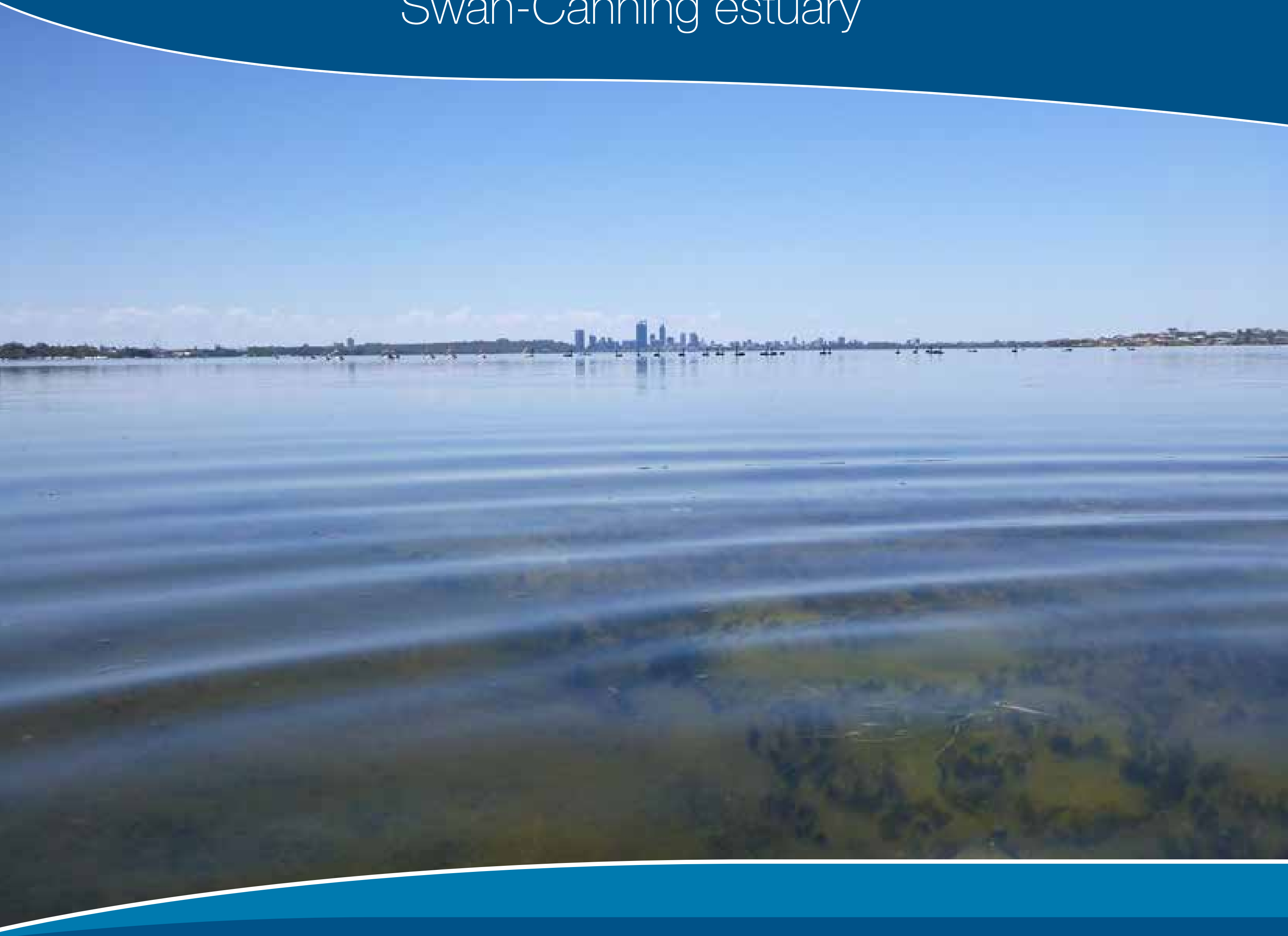
Government of **Western Australia**
Department of **Water**

Seagrass as an indicator of estuary condition for the Swan-Canning estuary



WaterScience
technical series

Report no. WST 62
January 2014

Government of **Western Australia**
Department of **Water**

Seagrass as an indicator of estuary condition for the Swan-Canning estuary

Department of Water

Water Science Technical Series

Report no. 62

January 2014

Department of Water
168 St Georges Terrace
Perth Western Australia 6000
Telephone +61 8 6364 7600
Facsimile +61 8 6364 7601

National Relay Service 13 36 77
www.water.wa.gov.au

January 2014

ISSN 1836-2869 (print)
ISSN 1836-2877 (online)

ISBN 978-1-922124-68-5 (print)
ISBN 978-1-922124-69-2 (online)

Acknowledgements

This project was funded by the Government of Western Australia through the Swan River Trust and the Department of Water. The Department of Water would like to thank the following for their contribution to this publication:

- Kieryn Kilminster and Vanessa Forbes for study design and report writing
- Artemis Kitsios, Peter Kretschmer, Helen Nice, Ben Marillier, Anne Brearley, Bronwyn Rennie, Zöe Goss, Sarah Evans, Georgina Evans, Frances Miller, Tim Storer, Karl Hennig, Dominic Heald, Belinda Quinton, Kelli O'Neill, Jeff Cosgrove and Kerry Trayler for field and lab work
- Lisa Dinnis and Mark Shackleton for assistance with data analysis
- Marianne Holmer, Jim Fourqurean, Kathryn McMahon, Paul Lavery, Catherine Collier, Mads Thomsen and Thomas Wernberg for their advice on study design at the project's outset
- Kingsley Dixon at the Botanic Gardens and Parks Authority for providing access to the Kings Park Laboratories and to Eric Bunn, Annette Johnson, Wolf Lewandrowski and Jason Stevens for their help while at Kings Park
- Marianne Holmer, Di Walker, Gary Kendrick, Marion Cambridge and Anne Brearley for their insightful discussions about our findings.
- Many thanks also to Malcolm Robb for his support of this project.
- Symbols for diagrams courtesy of the Integration and Application Network (ian.umces.edu/symbols).

Reference details

The recommended citation for this publication is: Kilminster & Forbes 2014, *Seagrass as an indicator of estuary condition in the Swan-Canning estuary,* Water Science Technical Series, report no. 62, Department of Water, Western Australia.

For more information about this report, contact
Kieryn Kilminster, Water Science Branch, Department of Water.

Cover photograph: Shallow seagrass meadow of *Halophila ovalis* overlain by macroalgae at Lucky Bay looking towards Perth city in the Swan-Canning estuary (photo credit: KL Kilminster).

Disclaimer

Contents

Appendices

Figures

Tables

Summary

Within many estuaries, seagrasses are foundation species – providing important habitat and performing important ecological functions within the ecosystem. Within the Swan-Canning estuary, the small species *Halophila ovalis* (common name paddleweed) dominates – providing approximately 403 ha of seagrass habitat. Monitoring of seagrass for effective management requires the integration of monitoring efforts at different scales. This report's purpose was to develop measures of seagrass condition at the physiological scale, which respond to individual environmental pressures. This targeted approach to understanding the mechanistic relationships between seagrass response and pressures will allow management policies to be specifically targeted at improving the resilience of seagrass in the Swan-Canning estuary.

Six sites were selected as representative of mono-specific seagrass meadows of *H. ovalis* in the Swan-Canning estuary and physiological monitoring was undertaken between October 2011 and May 2012.

The growth conditions for *H. ovalis* in 2011–12 were considered sub-optimal as growth was lower than had previously been reported and leaf nutrient concentrations were high (suggesting the seagrass was ready to grow rapidly if conditions were favourable). A possible explanation for this was the above-average summer rainfall of 2011–12, with associated cloud cover and increased turbidity in-situ resulting in fewer hours of saturating irradiance. This pattern of higher-than-average summer rainfall is expected to be more typical for the region into the future.

Macroalgal blooms (*Chaetomorpha linum*) in late spring to early summer significantly reduced the light available to seagrass at several sites. This species of macroalgae is considered a 'nuisance green alga' due to its capacity to grow rapidly in response to nutrient enrichment. It grows unattached and is moved around by winds and currents within the estuary. At sites where significant algae accumulated, the seagrass was light limited and unable to allocate sufficient resources to reproduction, so flowering and fruiting was significantly reduced. At most sites, *H. ovalis* flowering density was commonly highest in December, with maximum fruiting density reached one month later.

The period between January and March 2012 was the least likely to show constraints brought about by temperature, salinity and light conditions: in these months sediment conditions were found to constrain the growth of seagrass. Sediment conditions potentially toxic to seagrass are a secondary effect of eutrophication (nutrient enrichment) of the Swan-Canning estuary. A new 'sediment-stress' indicator has been developed from this study to inform on the relative effect of sediment conditions on seagrass growth.

Additional stresses in the estuary are related to invasive species. An estimated 5.2 billion *Batillaria australis* (a mud snail) are present within the *H. ovalis* meadows of the Swan-Canning estuary. Also noted during this study was the presence of introduced ascidians – including *Didemnum perlucidum*. While not explicitly explored in this study, negative effects on seagrass communities are generally noted with the introduction of non-native species.

Physiological indicators developed in this study include aspects that relate to seagrass performance and vulnerability. The refinement of these indicators across multiple years of study is necessary to capture the response range of these indicators given the high inter-annual variability expected of the ephemeral seagrass species. However, these process-scale indicators inform on *why* a change in seagrass population may be occurring, and provide early warning indicators of change before widespread loss of seagrass occurs.

1 Introduction

1.1 Seagrass fundamentals

Seagrasses are flowering plants (angiosperms) that grow completely submerged underwater – generally in the marine environment. The seagrass families form an ecological rather than a taxonomical group and, as such, are not necessarily closely related (den Hartog & Kuo 2006). Indeed, this ecological grouping has resulted in much debate as to which species to include as seagrasses, with *Ruppia* and *Zannichellia* problematic. Approximately 58 species of seagrass are recognised (Walker 1999), although that number depends on taxonomic versus genetic classification. About half of the known seagrass species are found in Australia, with the southern Australian bioregion home to many endemic species (approximately one quarter of those found worldwide) (Walker 1999).

Seagrasses are found in shallow coastal and estuarine environments worldwide (every continent except Antarctica). In Western Australia, the main habitats for seagrasses are sheltered coastal embayments, protected bays, lagoons enclosed by fringing reefs and estuaries. The endemic species growing in the state's coastal waters have very different life-traits to those species found within the estuaries. The seagrass functional form model first proposed by Walker et al. (1999) and more recently expanded by Carruthers et al. (2007) places the large seagrasses (such as *Posidonia*) and small seagrasses (such as *Halophila* and *Zostera*) on opposing ends of the scale. The large species are characterised by restricted but persistent distribution and slow regeneration, turnover, and responsiveness to perturbation. By contrast, the small species are widely distributed, reproduce quickly via seed banks, are ephemeral with rapid turnover, and respond rapidly to perturbation. The seagrasses found in estuaries worldwide tend to be these smaller seagrass species – perhaps better adapted to the dynamic conditions within estuaries. In the Swan-Canning estuary, *Halophila ovalis* is the most dominant seagrass, although a *Zostera* species, *Halophila decipiens* and *Ruppia megacarpa* are also observed in the lower estuary. The *Zostera* species is found in the most marine part of the Swan-Canning estuary, and has at various times been reported as *Heterozostera tasmanica, Zostera tasmanica, Zostera muelleri and Zostera mucronata* – a result of various clumping and splitting of species groupings in line with conflicting molecular and morphological studies. It is most recently acknowledged as the species *Zostera muelleri* (Jacobs et al. 2006).

Seagrasses grow in soft benthic sediments, with their roots and rhizomes below the sediment surface. Vegetative growth via these rhizomes allows the seagrass to colonise large areas, which are referred to as seagrass meadows. As flowering plants, they can also reproduce sexually and establish (with varying success among the different seagrass species) from seed. Seagrasses bridge the water/sediment interface, and can take up nutrients both from the water column and the sediment porewater. Seagrasses typically invest significantly in below-ground plant parts. This below-ground biomass has been described as both an asset and a burden (Hemminga 1998) – a burden since the growth and maintenance of roots and rhizomes has an associated energy cost and makes plants vulnerable to unfavourable sediment conditions.

1.2 Ecosystem value of seagrasses

Seagrasses are important components of aquatic ecosystems and their value specifically to the Swan-Canning estuary is summarised in Figure 1.

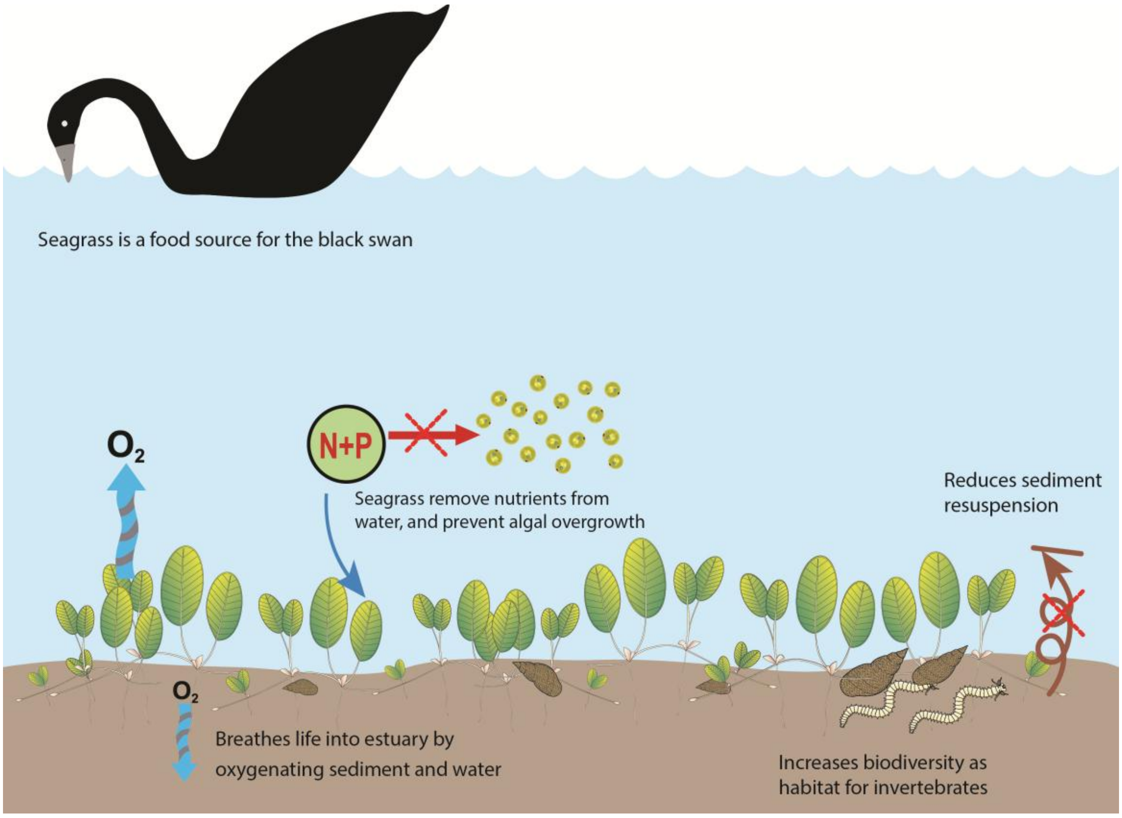


Figure 1 *Ecosystem value of seagrass in the Swan-Canning estuary*

Seagrasses are photosynthetic primary producers – meaning they convert energy from the sun and inorganic carbon into biomass. Although net primary production is extremely variable among seagrass species, location and seasons, they are thought to be some of the most productive groups of organisms in the world (Mateo et al. 2006). Average net primary production of seagrasses is thought to be twice that of forests, namely 817 gC $m^{-2}year^{-1}$ compared with 400 gC $m^{-2}year^{-1}$ (Mateo et al. 2006). More recently seagrass meadows have been acknowledged as significant carbon stores, with their protection being of global importance to mitigate climate change (Fourqurean et al. 2012a; Fourqurean et al. 2012b).

Halophila ovalis is a highly productive seagrass species. Net primary production of *H. ovalis* over the whole Swan-Canning estuary has been estimated at 500 gC $m^{-2}year^{-1}$ (1981–82: Hillman et al. 1995). The process of photosynthesis also produces oxygen as a by-product, and this may play a role in maintaining oxic conditions at the sediment/water interface.

Seagrass meadows are instrumental in increasing biodiversity of the benthic environment, and this is as true for the small seagrass species as for the medium to large seagrasses (Casares & Creed 2008). They stabilise sediments, which in turn reduces turbidity caused by sediment resuspension (Koch et al. 2007). They also support diverse and productive faunal assemblages when compared with unvegetated sediments (Orth et al. 1984; Valentine & Duffy 2006). The seagrass itself provides a physical substrate for epiphytic algae to attach, while the meadows are commonly cited as nurseries for juvenile fish (although these aspects are more important for the persistent large seagrass species than the smaller species). Seagrasses are also a food source for fish, dugong and manatees, as well as waterfowl. In the Swan-Canning estuary, the black swan *Cygnus atratus* feeds on both *H. ovalis* and *Ruppia megacarpa,* reportedly consuming up to 25% of the daily production of *H. ovalis* at sites suitable for grazing (Eklöf et al. 2009; Choney 2012).

Seagrasses act both as a source and a sink of nutrients (Hemminga et al. 1991), while also substantially influencing nutrient cycling processes. The sediment biogeochemistry is altered by seagrasses (by their inputs of carbon and oxygen), which in turn influences nitrogen, sulfur, iron and phosphorus cycling (Marbà et al. 2006). In the Swan-Canning estuary, the seagrass *H. ovalis* is a substantial sink for nutrients in the summer and autumn seasons, with >2500 kg N and >500 kg P (Connell & Walker 2001) tied up in plant biomass.

The area of *H. ovalis* in the Swan-Canning estuary has been estimated to be between 20 and 25% of the estuary's total area. Hillman et al. (1995) report 568 ha of seagrass meadows in the estuary in 1976 and 598 ha in 1982. Connell & Walker (2001) report an areal coverage of 461 ha in 1996. Recent work by the Department of Water estimates coverage at 403 ha in 2011. This pattern of decline unfortunately reflects the recognised global decline of seagrasses (Orth et al. 2006a). Most seagrass losses can be attributed to human activities in adjacent catchments leading to increased nutrient and sediment runoff (Walker & McComb 1992; Orth et al. 2006a).

1.3 Threats to seagrass

The threats to seagrasses pertinent to those found in the Swan-Canning estuary are discussed in the sections following and summarised in Figure 2.

Nutrients

Cultural eutrophication (or nutrient over-enrichment, especially with nitrogen and phosphorus) has been cited as a major cause of seagrass loss worldwide (Ralph et al. 2006; Burkholder et al. 2007). Shepherd et al. (1989) suggest that the mechanism for seagrass decline under eutrophic conditions is that of algal overgrowth (phytoplankton, epiphytes and unattached macroalgae). Seagrass decline under an increased nutrient regime involves 'a cascade of direct and indirect effects' including: reduction in light from enhanced growth of epiphytes and macroalgae, sediment loading and resuspension, water column hypoxia and sediment anoxia (potentially increasing exposure to toxic sediment sulfides), and trophic imbalances (Burkholder et al. 2007). Unfavourable sulfide-rich sediment conditions associated with anoxia may also develop as a secondary effect of eutrophication. Ecosystems do not respond to gradual change in a smooth fashion, but rather are subject to sharp shifts in regime (Folke et al. 2004). The flip from a seagrass-dominated to an algal-dominated state is considered undesirable for aquatic ecosystems. An example of an estuary that underwent this undesirable switch in states is the Peel-Harvey estuary.

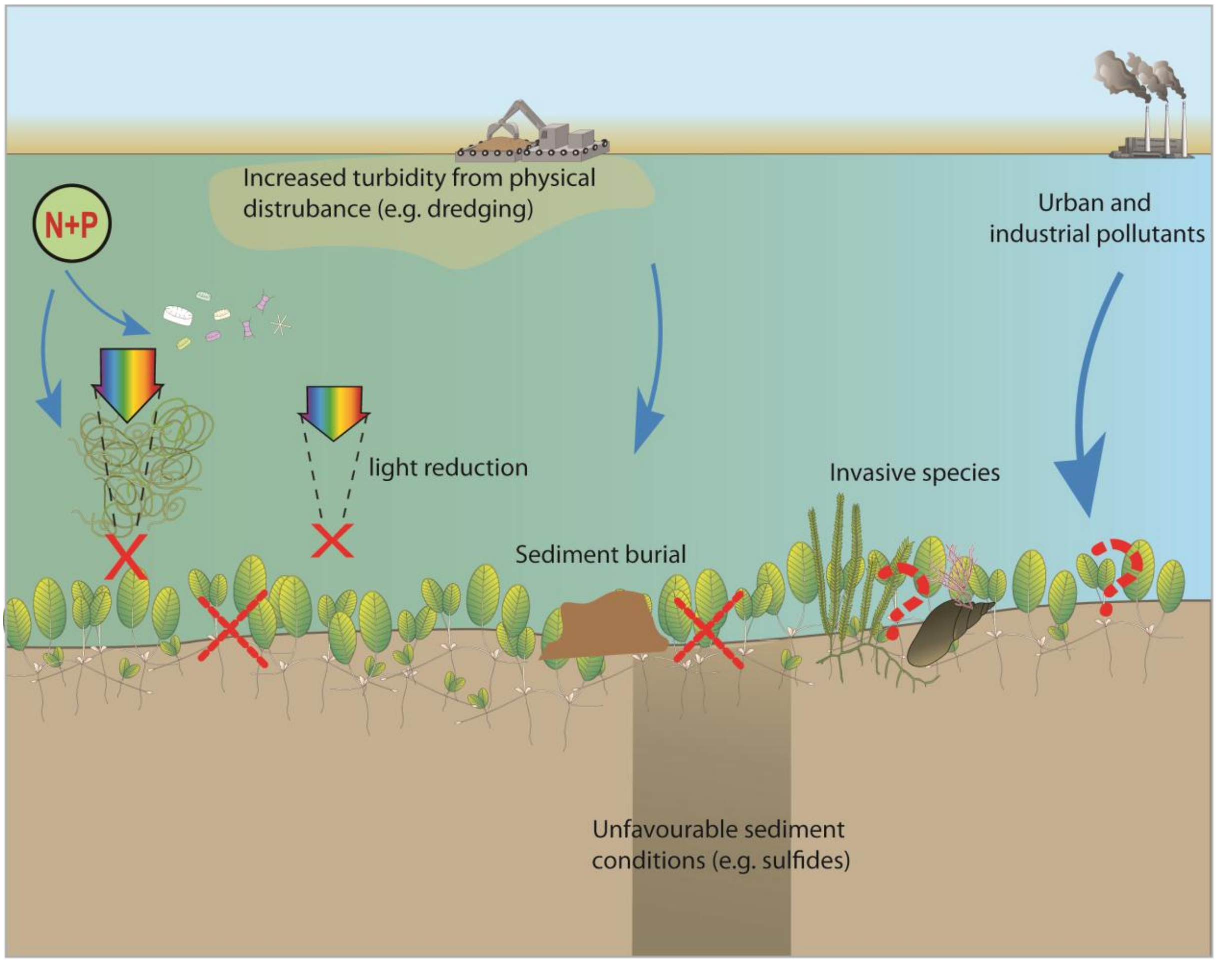


Figure 2 *Threats to seagrasses*

Light reduction

Seagrasses require some of the highest levels of light of any plant group worldwide. Light is one of the principal determinants of whether seagrass can survive in a given environment. The study of light and photosynthesis in aquatic ecosystems is a well-defined field of study – for more information please see Kirk (1983). Light transmitted from the sun passes through the atmosphere (where it is affected by scattering and absorption) before it hits the surface of the water, and is either transmitted through the water surface or reflected. As light passes through the water column it can be absorbed, scattered and/or affected by the processes of fluorescence and Raman scattering. These processes affect both the quantity and quality (wavelength) of light reaching the benthic environment. Light is diminished in an approximately exponential manner as it passes through the water column according to the Lambert-Beer equation, with short wavelengths (red spectrum) attenuating faster.

Photosynthetically active radiation is light of wavelength 400 to 700 nm, and comprises approximately 38% of the solar irradiance (Kirk 1983). Seagrasses require approximately 11% of the surface irradiance (Duarte 1991), although this is an average value from multiple species worldwide, with some species reported to require up to 37% of the surface irradiance

(Lee et al. 2007). These extremely high light requirements mean that seagrasses are acutely sensitive to changes in environmental conditions that affect light.

The minimum light requirement can be determined by establishing the relationship between photosynthesis and irradiance (*P-I* curves) and from these curves determine I_k and I_c (expressed as μmol photons $m^{-2}s^{-1}$): I_k describes conditions where light is saturating for photosynthesis and I_c where light is at the compensation point (i.e. where gross photosynthesis = respiration and no net growth occurs). Hillman et al. (1995) reported that *H. ovalis* from the Swan River had an I_k of 200 μmol photons $m^{-2}s^{-1}$ and I_c of 40 μmol photons $m^{-2}s^{-1}$. Average values of I_k determined from *P-I* curves for other seagrass species ranged from 36 to 468 μmol photons $m^{-2}s^{-1}$ (Lee et al. 2007). It should be noted, however, that seagrasses can physiologically acclimate to low light, resulting in local differences in light requirements for the same species – where seagrass established near the depth limit have higher photosynthetic efficiency (Lee et al. 2007).

Photosynthetic rates may be saturated at irradiances higher than the I_k, so more light does not equate to greater primary production. Increases in primary production are related to the period of time during which the seagrass can photosynthesise. Therefore, a useful measure of light availability for seagrass meadows is H_{sat} – the number of hours where irradiance is greater than the saturation irradiance for the seagrass. While generally more production occurs with more hours of light above saturation irradiance, this response is not necessarily linear. For example, *Zostera marina* was shown to show a saturation-type response where H_{sat} >10 hours did not enhance growth rates (expressed as leaf formation rates) (Dennison & Alberte 1985).

Algae and seagrasses

Epiphyte overgrowth has been implicated as a probable cause of seagrass decline associated with eutrophication (Ralph et al. 2006). This problem is more relevant for the longer-lived persistent species (e.g. *Posidonia*) than the species with fast turnover such as *H. ovalis. Halophila* species are reported to have an average leaf lifespan of ~25 days compared with an average leaf lifespan of ~170 days for *Posidonia* species (Hemminga et al. 1999) – as such epiphytes are not likely to have sufficient time to grow large enough on *H. ovalis* to cause significant shading (Hillman et al. 1995).

Free-floating macroalgae, however, have a much greater potential to shade *H. ovalis* beds, limiting the light available for photosynthesis. Nuisance macroalgae, such as *Chaetomorpha linum* (Figure 3), are fast-growing and can respond rapidly to favourable growth conditions. If these macroalgae accumulate in areas where seagrass beds are present, the reduced light conditions and possible increased organic load to sediments may cause stress to seagrass populations.

Figure 3 Chaetomorpha linum *accumulations near Heathcote in the Swan-Canning estuary*

Sediments

The sedimentary environment of seagrasses is important for their survival. While seagrasses are known to inhabit a range of sedimentary environments – calcareous sediment to organic-rich mudflats – it appears they are generally quite sensitive to perturbations in their sediment environment.

Burial of seagrasses may occur from changes in hydrology (resulting in erosion and deposition), dredging and the subsequent settlement of suspended solids, or the effects of floods or tropical storms (Cruz-Palacios & van Tussenbroek 2005). Several studies have explored the tolerance of seagrass to burial; for example, *Zostera noltii* shoots did not survive more than two weeks under complete burial (Cabaço & Santos 2007). The ability of seagrasses to survive burial appears to vary among seagrass species, being associated with the extent to which the seagrass shoots are subsidised by their interconnectedness (via rhizomes) with other non-buried plant shoots (Ooi et al. 2011). Ooi et al (2011) suggested that *H. ovalis* was a weak integrator, and did not show evidence of subsidising buried shoots.

Changes to the *quality* of the sedimentary environment may also influence seagrass persistence and survival. Organic matter enrichment has been shown to have limited benefit nutritionally to the seagrass *H. ovalis* (Kilminster et al. 2006), while the physiological responses of *Posidonia oceanica* to organic matter enrichment have been interpreted as adaptations to anoxia exposure (Pérez et al. 2007). Persistent anoxia may result from the addition of organic matter and a shift in sediment biogeochemical processes.

In anoxic sediments, organic matter is decayed primarily by the reduction of sulfate to sulfide. Hydrogen sulfide is toxic to plants: even a transient pulse of sulfide has been shown to significantly reduce growth rates of *H. ovalis* (Kilminster et al. 2008). Hydrogen sulfide enters plants through the below-ground parts, primarily at night (Pedersen et al. 2004) when radial oxygen loss from roots (a photosynthetically dependent process) is at its lowest (Connell et al. 1999; Jensen et al. 2005). Seagrass has some capacity to tolerate sulfide exposure, in that it re-oxidises sulfide that has entered the plant and may then accumulate elemental sulfur within the tissues (Holmer et al. 2005b), although this is likely to be energetically costly to the plant. The contribution of sediment-derived sulfide to seagrass tissues can be estimated using sulfur isotope analysis (e.g. Frederiksen et al. 2008). However, the availability of oxygen to re-oxidise the sulfide depends on oxygen created as a by-product of photosynthesis, so presumably the tolerance of seagrass to sulfide stress would also have a strong relationship with light availability.

Physical disturbances

Physical disturbances within the estuary or marine environment also have the potential to cause direct or indirect effects. Some examples include boat use (propeller and anchor scarring) which may cause direct damage to seagrass; and dredging through or near seagrass beds which may physically remove seagrass beds, resulting in loss from burial but also causing indirect effects due to increased turbidity or the release of sediment-bound nutrients or contaminants.

Invasive species

The introduction of exotic marine species may have harmful effects on the ecological function and biodiversity of seagrass communities. For example, dieback of the seagrass *P. oceanica* in the Mediterranean has been associated with the exotic macroalgae *Caulerpa taxifolia* (De Villèle & Verlaque 1995). In the Swan-Canning estuary, the invasive mud snail *Batillaria australis* has established and is now super-abundant within *H. ovalis* beds (Thomsen & Wernberg 2009). *B. australis* does not directly consume seagrass, however it is thought to negatively influence *H. ovalis* production due to physical disturbance of the sediment and interaction with the drift algae (the snail provides a hard substrate for the attachment of algal thalli) (Thomsen & Wernberg 2009). These findings agree with a recent review suggesting that predominantly negative effects are observed when non-native species are introduced into seagrass communities (Williams 2007).

Pollutants

As is common for an urbanised estuary, point and diffuse sources of pollution are recognised to be affecting the Swan-Canning. Sediment concentrations of some metals (zinc, lead, copper, mercury, selenium and manganese) and some organochlorine pesticides (dieldren and DDE) have been reported to exceed recommended guideline concentrations (Nice 2009). Water within estuaries have relatively long residence times so it is probable that exposure time to anthropogenic contaminants is higher for seagrasses in estuaries than those in coastal, nearshore environments.

Pollutants such as metals, antifoulants, petrochemicals, herbicides and pesticides are all considered potentially harmful to seagrasses (Ralph et al. 2006). Of these contaminants, the physiological effect of metals on seagrass is the best understood. Metals can cause disruption to photosynthetic pathways, however recovery from and acclimation to metal exposure has also been documented (Prange & Dennison 2000). To the authors' knowledge, no studies have directly assessed the effect of pollutants on seagrasses in the Swan-Canning estuary (and this study does not examine it either). It seems reasonable, however, to assume the known pollutants may contribute to a chronic reduction in seagrass productivity within the estuary via their impact on a range of metabolic pathways.

1.4 Management implications

Programs and plans developed by the Swan River Trust (and partners) to manage the Swan-Canning estuary include the Swan Canning Cleanup Program (1999), Healthy Rivers Action Plan (2008) and *Swan Canning water quality improvement plan* (2009). These aimed to improve the estuary's water quality primarily through nutrient control. Consequently, investment has focused on water quality monitoring. To date, little investment in tracking the estuary's health using biotic indicators has occurred. However, the new draft River Protection Strategy aspires to a more holistic management framework and includes the development of measures of ecosystem health.

Seagrass is a biological component of the lower estuary that assimilates conditions within the estuary. Seagrass can be viewed as a downstream integrator of the success or failure of catchment management. Monitoring of seagrass in the Swan-Canning estuary to date has been ad hoc. The resulting limitations on our understanding of seagrass in the estuary means it is difficult to predict whether its distribution, extent, abundance or condition has changed significantly in the period in which active management of the Swan-Canning has taken place.

A structured monitoring program for seagrasses should enable early detection of change and guide the implementation of policies to mitigate adverse effects. The development of seagrass-based ecological indicators (as pursued in this study) should form part of this monitoring program.

Ecological indicators

Estuaries are places of extraordinary natural change in both space and time, which adds complexities to the development of environmental indicators able to assess change objectively and efficiently (Bortone 2005). There are many purposes for which we can use ecological indicators to inform on the condition of the environment. Ecological indicators may be used to provide early warning of changes in the environment or as diagnostic tools for environmental problems, and they may address different scales of question. Dale & Beyeler (2001) assert that the most useful type of indicator is one with high sensitivity to a particular (perhaps subtle) stress, which can therefore behave as an early indicator of reduced environmental integrity.

It is critical to define the breadth of issue for which the indicator is attempting to inform. In terminology adopted by Jordan and Smith (2005), the broadest indicator will be an *ecosystem indicator* (Table 1). Adams and Bortone (2005) suggest that a future direction for estuarine indicator research relates to functional-level indicators and processes, essentially trying to understand why organisms or systems respond the way they do to environmental factors. Elucidating these mechanistic relationships between environmental stressors and biota should be considered important for effective management of estuaries and it is this type of indicator that this project is striving to develop.

Table 1 *Types of indicators used in ecological assessment, after Jordon and Smith (2005) and Adams and Bortone (2005)*

Indicator type	Example	Scale	Comment
Chemical	Water or sediment quality	Individual site level	Difficult to link to biological status
Single species	Abundance or distribution of keystone species (e.g. Swan River prawn or black swan)	System-scale but species specific	Species may recover due to changes in other management (e.g. fishing pressure), while estuary remains unhealthy
Community	Infaunal macrobenthic communities, fish communities, or seagrass coverage	Habitat type investigated	Tend to be specific to individual habitat type. Generally more robust than single species indicators
Functional or process level	Studies linking environmental stress and biotic responses	Site to system-scale	Mechanistic relationships between stressors and organisms
Ecosystem	Usually multi-variate derived measures which integrate multiple levels of biotic communities, trophic levels and possibly abiotic attributes too	System-scale	Principal weakness is that these are mathematical abstractions – finding ecological meaning and communicating that meaning is challenging

1.5 Seagrasses as biological indicators

Many attributes of seagrasses have been proposed as biological indicators within the scientific literature, leading to the assessment of some ~60 indicator variables for environmental monitoring in a study by Martínez-Crego et al. (2008). The complexity of the plants and their interaction with the natural environment can lead to differing responses in proposed indicators to the same apparent perturbation. For example, seagrasses commonly respond to light deprivation by reducing overall leaf size (which reduces the respiratory demand of the shoot), however the reverse can also be observed in the early stages of shading – where leaf width or leaf length increases (often with a concurrent increase in leaf thinness) (Grice et al. 1996; Ralph et al. 2007). A recent review suggests a set of 21 bioindicators for light reduction of seagrasses alone and range from the plant physiology-scale to the meadow-scale (McMahon et al. 2013). Bioindicators need to be sensitive to the scale of the environmental quality gradient, and often the selection of multiple indicators is required to reflect the multiple anthropogenic disturbances causing the gradient in environmental conditions (Martinez-Crego et al. 2008).

Halophila ovalis as an ecological indicator

The environmental stresses that we consider plausible for *H. ovalis* to inform on include deterioration of:

- water quality through light reduction
- water quality by nutrient pollution
- sediment quality.

The literature suggests a range of indicator metrics for seagrass that could be explored within the Swan-Canning estuary context. For example, the ratio of leaf nitrogen content to leaf mass has shown promise as a nutrient pollution indicator in *Zostera marina* (Lee et al. 2004; Burkholder et al. 2007); $\delta^{15}N$ within seagrass tissues may indicate likely nutrient sources (Fourqurean et al. 1997); and the morphometric traits of the seagrass might be useful (Lee et al. 2004). Additionally, models of *H. ovalis* productivity could be further explored (Hillman 1985; Kilminster et al. 2008).

This study was designed to capture sufficient information to test these and other mechanistic relationships between the seagrass *H. ovalis* and the environmental stressors to allow development of functional-level indicators for seagrass in the Swan-Canning estuary.

2 Project overview and site descriptions

2.1 Overview of sampling program

The Western Australian Minister for the Environment (also the Minister for Water) launched this project on 25 September 2011 to celebrate World Rivers Day. The project was a partnership between the Department of Water and Swan River Trust.

Sampling took place from early October 2011 through to the end of May 2012. It was a multi-faceted project, with measurements occurring across different temporal scales. Figure 4 describes the relationship of the project components and the main measurements undertaken in each part. Further details of the methodology used will be described later in the report and subsequent appendices.

