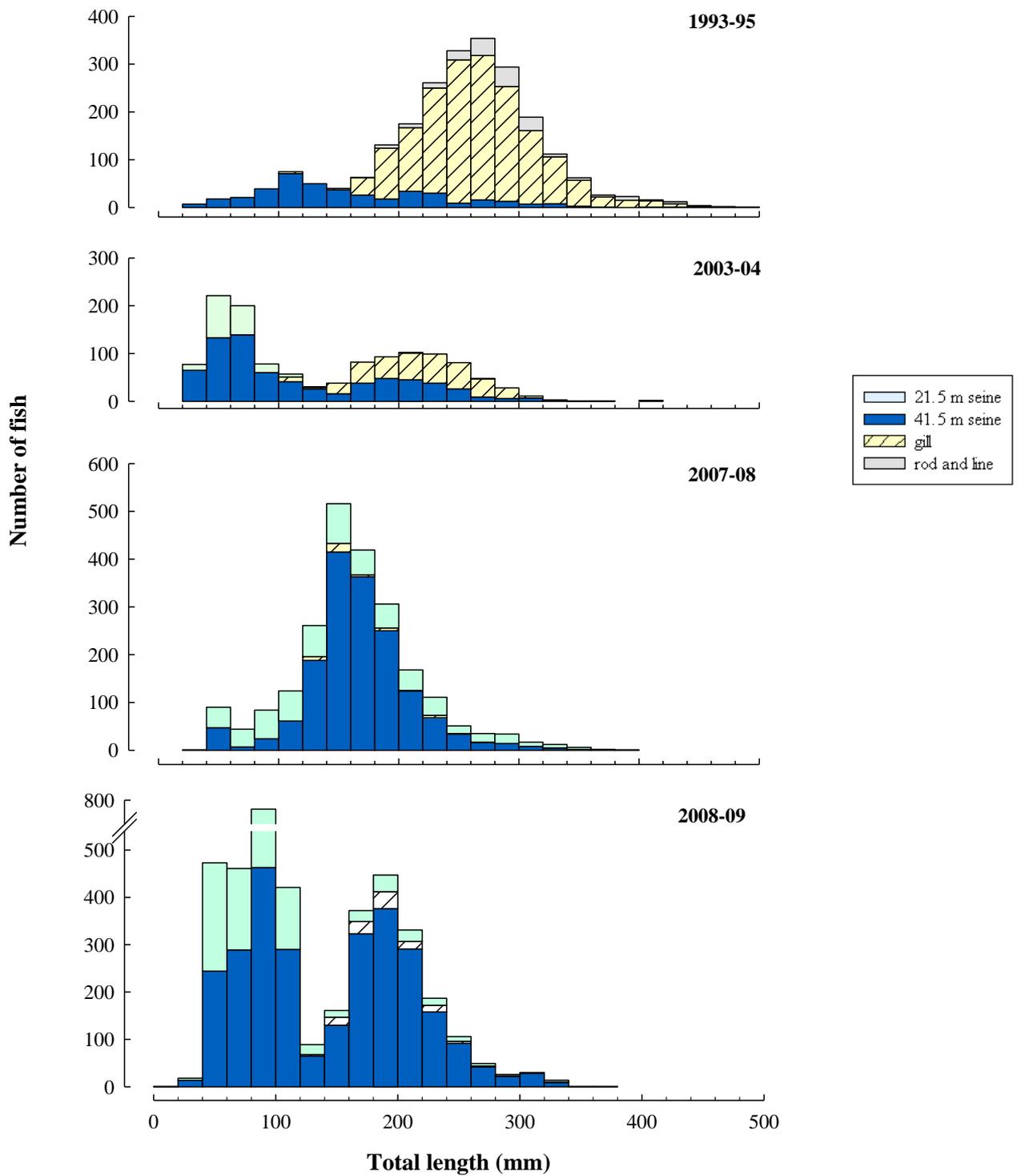
Figure 3.5: Length frequency data for *Acanthopagrus butcheri* collected by seine netting (21.5 m and 41.5 m), gillnetting and rod and line fishing in the Swan River Estuary in 1993-95 (Sarre 1999), 2003-04 (Hoeksema and Smith, unpubl. data) and 2007-09 (current study).

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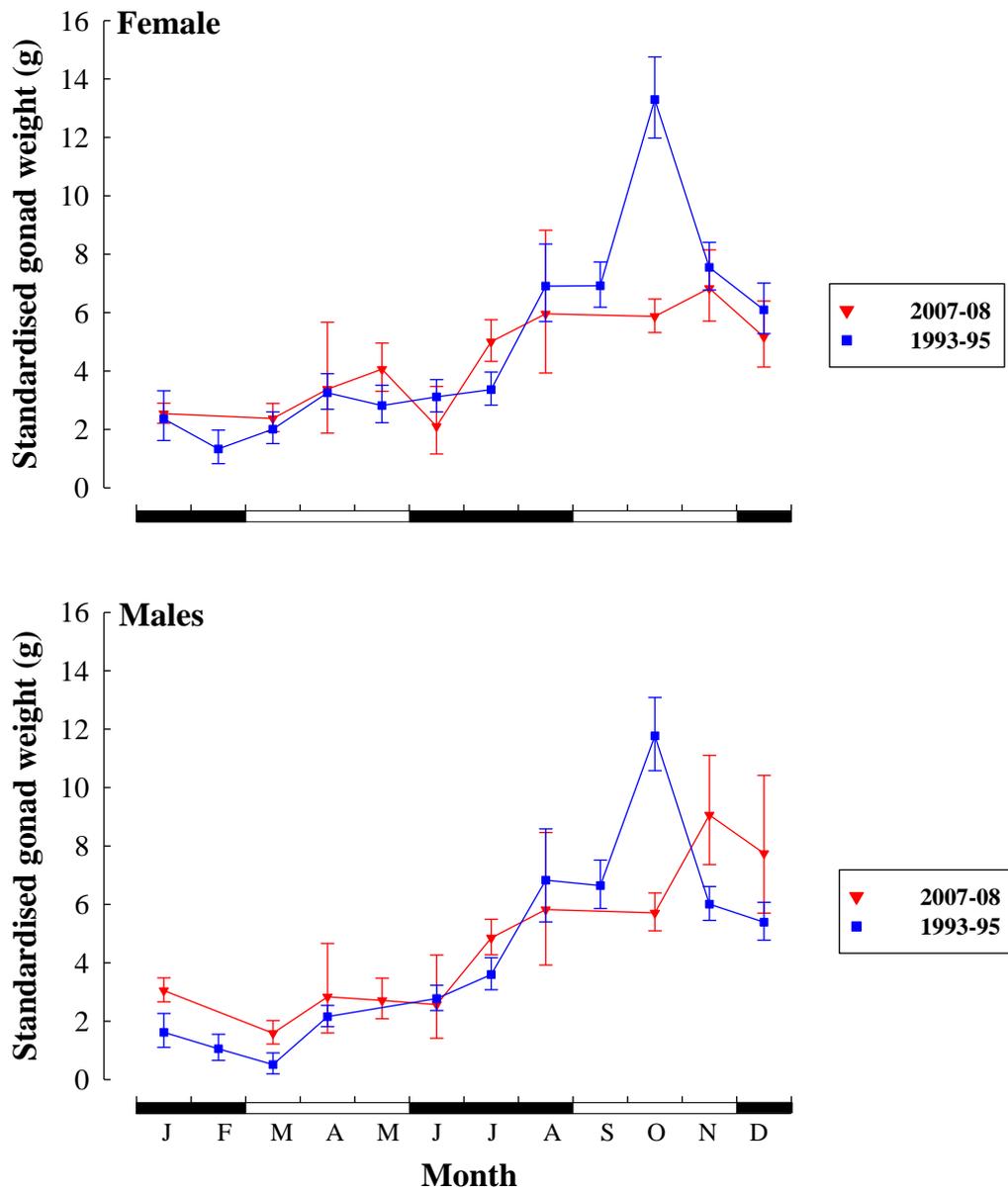
Figure 3.6: Gonad weights of female and male *Acanthopagrus butcheri* $> L_{50}$ s at maturity (see below) caught in the Swan River Estuary during 1993-95 (Sarre 1999) and 2007-08 (current study). The gonad weights for the two data sets have been standardised for fish of a common total length (238 mm) using ANCOVA.

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trends in prevalence of mature fish in sequential 20 mm length intervals in November and December of 1993 and 1994 differed substantially to the trends described for mature fish in 2007 (Fig. 3.8). Thus, in the former period, all fish <180 mm were immature and a substantial number of fish >200 mm were still immature. The situation for males was similar to that for females (Fig. 3.8).

The lengths by which 50% of females and males *A. butcheri* in 2007-08 had attained maturity (168 and 159 mm, respectively) were significantly less than those for the corresponding sexes in 1993-94 (196 and 201 mm, respectively) (both P <0.001) (Fig. 3.9). The age at which *A. butcheri* typically attained maturity in the Swan-Canning Estuary also varied between the two study periods (Fig. 3.9). During 2007-08, only 5% of females and 10% of males had matured by the end of their second year, whereas in 1993-94, as much as 64% of females and 62% of males had attained maturity by the end of their second year of life (Fig. 3.10).

3.3.4 Estimates of fish density and biomass production

The point estimate for the median density of *A. butcheri* (fish 100 m^{-2}), derived from the 41.5 m seine net data, was far less for 1993-95 (2.1) than for 2003-04, 2007-08 and 2008-09 (ranging from 13.4 – 17.5) (Table 3.3 and Fig. 3.11). The estimate for the per capita annual biomass production (kg fish^{-1} year^{-1}) for 1993-95 (0.19) was greater than for 2003-04, 2007-08 and 2008-09, *i.e.* 0.05, 0.04 and 0.02, respectively. The estimate for annual biomass production (kg 100 m^{-2} year^{-1}) was substantially greater for 2003-04 (0.95) than in 2007-08 (0.51), 2008-09 (0.35) and 1993-95 (0.39) (Table 3.3 and Fig. 3.11).

Table 3.3: Estimates of median density (fish 100 m^{-2}), per capita annual biomass production (kg fish^{-1} year^{-1}) and annual biomass production per unit area (kg 100 m^{-2} year^{-1}) and their associated 95% confidence limits for *Acanthopagrus butcheri* in the Swan-Canning Estuary, derived using 41.5 m seine net data for 1993-95 (Sarre 1999), 2003-04 (Hoeksema and Smith, unpubl. data) and 2007-08 and 2008-09 (current study).

41.5 m seine	1993-95 $\bar{x}$	Lower	Upper	2003-04 $\bar{x}$	Lower	Upper	2007-08 $\bar{x}$	Lower	Upper	2008-09 $\bar{x}$	Lower	Upper
Density	**2.1**	0.4	6.3	**17.5**	7.7	56.0	**13.4**	4.5	45.1	**15.3**	8.8	27.2
Per capita	**0.19**	0.14	0.24	**0.05**	0.05	0.06	**0.04**	0.02	0.08	**0.03**	0.01	0.06
Biomass	**0.39**	0.07	1.22	**0.95**	0.42	3.16	**0.58**	0.16	2.64	**0.45**	0.16	1.05
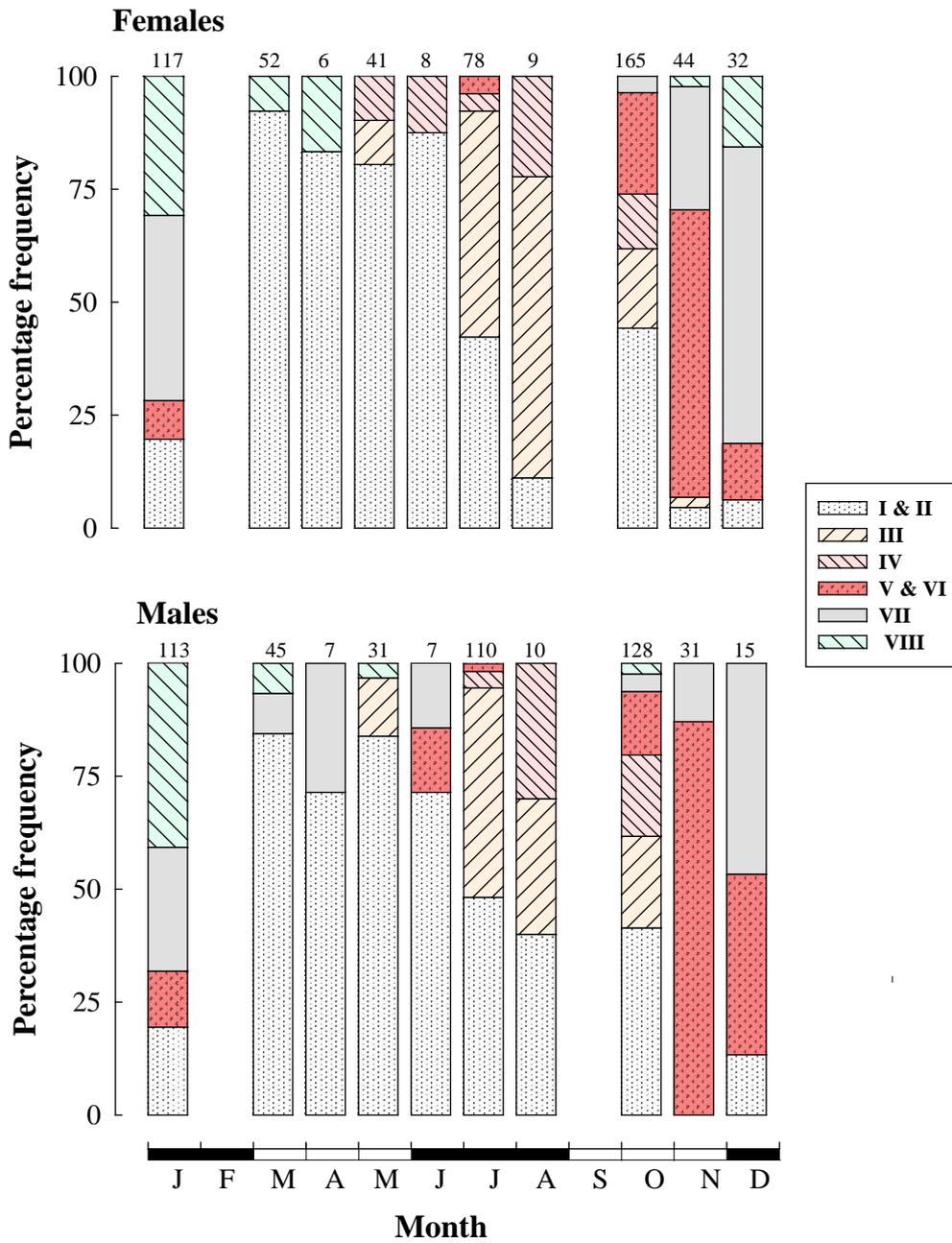
Figure 3.7: Percentage frequency of occurrence of sequential gonadal development stages in female and male *Acanthopagrus butcheri* greater than the length at maturity in the Swan-Canning Estuary between July 2007 and June 2008. Sample size given above each month. Shaded horizontal bars represent summer and winter, open horizontal bars denote autumn and spring.
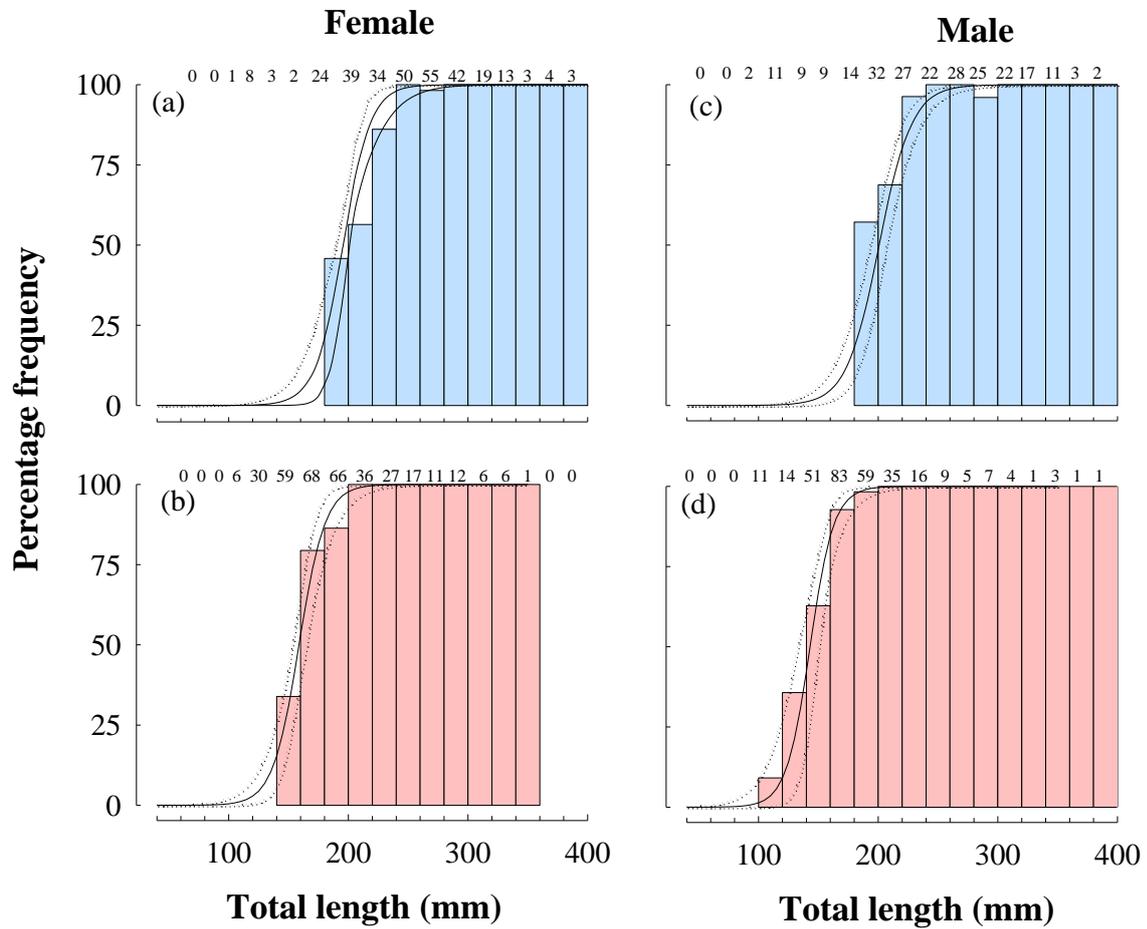
Figure 3.8: Percentage frequency of occurrence in successive 20 mm length classes of mature female and male *Acanthopagrus butcheri* (gonad stages V-VIII) in the Swan-Canning Estuary in (a,c) 1993-95 and (b,d) 2007-08. Logistic curves (solid lines) and 95% confidence limits (dotted lines) describing the probability of maturity of *A. butcheri* at each length are shown.
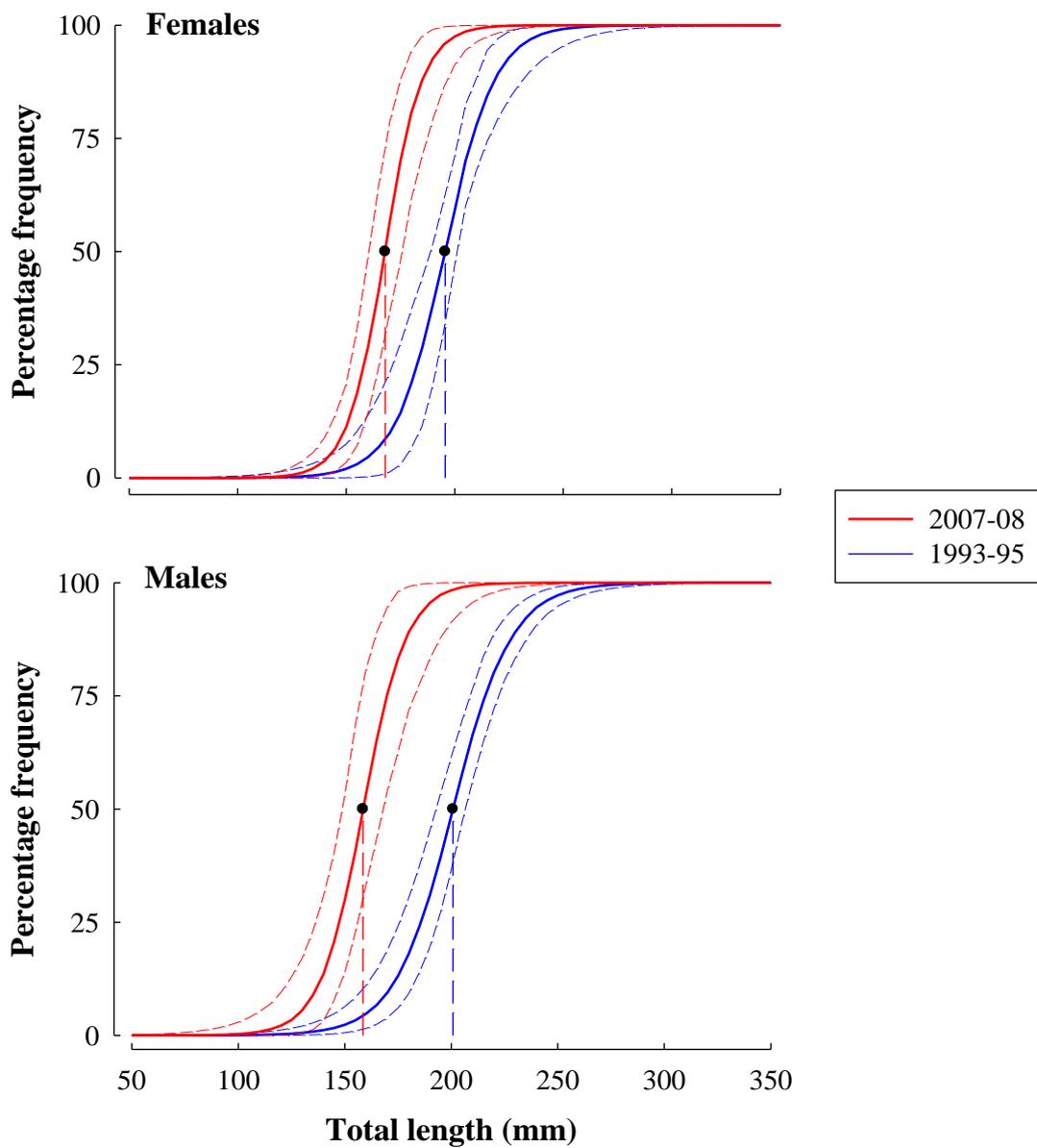
Figure 3.9: Comparison of lengths at which 50% of female and male *Acanthopagrus butcheri* collected in 1993-95 (Sarre 1999) and 2007-08 (current study) in the Swan-Canning Estuary attained maturity. Logistic curves (solid lines) and the 95% confidence limits (dotted lines) describing the probability of maturity for *A. butcheri* at each length. Vertical dashed lines highlight the L_{50}s at maturity in each study period.
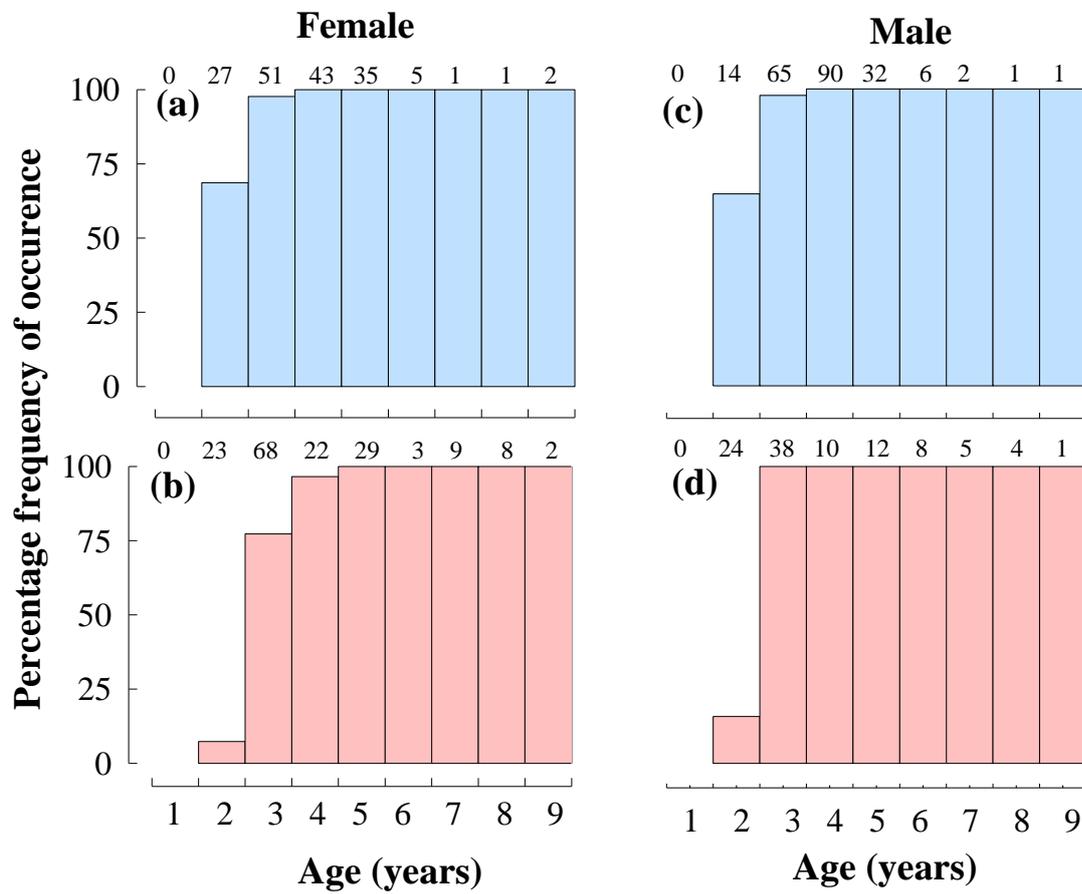
Figure 3.10: Percentage frequency of occurrence of mature *Acanthopagrus butcheri* collected in the Swan-Canning Estuary during the spawning season in 1993-95 (Sarre 1999) (a,c) and in 2007-08 (current study) (b,d).
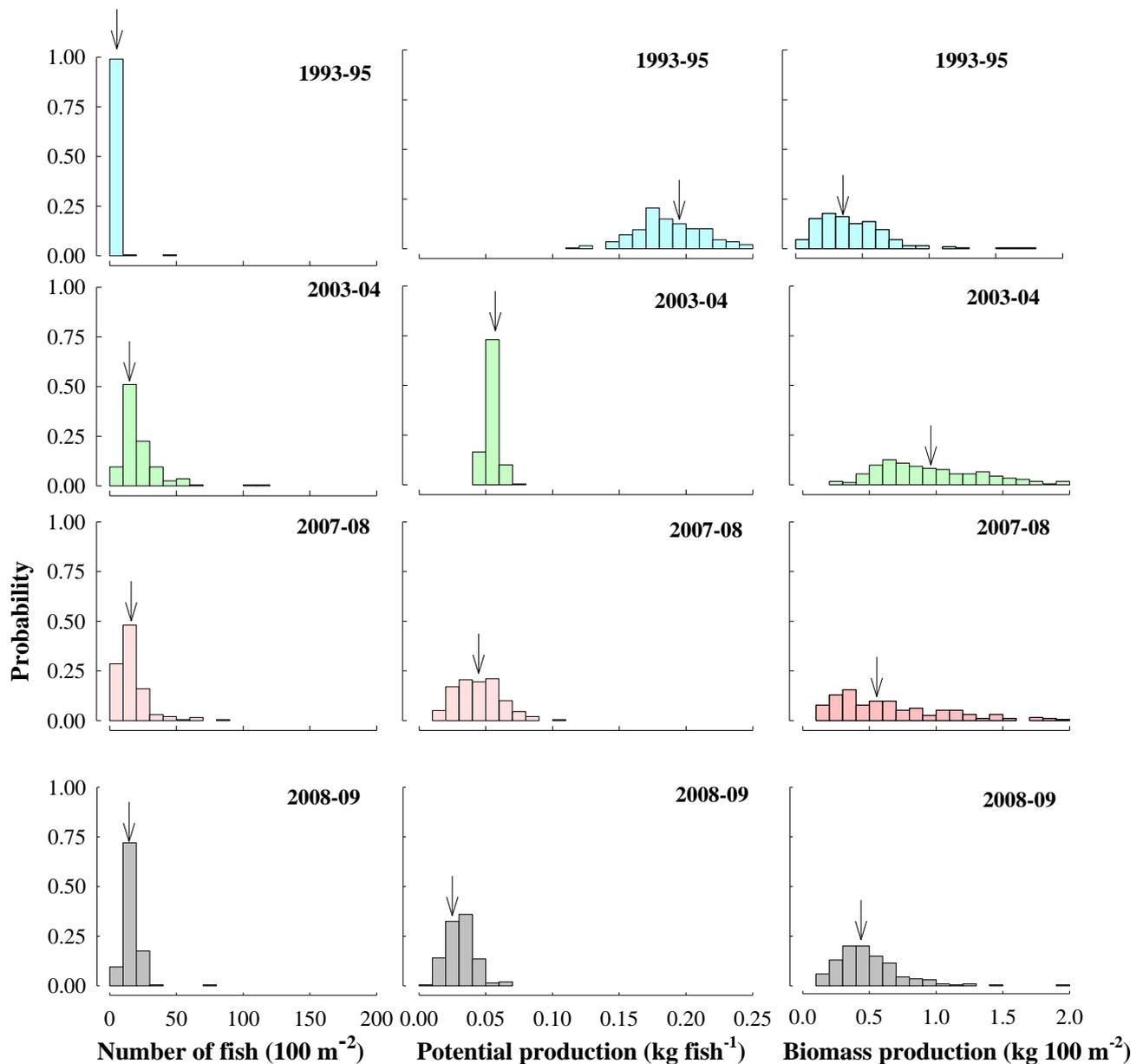
Figure 3.11: Probability distributions for estimates of densities (fish 100 m^{-2}), per capita biomass production (kg fish^{-1} year^{-1}) and biomass production per unit area (kg 100 m^{-2} year^{1}) for *Acanthopagrus butcheri* in the Swan-Canning Estuary, derived from 41.5 m seine net data during 1993-95 (Sarre 1999), 2003-04 (Hoeksema and Smith, unpubl. data) and 2007-08 and 2008-09 (current study). The distributions were determined by resampling using WinBUGS. Arrows denote median values.

3.4 Discussion

3.4.1 Changes in growth

Previous studies on Black Bream in south-western Australia have demonstrated that its pattern of growth varies markedly among estuaries (*e.g.* Sarre and Potter 2000, Hoeksema *et al.*, 2006). This study has shown that the growth of *A. butcheri* can change markedly over a relatively short period of time (~ 15 years) within the same estuary. As there is strong evidence that the growth of *A. butcheri* is influenced far more by environmental than genetic factors (Partridge *et al.* 2004) and, in particular, density dependent factors (Hoeksema *et al.* 2006), it would appear highly likely that the reduced growth performance of Black Bream in recent years is, at least to some extent, associated with its marked increase in abundance.

Our comparisons of the length and body weight data for Black Bream for different periods showed that there has been a decline in the mean weight at length of Black Bream in the Swan-Canning Estuary over the last 15 years. This apparent decline in body condition and in growth performance is likely to be related to one or a combination of the following factors including 1) a possible decline in the quantity and/or quality of suitable prey/food, 2) density-dependent factors leading to increased intra-specific competition for food and/or spatial resources and/or 3) the direct influence of environmental factors, *e.g.* water quality, on the growth of Black Bream. In considering these factors, it may be relevant that preliminary data from a current dietary study on Black Bream by PhD student Thea Linke (Murdoch University) suggest that this species in the upper Swan Estuary now consumes a far lesser volume of bivalves and a much greater volume of polychaetes than in 1993-95 (Sarre *et al.* 2000, T. Linke, unpubl. data). This finding is consistent with the results of studies on macro-benthic organisms in the same region of the estuary, showing that the abundance of the bivalve species *Xenostrobus securis* and *Fluviolanatus subtorta*, which were found by Sarre *et al.* (2000) to be key prey items of Black Bream in 1993-95, has reduced markedly (Kanandjembo *et al.* 2001b, Wildsmith 2007). Regular periods of poor water quality (anoxic conditions) in the upper Swan Estuary have been implicated as a key reason for the reduced abundances of these bivalve species (Wildsmith 2007). However, the far higher abundance of Black Bream in nearshore waters, as shown by this study, is also likely to have had an influence on the abundances of these prey. It is possible that polychaetes may represent a poorer food source for Black Bream than bivalves, particularly given the fact that this fish species exhibits a marked preference for the latter prey (Chapter 4). However, it has also been shown that Black Bream can grow rapidly on a largely vegetative diet, such as in Hamersley Inlet (Hoeksema *et al.*, 2006).