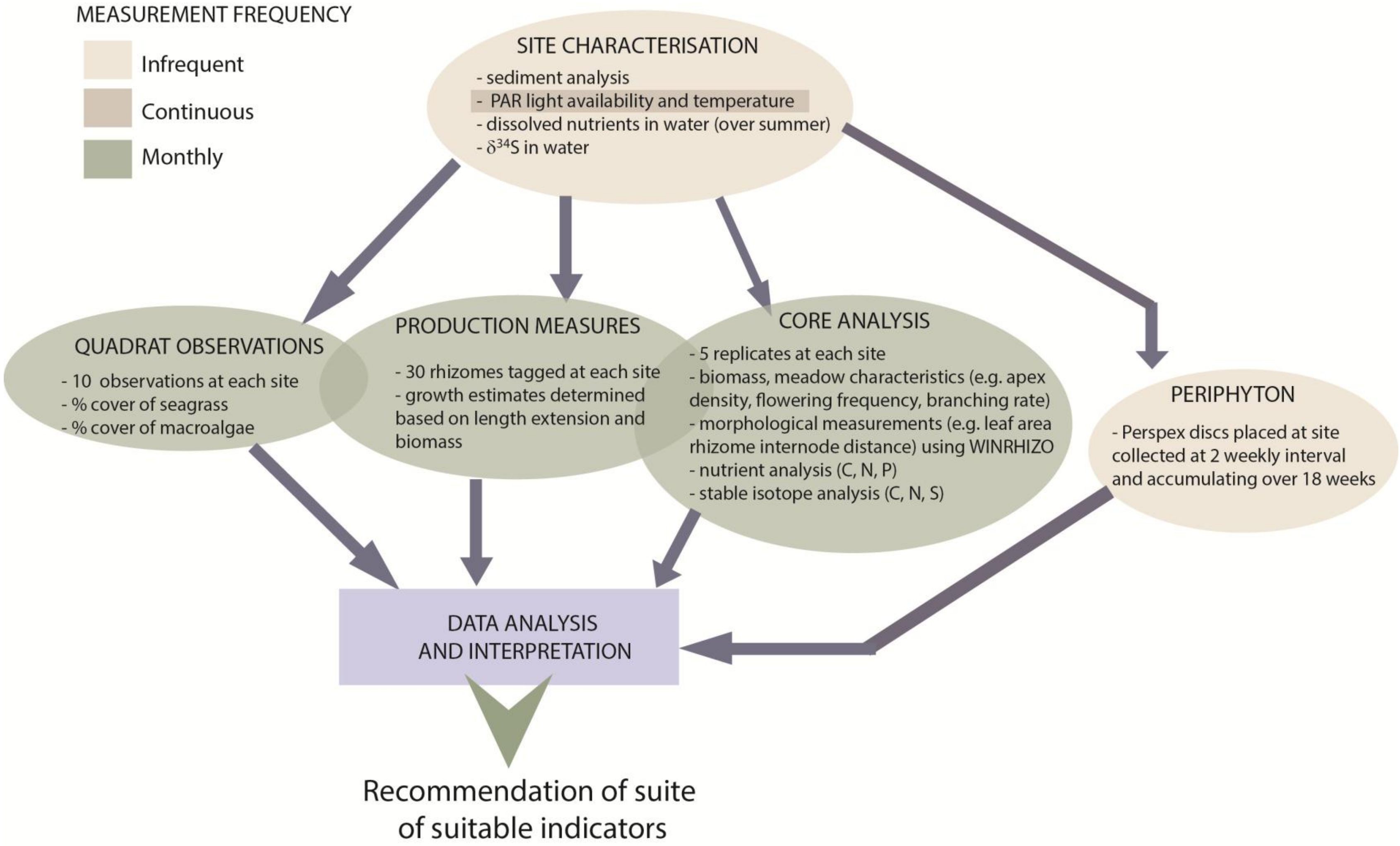


Figure 4 Overview of seagrass sampling project. Measurements were undertaken between October 2011 and May 2012.

2.2 Overview of sites

This study took place in the Swan-Canning estuary. Six sites were chosen to represent shallow seagrass meadows across almost the entire distribution range of *Halophila ovalis* within the estuary (Figure 5). *H. ovalis* is found further upstream in the Swan River – it has recently been observed in Perth water at approximately 1 m depth (unpublished data, DoW). Seagrass is also found further downstream than RCK, but these downstream meadows usually contain a mix of seagrass species.

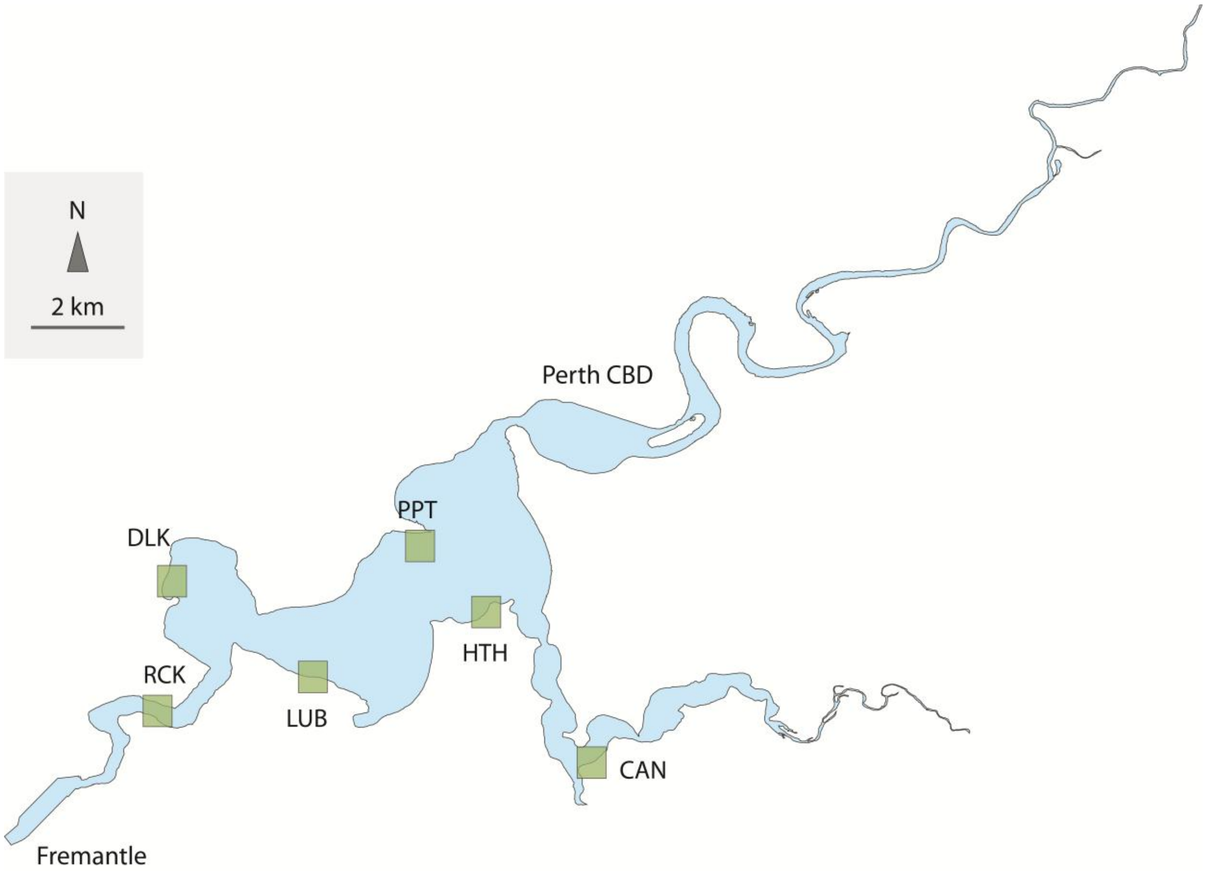


Figure 5 *Location of seagrass sites within the Swan-Canning estuary*

Site RCK

RCK is located at easting 384132 and northing 6456086 (grid zone 50) (Figure 6) on the western side of Point Roe. It is the site closest to the mouth of the Swan-Canning and therefore more influenced by marine biota than the other sites. There were significant dead shells of bivalves and molluscs observed in the site's sediment. This area of the river appears to be well used by kayakers, fishermen (in waders) and dogs; instrumentation at the site suffered some seemingly well-intentioned interference.

The seagrass meadow sampled was at -0.7 mAHD.

Figure 6 *Quadrat observations at site RCK*

Site DLK

DLK is located at easting 383943 and northing 6459045 (grid zone 50). The site is within Freshwater Bay, where a significant number of boats are moored (Figure 7). The bay's shoreline is also a popular picnic spot, with green lawns almost to the water's edge. Freshwater Bay is popular for a variety of uses and in-situ equipment was also interfered with (and placed neatly under a tree) at this site. Dolphins were commonly observed at the site, and seahorses took up residence on underwater equipment deployed here during the study.

The seagrass meadow sampled was at -0.8 mAHD.

Figure 7 *Site DLK with boats moored in the bay*

Site LUB

Site LUB (Lucky Bay) was located at easting 387650 and northing 6456773 (grid zone 50) and was within the Alfred Cove Marine Park (Figure 8). This site was the shallowest sampled and black swans were commonly seen grazing nearby. LUB is likely to be the site least affected by public use.

The seagrass meadow sampled was at -0.4 mAHD.

Figure 8 *Retrieving tagged rhizomes at site LUB*

Site HTH

HTH is located at easting 390447 and northing 6458489 (grid zone 50) to the west of Point Heathcote, within Waylen Bay. This site was probably the most exposed of all the sites: conditions depended on the direction of the wind, being particularly rough during southerly or south-westerly winds (Figure 9). The nuisance macroalga *Chaetomorpha linum* was problematic at the site and dolphins were regularly sighted here.

The seagrass meadow sampled was at -0.7 mAHD.

Figure 9 *The two extremes of conditions observed at site HTH*

Site CAN

CAN (Canning) was located at easting 392749 and northing 6455005 (grid zone 50), and accessed from the Rossmoyne foreshore of the Canning Estuary. This was the farthest upstream site and had tannin-stained waters (Figure 10). Seagrass establishment at this site appeared to depend on salinity, and was first observed mid-December 2011. Equipment at this site was sometimes affected by wash from boating activities.

The seagrass meadow sampled was at -0.54 mAHD.

Figure 10 *Site CAN along the Rossmoyne foreshore*

Site PPT

PPT was located at easting 388855 and northing 6460295 (grid zone 50) and was within the Pelican Point Marine Park (Figure 11). Kite and wind surfers use the area just beyond the marine park (marked by yellow buoys) extensively – however no human disturbance to the site was noted. The macroalga *Chaetomorpha linum* was prevalent at times and dolphins occasionally sighted nearby. In bare sand areas most notably, we often observed large accumulations of shells from the mud snail *Batillaria australis.*

The seagrass meadow sampled was at -0.6 mAHD.

Figure 11 *Sampling at PPT*

Water depth

Depth of the seagrass sites sampled was estimated relative to AHD using recent bathymetry (provided by SRT/DoT 2012). Occasionally, bathymetry did not extend to the shallows sampled in this study, so best estimates were made using the nearest known point and measurements taken in-situ. As the estuary is tidal, tide data from Fremantle (combined with estimates of the sites at AHD) were used to estimate the height of overlying water at each site over each month. The average minimum and average maximum heights of overlying water for the period October 2011 to May 2012 are shown in Table 2. There was seasonality to the tide heights, with lower minimums and maximums observed in October 2011 that steadily increased to approximately 20 cm higher by May 2012.

Table 2 *Average depth of seagrass meadows and estimates of overlying water from October 2011 to May 2012*

Site	mAHD	Water overlying at low tide (m)	Water overlying at high tide (m)
PPT	-0.6	0.53	1.08
RCK	-0.7	0.63	1.18
HTH	-0.7	0.63	1.18
LUB	-0.4	0.33	0.88
DLK	-0.8	0.73	1.28
CAN	-0.54	0.47	1.02

3 Site and estuary conditions

3.1 Rainfall

The daily rainfall recorded by the Bureau of Meteorology at Perth Metro (station 009225) is shown in Figure 12. The study period had particularly high summer rainfall in December to February (Table 3).

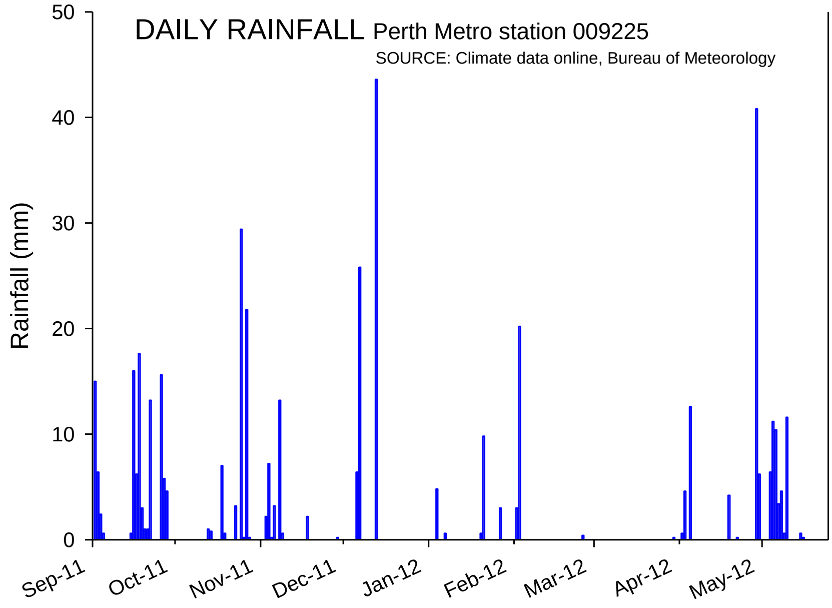


Figure 12 *Daily rainfall at Perth Metro (station 009225) for the study period taken from Climate data online, Bureau of Meteorology*

Table 3 *Rainfall summaries for the Perth region taken from Bureau of Meteorology monthly weather reviews <http://www.bom.gov.au/climate/mwr/>*

Month	**Rainfall for Perth region**
September 2011	Near average
October 2011	Below average
November 2011	Above average
December 2011	Very much above average
January 2012	Above average
February 2012	Above average
March 2012	Very much below average
April 2012	Near average
May 2012	Below average

3.2 Swan-Canning routine data

The Department of Water undertakes routine water quality sampling in partnership with the Swan River Trust. In-situ measurements of salinity, pH, dissolved oxygen and temperature, as well as chlorophyll *a* (chl *a*), phytoplankton identification and cell counts are obtained weekly, while water is analysed for nutrients on a fortnightly basis. The site locations for the routine Swan-Canning estuary monitoring close to the seagrass sampling locations are shown in Figure 13. The grey ovals show grouping of sites for the nutrient graphs presented later in this section.

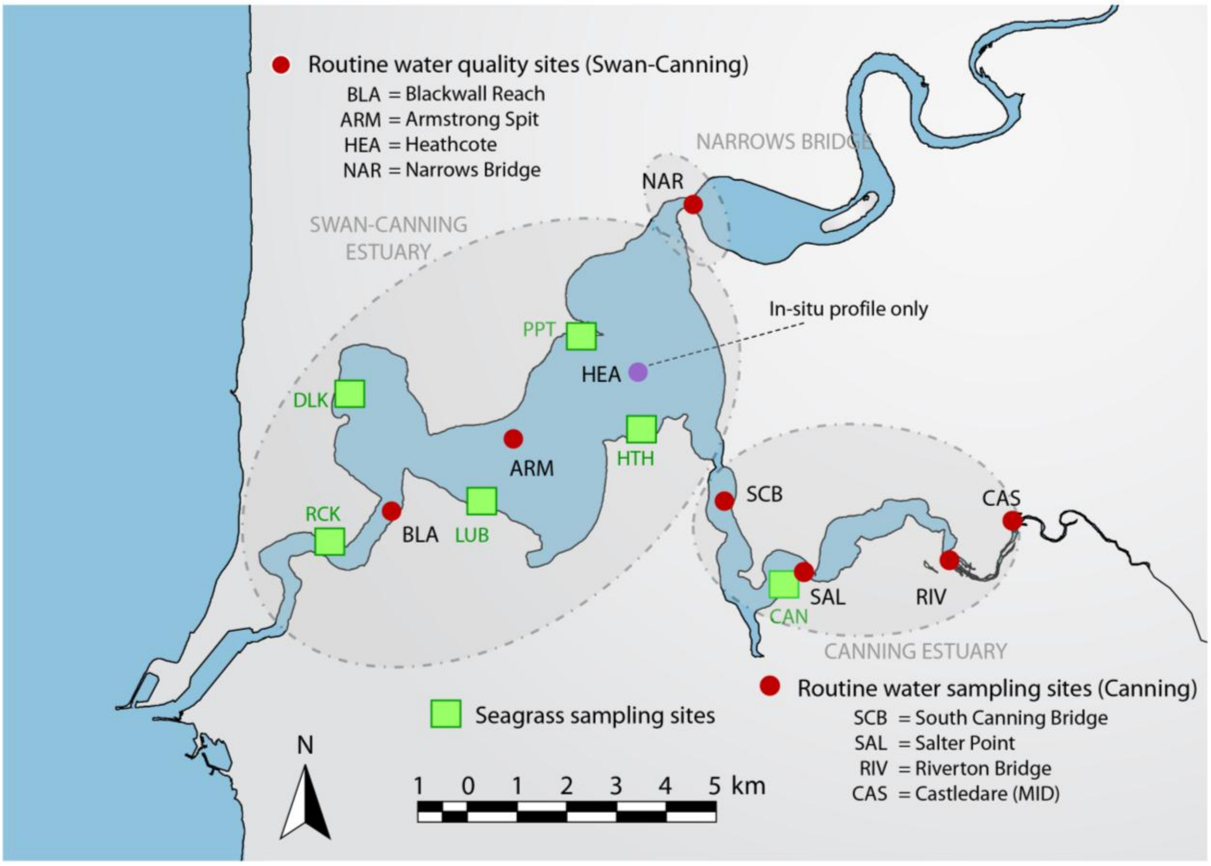


Figure 13 *Routine water quality sampling locations in the Swan-Canning estuary relative to seagrass sampling locations, with grey ovals showing site groupings for figures 16 to 18*

Temperature and salinity

Water column temperature measured at the routine sampling locations for 2011–12 is shown as interpolated plots in Figure 14. The seasonal patterns observed were very similar among all sites, with an average temperature (for the whole water column) of 24° C in the period December 2011 to 30 March 2012 at sites BLA, ARM, HEA and SAL, and 25° C for the same period at RIV. The productivity of *Halophila ovalis* increases approximately linearly between 10 and 25° C (Hillman et al. 1995) (note the effect on productivity above 25° C was not investigated in the study by Hillman).

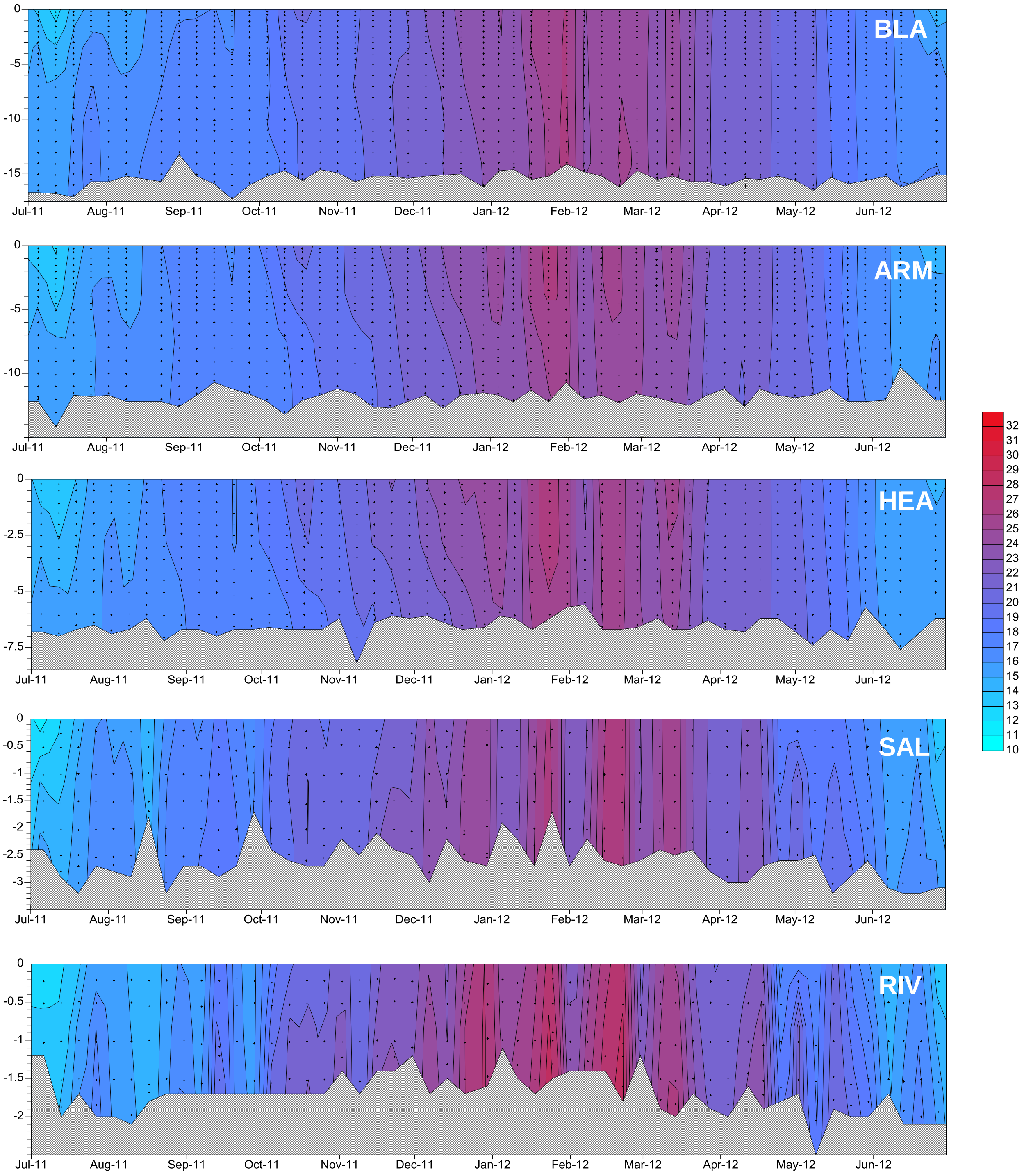


Figure 14 *Interpolated plots of temperature (10–32° C) from the weekly monitoring for sites BLA, ARM, HEA, SAL and RIV. Depth in metres is recorded on the vertical scale: note the different vertical scale for each site.*

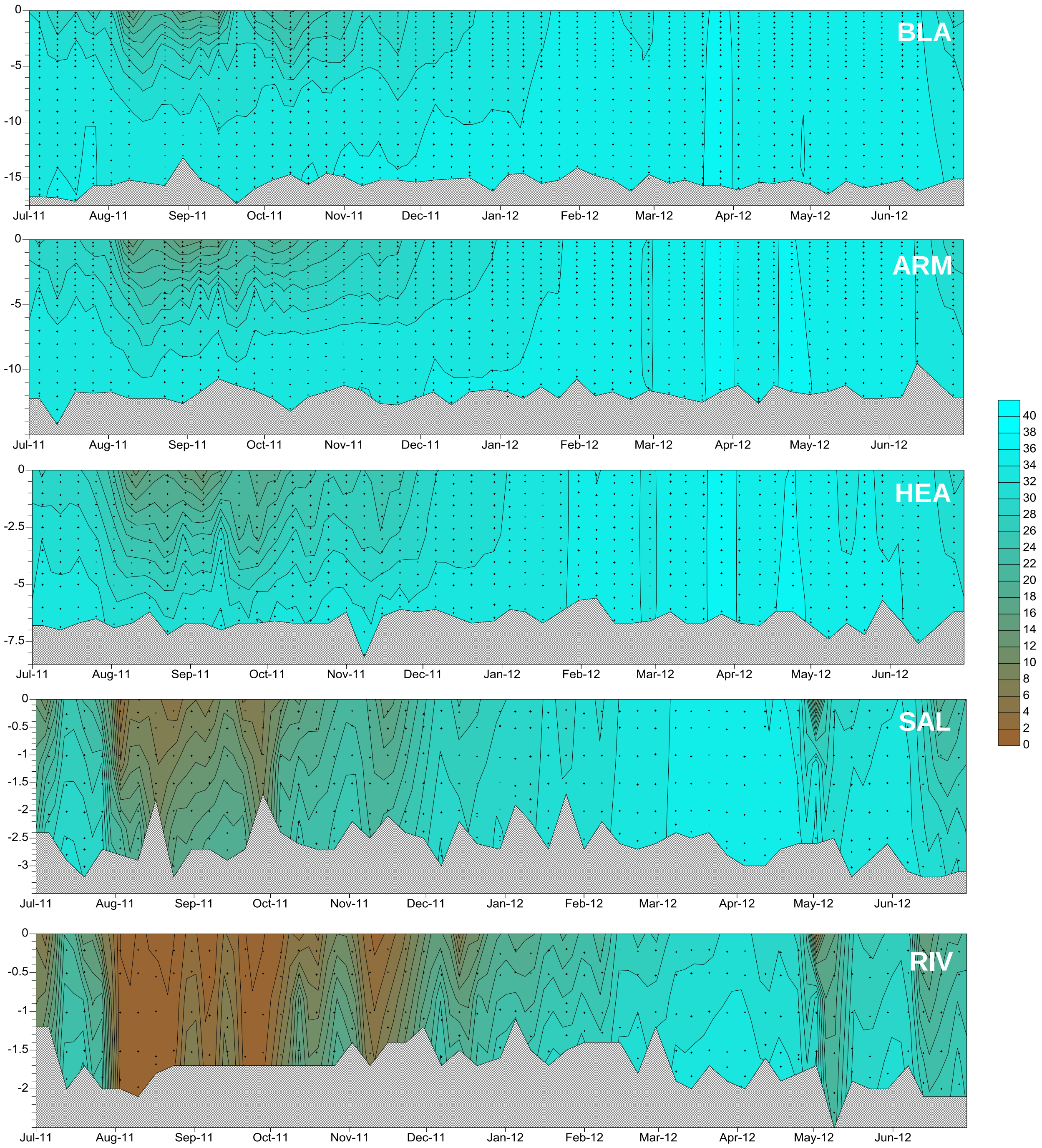


Figure 15 *Interpolated plots of salinity (0–40 psu) from the weekly monitoring for sites BLA, ARM, HEA, SAL and RIV. Depth in metres is recorded on the vertical scale: note the different vertical scale for each site.*

The salinity measured at the routine sampling locations for 2011–12 is shown as interpolated plots in Figure 15. The minimum salinity measured at HEA was 11.5 psu, at ARM 12.7 psu and at BLA 13.6 psu. In contrast, salinities <1 psu were measured at both SAL and RIV. The average salinity, measured over the whole year, was close to marine waters (31–32 psu) at HEA, ARM and BLA and somewhat more brackish at both SAL (26 psu) and RIV (19 psu).

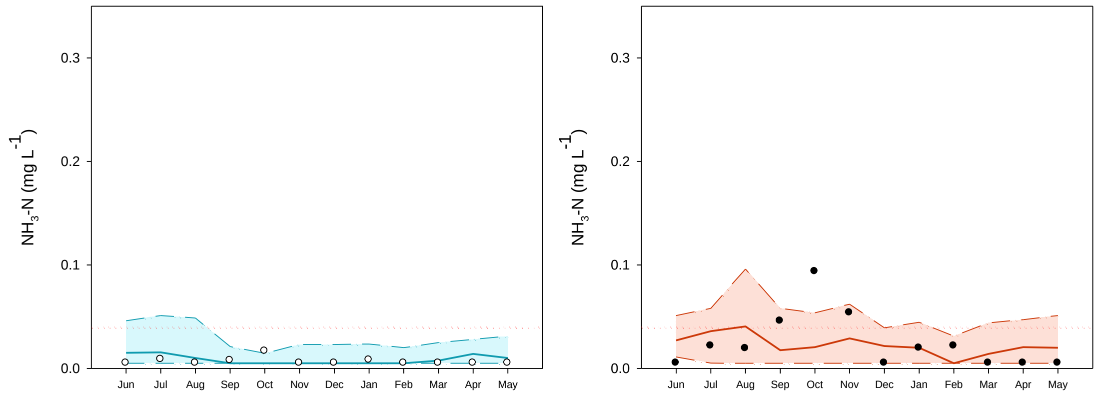
Salinity at CAN was likely to be the master environmental variable that determined whether seagrass was present at the site. Hillman et al. (1995) reported that *H. ovalis* growth was severely limited at salinities below 10 psu and considered optimal at salinities between 25 and 35 psu. Salinities consistently greater than 25 psu using data from SAL as a surrogate were unlikely to occur at site CAN until December 2011 – concurrent with the first sighting of *H. ovalis* at this site.

Water column nutrients

The monthly median nutrient concentrations measured in the three reaches of the Swan-Canning estuary are shown for Lower Swan Estuary, Canning Estuary and Narrows Bridge (see Figure 13 for sites in each grouping) in figures 16 to 18. These graphs show medians from each month (recorded in the June 2011 to May 2012 period) overlaying background (from June 2006 to May 2011) summary data (monthly median and 10th and 90th percentiles).

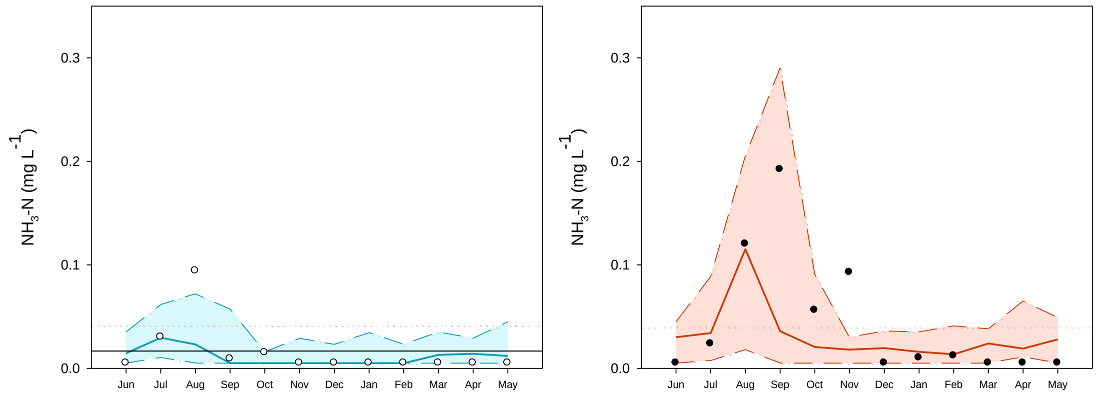
These summary plots suggest the largest proportion of dissolved inorganic nitrogen (DIN) is in the form ammonia/ammonium (NH_3/NH_4^+). Significant pulses of DIN are delivered to the estuary in the winter period (July to September), which are probably associated with rainfall.

By contrast, concentrations of soluble reactive phosphorus (SRP) appear to peak during the summer months, particularly in the Canning Estuary. Sediment release of phosphorus associated with low oxygen is the most likely cause of the summer SRP peak, where deoxygenation within the upper section of the reach is likely enhanced by higher temperatures accelerating microbial oxygen consumption rather than salinity stratification. Figure 19 shows a series of interpolated plots for site RIV for just the summer period (1 December 2011 to 30 March 2012), which illustrates the stronger relationship between temperature and deoxygenation (rather than salinity stratification).



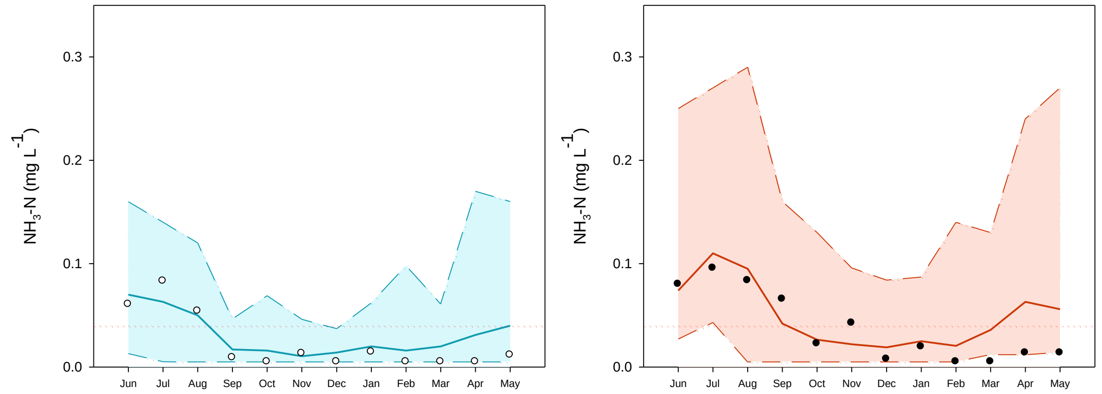
(a) **NH_3-N Lower Swan Estuary (Surface)**

(b) **NH_3-N Lower Swan Estuary (Bottom)**



(c) **NH_3-N Narrows Bridge (Surface)**

(d) **NH_3-N Narrows Bridge (Bottom)**



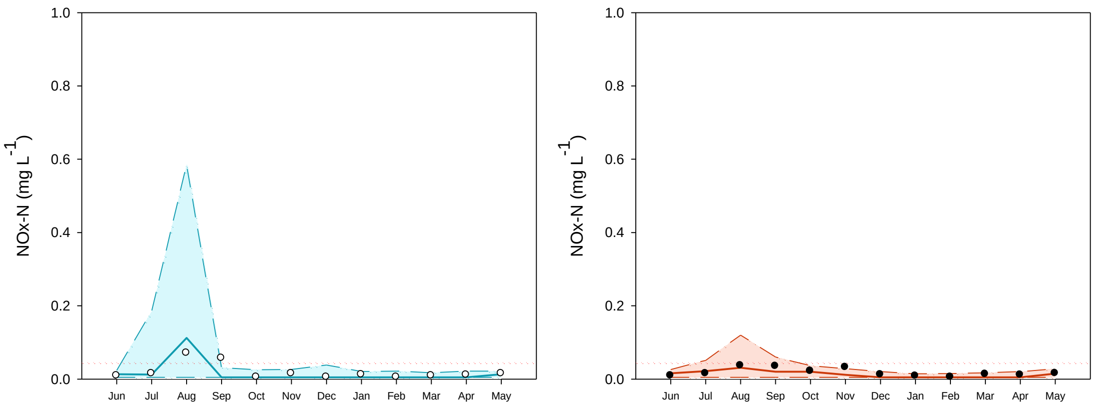
(e) **NH_3-N Canning Estuary (Surface)**

(f) **NH_3-N Canning Estuary (Bottom)**

▭ Monthly background 10th & 90th percentile
— Monthly background median
○ 11/12 monthly median
······ ANZECC & ARMCANZ guideline for SW estuaries

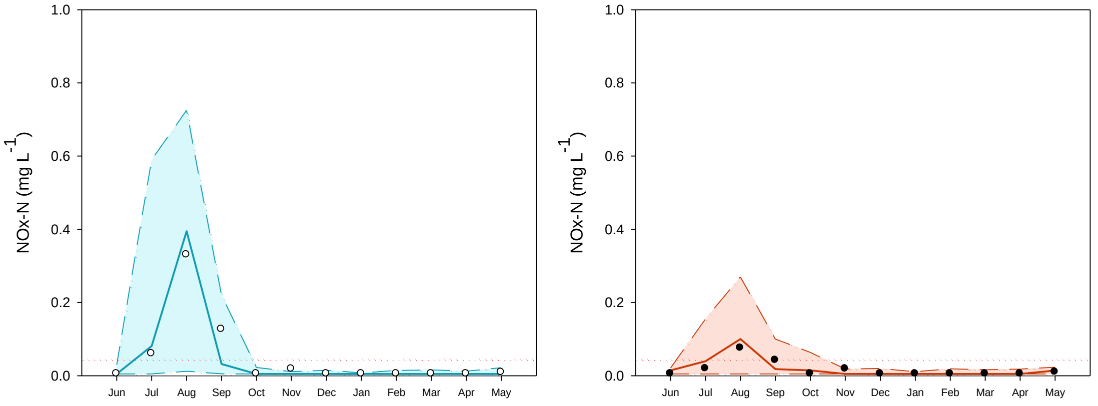
▭ Monthly background 10th & 90th percentile
— Monthly background median
● 11/12 monthly median
······ ANZECC & ARMCANZ guideline for SW estuaries

Figure 16 *Summary data for ammonia (NH_3-N; mg N L^{-1}) measured in surface and bottom water in lower Swan-Canning estuary. Median values from 2011–12 are shown by dots overlying the background (June 2006 to May 2011).*



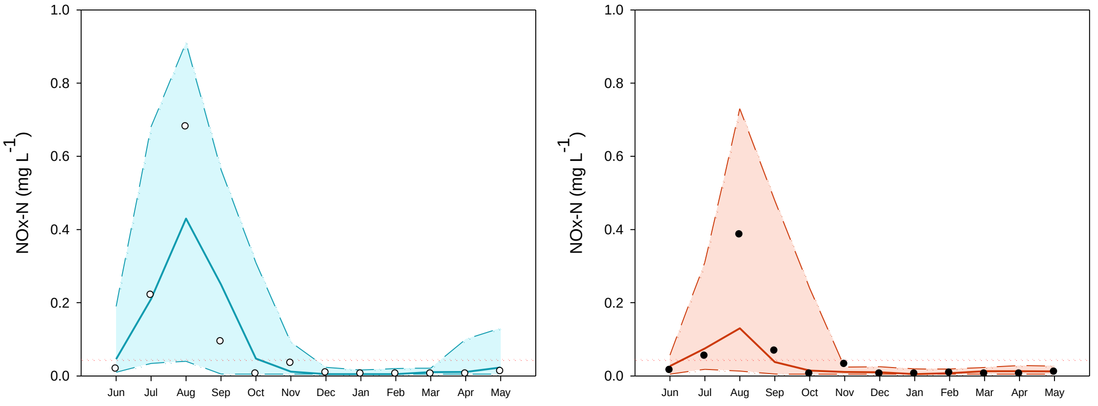
(a) NOx-N Lower Swan Estuary (Surface)

(b) NOx-N Lower Swan Estuary (Bottom)



(c) NOx-N Narrows Bridge (Surface)

(d) NOx-N Narrows Bridge (Bottom)



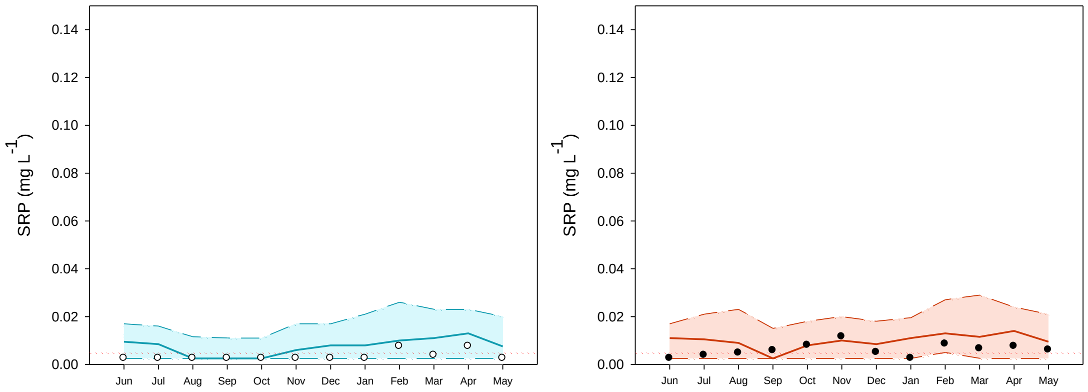
(e) NOx-N Canning Estuary (Surface)

(f) NOx-N Canning Estuary (Bottom)

▭ Monthly background 10th & 90th percentile
— Monthly background median
○ 11/12 monthly median
······ ANZECC & ARMCANZ guideline for SW estuaries

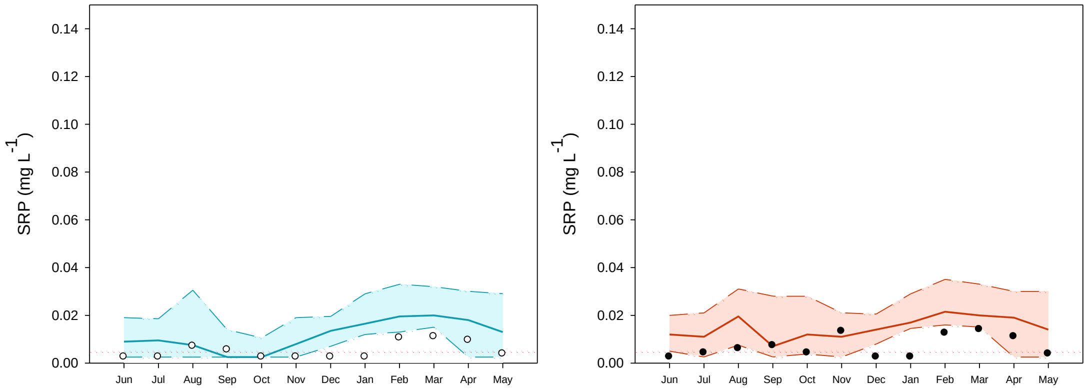
▭ Monthly background 10th & 90th percentile
— Monthly background median
● 11/12 monthly median
······ ANZECC & ARMCANZ guideline for SW estuaries

Figure 17 *Summary data for nitrate + nitrite (NO$_x$-N; mg N L^{-1}) measured in surface and bottom water in lower Swan-Canning estuary. Median values from 2011–12 are shown by dots overlying the background (June 2006 to May 2011).*



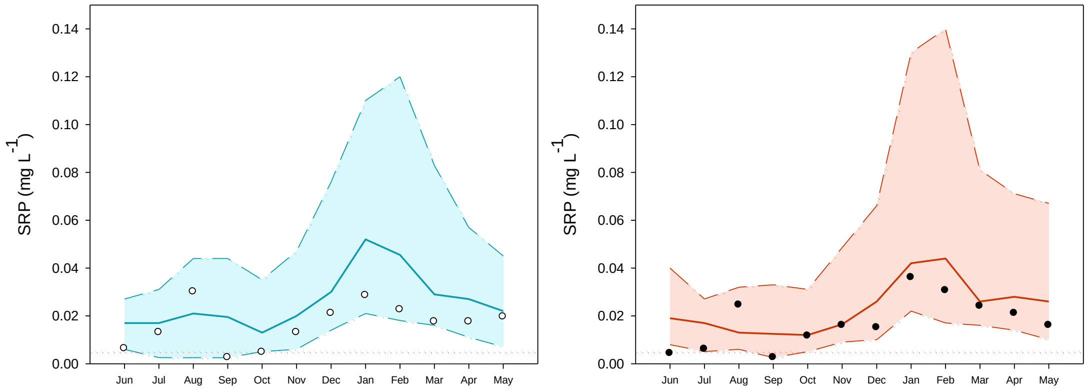
(a) SRP Lower Swan Estuary (Surface)

(b) SRP Lower Swan Estuary (Bottom)



(c) SRP Narrows Bridge (Surface)

(d) SRP Narrows Bridge (Bottom)



(e) SRP Canning Estuary (Surface)

(f) SRP Canning Estuary (Bottom)

🟦 Monthly background 10th & 90th percentile
— Monthly background median
○ 11/12 monthly median
⋯ ANZECC & ARMCANZ guideline for SW estuaries

🟥 Monthly background 10th & 90th percentile
— Monthly background median
● 11/12 monthly median
⋯ ANZECC & ARMCANZ guideline for SW estuaries

Figure 18 *Summary data for soluble reactive phosphorus (SRP; mg P L^{-1}) measured in surface and bottom water in lower Swan-Canning estuary. Median values from 2011–12 are shown by dots overlying the background (June 2006 to May 2011).*

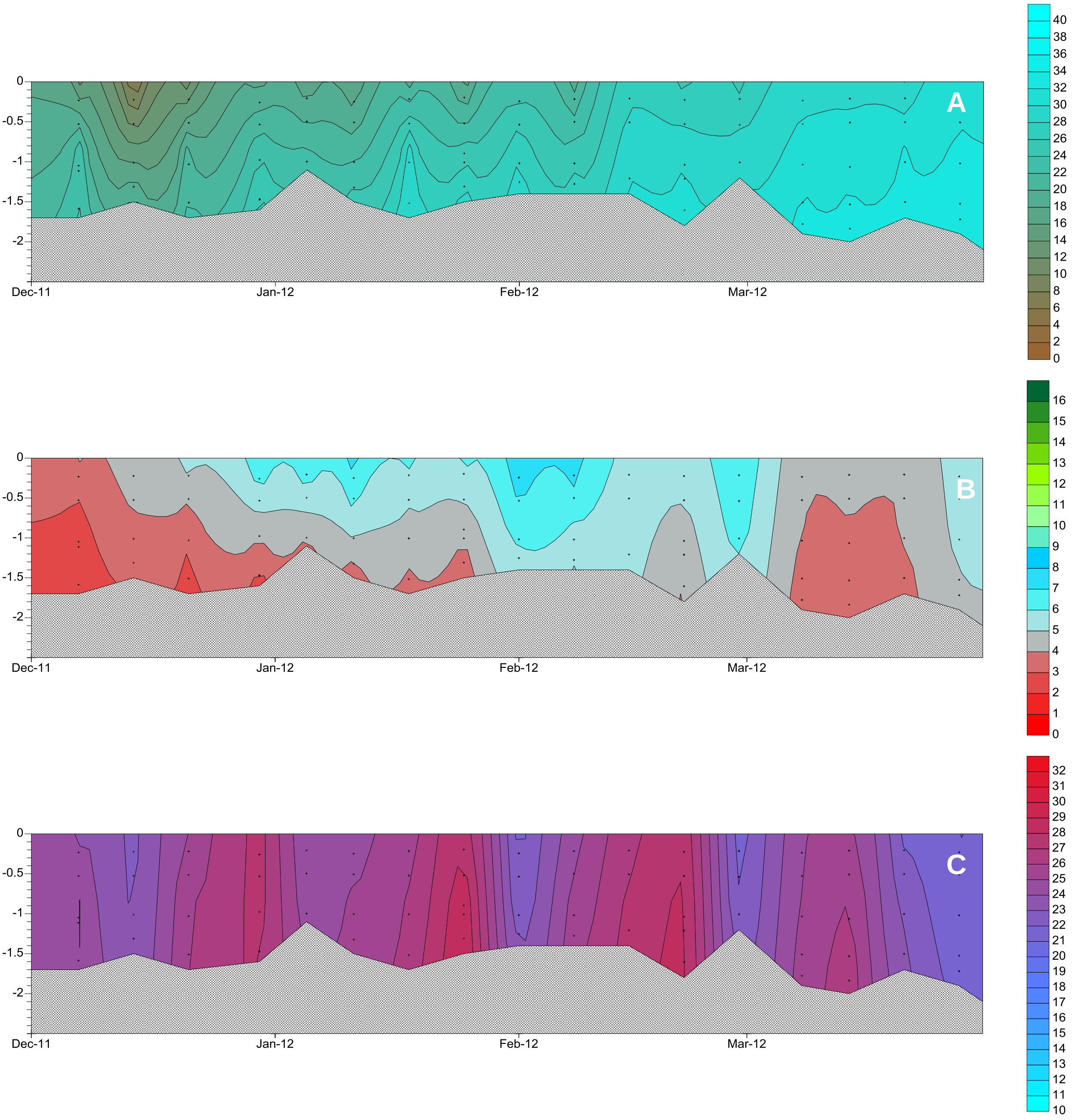


Figure 19 *Interpolated plot at site RIV of A) salinity (psu), B) DO (mg/L) and C) temperature (°C) for the 'summer period' of 1 December 2011 to 30 March 2012*

3.3 Characterisation of sediment type at each site

Four replicate sediment samples were collected from each of the six regular sites on 7 November 2011 and 29 May 2012. Sediment was collected in polycarbonate cores (96 mm ID). Water was decanted from the cores and the top 5 cm of sediment sampled and analysed at the Environmental Analysis Laboratory, Southern Cross University (NSW).

Results of sediment analysis (average and standard deviations) are shown in tables 4 and 5.

Table 4 *Average values obtained from sediment analysis (standard deviation (SD) in parentheses) for sediment collected on 7 November 2011*

SITE	Moisture (%)	TOC (%)	TN (%)	TP (mg/kg P)	AVS (% DW)	SCr (% DW)
PPT	22.5 (1.1)	0.27 (0.04)	0.03 (0.005)	57 (8.6)	0.01 (0.0002)	0.06 (0.008)
HTH	22.1 (1.6)	0.23 (0.03)	0.03 (0.003)	68 (4.7)	0.01 (0.006)	0.03 (0.015)
LUB	20.0 (0.8)	0.22 (0.04)	0.03 (0.004)	51 (3.5)	0.01 (0.007)	0.04 (0.005)
RCK	25.1 (2.5)	0.34 (0.07)	0.04 (0.010)	81 (14)	0.01 (0.01)	0.06 (0.015)
DLK	21.7 (0.8)	0.34 (0.04)	0.04 (0.005)	76 (9.5)	0.00 (0.006)	0.07 (0.024)
CAN	21.4 (1.1)	0.21 (0.01)	0.02 (0.005)	68 (6.2)	0 (0)	0.01 (0.006)

Table 5 *Average values obtained from sediment analysis (SD in parentheses) for sediment collected on 29 May 2012*

SITE	Moisture (%)	TOC (%)	TN (%)	TP (mg/kg P)	AVS (% DW)
PPT	24.6 (3.7)	0.20 (0.01)	0.06 (0.002)	60 (4.8)	0.01 (0.0002)
HTH	26.1 (2.2)	0.22 (0.05)	0.07 (0.006)	79 (7.5)	0.01 (0.006)
LUB	25.4 (2.9)	0.26 (0.07)	0.07 (0.009)	65 (12)	0.01 (0.0003)
RCK	28.0 (4.8)	0.26 (0.04)	0.07 (0.007)	80 (3.0)	0.02 (0.007)
DLK	25.9 (3.2)	0.18 (0.05)	0.06 (0.002)	66 (4.5)	0.01 (0.007)
CAN	23.2 (0.7)	0.18 (0.02)	0.07 (0.003)	72 (7.0)	0.01 (0.007)

Sediment data were analysed (Appendix A-1) using PRIMER 6 and PERMANOVA and R. A two-way ANOSIM was performed and showed that both *month* and *site* were significant factors (month: Global R=0.71, $p<0.0001$; site: Global R=0.48, $p<0.0001$). Figure 20 shows the significant variation between the samples collected on each sampling occasion (November 2011 and May 2012). Differences in month were mainly driven by differences in total nitrogen and moisture content, whereas differences between sites were mainly due to differences in total organic carbon. Results of the univariate analysis of sediment data are presented in Appendix A-2. There was a strong linear relationship between total organic carbon and total nitrogen in the sediments (Figure 21).

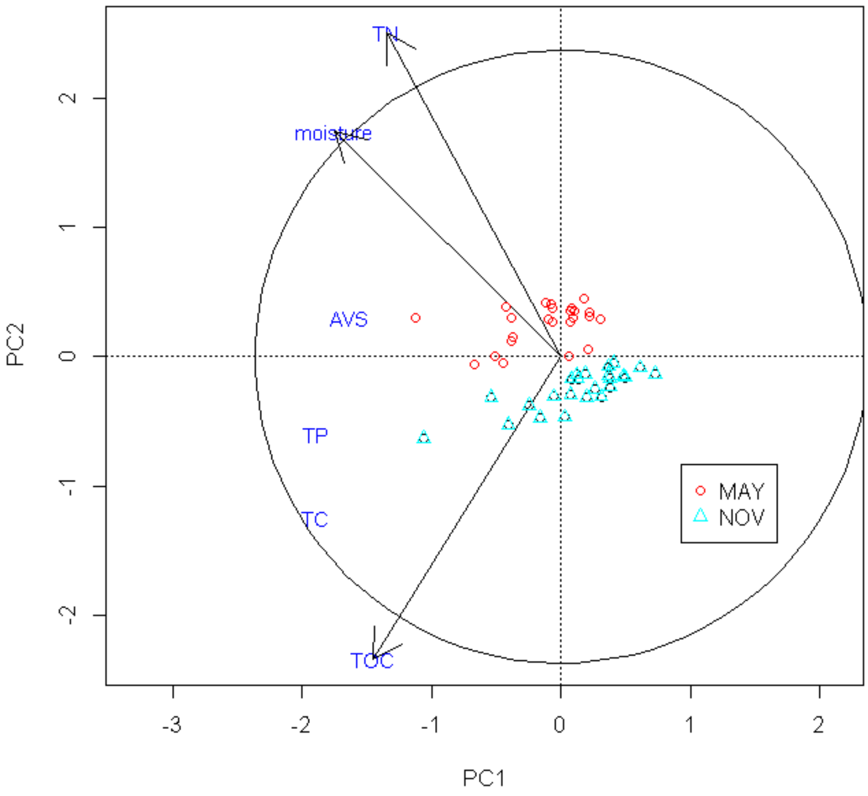


Figure 20 *Principal component analysis (PCA) for sediment data collected at each site on each sampling occasion*

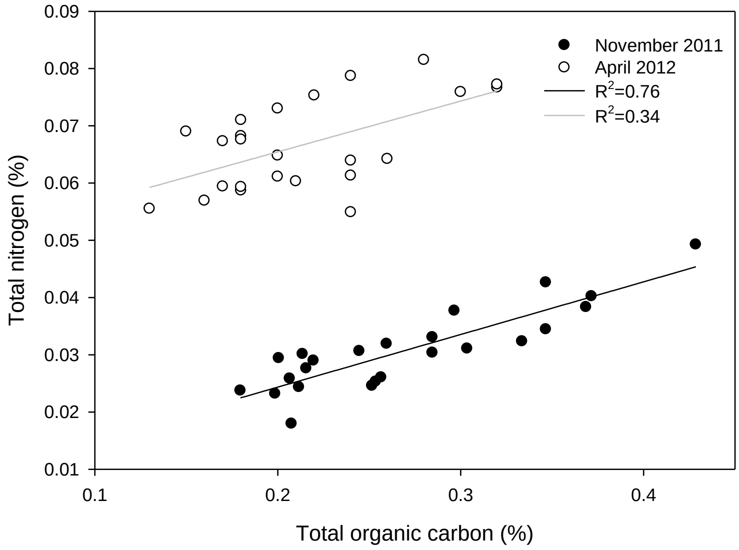


Figure 21 *Scatterplot of total nitrogen against total organic carbon for sediments collected on 7 November 2011 and 29 April 2012*

The reduced sulfur fraction of sediment was analysed for the $\delta^{34}S$[†] on two occasions in March and May 2012 following extraction by the chromium reducible sulfur method (Appendix B-1, B-3). Results obtained for each site (Table 6) were not significantly different (one-way ANOVA), so the average measured across all sites of -24.03 ‰ will be used for the sulfide intrusion calculations in Section 5.1.

Table 6 *Sulfur isotope ratio analysed in reduced inorganic sulfur (RIS) measured as chromium reducible sulfur (CRS) in sediment at each site (average, with standard deviation in parentheses; n=5)*

SITE	δ^{34}S in RIS
PPT	-23.9 (0.66)
HTH	-23.4 (0.99)
LUB	-24.3 (0.81)
RCK	-23.8 (1.4)
DLK	-24.4 (0.56)
CAN	-24.3 (0.41)

[†] Isotope ratios (e.g. reported as δ) are a ratio of ratios, e.g. $\delta^{34}S = \left(\frac{(ratio\ of\ 34S\ to\ 32S\ of\ sample)}{(ratio\ of\ 34S\ to\ 32S\ of\ standard)} - 1\right) * 1000$

4 Changes in environmental and biological variables over time

4.1 Temperature

Temperature loggers (Tidbit V2 underwater and HOBO Pendant) were deployed at each site for the duration of the experiment, attached to the PAR logger wiper units. Unfortunately loss of equipment and downloading practicalities meant that data collected were not continuous at sites CAN, RCK and LUB. All sites had a fairly uniform relationship for average temperature at any given sampling month – within 2° C of each other.

Temperature data were averaged over the rhizome-tagging period for each site for each month of sampling (Figure 22). Where data were missing due to instrument loss, temperature was estimated (by correlation with other similarly acting sites) for use in multi-variate data analysis.

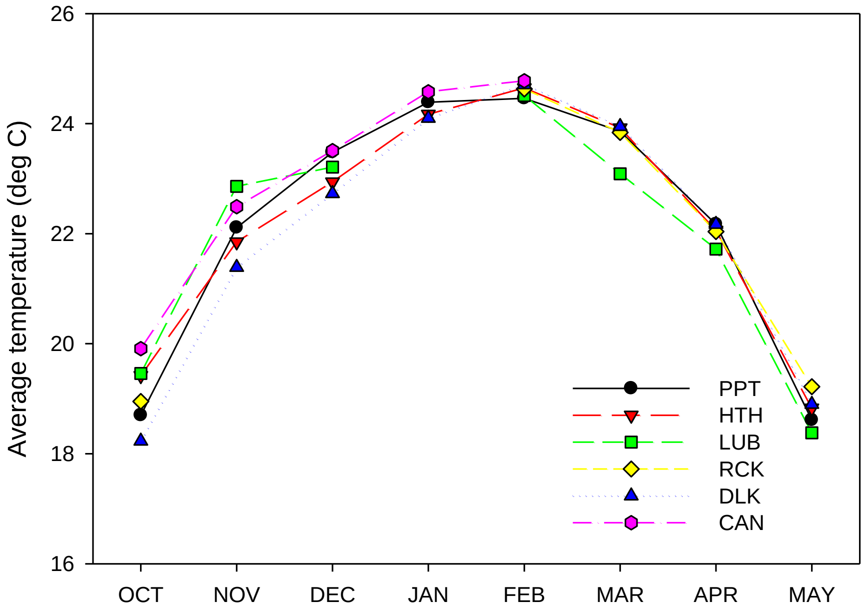


Figure 22 *Average temperature for the rhizome-tagging periods at each site*

4.2 Photosynthetically active radiation (PAR)

Two Odyssey PAR loggers with attached wiper units were deployed at each site – see <www.odysseydatarecording.com> for details. These were buried into the sediment such that the sensor was positioned at approximately seagrass canopy height (Figure 23). The depth of water overlying the sensors was recorded at various times during the deployment. An additional Odyssey PAR logger was located out of the water (at easting 390744, northing 6468652, grid zone 50) to record the daily irradiance from the sun (without influence from the water column) – this site is referred to as AIR henceforth. Odyssey PAR sensors record incident light in the 400 to 700 nm wavelength region: that which is believed to be most useable by photosynthetic organisms. Odyssey PAR sensors are low-cost and useful for applications where relative differences are more important than absolute readings. In this study, the sensor was programmed to accumulate pulse data over a 10-minute interval, and at the end of the scan time record the accumulated value. This data integration compensates for the high degree of fluctuation in solar irradiance. Loggers were calibrated against each other using (as recommended by Odyssey) the midday sun as a reference with a nominal irradiance of 2000 μmol photons $m^{-2}s^{-1}$ (note: 1 μmol photons $m^{-2}s^{-1}$ =1 μE $m^{-2}s^{-1}$). As such, the PAR measurements should be interpreted as relative rather than absolute.

An example of the 'continuous' (every 10 minutes) PAR data is shown for two sites (AIR and PPT) in Figure 24. A marked reduction in light available underwater occurred at most sites in April and May 2012 of the study period.

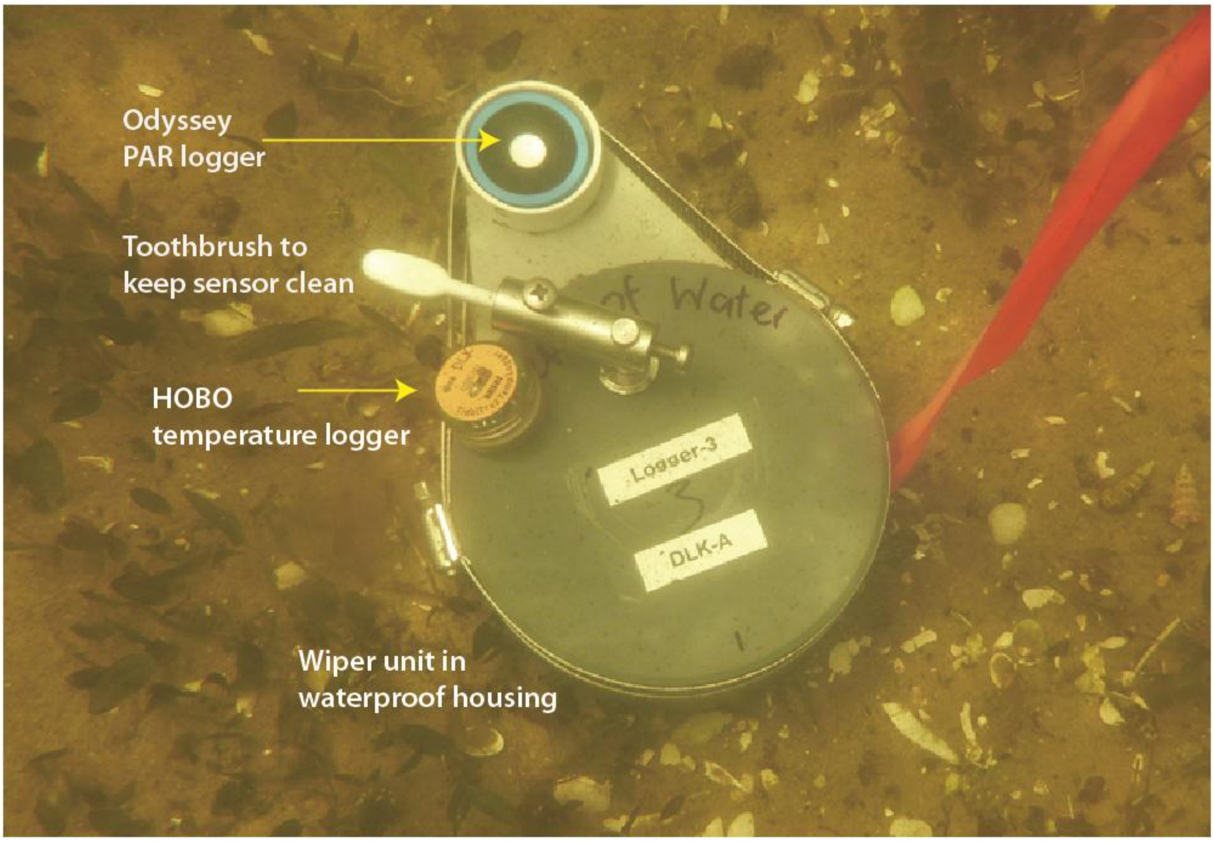


Figure 23 *Underwater set-up of the Odyssey PAR logger with wiper unit and the Hobo temperature logger*

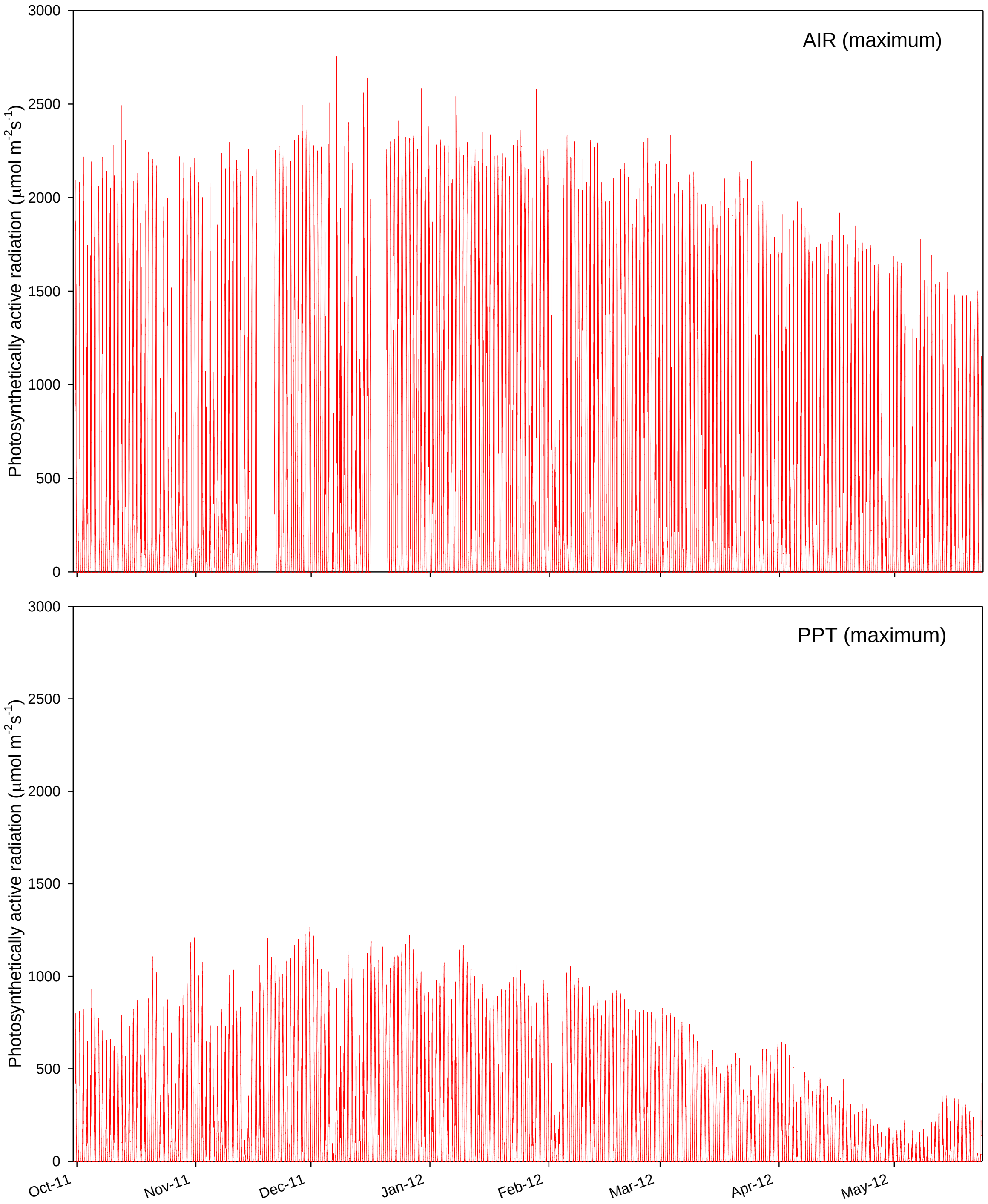


Figure 24 *Example of photosynthetically active radiation (PAR) data for the in-air (out of water) sensor (top), and the sensors deployed at site PPT (bottom). Where data exists for multiple sensors, the greatest (i.e. maximum) reading was used for further analysis. There was a marked reduction in the available light throughout late March to May 2012. Note: data gaps in AIR in November and December were due to the logger being offline while being downloaded.*

Several indicative parameters were derived from these data. These included the:

- monthly medians of the daily peak light received at each site, where peak light was defined as that received in the two hours before and after midday (i.e.10 am – 2 pm[‡])
- monthly medians of the percentage of surface irradiance (again calculated in the 10 am – 2 pm period)
- number of hours for which the site received PAR irradiance which was greater than the saturation irradiance – as reported in Hillman et al. (1995) – and reported monthly associated with the rhizome-tagging periods.

The peak light observed at each site and at the AIR reference is shown in Figure 25A. It shows the expected decrease in available light in the underwater environment moving towards winter (associated with increased angle of incidence). The peak light was highest at LUB at the start of the study period, which was expected given it was the shallowest site. CAN, the second shallowest site, had one of the lowest peak light measurements – likely indicative of turbidity derived from the catchment, and fitting with the observations of tannin and often turbid waters at the site. Site HTH was significantly affected by macroalgae in spring (as will be discussed in Section 4.4), and this had an immense impact on the light climate at the time. Unexpectedly, DLK had excellent light penetration during the autumn months; it is believed this was due to increased water clarity at the site (which was also noted during site visits). The increase in water clarity relative to other sites may be because of the site’s eastward orientation and it thereby remaining calm during south-westerly winds. Additionally, very little rain fell during March and April to increase the river’s turbidity (see Figure 12). The percentage of surface irradiance (% SI) is shown in Figure 25B, and shows much the same pattern as discussed above for peak light intensity. Interesting to note, however, is that at site HTH in December the % SI falls below 11%, the generalised threshold which Duarte (1991) suggests seagrass require to be sustained. Specific minimum light requirements (MLR) can be determined for each seagrass species (Collier et al. 2012), although a recent study suggests that MRL not only vary with species but also with the history of light conditions the seagrass has experienced (Yaakub et al.).

The daily hours above the saturating irradiance for *H. ovalis* were calculated for each of the rhizome-tagging periods for each month (see Figure 26). For the most part, these seagrass meadows received between seven and 10 hours of saturating irradiance during most of the months sampled. The striking exception is for site HTH in December, which received zero hours per day above the saturating irradiance due to a severe macroalgal accumulation. By May, most sites were receiving less than four hours of saturating irradiance per day.

[‡] Note: Deriving peak light as period between 10 am and 2 pm has been reported previously in Hirst et al. (2008).

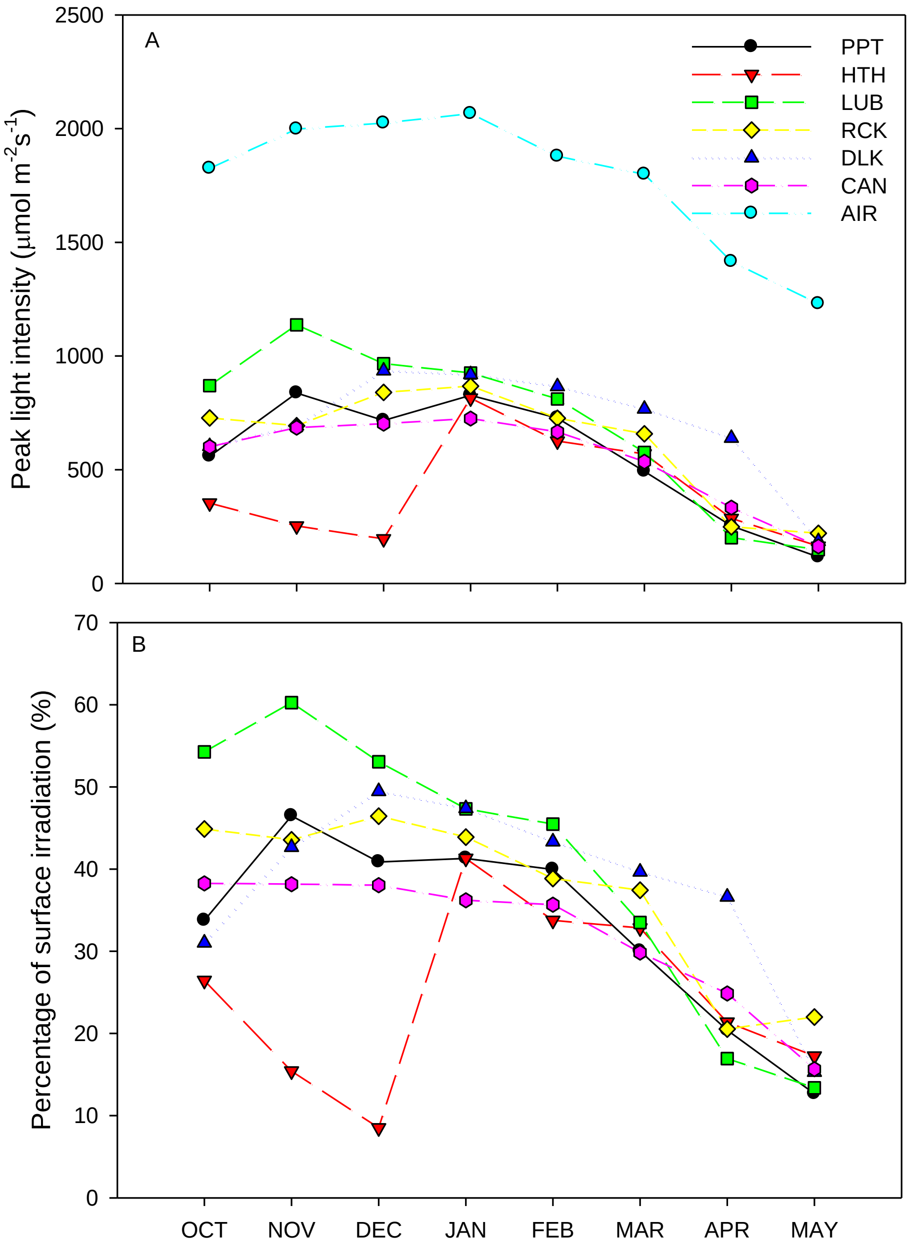


Figure 25 *A) Peak light intensity (defined as that measured in the four-hour window between 10 am and 2 pm) at the six sites and the in-air reference (medians); and B) Percentage of the surface irradiation measurable by in-situ PAR loggers at each site as medians. Percentage of surface irradiation calculated for 10-minute intervals for the period 10 am to 2 pm by comparing in-situ logger readings with in-air logger readings, and the median value of these across each month is plotted.*

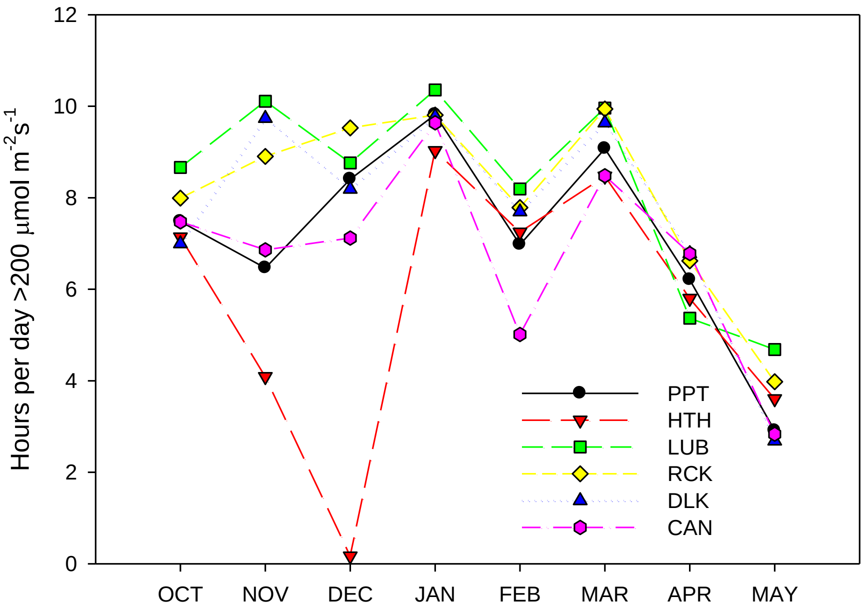


Figure 26 *Hours per day above the saturating irradiance of 200 μmol $m^{-2}s^{-1}$ recorded at each site during the tagging period*

4.3 Water quality

Bottom water samples were collected at each site at weekly intervals during the summer period (7 December 2011 to 17 April 2012). Water was filtered on-site (0.45 μm) and analysed for filtered nutrients (ammonium, nitrate + nitrite and SRP) by the National Measurement Institute.