The increased abundance of Black Bream in the Swan-Canning Estuary, as determined in this study, support the hypothesis that density-dependent factors/increased intra-specific competition, may also be important in influencing the growth performance of Black Bream. Finally, as low oxygen concentration is well known to be a factor that can impact the growth and feeding of fishes (*e.g.* Kramer 1987, Pichavant *et al.* 2001), algal blooms and associated

anoxic water conditions, which now occur regularly in the Swan-Canning Estuary as a result of eutrophication (Smith 2006), may also be important factors influencing Black Bream. Furthermore, as water quality conditions will influence a range of species in the food chain other than Black Bream, such changes in water quality are likely to exert a range of indirect effects on the growth and body condition of this sparid, *e.g.* such as through the influence of water quality on the abundance of prey species.

3.4.2 Abundances of different year classes

The data on the relative abundances of fish of the different year classes of Black Bream in catches demonstrated that inter-annual recruitment of *A. butcheri* varies markedly. For example, recruitment was very low in 2006 (and to a lesser extent in 2007) but high in 2005 and 2008. It may thus be relevant that 2006 corresponded to the lowest ever recorded annual rainfall for Perth (480 mm, recorded at Perth Airport), that rainfall in 2007 (693 mm) was below the annual average of 779 mm, and that rainfall in both 2005 and 2008 was well above average (820 and 828 mm, respectively) (BoM 2010). In the case of the 1999 year class, which was low in abundance, rainfall was slightly above average for that year (793 mm). However, in January of the following year, the greatest ever amount of rainfall for January was recorded (102 mm; average for January = 8.9 mm), which lead to large scale flooding of the upper estuary and then a very large toxic algal bloom (*Microcystis*) throughout much of the estuary (see Swan River Trust 2000b). Thus, it is very possible that this unseasonal rainfall strongly impacted on the survival of the early 0+ juveniles of the 1999 year class. In summary, these preliminary comparisons of year class strength with rainfall suggest that, in the Swan-Canning Estuary, good recruitment of Black Bream is typically associated with high winter rainfall and poor recruitment with low winter rainfall. Alan Cottingham will undertake a more detailed investigation of the factors influencing annual recruitment in Black Bream for his PhD.

3.4.3 Reproductive biology

The data collected in this study and in 1993-95 by Sarre and Potter (1999) for mean monthly GSIs and the monthly prevalances of Black Bream with gonads at different maturity stages demonstrated that spawning in the two study periods occurred at essentially the same time. Comparisons of the length and age at maturity data collected in the two study periods showed, however, that Black Bream now typically mature at a far smaller size than in 1993-95 (females = 169 *vs* 218 mm, respectively, males =159 *vs* 212 mm, respectively), and at an older age (*ca* 3 *vs* 2 years, respectively, for both sexes). The changes in these variables between the two study periods suggest that attainment of maturity by Black Bream is related neither solely to length nor to age. Attainment of maturity at a smaller size by individuals in the current population of Black Bream would help compensate for a loss of egg production that would otherwise occur because of the poorer growth performance of *A. butcheri* in the Swan-Canning Estuary than in the past. The results of this study further highlight the remarkable degree to which certain biological traits of Black Bream are plastic. Thus, not only do the lengths and ages at maturity of Black Bream vary markedly among estuaries

(*e.g.* Sarre and Potter 2000, Hoeksema *et al.* 2006), but they can also vary markedly over time in the same estuary. As the individuals of *A. butcheri* in the Swan-Canning Estuary now mature at a far smaller size than in 1993-95 and well below the MLL of 250 mm, they now potentially spawn over several (typically, at least 3) spawning seasons before they can be legally caught and retained, compared with only once during 1993-95. Thus, the current MLL now offers substantial protection for the breeding stock of Black Bream from fishing pressure.

3.4.4 Black Bream density and biomass production

The 41.5 m seine net data, derived for 1993-95, 2003-04, 2007-08 and 2008-09, indicate that densities of Black Bream in nearshore waters of the Swan-Canning Estuary increased from very low levels in the earliest period to sustained far higher levels since 2003-04. Although the estimates of median density were imprecise (due to the highly skewed distribution of catches and to the restricted number of sampling sites for which there were data for all study periods), the catches have almost certainly increased since 1993-95. The increased abundance of Black Bream is consistent with a marked reduction in commercial fishing in recent years.

As is consistent with the reduced growth performance of Black Bream in recent years, the per capita annual biomass production is estimated to have declined from it maxima in 1993-95, to its lowest level in 2008-09. The same trend was not followed, however, by the estimates of annual biomass production per unit area. Thus, this latter measure of production increased markedly from the level recorded for 1993-95 to a peak in 2003-04, before declining substantially in 2007-08 and 2008-09 to a level approaching that recorded in the early period. The above trends in density, growth performance and biomass production per unit area suggest that 1) between 1993-95 and 2003-04, there was a marked increase in density which led to a marked rise in biomass production of the stock and that 2), since 2003-04, densities remained high but the growth performance of Black Bream had continued declined from already a declining level in 2003-04, leading to an reduction in overall biomass production of the population.

This study has shown that estimates of annual biomass production, when considered in the context of changes in fish density and growth, can be useful for understanding the dynamics of a fish population. Information on biomass production provides a useful complement to other information, such as age composition data. In the case of Black Bream, the data on age composition demonstrates that, in all periods, the majority of fish were young, *i.e.* ≤ 5 years. As several studies have shown that Black Bream can live for over 20 years (Potter *et al.* 2008, Sarre and Potter 2000), the paucity of fish > 5 years in the Swan-Canning Estuary suggests that this population is being subjected to substantial mortality, either from fishing or from environmental factors such as, for example, fish kill events. In this context, it may be relevant that the abundance of fish in samples collected during or after 2003-04 and which belonged to year classes that were older than 2003 were always very low. As there was a large fish kill event in 2003, which was estimated to have killed several hundred thousand fish, most of which were Black Bream, it would appear likely that this event had a major impact on the abundance of this species in the estuary at that time. Continued long term

monitoring of year class abundance is likely to yield data which can help "tease out" the relative extents to which fishing *vs* environmental factors influence the abundance of Black Bream in the Swan-Canning Estuary.

3.4.5 Implications for management

Since Black Bream now exhibit far poorer growth than in 1993-95, and there has been a substantial decline in the length at which maturity is attained, individuals can now potentially spawn over several years before they reach the MLL, compared with only once, as in 1993-95. As the abundances of Black Bream have substantially increased, at least in nearshore waters, it would appear unlikely that egg production is decreasing. In these respects, the stock of Black Bream in the Swan-Canning Estuary might be considered as very "healthy". However, the poorer growth performance of Black Bream in more recent years means that there are fewer fish above the MLL, and thus, fewer fish are available to fishers. Thus, the quality of fishing is likely to have declined.

As certain biological attributes of Black Bream, *e.g.* growth, are highly plastic, such attributes are likely to be highly sensitive to environmental change and thus could be considered to be useful as "indicators" of the condition of the environment. In the case of growth, this is particularly likely to be so given that the observed differences among various populations of this species do not appear to have a strong genetic basis. However, the biological characteristics of Black Bream are likely to be influenced by a complex suite of factors including various environmental and biotic factors, as well as fishing pressure, the effects of which are difficult to disentangle. In this regard, further studies, probably involving modeling to elucidate the ways in which these various factors interact, are likely to provide further insights into the drivers of change in the population dynamics of estuarine fish species.

The approach taken in this study to measure the productivity of the Black Bream population in the Swan-Canning Estuary represents a valuable means for monitoring the health of this stock. Estimation of biomass production provides important information regarding the status of a stock and, in the case of Black Bream which has a highly variable biology, overcomes many of the problems, *i.e.* equilibrium assumptions, associated with using traditional stock assessment methods.

Future work will focus on improving aspects of the methods developed in the study for estimating biomass production and studying changes in growth performance. For his PhD, A. Cottingham plans to modify his methods to estimate the instantaneous rate of biomass production (rather than annual biomass production) and thereby remove any confounding influences that mortality might have on comparisons of biomass production between different periods. Alan is also working on a new technique for analysing inter-annual changes in growth. This technique, which is based on the von Bertalanffy growth model, uses information in typical length-at-age data sets, such as that produced in this and in previous studies for Black Bream, to produce growth parameter estimates specific to different years. If

successful, this method will be used to track more precisely how growth has changed since 1993-95, and produce more accurate estimates of biomass production.

Chapter 4. A preliminary food web for the upper Swan-Canning Estuary, with particular emphasis on four species of estuarine fishes, *Acanthopagrus butcheri*, *Leptatherina wallacei*, *Pseudogobius olorum* and *Papillogobius punctatus*

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Executive Summary

The fish faunas of the Swan-Canning Estuary have been studied by various researchers since the late 1970s, as have its benthic (bottom-dwelling) invertebrate faunas since the 1980s. These invertebrates have been shown to be a major food source for many fish species in this estuarine system. However, few studies have attempted to examine the more complex trophic (dietary) interactions between fish species and the various other components of the estuarine flora and fauna. Determining these relationships enables a food web of the system to be constructed, which provides an extremely valuable tool for predicting how changes in the abundance of one type of biota can impact those of various others in the ecosystem.

Traditionally, fish diets have been studied by examining their gut contents. This method is effective for determining the types and quantities of prey consumed at any particular place and time (hours or days). However, it is limited in that consumed prey are difficult to identify if they are well digested, and it does not distinguish between prey that are consumed directly *vs* indirectly, the latter of which may not actually provide any nutrition to the species of interest. Increasingly, biochemical methods are being used as complementary techniques to provide further information on trophic interactions in aquatic ecosystems. These include analyses of the natural abundances of the stable isotopes of carbon (C) and nitrogen (N) in the tissues of organisms, which tend to accumulate along trophic pathways. Measurement of these isotopic "signatures" provides insight into the types of food that are actually assimilated into the consumers' tissues over longer periods (*i.e.* weeks to months), the original food source for a trophic pathway and the trophic level at which each species feeds at. Analysis of the essential fatty acids in the tissues of consumers, which are conserved in their cell membranes, provides a further avenue for tracing trophic pathways, as all consumers must derive them originally from primary producers or from organisms higher up in the food chain.

The overall aim of this component of the study was to develop a food-web for the upper Swan-Canning Estuary using both traditional gut content analyses for selected fish species and complementary biochemical analyses of those species and their prey, *i.e.* stable isotope (δ^{13}C and δ^{15}N) and essential fatty acid analyses.

The fish species chosen for this study were the large benthic-feeding Black Bream (*Acanthopagrus butcheri*), the small pelagic (water column)-feeding Western Hardyhead (*Leptatherina wallacei*) and the small benthic-feeding Blue-spot Goby (*Pseudogobius olorum*) or Red-spot Goby (*Papillogobius punctatus*). These species were sampled seasonally in the shallows of the tidal part of the Swan River between summer and spring 2007 and in the tidal reaches of the Canning River between winter 2007 and autumn 2008 and summer

and spring 2009. Samples of the potential food sources for these fish species, including benthic macroinvertebrates, plankton, microphytobenthos (microscopic bottom-dwelling algae), macroalgae, seagrass, terrestrial fringing vegetation and terrestrial insects, were collected in the summer and winter of each sampling period in both regions. In addition to the gut content analyses undertaken for each of the above fish species in the two regions, the fish and prey samples collected from the Swan River were also subjected to stable isotope and fatty acid analyses, while those from the Canning River were only subjected to stable isotope analyses due to budget constraints.

Gut content analyses

Examination of the gut contents of *A. butcheri* collected from the Swan and Canning rivers showed that this species was highly omnivorous and opportunistic in its feeding behaviour, consuming a wide range of invertebrate and algal taxa and, on occasion, other fish species. The most dominant types of prey, however, were polychaetes, bivalve molluscs, amphipod crustaceans and algae. *Acanthopagrus butcheri* showed a marked tendency to consume bivalves when they were available, feeding mainly on the galeommatid bivalve *Arthritica semen* in the Swan River and on *Sanguinolaria biradiata* (Psammobiidae) in the Canning River. It is noteworthy that the dominant bivalve preyed on by *A. butcheri* in the Swan River in 2007 has changed from that recorded about two decades ago, when it consumed mainly *Xenostrobus securis*.

Pseudogobius olorum, which was caught consistently in the Swan River but not the Canning River (while the opposite was true for *P. punctatus*), preyed mainly on nereid polychaetes, green algae, amphipods and harpactacoid copepods. This also contrasts markedly with the diet recorded for this species in the Swan River about three decades ago, which was dominated by algae and mats of bacteria and fungi. *Papillogobius punctatus* caught in the Canning River between 2007 and 2009, however, were largely carnivorous, feeding mainly on amphipods, polychaetes and copepods.

Leptatherina wallacei in both the Swan and Canning rivers were highly omnivorous and opportunistic. While their diet was dominated by calanoid copepods, other taxa such as lysianassid amphipods, harpactacoid copepods, diptera insects and nereid polychaetes were also frequently consumed. Gastropods and algae also made notable contributions to the diet of this species in the Canning River.

In both regions of the upper Swan-Canning Estuary, the greatest differences in gut content composition typically occurred between *A. butcheri* and *L. wallacei*. Although both of these species were omnivorous and opportunistic in their feeding behaviour, they tended to select prey mainly from the benthos and water column, respectively. The diet of *P. olorum* in the Swan River tended to overlap that of *A. butcheri* to some extent, while this was not the case for *P. punctatus* in the Canning River, whose diet was highly dissimilar to that of *A. butcheri* in the same region. However, the level of interspecific competition increased when the availability of preferred prey was reduced, as demonstrated by the considerable overlap in the diets of *P. punctatus* and *L. wallacei* in the Canning River in winter 2007. This was the only case in which dietary composition did not differ significantly between species in a region.

Stable isotope analyses

Three trophic levels, from the lowest aquatic primary producer (typically phytoplankton) to the highest order consumer (a species of fish), were detected in the Swan and Canning rivers from the $\delta^{15}N$ signatures of the various plant and animal components of the ecosystem. In

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both of these regions, the $\delta^{15}N$ values for *A. butcheri*, *P. olorum*, *P. punctatus* and *L. wallacei* were always very similar and were typically also similar to that of the carid shrimp *Palaemonetes australis*. These findings suggest that this invertebrate species is also a highly opportunistic omnivore. It is noteworthy that the $\delta^{15}N$ values for each of the above species were greater than those of the same or similar species in the Nornalup-Walpole and Leschenault estuaries. This may be a consequence of (i) the shorter trophic pathways from primary producer to fish in the Nornalup-Walpole and Leschenault, and therefore a difference in fish feeding behaviour between these systems and the Swan-Canning Estuary and/or (ii) a greater exposure to anthropogenic sources of nutrients in the severely modified Swan-Canning Estuary compared to the largely unmodified Nornalup-Walpole Estuary.

Examination of the $\delta^{13}C$ values of the various ecosystem components in the Swan River strongly suggests that this system is supported mainly by carbon derived from freshwater/terrestrial sources. This was demonstrated by the fact that the $\delta^{13}C$ values of aquatic primary producers, invertebrates and fish in this region were typically similar and relatively depleted. In contrast, the Canning River is supported mainly by marine-derived carbon, as indicated by the relatively wide ranging and typically higher $\delta^{13}C$ values of the ecosystem components in this region.

Fatty acid analyses
The fatty acid compositions of muscle tissue from *A. butcheri*, *P. olorum* and *L. wallacei* in the Swan River were broadly similar, thus reflecting the opportunistic and omnivorous feeding behaviours of each of these species. Despite these overall similarities, examination of the full suite of fatty acids contributing > 1% to the total fatty acid content for each fish species showed that they do display some degree of preferential feeding.

Food webs
The following preliminary food webs have been constructed for the Swan and Canning rivers using the collective trophic information that was obtained during this study. While these food webs clearly depict how *A. butcheri*, *L. wallacei*, *P. olorum* and/or *P. punctatus* interact with primary producers and invertebrate species, they do not illustrate the pathways between primary producers and higher level consumers unless they are direct.

It is recommended that, in order for future studies to capture the wider structure and complexity of the food webs in this estuarine system, all food sources should be identified to the highest possible taxonomic level and that the greatest number of species should be examined. This will ensure that the intricacies of trophic interactions between species, which may not be apparent at coarser levels of examination, are able to be more fully revealed.

Preliminary food web for the tidal reaches of the Swan River, based on traditional gut content, stable isotope and fatty acid analyses of samples collected in summer to spring 2007. Direct trophic interactions with ecosystem components and *A. butcheri* depicted with a solid line, with *P. olorum* a short-dashed line and with *L. wallacei* a long-dashed line.

Preliminary food web for the tidal reaches of the Canning River, based on traditional gut content, stable isotope and fatty acid analyses of samples collected in winter 2007 to autumn 2008 and in summer to spring 2009. Direct trophic interactions with ecosystem components and *Acanthopagrus butcheri* depicted with a solid line, with *Papillogobius punctatus* a short-dashed line and with *Leptatherina wallacei* a long-dashed line.

4.1 Introduction

Estuaries are traditionally a focal point for a diverse range of anthropogenic activities. The catchments of these systems are commonly cleared for agricultural purposes, the land surrounding the estuary basins and lower tributaries are typically highly modified to accommodate urban development (*e.g.* McComb and Lukatelich 1995, Hodgkin and Hesp 1998) and the estuaries themselves often support substantial fisheries (*e.g.* Haedrich and Hall 1976, Lenanton and Potter 1987). As a consequence, very few estuaries remain unmodified and globally, are now considered the most degraded of all temperate marine ecosystems (Jackson *et al.* 2001), a fact that is particularly relevant in south-western Australia where only three estuarine systems remain classified as near pristine (Brearley 2005).

Despite this, estuaries are amongst the most productive of all ecosystems (Schelske and Odum 1961), supporting complex and diverse ecosystems, although the extent of this productivity is typically augmented by the degree of anthropogenic influence. Thus, for example, temperate estuaries constitute important nursery areas for a number of marine fish species, with the high productivity of these systems supporting rapid growth of juvenile fishes and thereby reducing their susceptibility to predation (Kennish 1990). Unlike most temperate estuaries elsewhere in the world however, those of south-western Australia also support a number of species that can complete their entire life-cycle within these systems and which are often highly abundant (*e.g.* Loneragan and Potter 1990, Potter and Hyndes 1999, Hoeksema and Potter 2006, Hoeksema *et al.* 2009).

The estuarine environment is highly dynamic, varying both temporally and spatially in its physico-chemical characteristics (*e.g.* Chuwen *et al.* 2009a) and this variability is often reflected in its fauna. As a consequence, the immigration of juveniles of marine fish species to estuaries and the recruitment of estuarine species occur at times when conditions within the estuary are most conducive to the survival and development of the individuals of these species (Potter and Hyndes 1999, Hoeksema and Potter 2006). Thus, the composition of fish faunas in temperate estuaries often undergo pronounced cyclical changes throughout the year due to the time-staggered recruitment and immigration and emigration of various species and can vary markedly between years when environmental conditions in those years differ conspicuously (*e.g.* Potter *et al.* 1986, Young and Potter 2003, Hoeksema and Potter 2006).

The estuaries in south-western Australia typically comprise a wide, central basin, a narrow entrance channel and the lower reaches of their tributary rivers (*e.g.* Potter and Hyndes 1999, Chuwen *et al.* 2009a, Potter *et al.* 2010) and these broad regions are often characterised by distinct ichthyofaunas. Thus, for example, the estuarine sparid *Acanthopagrus butcheri* and the marine mugilid *Mugil cephalus* have been shown to dominate the fish faunas of the deeper and more offshore waters of the tributary rivers of estuaries along the south coast of Western Australia (Chuwen *et al.* 2009b).

The ichthyofaunas of estuaries in south-western Australia have increasingly been the focus of scientific study over the past three decades (see Potter and Hyndes 1999). While these studies have extensively detailed the composition of these faunas and their temporal and spatial variability, few studies have attempted to describe the interactions between the various species that comprise these ichthyofaunas and the various other components that constitute the estuarine flora and fauna, *e.g.* primary producers and invertebrates (*e.g.* Svensson *et al.* 2007). Thus, for example, the composition of the fish fauna in the large, permanently-open Swan-Canning Estuary, has been examined on a number of occasions with varying degrees of spatial and temporal replication (*e.g.* Loneragan *et al.* 1989, Lonergan and Potter 1990, Kanadjembo *et al.* 2001a, Hoeksema and Potter 2006, Valesini *et al.* 2009), as has the composition of various components of the benthic fauna (*e.g.* Chalmer *et al.* 1976, Kanandjembo *et al.* 2001b, Valesini *et al.* 2009). Although two of these studies (Kanandjembo *et al.* 2001a, b, Valesini *et al.* 2009) have concurrently detailed the characteristics of multiple components of the estuarine fauna, *i.e.* fish and benthic invertebrates, neither has attempted to elucidate the interactions between these faunal components.

Furthermore, various aspects of the biology of a number of fish species that utilise estuarine systems in south-western Australia have been examined, with these species specific investigations sometimes also examining the diets of those species (*e.g.* Chubb *et al.* 1981, Chubb and Potter 1984, Chrystal *et al.* 1985, Nel *et al.* 1985, Potter *et al.* 1988, Laurenson *et al.* 1993, Wise *et al.* 1994, Chuwen *et al.*, 2007). Thus, for instance, the biology and diets of *A. butcheri* and two other highly abundant estuarine species, the gobiid *Pseudogobius olorum* and the atherinid *Leptatherina wallacei*, have been described in the Swan-Canning Estuary (Prince *et al.* 1982, Prince and Potter 1983, Gill and Potter 1993, Gill *et al.* 1996, Sarre and Potter 2000, Sarre *et al.* 2000). It should be noted however, that these detailed studies were conducted separately for each species and during vastly different periods, *i.e.* 1993-95, 1983-85 and 1978-80, respectively.

The application of traditional gut content analyses in such studies enabled the diet and thus the direct interactions between certain fish species and various other components of estuarine ecosystems to be determined. While this method is effective at demonstrating these direct interactions, it is limited in that consumed prey are often digested and excreted rapidly and thus the content of a given stomach may only represent prey ingested by a fish over a short period of time, *i.e.* hours or days, and soft-bodied prey may be underestimated. Furthermore, this technique cannot discriminate between prey that are targeted by a species and those that are consumed as a consequence of foraging behaviour and do not contribute to the nutrition of that species, *e.g.* algae consumed while foraging for associated invertebrates.

Increasingly, the examination of the natural abundance of the stable isotopes of carbon (C) and nitrogen (N) is being employed as a complementary technique to gut content analysis to further elucidate trophic interactions in aquatic ecosystems, *e.g.* Svensson *et al.* (2007), Abrantes and Sheaves (2008, 2009), Maier and Simenstad (2009) and Vorwerk and Froneman (2009). During the process of digestion and assimilation, the heavy isotopes of C

and N are discriminated against and, as a consequence, tend to be accumulated along trophic pathways (DeNiro and Epstein 1978, 1981), thus providing insight into the assimilation of material by consumers over a more protracted period, *i.e.* weeks to months (*e.g.* Peterson and Fry 1987). Thus, the signature for $\delta^{13}C$, which in essence is the ratio between the abundance of the C^{13} and C^{12} isotopes, varies little along trophic pathways, *i.e.* an enrichment of *ca* 1‰ with each increase in trophic level, and thus is a useful indicator of the original source of carbon for that pathway (DeNiro and Epstein 1978, Fry 2006). Unlike $\delta^{13}C$, the signature for $\delta^{15}N$, the ratio between the N^{15} and N^{14} isotopes, changes in a predictable manner along trophic pathways, increasing in a stepwise enrichment of *ca* 3.5‰ with each trophic level, and thus acts as a useful indicator of the trophic feeding level of an organism from a defined baseline, typically a primary producer (DeNiro and Epstein 1980, Vander Zanden and Rasmussen 1999, Fry 2006).

A further complimentary method that is increasingly being employed is that examining the composition of fatty acids in aquatic organisms, *e.g.* Richoux and Froneman (2008), Crawley *et al*. (2009) and Hanson *et al*. (2010). Unlike primary producers, that are able to synthesize fatty acids *de novo*, consumers derive all their lipid requirements either directly through the consumption of other organisms or indirectly through the transformation of proteins and carbohydrate precursors (Richoux and Froneman 2008). Lipids, particularly polyunsaturated fatty acids, are crucial for the effective functioning of organisms as they form fundamental structural components in cell membranes and perform vital functional roles within those membranes (Art 1999). Thus, fatty acids that cannot be synthesized by animals are considered essential and must be derived from primary producers or through trophic pathways and, as such, are highly conserved in aquatic ecosystems (Arts *et al*. 2001). Fatty acid composition can therefore be used, when considered conservatively, to trace trophic pathways in aquatic ecosystems and identify crucial trophic interactions (*e.g.* Dalsgaard *et al*. 2003).

In order to begin to elucidate the interactions between the various aquatic components of the Swan-Canning ecosystem, the general complexity of estuarine systems and the unique characteristics of estuaries in south-western Australia must first be taken into account. Thus, the current study selected a suite of fish species that complete their life cycles within the Swan-Canning Estuary and which are abundant within the upper reaches of that system throughout the year. Furthermore, the main biotic components that comprise the Swan-Canning ecosystem, and which may also represent elements of the trophic pathways of the selected fish species, were sampled concurrently. The two morphologically distinct regions that constitute the upper estuary, *i.e.* the saline, lower reaches of the Swan and Canning rivers, were sampled seasonally and, where possible, over a similar period, to further reduce the influence of spatial and temporal variability. Lastly, the complimentary methods of traditional gut content, stable isotope and fatty acid analyses were employed together, where possible, to detail the biotic interactions within the Swan-Canning estuarine ecosystem.

The aims of this study were thus as follows.

(1) Determine the diets of *Acanthopagrus butcheri*, *Leptatherina wallacei* and *Pseudogobius olorum* in the Swan River, and of the former two species and *Papillogobius punctatus* in the Canning River, using traditional gut content analyses. These data will be used to examine how those diets are influenced by season and, where relevant, region.

(2) Determine the trophic levels of the above four fish species and their potential prey, and the ultimate sources of carbon that form the basis of the Swan and Canning river ecosystems, using stable isotopes $\delta^{13}C$ and $\delta^{15}N$.

(3) Identify those components of the diets of the above fish species in the Swan River that are assimilated over longer periods of time, as indicated by the fatty acid compositions of their tissues.

(4) Construct preliminary food webs for the tidal reaches of the Swan and Canning rivers using the collective information derived from the above three aims.

4.2 Materials and Methods

4.2.1 Sampling of fish and their prey

Fish were sampled seasonally by seine net from three replicate sites in the nearshore, shallow waters of two regions of the Swan-Canning Estuary between summer and spring 2007 in the upper Swan River, and in the Canning River between winter 2007 and autumn 2008 and summer and spring 2009 (Fig. 4.1). For convenience, these three sampling periods will hereafter be referred to as the periods Swan River 2007 and Canning River 2007/08 and 2009, respectively. The seine net, which consisted of two 10 m long wings (6 m of 9 mm mesh and 4 m of 3 mm mesh) and a 1.5 m wide bunt made of 3 mm mesh, was laid parallel to the shore and then hauled on to the bank, fishing to a maximum depth of 1.5 m and sweeping an area of 116 m^2. Upon hauling the net, up to 10 individuals of each target species of fish were immediately placed in an ice slurry before being returned to the laboratory, with the remainder of the catch being released live back into the water.

The fish species targeted in both the upper Swan and Canning rivers were the large, benthic-feeding Black Bream *Acanthopagrus butcheri* and the small pelagic-feeding Western Hardyhead *Leptatherina wallacei*. Due to inconsistent catches between the two regions of a small benthic-feeding species of fish, the Blue-spot Goby *Pseudogobius olorum* was targeted in the upper Swan River and another gobiid *Papillogobius punctatus* was collected from the Canning River. Both of these gobiids are small and share similar life-history traits (*cf* Gill and Potter 1993, Gill 1996).

Prey items were collected in the summer and winter of each period from the upper Swan and Canning rivers, with individual items being collected from across the three replicate sites to collectively represent each region. Benthic macroinvertebrates were collected using an 11 cm diameter cylindrical steel sediment corer, which sampled sediment to a depth of 10 cm and covered a surface area of 96 cm^2. Benthic macroinvertebrate samples were immediately wet-sieved through a 500 μm mesh to discard any fine sedimentary material and placed in an ice slurry until being separated further under a dissecting microscope in the laboratory. The hyperbenthic fauna was sampled using a benthic sled that comprised a rectangular steel frame (50 cm wide x 25 cm high) mounted on two runners that maintained its base at *ca* 3 cm above the substrate surface, and which had a steel 'lip' at its entrance to effectively collect fauna resting on the substrate. A plankton net, that was 1.45 m in length and consisted of 150 μm mesh, was attached to the frame and tapered gradually from its mouth to a cod-end comprising an 11 cm diameter Perspex cylinder with a 150 μm mesh draining port. The sled was towed manually for 50 m along a subtidal transect that lay parallel to the shoreline. A General Oceanics flowmeter was attached at the entrance of the net to record the volume of water filtered during each replicate tow.

Plankton samples were collected by pouring *ca* 100 L of water from each site through a series of sieves of sequentially decreasing mesh sizes, *i.e.* 250, 74, 50, 20 and 5 μm mesh. The
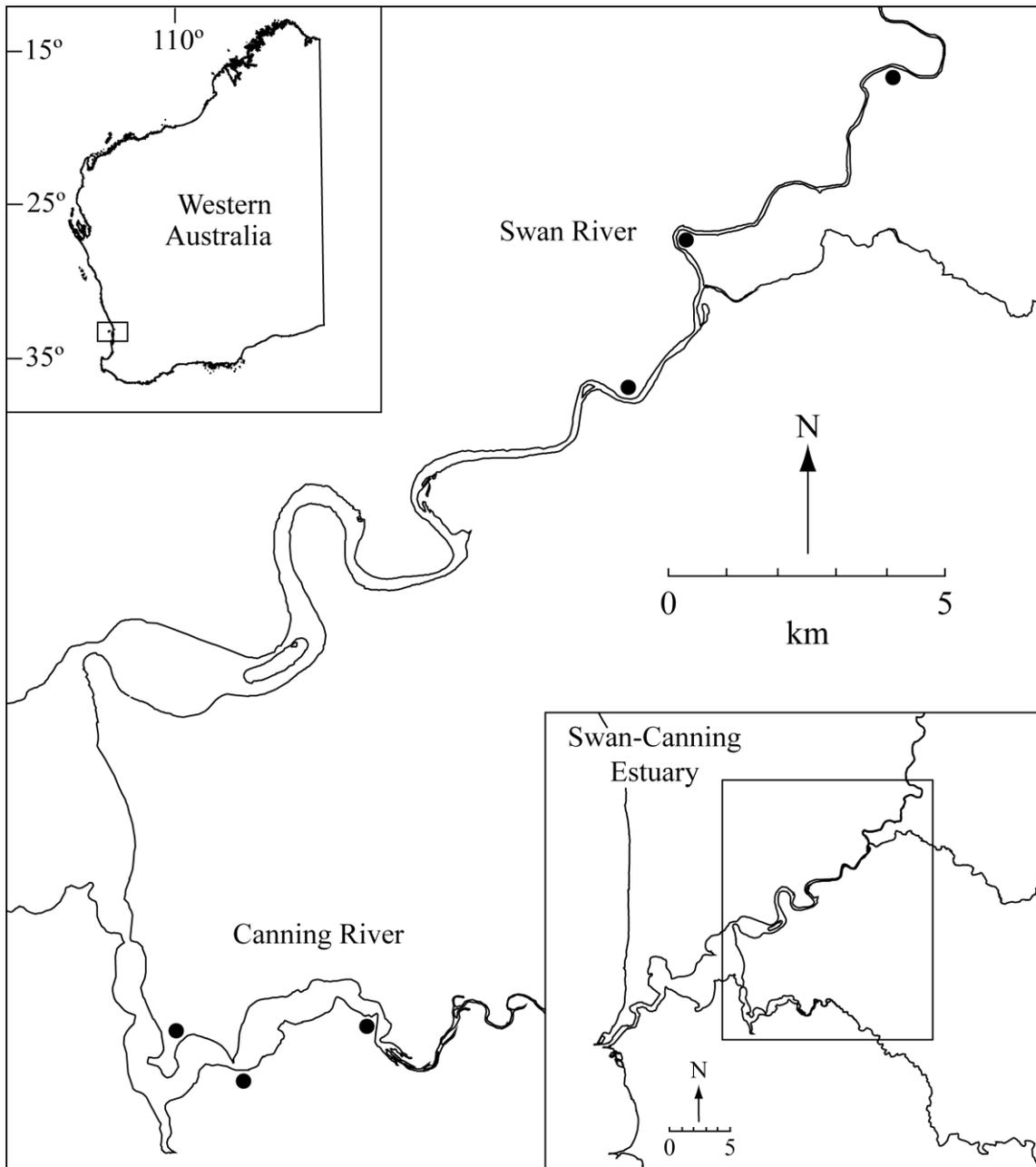
Figure 4.1: Map showing the location of the sampling sites in the Swan and Canning rivers (●) and the location of the sampling regions within the Swan-Canning Estuary (inset box lower right) and of the estuary in Western Australia (inset box upper left).

material retained on each sieve was then washed with distilled water onto Glass Fiber/F (GF/F) filters. Microphytobenthos was collected by scraping the top layer (*ca* 2 cm) of sediment into a container, agitating the contents and filtering the supernatant onto GF/F filters. Filter papers holding plankton and microphytobenthos samples were sealed and stored in aluminum foil.

Macrophyte and seagrass samples were collected by hand when present at a site, as were samples of terrestrial fringing vegetation. Samples were rinsed with distilled water before being stored. Terrestrial insects were collected at night using a 60 L barrel that was lined with plastic and filled with loosely balled paper that prevented insects from escaping once they had entered the barrel. A fluorescent light was placed inside the barrel as an attractant.

On each sampling occasion and at each site, salinity, water temperature and dissolved oxygen concentrations were measured at the middle of the water column using a Yellow Springs Instrument 556 water quality meter.

4.2.2 Gut content analyses

4.2.2.1 Laboratory procedures
The total length (TL) of each fish was measured to the nearest mm and the weight of each individual measured to the nearest g. To avoid pronounced variability due to onotogenic shifts in diet, larvae and early juveniles were not selected for gut content analyses (as with stable isotope and fatty acid analyses). As such, only individuals that appreciably exceeded the known size of settlement for *A. butcheri*, *i.e.* 11.7 mm, *P. olorum*, *i.e.* 8.2 mm, and *P. punctatus*, *i.e.* 10 mm (Neira *et al*., 1998) where included and in the case of *L. wallacei*, for which the size at settlement is not documented, the smallest size recorded in detailed biological or ecological studies of the species, *i.e.* 11 mm (e.g. Prince and Potter, 1983; Hoeksema and Potter, 2006). Furthermore, *A. butcheri* less than 80 mm were excluded due to a developmental difference in eye morphology that may influence feeding behaviour (Shand *et al*., 2000) and therefore diet. The length of *A. butcheri* examined for gut content analyses (and also stable isotope and fatty acid analyses) thus ranged from 80 – 330 mm, that of *P. olorum* from 14 – 59 mm, *L. wallacei* from 22 – 64 mm and *P. punctatus* from 17 – 62 mm.

The stomach, in the case of *A. butcheri*, and the whole digestive tract (stomach and intestine), in the case of *L. wallacei*, *P. olorum* and *P. punctatus*, was removed from each fish and stored in 70% ethanol, before its contents were examined under a dissecting microscope. The fullness of each gut was scored on a scale from 0 (empty) to 10 (fully distended). Note that empty stomachs or those that contained only sediment or unidentifiable material were not considered for dietary analyses. The contents of each gut were identified to the lowest possible taxon and then allocated to one of a number of broader taxonomic groups, subsequently referred to as dietary categories, and also to a dietary subcategory, typically an Order or Family.

The frequency of occurrence of each dietary category and subcategory in the gut of each fish (%F) was recorded and, using the percentage cover method (Hyslop 1980), the contribution by volume of each category to the total volume of the gut contents of each fish (%V) was determined.

4.2.2.2 Statistical analyses

The overall mean percent volumetric contributions of the main dietary categories to the diets of each species of fish in the different regions and sampling periods were calculated, their sum adjusted to 100% and plotted as histograms. Main dietary categories were considered those that consistently contributed to the diets of fish and which made a substantial (>2.5%) contribution to the diet of at least one species in a given region and sampling period, *i.e.* Polychaeta, Bivalvia, Gastropoda, Copepoda, Amphipoda, Insecta, Teleostei and algae. The same procedure was then applied to the mean seasonal diets of each species in the Swan and Canning rivers in each sampling period.

The suite of dietary categories subjected to multivariate analyses was expanded to include other dietary categories or sub-categories that made an appreciable (>1.0%) contribution to the diet of at least one species in a given region and sampling period or which made a unique contribution to the diet of a species. This suite thus included 21 dietary categories or sub-categories, *i.e.* Rotifera, Polychaeta, Bivalvia, Gastropoda, Copepoda, Ostracoda, Amphipoda, Isopoda, Tanaidacea, Caridea, Penaeidae, Brachyura, Crustacean larvae, Araneae, Insecta, Teleostei, algae, terrestrial plants, Cyanobacteria, seagrass and detritus.

Before being subjected to multivariate analyses, gut content data were randomly averaged, employing three individuals of a species in a given region and season, to produce the "replicate samples" used in all subsequent analyses. This procedure was employed as the guts of individual fish frequently contained only a few of the 21 dietary categories or sub-categories and thus, when considered separately, considerably increased the variability of a species' diet. Given the short feeding period the contents of an individual fish's gut represents and the inherent within species variability this creates, the randomisation procedure was considered appropriate to examine, in a statistically robust manner, dietary differences between species.

The percent volumetric contributions of each dietary category/sub-category in each replicate sample of the Swan River and Canning River in winter and spring 2007 were square root transformed and used to construct a Bray-Curtis resemblance matrix using PRIMER v6.1.2 (Clarke and Gorley 2006). The resultant matrix was subjected to three-way permutational analysis of variance (PERMANONVA) (Anderson *et al.* 2008) to facilitate a preliminary exploration of the influence of species, region and season on dietary composition and whether there were any interactions between those main effects. That matrix was then subjected to non-metric multidimensional scaling (nMDS) ordination (Clarke 1993) to visually explore any significant effects and interaction terms. On the basis of the results of the PERMANOVA analysis and given the limited overlap in sampling periods, subsequent analyses were

conducted separately for each region and sampling period, *i.e.* Swan River 2007, Canning River 2007/08 and Canning River 2009.

Separate matrices were thus constructed from the square root transformed percent volumetric contributions of each dietary category/sub-category in each replicate sample of the Swan River between summer and spring 2007, the Canning River between winter 2007 and autumn 2008 and the Canning River between summer and spring 2009. In each region and period, two-way crossed Analyses of Similarity (ANOSIM) tests (Clarke 1993) were used to determine whether the dietary composition of the three species were significantly different and whether these differences were significantly related to season. Particular emphasis was placed on the *R*-statistic (*R*) in all ANOSIM tests, with *R* values close to unity demonstrating that the composition of *a priori* groups of samples are very different and those close to 0 showing that such groups are highly similar. When pairwise comparisons in an ANOSIM test detected significant differences between the *a priori* levels of the above factors, those differences were illustrated by selectively subjecting matrices refined by factor to nMDS ordination and described by subjecting the same matrices to Similarity Percentages (SIMPER) in order to determine the dietary categories/sub-categories that best typified the diet of those *a priori* groups and those that best distinguished them (Clarke 1993).

4.2.3 Stable isotope analyses- laboratory procedures

White muscle tissue was dissected from the dorsal surface of six individuals of each target fish species in each season of each period and placed in a plastic eppendorf vial that was transferred directly to a drying oven for 24 hours at 60°C. Samples were then sealed and stored in an air-tight glass desiccator that contained silica gel dry-beads until further stable isotope analyses. The same procedure was applied to all prey items collected in the summer and winter of each period, with the exceptions that a sample of muscle tissue was collected from all bivalve, gastropod and caridea species following the removal of shells or exoskeletons, smaller prey species, *e.g.* copepods, were used whole and often pooled from a site to produce an appropriate sample size and a portion of primary producers was isolated and used as a sample. For the purpose of stable isotope analyses, when collected, terrestrial plants were separated into small riparian vegetation (SRV), *i.e.* reeds and sedges, and large riparian vegetation (LRV), *i.e.* fringing shrubs and trees.

Plankton samples were fumigated with 32% HCl in a desiccator for 24 hours prior to further analyses to remove inorganic sources of carbon. This procedure was shown to be required only for plankton samples, by subjecting a range of different dietary items, *e.g.* decapods, isopods, microphytobenthos, detritus and the various plankton fractions, to $\delta^{13}C$ analysis prior to and following acid treatment and then employing ANOVA to detect significant differences in $C^{13/12}$ signatures (data not shown) (see Yamamuro *et al.* 1993). As acid treatment can influence the nitrogen stable isotope content of a sample, a sub-sample of each plankton sample was removed prior to fumigation and prepared separately for $\delta^{15}N$ analysis (see Rolff 2000).

After drying, samples (excluding those on GF/F filter papers) were ground to a fine powder in a mortar and pestle, weighed (animal tissue: 1 mg, plant tissue: 2-3 mg, sediment: 10-75 mg) and enclosed in tin capsules for stable isotope analysis. The stable isotopic ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ for all samples were determined using a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the University of California Davis Stable Isotope Facility, Davis, California, USA. All stable isotope results were reported as $\delta^{13}C$ and $\delta^{15}N$ relative to Pee Dee belemnite and atmospheric nitrogen, respectively (Paul et al. 2007), according to the following equation.

$$\delta^H X = \left[\left(\frac{R_{SAMPLE}}{R_{STANDARD}} - 1\right)\right] * 1000$$

where X = the element being measured, H = the heavy isotope mass, R = ratio of the heavy and light isotopes of the element being measured, *e.g.* $^{13}C/^{12}C$ or $^{15}N/^{14}N$ and 1000 = an amplification coefficient.

4.2.4 Fatty acid analyses

4.2.4.1 Laboratory procedures
Samples for fatty acid analyses were collected from the Swan River in the summer and winter of 2007 following the same procedures as those described for stable isotope samples. Samples were stored at -80°C in glass vials with teflon coated lids, before being freeze-dried (lyophilisation) for 24 hours and transferred, in thick-walled styrofoam containers filled with dry ice, to the Institute for Hydrobiology and Fisheries Science at the University of Hamburg, Germany, for further processing.

Samples were again lyophilised, before their dry mass was determined using a Sartorius micro-balance (±2μg). During the weighing procedures, samples were stored in a desiccator to prevent unequal condensation on the dried tissue.

Lipids were quantitatively extracted from samples following a modification of the method of Folch *et al.* (1957), as described in Hagen (2000), using an ultrasonic disruption in a 2:1 (v:v) solution of dichloromethane:methanol and a washing procedure using aqueous KCl solution (0.88%). In the case of large tissue samples (>10 mg), 8 ml of dichloromethane:methanol and 2 ml of KCl solution were used, while for samples <10 mg, only 4 and 1 ml of each solution, respectively, was required. Prior to extraction, tricosanoic acid was added to samples, as an internal standard, to enable fatty acids to be quantified. In the case of fish tissue samples, a Potter homogeniser was also employed prior to ultrasonic disruption to ensure the effective extraction of lipids. Following disruption of the tissue samples, the supernatant was separated, transferred to a clean vial and placed in a centrifuge for 10 min at 2500 r/m and 2°C. The resultant lower, lipid-containing phase was then isolated and

evaporated under nitrogen, before being combined with 1 ml of dichloromethane:methanol solution.

For fatty acid analyses, a subsample (100 µl) of the total lipid solution was hydrolysed and the fatty acids converted to their methyl ester derivatives in methanol containing 3% concentrated sulfuric acid at 80°C for 4 h (Kattner and Fricke 1986). After cooling, 2 ml of Aqua bidest. was added and three repeated extractions of the fatty acid methyl esters (FAMEs) conducted using 1 ml hexane. Samples were analysed using a gas chromatograph (HP 6890A) equipped with a DBFFAP column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness) that was operated by a defined temperature program and utilised helium as carrier gas. Samples were injected using a hot split/splitless inlet (250°C, split mode 1:20) or a programmable temperature vaporiser injector (solvent vent mode). FAMEs and fatty alcohols were detected by flame ionisation and identified by comparing the retention times of samples against those derived from standards of known composition. The accuracy of substance identifications was checked for selected peaks using gas chromatographic-mass spectrometry (GC-MS).

The abbreviated fatty acid nomenclature used, *i.e.* z:y(n-x), follows that assigned by the IUPAC-IUB Commission on Biochemical Nomenclature (1978) where z = number of carbon atoms, y = number of double bonds, n = chain length and x = position of the double bond closest to the terminal methyl group.

4.2.4.2 Statistical analyses

The percent contributions of each fatty acid to the total fatty acid content in each replicate sample of tissue from *A. butcheri*, *P. olorum* and *L. wallacei* in the Swan River in summer and winter 2007 were square root transformed and used to construct a Bray-Curtis resemblance matrix using PRIMER v6.1.2 (Clarke and Gorley 2006). Two-way crossed ANOSIM tests (Clarke 1993) were used to determine whether the compositions of fatty acids in the three species were significantly different and whether these differences were significantly related to season. When pairwise comparisons in an ANOSIM test detected significant interspecific or seasonal differences, those differences were illustrated subjecting matrices, refined by factor, to nMDS ordination and described by subjecting the same matrices to SIMPER to determine the suite of fatty acids that best typified the fatty acid content of the three species and that which best distinguished them (Clarke 1993).

4.3 Results

4.3.1 Environmental variables

Salinity followed a similar trend in the Swan River in 2007 and in the Canning River in 2007/08 reaching its maximum in summer and decreasing to its minimum in the winter of both periods. The maximum salinity attained in the Canning River, however, was greater than that of the Swan River, *i.e.* 33 *vs* 25, as was the minima attained in winter, *i.e.* 13 *vs* 5. Salinity in the Canning River in 2009 was high in summer, but reached its peak of 35.9 in autumn and only decreased slightly in winter before a precipitously decline to its minima of 6.9 in spring (Fig. 4.2).

Water temperature followed a similar trend in both the Swan and Canning rivers in each period, attaining its maximum of between 24.4 to 27.9°C in summer and its minimum of between 14.6 and 16.3°C in winter. Trends in dissolved oxygen concentration however, varied markedly between the three periods. Thus, concentrations in the Swan River in 2007 were greatest in winter (9.4 mg L^{-1}) and least in summer (4.9 mg L^{-1}), while the reverse pertained in the Canning River in 2009 and those in the Canning River in 2007/08 varied little throughout the year.

4.3.2 Diets of *A. butcheri, L. wallacei* and *P. olorum* or *P. punctatus* in the Swan and Canning rivers in 2007-08

4.3.2.1 Diets of A. butcheri, P. olorum *and* L. wallacei *in the Swan River in 2007*

The diet of *Acanthopagrus butcheri* in the Swan River in 2007 was largely dominated by three main dietary categories, *i.e.* Polychaeta, Bivalvia and Amphipoda (Table 4.1, Fig. 4.3). While this species consumed a range of different polychaetes, *i.e.* Nereididae, Orbiniidae and Serpulidae, the first of these made by far the greatest contribution (13.6%) to the overall diet and was present in over 40% of guts examined (Table 4.1). *Acanthopagrus butcheri* also consumed a number of different bivalves, with the Galeommatidae being consumed regularly and contributing 9.8% to the overall diet of this species, but with the Mytilid and Tellinid bivalves also making appreciable contributions (Table 4.1). Furthermore, Corophiid and Aorid amphipods also made appreciable contributions to the diet of *A. butcheri*, *i.e.* 7.4 and 5.6%, respectively. It is also noteworthy that, collectively, algae contributed 7.8% to the diet of *A. butcheri* and a small contribution was made by teleosts (Fig. 4.3).

Although polychaetes, bivalves and amphipods were each consumed by *A. butcheri* throughout the year, the relative contribution of each of these main dietary categories differed between seasons. Thus, while polychaete and amphipod consumption peaked in autumn, that of bivalves was least in that season (Fig. 4.4). Furthermore, algae consumption was greatest in summer and spring, while the few teleosts were consumed mostly in winter and spring (Fig. 4.4).

Table 4.1: Percent frequency of occurrence (%F) and percent volumetric contribution (%V) of dietary categories and sub-categories to the overall mean diets of *Acanthopagrus butcheri*, *Pseudogobius olorum* and *Leptatherina wallacei* in the Swan River in 2007 and of *A. butcheri*, *Papillogobius punctatus* and *L. wallacei* in Canning River in 2007-08 and 2009.

	Swan River						Canning River											
	Summer – Spring 2007						Winter 2007 – Autumn 2008						Summer – Spring 2009					
	A. butcheri		P. olorum		L. wallacei		A. butcheri		P. punctatus		L. wallacei		A. butcheri		P. punctatus		L. wallacei	
	%F	%V	%F	%V	%F	%V	%F	%V	%F	%V	%F	%V	%F	%V	%F	%V	%F	%V
Rotifera*	-	-	-	-	4.5	0.1	-	-	-	-	8.0	2.0	-	-	-	-	-	-
Annelida	63.3	25.1	49.2	24.1	32.4	5.7	32.3	8.7	40.2	15.1	17.7	4.3	29.9	8.7	30.9	10.5	-	-
Polychaeta*	63.3	25.1	49.2	24.1	32.4	5.7	32.3	8.7	40.2	15.1	17.7	4.3	29.9	8.7	30.9	10.5	-	-
Nereididae	41.8	13.6	22.9	13.1	1.8	1.3	18.3	5.9	11.8	5.8	4.4	1.7	18.2	4.4	23.6	8.5	-	-
Orbiniidae	6.1	3.2	-	-	-	-	8.6	1.5	-	-	-	-	5.2	0.6	0.9	0.1	-	-
Serpulidae	5.1	1.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
unid. polychaetes	18.4	6.5	28.0	11.0	30.6	4.4	11.8	1.3	28.4	9.2	13.3	2.6	9.1	3.7	6.4	1.9	-	-
Nematoda	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.6	<0.1	1.0	<0.1
Mollusca	63.3	19.7	8.5	1.6	30.6	2.9	65.6	37.4	1.0	<0.1	30.1	5.4	49.4	25.4	0.9	0.1	44.2	1.7
Bivalvia*	61.2	19.4	8.5	1.6	29.7	2.8	64.5	37.2	1.0	<0.1	9.7	1.3	48.1	25.2	0.9	0.1	-	-
Galeommatidae	45.9	9.8	6.8	1.4	28.8	2.4	7.5	0.7	1.0	<0.1	9.7	1.3	2.6	0.3	0.9	0.1	-	-
Mytilidae	15.3	4.1	1.7	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tellinidae	10.2	3.1	-	-	-	-	11.8	5.5	-	-	-	-	2.6	0.4	-	-	-	-
Psammobiidae	-	-	-	-	-	-	36.6	24.9	-	-	-	-	27.3	18.6	-	-	-	-
Mactridae	5.1	2.0	-	-	-	-	3.2	0.5	-	-	-	-	2.6	0.9	-	-	-	-
unid. bivalves	5.1	0.4	-	-	1.8	0.4	14.0	5.4	-	-	-	-	14.3	5.0	-	-	-	-
Gastropoda*	3.1	0.3	-	-	3.6	0.1	2.2	0.2	-	-	27.4	4.1	2.6	0.2	-	-	-	-
Hydrobiidae	1.0	<0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Batillaridae	-	-	-	-	-	-	-	-	-	-	-	-	2.6	0.2	-	-	-	-
unid. gastropods	3.1	0.2	-	-	3.6	0.1	2.2	0.2	-	-	27.4	4.1	-	-	-	-	-	-
juvenile molluscs*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	44.2	1.7
Arthropoda	73.5	24.9	74.6	36.6	82.9	46.0	45.2	12.1	93.1	64.1	94.7	62.3	66.2	19.7	88.2	51.7	74.0	39.8
Crustacea	72.4	23.9	74.6	34.9	79.3	38.0	44.1	10.9	93.1	64.1	89.4	51.2	62.3	18.3	88.2	51.7	72.1	29.2
Copepoda*	-	-	43.2	7.3	57.7	17.9	-	-	43.1	14.5	63.7	19.0	-	-	48.2	16.3	58.7	20.1
Calanoida	-	-	7.6	0.3	45.9	15.9	-	-	24.5	8.5	31.9	11.1	-	-	14.5	6.0	36.5	13.8
Cyclopoida	-	-	1.7	0.1	-	-	-	-	13.7	4.9	6.2	2.7	-	-	32.7	4.7	28.8	2.7
Harpacticoida	-	-	38.1	6.8	20.7	2.0	-	-	16.7	1.1	38.1	5.3	-	-	32.7	4.7	28.8	2.7
unid. copepods	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.5	0.8	5.8	0.4
copepod eggs/nauplii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.9	<0.1	18.3	0.6
Ostracoda*	-	-	12.7	2.8	1.8	0.3	-	-	1.0	0.7	10.6	0.7	-	-	3.6	0.6	8.7	0.7
Amphipoda*	61.2	19.3	28.0	10.5	9.9	5.9	33.3	7.9	46.1	30.0	23.9	13.2	61.0	16.6	63.6	34.0	18.3	7.3

	Swan River						Canning River											
	Summer – Spring 2007						Winter 2007 – Autumn 2008						Summer – Spring 2009					
	A. butcheri		*P. olorum*		*L. wallacei*		*A. butcheri*		*P. punctatus*		*L. wallacei*		*A. butcheri*		*P. punctatus*		*L. wallacei*	
	%F	%V	%F	%V	%F	%V	%F	%V	%F	%V	%F	%V	%F	%V	%F	%V	%F	%V
Lysianassidae	4.1	0.2	1.7	0.3	7.2	3.2	1.1	0.1	-	-	-	-	-	-	-	-	-	-
Corophiidae	43.9	7.4	11.9	3.1	3.6	1.1	19.4	2.4	6.9	1.4	5.3	1.2	31.2	4.1	9.1	5.3	1.9	0.5
Aoridae	21.4	5.6	-	-	-	-	6.5	0.5	3.9	0.8	5.3	2.1	-	-	-	-	-	-
unid. amphipods	26.5	6.0	17.8	7.1	4.5	1.7	22.6	4.9	43.1	27.8	20.4	9.9	41.6	12.5	60.0	28.7	16.3	6.9
Isopoda*	6.1	0.4	5.1	1.1	5.4	0.6	-	-	-	-	0.9	0.5	1.3	0.1	-	-	1.9	0.9
Tanaidacea*	-	-	-	-	-	-	1.1	<0.1	-	-	-	-	-	-	-	-	-	-
Caridea*	-	-	-	-	0.9	0.3	-	-	-	-	-	-	2.6	1.6	-	-	-	-
Penaeidae*	2.0	0.7	-	-	-	-	3.2	1.2	-	-	-	-	-	-	-	-	-	-
Brachyura*	1.0	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Crustacean larvae*	-	-	-	-	6.3	1.9	-	-	-	-	0.9	0.1	-	-	-	-	-	-
unid. crustaceans	13.3	3.0	32.2	13.1	32.4	11.1	10.8	1.8	32.4	18.9	44.2	17.6	1.3	<0.1	0.9	0.9	1.0	0.1
Chelicerata	**1.0**	**0.5**	**0.8**	**0.3**	**2.7**	**0.2**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Araneae*	1.0	0.5	0.8	0.3	2.7	0.2	-	-	-	-	-	-	-	-	-	-	-	-
Insecta*	**12.2**	**0.5**	**8.5**	**1.5**	**24.3**	**7.9**	**2.2**	**1.2**	**-**	**-**	**19.5**	**11.1**	**3.9**	**1.4**	**-**	**-**	**26.0**	**10.7**
Diptera	-	-	0.8	0.1	6.3	1.4	2.2	0.7	-	-	15.0	7.6	-	-	-	-	18.3	7.8
Hymenoptera	1.0	<0.1	0.8	0.1	3.6	0.8	0.0	0.0	-	-	4.4	1.7	-	-	-	-	5.8	1.4
Lepidoptera	-	-	-	-	0.9	0.3	-	-	-	-	-	-	-	-	-	-	-	-
Thysanoptera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.0	<0.1
Cicadidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.0	0.3
unid. insects	2.0	<0.1	-	-	14.4	3.2	-	-	-	-	2.7	0.7	-	-	-	-	3.8	1.2
insect larvae	11.2	0.4	7.6	1.3	8.1	2.2	2.2	0.5	-	-	1.8	1.1	3.9	1.4	-	-	-	-
Chordata	**6.1**	**1.3**	**-**	**-**	**-**	**-**	**1.1**	**1.0**	**-**	**-**	**-**	**-**	**2.6**	**2.5**	**0.9**	**0.2**	**-**	**-**
Teleostei*	6.1	1.3	-	-	-	-	1.1	1.0	-	-	-	-	2.6	2.5	0.9	0.2	-	-
Algae*	**22.4**	**7.8**	**33.1**	**10.7**	**9.0**	**0.8**	**40.9**	**23.4**	**2.9**	**1.1**	**7.1**	**1.7**	**23.4**	**13.2**	**0.9**	**0.1**	**51.9**	**12.3**
Bacillariophyceae	7.1	1.0	12.7	1.1	8.1	0.6	2.2	0.1	1.0	<0.1	6.2	0.3	-	-	-	-	2.9	0.1
Cladophoraceae	15.3	5.7	25.4	9.6	-	-	1.1	0.9	2.0	1.1	0.9	0.8	5.2	1.2	-	-	-	-
Ulvaceae	4.1	0.6	-	-	-	-	5.4	2.2	-	-	-	-	-	-	-	-	-	-
Cystocloniaceae	-	-	-	-	-	-	29.0	16.2	-	-	-	-	-	-	-	-	-	-
Rhodomelaceae	2.0	0.4	1.7	0.1	0.9	0.2	9.7	3.9	-	-	1.8	0.7	-	-	-	-	-	-
Dictyotaceae	-	-	-	-	-	-	1.1	0.2	-	-	-	-	-	-	-	-	-	-
Chrysophyceae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	35.6	9.7
Gracilariaceae	-	-	-	-	-	-	-	-	-	-	-	-	19.5	10.7	-	-	-	-
unid. algae	-	-	-	-	-	-	-	-	-	-	-	-	1.3	1.3	0.9	0.1	-	-
unid. phytoplankton	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	37.5	2.5
Other	**80.6**	**21.3**	**71.2**	**26.9**	**75.7**	**44.4**	**68.8**	**17.2**	**63.7**	**19.7**	**62.8**	**23.4**	**67.5**	**29.9**	**90.9**	**39.3**	**80.8**	**46.8**

| | Swan River Summer – Spring 2007 | | | | | | Canning River Winter 2007 – Autumn 2008 | | | | | | Canning River Summer – Spring 2009 | | | | | |
| | A. butcheri | | P. olorum | | L. wallacei | | A. butcheri | | P. punctatus | | L. wallacei | | A. butcheri | | P. punctatus | | L. wallacei | |
	%F	%V	%F	%V	%F	%V	%F	%V	%F	%V	%F	%V	%F	%V	%F	%V	%F	%V
terrestrial plants*	24.5	4.6	11.9	0.6	2.7	0.2	-	-	-	-	0.9	<0.1	-	-	-	-	-	-
Cyanobacteria*	13.3	3.2	7.6	1.8	0.9	0.3	3.2	0.4	-	-	-	-	-	-	-	-	-	-
seagrass*	2.0	0.2	-	-	-	-	-	-	-	-	-	-	3.9	2.3	-	-	-	-
detritus*	29.6	3.5	4.2	0.4	4.5	0.2	16.1	2.5	2.9	0.1	4.4	0.2	-	-	-	-	-	-
sediment	52.0	3.3	29.7	3.6	10.8	0.2	57.0	7.3	34.3	2.4	15.9	1.1	14.3	1.4	6.4	0.5	5.8	0.8
unid. eggs	-	-	2.5	0.4	25.2	6.7	-	-	5.9	0.4	26.5	6.7	-	-	-	-	-	-
unid. material	22.4	6.6	52.5	20.1	65.8	36.8	19.4	7.0	37.3	16.8	37.2	15.4	55.8	26.2	90.0	38.8	80.8	46.1
Total number of guts	**111**		**119**		**112**		**103**		**102**		**113**		**77**		**110**		**104**	
Mean gut fullness (±SE)	**5.3 ±0.3**		**5.4 ±0.2**		**6.7 ±0.2**		**5.9 ±0.3**		**4.7 ±0.3**		**5.4 ±0.2**		**5.1 ±0.3**		**6.5 ±0.2**		**5.9 ±0.2**	
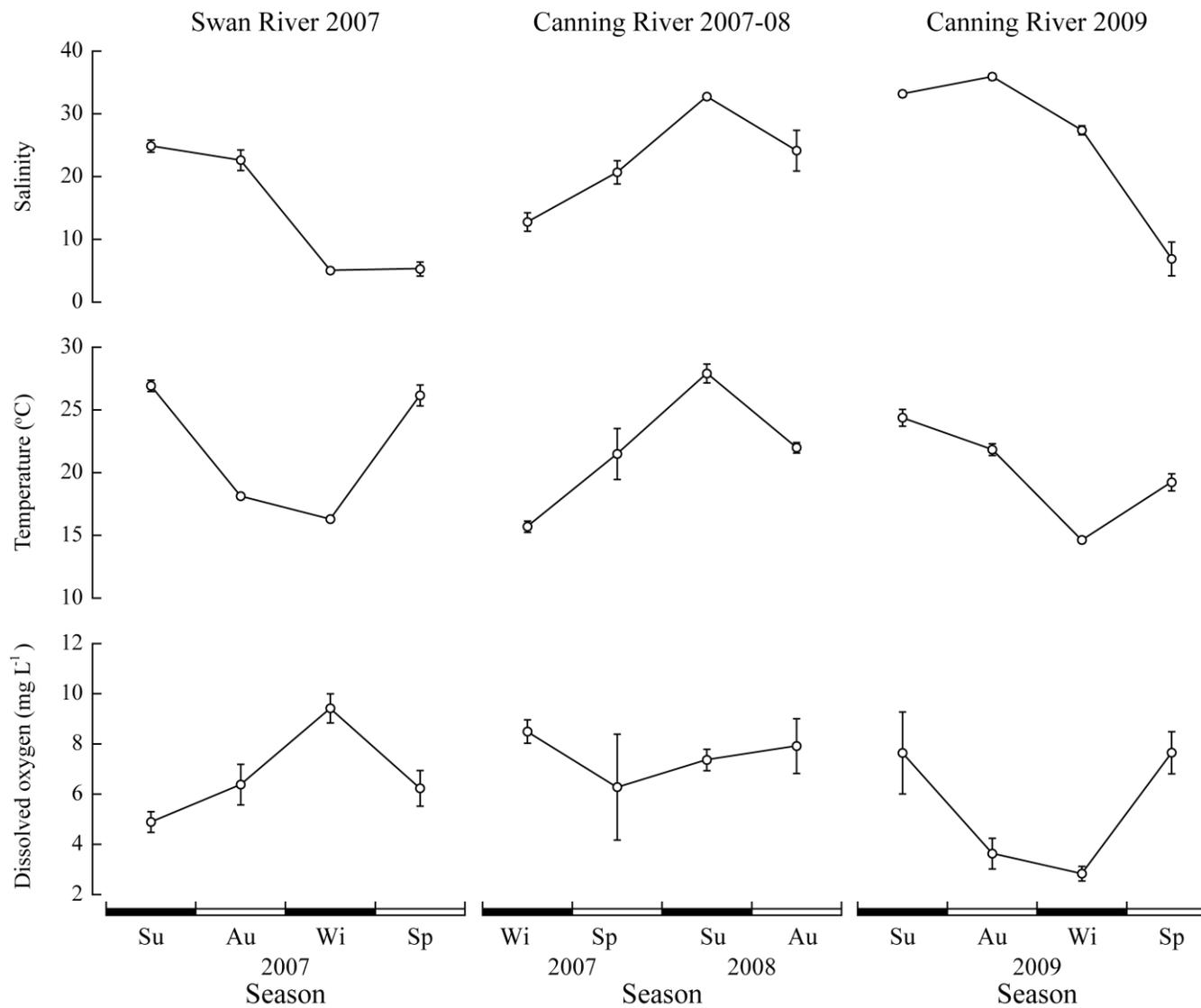
Figure 4.2: Mean seasonal salinities, temperature (°C) and dissolved oxygen concentrations (mg l^{-1}) in the Swan River between summer and spring 2007 and in the Canning River between winter 2007 and autumn 2008 and between summer and spring 2009.
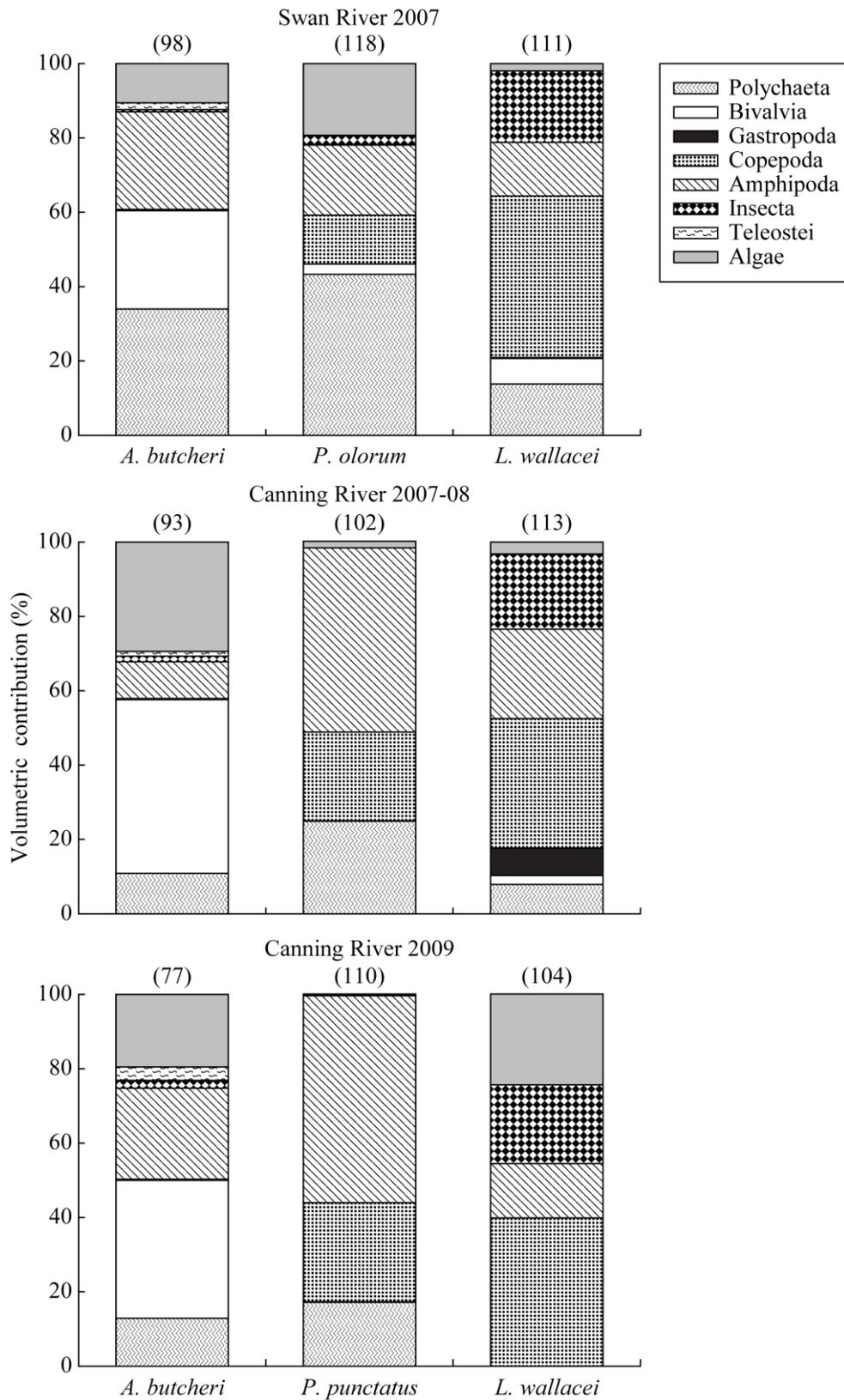
Figure 4.3: Stacked histograms of the relative volumetric contribution (%) of main dietary categories, *i.e.* Polychaeta, Bivalvia, Gastropoda, Copepoda, Amphipoda, Insecta, Teleostei and Algae, to the overall mean diets of *Acanthopagrus butcheri*, *Pseudogobius olorum* and *Leptatherina wallacei* in the Swan River in 2007 and of *A. butcheri*, *Papillogobius punctatus* and *L. wallacei* in Canning River in 2007-08 and 2009. Sample size (n) is given in parentheses.