Dissolved nitrogen concentrations (ammonium and nitrate + nitrite) in bottom waters at each of the six sites during the summer months are shown in Figure 27. Dissolved phosphorus concentrations (as SRP) in bottom waters for the same sites and period are shown in Figure 28. Dissolved nutrient concentrations were generally highest at CAN, the site closest to riverine nutrient inputs from the catchment. Higher nutrient concentrations were generally observed with rainfall. However, nutrients within the estuary also increased in late summer/ early autumn – a period when rainfall was not recorded. It is possible these nutrients are a result of internal recycling of seagrass biomass within the estuary. Seagrass biomass is suggested as the source rather than macroalgae, given the macroalgal biomass appeared to peak in late spring and decline in early summer, whereas the seagrass biomass was declining in late summer/early autumn. Recycling of sediment organic matter (releasing nutrients) is likely to be greatest during summer when temperatures are highest, so does not explain the late summer/autumn peak in nutrients.

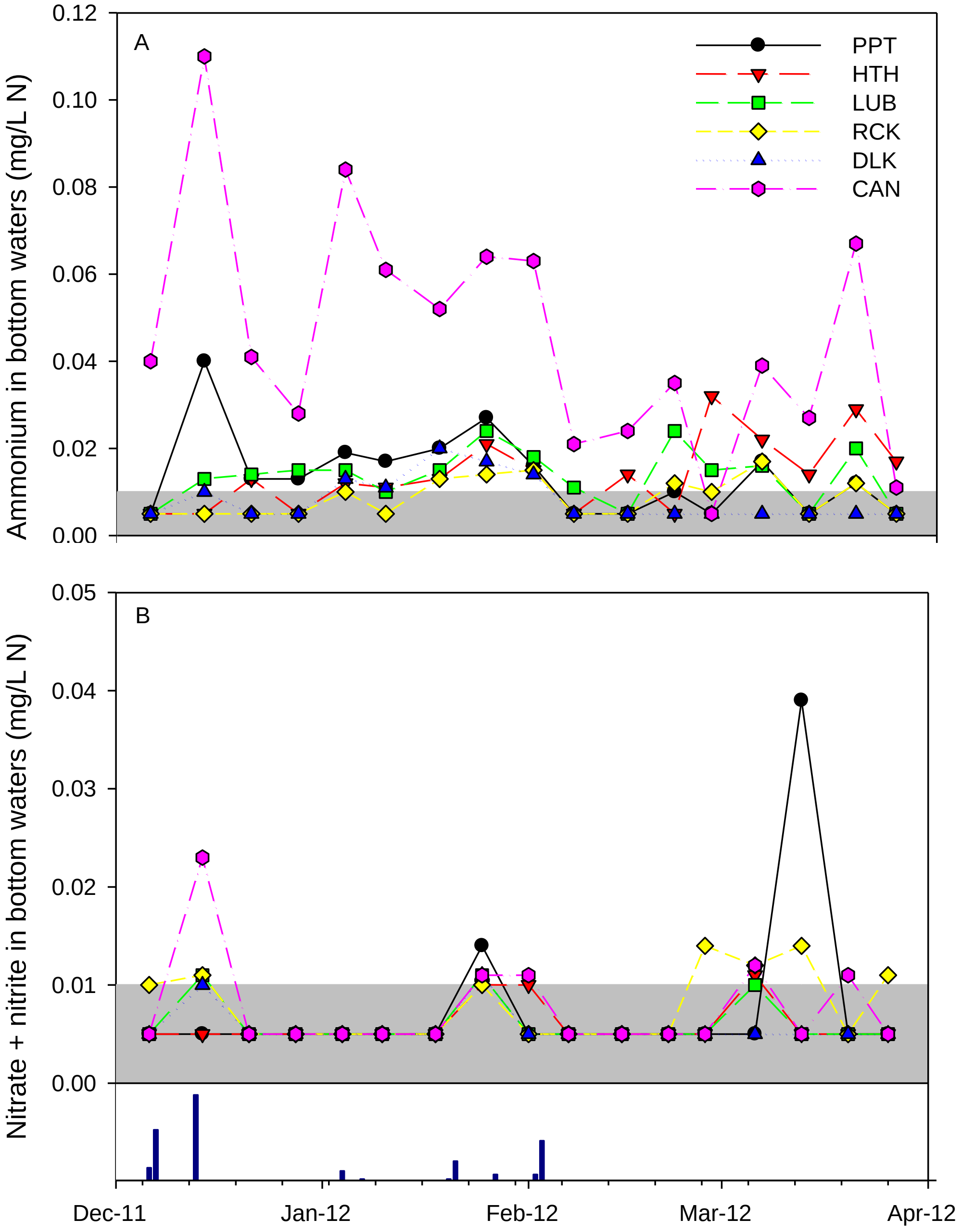


Figure 27 *Dissolved nitrogen concentrations A) ammonium and B) nitrate + nitrite measured weekly in bottom water at the six sites during the summer period. The grey box in each chart indicates the limit of reporting (LOR), and results shown within this box have been given the nominal value of half the LOR. The dark blue vertical bars show rainfall events during this period (from Figure 12).*

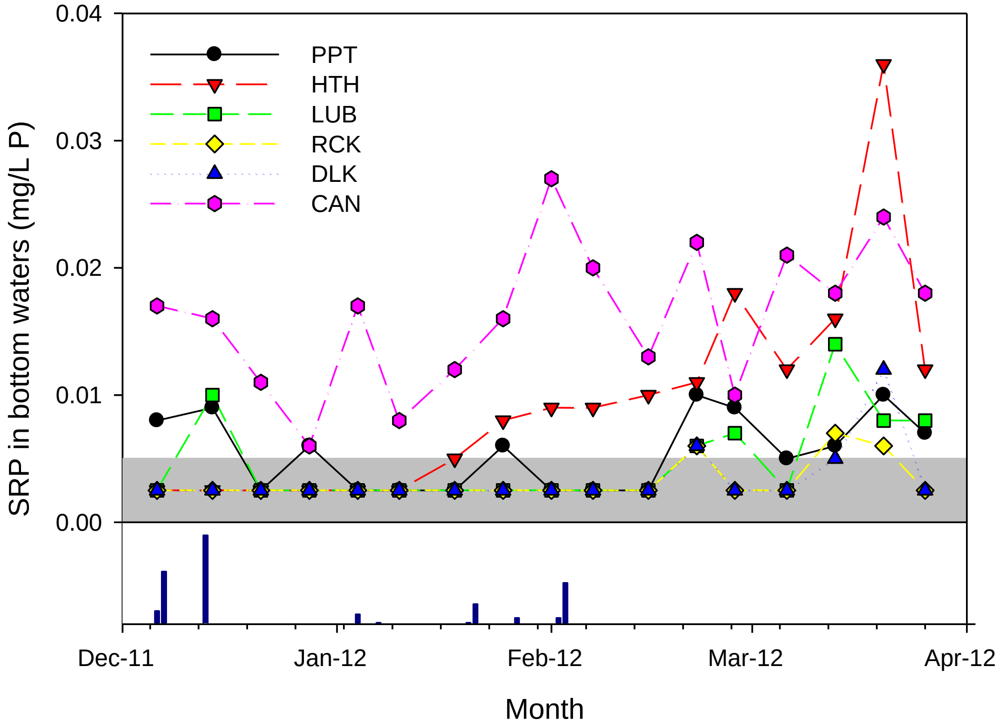


Figure 28 *Dissolved phosphorus concentrations (measured as soluble reactive phosphorus) sampled weekly in bottom water at the sites during the summer period. The grey box indicates the limit of reporting (LOR), and results shown within this box have been given the nominal value of half the LOR. The dark blue vertical bars show rainfall events during this period (from Figure 12).*

Bottom water samples were also collected for $\delta^{34}S$ analysis from each site on five occasions during the study period. Samples were filtered on-site (0.45 µm), preserved with HNO_3 and analysed at the Environmental Analysis Laboratory, Southern Cross University. Sulfur isotope analysis was carried out on sulfate precipitated from these water samples following the addition of $BaCl_2$ (Appendix B-2, B-3).

Bottom water had similar sulfate isotopic ratios for each site – ranging from 19.2 ‰ to 22.4 ‰. Slightly less positive values were observed at the start of the sampling period (19.8 ‰, November 2011) compared with the remaining period (average of 21.8 ‰). As there was no significant difference in the $\delta^{34}S$ value between sites (one-way ANOVA), the average of all samples across all sites (21.4 ‰) will be used for calculating sulfide intrusion into seagrass (see Section 5.1).

4.4 Field observations of macroalgae and seagrass

At each site (eight time periods from October 2011 to May 2012) seagrass cover and macroalgae cover were estimated using randomly placed quadrats (30 x 30 cm; 10 replicates). Observations of both seagrass and macroalgae percentage cover were categorised (to reduce operator bias) into cover classes of none, 0–10% cover, 10–25% cover, 25–50% cover, 50–75% cover, 75–90% cover and 90–100% cover. Summary statistics were obtained from these ‘cover classes’ by taking the mid-point of the category as the reported value, allowing average percentage cover and standard deviation for the sites to be calculated.

Macroalgae was observed in 37% of quadrats (n=480). It was most abundant in November and December across most sites. The most common macroalga observed was *Chaetomorpha linum* (30% of quadrats). *Chaetomorpha* caused nuisance blooms at HTH in particular, where it was observed completely filling the water column (approximately 1 m deep). The *Chaetomorpha* present in November and December at PPT could also be described as a nuisance bloom, but it had significantly less biomass than at HTH for the same period. *Gracilaria comosa* was the next most common macroalga, but was only observed in 3.5% of quadrats. Mixed consortia of macroalgae were observed in the remaining 3.5% of quadrats. Macroalgae was not observed at the site in the Canning Estuary (CAN).

Sites CAN, PPT and HTH were the patchiest in terms of seagrass coverage. The ratios of presence to absence recorded for the whole study period are shown in Table 7. These observations showed that seagrass was most sparse at CAN, with no seagrass able to be sampled until January 2012.

The estimates of seagrass and macroalgal cover were analysed (pre-treatment as per Appendix A-1) using the multi-variate analysis package PRIMER 6 and PERMANOVA. A two-way ANOSIM was performed, showing that both site and month were significant factors (site: Global R=0.465, p<0.00001; month: Global R=0.324, p<0.00001).

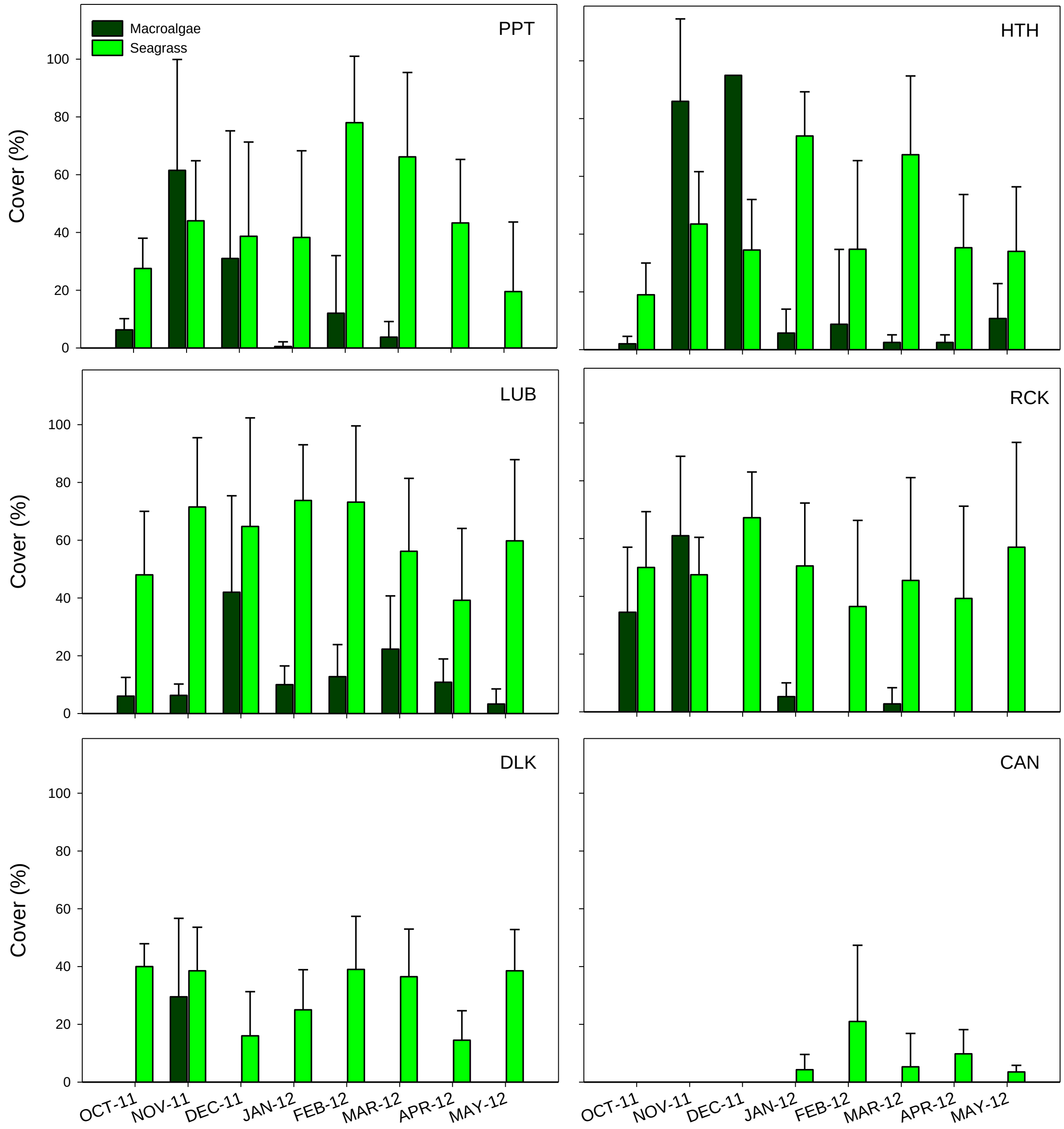


Figure 29 *Percentage cover observed in quadrats for macroalgae and seagrass at the six sites in the Swan-Canning estuary (mean + SD)*

Table 7 *Number of instances where zero seagrass coverage was recorded at each site, with the ratio of presence to absence for the total period recorded in the final column*

	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	Presence/absence
PPT	0	0	2	3	1	1	1	3	0.862
HTH	0	0	0	3	2	0	0	1	0.925
LUB	0	0	0	0	0	0	0	0	1
RCK	0	0	0	0	0	0	1	0	0.988
DLK	0	0	0	0	0	0	0	0	1
CAN	10	10	10	4	4	6	3	3	0.375

4.5 Seagrass production measures

Seagrass growth rates were estimated using the rhizome-tagging method, following Short and Duarte (2001). Leaf production rate, rhizome extension rate and production rate (per apex) are shown in Figure 30. These measures of growth rates and production were quite variable across sites and months (significant for site, month and site x month – see Appendix A-4), although a generalised pattern of higher growth rates was apparent during the summer months compared with spring or autumn. For site DLK, the highest growth rate (leaf formation, rhizome extension and total production) was observed in February 2012, whereas site PPT had its highest leaf formation and total production rates in December 2011 (with highest rhizome extension rate observed in January 2012). It is likely that growth rates at each site were significantly influenced by site-specific environmental variables (e.g. temperature, light, floating macroalgae and nutrients).

Seagrass growth metrics were examined using multi-dimensional scaling (MDS) and cluster analysis (with a SIMPROF test to show samples with statistically similar production rates). Figure 31 shows the MDS with SIMPROF groupings (top) and the same analysis showing the relative light climate of the site at the time of sampling (bottom). Not too surprisingly, the environmental variables (associated with light, temperature and water depth) were found to be statistically significantly related to the seagrass production measures (using RELATE: Rho=0.197, $p<0.05$ – see Appendix A-5 for further detail).

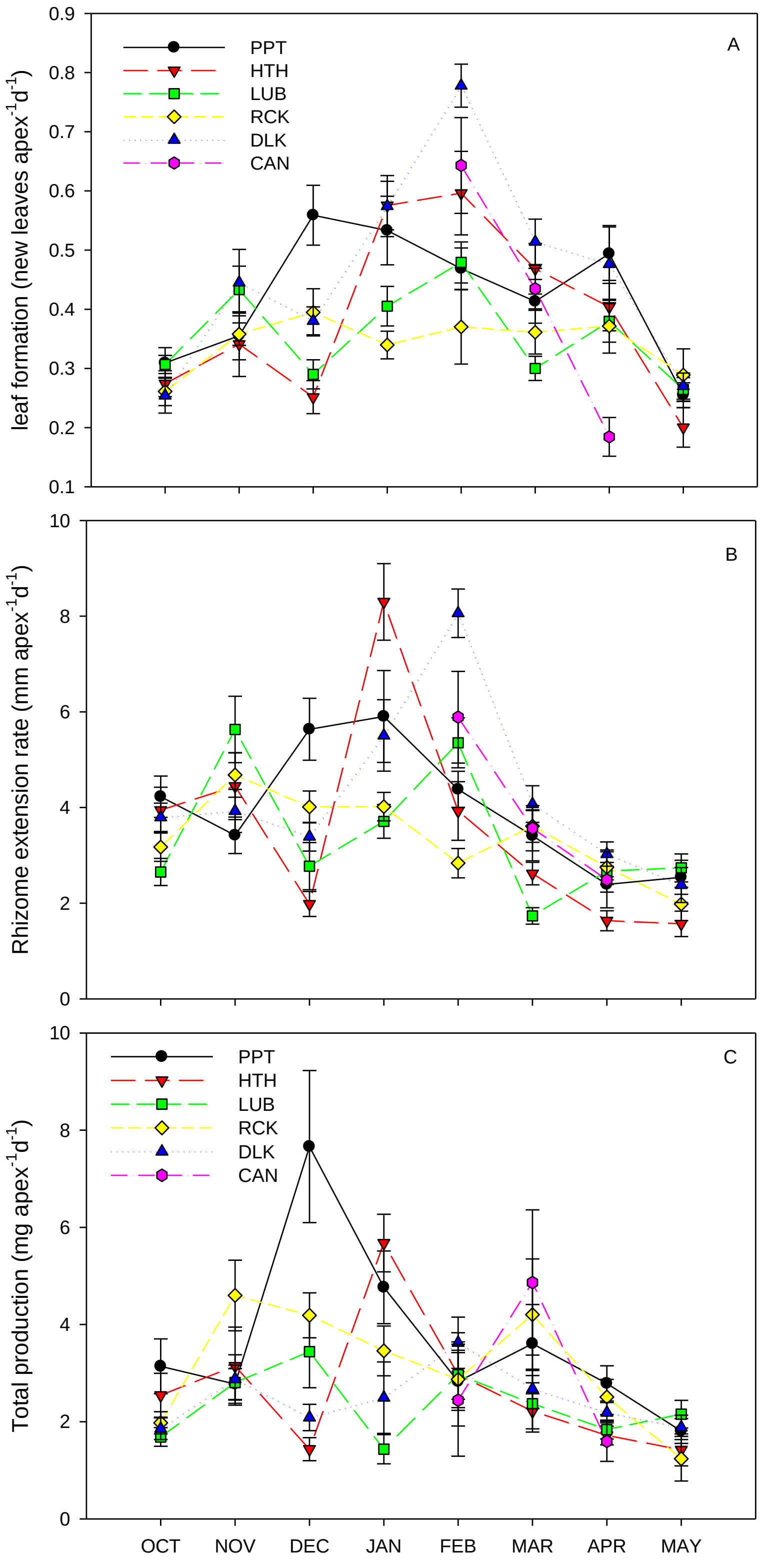


Figure 30 *Seagrass production measures for each site at each time period of A) leaf formation rate (mean +/- SE, n=8-28); B) rhizome extension rate (mean +/- SE, n=8-28); and C) total production rate (mean +/- SE, n=4-24)*

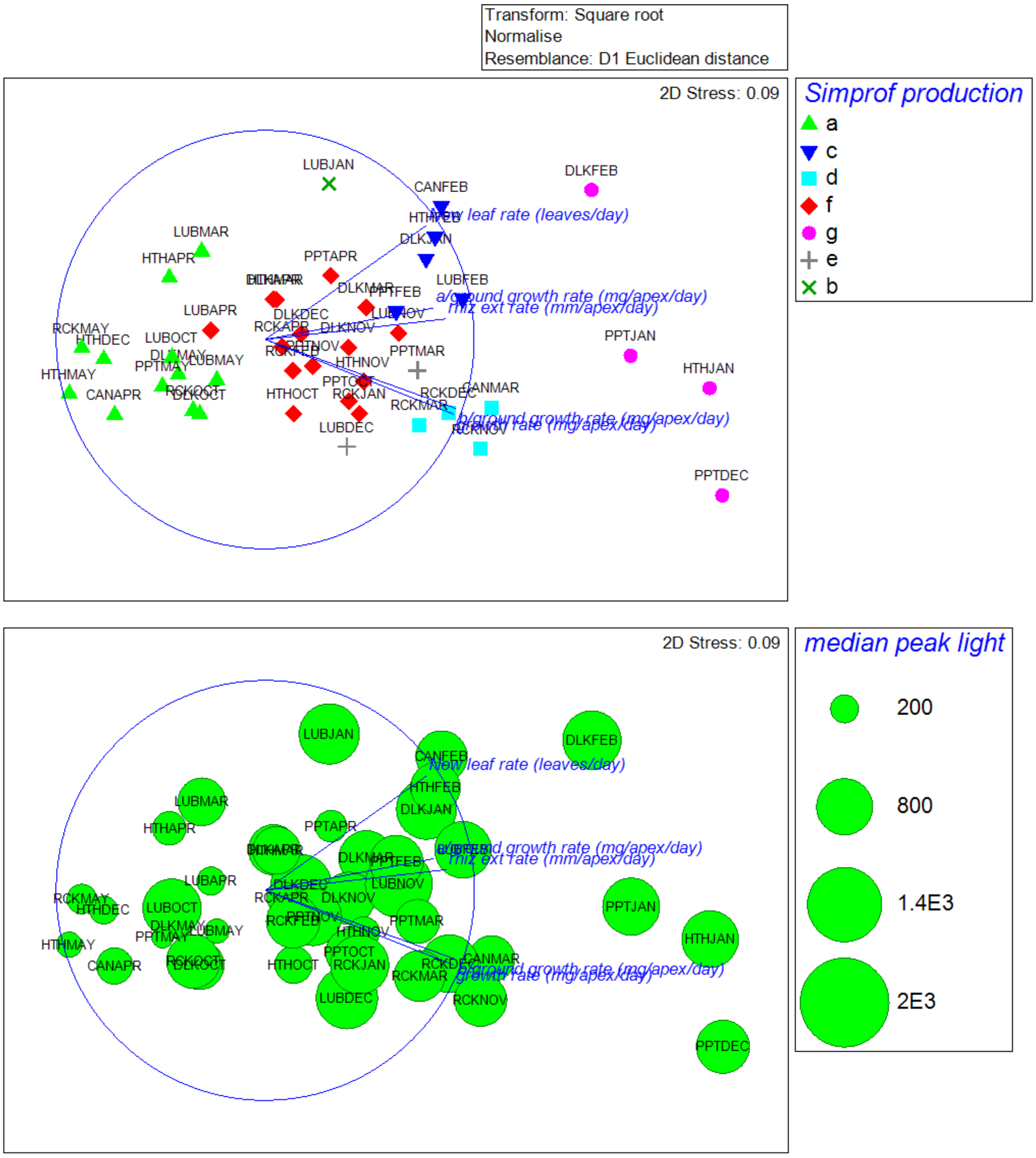


Figure 31 *Multi-dimensional scaling (MDS) plots of production data (variables of new leaf growth rate, above- and below-ground growth rate, total growth rate and rhizome extension rate) showing groupings determined as significant (p<0.05) by SIMPROF test (top) and relationship to available light in-situ measured as median peak light (as described in Section 4.2) (bottom). Note: data was pre-treated as per Appendix A-1.*

4.6 Seagrass meadow measurements (from cores)

Five replicate samples were obtained for each site at each time period. For each replicate sample, seagrass was collected by coring with two 96 mm (ID) cores placed adjacent to each other and pushed approximately 15 cm into the sediment (to capture the vast majority of root biomass). Samples were then sieved in the field (approximately 1.5 mm holes) to remove most of the unattached sediment. Snails and seagrass were then transferred into wet calico bags and kept on ice until processing at Kings Park Science Laboratories.

By sampling a known area (approximately 0.0145 m^2), measurements were then scaled to a meadow-scale (per square metre). This method, however, assumed the meadow was continuous, which was not always the case (as shown in Section 4.4) – and this needs to be kept in mind during interpretation of data. From these cores, measurements of branching density, apex density, leaf density, average leaf mass and total biomass were determined.

Branches were recorded as present in the core sample's material when the branching segment showed development of one or more leaf pairs. Branching density (Figure 32A) varied from 120 to 1200 branches per square metre, but showed no obvious temporal pattern.

Apex density (the number of growing tips) was highly variable, with averages recorded between 550 and 2100 per square metre (Figure 32B). While no overall trend in apex density among sites was observed temporally, sites HTH and PPT showed similar trends – with a reduction in apex density during November/December when *Chaetomorpha* was present at these sites.

Many of the sites showed a decrease in leaf density in November and December (relative to the October period) (Figure 32C). LUB was the only site where leaf density increased from October to November. The density of leaves ranged from 260 to 1900 per square metre and the peak leaf density occurred in January or February for most sites (although this was still very variable). Average leaf dry weight ranged from 0.18 to 1.15 mg and showed no consistent temporal pattern across sites (Figure 32D).

Above-ground biomass and total biomass are shown in Figure 32E and F. Site CAN had the least biomass present (44 – 90 g DW m^{-2}), and RCK had at times the highest biomass measured (up to 292 g DW m^{-2}). The ratio of above- to below-ground biomass (data not shown) was low during November and December sampling for sites PPT and DLK because storms in these months stripped significant quantities of leaves from the meadow (and left viable rhizome beneath the sediment which subsequently re-grew).

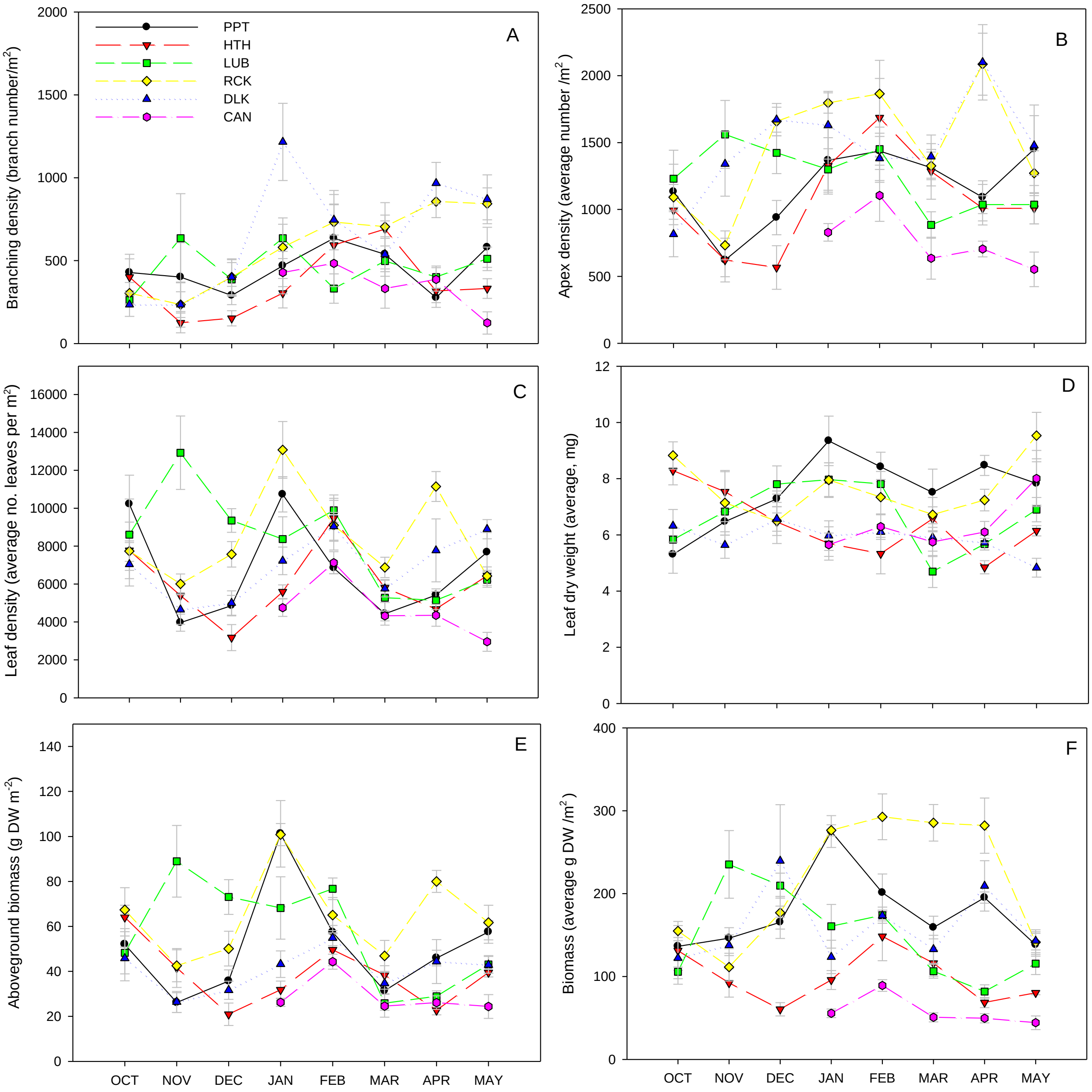


Figure 32 *Seagrass meadow measurements from cores of* H. ovalis *at six sites in the Swan-Canning including A) branching density; B) apex density; C) leaf density; D) leaf dry weight; E) above-ground biomass; and F) total biomass (mean +/- SE)*

4.7 Reproduction

Halophila ovalis is a dioecious plant (i.e. distinct male and female organisms) with sub-marine pollination. Each fruit is reported to contain seven to 60 seeds, with each seed 0.2 to 1 mm – the smallest reported for the seagrass group (Orth et al. 2006b).

Figure 33 *A) Female* Halophila ovalis *flower, B) fruit and C) fruit dissected in half to show seeds inside*

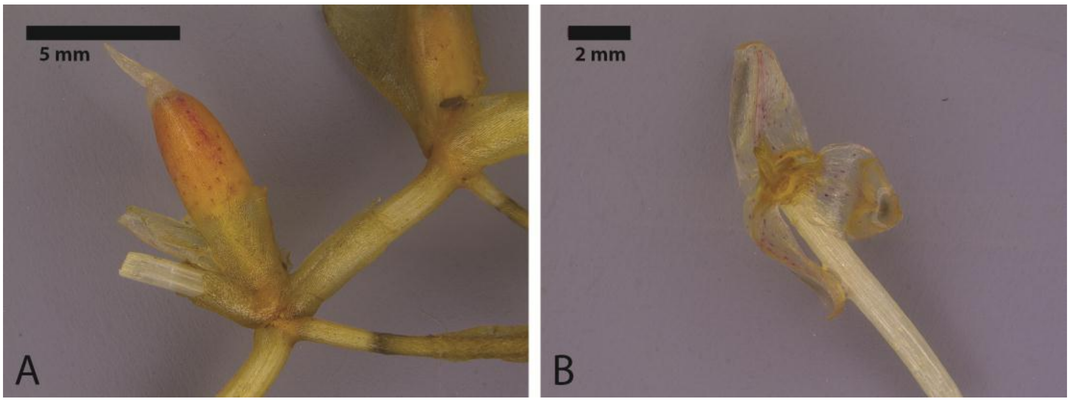


Figure 34 *Male flowers of* Halophila ovalis *A) immature, B) mature*

The flowering and fruiting frequency of *H. ovalis* was quite variable among the six sites (Figure 35). Most of the sites began flowering in November, with peak flowering intensity in December 2011. Peak fruiting typically occurred one month after peak flowering. Sites CAN and HTH were the exception to this trend. Flowering at CAN was temporally offset from the other sites, but this was due to seagrass only establishing at CAN in mid-December 2011. It is likely that low salinities (<10 psu) at this site prevented seagrass establishment before mid-December, however sexual reproduction appeared accelerated at the CAN site.

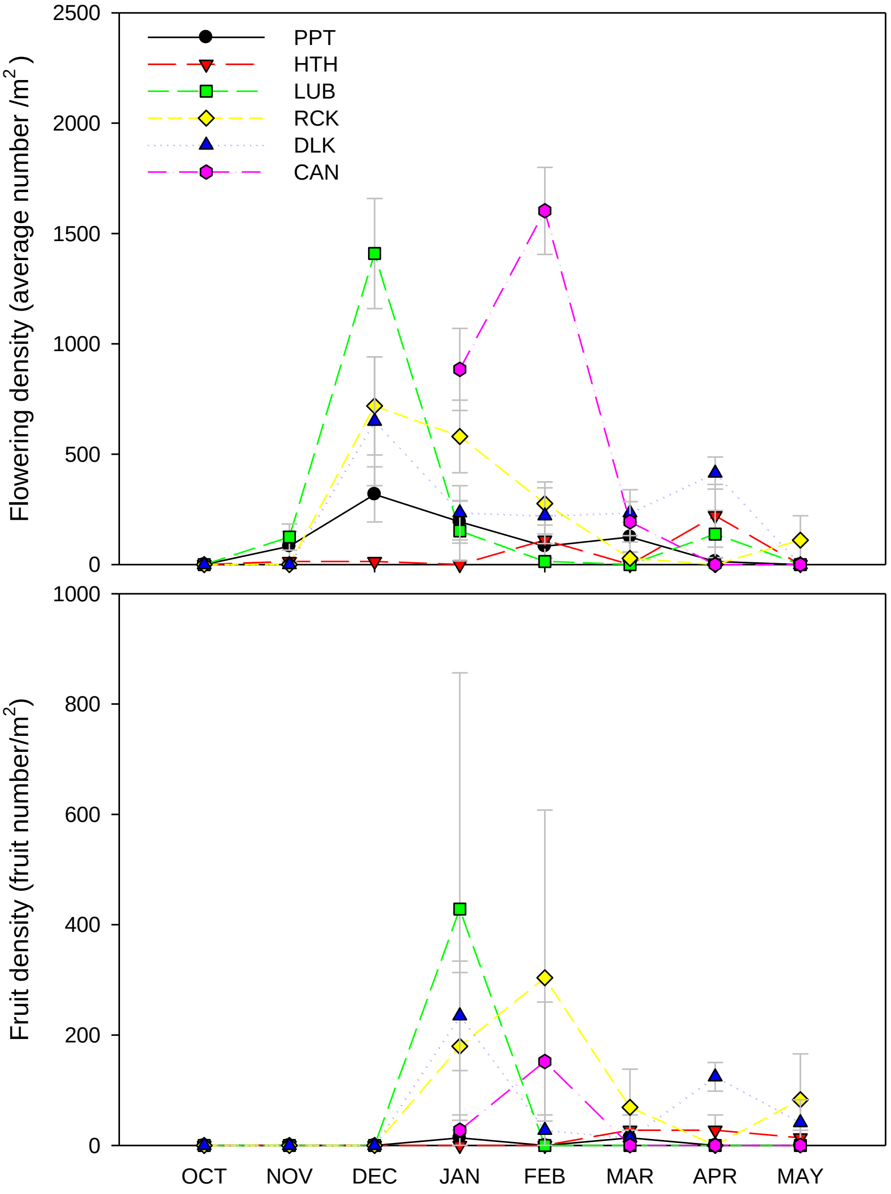


Figure 35 *Reproduction measures of* H. ovalis *at six sites in the Swan-Canning estuary over the study period October 2011 to May 2012: A) flowering density and B) fruiting density (mean +/- SE)*

At the CAN site the seagrass established and started flowering within a month of first appearing and the peak in flowering and fruiting co-occurred in the same month (February 2012). At CAN it was also reasonably common to observe a segment of *H. ovalis* with an immature female flower on the first leaf pair from the apex, a mature female flower on the next leaf pair and a fruit on the third leaf pair. The production rate measured at CAN (by rhizome tagging) indicated that a new leaf pair was produced every three days in February – suggesting that within 10 days *H. ovalis* could flower, be pollinated and develop fruit. This is incredibly fast compared with *Enhalus acoroides*, another seagrass species, which takes five months to develop fruit (Rollón et al. 2003).

Flowering at HTH appeared suppressed temporally and the density of flowering was much reduced, most likely due to the presence of *Chaetomorpha linum* substantially reducing light. The seagrass at HTH had its peak flowering event during April 2012, with only 220 flowers per square metre on average.

More than 600 flowers were recorded at the six sites within the Swan-Canning estuary between October 2011 and May 2012 (Table 8). Of these flowers, 40% were identified as male, 25% as female and for the remainder the sex was unknown (flowers were immature). Sites LUB and CAN had the highest density of flowering and PPT and HTH appeared to have the least reproductive success (producing the least number of fruits for their flowering effort). Although highly variable, the approximate success rate across all sites for converting flowers to fruits was 31% (as determined by linear regression); however, since only female flowers can become fruit (and total flower count included both sexes), the fertilisation success could realistically be as high as 46% (if we assume that half of the immature flowers recorded were female).

Table 8 *Total observations of* Halophila ovalis *reproduction across six sites in the Swan-Canning estuary*

Site	**Flowers (total)**	**Male flowers**	**Female flowers**	**Unknown immature flowers**	**Fruit**	**Ratio of fruit to flower**	**Annual* fruit production per m^2**
CAN	194	107	43	44	13	0.07	35.9
DLK	127	32	41	54	32	0.25	88.4
HTH	26	17	6	3	5	0.19	13.8
LUB	133	64	11	58	31	0.23	85.7
PPT	59	11	22	26	2	0.03	5.5
RCK	124	37	40	47	46	0.37	127
Total	*663*	*268*	*163*	*232*	*129*		

*** Annual production calculated based on area sampled between January 2012 and May 2012 (when fruits were present)**

Scaling the number of fruit observed to an annual scale (assuming that all fruit to be produced were produced by May 2012), we see that RCK was the most successful sexual reproducer – producing 127 fruit per m^2 – followed by DLK and LUB with >85 fruit per m^2 each. Sites HTH and PPT were poor, producing less than 15 fruit per m^2 (Table 8). Kuo and Kirkman (1992) report an average of 7.4 seeds per fruit of *H. ovalis* from south-west Australia. The potential seed bank generated in the 2011–12 year (assuming 7.4 seeds per fruit) was 940 seeds per m^2 at RCK and as low as 110 seeds per m^2 at HTH and PPT.