Figure 4.4: Stacked histograms of the relative volumetric contribution (%) of main dietary categories, *i.e.* Polychaeta, Bivalvia, Gastropoda, Copepoda, Amphipoda, Insecta, Teleostei and Algae, to the mean seasonal diets of *Acanthopagrus butcheri*, *Pseudogobius olorum* and *Leptatherina wallacei* in the Swan River between summer and spring 2007. Sample size (n) is given in parentheses.

Polychaetes and amphipods made a substantial contribution to the diet of *Pseudogobius olorum*, as did algae and copepods (Table 4.1, Fig. 4.3). Collectively, polychaetes contributed 24.1% to the overall diet of *P. olorum*, with the nereid polychaetes contributing 13.1% of that total. Amphipods made an overall contribution of 10.5% to the diet of *P. olorum* with 3.1% of that total being represented by the Corophiidae (Table 4.1). The green filamentous algae Cladophora made a substantial contribution (9.6%) to the diet of *P. olorum* in the Swan River in 2007, as did harpacticoid copepods, *i.e.* 6.8% (Table 4.1).

Polychaetes, amphipods, algae and copepods were observed in the diet of *P. olorum* year round, with the relative contribution of polychaetes being > 50% in summer and autumn, that of amphipods and algae peaking in spring and of copepods being greatest in winter (Fig. 4.4).

The diet of *Leptatherina wallacei* in the Swan River was dominated to a large extent by copepods, but with the main dietary categories Insecta, Amphipoda and Polychaeta also making appreciable contributions (Table 4.1, Fig. 4.3). Calanoid copepods made by far the greatest contribution to the overall diet of *L. wallacei*, *i.e.* 15.9%, followed by the Lysianassid amphipods (3.2%), Harpactacoid copepods (2.0%), Diptera insects (1.4%) and Nereid polychaetes (1.3%). It should be noted that a substantial proportion of the contributions made by the latter three main dietary categories were due to material that could either not be identified beyond Class or Order or that were larvae (Table 4.1).

In each season, the diet of *L. wallacei* was typically dominated by one or two main dietary items. Thus, in summer, the majority of this species diet comprised polychaetes, in autumn and spring copepods and in winter insects and amphipods (Fig. 4.4).

4.3.2.2 Diets of A. butcheri, P. punctatus *and* L. wallacei *in the Canning River in 2007/08*
The diet of *A. butcheri* in the Canning River 2007/08 comprised mainly bivalves and algae, with smaller contributions being made by amphipods and polychaetes (Table 4.1, Fig. 4.3). *Acanthopagrus butcheri* consumed a range of different bivalves, *i.e.* Galeommatidae, Tellinidae, Psammobiidae and Mactridae, with the contribution made by Psammobiidae being by far the greatest (24.9%) and most regular, occurring in 36.6% of all stomachs (Table 4.1). Of the algae consumed, the majority comprised Cystocloniaceae (16.2%), with appreciable volumes of Rhodomelaceae (3.9%) and Ulvaceae (2.2%) also being consumed (Table 4.1). Nereid and Orbiniid polychaetes contributed 5.9 and 1.5%, respectively, with Corophiid amphipods contributing 2.4%, Penaeids 1.2% and teleosts 1.0% to the diet of *A. butcheri*.

While bivalves, algae, amphipods and polychaetes were all consumed by *A. butcheri* in each season, the diet of this species was dominated by bivalves in winter and autumn and by algae in summer (Fig. 4.5). Furthermore, insects were consumed primarily in spring and teleosts in summer (Fig. 4.5).

Amphipods made by far the greatest contribution to the diet of *Papillogobius punctatus*, with substantial contributions also being made by polychaetes and copepods (Table 4.1, Fig. 4.3). While Corophiidae and Aoridae were consumed by *P. punctatus*, highly macerated
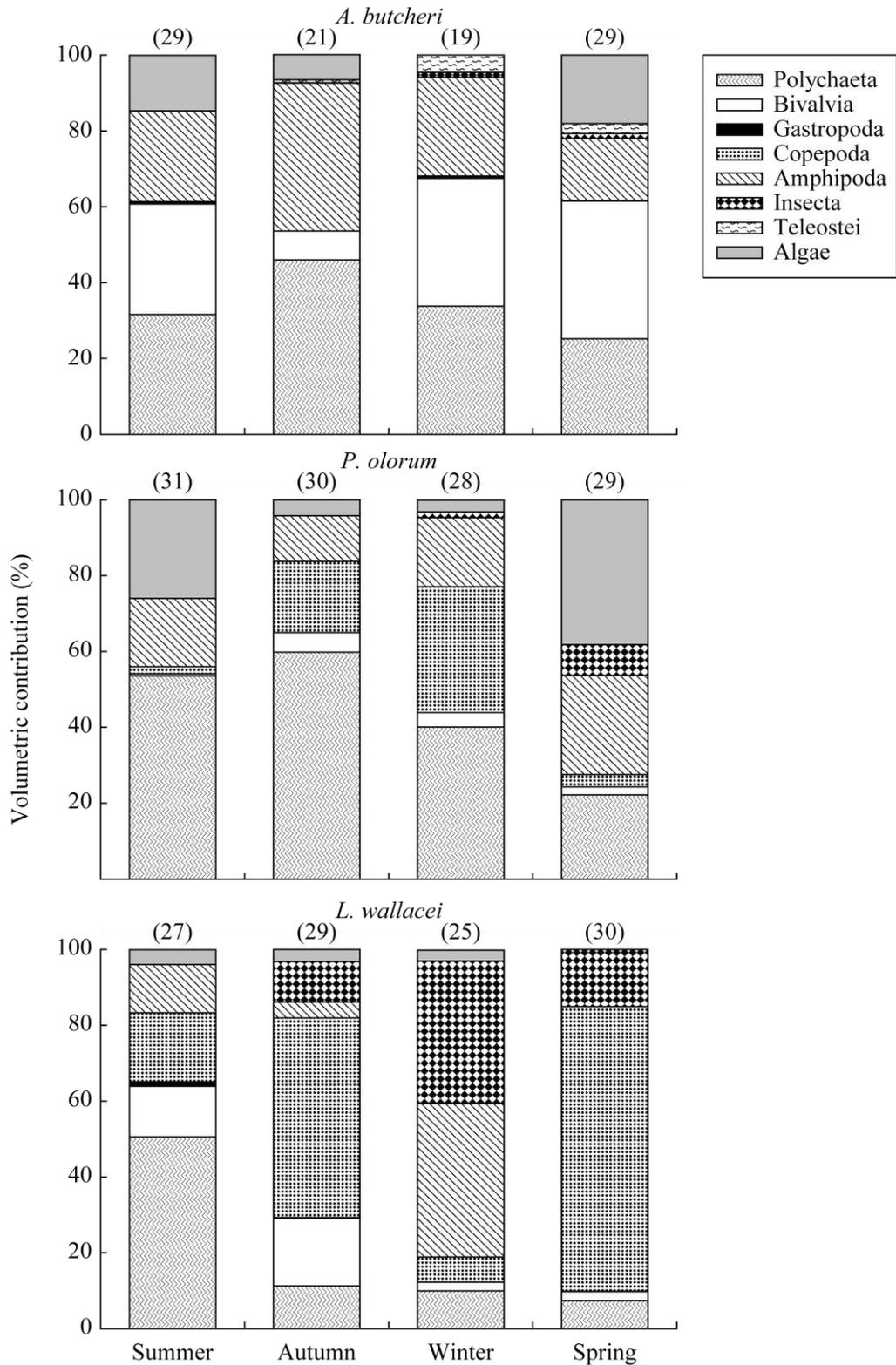
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Figure 4.5: Stacked histograms of the relative volumetric contribution (%) of main dietary categories, *i.e.* Polychaeta, Bivalvia, Gastropoda, Copepoda, Amphipoda, Insecta, Teleostei and Algae, to the mean seasonal diets of *Acanthopagrus butcheri*, *Papillogobius punctatus* and *Leptatherina wallacei* in the Canning River between winter 2007 and autumn 2008. Sample size (n) is given in parentheses.

amphipods were observed in almost half of all guts examined and unidentified amphipods accounted for 27.8% of this species' diet (Table 4.1). Similarly, while Nereid polychaetes contributed 5.8% to the diet of *P. punctatus*, unidentified polychaetes contributed 9.2%. Calanoid, cyclopoid and harpacticoid copepods all made appreciable contributions to the diet of *P. punctatus*, comprising 8.5, 4.9 and 1.1% of the diet of this species (Table 4.1).

The diet of *P. punctatus* was comprised largely of amphipods in each season, with the seasonal contribution of polychaetes and copepods varying. Thus, polychaetes made their greatest relative contribution to the diet of *P. punctatus* in summer and spring, while copepods contributed most in winter and autumn (Fig. 4.5).

In the Canning River in 2007/08, the diet of *L. wallacei* was largely dominated by copepods, with substantial contributions by amphipods and insects (Table 4.1, Fig. 4.3). Calanoid copepods made the greatest contribution to the diet of this atherinid, *i.e.* 11.1%, with harpacticoid and cyclopoid copepods contributing 5.3 and 2.7%, respectively. The majority of amphipods consumed (9.9%) could not be identified further, but of those that could Aoriidae contributed 2.1% and Corophiidae 1.2% (Table 4.1). Of the insects consumed, Diptera made the greatest contribution of 7.6% to the diet, while Hymenoptera contributed 1.7%. Polychaetes and gastropods each contributed *ca* 4% to the diet of *L. wallacei*, while algae contributed 1.7% and Galeommatid bivalves 1.3% (Table 4.1).

The diet of *L. wallacei* was typically dominated by a single dietary category in any given season. Thus, in winter, the majority of this species diet comprised amphipods, in spring and summer copepods and in autumn insects (Fig. 4.5).

4.3.2.3 Diets of A. butcheri, P. punctatus *and* L. wallacei *in the Canning River in 2009*
Bivalves, followed by amphipods, algae and polychaetes, made the greatest contribution to the diet of *A. butcheri* in the Canning River in 2009 (Table 4.1, Fig. 4.3). Of the bivalve species consumed, psammobiids contributed the most by volume (18.6%). While 4.1% of the diet of *A. butcheri* comprised Corophiid amphipods, the majority of amphipods could not be identified further, with these macerated individuals constituting 12.5% of the diet (Table 4.1). Gracilaria contributed 10.7% and nereid polychaetes 4.4% to the diet of this species, with teleosts (2.5%), seagrass (2.3%), carid shrimp (1.6%) and insects (1.4%) also making appreciable contributions (Table 4.1).

The contribution made by bivalves and polychaetes to the diet of *A. butcheri* was greatest in winter, that made by amphipods in spring and by algae in summer (Fig. 4.6). Furthermore, teleosts were primarily consumed in autumn and insects in spring.

The diet of *P. punctatus* in the Canning River in 2009 largely comprised amphipods, with substantial volumes of copepods and polychaetes also being consumed (Table 4.1, Fig. 4.3). While Corophiid amphipods constituted 5.3% of this species diet, a further 28.7% comprised amphipods that could not be identified further (Table 4.1). Calanoid copepods comprised 6.0% and cyclopoid and harpacticoid copepods each 4.7% of the diet of *P. punctatus*, while
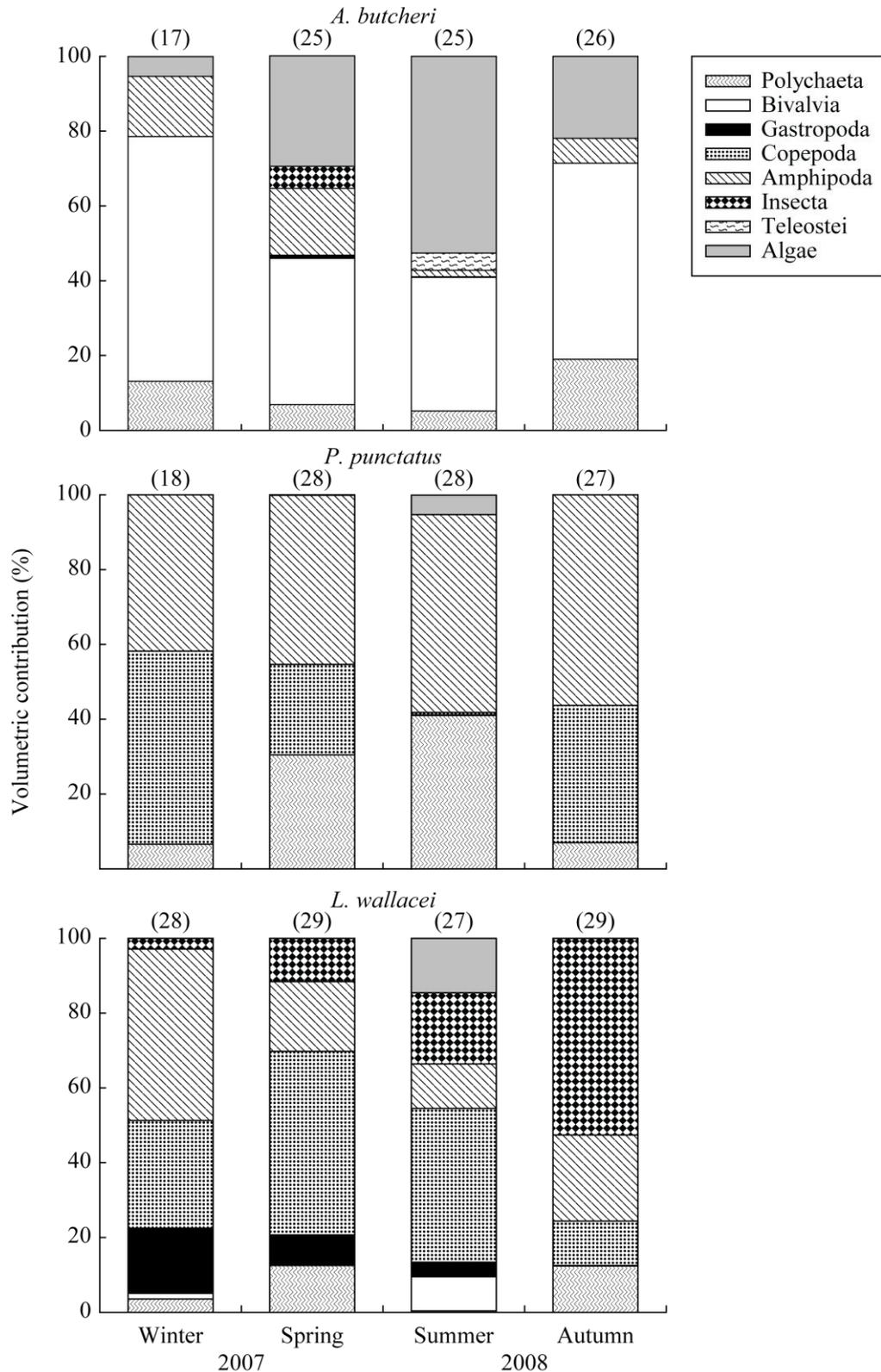
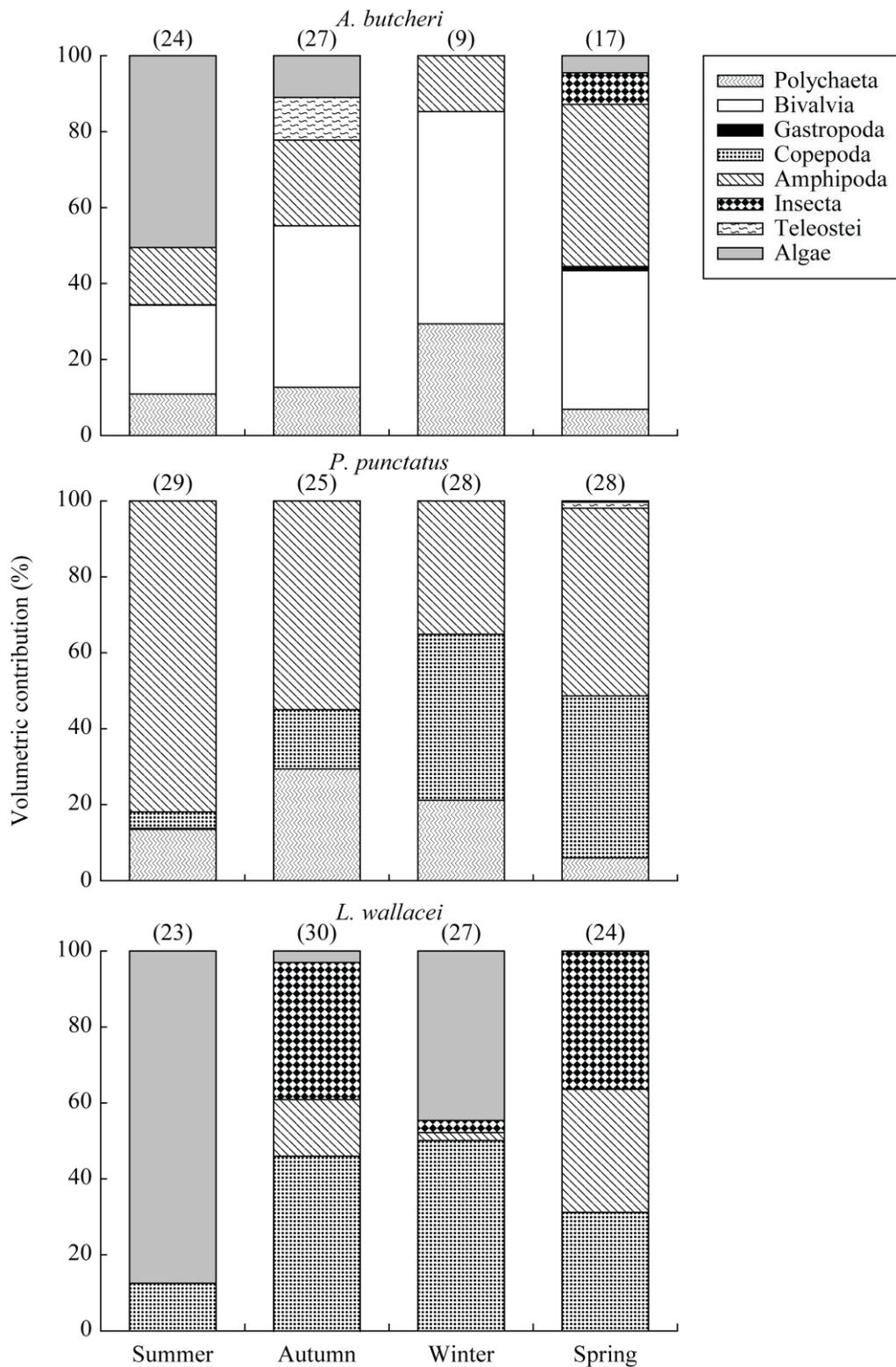**Figure 4.6:** Stacked histograms of the relative volumetric contribution (%) of main dietary categories, *i.e.* Polychaeta, Bivalvia, Gastropoda, Copepoda, Amphipoda, Insecta, Teleostei and Algae, to the mean seasonal diets of *Acanthopagrus butcheri*, *Papillogobius punctatus* and *Leptatherina wallacei* in the Canning River between summer and spring 2009. Samples size (n) is given in parentheses.

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nereid polychaetes contributed 8.5% (Table 4.1). An infrequent (F = 0.9%) and small (V = 0.2%) contribution was also made to the diet of this small gobiid species by teleosts (Table 4.1, Fig. 4.3).

Collectively, the contributions made by amphipods, copepods and polychaetes constituted the vast majority of the diet of *P. punctatus* in each season. Amphipods were the primary source of food in summer, while the contributions made by copepods were greatest in winter and spring and by polychaetes in autumn (Fig. 4.6).

Copepods, followed by algae, insects and amphipods, constituted the majority of the diet of *L. wallacei* in the Canning River in 2009 (Table 1, Fig. 2). Calanoid copepods made by far the greatest contribution (13.8%) to this species diet, but it also fed, to a far lesser extent, on cyclopoid and harpacticoid copepods, *i.e.* both 2.7%. The unicellular algae Chrysophyceae made a substantial contribution (9.7%) to the diet of *L. wallacei*, as did Dipterain insects (7.8%) and unidentified amphipods (6.9%). Although juvenile molluscs only made a small contribution by volume to the diet of *L. wallacei* (1.7%), this dietary item was consumed regularly, being found in almost half the guts examined (Table 4.1).

Of the main dietary items consumed by *L. wallacei*, only copepods were preyed upon in each season. Algae comprised the vast majority of the diet for this species in summer, while insects and amphipods contributed largely to its diet in both autumn and spring (Fig. 4.6).

4.3.3 Dietary composition of *A. butcheri* and *L. wallacei* in the Swan and Canning rivers
A preliminary analysis employing PERMANOVA demonstrated that the composition of the diets of *A. butcheri* and *L. wallacei* in the Swan and Canning rivers in the winter and spring of 2007 were significantly influenced by species, region and season (all $P < 0.001$) and that all two- and three-way interactions between these factors were significant (Table 4.2). The components of variation were greatest for species, followed by the region x species and species x season interactions, which in turn were greater than the region x species x season interaction, the region and species main effects and the region x season interaction, respectively (Table 4.2).

When the mean dietary composition data for *A. butcheri* and *L. wallacei* in winter and spring 2007 were subjected to ordination, the seasonal samples for each species in each region tended to group together and showed no overlap (Fig. 4.7). Given that region always strongly influenced the composition of the diet of these two species, it was considered valid to conduct all subsequent analyses on dietary composition separately for the Swan and Canning rivers.

4.3.3.1 Dietary composition of fish in the Swan River in 2007
Two-way crossed ANOSIM demonstrated that the composition of the diets of *A. butcheri*, *P. olorum* and *L. wallacei* in the Swan River in 2007 were significantly related ($P = 0.001$) to both species and season, with the *R*-statistic for the former far exceeding that of the latter, *i.e.* 0.600 and 0.328, respectively. Pairwise comparisons demonstrated that, across all species, dietary composition differed significantly between seasons (all $P = 0.001$) and that the

Table 4.2: Pseudo-F, components of variation and significance levels for three-way PERMANOVA of the dietary composition of *Acanthopagrus butcheri* and *Leptatherina wallacei* in the Swan and Canning rivers in winter and spring 2007. **$P < 0.01$, ***$P < 0.001$.

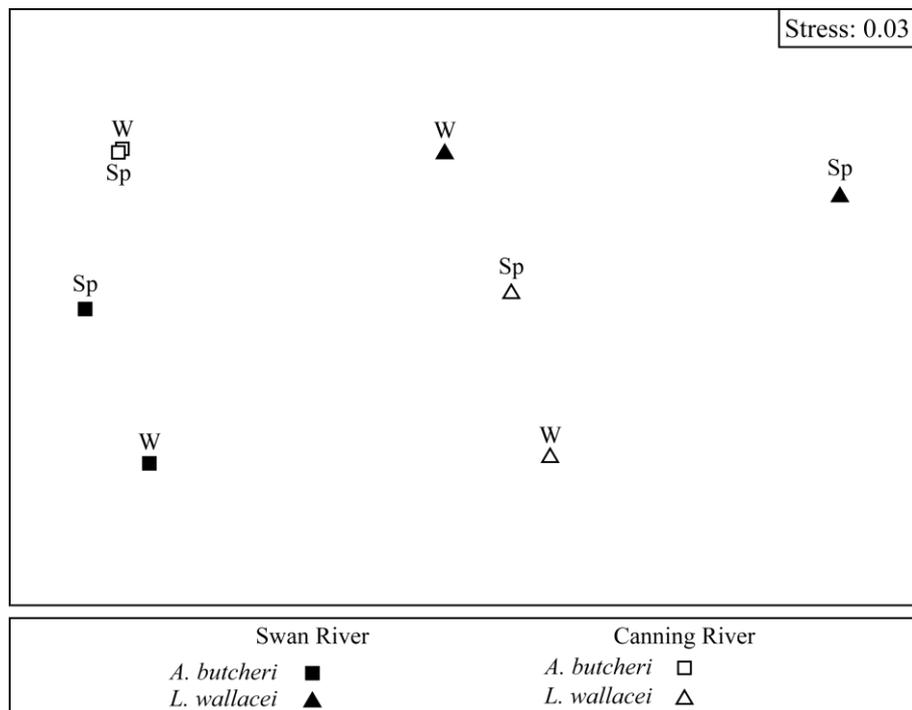|	**Main effects**			
	Region (Re)	Species (Sp)	Season (Se)	Residual
df	1	1	1	56
Pseudo- F	8.190***	50.164***	7.286***	-
CoV	15.373	40.199	14.374	31.650
	Interactions			
	Re x Sp	Re x Se	Sp x Se	Re x Sp x Se
df	1	1	1	1
Pseudo- F	7.020***	3.7049**	6.714***	3.223**
CoV	19.897	13.334	19.381	17.095

Figure 4.7: Non-metric multidimensional scaling ordination plot derived from the matrix constructed using the mean seasonal dietary composition of *Acanthopagrus butcheri* and *Leptatherina wallacei* in the Swan and Canning rivers in winter (W) and spring (Sp) 2007.

greatest seasonal differences were those for autumn and winter *vs* spring, *i.e.* $R = 0.441$ and 0.438, respectively, and least between summer and autumn ($R = 0.191$). While dietary composition (across all species) was typified in each season by polychaetes, in autumn, winter and spring also by copepods and in winter also by amphipods, that in spring was distinguished from those in autumn and winter by consistently higher contributions by copepods and bivalves and lower contributions by polychaetes. The consistent and greater consumption of insects in spring also distinguished diet in spring from that in autumn, while the reverse was true of amphipods in spring and winter.

When the data for each season was considered separately, dietary composition was significantly related to species in each of those seasons (all $P = 0.001$), with the R-statistic being greatest in spring (0.891), followed by autumn (0.580), which in turn was greater than both summer (0.439) and winter (0.430). On the ordination plots for spring and autumn, derived from the matrices of the dietary composition of the three fish species in each of those seasons, the samples for *A. butcheri*, *P. olorum* and *L. wallacei* formed discrete groups, with those for the former two species tending to lie closer but not to overlap (Fig. 4.8b, d). Pairwise comparisons confirmed that these interspecific differences were significant ($P = 0.001-0.013$) and that, in both autumn and spring, the difference between *A. butcheri* and *L. wallacei* was greatest, *i.e.* $R = 1.000$ and 0.952, respectively, and that between *A. butcheri* and *P. olorum* least, *i.e.* $R = 0.566$ and 0.271, respectively (Table 4.3). While the samples for *A. butcheri* and *L. wallacei* also formed discrete groups on the ordination plots for summer and winter, those for *P. olorum* displayed some overlap with the former species in summer and the latter species in winter (Fig. 4.8a, c). Pairwise comparisons demonstrated that in both summer and winter, the greatest interspecific differences in dietary composition were between *A. butcheri* and *L. wallacei* (both $P = 0.001$; $R = 0.605$ and 0.572, respectively), but were least between *A. butcheri* and *P. olorum* in summer ($P = 0.001$; $R = 0.354$) and that the dietary compositions of *P. olorum* and *L. wallacei* did not differ significantly in winter ($P = 0.004$; $R = 0.290$).

In each season, the diet of *A. butcheri* was typified by bivalves and polychaetes, while that of *P. olorum* was typified by polychaetes and algae, and that of *L. wallacei* by copepods and insects, in at least two of the seasons (Table 4.3). The pronounced differences in the composition of the diets of the three species in spring, for example, were due to the consistently greater consumption of bivalves by *A. butcheri* than by *P. olorum* and *L. wallacei*, and also of polychaetes than by *L. wallacei*. The diet of *P. olorum* was further distinguished from that of *A. butcheri* and *L. wallacei* by the greater consumption of algae and amphipods and also from the latter species by polychaetes, while *L. wallacei* consistently consumed more copepods than either of the other species (Table 4.3).

4.3.3.2 Dietary composition of fish in the Canning River in 2007-08
The composition of the diets of *A. butcheri*, *P. punctatus* and *L. wallacei* in the Canning River between winter 2007 and autumn 2008 were shown, by two-way crossed ANOSIM, to be significantly related ($P = 0.001$) to both species and season, with the R-statistic for species (0.720) far exceeding that of season (0.290). Pairwise comparisons demonstrated that, when

Table 4.3: Global *R*-statistic values and significance levels (bold) for ANOSIMs, and *R*-statistic values and significance levels (light shaded boxes) for pairwise comparisons, of the dietary compositions of *Acanthopagrus butcheri*, *Pseudogobius olorum* and *Leptatherina wallacei* in the Swan River in summer to spring 2007. Dietary categories/sub-categories determined by SIMPER as most responsible for typifying the dietary composition of each species (dark shaded boxes) and for distinguishing between the dietary compositions of the three species in each paired comparison (unshaded boxes). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. [Ab] = *A. butcheri*, [Po] = *P. olorum*, [Lw] = *L. wallacei* denote the species in which the dietary category/sub-category made the greater contribution to the dietary composition.

Summer $R = 0.439$***	A. butcheri	P. olorum	L. wallacei
A. butcheri	Polychaeta Bivalvia Amphipoda Algae	0.354***	0.572***
P. olorum	Bivalvia[Ab] Amphipoda[Ab] Polychaeta[Po] Algae[Po]	Polychaeta Algae	0.474***
L. wallacei	Amphipoda[Ab] Bivalvia[Ab] Polychaeta[Ab] Algae[Ab]	Polychaeta[Po] Algae[Po]	Bivalvia

Autumn $R = 0.580$***	A. butcheri	P. olorum	L. wallacei
A. butcheri	Polychaeta Amphipoda	0.271*	0.952***
P. olorum	Amphipoda[Ab] Polychaeta[Ab] Copepoda[Po] Bivalvia[Ab]	Polychaeta	0.483***
L. wallacei	Amphipoda[Ab] Polychaeta[Ab] Copepoda[Lw]	Polychaeta[Po] Copepoda[Lw]	Copepoda Polychaeta

Winter $R = 0.430$***	A. butcheri	P. olorum	L. wallacei
A. butcheri	Bivalvia Amphipoda Terr. plants Polychaeta	0.455**	0.605**
P. olorum	Bivalvia[Ab] Copepoda[Po] Terr. plants[Ab] Polychaeta[Ab] Amphipoda[Ab]	Copepoda	0.290**
L. wallacei	Bivalvia[Ab] Terr. plants[Ab] Insecta[Lw] Polychaeta[Ab] Amphipoda[Ab]	Insecta[Lw] Amphipoda[Lw] Polychaeta[Po] Copepoda[Po]	Insecta

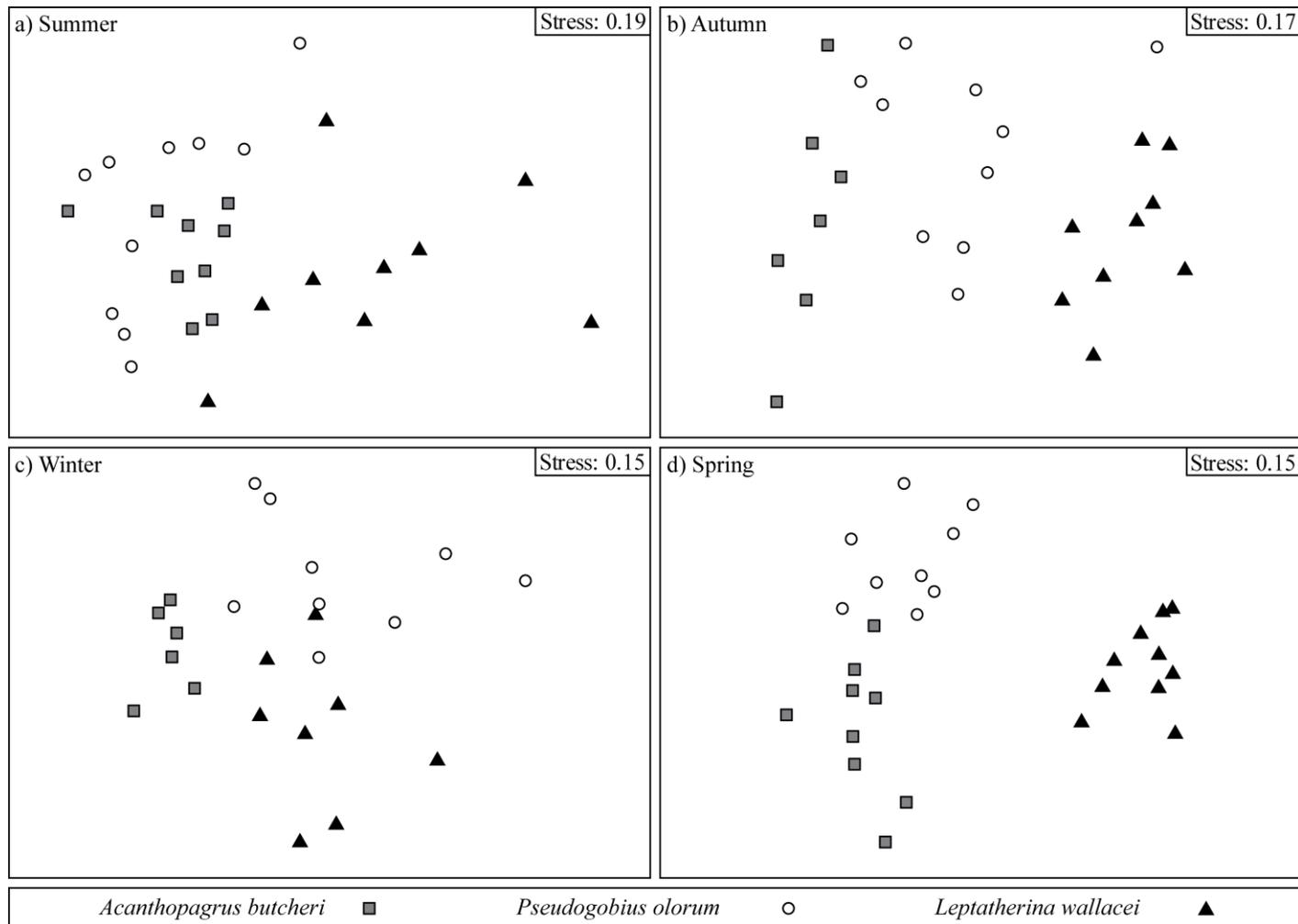| Spring $R = 0.891$***	A. butcheri	P. olorum	L. wallacei
A. butcheri	Bivalvia Polychaeta	0.566***	1.000***
P. olorum	Bivalvia[Ab] Algae[Po] Amphipoda[Po]	Algae Amphipoda	0.974***
L. wallacei	Copepoda[Lw] Bivalvia[Ab] Polychaeta[Ab]	Copepoda[Lw] Algae[Po] Amphipoda[Po] Polychaeta[Po]	Copepoda Insecta

Figure 4.8: Non-metric multidimensional scaling ordination plots derived from the matrices constructed using replicate samples of the dietary composition of *Acanthopagrus butcheri*, *Pseudogobius olorum* and *Leptatherina wallacei* in the Swan River in a) summer, b) autumn, c) winter and d) spring 2007.

considered across all species, dietary composition differed significantly between seasons (all $P = 0.001$-0.003) and that the greatest seasonal differences were those for summer *vs* autumn and winter, *i.e.* $R = 0.374$ and 0.437, respectively, and least between those latter two seasons ($R = 0.195$). While dietary composition across the three species was typified in winter and spring 2007 by copepods and in the latter season also by amphipods and polychaetes, no single dietary category/sub-category consistently typified the diets of all three species in summer and autumn 2008. Both amphipods and copepods were consistently consumed in greater quantities, across all species, in autumn and winter, and also polychaetes in autumn, than in summer.

When considered separately, dietary composition in each season was significantly related to species (all $P = 0.001$), with the *R*-statistic being high in spring 2007 and summer and autumn 2008, *i.e.* 0.775, 0.795, 0.708, respectively, and slightly less in winter 2007 (0.531). On the ordination plots, derived from the matrices of the dietary composition of the three fish species in each season, the samples for *A. butcheri* always formed discrete groups. While this was also the case for *P. punctatus* and *L. wallacei* in summer 2008, there was limited overlap between these species in autumn 2008 and the samples for these two species intermingled in both winter and spring 2007 (Fig. 4.9a-d). Pairwise comparisons demonstrated, however, that the composition of the diets of the three species were significantly different in each season ($P = 0.001$-0.002), with the exception of *P. punctatus* and *L. wallacei* in winter 2007, *i.e. P* $= 0.149$. Interspecific differences in dietary composition were high on each occasion ($R = 0.688$-1.000), with the exceptions of those between *P. punctatus* and *L. wallacei* in winter and spring 2007 and autumn 2008, *i.e.* 0.092, 0.357 and 0.446, respectively (Table 4.4).

The diet of *A. butcheri* was typified by bivalves, algae, amphipods and polychaetes in at least two of the four seasons, while the same was true of polychaetes, amphipods and copepods for *P. punctatus* and of copepods for *L. wallacei* (Table 4.4). The pronounced differences between the dietary compositions of *A. butcheri* and both *P. punctatus* and *L. wallacei* were due, in each season, to the consistently greater contribution made to the diet of *A. butcheri* by bivalves and also by algae and polychaetes in each season except winter 2007 and summer 2008, respectively (Table 4.4). In spring 2007 and summer and autumn 2008, when the composition of the diet of *P. punctatus* differed significantly from that of *L. wallacei*, these differences could always be attributed to a greater prevalence of amphipods in the diet of *P. punctatus* and also often to a greater contribution by polychaetes and lower contribution by insects (Table 4.4).

4.3.3.3 Dietary composition of fish in the Canning River in 2009

Two-way crossed ANOSIM demonstrated that the composition of the diets of *A. butcheri*, *P. punctatus* and *L. wallacei* in the Canning River in 2009 were significantly related ($P = 0.001$) to both species and season, with the *R*-statistic for species far exceeding that of season, *i.e.* 0.809 and 0.373, respectively. Pairwise comparisons demonstrated that, across all species, dietary composition differed significantly between seasons (all $P = 0.001$-0.003) and that seasonal differences were high between summer and each other season (autumn $R = 0.414$; winter $R = 0.495$; spring $R = 0.480$) and least between autumn and spring ($R = 0.183$). While

Table 4.4: Global *R*-statistic values and significance levels (bold) for ANOSIMs, and *R*-statistic values and significance levels (light shaded boxes) for pairwise comparisons, of the dietary compositions of *Acanthopagrus butcheri*, *Papillogobius punctatus* and *Leptatherina wallacei* in the Canning River in winter 2007 to autumn 2008. Dietary categories/sub-categories determined by SIMPER as most responsible for typifying the dietary composition of each species (dark shaded boxes) and for distinguishing between the dietary compositions of the three species in each paired comparison (unshaded boxes). *P < 0.05, **P < 0.01, ***P < 0.001. [Ab] = *A. butcheri*, [Po] = *P. punctatus*, [Lw] = *L. wallacei* denote the species in which the dietary category/sub-category made the greater contribution to the dietary composition.

Winter 2007 $R = 0.531^{***}$	*A. butcheri*	*P. punctatus*	*L. wallacei*
A. butcheri	Bivalvia Amphipoda Polychaeta	1.000**	0.747***
P. punctatus	Bivalvia ^{Ab} Copepoda ^{Pp} Amphipoda ^{Pp} Polychaeta ^{Ab}	Copepoda Amphipoda	0.092^{NS}
L. wallacei	Bivalvia ^{Ab} Copepoda ^{Lw} Amphipoda ^{Lw} Polychaeta ^{Ab} Gastropoda ^{Lw}	NS	Copepoda

Spring 2007 $R = 0.775^{***}$	*A. butcheri*	*P. punctatus*	*L. wallacei*
A. butcheri	Bivalvia Algae Amphipoda	0.954***	0.970***
P. punctatus	Bivalvia ^{Ab} Algae ^{Ab} Copepoda ^{Pp} Polychaeta ^{Pp} Amphipoda ^{Pp}	Polychaeta Amphipoda Copepoda	0.357**
L. wallacei	Copepoda ^{Lw} Bivalvia ^{Ab} Algae ^{Ab}	Copepoda ^{Lw} Amphipoda ^{Pp} Polychaeta ^{Pp} Gastropoda ^{Lw}	Copepoda Polychaeta Gastropoda

Summer 2008 $R = 0.795^{***}$	*A. butcheri*	*P. punctatus*	*L. wallacei*
A. butcheri	Algae	0.845***	0.688***
P. punctatus	Algae ^{Ab} Amphipoda ^{Pp} Bivalvia ^{Ab} Polychaeta ^{Pp}	Polychaeta Amphipoda	0.839**
L. wallacei	Algae ^{Ab} Bivalvia ^{Ab} Copepoda ^{Lw} Insecta ^{Lw}	Polychaeta ^{Pp} Amphipoda ^{Pp} Copepoda ^{Lw} Insecta ^{Lw}	Copepoda

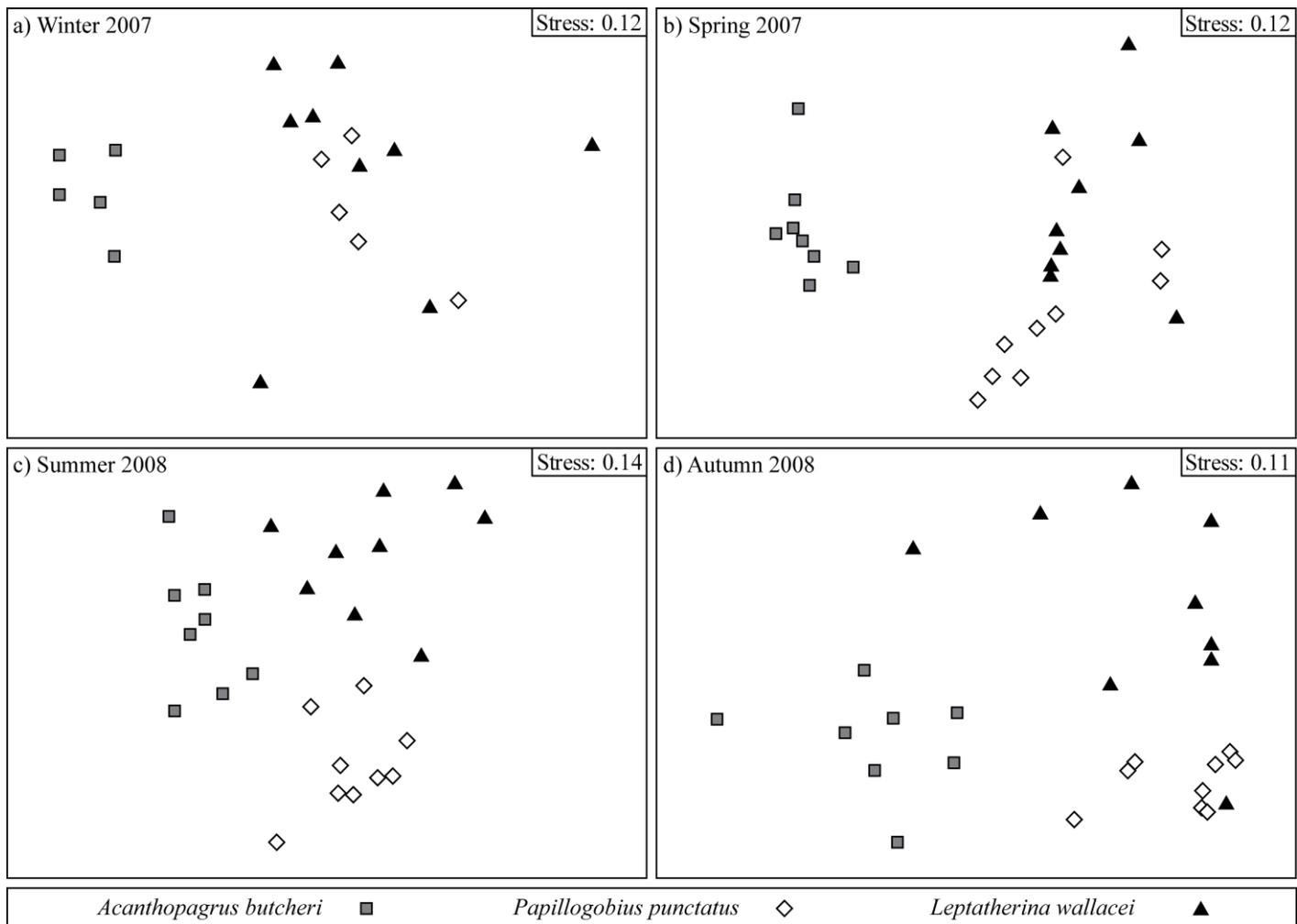| **Autumn 2008** $R = 0.708^{***}$	*A. butcheri*	*P. punctatus*	*L. wallacei*
A. butcheri	Bivalvia Polychaeta	0.983***	0.780***
P. punctatus	Bivalvia ^{Ab} Copepoda ^{Pp} Amphipoda ^{Pp} Algae ^{Ab} Polychaeta ^{Ab}	Amphipoda Copepoda	0.446***
L. wallacei	Bivalvia ^{Ab} Insecta ^{Lw} Algae ^{Ab} Polychaeta ^{Ab}	Insecta ^{Lw} Amphipoda ^{Pp} Copepoda ^{Pp}	Insecta

Figure 4.9: Non-metric multidimensional scaling ordination plots derived from the matrices constructed using replicate samples of the dietary composition of *Acanthopagrus butcheri*, *Papillogobius punctatus* and *Leptatherina wallacei* in the Canning River in a) winter and b) spring 2007 and c) summer and d) autumn 2008.

amphipods and/or copepods commonly typified the dietary composition (across all species) in each season, that in summer was distinguished from each of the other season by a consistent and greater consumption of amphipods and from both winter and spring also by a consistently lower consumption of copepods.

When dietary composition in each season was considered separately, ANOSIM demonstrated that it was significantly related to species (all $P = 0.001$) on each occasion and that the R-statistic was high in summer, autumn and winter, *i.e.* 0.893, 0.816, 0.933, respectively, and 0.580 in winter 2007. On the ordination plots, derived from the matrices of the dietary composition of the three fish species in each season, the samples for each species always formed discrete groups, with those for *A. butcheri* and *L. wallacei* tending to lie apart and those for *P. punctatus* either between and/or below those former two groups (Fig. 4.10a-d). Pairwise comparison demonstrated that the diets of three species were always significantly different ($P = 0.001$-0.005) and that these differences were typically substantial, *i.e.* R = 0.468-1.000 (Table 4.5).

The diet of *A. butcheri* was frequently typified by bivalves and amphipods, that of *P. punctatus* by amphipods, copepods and/or polychaetes and that of *L. wallacei* always by copepods but also by algae, insects and juvenile molluscs in two of the four seasons (Table 4.5). In each season, the diet of *A. butcheri* was distinguished from that of both *P. punctatus* and *L. wallacei* by a greater prevalence of bivalves, and also of algae in summer, of polychaetes in winter and amphipods in spring (Table 4.5). The composition of the diet of *P. punctatus* differed from that of *L. wallacei* in that, in each season, it consistently comprised more amphipods and, in summer, autumn and winter, also polychaetes, while in summer and winter *L. wallacei* fed more on algae and in autumn and spring more on insects than did *P. punctatus* (Table 4.5).

4.3.4 Isotopic characteristics of fish and their potential prey in the Swan and Canning rivers in 2007-08

4.3.4.1 Isotopic characteristics of fish and their potential prey in the Swan River in 2007
In the Swan River, the mean $\delta^{13}C$ and $\delta^{15}N$ values for *A. butcheri*, *P. olorum* and *L. wallacei* were very similar, with those for $\delta^{13}C$ ranging only from -25.6 to -28.0‰ in summer and from -26.6 to -28.4‰ in winter, and those for $\delta^{15}N$ ranging only from 14.8 to 15.9‰ in summer and 14.4 to 15.8‰ in winter (Table 4.6; Fig. 4.11). The isotopic signatures of aquatic primary producers, *i.e.* phytoplankton, cyanobacteria and microphytobenthos (MPB), had similar mean $\delta^{13}C$ values ranging from -24.9 to -26.7‰ in summer and -25.3 to -26.4‰ in winter, and also similar mean $\delta^{15}N$ values ranging from 6.3 to 8.3‰ in summer and 5.2 to 8.0‰ in winter. These values however, were often associated with large standard errors and, in particular, those for $\delta^{13}C$ of MPB in summer and for $\delta^{15}N$ of phytoplankton and MPB in winter (Table 4.6; Fig. 4.11). The increase in $\delta^{15}N$ values from the lowest value aquatic

Table 4.5: Global *R*-statistic values and significance levels (bold) for ANOSIMs, and *R*-statistic values and significance levels (light shaded boxes) for pairwise comparisons, of the dietary compositions of *Acanthopagrus butcheri*, *Papillogobius punctatus* and *Leptatherina wallacei* in the Canning River in summer to spring 2009. Dietary categories/sub-categories determined by SIMPER as most responsible for typifying the dietary composition of each species (dark shaded boxes) and for distinguishing between the dietary compositions of the three species in each paired comparison (unshaded boxes). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. ^{Ab} = *A. butcheri*, ^{Po} = *P. punctatus*, ^{Lw} = *L. wallacei* denote the species in which the dietary category/sub-category made the greater contribution to the dietary composition.

Summer **$R = 0.893$*****	A. butcheri	P. punctatus	L. wallacei
A. butcheri	Algae Amphipoda	0.818***	0.802***
P. punctatus	Algae ^{Ab} Amphipoda ^{Pp} Bivalvia ^{Ab} Polychaeta ^{Pp}	Amphipoda	1.000***
L. wallacei	Algae ^{Ab} Bivalvia ^{Ab} Amphipoda ^{Ab} J. molluscs ^{Lw}	Amphipoda ^{Pp} Algae ^{Lw} Polychaeta ^{Pp}	Algae J. molluscs Copepoda

Autumn **$R = 0.816$*****	A. butcheri	P. punctatus	L. wallacei
A. butcheri	Bivalvia Amphipoda	0.582***	0.925***
P. punctatus	Bivalvia ^{Ab} Polychaeta ^{Pp} Copepoda ^{Pp} Amphipoda ^{Pp}	Amphipoda Polychaeta Copepoda	0.960***
L. wallacei	Bivalvia ^{Ab} Copepoda ^{Lw} Insecta ^{Lw} Amphipoda ^{Ab}	Amphipoda ^{Pp} Insecta ^{Lw} Polychaeta ^{Pp} Copepoda ^{Lw}	Insecta Copepoda J. molluscs

Winter **$R = 0.933$*****	A. butcheri	P. punctatus	L. wallacei
A. butcheri	Bivalvia Polychaeta	0.934**	1.000**
P. punctatus	Bivalvia ^{Ab} Copepoda ^{Pp} Amphipoda ^{Pp} Polychaeta ^{Ab}	Copepoda Amphipoda Polychaeta	0.913***
L. wallacei	Bivalvia ^{Ab} Copepoda ^{Lw} Algae ^{Lw} Polychaeta ^{Ab}	Algae ^{Lw} Amphipoda ^{Pp} Polychaeta ^{Pp} Copepoda ^{Lw}	Algae Copepoda

Spring **$R = 0.580$*****	A. butcheri	P. punctatus	L. wallacei
A. butcheri	Amphipoda Bivalvia	0.671**	0.691***
P. punctatus	Bivalvia ^{Ab} Copepoda ^{Pp} Amphipoda ^{Ab}	Copepoda Amphipoda	0.468**
L. wallacei	Bivalvia ^{Ab} Copepoda ^{Lw} Insecta ^{Lw} Amphipoda ^{Ab}	Insecta ^{Lw} Amphipoda ^{Pp} Copepoda ^{Pp}	Insecta Copepoda

Table 4.6: Mean (±1SE) values of δ^{13}C and δ^{15}N (‰) for *Acanthopagrus butcheri*, *Papillogobius punctatus* and *Leptatherina wallacei*, and for the various components of the macrofauna, meiofauna and primary producers, and for detritus in the Swan River in summer and winter 2007, and in the Canning River in winter 2007 and summer 2008 and in summer and winter 2009.

	Swan River 2007				Canning River 2007-08				Canning River 2009			
	Summer		Winter		Winter		Summer		Summer		Winter	
	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N
Teleostei												
Acanthopagrus butcheri	-25.6 ±0.6	15.7 ±0.2	-26.6 ±0.3	15.8 ±0.1	-22.0 ±0.3	16.0 ±0.1	-21.5 ±0.2	15.9 ±0.1	-21.6 ±0.3	16.2 ±0.1	-21.7 ±0.3	16.1 ±0.1
Pseudogobius olorum	-26.7 ±0.2	14.8 ±0.1	-26.8 ±0.4	14.4 ±0.2	-	-	-	-	-	-	-	-
Papillogobius punctatus	-	-	-	-	-20.6 ±0.3	15.2 ±0.1	-20.0 ±0.2	15.0 ±0.1	-20.4 ±0.2	15.4 ±0.1	-21.0 ±0.2	15.7 ±0.1
Leptatherina wallacei	-28.0 ±0.3	15.9 ±0.1	-28.4 ±0.2	15.3 ±0.2	-23.9 ±0.3	15.3 ±0.2	-22.8 ±0.2	15.6 ±0.1	-23.2 ±0.2	15.6 ±0.1	-24.1 ±0.2	15.6 ±0.1
Macrofauna												
Polychaeta	-25.8 ±0.5	11.4 ±0.3	-25.7 ±0.4	11.9 ±0.4	-19.6 ±0.9	11.8 ±0.5	-19.7 ±0.9	12.1 ±0.4	-	-	-19.3 ±0.3	11.6 ±0.3
Bivalvia	-28.0 ±0.3	10.4 ±0.5	-	-	-22.6 ±0.7	10.0 ±0.5	-21.8 ±0.4	9.8 ±0.4	-22.9 ±0.7	11.8 ±0.1	-19.4 ±3.3	11.6 ±0.7
Gastropoda	-24.6 ±0.1	10.2 ±0.1	-	-	-18.0 ±0.8	11.3 ±0.7	-17.7 ±0.9	12.9 ±0.5	-	-	-16.7 ±1.4	12.6 ±0.3
Ostracoda	-	-	-21.1	8.8	-	-	-	-	-	-	-	-
Amphipoda	-25.0 ±0.6	8.7 ±0.6	-27.5 ±1.0	9.5 ±0.5	-19.9 ±1.0	9.4 ±0.2	-17.2 ±0.8	9.1 ±0.1	-18.1 ±1.0	10.4 ±0.1	-18.8 ±0.9	10.3 ±0.3
Isopoda	-22.2 ±0.8	12.9 ±1.3	-22.4	11.2	-20.4	9.9	-	-	-	-	-	-
Caridea	-27.2 ±0.5	15.6 ±0.8	-25.6 ±1.4	15.2 ±0.9	-18.7 ±0.3	13.0 ±0.2	-23.9 ±0.7	14.3 ±0.1	-	-	-23.3 ±0.3	15.1 ±0.2
Insecta	-23.8 ±1.2	7.0 ±0.7	-18.4 ±4.1	7.5 ±1.9	-	-	-21.6 ±1.5	3.0 ±1.7	-22.9 ±0.6	9.8 ±1.3	-	-
Meiofauna												
Calanoida	-31.7 ±3.6	5.3 ±0.1	-34.6 ±1.0	12.9 ±1.3	-26.3 ±0.6	8.7 ±0.3	-22.8 ±0.9	11.0 ±0.7	-24.9 ±1.4	11.2 ±0.8	-26.7 ±0.1	10.5 ±0.5
Cyclopoida	-26.5	4.5	-	-	-	-	-	-	-24.6 ±0.6	9.1 ±0.9	-26.2	8.9
Harpacticoida	-	-	-28.4	5.0	-	-	-	-	-20.2	9.0	-26.6	2.2
Primary producers												
Large riparian vegetation	-28.8 ±0.6	4.2 ±0.4	-	-	-	-	-28.6 ±0.4	3.4 ±1.1	-	-	-29.9 ±0.5	0.7 ±0.2
Small riparian vegetation	-28.9 ±0.4	5.1 ±0.5	-	-	-	-	-29.9	7.4	-	-	-28.6 ±0.7	7.4 ±1.0
Macroalgae	-	-	-	-	-20.1 ±1.0	10.3 ±0.4	-21.6 ±1.5	12.2 ±2.3	-25.4	9.7	-22.4	10.1
Seagrass	-	-	-	-	-12.0	7.6	-12.9 ±0.1	7.2 ±0.1	-13.6	8.9	-	-
Phytoplankton	-26.7 ±0.5	8.3 ±0.7	-26.4 ±0.8	5.2 ±1.0	-25.2 ±0.3	6.0 ±0.6	-23.6 ±0.3	8.0 ±1.2	-	-	-26.7 ±0.5	4.9 ±1.1
Cyanobacteria	-24.9 ±0.5	6.3 ±0.5	-25.9	8.0	-17.0	8.5	-18.1	6.6	-	-	-	-
Microphytobenthos	-22.7 ±2.0	6.8 ±0.8	-25.3 ±0.2	6.3 ±0.8	-23.5 ±0.5	9.0 ±0.6	-22.6 ±0.2	8.9 ±1.0	-	-	-25.9 ±0.7	5.1 ±1.6
Other												
Detritus	-25.9 ±0.2	8.5 ±1.6	-24.6 ±1.1	3.8 ±1.2	-26.2 ±0.6	3.3 ±1.5	-26.9 ±1.4	7.5 ±0.7	-	-	-27.4 ±0.6	2.5 ±2.1
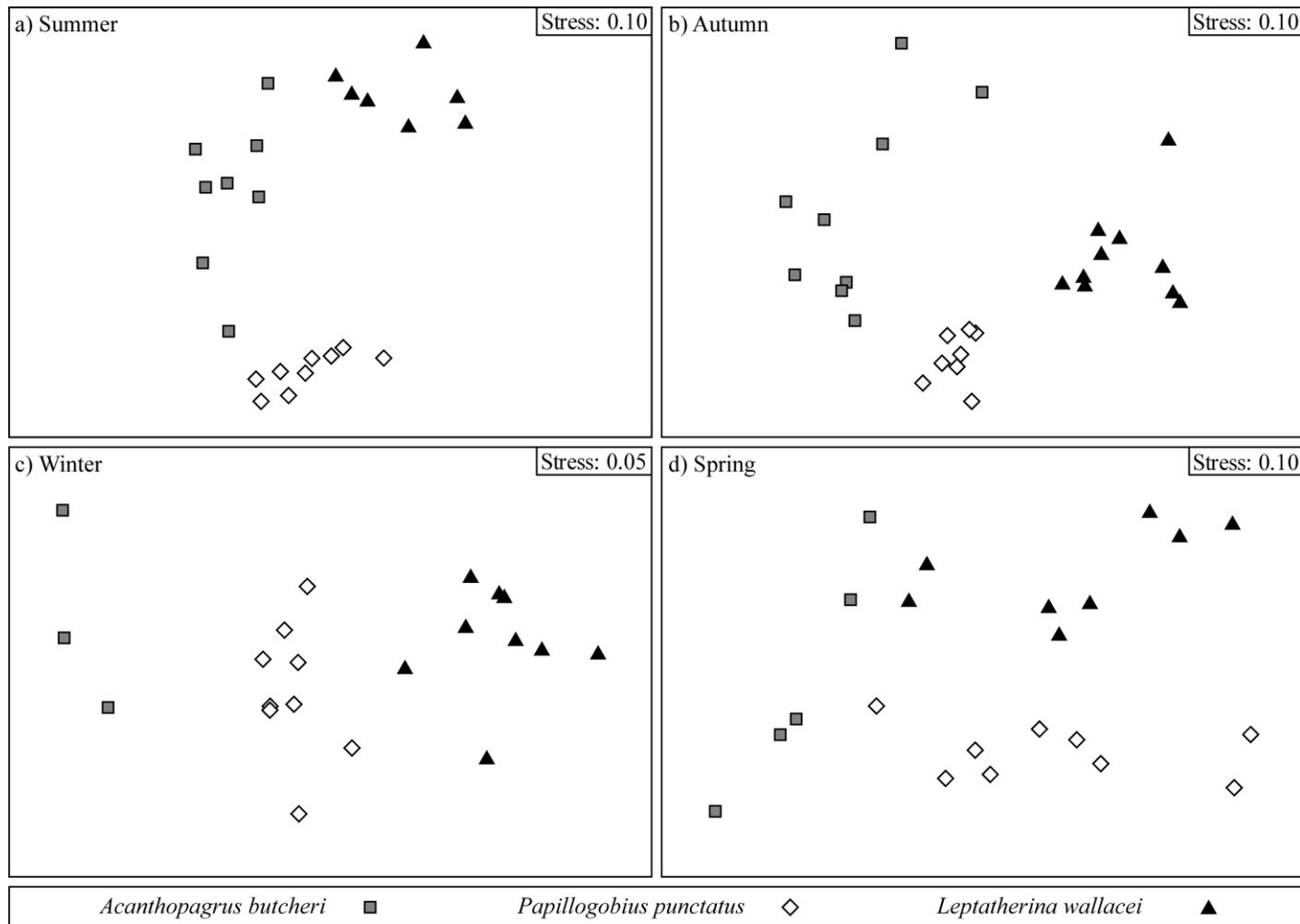
Figure 4.10: Non-metric multidimensional scaling ordination plots derived from the matrices constructed using replicate samples of the dietary composition of *Acanthopagrus butcheri*, *Papillogobius punctatus* and *Leptatherina wallacei* in the Canning River in a) summer, b) autumn, c) winter and d) spring 2009.