4.8 Seagrass chemical analysis

Halophila ovalis samples from cores were analysed for nutrients (C, N, P, S) and stable isotopes (C, N, S) as shown in Figure 36. Seagrass samples were sectioned then dried at 60° C for >48 hours (until constant weight) and ground before analysis. Where epiphytic fuzz was observed leaves were cleaned as best as possible by scraping with a razor (but were discarded from the chemical analysis sample if fouling was too severe). The youngest parts of the plants (first two internodes) were used for the sulfur analysis and, as significantly more biomass was required for the P analysis, the remainder of the leaves within the sample were used for the C, N and P analyses. Older roots and rhizomes (plus any discarded leaves) were dried and weighed to determine total biomass measurements – but these were not analysed.

The Marine and Freshwater Research Laboratory at Murdoch University, Western Australia, analysed the plant material for total phosphorus by the molybdenum blue method on a Lachat automated flow injection analyser. Aqueous calibration standards, blanks and an internal plant standard (*Chaetomorpha* from the Peel-Harvey estuary) were also analysed. Stable isotope analysis (and percentage C, N and S) was undertaken at the Southern Denmark University using EA-IRMS (instrument was Delta V Advantage Isotope Ratio MS with an organic elemental analyser from Thermo Scientific) (see Appendix B-4 for further detail).

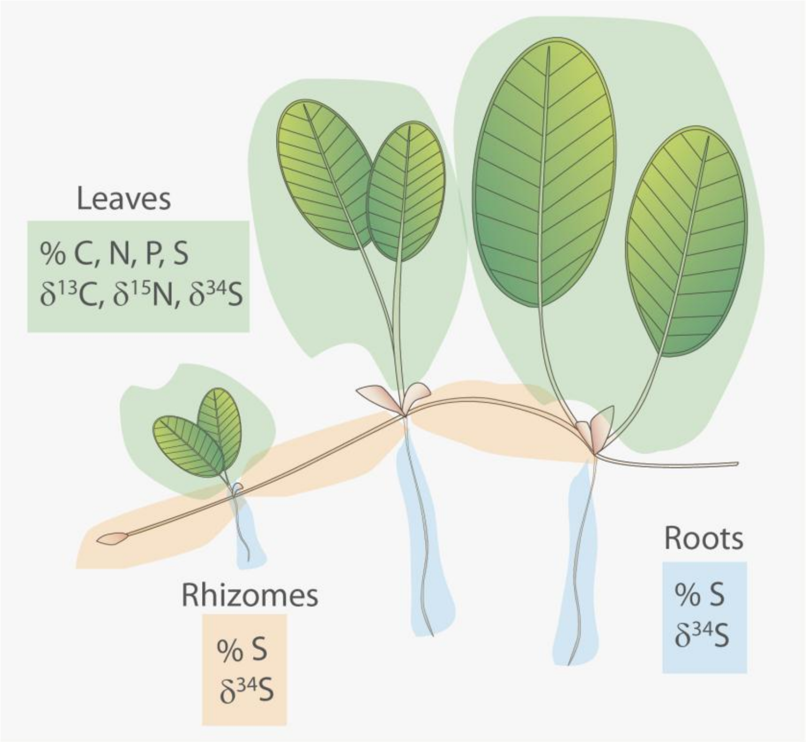


Figure 36 *Division of plant material for nutrient and stable isotope analysis*

The δ^{13}C and δ^{15}N results for *H. ovalis* leaves are shown in Figure 37. The range of δ^{13}C values in leaves (average of five replicate samples per site/time) was -15.3 ‰ to -9.3 ‰, which is within the range reported in the review of stable carbon isotopes in seagrasses by Hemminga & Mateo (1996). A general trend of increasing δ^{13}C occurred across all sites during the sampling period, with more negative δ^{13}C values in October to December than in March to May. The range of δ^{15}N in leaves was 6.3 ‰ to 10.8 ‰. There were clear site differences in stable isotope ratios, with CAN followed by HTH having the most negative leaf δ^{13}C, and site LUB having the most positive δ^{15}N. The percentage of carbon, nitrogen and phosphorus in *H. ovalis* leaves is shown in Figure 38, and ranged from 26 to 32% for carbon, 1.5 to 2.5% for nitrogen and 0.17 to 0.44% for phosphorus.

The carbon stable isotope ratios measured in leaves of *H. ovalis* showed a significant interaction between site and month when comparing data from five sites (PPT, HTH, LUB, RCK, DLK) across all sampling months. A similar result was obtained if a subset including CAN was analysed (for a five-month period). In fact overall univariate analysis of nitrogen stable isotopes, %C, %N and %P gave similar results by two-way ANOVA for site and month – see Appendix A-1–6.

The carbon isotope signal measured in seagrass leaves is likely to reflect the source of carbon available to the seagrass, or an environmental aspect related to the uptake of carbon. Seagrasses fix carbon from the aquatic environment both as CO_2 and as HCO_3^- (Beer et al. 2002). Dissolved CO_2 typically has δ^{13}C of -9 ‰, while HCO_3^- is circa 0 ‰, which means that plants using both HCO_3^- and CO_2 have less depleted carbon isotope ratios than those plants using CO_2 only (Hemminga & Mateo 1996). The equilibrium of $CO_2 \leftrightarrow HCO_3^- \leftrightarrow H_2CO_3^{2-}$ is pH dependent, and there was a trend in pH observed along the estuary (average for the sampling period July 2011 to June 2012). Slightly more neutral pH of 7.73 was measured at SAL (near CAN) than the more basic pH of 8.05 measured at BLA (nearest RCK). At pH 6.4, approximately half the dissolved inorganic carbon would be present in the water as CO_2 and the rest as HCO_3^-. As the pH increases, the relative amount of CO_2 decreases exponentially until there is no CO_2 fraction dissolved in water at pH 8.3. It seems likely the availability of CO_2 (which has a more depleted δ^{13}C signal than HCO_3^-) was greater at site CAN and then HTH relative to the other sites closer to the mouth of the Swan-Canning estuary. Other factors that could cause variability in δ^{13}C include inputs of ^{13}C-depleted carbon derived from terrestrial organic matter and light availability affecting the δ^{13}C of aquatic plants (Hemminga & Mateo 1996). Decreased isotopic discrimination occurs with increasing irradiance (i.e. δ^{13}C values would become more positive with greater light). It seems likely the carbon stable isotope ratio of the seagrass is a combination of the physical location within the estuary (and pH of the water accordingly) and the light that the site receives.

The nitrogen isotope signal measured in the seagrass leaves is also likely to reflect the source, although for nitrogen, the influence of fertilisers or wastewater/sewage are the dominant sources to consider and usually result in an enrichment in δ^{15}N values. Most sites are located near recreational parks and foreshore grassed areas. The two sites that may have less influence are LUB, located 150 m from the foreshore, and PPT, located 75 m from the foreshore and adjacent to a nature reserve. These sites are compared with DLK and HTH, located just 25 m and 42 m from the foreshore. Seagrass from LUB had the most isotopically enriched ^{15}N signal, with PPT slightly more enriched than the rest of the sites. The possible reasons for these variations are discussed further in Section 5.2.

The sulfur isotope ratio can be used to trace the influence of sediment sulfides because sulfur derived from sediment sources has a much more negative δ^{34}S (typically -20 to -30 ‰) compared with seawater (~20 ‰). δ^{34}S and %S results for leaves, rhizomes and roots are shown in figures 39 and 40. The range of δ^{34}S values (average of five replicate samples per site/time) obtained for leaves was between 10.6 ‰ and 16.8 ‰; for rhizomes between 2.1 ‰ and 16.1 ‰; and for roots, which were the most negative, between -18.5 ‰ and -0.9 ‰. Average sulfur concentration was greatest in leaves (0.67%) and similar between rhizomes and roots (0.49% and 0.46% respectively).

Two-way ANOVA of the sulfur stable isotope ratios and %S measured in leaves, roots and rhizomes showed that site, month and site x month interaction were typically significant factors. This analysis was carried out for data from five sites (PPT, HTH, LUB, RCK, DLK) across all sampling months, and for the subset of data from the six sites for January to May (as no seagrass was present at CAN between October and December). Full results of these analyses can be found in Appendix A-1–6.

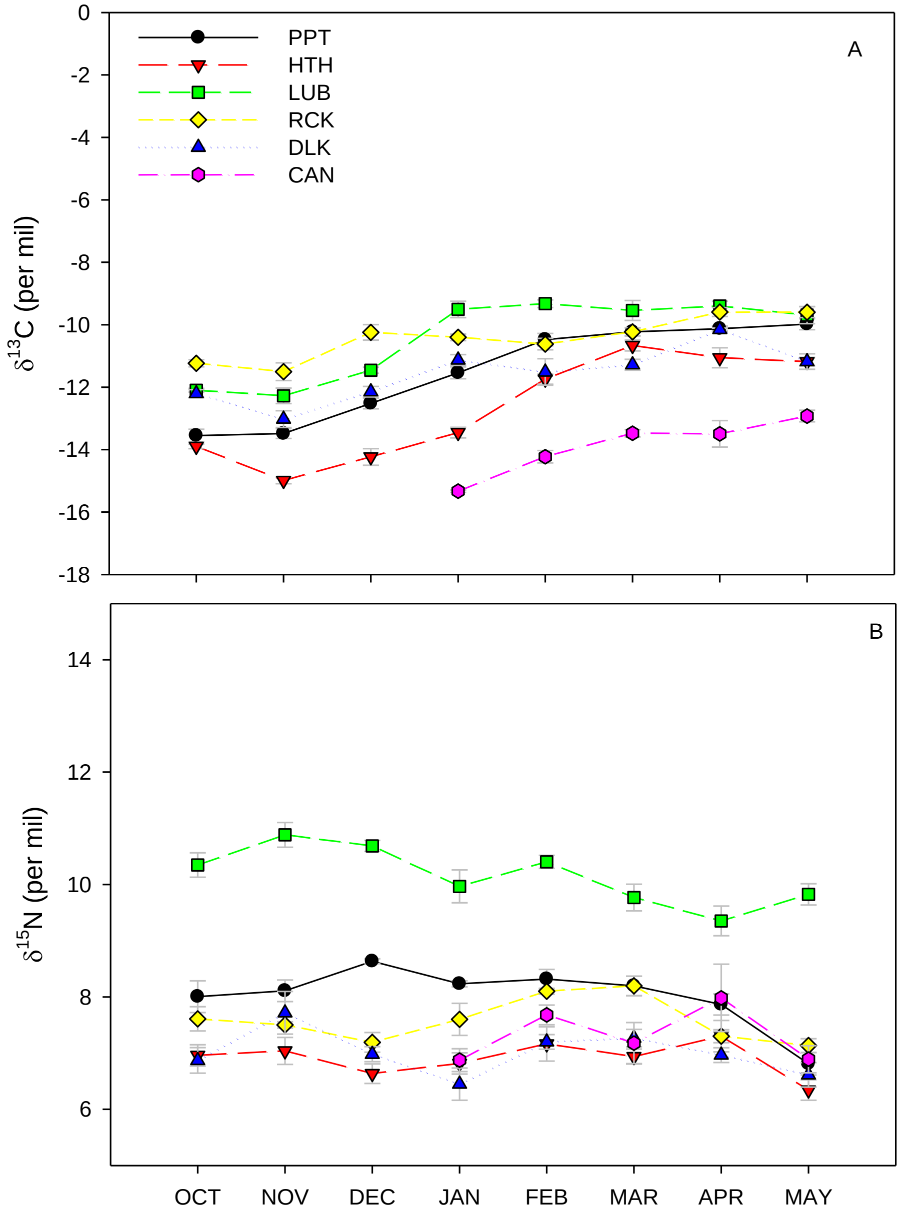


Figure 37 *Stable isotope ratios in* H. ovalis *leaves for A) carbon and B) nitrogen for six sites in the Swan-Canning estuary from October 2011 to May 2012 (mean +/- SE)*

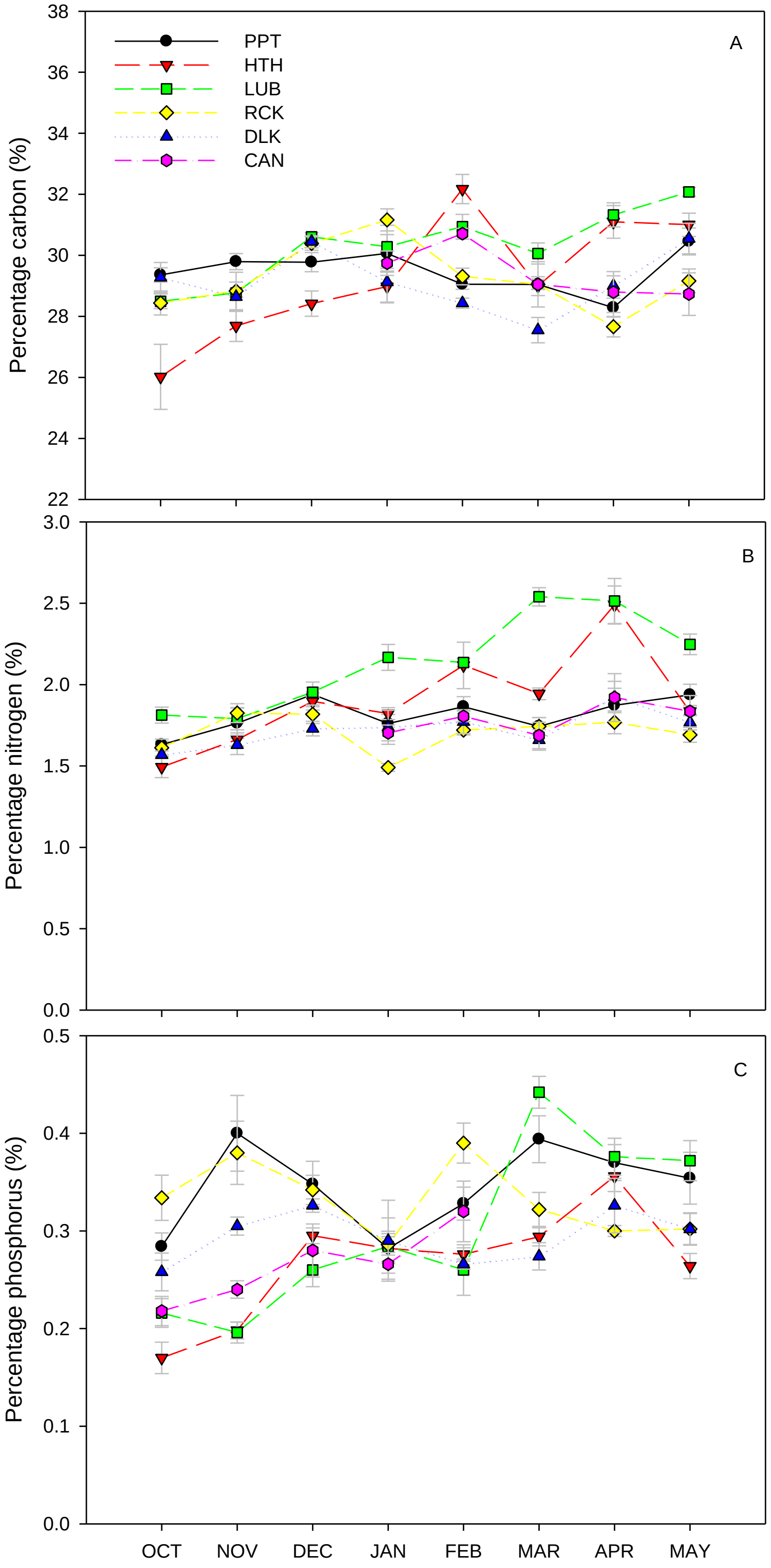


Figure 38 *Percentage of A) carbon, B) nitrogen and C) phosphorus in* H. ovalis *leaves at six sites from October 2011 to May 2012 (mean +/- SE)*

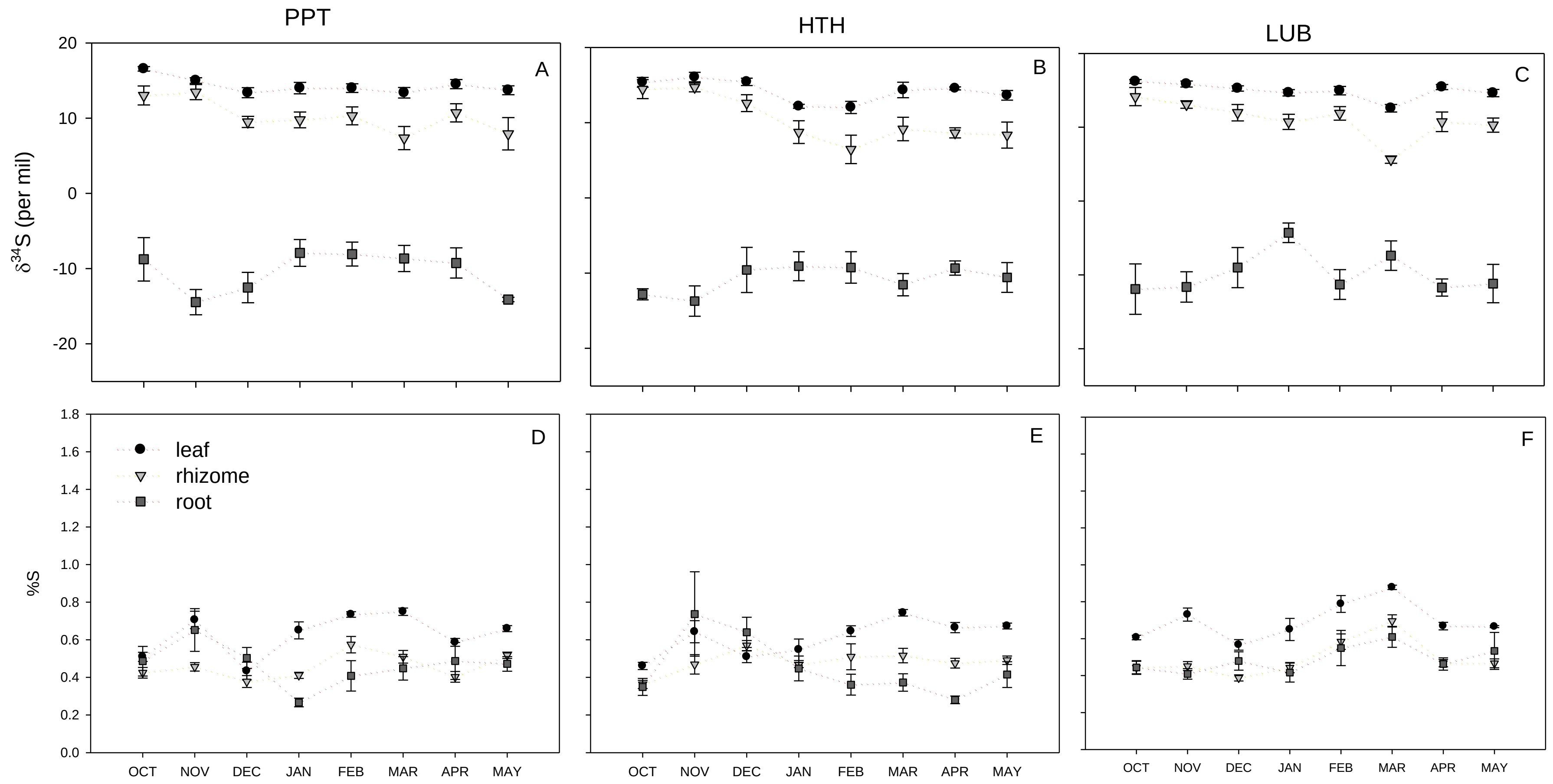


Figure 39 *Sulfur stable isotopes in leaf, rhizome and root for A) PPT, B) HTH and C) LUB; and percentage sulfur in leaf, rhizome and root for D) PPT, E) HTH and F) LUB (mean +/- SE)*

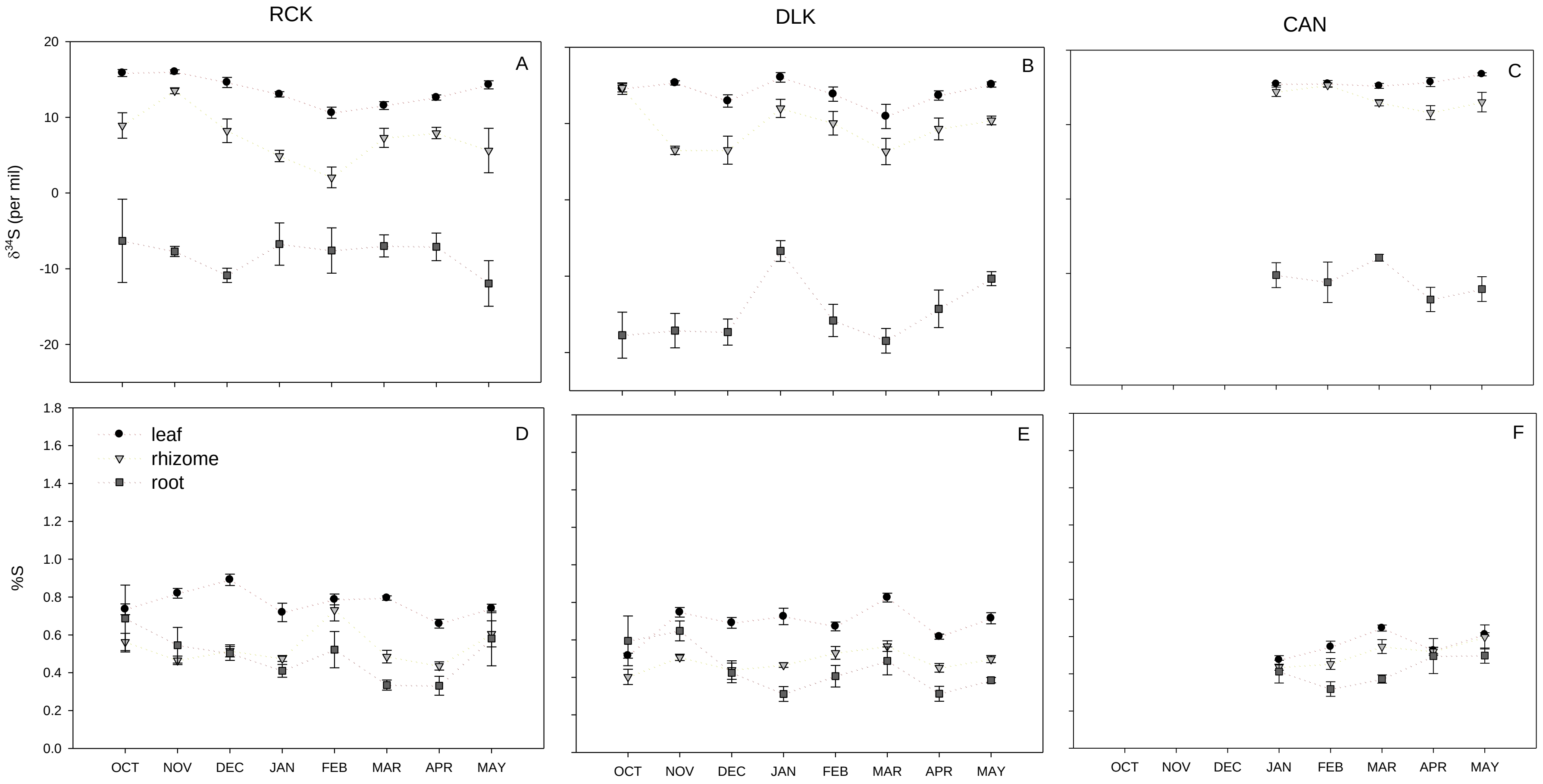


Figure 40 *Sulfur stable isotopes in leaves, rhizomes and roots for A) RCK, B) DLK and C) CAN; and percentage sulfur in leaves, rhizomes and roots for D) RCK, E) DLK and F) CAN (mean +/- SE)*

4.9 Seagrass biometrics (WinRHIZO)

Biometric measurements of *Halophila ovalis* were made with the image analysis tool WinRHIZO Pro <http://www.regent.qc.ca/products/rhizo/WinRHIZO.html> at the Kings Park Science Laboratories (Botanic Garden Park Authority). From each core replicate sample approximately two to four linear segments of *H. ovalis* were scanned and analysed with WinRHIZO as per Figure 41. Where possible, leaf pairs were scanned and the data obtained before they were reduced to the individual leaf scale. Root segments were scanned by placing them in a small amount of water. Roots were not analysed as frequently as the leaves and rhizome sections because the fine roots were often broken during laboratory sorting. An example of a leaf and rhizome scan is shown in Figure 42. By selecting each leaf pair, or rhizome section individually (and giving it an informative name), the program then calculated parameters such as leaf length, leaf area, rhizome diameter and internode distance (length of the rhizome sections between leaves). Some post-processing of data was necessary, especially of leaf segments. Global leaf lengths were over-estimated, and consequently the total leaf length was taken as the sum of lengths measuring over 0.8 mm. This adjustment corresponded well with the manual measurement checks for leaves. No such problems were observed with the measurement of the linear-shaped rhizome and root segments. We measured more than 2500 leaves, rhizomes and roots using WinRHIZO during the study.

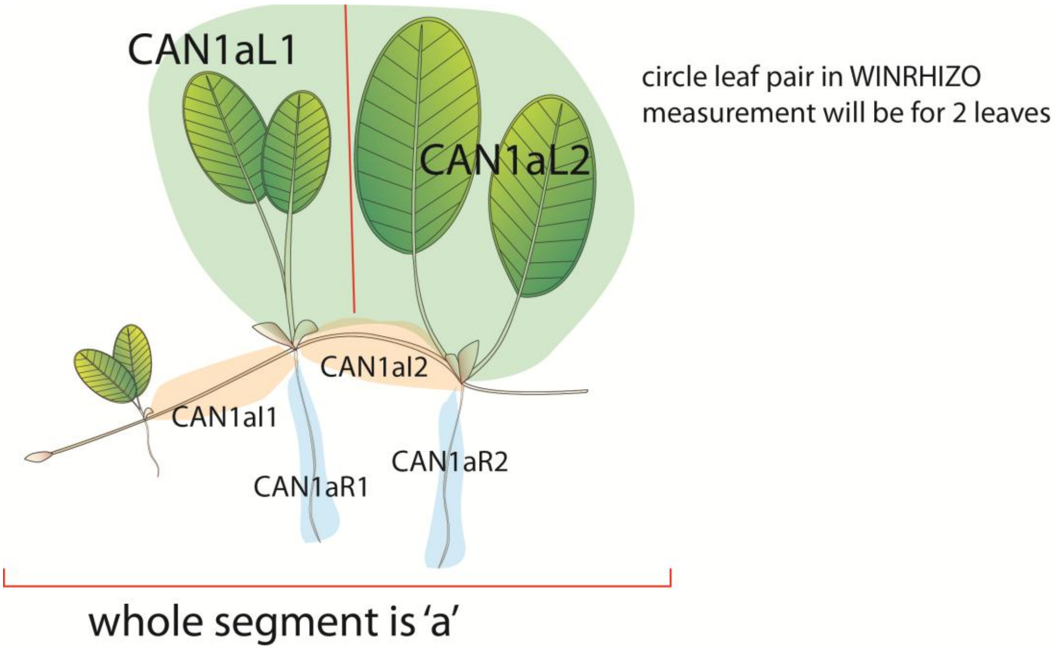


Figure 41 *Segment of* H. ovalis *scanned and analysed with WinRHIZO to obtain measurements of leaves, rhizome diameters, internode distances and root lengths*

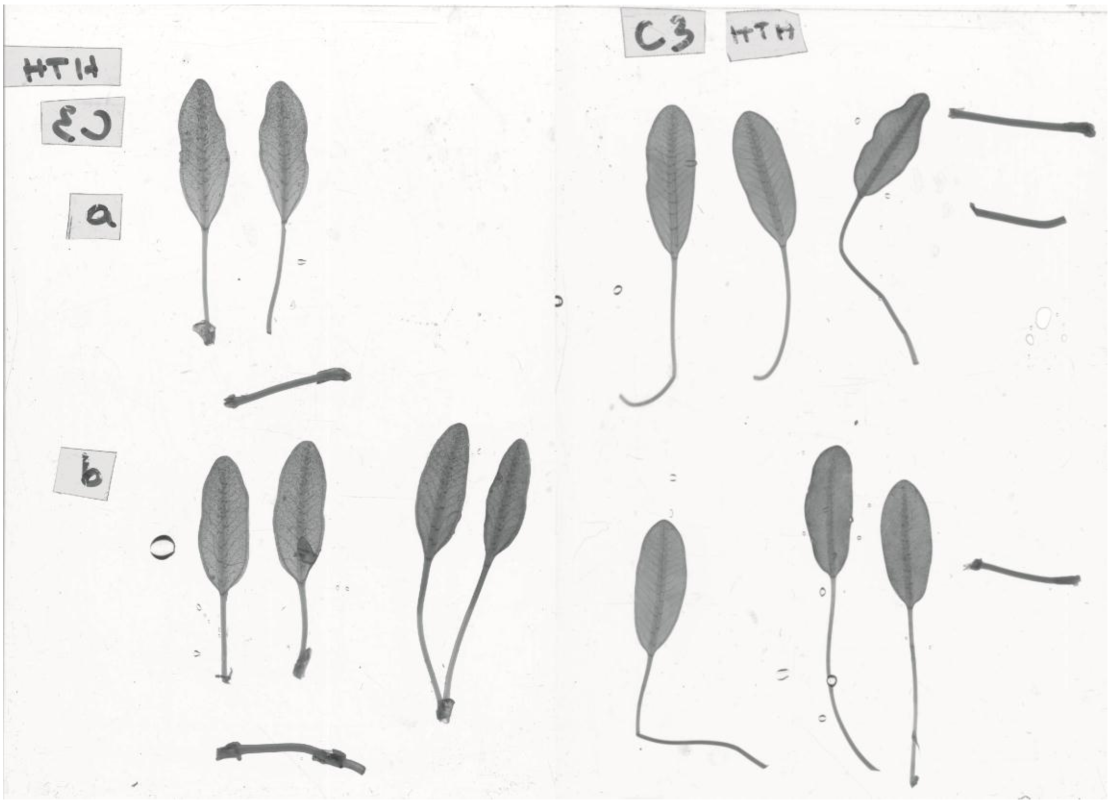


Figure 42 *Leaf and rhizome sections scanned for WinRHIZO analysis from site HTH in October (left) and December (right)*

The summary data obtained by site for each month are shown in Figure 43. Generalised trends were evident, including leaf length and leaf area peaking in the summer period for most sites, and internode distances and internode diameters showing a decreasing trend over the study period. Sites HTH and LUB, however, increased their internode distances between October and November.

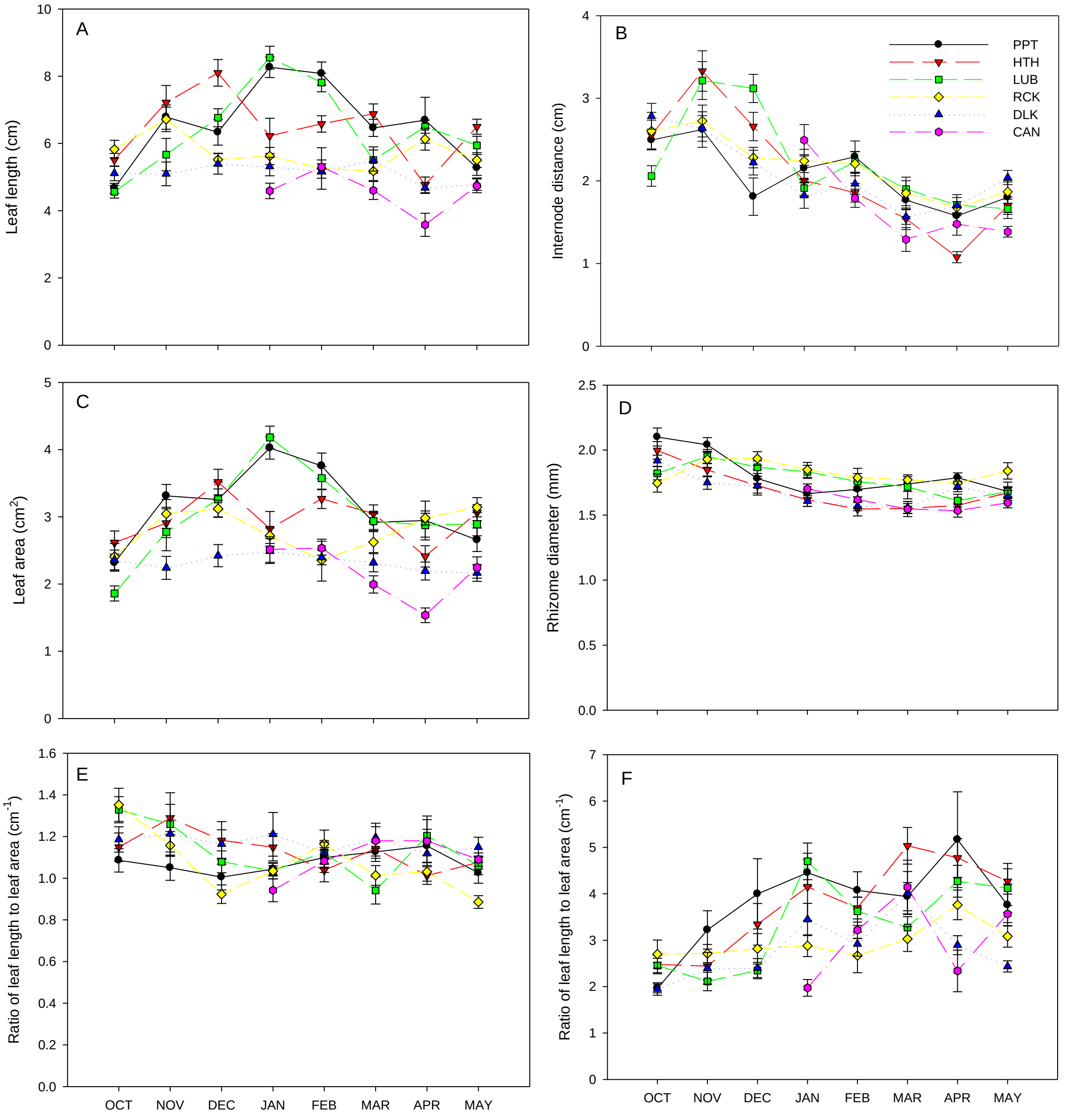


Figure 43 *Summary measurements by WinRHIZO of A) leaf length (L1 and L2); B) internode distance (between 1st – 2nd and 2nd – 3rd leaf pairs); C) leaf area (L1 and L2); D) rhizome diameter (I1 and I2); E) ratio of leaf length to leaf area for L1 and L2; F) ratio of leaf length to internode distance for L1/I1 and L2/I2 (mean +/- SE)*

4.10 *Batillaria australis* density

Gastropods and molluscs were separated from the seagrass core samples, and empty shells discarded. The mud snail *Batillaria australis* was found in extremely large numbers within the seagrass meadows sampled – a maximum of 6900 individuals per square metre, and a median of 1300 individuals per square metre. The abundance this non-native snail (*B. australis*) was significantly greater than the native snails and gastropods, or the occasional hermit crab (often in *B. australis* shells).

A strong seasonal trend did not seem to be associated with the abundance of snails found, but rather the highest number of snails was observed at the sites closest to the marine end of the estuary (e.g. RCK and DLK). The sites with the lowest density of snails were CAN and LUB (Figure 44).

Changes in the size distribution of *B. australis* did show a seasonal effect, with the greatest numbers of juveniles present in May 2012. Figure 45 shows the numbers of *B. australis* smaller than 1.5 cm increased during the summer to autumn months (January to May), suggesting that recruitment took place during summer. Figures 46 and 47 show a replicate core collected in November 2011 and May 2012 from site RCK – demonstrating few (if any) juveniles in November, and an abundance of juveniles in May.