Figure 4.11: Mean (±1SE) values of $\delta^{13}C$ and $\delta^{15}N$ (‰) for *Acanthopagrus butcheri* (△), *Pseudogobius olorum* (▲) and *Leptatherina wallacei* (▲), and for the various components of the macrofauna (□'s), meiofauna (○'s) and primary producers ($\vdots$'s), and for detritus (◆) in the Swan River in summer and winter 2007.

primary producer to the highest value fish species was therefore equivalent to *ca* 3 trophic levels in the Swan River in both summer and winter 2007.

Terrestrial primary producers, *i.e.* small riparian vegetation (SRV) and large riparian vegetation (LRV), differed from aquatic primary producers in that their mean δ^{13}C and δ^{15}N values in summer ranged from only -28.8 to -28.9‰ and 4.2 to 5.1‰, respectively. The isotopic values for detritus were similar to those of aquatic primary producers for δ^{13}C, however, in both summer (-25.9‰) and winter (-24.6‰), but varied more widely for δ^{15}N, *i.e.* 8.5 and 3.8‰, respectively, and these values were typically associated with high error (Table 4.6; Fig. 4.11).

While isotopic values for cyclopoid (δ^{13}C = -26.5‰; δ^{15}N = 4.5‰) and harpacticoid (δ^{13}C = -28.4‰; δ^{15}N = 5.0‰) copepods were only determined in summer and winter, respectively, they were similar. Calanoid copepods, however, differed in their isotopic values, particularly those for δ^{15}N, between summer and winter, *i.e.* δ^{13}C = -31.7 *vs* -34.6‰ and δ^{15}N = 5.3 *vs* 12.9‰, respectively, and these seasonal values were associated with high variability (Table 4.6; Fig. 4.11).

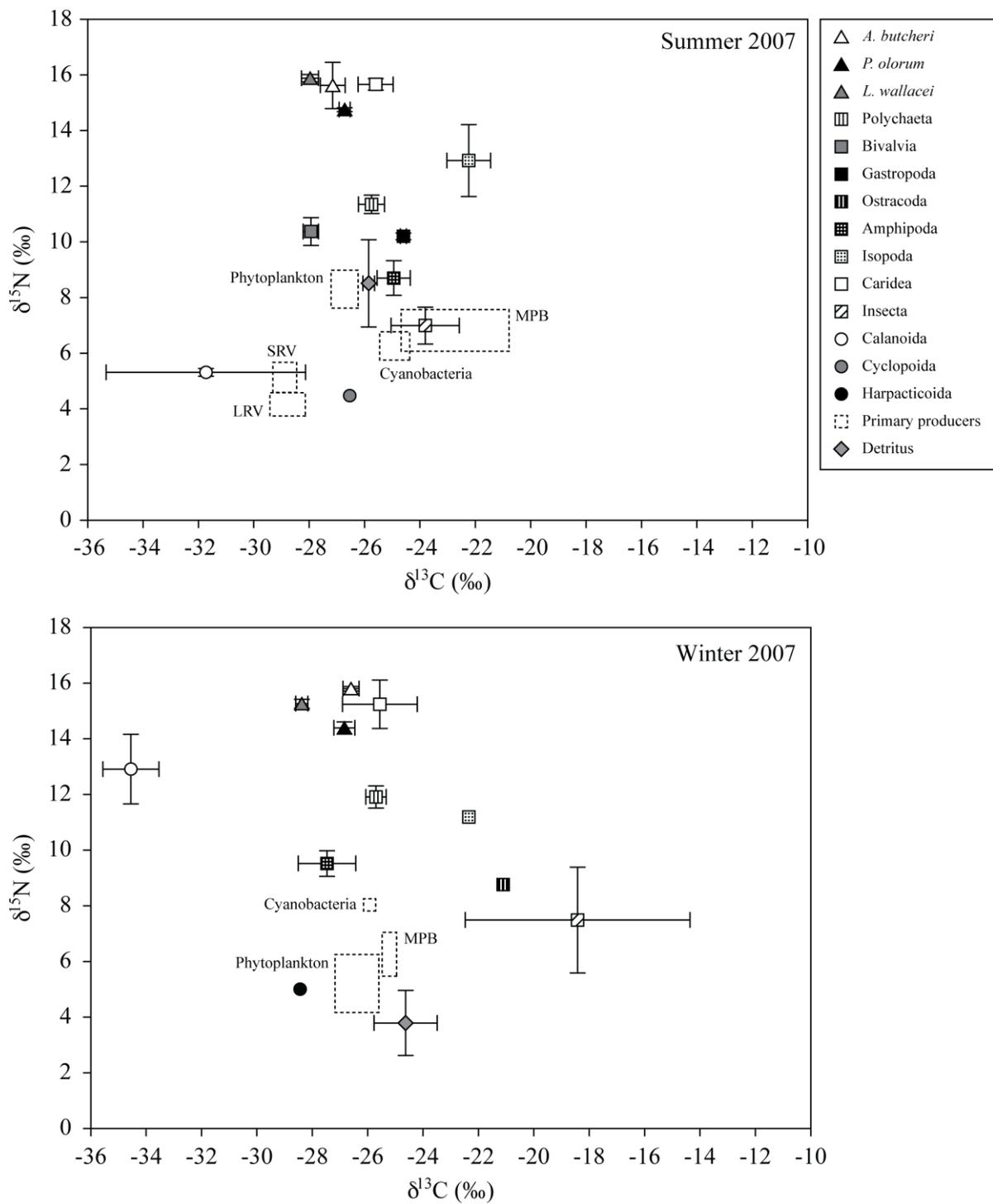The various components of the macrofauna varied markedly in isotopic characteristics, ranging in summer from -22.2 to -28.0‰ for δ^{13}C and 7.0 to 15.6‰ for δ^{15}N and in winter from -18.4 to -27.5‰ for δ^{13}C and 7.5 to 15.2‰ for δ^{15}N, with the values for insects in winter being particularly variable (Table 4.6; Fig. 4.11). In each season, carid shrimp had the highest δ^{15}N value, a value equivalent to those of the three species of fish, followed by isopods, polychaetes, amphipods and insects, with bivalves and gastropods having intermediate values in summer and ostracods a low value in winter (Table 4.6; Fig. 4.11). Macrofauna typically had δ^{13}C values within the range of those of aquatic primary producers, with the exceptions of isopods, ostracods and insects in winter.

4.3.4.2 Isotopic characteristics of fish and their potential prey in the Canning River in 2007-08

The mean δ^{13}C and δ^{15}N values for *A. butcheri*, *P. punctatus* and *L. wallacei* in the Canning River were highly similar, with those for δ^{13}C ranging from only -20.6 to -23.9‰ in winter 2007 and from -20.0 to -22.8‰ in summer 2008, and those for δ^{15}N ranging from only 15.2 to 16.0‰ in winter and 15.0 to 15.9‰ in summer (Table 4.6; Fig. 4.12). The isotopic values for aquatic primary producers (phytoplankton, cyanobacteria, MPB, macroalgae and seagrass) varied markedly, with mean δ^{13}C values ranging from -12.0 to -25.2‰ in winter and -12.9 to -23.6‰ in summer, and with mean δ^{15}N values ranging from 6.0 to 10.3‰ in winter and 6.6 to 12.2‰ in summer. Phytoplankton, MPB and particularly macroalgae in summer 2008 displayed the greatest variation in isotope values for primary producers (Table 4.6; Fig. 4.12). The increase in δ^{15}N values from the lowest value aquatic primary producer to the highest value fish species was therefore equivalent to *ca* 3 trophic levels in the Canning River in both winter 2007 and summer 2008.

Figure 4.12: Mean (±1SE) values of $\delta^{13}C$ and $\delta^{15}N$ (‰) for *Acanthopagrus butcheri* (△), *Papillogobius punctatus* (▲) and *Leptatherina wallacei* (▲), and for the various components of the macrofauna (□'s), meiofauna (○'s) and primary producers (⊡'s), and for detritus (◆) in the Canning River in winter 2007 and summer 2008.

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Terrestrial primary producers, *i.e.* SRV and LRV, differed from aquatic primary producers in that their mean δ^{13}C values in summer 2008 ranged from -28.6 to -29.9‰, and also varied markedly in their δ^{15}N values with that of SRV (7.4‰) being far greater than that of LRV (3.4‰). The δ^{13}C values for detritus were depleted compared to those of aquatic primary producers in both winter 2007 (-26.2‰) and summer 2008 (-26.9‰), but differed widely for δ^{15}N, *i.e.* 3.3 and 7.5‰, respectively (Table 4.6; Fig. 4.12).

The isotopic values for calanoid copepods were similar in winter 2007 and summer 2008, *i.e.* δ^{13}C = -26.3 *vs* -22.8‰ and δ^{15}N = 8.7 *vs* 11.0‰, respectively (Table 4.6; Fig. 4.12). Macrofauna in the Canning River varied in isotopic characteristics, ranging in winter 2007 from -18.0 to -22.6‰ for δ^{13}C and 8.7 to 13.0‰ for δ^{15}N and in summer 2008 from -17.2 to -23.9‰ for δ^{13}C and 3.0 to 14.3‰ for δ^{15}N. It should be noted however, that insects (δ^{13}C = -21.6‰; δ^{15}N = 3.0‰) differed markedly from aquatic macrofauna in the later season (Table 4.6; Fig. 4.12). In both seasons, carid shrimp had the highest δ^{15}N value of any component of the macrofauna, but differed markedly in its δ^{13}C signature between winter 2007 and summer 2008, *i.e.* -18.7 *vs* -23.9‰. Polychaetes and gastropods tended to have higher δ^{15}N in both seasons than, isopods, amphipods and bivalves, with the latter tending to have δ^{13}C values similar to MPB and the remaining macrofauna to macroalgae and cyanobacteria (Table 4.6; Fig. 4.12).

4.3.4.3 Isotopic characteristics of fish and their potential prey in the Canning River in 2008-09

In the Canning River in 2009, the mean δ^{13}C and δ^{15}N values for *A. butcheri*, *P. punctatus* and *L. wallacei* were very similar, with δ^{13}C ranging from -20.4 to -23.2‰ in summer and from -21.0 to -24.1‰ in winter, and δ^{15}N ranging from 15.4 to 16.2‰ in summer and 15.6 to 16.1‰ in winter (Table 4.6; Fig. 4.13). The δ^{13}C value for macroalgae (-25.4‰) differed markedly from that of seagrass (-13.6‰) in summer, but these two aquatic primary producers shared similar δ^{15}N values, *i.e.* 9.7 and 8.9‰, respectively. Phytoplankton and MPB shared similar isotopic values in winter (δ^{13}C = -26.7 and -25.9‰ and δ^{15}N = 4.9 and 5.1‰, respectively), although both displayed considerable variability with respect to δ^{15}N, and differed from that of macroalgae in that season, *i.e.* δ^{13}C = -22.4‰ and δ^{15}N = 10.1‰ (Table 4.6; Fig. 4.13). The increase in δ^{15}N values from the lowest value aquatic primary producer to the highest value fish species was thus equivalent to *ca* 2 trophic levels in the Canning River in summer and *ca* 3 in winter. Although, it should be noted that in the Canning River in summer 2009, a considerable number of the samples could not be included in the analyses due to their small size and therefore unreliable estimates of δ^{13}C and δ^{15}N.

In winter, terrestrial primary producers, *i.e.* SRV and LRV, ranged in mean δ^{13}C values from only -28.6 to -29.9‰, differed markedly in their δ^{15}N values with that of SRV (7.4‰) far exceeding than that of LRV (0.7‰). The isotopic values for detritus in winter were δ^{13}C = -

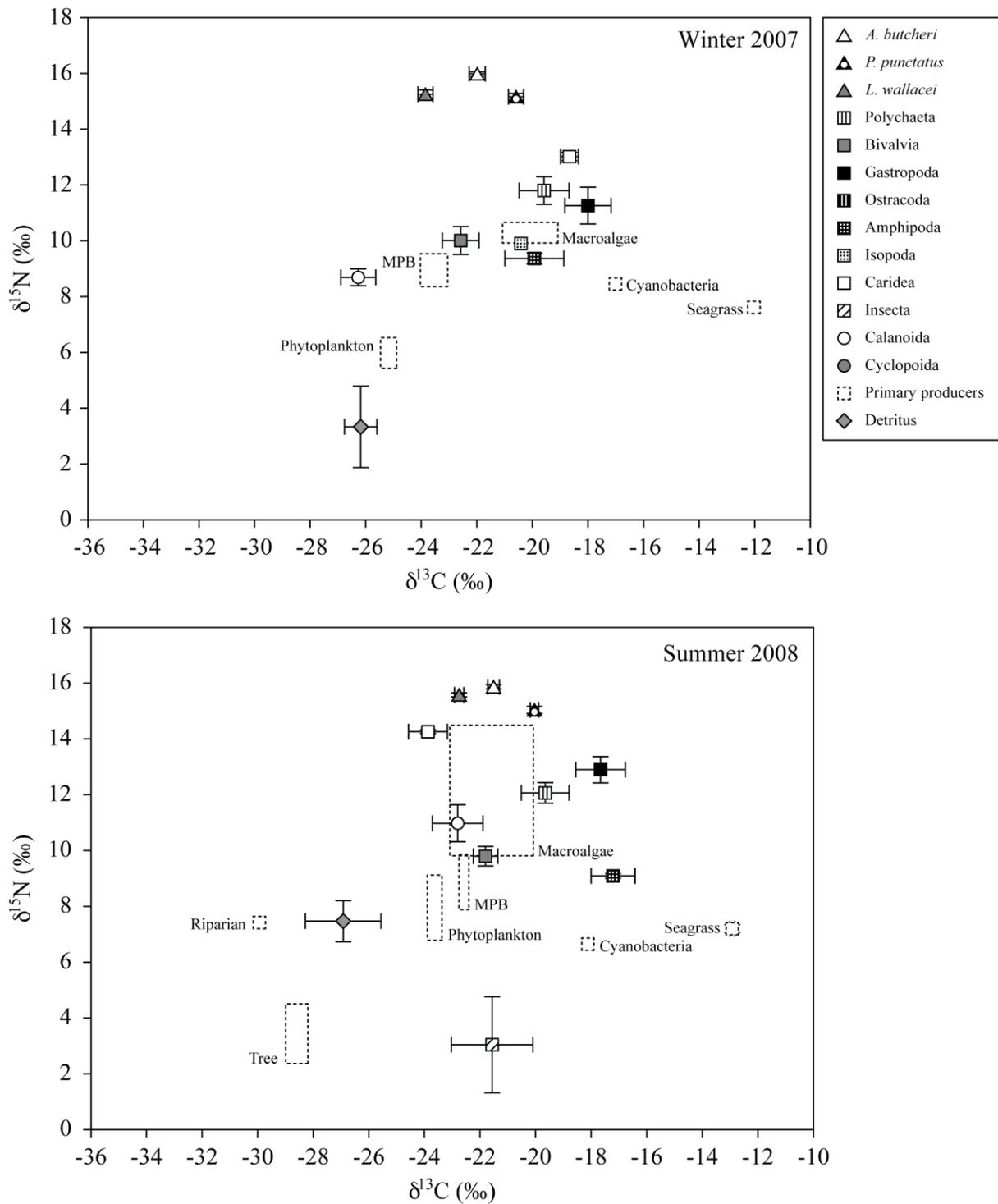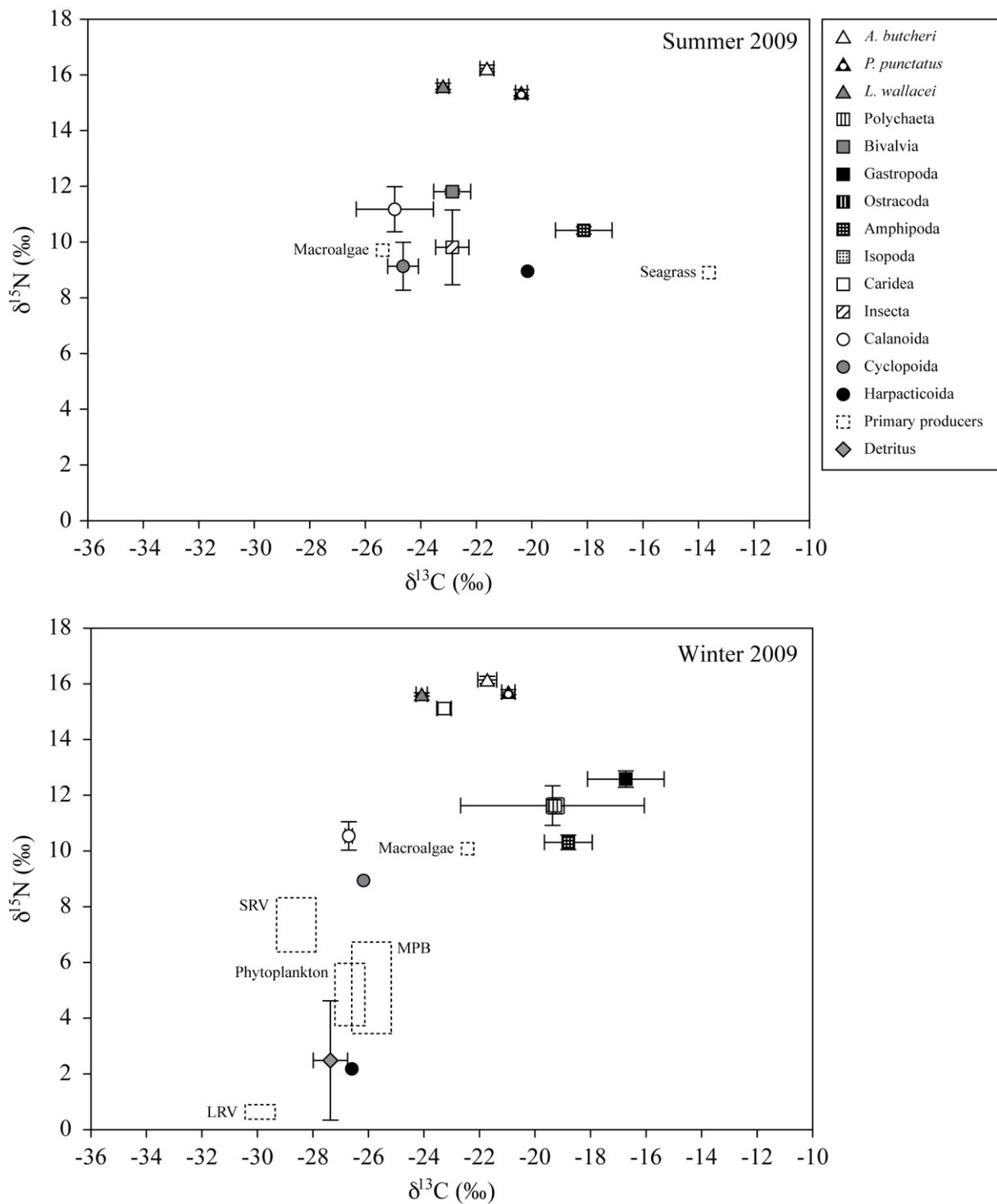**Figure 4.13:** Mean (±1SE) values of $\delta^{13}C$ and $\delta^{15}N$ (‰) for *Acanthopagrus butcheri* (△), *Papillogobius punctatus* (△) and *Leptatherina wallacei* (▲), and for the various components of the macrofauna (□'s), meiofauna (○'s) and primary producers (⊡'s), and for detritus (◆) in the Canning River in summer and winter 2009.

27.4‰ and $\delta^{15}N$ = 2.5‰, but showed considerably variability about those means, particularly with respect to $\delta^{15}N$ (Table 4.6; Fig. 4.13).

The $\delta^{13}C$ values for calanoid and cyclopoid copepods, *i.e.* -24.9 and -24.6‰, respectively, differed from that of harpacticoids (-20.2) and range in $\delta^{15}N$ from 9.0 to 11.2‰. In winter however, the $\delta^{13}C$ values for these copepods were highly similar, *i.e.* -26.2 to -26.7‰, but both calanoid and cyclopoid copepods differed markedly from harpacticoids with respect to $\delta^{15}N$, *i.e.* 10.5 and 8.9 *vs* 2.2‰ (Table 4.6; Fig. 4.13).

In the Canning River in summer 2009, the isotopic characteristics of the macrofauna ranged from -18.1 to -22.9‰ for $\delta^{13}C$ and from 9.8 to 11.8‰ for $\delta^{15}N$ (Table 4.6; Fig. 4.13). In the winter of that year, isotopic values for carid shrimp were equivalent to those of the three fish species, *i.e.* $\delta^{13}C$ = -23.3‰; $\delta^{15}N$ = 15.1‰, with those for bivalves, polychaetes, amphipods and gastropods ranging from -18.8 to -23.3‰ for $\delta^{13}C$ and from 10.3 to 15.1‰ for $\delta^{15}N$ (Table 4.6; Fig. 4.13).

4.3.5 Fatty acid composition of fish and their potential prey in the Swan River in 2007

A total of 14 different fatty acids were identified that contributed greater than 1% to the total fatty acid content of *A. butcheri*, *P. olorum* and *L. wallacei* in the Swan River in 2007 (Table 4.7). Overall, the contributions made by saturated, monounsaturated and polyunsaturated fatty acids were similar in each species and in both seasons. Irrespective of season, polyunsaturated fatty acids typically dominated the fatty acid content of each species, contributing between 42.5 and 51.0% to the total, with 22:6(n-3) being by far the most prevalent polyunsaturated fatty acid in each species (Table 4.7; Fig. 4.14). Saturated fatty acids, in both summer and winter 2007, also made a substantial contribution to the fatty acid content of each species, contributing between 32.4 and 36.7%, and of these fatty acids, 16:0 always made, by far, the greatest contribution. Monounsaturated fatty acids made a consistently lower contribution to the total fatty acid content of the three species, ranging between 12.7 and 16.0%, and, of the three monounsaturated fatty acid identified, 18:1(n-9) was the most prevalent (Table 4.7; Fig. 4.14).

Despite these overall similarities, when the matrix, derived from the percent contribution of each fatty acid to the composition of each replicate sample of fish tissue in each season, was subjected to ANOSIM, fatty acid composition was shown to be significantly related (both *P* = 0.001) to both species (*R* = 0.675) and season (*R* = 0.181). Thus, when the data was considered separately for each season, the samples for each species formed distinct groups on the seasonal ordination plots and showed only limited overlap (Fig. 4.15). Pairwise comparisons demonstrated that, in each season and in each case, these interspecific differences were significant (all *P* = 0.001) and large, with the greatest difference in fatty acid composition being between *P. olorum* and *L. wallacei* in summer (*R* = 0.876) and between *A. butcheri* and *L. wallacei* in winter (*R* = 0.732).

Table 4.7: The mean percentage contributions (± 1SE) of fatty acids and total mean contributions (± 1SE) of saturated, monounsaturated and polyunsaturated fatty acids to the total fatty acid content (>1%) of *Acanthopagrus butcheri*, *Pseudogobius olorum* and *Leptatherina wallacei* in the Swan River in summer and winter 2007.

	A. butcheri		P. olorum		L. wallacei	
	Summer	Winter	Summer	Winter	Summer	Winter
Saturated						
14:0	0.7 ±0.2	0.6 ±0.2	1.7 ±0.6	1.1 ±0.1	1.8 ±0.2	1.3 ±0.1
16:0	23.0 ±0.5	24.3 ±0.3	20.6 ±0.3	19.5 ±0.5	25.1 ±0.4	22.1 ±0.3
17:0	1.1 ±0.1	0.6 ±0.1	1.8 ±0.1	1.5 ±0.1	1.1 ±0.1	1.0 ±0.1
18:0	8.2 ±0.2	7.7 ±0.2	12.0 ±0.2	10.4 ±0.2	8.6 ±0.2	7.9 ±0.2
Total	**33.0 ±0.9**	**33.1 ±0.8**	**36.1 ±1.2**	**32.5 ±0.9**	**36.7 ±0.9**	**32.4 ±0.6**
Monounsaturated						
16:1(n-7)	4.3 ±0.2	3.3 ±0.2	3.1 ±0.2	3.5 ±0.2	3.3 ±0.2	3.1 ±0.2
18:1(n-7)	2.8 ±0.1	2.4 ±0.1	3.4 ±0.1	4.4 ±0.2	2.8 ±0.1	3.2 ±0.2
18:1(n-9)	8.9 ±0.3	7.0 ±0.3	8.2 ±0.2	7.0 ±0.2	8.5 ±0.3	8.2 ±0.5
Total	**16.0 ±0.6**	**12.7 ±0.6**	**14.6 ±0.5**	**14.9 ±0.5**	**14.6 ±0.6**	**14.5 ±0.9**
Polyunsaturated						
18:2(n-6)	1.8 ±0.1	1.5 ±0.1	0.9 ±0.1	1.6 ±0.2	1.9 ±0.1	3.7 ±0.5
18:3(n-3)	0.2 ±0.1	0.1 ±0.1	0.2 ±0.1	0.5 ±0.1	1.3 ±0.2	1.7 ±0.1
18:4(n-3)	0.1 ±0.1	-	0.1 ±0.1	0.2 ±0.1	1.7 ±0.2	1.0 ±0.2
20:4(n-6)	6.4 ±0.3	7.0 ±0.3	6.2 ±0.3	5.8 ±0.3	4.1 ±0.4	3.6 ±0.2
20:5(n-3)	10.3 ±0.4	8.9 ±0.3	13.4 ±0.3	13.6 ±0.6	5.3 ±0.2	5.7 ±0.4
22:5(n-3)	4.7 ±0.1	5.2 ±0.1	4.9 ±0.1	5.2 ±0.2	4.9 ±0.1	5.2 ±0.2
22:6(n-3)	21.0 ±0.6	26.9 ±0.9	16.9 ±0.7	17.7 ±1.2	26.5 ±0.9	30.1 ±1.0
Total	**44.6 ±1.7**	**49.6 ±1.8**	**42.5 ±1.7**	**44.6 ±2.7**	**45.8 ±2.2**	**51.0 ±2.6**
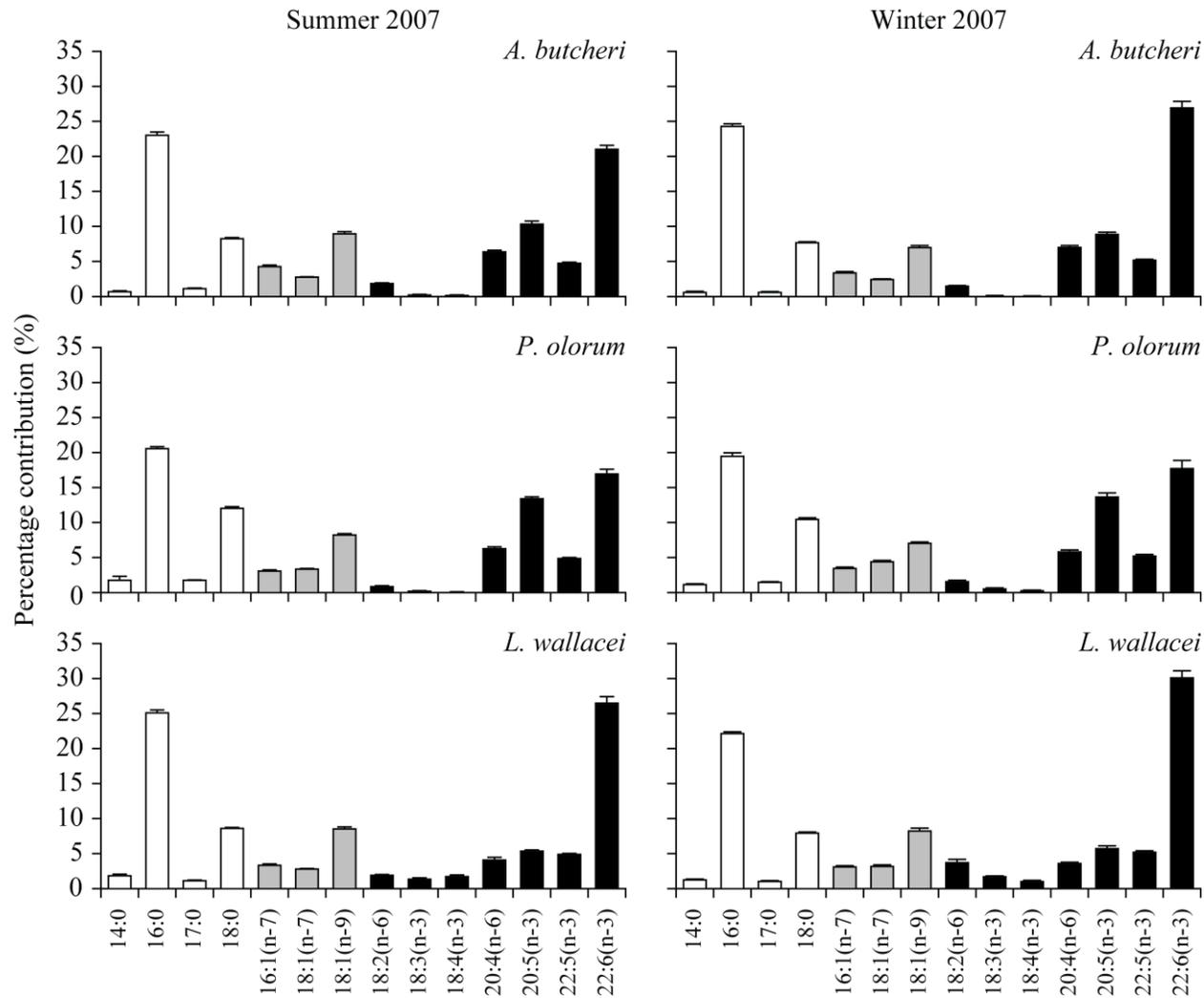
Figure 4.14: The percentage contributions (mean and SE) of fatty acids that contributed >1% to the total fatty acid content of *Acanthopagrus butcheri*, *Pseudogobius olorum* and *Leptatherina wallacei* in the Swan River in summer and winter 2007. Saturated fatty acids = white, monounsaturated fatty acids = grey, polyunsaturated fatty acids = black.

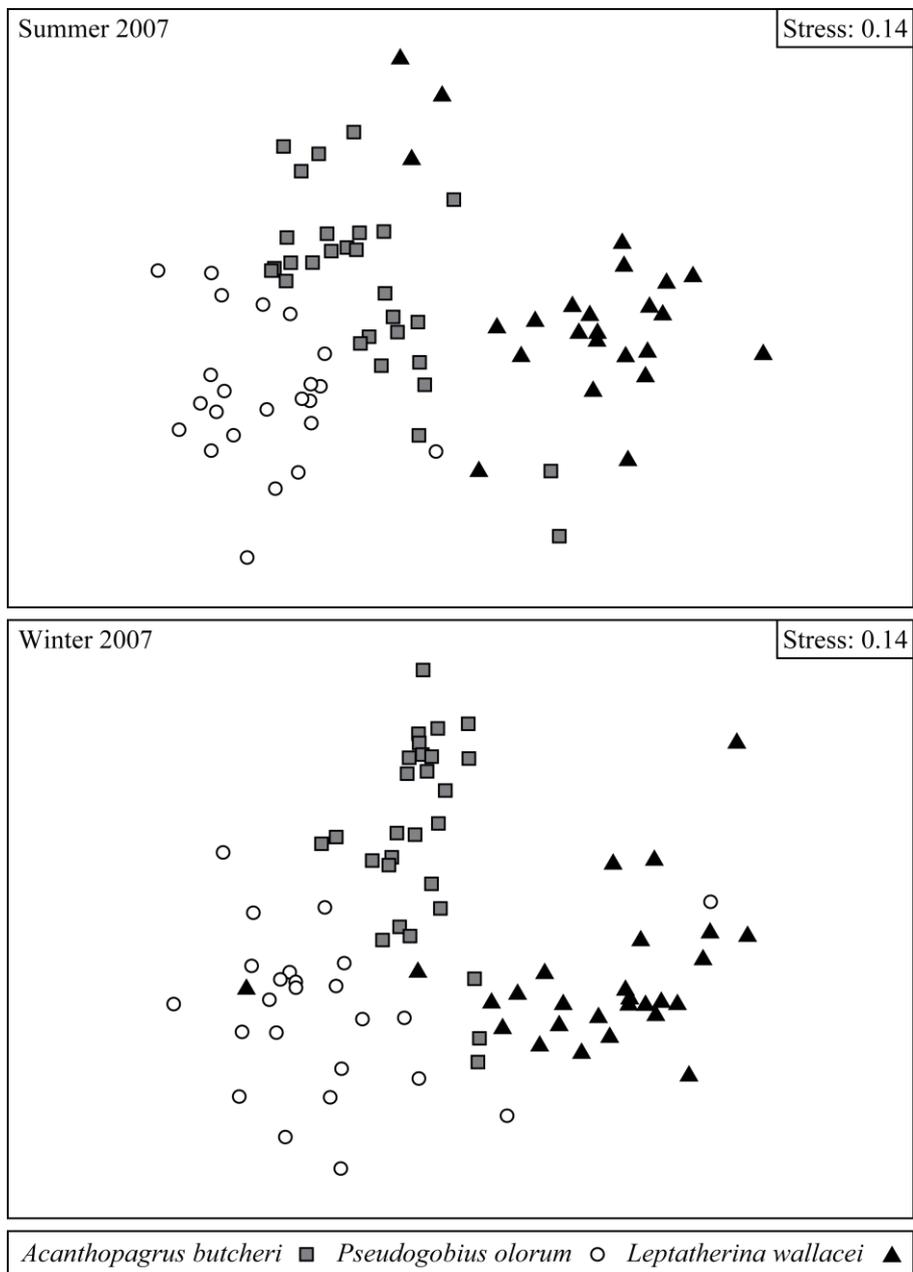**Figure 4.15:** Non-metric multidimensional scaling ordination plots derived from the matrices constructed using replicate samples of the fatty acid composition (all fatty acids that contributed >1% to total fatty acid content) of *Acanthopagrus butcheri*, *Pseudogobius olorum* and *Leptatherina wallacei* in the Swan River in summer and winter 2007.

In both seasons, the fatty acid composition of *A. butcheri*, *P. olorum* and *L. wallacei*, were typified by the saturated fatty acid 16:0 and the polyunsaturated 22:6(n-3), with that of the former two species also being typified by the polyunsaturated 20:5(n-5) and that of *A. butcheri* also by the monounsaturated fatty acid 18:1(n-9) in summer and that of *P. olorum* also by the saturated 18:0 in both season (Table 4.8).

In summer, the fatty acid composition of *A. butcheri* was distinguished from those of both *P. olorum* and *L. wallacei* by a greater precent contribution by 22:6(n-3) and from that of *P. olorum* also by 18:2(n-6) and consistently lower contributions by 14:0, 18:0, and from that of *L. wallacei* also by consistently lower contributions of 18:4(n-3), 18:3(n-3) and 14:0 (Table 4.8). The fatty acid composition of *P. olorum* was distinguished from that of *L. wallacei* in summer by a consistently greater contribution by 20:5(n-3) and by lower contributions of 18:4(n-3), 22:6(n-3) and 18:3(n-3). In winter, similar suites of fatty acids distinguished between the species, with *A. butcheri* being distinguished from *P. olorum* by a greater contribution by 22:6(n-3) and lower contributions by 20:5(n-3) and 14:0, from *L. wallacei* by consistently lower percent contributions of 18:3(n-3) and 18:4(n-3) and with *P. olorum* being distinguished from *L. wallacei* by consistently greater contributions of 20:5(n-3) and lower contributions of 22:6(n-3) and 18:3(n-3) (Table 4.8).

In the Swan River in 2007, pooled across seasons, the fatty acid content of primary producers varied markedly. Thus, while the fatty acid content of macroalgae, phytoplankton, cyanobacteria and MPB were dominated by saturated fatty acids, in particular 16:0 which ranged from 23.0 to 33.6%, that of LRV was dominated by polyunsaturated fatty acids, largely 18:3(n-3), *i.e.* 42.8% (Table 4.9; Fig. 4.16). The fatty acid content of detritus was comprised of similar contributions of both saturated and polyunsaturated fatty acids, but while 16:0 was the dominant saturated fatty acid, 22:5(n-3) was the dominant polyunsaturated fatty acid, with 18:3(n-3) not contributing greater than 1% to detrital matter (Table 4.9). Monounsaturated fatty acid contributions varied markedly, ranging from 3.7% for LRV to 34.2% in cyanobacteria. Unique contributions to primary producers (excluding detritus) were made by 20:0 to LRV, 18:4(n-3) and 20:4(n-3) to phytoplankton and by 20:4(n-6) to MPB (Table 4.9; Fig. 4.16).

The fatty acid content of the macrofauna in the Swan River in 2007 (pooled across seasons) varied markedly. Thus, the dominant contribution to total fatty acid content in bivalves and ostracods was from saturated fatty acids, in insects was from monounsaturated fatty acids and in polychaetes, gastropods, amphipods and carid shrimp from polyunsaturated fatty acids (Table 4.10). Thus, the single most prevalent fatty acid in bivalves and ostracods was 16:0, in insects was 18:2(n-6), in polychaetes and amphipods was 20:5(n-3), gastropods 20:4(n-6) and in carid shrimp, despite the overall dominance of polyunsaturated fatty acids, the saturated fatty acid 16:0 (Table 4.10; Fig. 4.17). Unique contributions were made to bivalves by 16:1(n-5), to ostracods by 20:4(n-3), to amphipods by 16:3(n-4) and 20:3(n-3) and to carid shrimps by 20:3(n-6), however these contributions were always small and typically highly variable (Table 4.10; Fig. 4.17).

Table 4.8: Global *R*-statistic values and significance levels (bold) for ANOSIMs, and *R*-statistic values and significance levels (light shaded boxes) for pairwise comparisons, of the dietary compositions of *Acanthopagrus butcheri*, *Pseudogobius olorum* and *Leptatherina wallacei* in the Swan River in summer and winter 2007. Dietary categories/sub-categories determined by SIMPER as most responsible for typifying the dietary composition of each species (dark shaded boxes) and for distinguishing between the dietary compositions of the three species in each paired comparison (unshaded boxes). ***$P < 0.001$. [Ab] = *A. butcheri*, [Po] = *P. olorum*, [Lw] = *L. wallacei* denote the species in which the dietary category/sub-category made the greater contribution to the dietary composition.

Summer **R = 0.662*****	*A. butcheri*	*P. olorum*	*L. wallacei*
A. butcheri	16:0 22:6(n-3) 20:5(n-5) 18:1(n-9)	0.471***	0.668***
P. olorum	14:0 [Po] 18:2(n-6) [Ab] 18:0 [Po] 22:6(n-3) [Ab]	16:0 22:6(n-3) 20:5(n-3) 18:0	0.876***
L. wallacei	18:4(n-3) [Lw] 20:5(n-3) [Ab] 18:3(n-3) [Lw] 14:0 [Lw]	20:5(n-3) [Po] 18:4(n-3) [Lw] 22:6(n-3) [Lw] 18:3(n-3) [Lw]	22:6(n-3) 16:0

Winter **R = 0.687*****	*A. butcheri*	*P. olorum*	*L. wallacei*
A. butcheri	22:6(n-3) 16:0 20:5(n-3)	0.635***	0.732***
P. olorum	22:6(n-3) [Ab] 20:5(n-3) [Po] 14:0 [Po]	16:0 22:6(n-3) 20:5(n-3) 18:0	0.693***
L. wallacei	18:3(n-3) [Lw] 18:4(n-3) [Lw]	22:6(n-3) [Lw] 20:5(n-3) [Po] 18:3(n-3) [Lw]	22:6(n-3) 16:0

Table 4.9: The mean percentage contributions (± 1SE) of fatty acids and total mean contributions (± 1SE) of saturated, monounsaturated and polyunsaturated fatty acids to the total fatty acid content (>1%) of primary producers, *i.e.* large riparian vegetation (LRV), macroalgae, phytoplankton, cyanobacteria, microphytobenthos (MPB), and of detritus in the Swan River in 2007 (pooled for season).

	LRV	Macroalgae	Phytoplankton	Cyanobacteria	MPB	Detritus
Saturated						
14:0	1.4 ±0.6	4.5	3.1 ±0.3	11.3 ±3.9	3.8 ±0.5	2.0 ±1.0
15:0	-	2.7	-	1.8 ±0.2	4.4 ±1.1	-
16:0	15.9 ±1.4	33.6	23.0 ±0.7	25.8 ±1.4	28.1 ±1.8	16.4 ±4.6
18:0	1.7 ±1.0	15.4	7.6 ±0.4	8.9 ±1.3	4.6 ±0.6	6.4 ±0.9
20:0	3.6 ±2.0	-	-	-	-	2.7 ±1.4
Total	**22.5 ±5.0**	**56.2**	**33.8 ±1.4**	**47.7 ±6.8**	**40.9 ±3.9**	**27.4 ±8.0**
Monounsaturated						
16:1(n-7)	-	1.7	6.3 ±0.5	19.2 ±5.4	18.1 ±1.7	5.0 ±1.6
16:1(n-9)	-	5.3	-	3.6 ±1.8	-	-
18:1(n-7)	-	2.2	2.5 ±0.2	2.9 ±0.3	3.4 ±0.6	2.7 ±0.9
18:1(n-9)	2.6 ±0.6	16.3	7.9 ±0.5	8.6 ±2.7	6.8 ±1.0	7.9 ±1.0
20:1(n-7)	1.1 ±0.7	-	-	-	-	-
Total	**3.7 ±1.3**	**25.5**	**16.6 ±1.2**	**34.2 ±10.2**	**28.3 ±3.2**	**15.5 ±3.4**
Polyunsaturated						
16:3(n-4)	-	-	2.0 ±0.4	-	2.3 ±0.6	-
18:2(n-6)	12.6 ±1.2	5.1	3.9 ±0.3	3.0 ±1.0	1.7 ±0.5	3.0 ±1.0
18:3(n-3)	42.8 ±5.3	2.8	2.2 ±0.8	-	-	-
18:4(n-3)	-	-	4.6 ±0.6	-	-	-
20:2(n-6)	-	2.0	1.4 ±0.3	-	1.1 ±0.5	-
20:4(n-3)	-	-	1.3 ±0.3	-	-	-
20:4(n-6)	-	-	-	-	2.2 ±0.3	1.1 ±1.1
20:5(n-3)	1.3 ±0.7	-	7.2 ±0.6	2.0 ±1.0	5.5 ±1.0	6.3 ±2.0
22:5(n-3)	1.2 ±0.7	-	1.6 ±0.3	-	2.5 ±0.4	11.6 ±2.4
22:6(n-3)	-	-	5.9 ±0.7	2.5 ±0.5	1.3 ±0.5	2.5 ±2.5
Total	**57.9 ±7.9**	**9.9**	**30.3 ±4.3**	**7.5 ±2.6**	**16.6 ±3.8**	**24.5 ±8.9**

Table 4.10: The mean percentage contributions (± 1SE) of fatty acids and total mean contributions (± 1SE) of saturated, monounsaturated and polyunsaturated fatty acids to the total fatty acid content (>1%) of macrofauna, *i.e.* Polychaeta, Bivalvia, Gastropoda, Ostracoda, Amphipoda, Isopoda, Caridea, and of meiofauna, *i.e.* Calanoida and Cyclopoida, in the Swan River in 2007 (pooled for season).

	Macrofauna								Meiofauna	
	Polychaeta	Bivalvia	Gastropoda	Ostracoda	Amphipoda	Isopoda	Caridae	Insecta	Calanoida	Cyclopoida
Saturated										
14:0	-	1.3 ±0.7	-	3.4	1.5 ±0.3	4.4	2.2 ±0.6	-	3.4 ±0.3	2.1 ±2.1
15:0	-	-	-	-	-	-	-	-	1.1 ±0.4	1.7
16:0	12.1 ±0.8	22.9 ±3.2	14.1	21.8	18.5 ±1.1	25.7	22.2 ±1.6	17.0 ±3.4	27.8 ±2.4	20.2 ±6.6
17:0	2.6 ±0.2	2.2 ±0.2	3.4	-	-	-	1.3 ±0.1	-	2.4 ±0.8	2.4 ±0.4
18:0	7.7 ±0.3	10.1 ±1.0	8.5	12.1	6.1 ±0.5	4.4	8.3 ±0.7	6.5 ±1.0	10.3 ±1.2	17.6 ±0.6
Total	**22.5 ±1.3**	**36.5 ±5.0**	**26.0**	**37.4**	**26.0 ±1.9**	**34.5**	**34.0 ±3.0**	**23.5 ±4.4**	**44.9 ±5.0**	**44.1 ±9.6**
Monounsaturated										
16:1(n-5)	-	1.3 ±0.6	-	-	-	-	-	-	-	-
16:1(n-7)	5.3 ±0.7	5.1 ±1.1	4.3	5.0	5.9 ±0.9	2.3	6.4 ±0.9	9.0 ±4.8	3.2 ±1.8	1.5 ±0.1
16:1(n-9)	-	1.2 ±0.6	3.7	4.2	-	-	-	-	1.3 ±0.5	6.9 ±2.1
18:1(n-7)	5.5 ±0.5	4.7 ±1.3	2.6	6.9	4.8 ±0.5	2.2	5.7 ±1.3	1.2 ±0.8	1.9 ±0.4	3.9 ±0.5
18:1(n-9)	3.7 ±0.3	5.1 ±1.4	2.7	12.7	10.9 ±0.9	16.2	10.6 ±0.4	37.2 ±8.1	6.0 ±1.4	11.6 ±3.2
20:1(n-7)	-	-	-	-	-	-	-	-	-	1.2 ±1.2
20:1(n-11)	2.8 ±0.3	1.8 ±1.0	6.5	-	-	-	-	-	-	-
24:1(n-13)	-	-	-	-	-	-	-	-	1.6 ±0.6	-
Total	**17.3 ±1.8**	**19.3 ±6.1**	**19.9**	**28.7**	**21.6 ±2.3**	**20.7**	**22.7 ±2.6**	**47.5 ±13.7**	**14.0 ±4.6**	**25.1 ±7.0**
Polyunsaturated										
16:2(n-4)	-	-	-	-	-	-	-	-	-	1.2 ±1.2
16:3(n-4)	-	-	-	-	1.5 ±0.6	-	-	-	-	-
18:2(n-6)	1.8 ±0.5	1.0 ±0.5	2.2	3.5	2.1 ±0.3	-	2.6 ±0.5	19.5 ±4.9	3.3 ±0.6	4.1 ±0.3
18:3(n-3)	-	-	-	-	-	-	1.5 ±0.6	1.7 ±1.0	3.5 ±1.3	-
18:4(n-3)	-	1.8 ±0.9	-	-	2.6 ±1.2	-	1.0 ±0.4	-	2.4 ±0.6	-
20:2(n-6)	-	1.4 ±0.7	-	-	-	-	-	-	-	1.3 ±1.3
20:3(n-3)	-	-	-	-	1.5 ±1.5	-	-	-	-	-
20:3(n-6)	-	-	-	-	-	-	1.6 ±1.6	-	-	-
20:4(n-3)	-	-	-	1.5	-	-	-	-	-	-
20:4(n-6)	4.6 ±0.6	1.8 ±0.4	16.1	3.5	4.4 ±0.8	2.8	2.9 ±0.6	2.1 ±0.5	-	1.0 ±1.0
20:5(n-3)	17.7 ±1.1	5.3 ±1.7	8.9	10.5	19.4 ±2.5	14.2	14.2 ±2.3	3.3 ±0.8	6.8 ±0.9	6.0 ±3.1
22:5(n-3)	4.1 ±0.4	2.8 ±0.5	6.2	2.2	-	2.7	-	-	1.4 ±0.5	3.0 ±0.7
22:6(n-3)	3.0 ±0.5	13.0 ±5.1	3.2	2.3	10.9 ±1.3	25.1	13.8 ±1.8	-	17.6 ±6.3	4.7 ±1.9
Total	**31.2 ±3.0**	**27.0 ±9.8**	**36.7**	**23.6**	**42.4 ±8.1**	**44.8**	**37.7 ±7.6**	**26.6 ±7.1**	**34.9 ±10.1**	**21.3 ±9.4**
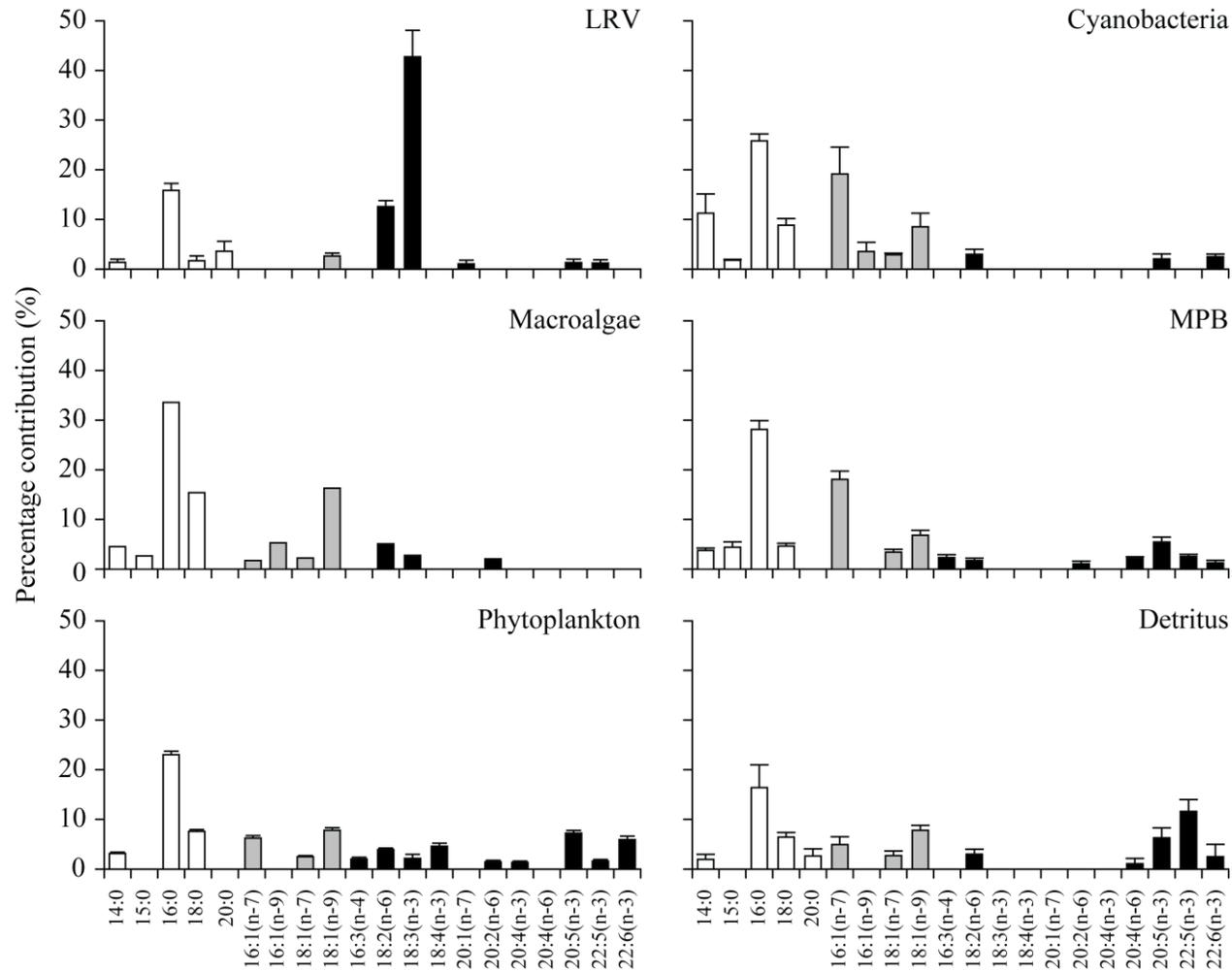
Figure 4.16: The percentage contributions (mean and SE) of fatty acids that contributed >1% to the total fatty acid content of primary producers, *i.e.* large riparian vegetation (LRV), macroalgae, phytoplankton, cyanobacteria, microphytobenthos (MPB), and of detritus in the Swan River in 2007 (pooled for season). Saturated fatty acids = white, monounsaturated fatty acids = grey, polyunsaturated fatty acids = black.
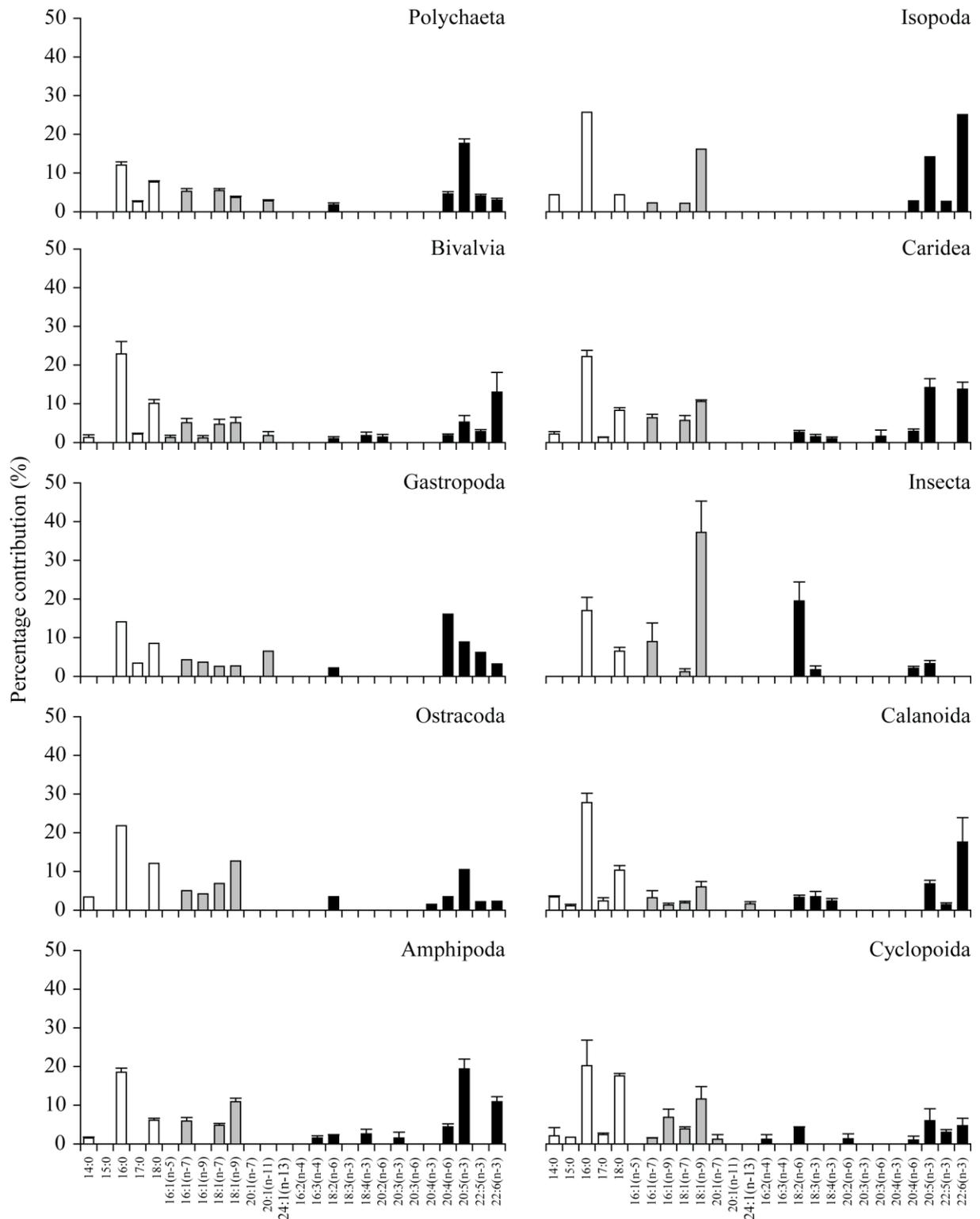
Figure 4.17: The percentage contributions (mean and SE) of fatty acids that contributed >1% to the total fatty acid content of macrofauna, *i.e.* Polychaeta, Bivalvia, Gastropoda, Ostracoda, Amphipoda, Isopoda, Caridea, and of meiofauna, *i.e.* Calanoida and Cyclopoida, in the Swan River in 2007 (pooled for season). Saturated fatty acids = white, monounsaturated fatty acids = grey, polyunsaturated fatty acids = black.

The fatty acid content of calanoid and cyclopoid copepods were similar in that they were both dominated by saturated fatty acids, but differed in that polyunsaturated fatty acids also made a substantial contribution to calanoids and monounsaturated fatty acids to cyclopoids (Table 4.10). While 16:0 made the single greatest contribution to the fatty acid content in both calanoid and cyclopoid copepods, the next highest contributor was 22:6(n-3) in calanoid copepods and 18:1(n-9) in cyclopoid copepods. Unique, but small and often highly varied, contributions were made to calanoid copepods by 24:1(n-13) and to calanoid copepods by 20:1(n-7) and 16:2(n-4) (Table 4.10; Fig. 4.17).

4.4 Discussion

4.4.1 The diets of *Acanthopagrus butcheri, Pseudogobius olorum, Papillogobius punctatus* and *Leptatherina wallacei* in the Swan-Canning Estuary

In the Swan River in 2007, the diet of *Acanthopagrus butcheri* was highly omnivorous, with this species consuming a wide range of invertebrate and algae species and, on occasion, also other teleost species. In particular, polychaetes, bivalve molluscs and amphipod crustaceans were consumed by this sparid and thus, in broad terms, the diet of *A. butcheri* in the Swan River in 2007 was similar to that of this species in the same estuary *ca* two decades ago, *i.e.* 1993-1995 (Sarre *et al.* 2000). However, while nereid polychaetes, galeommatid bivalves and corophiid and aorid amphipods made substantial contributions to the diet of *A. butcheri* in 2007, the mytilid bivalve *Xenostrobus securis* made by far the greatest contribution to the diet of *A. butcheri* in that earlier period (Sarre *et al.* 2000). Indeed, *X. securis* comprised *ca* 40% of the diet of *A. butcheri* in the 1990s compared with 19.4% collectively for bivalves in the current period and with no single dietary category contributing more than 13.6%, *i.e.* nereid polychaetes, in that later period. It therefore appears relevant that while both *Xenostrobus securis* and the galeommatid bivalve *Arthritica semen* were abundant in the Swan River in 1995-97, only the second bivalve remained abundant in 2005-08, with *X. securis* not being observed during extensive sampling of the Swan River in that later period (*cf* Kanandjembo *et al.* 2001b, Valesini *et al.* 2009).

Collectively, algae made similar contributions to the diet of *A. butcheri* in the two periods, but while Cladophora contributed only 0.3% by volume to the diet in the 1990s, it contributed 5.7% in the current period. Cladophora species were prevalent in the diets of *A. butcheri* in three estuaries along the south-coast of Western Australia, *i.e.* Stokes, Culham and Hamersley inlets, that were variably and often markedly hypersaline and within which the diversity of available food was therefore limited (Chuwen *et al.* 2007). Indeed, the collective contributions of algae to the diet of *A. butcheri* in these systems ranged from 38.4 to 62.8% and were thus far greater than those in the Swan River at any time, *i.e.* 7.8 to 8.3% (Sarre *et al.* 2000, Chuwen *et al.* 2007).

As in the Swan River, the diet of *A. butcheri* in the Canning River comprised mainly of bivalves, amphipods, polychaetes and algae, with other species of teleost being consumed only infrequently. However, unlike the Swan River the dominant bivalve consumed in the Canning River in both 2007-08 and 2009 was *Sanguinolaria biradiata* (Psammobiidae). Indeed, psammobiid bivalves contributed 24.9 and 18.6% to the diet of *A. butcheri* in the Canning River in 2007-08 and 2009, respectively, contributions that far exceed that of the red algae Cystocloniaceae (16.2%) in 2007-08 and those of unidentified amphipods (12.5%) and the green algae Gracilariaceae (10.7%) in 2009, the only other dietary taxa to contribute >10% in their respective years. While *S. biradiata* did not contribute to the diet of *A. butcheri* in the Swan River in 1993-95, it made a substantial contribution to the diet of this species in the Moore River Estuary (25.2%), *ca* 80 km north of the Swan-Canning Estuary, and a minor

contribution in the Nornalup-Walpole Estuary on the south coast of Western Australia, during the same period (Sarre *et al.* 2000). Interestingly, a study of the benthic fauna of the Swan-Canning Estuary in 1995-97 did not collect any psammobiid bivalves (Kanandjembo *et al.* 2001b), but *S. biradiata* was found to be abundant at some locations in the lower reaches of this system in 2005-08, although that later study did not examine benthic invertebrates in the Canning River (Valesini *et al.* 2009).

While polychaetes collectively made a consistent contribution to the diet of *A. butcheri* in the Canning River in 2007-08 and 2009, algae made a far greater contribution in 2007-08 than 2009, while the reverse was true of amphipods, presumably reflecting variations in the abundance of that latter taxa between the two periods and the opportunist feeding behaviour of this species (*e.g.* Sarre *et al.* 2000, Chuwen *et al.* 2007). Neither in the Swan River in 2007, nor in the Canning River in either period, did *A. butcheri* consume calanoid, cyclopoid or harpacticoid copepods, a trend reflected in most other estuaries in south-western Australia in which the diet of this species has been examined, with the exceptions of Nornalup-Walpole and Wellstead estuaries where the calanoid copepod *Gladioferens imparipes* made very minor contributions (Sarre *et al.* 2000, Chuwen *et al.* 2007).

In the Swan River in 2007, the diet of *Pseudogobius olorum* was dominated by nereid polychaetes and the green algae cladophora, which contributed 13.1 and 9.6%, respectively, to the overall diet of this species. Collectively, amphipods comprised 10.5% of the diet of *P. olorum*, while harpactacoid copepods comprised *ca* 7%. This contrasts markedly with the diet determined for this species in the Swan River *ca* three decades ago, *i.e.* 1983-85, which was dominated by algae and mats of bacteria and fungi (Gill and Potter 1993). While polychaetes, algae, amphipods and copepods were consumed in the Swan River throughout 2007, the relative contributions of these dietary categories varied somewhat throughout the year, presumably reflecting changes in availability of those food sources. Thus, for example, consumption of polychaetes was greatest in autumn and that of algae in summer and spring, although it should be noted that the relative contribution of the algae never exceeded *ca* 38%. The prevalence of animal matter in the diet of this species throughout 2007 is in stark contrast to the diet of this species in the 1980s, when animal matter was only consumed in considerable quantities during winter when the abundance of plant material in the Swan River was presumably at its lowest (Gill and Potter 1993).

As *P. olorum* could not be consistently caught in the Canning River in either 2007-08 or 2009, the diet of another small and short-lived gobiid, *Papillogobius punctatus*, was determined in that region in both periods. As with *P. olorum* in the Swan River, the diet of *P. punctatus* in the Canning River consisted of amphipods, polychaetes and copepods, but unlike *P. olorum* whose diet was more varied, these three dietary categories largely dominated the diet of *P. punctatus*. Thus, collectively these three dietary categories contributed *ca* 60% of the diet of *P. punctatus* in each period, although, it should be noted that various unidentified material comprised most of the remainder of the gut contents of this species. Unlike *P. olorum*, the diet of *P. punctatus* was primarily carnivorous, with algae making only a minor contribution to its diet in 2007-08 and with algae not being consumed in

2009. The diet of *P. punctatus* was therefore more similar to that of another, and closely related (Gill 1996), gobiid *Favonigobius lateralis*, whose diet concentrated on a single species of polychaete, a tanaid and an amphipod (Gill and Potter 1993). Differences in the diets of the carnivorous *F. lateralis* and the omnivorous *P. olorum* have been attributed primarily to the large terminal mouth, containing prominent caniniform teeth, of the former species and the inferior mouth, containing small villiform teeth, of *P. olorum* (Gill and Miller 1990, Gill and Potter 1993). Given the similarities in jaw morphologies of *P. punctatus* and *F. lateralis*, it is likely that differences in the diet of *P. olorum* and *P. punctatus* are the result of mouth morphology, rather than differences in the availability of prey between the Swan and Canning rivers.