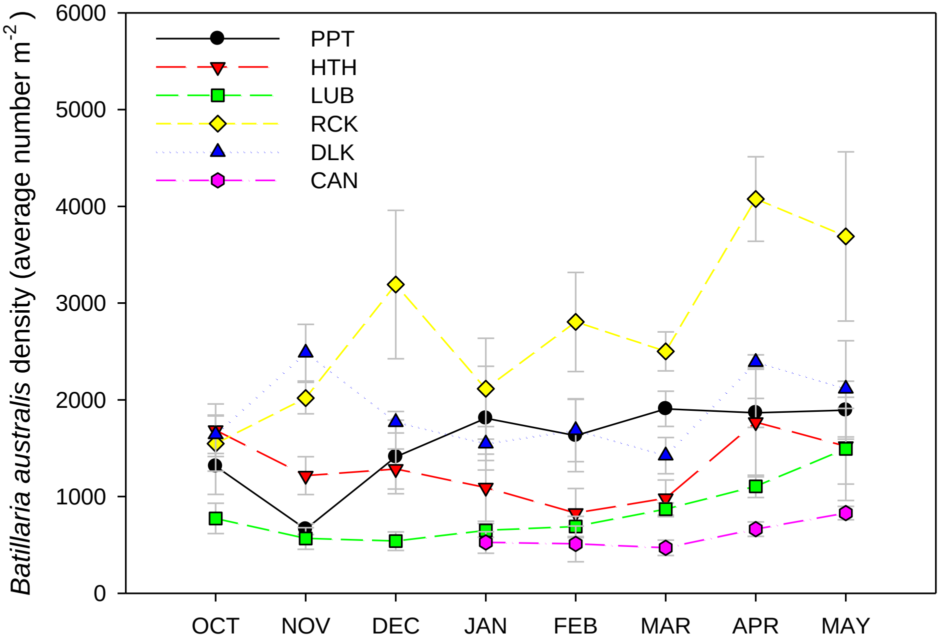


Figure 44 *Average density of* Batillaria australis *within seagrass meadows (mean +/- SE)*

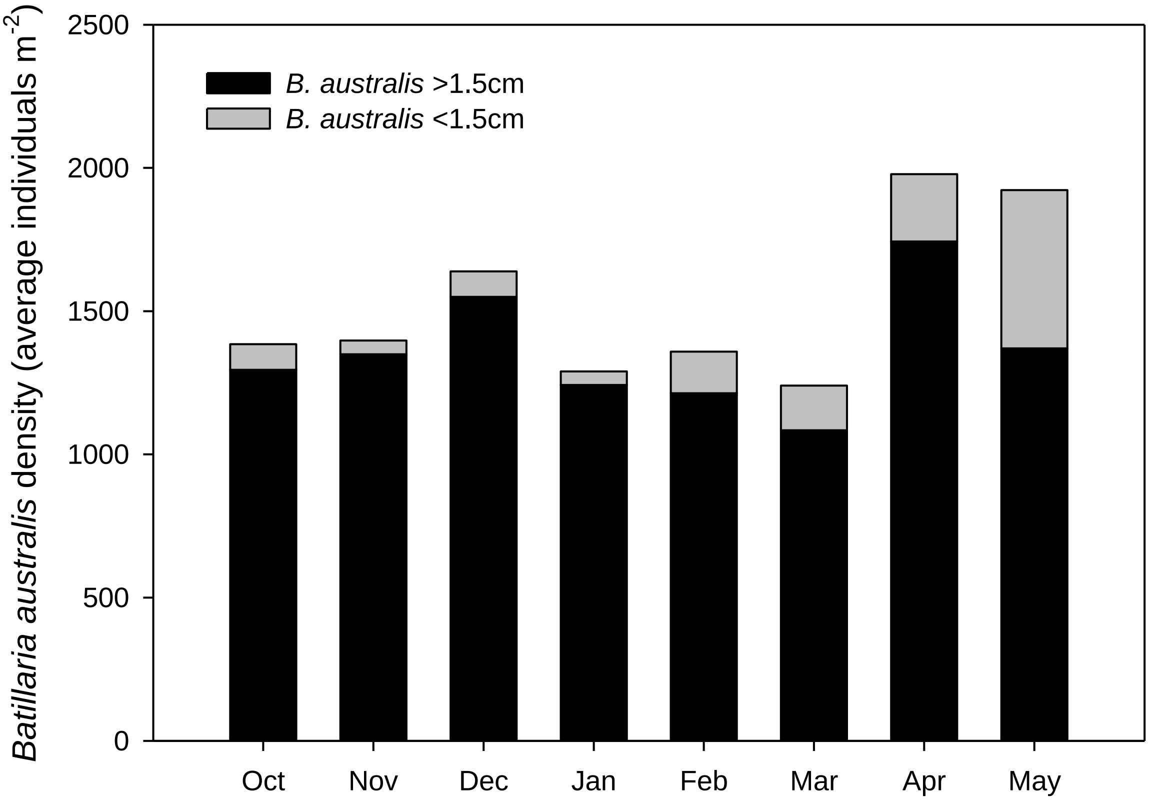


Figure 45 *Monthly density of* B. australis *for all sites pooled and separated by size class*

Figure 46 *Photograph of* B. australis *collected in one replicate sample in November 2011 from site RCK*

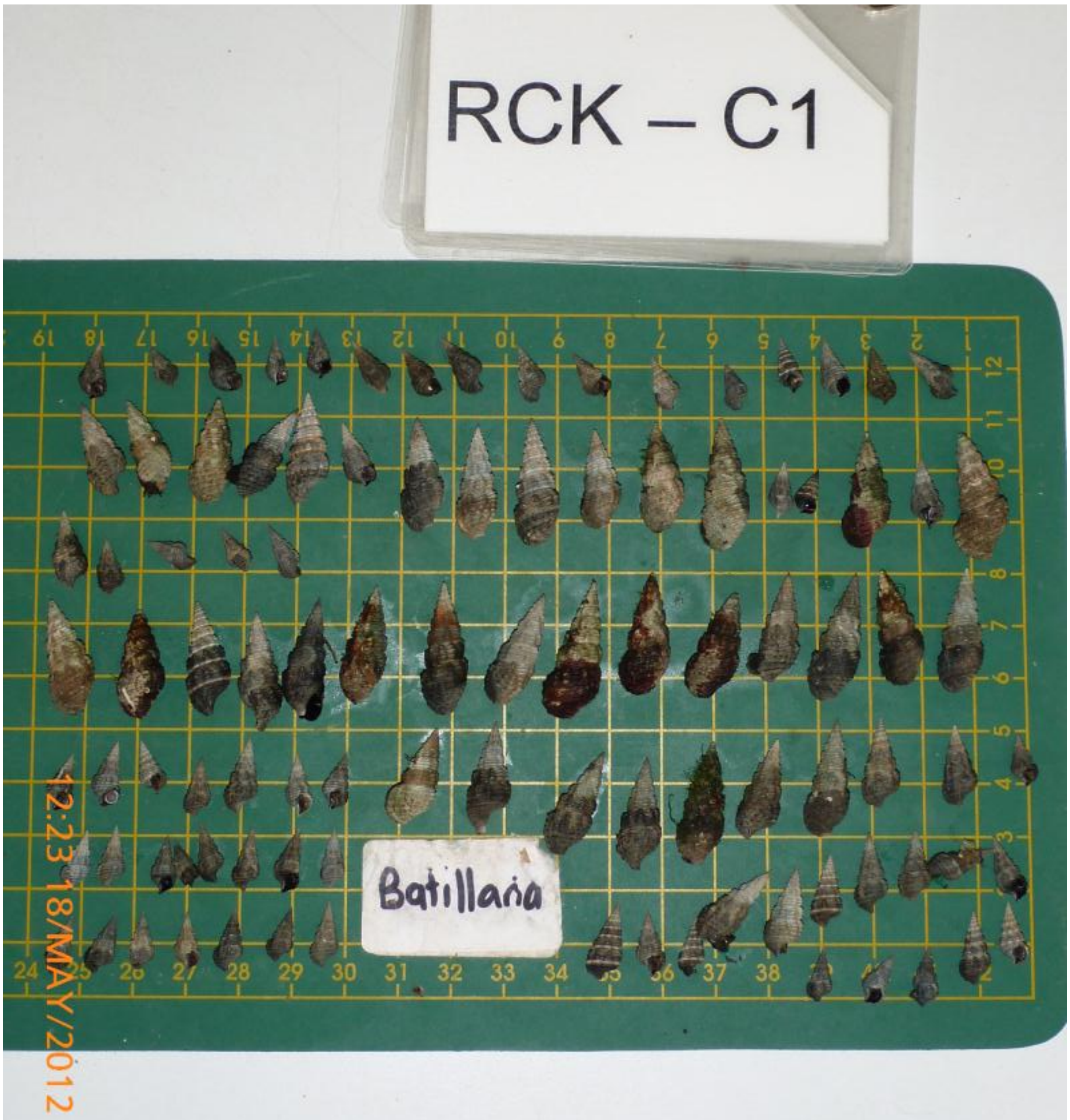
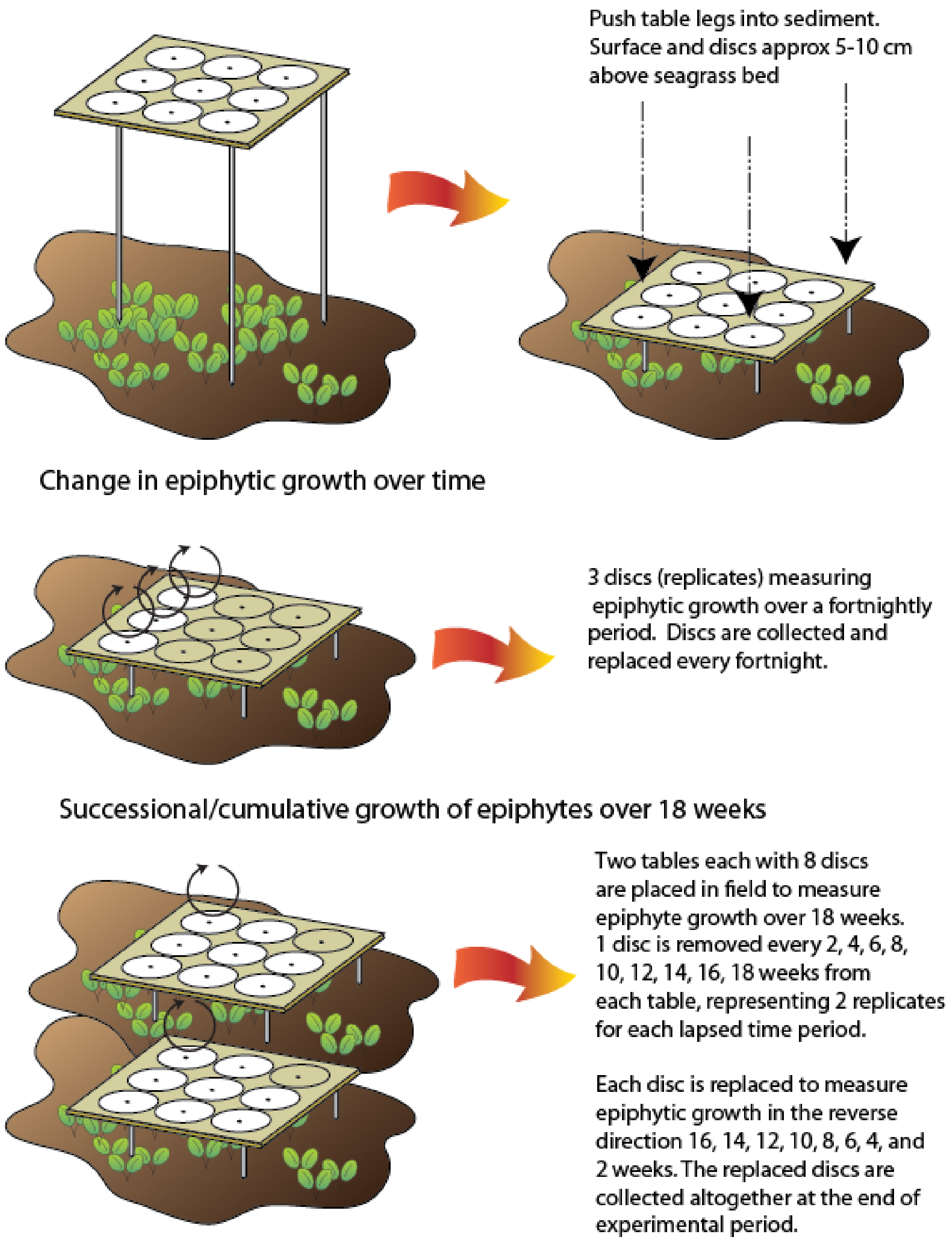


Figure 47 *Photograph of* B. australis *collected in one replicate sample in May 2012 from site RCK*

4.11 Periphyton growth

Periphyton growth was monitored at each site as a surrogate for epiphytic algal growth in the seagrass meadow. Epiphytes are known to affect the health of seagrasses by limiting the nutrients and light they require to grow.

Collection plates (Perspex roughened with sandpaper) were secured to tables and the tables positioned 5 to 10 cm above the seagrass meadow. The experimental design allowed for temporal (fortnightly) and cumulative periphyton growth to be assessed (figures 48 and 49). Dry weight biomass (g DW) was used as the measure of change in periphyton growth. Compositional changes (type and growth form) were also noted.



Push table legs into sediment. Surface and discs approx 5-10 cm above seagrass bed

Change in epiphytic growth over time

3 discs (replicates) measuring epiphytic growth over a fortnightly period. Discs are collected and replaced every fortnight.

Successional/cumulative growth of epiphytes over 18 weeks

Two tables each with 8 discs are placed in field to measure epiphyte growth over 18 weeks. 1 disc is removed every 2, 4, 6, 8, 10, 12, 14, 16, 18 weeks from each table, representing 2 replicates for each lapsed time period.

Each disc is replaced to measure epiphytic growth in the reverse direction 16, 14, 12, 10, 8, 6, 4, and 2 weeks. The replaced discs are collected altogether at the end of experimental period.

Figure 48 *Overview of experimental design to assess changes in periphyton growth*

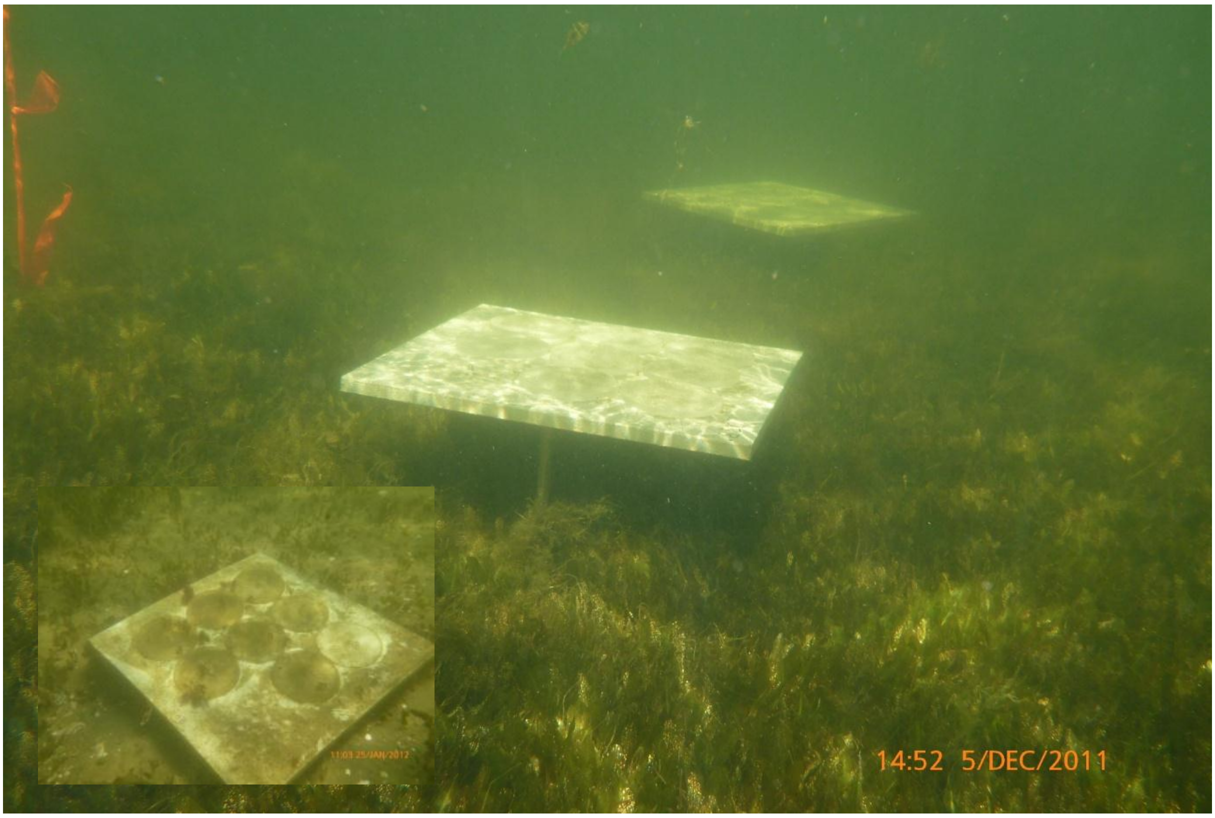


Figure 49 *Placement of tables and periphyton collection plates (see insert) in the seagrass meadow*

Fortnightly changes in periphyton growth

Three replicate samples were collected fortnightly from each site for 16 weeks between December 2011 and April 2012. Results from the dry weight of periphyton (mean and standard error of the mean) collected from each site are shown in Figure 50.

Mean dry weight of periphyton collected over the sampling period ranged between 0.02 g and 5.2 g. Periphyton growth at PPT, DLK and HTH was fairly constant and unaffected by the time of year that samples were collected. In contrast, sites CAN, RCK and LUB were slightly variable with a rise in growth in early summer (December/January) at CAN, RCK and LUB and again in early autumn at LUB and RCK. The highest mean dry weight (5.2 g) was recorded at CAN in December 2012 and may be related to high nutrients in the Canning River in spring and summer, specifically nitrate concentration (see figures 27 and 28) that was otherwise low over the rest of the period. Periphyton growth was generally highest by dry weight at the RCK and LUB sites. Periphyton growth at RCK included encrusting algal types that may have contributed more to the dry weight at this site. Filamentous algal growth, often associated with nutrient enrichment, was more typical at LUB, HTH and PPT. Periphyton growth at CAN was often fouled with flocculent (possibly diatomaceous) material (figures 51 and 52).

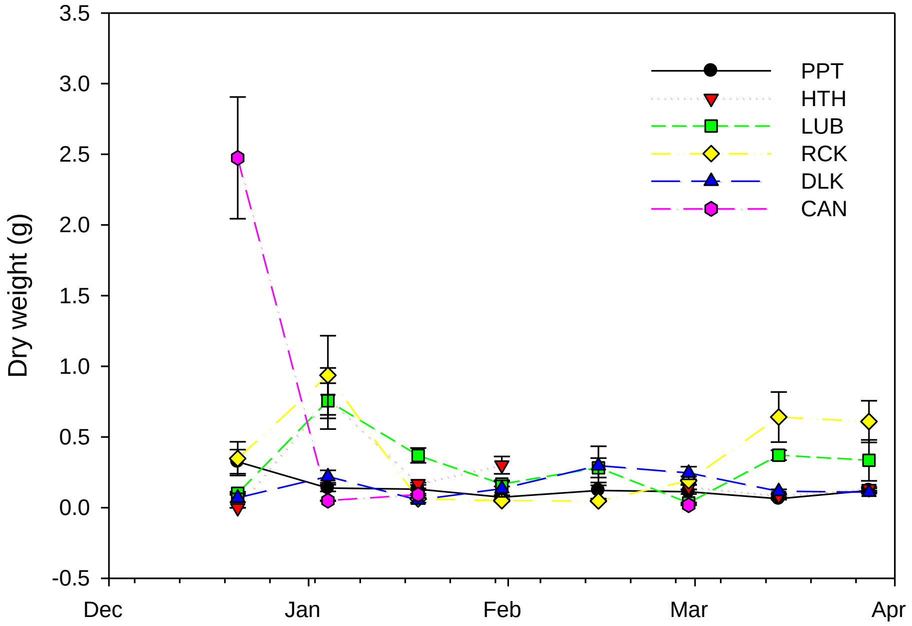


Figure 50 *Mean dry weight (± standard error) of periphyton collected fortnightly at each site (n=3) between December 2011 and April 2012*

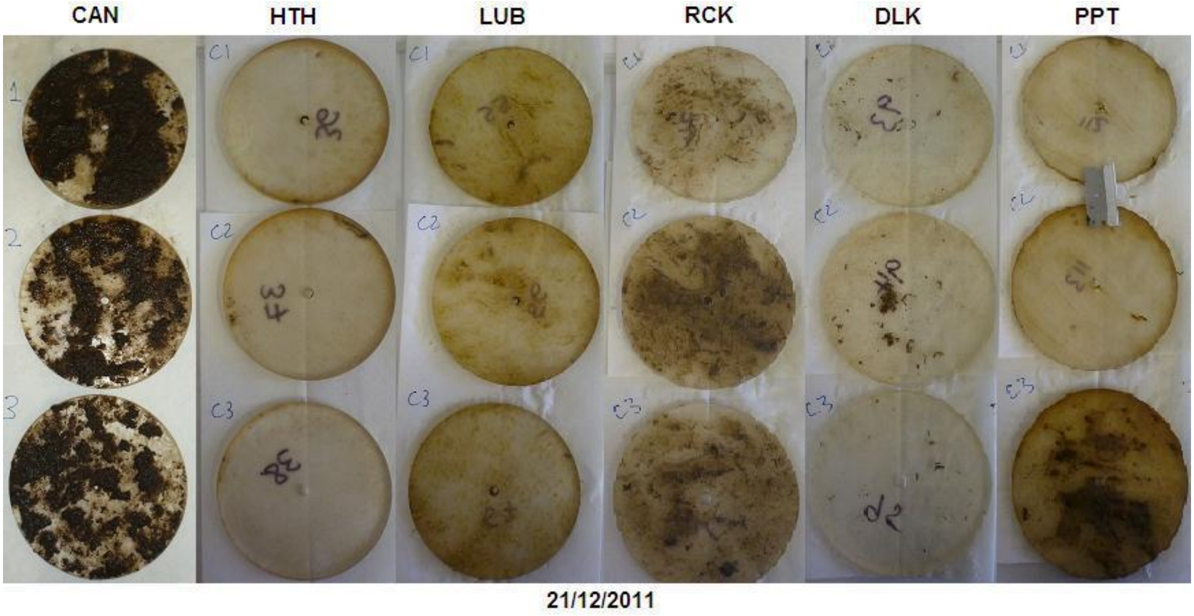


Figure 51 *Replicate periphyton plates collected after one fortnight at each site in December 2011*

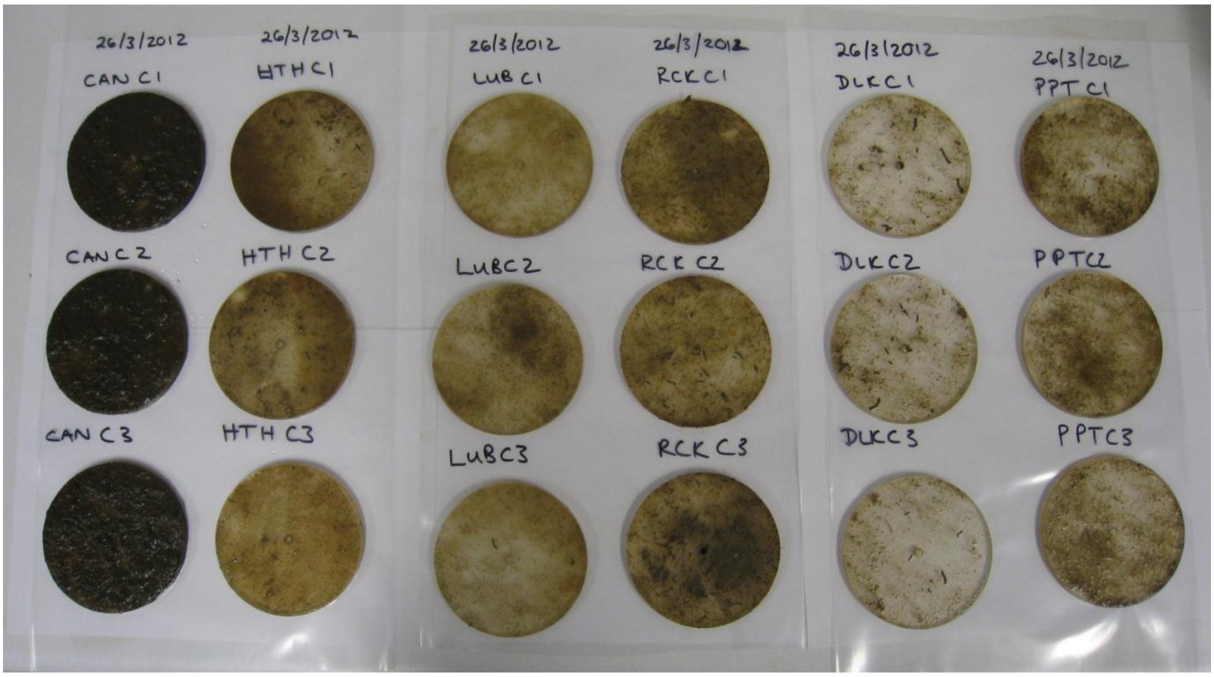


Figure 52 *Replicate periphyton plates collected after one fortnight* at each site in March 2012 (*CAN replicates represent one month's growth in this instance)*

Accumulative growth of periphyton

Two tables, each with eight plates, were placed at each site at the start of the sampling period. Every fortnight at each site, two replicate samples (one from each table) were collected and replaced with clean plates. The samples collected every fortnight represented the forward time-series and the replacement plates (all collected at the sampling period's conclusion – eight plates for each table) the reverse time-series of periphyton growth. Samples were collected between December 2011 and April 2012. Results from the dry weight of periphyton (mean only) collected from each site are shown in Figure 53 for the forward time-series and Figure 55 for the reverse time-series. Collection plates at HTH and CAN were excluded from the dataset as plates were either lost, disturbed or removed from these sites at various times over the sampling period.

The results from these collections show mean dry weight of periphyton growth at RCK and DLK increased over time for both the forward and reverse time-series. An early increase in growth was recorded at RCK and PPT in January (see plates in Figure 54). This was also reflected in the plates collected at RCK and LUB in the fortnightly collections for the same date (Figure 50), and considered to be a response to nutrients in the estuary. For the remaining period periphyton growth remained constant at PPT and LUB.

Observations suggest successional growth of thallus and encrusted algal forms at RCK and DLK (located nearer the mouth or marine extent of the estuary) – the gradual increase in the dry weight resulting from the establishment and growth of more complex algal communities on the plates. Algal forms at PPT and LUB were typically filamentous species that have a high turnover rate because of their simple structure – the constant dry weight at these sites a consequence of the continual turnover of these algal growth forms.

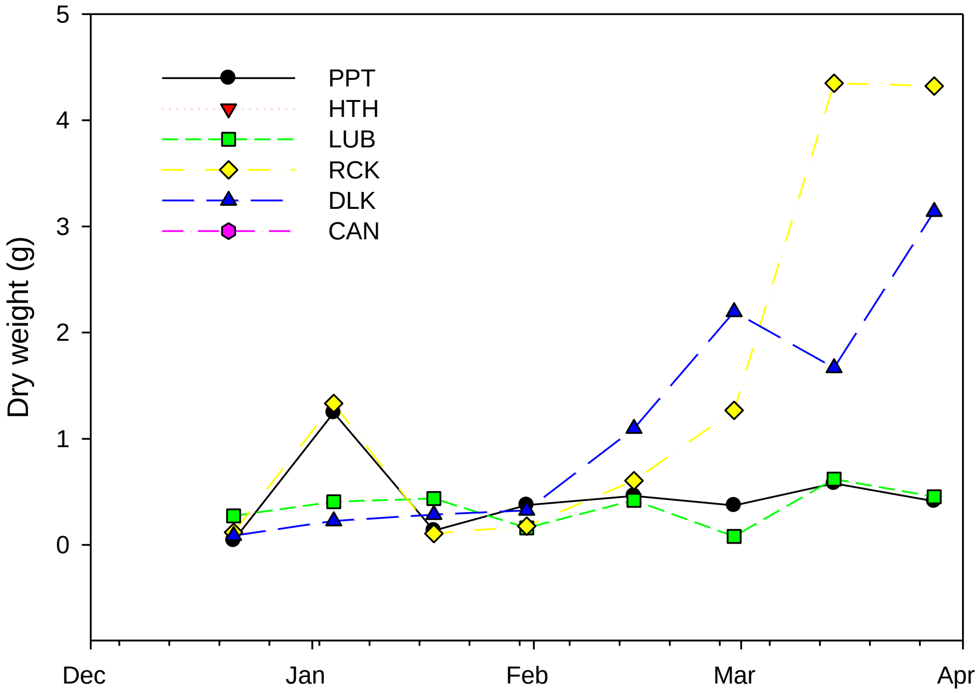


Figure 53 *Mean dry weight of accumulative periphyton growth (forward series) collected in fortnightly intervals from each site (n=2) between December 2011 and April 2012*

Figure 54 *Periphyton plates collected in January 2012 which correspond with the peak in periphyton growth at PPT and RCK*

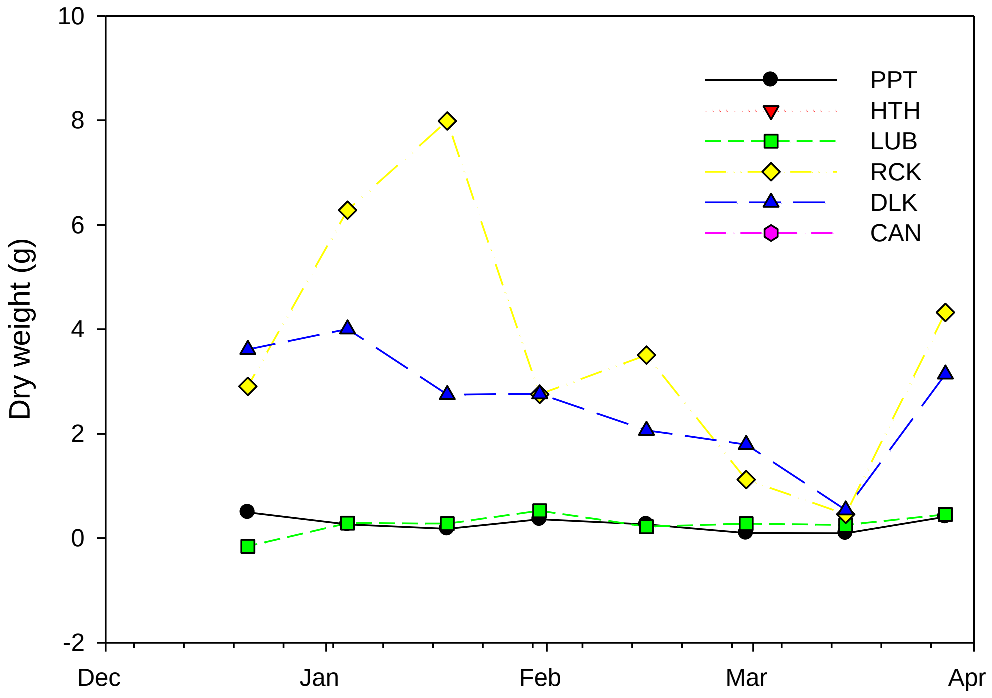


Figure 55 *Mean dry weight of accumulative periphyton growth (reverse series) of plates placed in the field in fortnightly intervals at each site (n=2) between December 2011 and April 2012*

Observations of other colonising communities

Apart from a few rocky outcrops and reefs, the riverbed of the Swan-Canning estuary is predominantly a soft-bottomed sediment environment. The benthic flora and fauna reflect this, with seagrasses and associated fauna dominating the shallow sandy substratum while macroalgal communities are restricted to the limited available rocky substratum.

The introduction of hard structures to soft-bottomed environments provides an artificial base and new habitat for colonisation by epifaunal communities. The lack of hard surface for algal attachment is highlighted on a micro-scale by the attachment of *Gracilaria comosa* thalli to the shell of the gastropod *Batillaria australis*. The tables securing the periphyton plates provided such a base, and over the study's duration the underside of the tables became almost completely overgrown by ascidian communities.

Figure 56 *The underside of a collection table located at DLK*

Figure 57 *The underside of a collection table located at PPT*

5 Interactions between variables

5.1 Sulfide intrusion into plant tissues

The exposure of plant tissues to sediment sulfides can be inferred from sulfur isotope data measured in each plant part as per Frederiksen et al. (2006) by calculating $F_{sulfide}$ values.

$$F_{sulfide} = \frac{\delta^{34}S_{tissue} - \delta^{34}S_{sulfate}}{\delta^{34}S_{sulfide} - \delta^{34}S_{sulfate}} \cdot 100$$

where $\delta^{34}S_{tissue}$ is the value measured in the plant part, $\delta^{34}S_{sulfate}$ = 21.4‰ (relating to the average value obtained in sulfate from the water column – see Section 4.3) and $\delta^{34}S_{sulfide}$ = -24.03‰ (relating to the average values measured in the sediment reduced sulfide pool – see Section 3.3).

It is hypothesised that plant tissue under greater physiological stress (i.e. increased organic loading, decreased light – which consequently reduces photosynthetically produced oxygen) would have a greater proportion of sulfide intrusion. Porewater sulfide concentrations are likely to be highest in summer (Frederiksen 2006), perhaps resulting in greater sulfide intrusion during this time. Nevertheless this is also the time of greatest growth and therefore possibly the greatest 'protection'.

The fraction of sulfides entering plant tissue from sediment sources is shown in Figure 58. Across all months and sites, the average sediment-derived sulfur for roots was 70%, for rhizomes 24% and for leaves 15%. There was a significant effect for site, month of sampling and the interaction site x month (Appendix A-7).

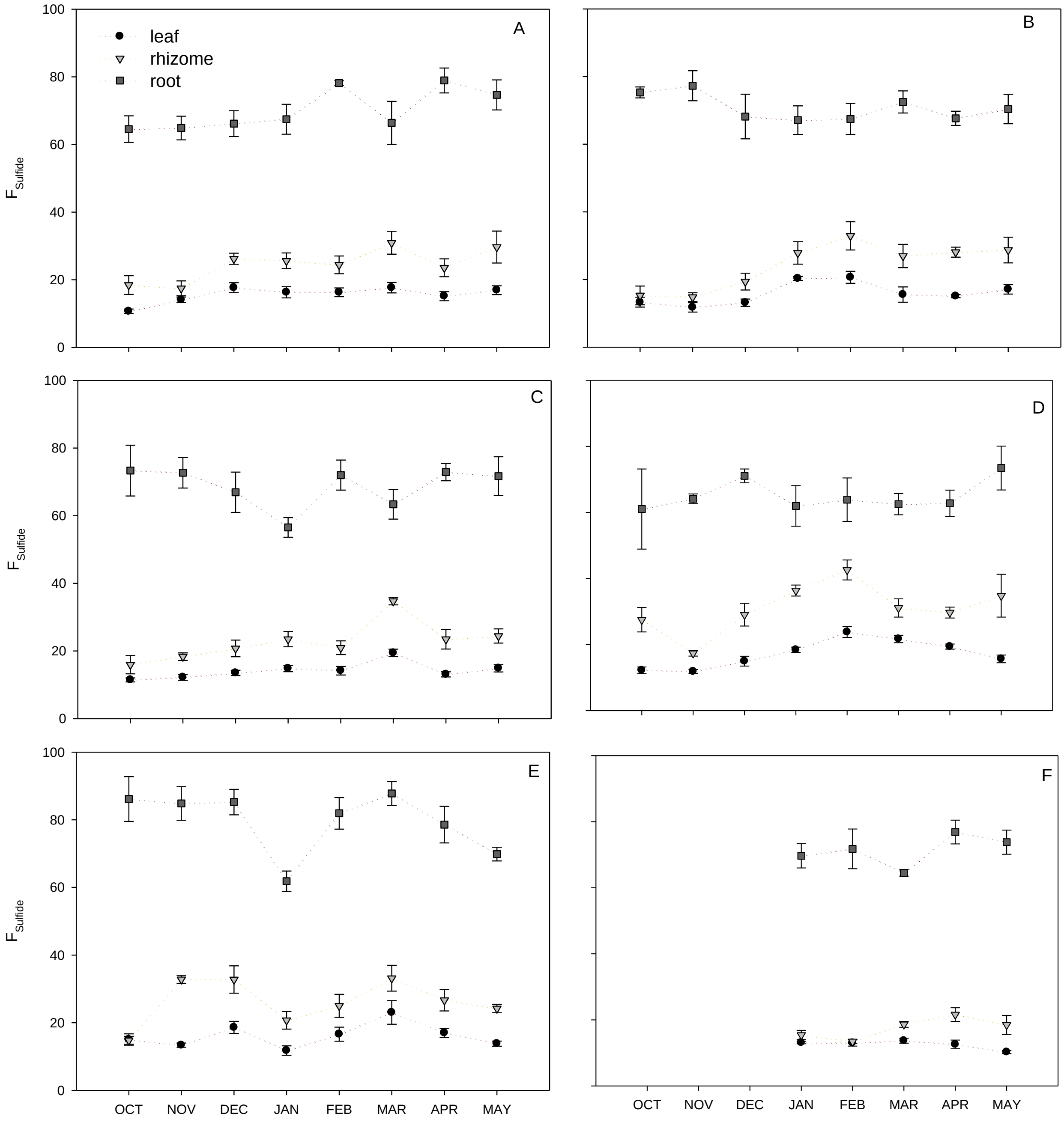


Figure 58 Percentage of sulfur derived from sediment as F$_{sulfide}$ for leaves, roots and rhizomes of H. ovalis at sites A) PPT, B) HTH, C) LUB, D) RCK, E) DLK and F) CAN from October 2011 to May 2012

5.2 Nutrient sinks and sources

The nitrogen to phosphorus atomic ratios (N:P) were determined for bottom water quality for the dissolved constituents (NH_4^+, NOx and SRP) and ranged from 18 to 0.9 (Figure 59). Larger N:P ratios indicate relatively more nitrogen available than phosphorus, and smaller N:P ratios indicate relatively more phosphorus available than nitrogen; that is, a N:P ratio of 10 indicates that 10 N atoms are present for every 1 P atom. The 'Redfield ratio' (16:1) is often used as a comparative point and is indicative of the nutrient ratio often observed for marine phytoplankton. The study of stoichiometric relationships which constrain the abundance and distribution of organisms has developed into a field of study in its own right: 'ecological stoichiometry' (Sterner & Elser 2002).