The diet of *Leptatherina wallacei* in the Swan River in 2007 was dominated by calanoid copepods, with lysianassid amphipods, harpactacoid copepods, diptera insects and nereid polychaetes also frequently being consumed. A comparative study of the mouth morphology of three co-occurring species of atherinid, *i.e. L. wallacei*, *Atherinosoma elongata* and *Leptatherina presbyteroides*, in the Swan-Canning Estuary concluded that the degree of jaw protrusion and tooth size dictated the height in the water column at which those species fed. The intermediate characteristics of the mouth of *L. wallacei* thus suggested that it fed primarily in the mid-water column or near the benthos (Humphries 1993). The fact that calanoid copepods are typically a dominant component of estuarine zooplankton (*e.g.* Gaughan and Potter 1995, Valesini *et al.* 2009) and that, although primarily benthic, lysianassid amphipods (*e.g.* Sainte-Marie 1986) and nereid polychaetes (Clark and Tritton 1970) both exhibit strong swimming behaviour, supports the feeding behaviour of *L. wallacei* being primarily in the mid-water column or near the benthos. However, the fact that harpactacoid copepods and insects were also consumed, suggests that this species can also selectively feed on or near the benthos or at the surface of the water column.

While a similar suite of prey was consumed by *L. wallacei* in the Canning River in both 2007-08 and 2009, gastropods made a notable contribution to the diet of this species in winter 2007 and algae a substantial contribution in summer 2009. The apparent highly opportunist omnivorous diet of *L. wallacei* in the Swan and Canning river between 2007 and 2009 thus parallels that determined for this species in the Swan-Canning Estuary in 1979-80, which comprised primarily of planktonic crustaceans, flying insects, polychaetes and unicellular algae (Prince *et al.* 1982).

ANOSIM tests demonstrated that the composition of the diets of *A. butcheri*, *P. olorum* and *L. wallacei* were always significantly different in the Swan River in 2007. In each season, the greatest interspecific difference in diet was between *A. butcheri* and *L. wallacei* and this was typically the result the consistent and greater consumption of bivalves, amphipods and/or polychaetes by *A. butcheri* and also, in some seasons, to the greater prevalence of copepods and insects in the diet of *L. wallacei*. Thus, although both species exhibited opportunist omnivorous feeding behaviour, they displayed a marked tendency to select prey primarily from the benthos and the water column, respectively. It is therefore relevant that, in *A. butcheri* greater than 80 mm in length, the areae centrales, the region of peak cell density

in the eye, was always located in the dorsal region of the dorso-temporal retinal quadrant, and that this configuration of the eye, essentially turned downward, was predominantly associated with benthic feeding behaviour by individuals of this species (Shand *et al.* 2000).

Although, the diet of the small and benthic gobiid *P. olorum* in the Swan River was always significantly different from that of *A. butcheri* and *L. wallacei*, the interspecific differences with the former species were typically low and were similarly low with *L. wallacei* in winter 2007. These results suggest that while diet differed significantly between these species, certain dietary items, on occasion, made highly similar contributions to the diets of multiple species. Thus, for example, in autumn 2007, while polychaetes consistently contributed less to the diet of *P. olorum* than to that of *A. butcheri*, polychaetes were by far the dominant dietary item in the diets of both species, representing 39.7 and 31.6%, respectively, of all prey consumed by those species in that season. It may therefore be pertinent, that in the Swan River in 2005, both the mean density of benthic macroinvertebrates and the mean taxonomic distinctness of the benthic fauna were influenced by season and were least in the summer and autumn of that year (Valesini *et al.* 2009). As such, in those season when the density and diversity of available prey items is diminished, interspecific competition for food resources may be increased, as reflected by the reduced dissimilarity in the diets of the various fish species at those times.

As in the Swan River, the composition of the diets of *A. butcheri* and *L. wallacei* in the Canning River in both 2007-08 and 2009 were always very different, but unlike that former region, the composition of the diet of *A. butcheri* was also highly dissimilar to that of the gobiid species, *i.e. P. punctatus*. In every season of the two periods, this difference could always largely be attributed to the consistently greater consumption of bivalves by *A. butcheri* than by the other two species. This observation, together with the results of other previous studies on the diet of *A. butcheri* in south-western Australia (Sarre *et al.* 2000, Chuwen *et al.*, 2007), suggests that, despite its omnivorous and opportunist feeding behaviour, this species shows a marked tendency to preferentially consume bivalves when this prey is available.

The diet of *P. punctatus* in the Canning River was primarily carnivorous, while that of *L. wallacei* was omnivorous, and these differences in feeding behaviour were reflected in the composition of the diets of these two species typically being highly dissimilar, due largely to the greater consumption of amphipods and also often polychaetes by *P. punctatus* and of either insects or algae by *L. wallacei*. In winter 2007, however, the dietary composition of these two species did not significantly differ. In that season, the diets of both *P. punctatus* and *L. wallacei* were overwhelmingly dominated by both copepods, *i.e.* 32.9 and 26.7%, respectively, and amphipods, 26.7 and 27.5%, respectively. The considerable overlap in the composition of the diets of these species in winter 2007 and the concentration on only a restricted suite of prey could be a consequence of the density of benthic macroinvertebrates in the Canning River tending to be least in winter, as observed between 1995-97 due largely to a decline in the abundance of the various polychaete species (Kanadjembo *et al.* 2001b), and that the abundance of algae and insects are also likely to be greatly reduced in that season.

Thus, at times when the availability of preferred dietary items is reduced, interspecific competition between co-occurring species can increase.

4.4.2 Trophic structure of the Swan and Canning rivers and the ultimate source of carbon in these two morphological distinct regions of the Swan-Canning Estuary

In the Swan River in 2007 and the Canning River in both 2007-08 and 2009, the various components of the ecosystem sampled for stable isotope analysis represented, on the basis of their $\delta^{15}N$ signatures, *ca* three trophic levels from the lowest aquatic primary producer, typically phytoplankton, to the highest order consumer, which invariably was a species of fish.

In the Swan and Canning rivers during each period, the $\delta^{15}N$ values for *A. butcheri*, *P. olorum*, *P. punctatus* and *L. wallacei*, were always very similar, ranging from only 14.4 to 16.2‰, but with that of *A. butcheri* tending to, but not always, be the highest. The $\delta^{15}N$ values for *A. butcheri* however, were considerably higher than those derived for the same species in the Nornalup-Walpole Estuary on the south coast of Western Australia, *i.e.* 10.2‰ (Svensson *et al.* 2007). Furthermore, the $\delta^{15}N$ values for the gobiid *F. lateralis*, which has a similar carnivorous diet to that of *P. punctatus*, and the atherinid *Leptatherina presbyteroides*, a congener of *L. wallacei*, in Nornalup-Walpole Estuary also had greatly depleted $\delta^{15}N$ values relative to the gobiid and atherinid species of the Swan-Canning Estuary, *i.e.* 7.3 and 7.9‰, respectively (Svensson *et al.* 2007).

This disparity in $\delta^{15}N$ values maybe a consequence of the shorter trophic pathways from primary producer to the gobiid and atherinid species and *A. butcheri* in the Nornalup-Walpole Estuary, *i.e.* 0.6 and 1.25 trophic levels, respectively, and therefore an altered feeding behaviour from that in the Swan-Canning Estuary. In the case of *A. butcheri*, the diet of this species in Nornalup-Walpole Estuary in 1993-95 differed markedly from that in the Swan-Canning Estuary in that it contained atypically high volumes of the seagrass *Ruppia megacarpa*, but also contained even far greater volumes of teleosts (Sarre *et al.* 2000), and therefore is unlikely to have resulted in relative depletion of $\delta^{15}N$ values. Furthermore, a study on the diets of gobiid and atherinid species in a nearby, seasonally-open estuary on the south coast of Western Australia, Wilson Inlet, determined that the diets of *P. olorum*, *F. lateralis* and *L. wallacei* were similar to those in the Swan-Canning Estuary during a similar period (*cf* Prince *et al.* 1982, Humphries and Potter 1993, Humphries 1993).

Alternatively, $\delta^{15}N$ values can be enriched in aquatic trophic pathways as a consequence of exposure to anthropogenically-derived sources of nutrients (*e.g.* McClelland and Valiela, 1997, 1998). Thus, for example, highly enriched values of $\delta^{15}N$ were detected in the Sydney rock oyster, *Saccostrea glomerata*, collected within the vicinity of a treated sewage effluent outflow in an estuary on the east coast of Australia (Piola *et al.* 2006). It is therefore relevant that while the Nornalup-Walpole Estuary is classified as largely unmodified and its catchment remains largely vegetated with native flora, the Swan-Canning Estuary is

considered severely modified, with the catchment of this system being substantially cleared of native vegetation for agricultural and urban uses (Brearley 2005). It is further relevant that, in the Leschenault Estuary, a large and permanently open estuary on the west coast of Western Australia that is also classified as severely modified and whose catchment is considerably cleared for primarily agricultural but also urban uses (Brearley 2005), the δ^{15}N values for both *F. lateralis* and *L. presbyteroides* are likewise enriched, *i.e.* 9.1 and 10.7‰, respectively, relative to those in the Nornalup-Walpole Estuary (Svensson *et al.* 2007), but are still less than those for gobiid and atherinid species in the Swan-Canning Estuary. Furthermore, while the overall mean δ^{15}N values of primary producers, such as phytoplankton, in the Swan-Canning Estuary and Nornalup-Walpole Estuary were similar, *i.e.* 6.5 and 5.8‰, respectively, those of a primary consumer, bivalve molluscs, differed markedly, *i.e.* 10.7 *vs* 5.0‰ (Svensson *et al.* 2007), further suggesting external sources of nutrient input and therefore δ^{15}N enrichment of the trophic pathways in the former estuary.

The δ^{15}N values for the carid shrimp, *Palaemonetes australis*, were always the highest of any invertebrate species and were comparable to those of the fish species in the Swan River in both seasons and in the Canning River in summer 2008 and winter 2009. The high δ^{15}N values of this species in the Swan-Canning Estuary suggest that, like particularly *A. butcheri*, *P. olorum* and *L. wallacei*, this species is an opportunist omnivore in that system. This parallels the feeding behaviour of the congeneric *Palaemonetes pugio*, whose diet is known to include seagrass epiphytes, microalgae, detritus, carrion and invertebrate prey (*e.g.* Morgan 1980, Quinones-Rivera and Fleeger 2005). The enriched δ^{15}N values for this species in the Swan-Canning Estuary however, contrast the situation for other carid shrimps, *Palaemon* sp., in the Nornalup-Walpole and Leschenault estuaries whose δ^{15}N values, like those of the fish in those systems, were relatively depleted, *i.e.* 6.6 and 9.0‰, respectively (Svensson *et al.* 2007).

Aside from the carid shrimp, δ^{15}N values of the other invertebrates and also detritus, which was often highly variable in its isotopic signature, tended not to show any consistent trends between the Swan and Canning rivers and between the different periods. However, when sampled concurrently, the δ^{15}N values of calanoid copepods were greater than those for cyclopoid copepods, which in turn were greater than those for harpacticoids that were always similar to the δ^{15}N values for detritus.

In the Swan River in summer and winter 2007, aquatic primary producers, invertebrates and fish species typically had similar δ^{13}C values, ranging from only -28.0 to -24.6‰. Calanoid copepods and isopods, which, in both seasons, were relatively depleted and enriched, respectively, in their δ^{13}C signatures and microphytobenthos in summer and ostracods in winter, which also possessed relatively enriched δ^{13}C values, were exceptions. It should be noted, however, that a number of those exceptions were associated with high errors, which often overlapped with the δ^{13}C range of the other ecosystem components. The δ^{13}C values of aquatic primary producers, invertebrates and fish species in the Canning River in 2007-08, and which were comparable to those sampled in the Swan River, ranged widely, *i.e.* -26.2 to -

18.8, but were typically greater than -24.0‰. Similarly, those for the Canning River in 2009 ranged markedly, *i.e.* -27.4 to -16.7‰, and were also generally greater than -24.0‰.

The stable isotope characteristics of aquatic ecosystems, particularly those of δ^{13}C, can be related to the original source(s) of nutrient input to those systems (*e.g.* Fry 2006). Thus, marine ecosystems tend to be more enriched in δ^{13}C relative to freshwater biomes and/or those strongly influenced by terrestrial inputs (*e.g.* Peterson and Fry 1987). In the current study, this relationship was demonstrated by the δ^{13}C of seagrass and of small and large riparian vegetation, which, when sampled concurrently with other primary producers, always had the highest and lowest δ^{13}C values, respectively. The relative depletion of δ^{13}C values of ecosystem components in the Swan River therefore strongly suggests that this system is supported primarily by carbon derived from freshwater/terrestrial sources and that of the Canning River, due to its relative enrichment, by marine derived carbon. It is therefore pertinent that the Swan River had a total annual discharge of 140,200 ML in 2007, while that of the Canning River between the winter of 2007 and autumn 2008 was only *ca* 10,800 ML and in 2009 only 10,450 ML (as measured at Walyunga on the Swan River and at Seaforth on the Canning River; [http://kumina.water.wa.gov.au/waterinformation/telem/616011/han.htm ; http://kumina.water.wa.gov.au/waterinformation/telem/616027/mtl.htm]). Furthermore, the flow of the Canning River is disrupted *ca* 10 km upstream of the point where this river discharges into the estuary basin by the Kent St Weir and, as such, the waters below the weir are influenced more by tidal than freshwater influences for much of the year. Such tidal influence is consistent with salinities in the Canning River in the summer and autumn of 2008 and particularly 2009, being highly elevated and often close to that of full-strength sea water and not declining to as marked a minima as that in the Swan River 2007 in winter of each year. Given the high variability of δ^{13}C signatures in the Canning River and especially that of primary producers, which on occasion possessed appreciably depleted δ^{13}C values, the input of carbon may be more diffuse in this river, being derived from both marine and freshwater/terrestrial sources and may be highly dependent of freshwater discharge in a given year.

4.4.3 Fatty acid composition of *A. butcheri, P. olorum* and *L. wallacei* and their potential prey in the Swan River

In broad terms, the fatty acid composition of muscle tissue from *A. butcheri*, *P. olorum* and *L. wallacei* in the Swan River in both summer and winter 2007 were similar and thus reflected the similarly opportunist and omnivorous feeding behaviours of each of these species. Thus, irrespective of season, polyunsaturated fatty acids typically dominated the fatty acid content of each species, due largely to the substantial contribution made by the essential fatty acid 22:6(n-3) (docosahexaenoic acid), while saturated fatty acids, particularly hexadecanoic acid (16:0), also contributed greatly. Furthermore, in each species, monounsaturated fatty acids consistently made a lower contribution to the total fatty acid content than those of either saturated or polyunsaturated fatty acids, but of the monounsaturated fatty acids, oleic acid 18:1(n-9) was always the most prevalent.

In the Swan River in 2007, only phytoplankton, cyanobacteria and microphytobenthos produced 22:6(n-3) and, other than the fish species, this essential fatty acid was observed only in aquatic invertebrates. It therefore appears that docosahexaenoic acid, is particularly important in the aquatic ecosystems such as the Swan River. This fatty acid is typically considered diagnostic of dinoflagellates (*e.g.* Hanson *et al.* 2010) and although the resolution with which the Swan River was examined in the current study did not allow this to be confirmed, it does appear relevant that only unicellular primary producers were observed with this particular essential fatty acid. Unlike docosahexaenoic acid, the fatty acids 16:0 and 18:1(n-9), were common in all primary producers and invertebrates, both aquatic and terrestrial.

Despite their broad similarities, when the full suite of fatty acids that contributed greater than 1% to the total fatty acid content of the muscle tissue of the three fish species was subject to ANOSIM tests, the compositions of fatty acids in the three species were significantly different. These tests thus further suggest that, while all three species are opportunistic omnivorous, *A. butcheri*, *P. olorum* and *L. wallacei* display a certain degree of preferential feeding. These interspecific differences however, were typically due to differences in the relative abundances of fatty acids that were common in both primary producers and invertebrate species and thus, at least at this level of resolution, were unable to yield any further information about the trophic interactions of the three species of fish.

4.4.4 Food webs of the Swan and Canning Rivers

Based solely on the results of this study, preliminary food webs have been constructed for both the Swan and Canning rivers (Figs 4.18, 4.19). While these food webs clearly depict the interactions between *A. butcheri*, *P. olorum* and *L. wallacei* with primary producers and invertebrate species in both of these rivers, they do not illustrate the trophic pathways that exist within these systems from primary producers to higher level consumers unless those pathways are direct.

It is clear from the current study that, given the high sensitivity of stable isotope and particularly fatty acid analyses, a broad-spectrum approach, such as that employed in this study, can not be used to accurately trace trophic pathways in highly complex aquatic systems such as the Swan-Canning Estuary. It is therefore recommended that, in order for future studies to capture the full structure and complexity of the food webs of estuarine systems, all food sources should be identified to the highest possible taxonomic level and the greatest number of species should be examined. This will ensure that a greater range of interactions between species can be elucidated, the intricacies of which may not be detectible at coarser levels of taxonomic resolution (*e.g.* Abrantes and Sheaves 2009).
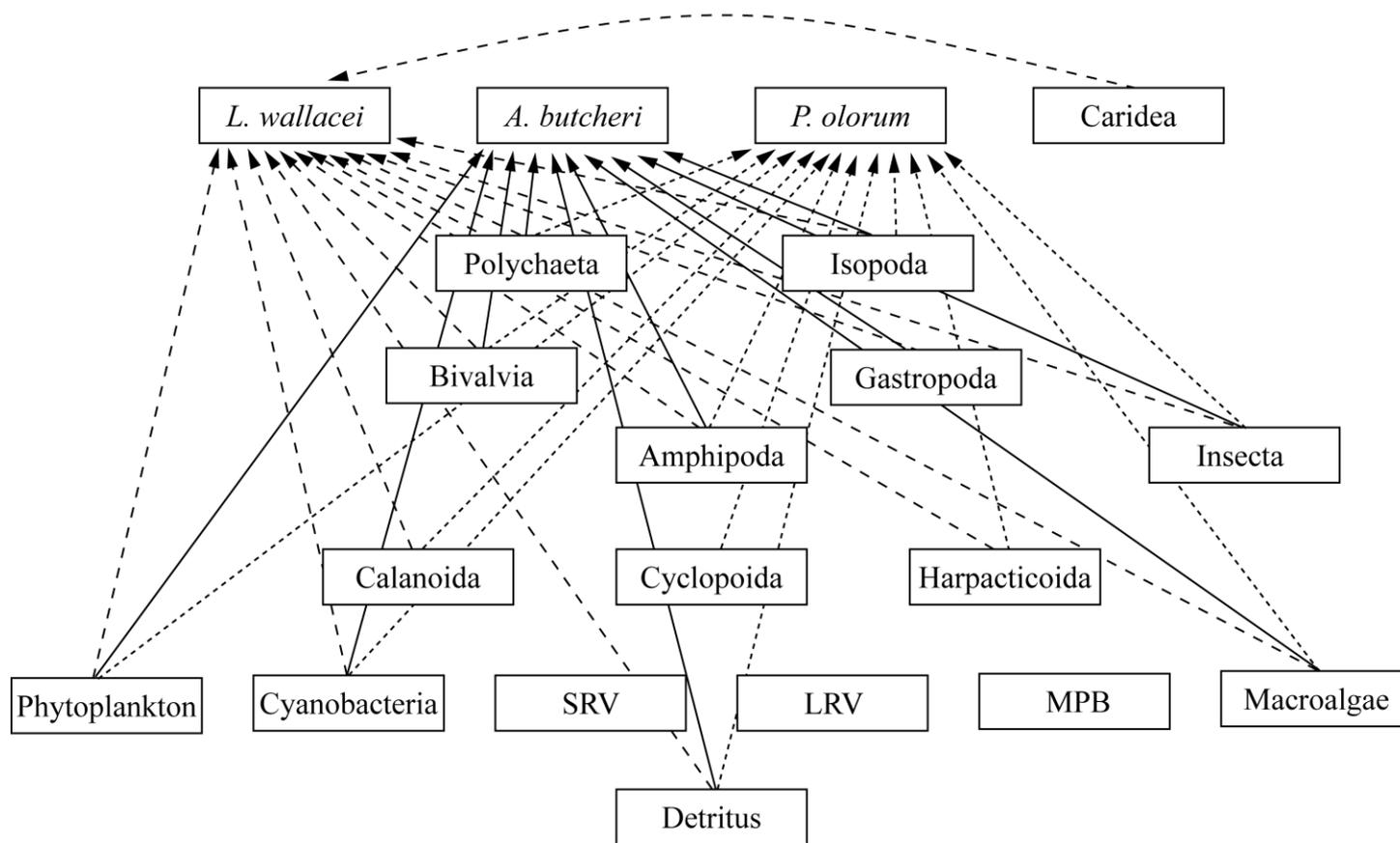
Figure 4.18: Preliminary food web for the tidal reaches of the Swan River, based on traditional gut content, stable isotope and fatty acid analyses of samples collected in summer to spring 2007. Direct trophic interactions with ecosystem components and *A. butcheri* depicted with a solid line, with *P. olorum* a short-dashed line and with *L. wallacei* a long-dashed line.
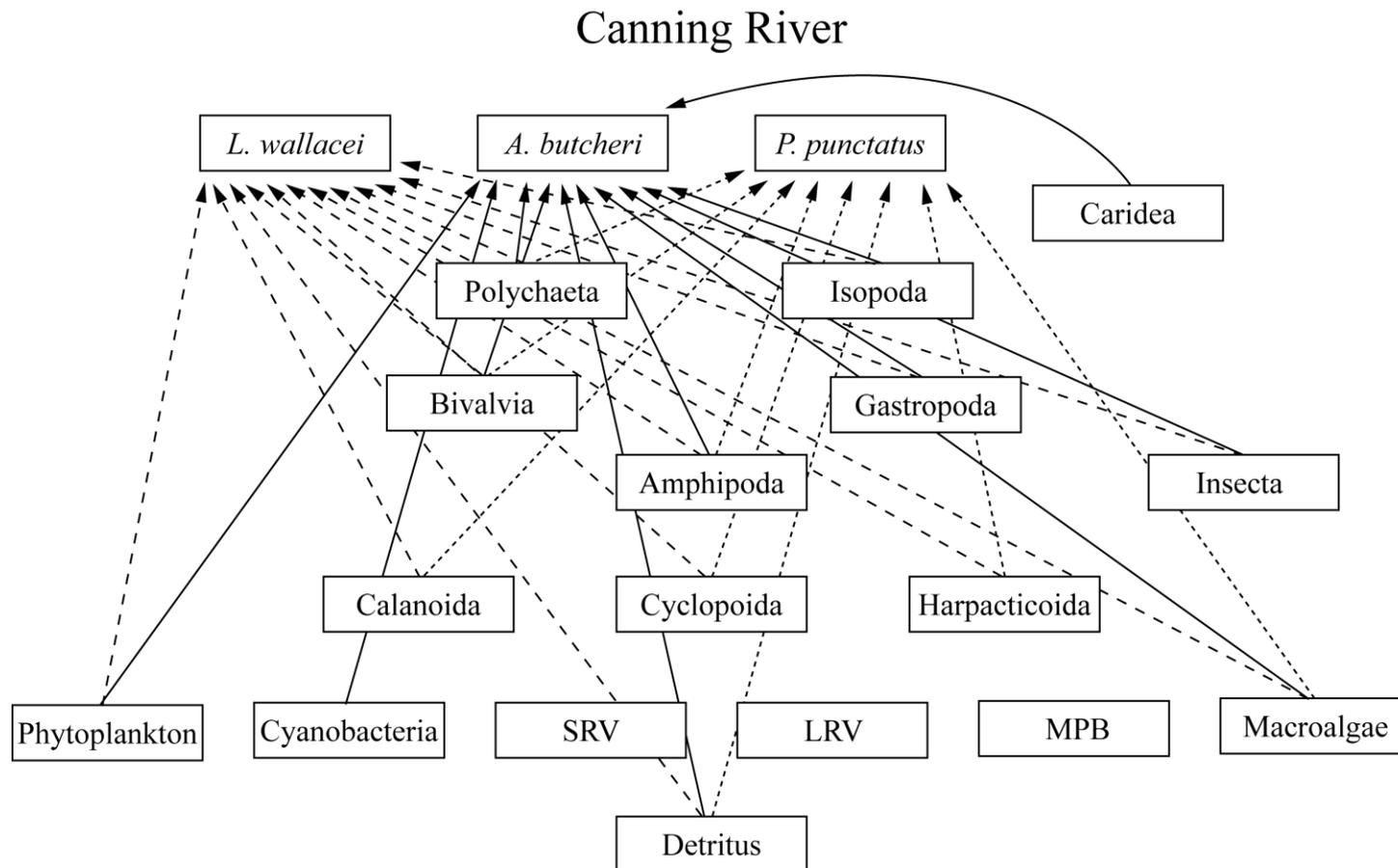
Figure 4.19: Preliminary food web for the tidal reaches of the Canning River, based on traditional gut content, stable isotope and fatty acid analyses of samples collected in winter 2007 to autumn 2008 and in summer to spring 2009. Direct trophic interactions with ecosystem components and *Acanthopagrus butcheri* depicted with a solid line, with *Papillogobius punctatus* a short-dashed line and with *Leptatherina wallacei* a long-dashed line.

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Chapter 5. General conclusions and recommendations for management

5.1 General conclusions

This study has firstly provided managers of the Swan-Canning Estuary with two valuable and comprehensive approaches for assessing the health status of this system and its key fish stocks, both of which could readily be applied to any other estuary. The first of these approaches comprises a multimetric biotic index of estuarine health constructed from various fish assemblage characteristics recorded throughout the system over the last 30 years. The second has employed biological and abundance data for a fish population to estimate annual biomass production, and is ideally suited to understanding the population dynamics of recreationally-important fish stocks in estuaries when the biology and recruitment of those species are highly variable and reliable catch-per-unit-effort (CPUE) data are unavailable, such as, in this case, for Black Bream (*Acanthopagrus butcheri*) in the Swan-Canning Estuary.

The multimetric index is the first biotic indicator of ecological health to be developed for estuaries in Western Australia, although similar indices have been adopted for estuarine management in many other areas of the world. Several novel techniques were also developed during construction of this index, such as those for metric selection and data standardisation to account for gear-induced bias, which represent substantial advances on existing published methods. Moreover, given the above characteristics of Black Bream in the Swan-Canning Estuary, the approach developed in this study for assessing the population status of this species is far more appropriate than a range of other, more commonly-used stock assessment methods.

In addition to the above approaches, this study has also provided the first integrated and quantitative assessment of the trophic relationships between particular fish species and their prey in the Swan-Canning Estuary, and of the pathways of energy transfer and the likely energy sources sustaining this system. Such information is crucial for understanding the intermediary pathways of ecosystem function, and thus those that are important in driving changes in ecosystem health.

The findings of this study have provided the following indications that the ecological health of the Swan-Canning Estuary has declined since at least the late 1970s, particularly in its deeper offshore waters.

- The offshore multimetric biotic index has declined consistently from 1978/79 to 2008/09, resulting in the health status of those waters being classified as poor in the most recent study period, as opposed to fair in all other monitoring periods since the late 1970s.

- The variability of the scores for the offshore index among replicate sites, seasons (particularly at sites of lower ecological quality) and between the consecutive years of

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the current study were notably greater than those of the nearshore index. The greater variability in offshore index scores in recent periods was mirrored by greater inconsistencies in the offshore fish faunal composition among replicate samples in 2007-09 than in earlier monitoring periods, which was also detected in the nearshore fish assemblages to some extent. Such variability is typically reflective of stressed faunal assemblages, and may thus provide further indications of declining ecosystem integrity.

- The mean catch-rate, number of species and species diversity of fish in the offshore waters have undergone pronounced declines since the early to mid 1990s.

- Two of the fish species that were consistently less prevalent in the offshore waters during later than earlier monitoring periods, and were often mainly responsible for the significant inter-period differences in offshore fish assemblage composition, *i.e.* Perth Herring (*Nematalosa vlaminghi*) and Sea Mullet (*Mugil cephalus*), also exhibited similar trends in the nearshore waters. However, the other species that consistently declined in abundance from earlier to later periods in the offshore waters, *i.e.* Black Bream, displayed the opposite trend in the nearshore waters. Such findings may reflect the movement of this species from deeper to shallower habitats to avoid the less favourable environmental conditions, including lower dissolved oxygen concentrations, that were detected in the bottom waters of the middle to upper estuary. They may also be indicative of a range of other factors, such as greater prey availability in the benthos of the nearshore than offshore areas. Irrespectively, it is suggested that such potential onshore movement of species such as Black Bream may be at least partly responsible for the observed increase in the nearshore health index since the mid 2000s.

- The growth performance and per capita annual biomass production of Black Bream has declined markedly since the early to mid 1990s. Furthermore, annual biomass production per unit area is now also in decline. It is suggested that these findings are related, via density-dependent effects, to the greater abundance of Black Bream in the nearshore waters during more recent than earlier periods. Fish now take about twice as long to reach the minimum legal length for retention than in 1993-95 and, at a given length, are now considerably lighter, indicating that body condition has also declined. The poor growth performance of Black Bream in recent years has profound implications for the quality of recreational fishing in the Swan-Canning Estuary, as relatively fewer fish are now of a size where they can be legally caught and retained by fishers.

- The vast majority of Black Bream in the estuary are young (<6 yrs), indicating that the population is experiencing, or has relatively recently experienced, high mortality. However, as fish can now potentially spawn for several years before they are legally able to be caught and retained, it would appear that recreational fishing currently presents a low risk to the sustainability of the population in the Swan-Canning Estuary. The high mortality of the Black Bream population is highly likely to reflect,

at least in part, the deteriorating environmental conditions within the estuary, but determining the extent to which this is the case will require further monitoring of this population.

5.2 Recommendations for management

In order to work towards a better understanding of the characteristics and drivers of the apparent decline in the ecosystem health of the Swan-Canning Estuary, and to develop an effective monitoring program that employs, among other assessment tools, the fish-based indices developed in this study, the following recommendations for management are provided.

- The current ecosystem health indices represent working models which require further testing and refinement before they can be implemented as robust monitoring tools for the Swan-Canning Estuary. Thus, a further one year study is required to validate index sensitivity to ecological degradation and ascertain the effects of spatio-temporal sampling intensity on index precision (see subsection 2.4.2.4 for further details).

- The outcomes of the above study are also essential for designing a robust and cost-effective monitoring regime to enable the health of the Swan-Canning Estuary to be quantified into the future using the current indices. It is envisaged that this regime will also facilitate the future monitoring of Black Bream to enable reliable estimates of biomass production, density, growth, age and length-weight relationships to be obtained. It is imperative that any such monitoring regime is undertaken on an annual basis, at least for the ecosystem health index, such that temporal trends in index values can be interpreted reliably without the potentially misleading influence of gaps in the data record. Moreover, any future monitoring must employ standardised methods for sampling and analysis to avoid the confounding influence of sampling bias and inconsistencies in data interpretation.

- Various other biotic and abiotic stressors need to be monitored at comparable spatio-temporal scales to the fish fauna in order to (i) better elucidate the drivers of trends in the fish-based indices, *i.e.* by providing an independent means of index validation and/or (ii) form complementary indices of ecosystem health as part of a broader ecosystem assessment framework. One critical biotic component for which regular monitoring is strongly suggested is the benthic macroinvertebrate assemblages, which not only provide a key food source for many fish species in the estuary and would thus greatly assist in interpreting shifts in fish assemblage composition, biology and trophic interactions, but which are also known to be highly informative indicators of ecosystem health (Diaz *et al.* 2004). Critical abiotic components include water and sediment quality, both of which have numerous direct and indirect impacts on fish and other biota such benthic invertebrates, and provide key indications of environmental integrity. While the Department of Water already undertakes an extensive water quality monitoring program throughout the estuary each week, the spatial resolution of monitoring sites in some regions of the estuary is broader than that at which fish

will probably need to be collected in a future monitoring program. This disparity causes difficulties for testing the extent to which trends in water quality are influencing those of the fish-based indices.

- Identification of the causal pressures impacting on the health of the Swan-Canning Estuary will require indicators of anthropogenic stressors to be developed at local to catchment-wide scales. These may include those ranging from basin-scale measures of catchment clearance and riparian vegetation integrity to site-scale indices of instream habitat quality.

- Further work is needed to build on the knowledge of the trophic interactions in the Swan-Canning Estuary. While the current study has provided an understanding of (i) the trophic links among three common fish species and their prey in the upper estuary and (ii) the major sources of energy sustaining this part of the system, this work needs to be extended to include other regions of the estuary and a greater suite of secondary consumers, namely other fish species and ideally also birds and dolphins. Furthermore, in future studies, the supporting primary producers and consumers comprising the food sources for secondary consumers should be identified to the highest possible taxonomic level to enable more detailed interpretation of the complex trophic interactions within this system. It is also recommended that any such future work is undertaken at a spatial scale that is comparable with the above suggested monitoring programs for fish and benthic macroinvertebrates, and that it is repeated at least every 10 years to enable detection of any major shifts in ecosystem structure and function.

6. References

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