The N:P ratio observed in sea water is fairly consistent (and more closely reflects the Redfield ratio) compared with the N:P ratio observed within the Swan-Canning estuary – which appears to decrease in March and April (relative to earlier in the summer). These data (and figures 27 and 28) suggest the catchment is a significant source of dissolved N input (associated with rainfall), resulting in relatively more N available in December to February; whereas dissolved P appears less related to rainfall (likely internal recycling or sediment diagenesis). The drop in the N:P ratio observed after February suggests preferential nitrogen uptake by primary producers (internal nutrient cycling within the estuary).

Connell (1999) reported the N:P ratio of surface water in Lower Swan sites to be between 18:1 and 8:1, with sediment porewater ranging from 6:1 to 3:1 during October 1995 to May 1996. These data also suggest that sediment is an important source of phosphorus. The average N:P ratio of bottom water for Lower Swan sites (i.e. excluding CAN) was 6:1.

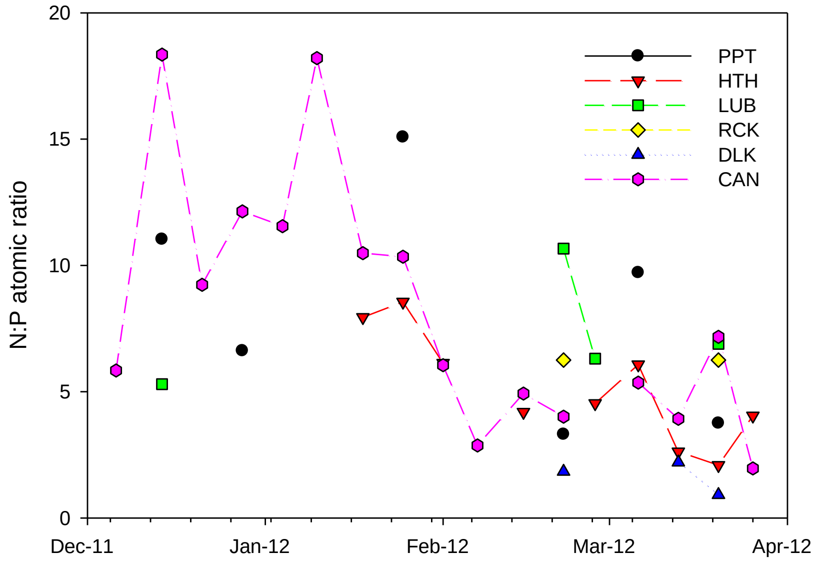


Figure 59 *The nitrogen to phosphorus atomic ratio of dissolved nutrients in bottom water from start of December 2011 to end of March 2012*

The C:N and N:P ratios of leaves of *H. ovalis* at each site (at each time period) were also determined (Figure 60). Higher C:N ratios indicate relatively more carbon than nitrogen within the tissues, and when considered in conjunction with the data presented in Figure 38, we can determine whether the change in ratio is due to an increase in carbon or a decrease in nitrogen. For example, the lower C:N ratio observed for LUB is due to relatively more nitrogen within the tissues at that site than at the others. Similarly, higher N:P ratios indicate relatively more nitrogen than phosphorus. For example, in October sites LUB and HTH had N:P ratios between 19 and 20:1, whereas PPT and DLK had N:P ratios between 13 and 14:1 – indicating that sites LUB and HTH had a relatively greater concentration of nitrogen (or less phosphorus) than sites PPT and DLK.

The range in N:P ratio within leaves of *H. ovalis* in this study was within the same range as that reported by Connell (1999) – where mean N:P ratios ranged from 21:1 to 7:1 between November 1995 and September 1996.

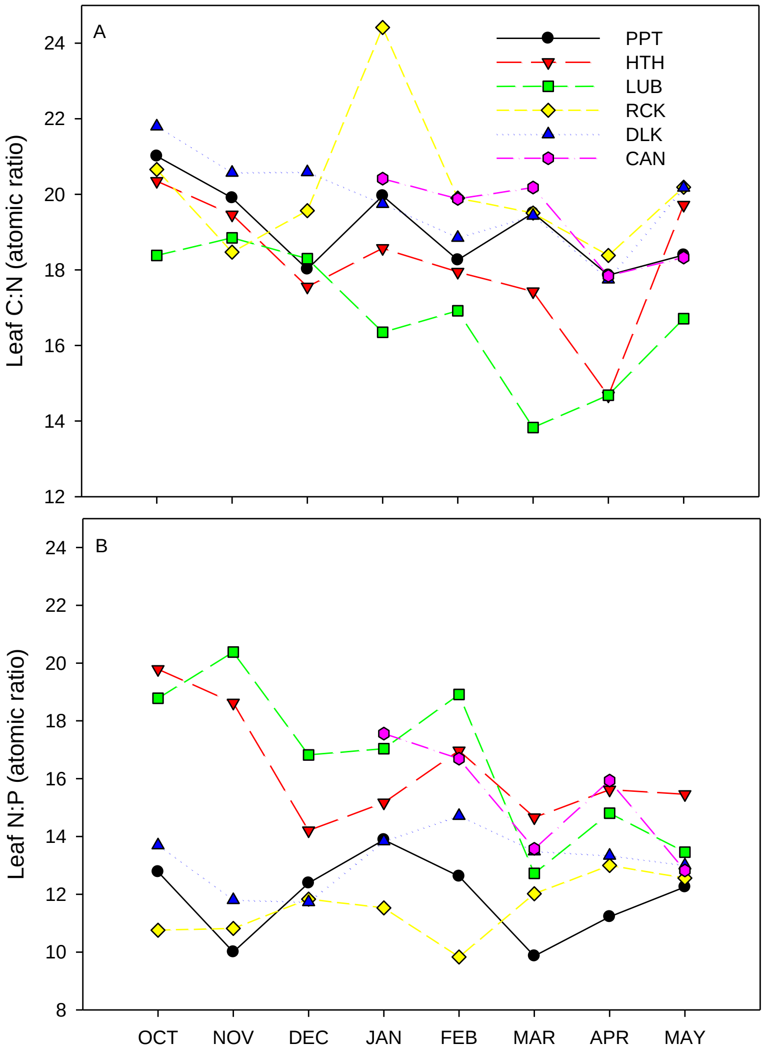


Figure 60 *Elemental ratios in* H. ovalis *leaves of A) carbon to nitrogen; and B) nitrogen and phosphorus*

The standing stock or 'sink' of nitrogen and phosphorus contained in the meadow at each site was determined by multiplying the concentration of nutrient measured in leaves by the density of leaves (g m^{-2}) and is shown by site in Figure 61. There was only a slight seasonal trend in the nutrient sink, compared with that previously reported by Hillman (1985) and Connell & Walker (2001). Using the average nitrogen and phosphorus concentrations (1.85% and 0.29% respectively) and average leaf biomass (55 g m^{-2}) we estimate that for 403 ha of seagrass, the summer sink of nutrients could be as high as 4100 kg of nitrogen and 640 kg of phosphorus. It is likely that this is an overestimate, as the seagrass in this study was sampled from shallow meadows (which were likely to be denser than those found deeper but nonetheless included in the 403 ha of total meadows in the estuary). The nutrient concentrations were quite similar to those used by Hillman et al. (1995) – whose calculation for whole-of-estuary sink used nutrient concentrations of 1.8% N and 0.23% P within leaves.

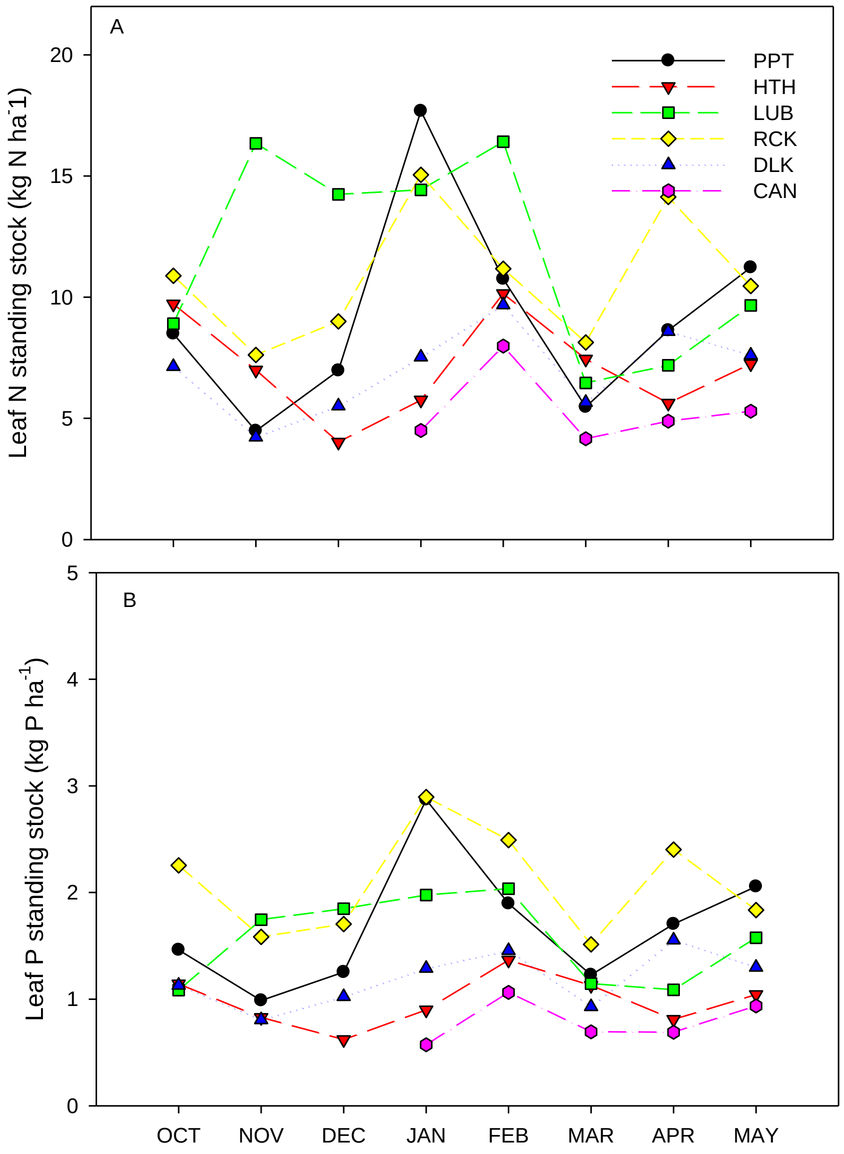


Figure 61 *Sink of nutrients within leaves of* H. ovalis *for A) nitrogen and B) phosphorus*

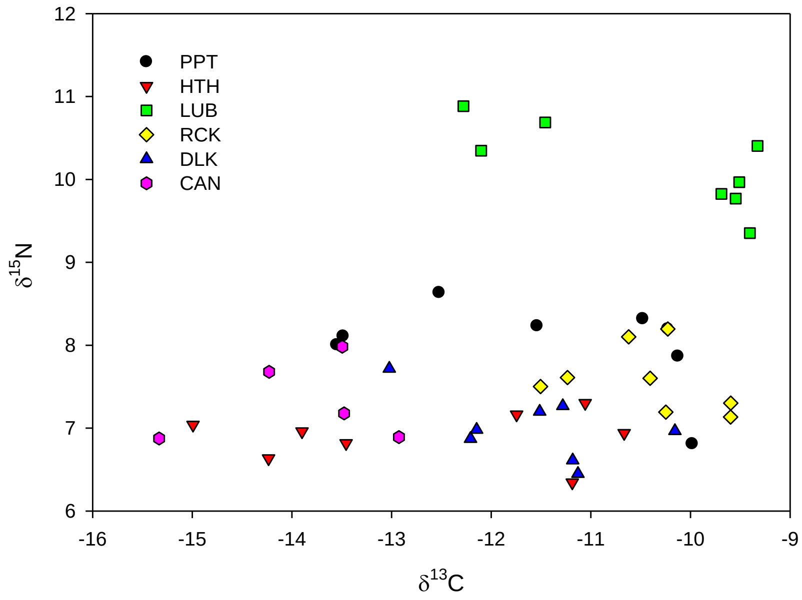


Figure 62 *Isotope ratios of carbon and nitrogen (δ^{13}C versus δ^{15}N) within* H. ovalis *leaves for six sites (average values obtained each month of sampling)*

Nitrogen isotope analysis has previously been used to identify wastewater input to estuaries through groundwater (McClelland et al. 1997). Groundwater containing nitrate from atmospheric deposition typically has a δ^{15}N value from +2 to +8 ‰., while nitrate derived from human and animal wastes is more enriched (+10 to 20 ‰); fertiliser (ammonium or nitrate based) reportedly has δ^{15}N around 0‰ (McClelland et al. 1997; Fry 2006). Nitrogen isotope ratios within macrophytes and macroalgae have previously been used to indicate land-derived wastewater input to estuaries (Cole et al. 2005), as well as to monitor for anthropogenic nutrient increases in seagrasses near coral reef ecosystems (Yamamuro et al. 2003).

The average isotopic ratios for carbon and nitrogen measured in leaves at each of the six sites (and eight sampling occasions) are plotted in Figure 62. This plot clearly shows seagrass from LUB had a more isotopically enriched δ^{15}N signal than the other sites. Leaf nitrogen concentrations at LUB were greater than the other sites (as seen in Figure 38), and the nitrogen isotopic ratio measured also suggests a different source of nitrogen. Overall, the δ^{15}N values measured in seagrass in this study reflect that of a eutrophic estuary, with average δ^{15}N values ranging from approximately 6.5 to 11 ‰. Grice et al. (1996) found higher δ^{15}N values in seagrass leaves at a more eutrophic site (8.6–8.8 ‰) compared with a site further from anthropogenic influences within Moreton Bay (2.6–4.5 ‰). In contrast to our study, the δ^{15}N values measured in *H. ovalis* leaves from Fiji and Green Island, Australia ranged from -1.4 to 2 ‰ in a study by Yamamuro et al. (2003).

In the study by Yamamuro et al. (2003), a significant positive correlation was found between nitrogen concentration and δ^{15}N in seagrass leaves (r=0.66). In our study, a positive correlation was also observed between %N and δ^{15}N in seagrass leaves (r=0.34, p<0.00001), and this relationship could be improved slightly when excluding samples from sites which received less than 4.5 hours of saturating irradiance each day (r=0.39). By considering the relationship between these variables independently for each month, significant correlations were observed for October (r=0.7), January (r=0.37), March (r=0.54), April (r=0.54) and May (r=0.66). Seagrass are known to exhibit 'luxury' uptake of nutrients, and it is likely the interaction between luxury uptake and dilution of nutrients with growth of seagrass affected the relationship between %N and δ^{15}N.

6 Determining uniqueness of individual measurements

6.1 Inverse SIMPROF

Many variables were measured within this study, and we attempted to ascertain which variables (if any) were co-varying, and therefore could be trimmed from future sampling efforts. By importing data into PRIMER 6 and telling it the samples were variables (and vice versa), cluster analysis with a sequence of SIMPROF tests could be used to determine which variables were not significantly different from each other (in multi-dimensional space) (B. Clarke, pers. comm.). This type 3 SIMPROF analysis (Somerfield & Clarke 2013) was applied to the 40+ variables collected as part of this study to ascertain which, if any, were redundant (and could be trimmed from future sampling efforts).

Data were split into three categories: *primary* – being variables relating to seagrass measured directly either in the field or the laboratory; *derived* – being variables which were derived from the primary variables, usually ratios of primary variables; and *environmental* – variables describing the meadow depth, water depth, light climate and temperature experienced by the seagrass during the study. 'Variables' were square-root transformed and normalised before analysis. The outputs of these analyses are shown in figures 63 to 65.

For the primary variables, apex density and *Batillaria* density were coherently co-varying (in multi-dimensional space). That these variables were not significantly different by the inverse SIMPROF test does not necessarily suggest they are strongly correlated in two-dimensional space: in fact they are only weakly although significantly correlated in two-dimensional space (Pearson correlation r=0.24; p<0.0001). Similarly the following primary variables also coherently co-varied in multi-dimensional space: average leaf weight and nitrogen stable isotope ratio within leaves (Pearson correlation r=0.15, p<0.05); above-ground growth rate and below-ground growth rate (Pearson correlation r=0.62, p<0.0001); rhizome extension rate and total growth rate (Pearson correlation r=0.62, p<0.0001); carbon stable isotope ratio in leaves and % carbon in leaves (Pearson correlation r=0.24, p<0.0001); leaf biomass per m^2 and sulfur stable isotope ratio in leaves (Pearson correlation not sig.).

For the derived data, the ratio of leaf length over leaf area and leaf area over leaf mass were coherently co-varying (in multi-dimensional space; Pearson correlation not significant). Similarly standing stock of P (leaf P as g/m^2) and the ratio of %P over leaf mass (Pearson correlation r=-0.19, p<0.01) and carbon to nitrogen ratio in leaves and Fsulfide in leaves (fraction of sulfur derived from sediment sulfide source) (Pearson correlation not significant) also were coherently co-varying.

For the environmental data, coherently co-varying variables were: the average daily temperature and minimum daily temperature (Pearson correlation r=0.59, p<0.0001); water depth at high tide and depth of site relative to Australian height datum (r=-0.95, p<0.0001); and average peak light and median peak light (peak light defined as light received between 10 am and 2 pm daily) (Pearson correlation r=0.94, p<0.0001).

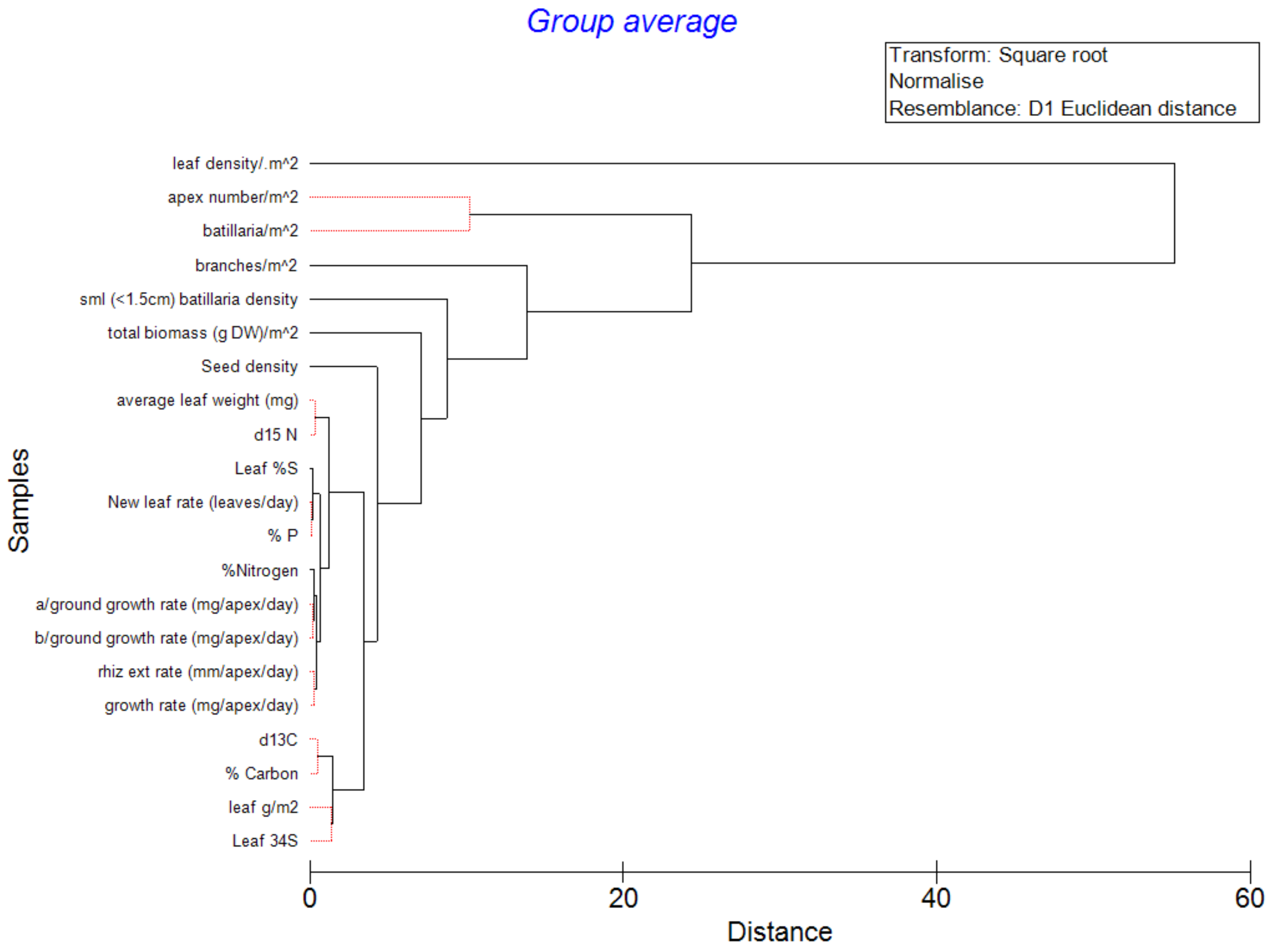


Figure 63 *Cluster analysis with SIMPROF test on the 'inverse' primary variables data (inverse as variables rather than samples)*

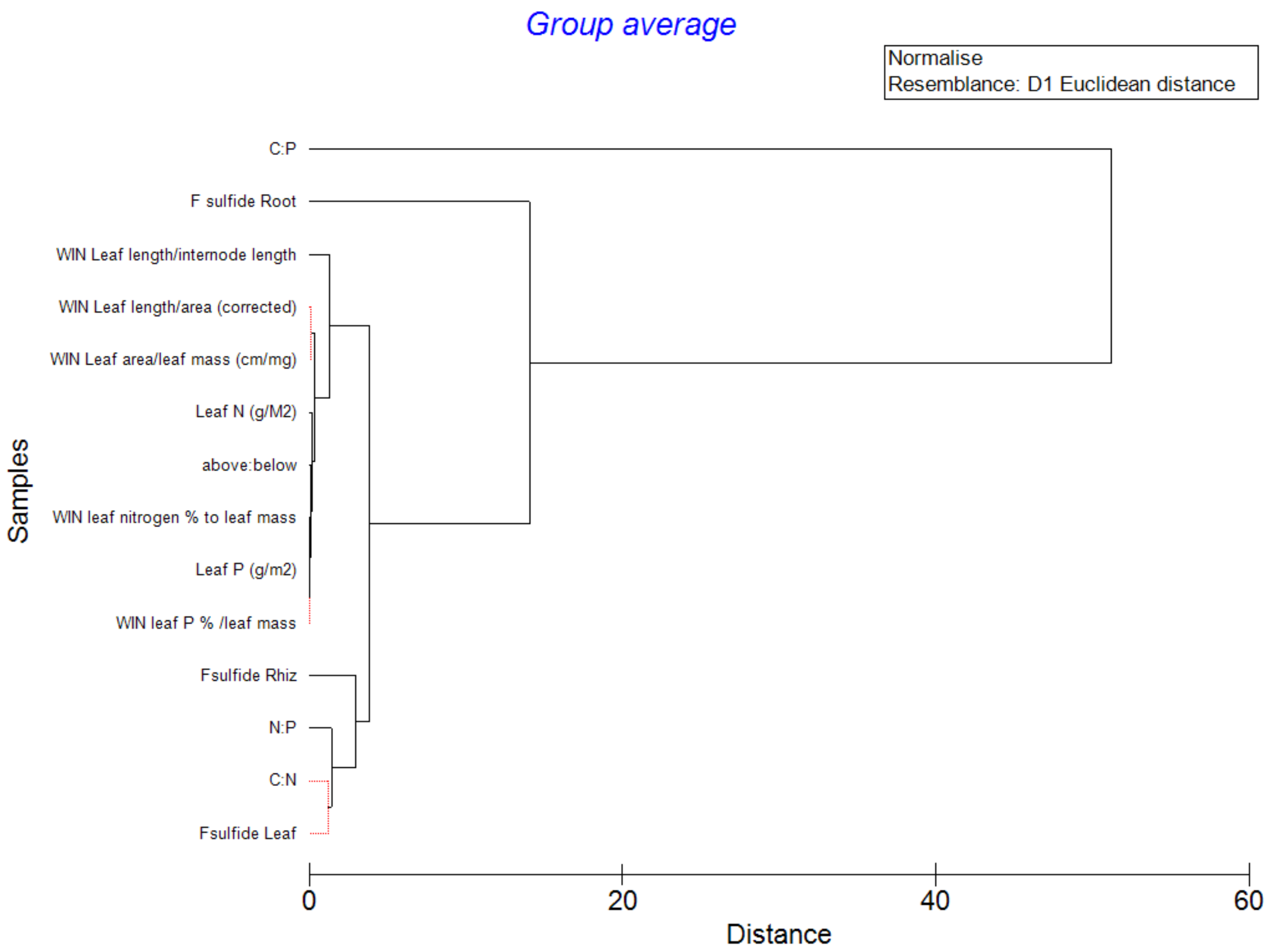


Figure 64 *Cluster analysis with SIMPROF test on the 'inverse' derived variables data (inverse as variables rather than samples)*

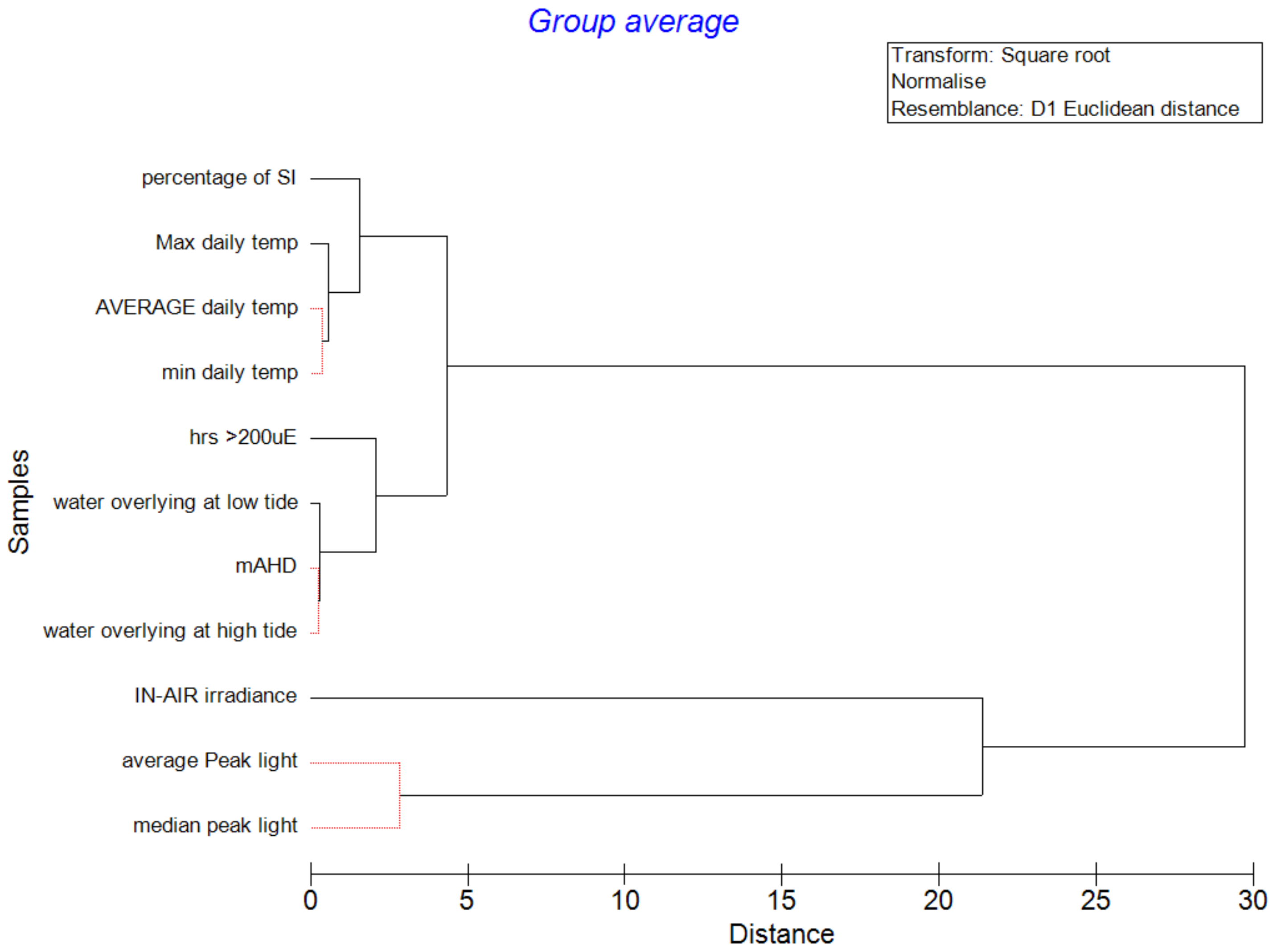


Figure 65 *Cluster analysis with SIMPROF test on the 'inverse' environmental variables data (inverse as variables rather than samples)*

The analysis of primary, derived and environmental data by inverse SIMPROF testing showed there were some relationships in how the data behave in multi-variate space which could be exploited to potentially simplify or reduce the data collection effort. However, there were very few cases where strong direct linear relationships existed between variables (Pearson correlation). The relationships between the different measures of production (e.g. rhizome extension and growth rates) were probably the only area in which the data collection effort could be reduced without significantly compromising on the information gathered.

7 How have estuary conditions and seagrasses changed in the past 30 years?

This section collates information from studies on *Halophila ovalis* and its productivity in the Swan-Canning estuary. The comparison includes information on estuary conditions and *H. ovalis* from two PhD theses (Hillman 1985; Connell 1999) and the papers from these studies (Hillman et al. 1995; Connell et al. 1999; Connell & Walker 2001), as well as the seagrass surveys and routine water quality monitoring of the Department of Water in partnership with the Swan River Trust.

7.1 Estuary conditions

Conditions in the Swan-Canning estuary have changed during the past few decades as a result of estuary management strategies being put in place, alongside deviations in rainfall and river flow patterns as a consequence of climate change. Nutrient concentrations in the estuary surface waters have substantially reduced in the Lower Swan during the past 30 years (Table 9). Surface water ammonium concentrations have decreased by 150 times, nitrate/nitrite concentrations by 100 times and soluble phosphate concentrations by 10 times since 1982. The reduction in surface water nutrient concentrations partly reflects the results of the Swan-Canning Cleanup Program and the Healthy Rivers Action Plan that were implemented during that time. These programs specifically targeted the reduction of nutrient inputs to the estuary through better land use management in the catchment, and remediation and intervention programs to improve conditions in the estuary. However, nutrient reductions in the estuary can also be linked to the lower total volume of river flow to the estuary as a result of reduced rainfall (climate change), which has reduced the nutrient load to the estuary.

Table 9 *Changes in the inorganic nutrient concentrations from 1980 to 2012. The data presented shows the maximum or the range of maximums recorded across sites during the different studies.*

	1980–82	**1995–96**	**2006–11**[3]	**2011–12**[4]
Surface				
NH_4 mg/l	~2.5	1.5	0.017	-
NO_x mg/l	3.5	0.3	0.11	-
SRP mg/l	0.11–0.15	0.05	0.013	-
Bottom				
NH_4 mg/l	2.5–8	-	0.045	0.1
NO_x mg/l	1–3.5	-	0.031	0.043
SRP mg/l	0.08–0.1	-	0.014	0.01

---

[3] Data from 90th percentile from HRAP monitoring for Lower Swan as per figures 16 to 18.

[4] Data from bottom water samples taken at seagrass monitoring sites (this study) as per figures 27 and 28.

Nutrient concentrations in the bottom waters of the lower estuary have also decreased over the past 30 years but this decrease is much smaller (Table 9). Bottom water ammonium concentrations are up to 80 times less, while phosphate concentrations have decreased by up to 10 times. Flushing events, driven by river flow, have been more infrequent in the Swan-Canning estuary – consequently nutrients and organic material entering the estuary are more likely to stay in the estuary. This has meant an increase in the importance of internal recycling of nutrients and organic material in the estuary.

7.2 Seagrass populations

A gradual decline in the distribution of *Halophila ovalis* has occurred in the Swan-Canning estuary during the past 30 years (Table 10). *H. ovalis* is still predominantly found in the shallow waters (< 2 m) of the estuarine basin, as well as deeper waters (up to 4 m) closer to the estuary mouth as light conditions improve. Similar to 1980–81 distribution patterns, *H. ovalis* did occupy a few areas in the Lower Canning River in 2011 and 2012 where distribution is considered to be more ephemeral. During the current study, *H. ovalis* only appeared at CAN in early December 2012 as salinities increased; peak productivity (see below) was also achieved later than at other sites. Late spring and early summer rainfall and runoff events prolonged fresh and turbid conditions in the upper estuary, likely delaying the establishment of *H. ovalis* at the CAN site.

Table 10 *Comparison of the distribution, productivity and biomass of* H. ovalis *in the Swan-Canning estuary 1976 to 2012*

Source	**Year**	**Distribution (ha)**	**Peak productivity (gDW.m^{-2}.day^{-1})**	**Peak biomass (g DW.m^{-2}) (recorded at sites)**
Allender	1976	568	-	-
Hillman et al. (1995)	1980–81		12 (December)	~60–120 (January)
	1981–82	598	50 (February)	~80–120 (April)
Connell (1999)	1995–96	461	-	~50–125 (January)
Department of Water	2010–11	403 (unpubl.)	8 (December)	~50–296 (February)

Past and current studies show the productivity of *H. ovalis* is extremely variable (Table 10). Differences in productivity can occur between years and across locations. In 1980–81 seagrass productivity was at its peak in December 1980 compared with February 1982 in 1981–82 (Hillman et al 1995). Productivity rates were also much higher in 1981–82 (Hillman et al. 1995). In the current study, average peak productivity values were comparable with the values recorded in 1980–81, but the timing in which sites achieved this peak varied. Sites RCK, LUB and PPT reached peak productivity in November/December, site HTH in January, site DLK in February and site CAN in March (Figure 30).

The productivity of *H. ovalis* was extremely high during 1981–82, in contrast to the previous year (1980–81) and the current study. The much-reduced productivity of *H. ovalis* in 1980–81 was attributed to the effect of high river discharge on salinities and light conditions in the estuary (Hillman et al. 1995). Lower productivity of *H. ovalis* in the current study may also be attributed to poorer light conditions as a result of turbidity effects from above-average rainfalls during the summer months.

As expected, peak *H. ovalis* biomass was generally achieved one to two months after production reached its peak. Biomass measures in the current study were higher than in 1980–81 and 1981–82. In particular, plant biomass at sites RCK and PPT were pronounced by higher leaf densities and above-ground biomass.

8 Indicators of stress

8.1 Overview

This section explicitly explores the data to look for possible indicators for individual environmental stressors. Those that we considered plausible for *Halophila ovalis* to inform on included:

- changes in water quality resulting in light reduction
- changes in water quality by nutrient pollution
- changes in sediment quality.

Additionally, we noted that seagrass was affected by the estuary's salinity at the site in the uppermost reach (within the Canning Estuary), and details of how it was affected will also be described in this section.

8.2 Light

Too much or too little light will constrain the productivity of *H. ovalis*. Both of these scenarios were observed during the study. At site HTH, light was significantly reduced during spring due to a *Chaetomorpha* macroalgal bloom. At site LUB, which was the shallowest of the study sites, extremely low tides meant that seagrass was exposed to very high light.

Seagrasses are responsive to their environment, and are able to alter plant morphology to suit the light climate. Some of the acclimations previously reported include:

- increase in lacunal (gas spaces) area within leaves at high light (which would concurrently be seen as a decrease in leaf thickness in low light) (Grice et al. 1996)
- increase in chlorophyll concentrations within leaves (Longstaff et al. 1999)
- increase in leaf length, which is commonly reported for the smaller genera of seagrasses (Longstaff & Dennison 1999; Ralph et al. 2007)
- increase in UV-absorbing pigments (often the red pigment anthocyanin) at high irradiances (McMillan 1983; Dawson & Dennison 1996; Gavin & Durako 2011; Novak & Short 2011).

Typically the depth of water overlying the seagrass at the shallowest study site LUB was between 25 and 45 cm at low tide. However there were several days in October and November where the water overlying the seagrass was estimated to only be ~4 cm at low tide. Additionally, for three days in December the tide was so low it was estimated the seagrass was completely exposed at low tide. We observed that the seagrass leaves did increase in redness (likely due to an increase in the UV-absorbing pigments) during this time (Figure 66), and measurements of growth (see Figure 30) suggest that production was constrained.

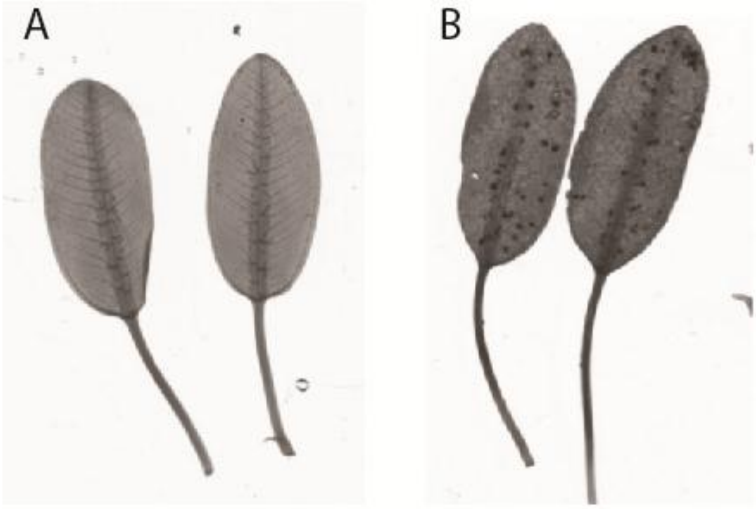


Figure 66 *Seagrass leaves from* H. ovalis *at A) RCK and B) LUB from December 2011 showing increase in red pigmentation (seen as dark spots in scanned image) at site LUB*

At HTH, the nuisance macroalga *Chaetomorpha linum* substantially reduced light that reached the seagrass meadow, particularly in November and December 2011 (Figure 67). Seagrass production was lower in December 2011 (see Figure 30), and many of the characteristics of the meadow (branching density, apex density, leaf density, above-ground biomass and total biomass) were also lower in December 2011 compared with the other months (see Figure 32). Plant morphology was also altered by the low light environment, with leaf length and leaf area increasing in December 2011 (compared with the other months). Considering data only from the spring/early summer (October to December 2011), a negative correlation was observed between leaf length and H_{sat} (hours above saturating irradiance) for all sites ($r=-0.33$, $p<0.005$).

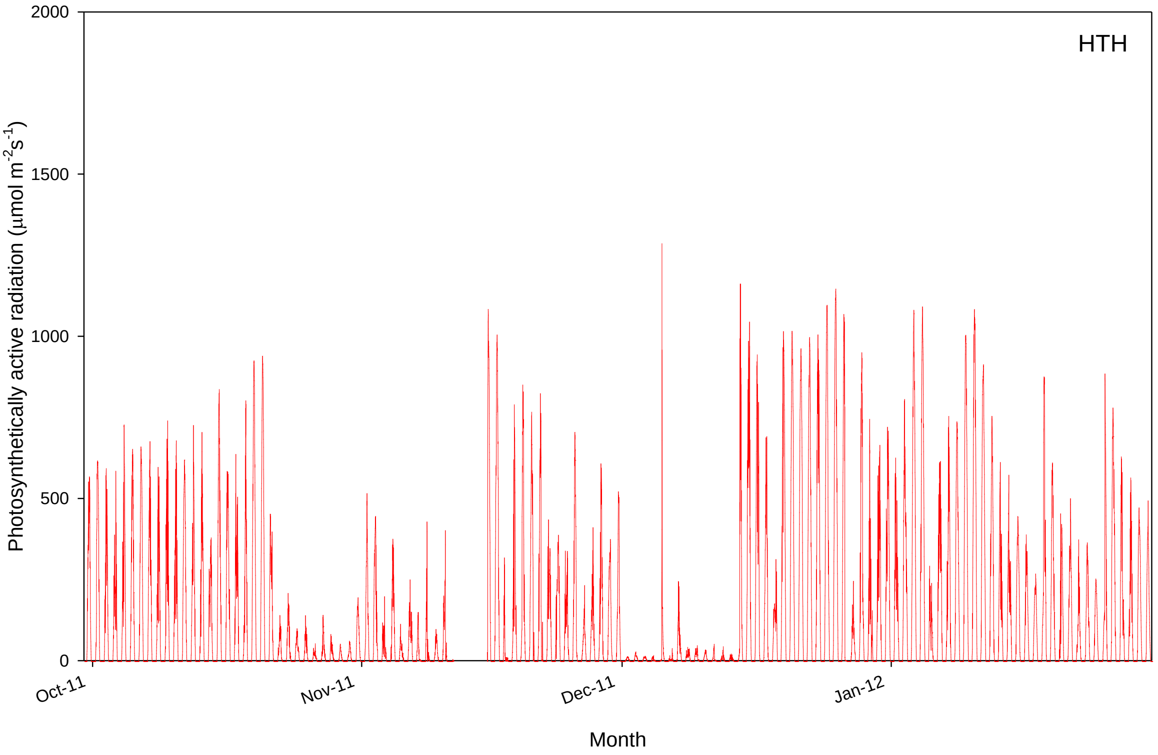


Figure 67 *Photosynthetically active radiation measured at seagrass canopy height within the underwater environment at site HTH from October 2011 to end of January 2012. Substantial reduction in PAR during November and December was due to accumulation of the macroalga* Chaetomorpha linum*.*

The number of hours above saturating irradiance is commonly related to the growth rate of seagrass, with the rate of growth commonly showing a saturation-type response. For example, growth of the seagrass *Zostera marina* was significantly reduced below 10 hours of saturating irradiance and values above 10 hours did not enhance seagrass growth (Dennison & Alberte 1985). The minimum H_{sat} required by seagrass to survive also appears to depend on seagrass species and environmental condition (particularly depth) (Dennison & Alberte 1985; Lee et al. 2007; Collier et al. 2012). Collier et al (2012) report that greater than four hours of saturating irradiance was required by *Halodule uninervis*.

Our study did not show a saturation-type growth response in relation to increasing hours above saturation, however there was a positive correlation between areal production and H_{sat} ($r=0.44$, $p<0.0001$; Figure 68). Below six hours of saturating irradiance resulted in an areal production rate typically less than 4 g m^{-2} d^{-1}. Variance of the production rate increased with increasing saturating light periods, which suggests other factors constrained the growth of *H. ovalis* in our study.

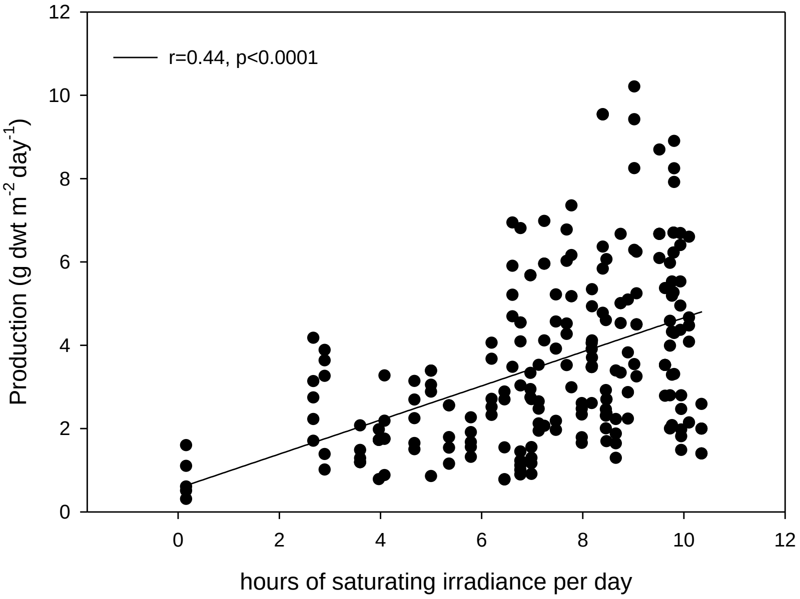


Figure 68 *Scatterplot of production of seagrass meadows against the number of hours of saturating irradiance per day (H_{sat})*

The strongest impact of light limitation from the *Chaetomorpha linum* accumulations was a suppression of the flowering success of the meadow. Flowering at HTH was almost non-existent during December, the month where most other sites had their highest flowering density. *Chaetomorpha linum* accumulations at this site reduced the H_{sat} to zero hours in December. The development of fruit was also significantly reduced at HTH.

The ecological importance of sexual reproduction for seagrass has often been questioned since seagrass also readily colonise clonally (Ackerman 2006). *H. ovalis* has been documented as a successful sexual coloniser in a experimental clearing study of a mixed seagrass meadow near Cairns (north Queensland, Australia) (Rasheed 2004). *H. ovalis* was the most successful sexual coloniser after clearing, but it was rapidly displaced (within 10 months) by asexual growth of the other seagrass species (*Halodule uninervis, Cymodocea rotundata, C. Serrata* and *Syringodium isoetfolium*). The success of *H. ovalis* in reproducing sexually may provide a stress subsidy in estuarine systems, allowing it to re-establish once environmental conditions return to those that are favourable. Further, without inter-species competition, *H. ovalis* can dominate. *H. ovalis* fruit from southern Western Australia contain up to 16 seeds (7.4 on average) (Kuo & Kirkman 1992), but little is known about the persistence of seed within the estuary. Successful sexual reproduction should improve the resilience of the seagrass population, and may be particularly important in areas such as the Canning that are thought to establish annually from seed. To the authors' knowledge, there have been no studies of *H. ovalis* seed banks within the Swan-Canning estuary.

8.3 Nutrient pollution

Although nutrient concentrations within the water column of the Lower Swan Estuary have been reduced dramatically since the 1980s, nutrient limitation of *H. ovalis* was not evident within the Swan-Canning estuary. The nutrient concentrations within seagrass leaf tissues were within reported ranges by both Hillman et al. (1995; mean of 1.8% N and 0.23% P dry weight) and Connell (1999; range for N 1.0–2.2% and 0.2–0.35% for the months October to May – as in current study). In fact, the nutrient concentrations for leaves measured in this study (mean of 1.85% N and 0.29% P – see histograms of %C, %N and %P in Figure 69) suggest sufficient nutrient concentrations to support enhanced growth.

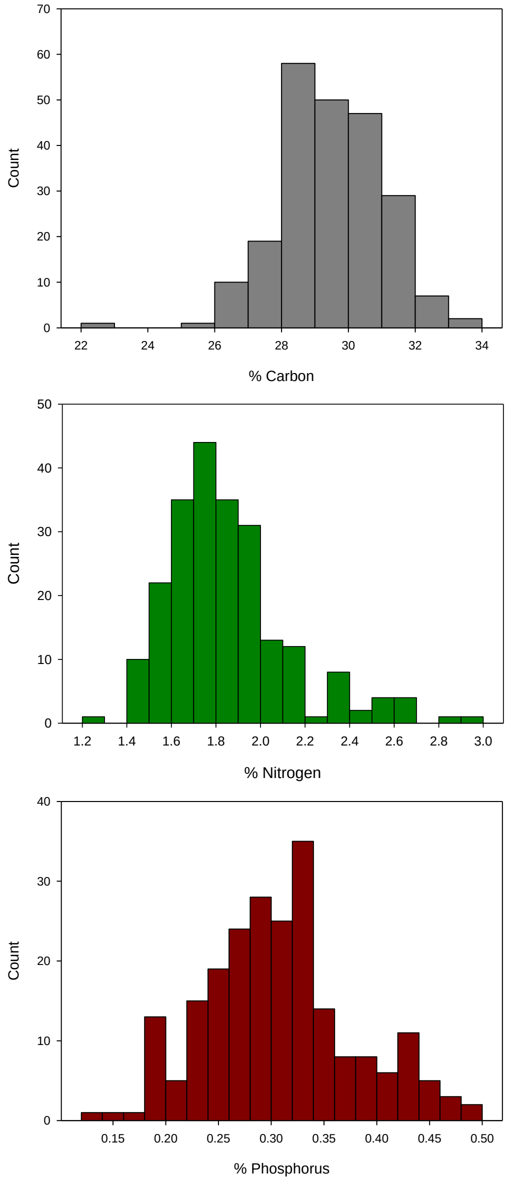


Figure 69 *Histograms of percent nutrient content of* H. ovalis *leaves collected across the six sites within the Swan-Canning estuary, October 2011 to May 2012*

H. ovalis has been reported to exhibit 'luxury uptake' of nutrients; that is, where nutrients are taken up beyond the immediate growth requirements of the plant, but can be used when conditions for photosynthesis improve. Nutrient concentrations are then 'diluted' over summer as the production of seagrass reaches its peak. This pattern is observable in data reported previously, where nitrogen concentrations reduce from a maximum of 2.8% (dry weight) in winter to only 1.0% in summer and phosphorus concentrations reduce from 0.35% in winter to 0.20% in summer (Hillman et al. 1995; Connell 1999). Nutrient concentrations in the current study did not show a similar seasonal trend, and the lowest concentrations were higher than those reported previously. These data, along with the evidence of reduced productivity (compared with data from 1981–82) suggest the seagrass growth was not limited by nutrient supply.

Evidence was found, however, of differing sources of nutrients throughout the estuary, as well as some evidence of localised nutrient enrichment. Seagrass from site LUB had a consistently different $\delta^{15}N$ signal within its tissues (compared with other sites), and a higher nitrogen concentration within the leaf tissues than other sites. Further investigation is warranted to reliably assess the source of the nitrogen input, although the enriched signal could be due to sewage outfalls, historic septic tanks, groundwater nitrate, or possibly defecation from waterfowl.

8.4 Sediment stress

Deteriorated sediment condition may result from organic matter loading to the estuary, whether delivered from terrestrial sources or produced internally (from the conversion of inorganic nutrients to organic material, such as phytoplankton primary production). Combined with climatic changes which mean the estuary does not ‘flush’ with winter rains and scour sediments – these changes in sediment condition are an emerging concern for the estuary.

Within sediments, sulfur-reducing bacteria mineralise organic matter using the sulfate ion as an electron acceptor – creating sulfide. Sulfide is toxic to plants (Raven & Scrimgeour 1997) and it is likely that seagrass growth and survival is constrained by sediment conditions (Terrados et al. 1999). Seagrasses can re-oxidise the sulfide to the non-toxic sulfate by releasing oxygen through their roots, which often results in the accumulation of elemental sulfur within tissues (Holmer et al. 2005b). Seagrasses show intrusion of sulfides via below-ground parts under both pristine and organic-enriched conditions (Holmer et al. 2003; Frederiksen et al. 2006; Frederiksen et al. 2008; Holmer & Kendrick 2013). Sulfide stress related to organic matter enrichment has been shown to influence seagrass growth for several seagrass species (Holmer et al. 2005a; Mascaró et al. 2009). However, the relationship with growth can be a complex one – related to light availability, productivity, temperature, seagrass species and other aspects of sediment condition (see Figure 70).

In this study, we used the sulfur stable isotope ratio and total sulfur within the tissues of the seagrass *H. ovalis* as an indicator of ‘sediment stress’ – or more precisely ‘sulfide stress’. Sulfide derived from sediment sources has a distinct isotopic signature (Trust & Fry 1992), which makes it possible to use isotopic analysis of seagrass tissue to trace sulfide intrusion into seagrasses (Frederiksen et al. 2006). The detailed dataset allows us to explore the relationship of these measurements with seagrass productivity.

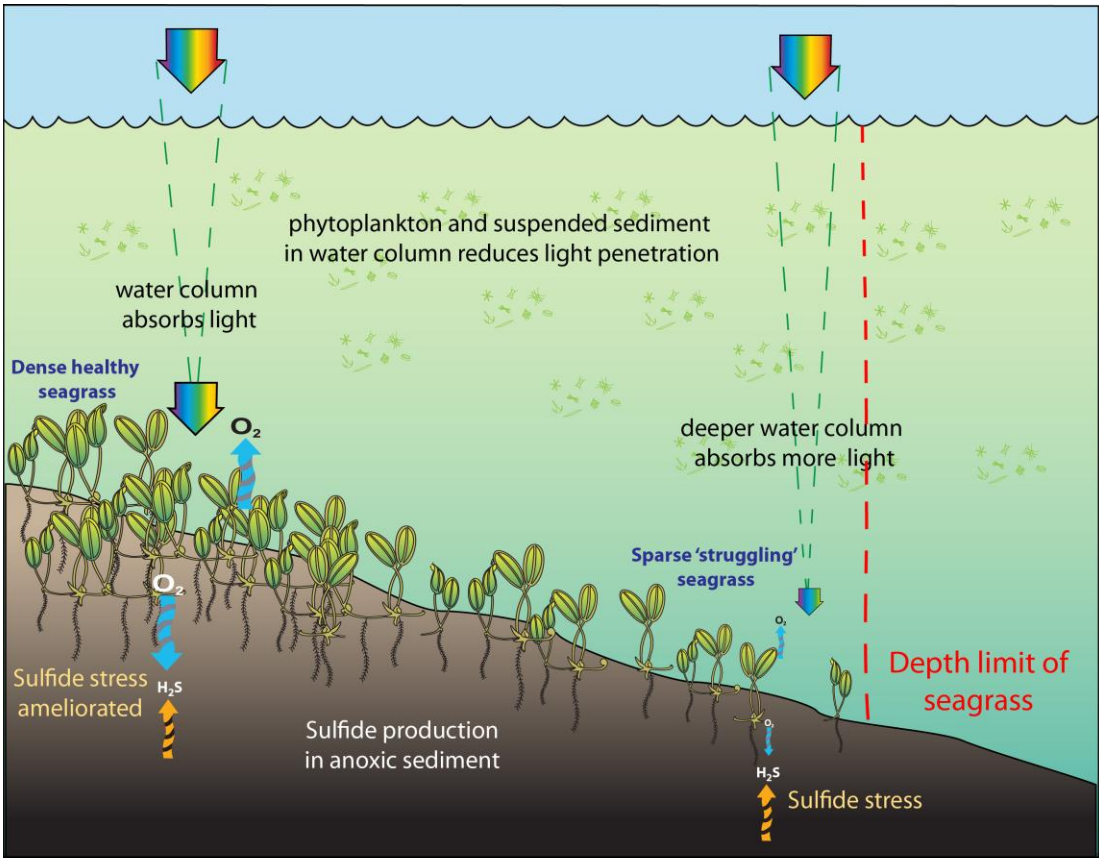


Figure 70 *Conceptual diagram of interaction of sediment condition and seagrass production*

A subset of data was selected relating to the period where productivity was likely to be at its peak, not limited by either light or temperature (Figure 71). The months of January, February and March were chosen as these had the highest average daily temperature (all >23.7 °C). Light availability was also relatively high across these months, and individual sites were not affected by *Chaetomorpha* accumulations at this time. Sulfate reduction rates are faster at higher temperatures (Devereux et al. 2011) and within seagrass-vegetated areas they are higher in summer than winter (Welsh et al. 1996; Holmer et al. 2003). So an additional rationale for choosing the hottest months is that sulfur-reducing bacterial activity is likely to be at a maximum, potentially increasing the pools of sulfide in the sediment. It might then be assumed that variation in productivity across sites/months might be caused by aspects relating to the sediment stress the seagrass was exposed to.

The relationship of sulfur dynamics to seagrass productivity was explored to derive potential sediment-stress metrics; that is, metrics which describe how sediment conditions (specifically sulfide) constrain seagrass growth. The strongest relationship was observed for the leaf component, where reduced productivity was observed with an increase in the ratio ((δ^{34}S +30[5]) / %S) – Pearson correlation of 0.43 and 0.5 for leaf formation and rhizome extension

---

[5] Note: to each δ^{34}S value we added 30 to ensure that the ratio was always positive.

rate respectively. This fits with our understanding of what might be occurring to limit seagrass growth – where a decrease in the ratio ((δ^{34}S +30) / %S) indicates either a decrease in the δ^{34}S signal (i.e. more sediment-derived sulfur within seagrass tissues via sulfide intrusion) or an increase in %S.

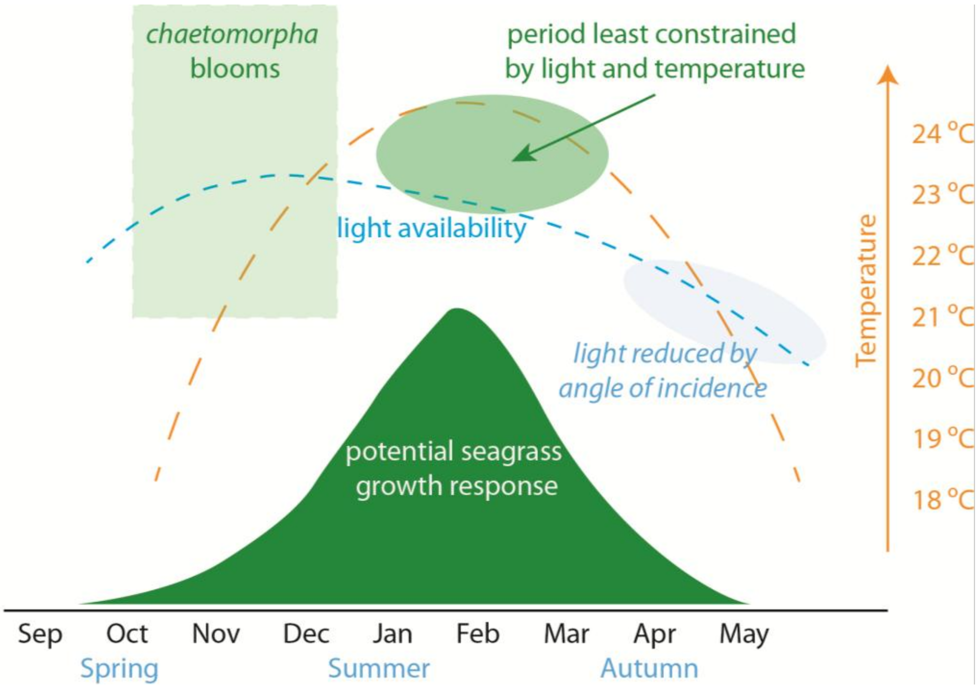


Figure 71 *Diagram depicting potential constraints on growth of seagrass in the Swan-Canning estuary (note: low salinity is also thought to constrain seagrass growth at site CAN in the Canning Estuary)*

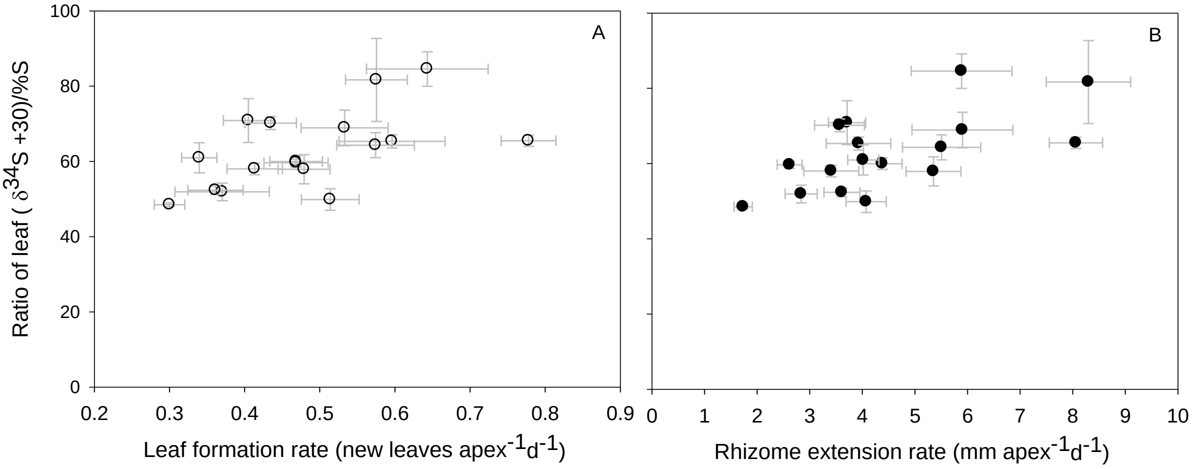


Figure 72 *Scatterplot of the ((δ^{34}S +30) / %S) in leaves versus A) leaf formation rate and B) rhizome extension rate*

.

We suggest that the ratio ((δ^{34}S +30) / %S) in *H. ovalis* leaves might be used as a potential sediment-stress indicator. Ranking the sites based on their average sediment-stress indicator value we get RCK<LUB<DLK<PPT<HTH<CAN, where seagrass growth at RCK is most constrained by sediment conditions and CAN is the least constrained. This indicator fits with our ecological understanding of the interaction of seagrass with their sediment environment, where organic matter enrichment can lead to sulfide build-up, inhibiting seagrass productivity. Further discussion of the proposed sediment-stress indicator can be found in Kilminster et al. (2014).

8.5 Salinity stress

Halophila ovalis has been shown to respond to salinity stress on a cellular, morphological and distributional level in the Swan-Canning estuary (Hillman et al. 1995; Benjamin et al. 1999).

In this study the leaves of *H. ovalis* were morphologically quite different at CAN compared with the leaves at sites in the estuary basin. Leaves were thicker, darker and had a wrinkly leaf margin at CAN (Figure 73). Leaf size was also smaller but leaf weight was relatively unaffected.

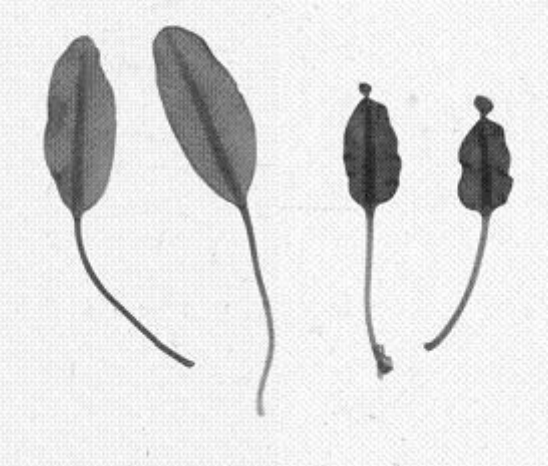


Figure 73 *Two leaves from RCK (left) and two leaves from CAN (right) showing the smaller leaf size and wrinkly leaf margin at CAN from the more typical leaf appearance*

Similar to surveys conducted by Hillman et al. (1995), the distribution of *H. ovalis* habitats in the Canning River (CAN) appeared to be more transient than in the lower estuary basin. *H. ovalis* has been completely absent from the Lower Canning River as a result of lower salinities and poorer light conditions due to above-average rainfall and river flow events, such as in 1981. Prolonged periods of low salinities (< 15 PSU) have been shown to cause severe declines in *H. ovalis* biomass (Hillman et al. 1995). Salinities in the Canning River remained low until December due to high spring/summer rainfall events. *H. ovalis* only appeared to establish at CAN in mid-December when salinities increased to those similar in the lower basin.

9 Conclusions and recommendations

9.1 Growth conditions for *Halophila ovalis*

The summer of 2011–12 had sub-optimal environmental conditions for seagrass growth. The growth rates at a per-shoot level were significantly lower than the rate previously reported for *Halophila ovalis* in the Swan-Canning estuary (Hillman et al. 1995). Additionally, the nutrient concentrations measured within seagrass suggested they were ready to grow rapidly once conditions became favourable. Climatic factors were likely to be a strong contributor to slower growth, with seagrass (on average across all sites) receiving between seven and 9.7 hours of saturating irradiance between October 2011 and March 2012, after which the available light dropped significantly (6.2 hours in April and 3.4 hours in May). The 2011–12 summer had above-average rainfall, with 11 days of rainfall recorded for December 2011 to February 2012. Along with these periods of rainfall, significant leaf shedding was observed (see Figure 74 showing reduction in leaf density, particularly in November and December).

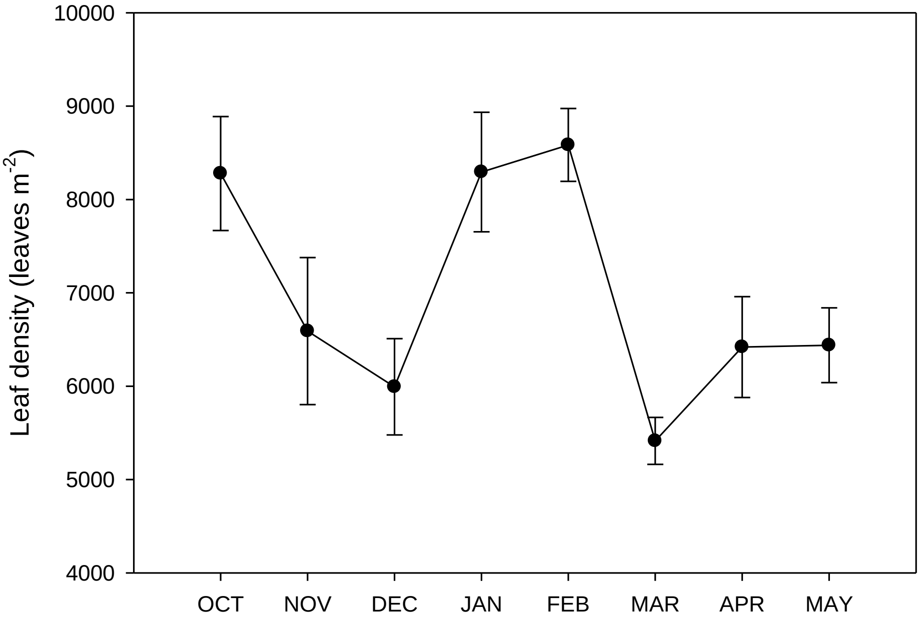


Figure 74 *Leaf density of* H. ovalis *averaged across all sites in the Swan-Canning estuary between October 2011 and May 2012*

The total biomass (or standing stock) for *H. ovalis* reached its maximum in February, with this period also being associated with the highest standing stock of nitrogen and phosphorus bound within seagrass leaves (Figure 75). Using the average nitrogen and phosphorus concentrations (1.85% and 0.29% respectively) and average leaf biomass (55 g m^{-2}), we estimate that for 403 ha of seagrass, the summer sink of nutrients could be as high as 4100 kg of nitrogen and 640 kg of phosphorus within seagrass leaves. However, we must keep in mind when scaling these measurements to a whole-of-estuary scale, that this study targeted the estuary's shallow seagrass meadows, and that these are likely to be denser per area (therefore also containing more nutrients) than the deeper seagrass meadows.

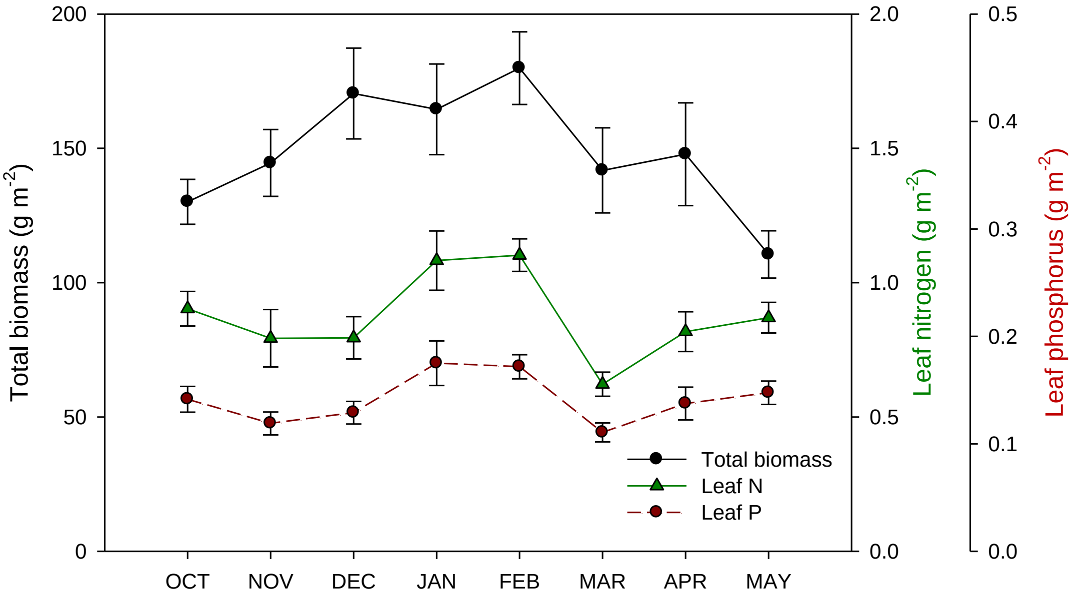


Figure 75 *Biomass, leaf nitrogen and leaf phosphorus averaged for all sites between October 2011 and May 2012*

As in previous studies only a very weak correlation was observed between total biomass and productivity. Kilminster et al. (2008) noted that biomass was not a sensitive metric to environmental stress and should not be used alone to indicate a 'healthy seagrass population'. This current study supports that suggestion – if we want to know how well seagrass are growing, we need to measure growth.

9.2 Nuisance macroalgae

The species *Chaetomorpha linum* formed macroalgal blooms at several sites in late spring/ early summer (see Figure 76). The blooms of macroalgae were most likely associated with the supply of nutrients from catchment runoff and the start of warmer temperatures favourable for growth. Sites HTH, PPT and RCK were most affected by the *Chaetomorpha* and it is likely that wind direction played a role in where it accumulated.

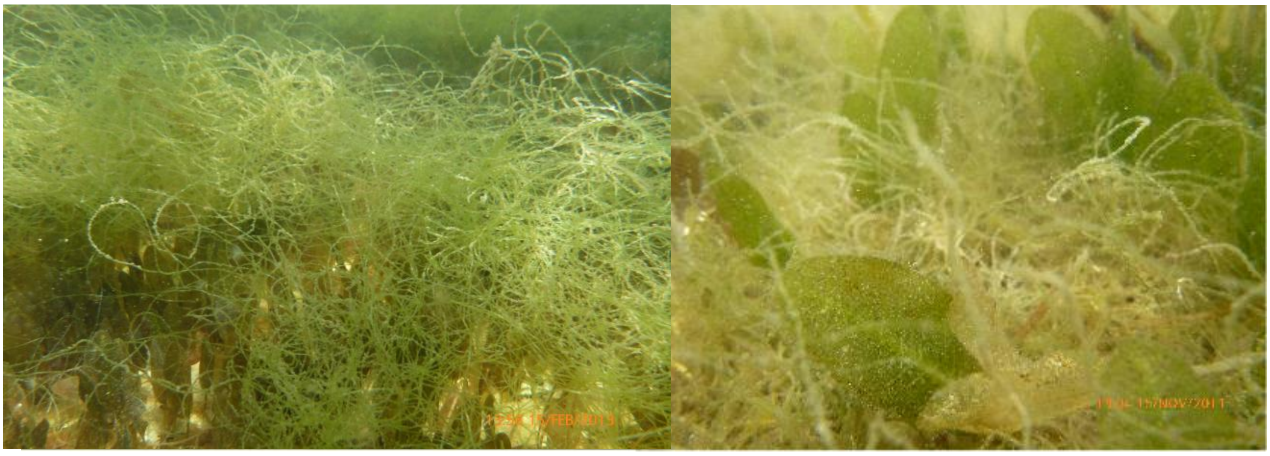


Figure 76 *Examples of* Chaetomorpha linum *accumulations smothering seagrass beds*

These macroalgal accumulations resulted in significant reductions in light and reduced seagrass production. At site HTH (the one most affected by *Chaetomorpha*) seagrass leaves also showed morphological acclimation, with longer leaves produced in the initial stages of shading, combined with a thinning of the seagrass meadow (decrease in leaf density). However, seasonal variability swamps the ability to use morphometric changes as an indicator.

The most significant effect of the *Chaetomorpha* on seagrass was that large macroalgal accumulations in November and December (as was seen at HTH) suppressed the ability of the seagrass to flower and reproduce. While seagrass can reproduce clonally, the annual production of seeds is expected to impart increased resilience to the seagrass population (e.g. through genetic diversity and ability to expand distribution). It seems likely from our study that establishment from seed is more important in some locations than others within the Swan-Canning estuary. Our data suggest that large *Chaetomorpha* accumulations are likely to reduce the annual seed bank, but to date *H. ovalis* seed banks within the Swan-Canning estuary have not been studied (to the authors' knowledge).

9.3 Invasive species

Generally negative effects are observed when non-native species are introduced into seagrass communities (Williams 2007). Several non-native, invasive species were noted in the estuary during the course of this project. The most abundant of these was, by far, the mud snail *Batillaria australis.* This snail is endemic to the east coast of Australia, but is believed to be invasive in Western Australia (only found in the Swan-Canning estuary and Cockburn Sound) (Thomsen & Wernberg 2009). It is staggering to consider the potential effect of these invasive snails on the Swan-Canning ecosystem.

In this study, a median count of more than 1300 snails per square metre was found within the seagrass beds. With an estimated 403 ha of seagrass beds in the estuary, this would suggest the existence of more than 5.2 billion snails within seagrass-vegetated areas. This number does not take into account snails found in bare sand, yet is almost twice the number previously reported by Thomsen & Wernberg (2009).

Sites closest to the river mouth had the greatest number of *B. australis.* Site RCK had the most snails observed in any sample, equating to 6900 snails per square metre. A seasonal trend in the abundance of juveniles was apparent, with more appearing late summer into autumn. This fits with what has been reported for *B. australis* on the east coast, where breeding takes place in summer by cohorts that are two to three years old with few snails surviving to their fourth year (Beechey 2005).

Thomsen & Wernberg (2009) attempted to answer the question of *what impact approximately 3.6 billion snails have in the Swan River.* The estimates included:

- moving 450 000 m^3 of sediment per day
- releasing 18 tonnes of nitrogen per year
- filtering 3 billion litres of water per day
- creating 1.7 billion shells per year
- creating 1.8 million m^2 of hard substratum for the attachment of 36.5 million algal thalli.

Thomsen & Wernberg (2009) also suggested the *B. australis* population may not have reached its climax in 2008–09: certainly our estimate of at least 5.2 billion snails occurring within the seagrass-vegetated areas alone in 2011–12 support the hypothesis that the abundance of this gastropod is still increasing.

Other potentially invasive species were also noted in the sessile communities that attached themselves to the underside of the periphyton plates. Site DLK – near Royal Freshwater Bay Yacht Club – had the greatest diversity of species settled, with an assortment of colonial ascidians, a starfish and possibly some sponges too. Tentative identifications (from photos) of invasive species included the introduced ascidians *Didemnum perlucidum* and *Styela* species and possibly worm casings from a *Hydroides* species (J. McDonald, pers. comm.). In contrast, a less diverse assemblage had formed at PPT site (near UWA) – with predominantly solitary *Styela* species (J. McDonald, pers. comm.). As the Swan-Canning is predominantly a soft-bottom environment, the presence of moorings and boats within Freshwater Bay are likely to contribute to the diversity of species observed at DLK by providing a hard substrate for attachment. Additionally, *Didemnum* species are known to be transported via vessel hulls and ballast, and have been shown to increase in dominance in response to water warming (Smale et al. 2011).

9.4 Implementing the seagrass indicator

Halophila ovalis is a keystone species in the Swan-Canning estuary and is ecologically beneficial. It is the most dominant aquatic benthic vegetation and also a food source for the iconic black swan. Understanding the condition of this species of seagrass also informs on the condition of the estuary as a whole. It is important to monitor seagrass at multiple, integrated scales because a single scale is not sufficient for effective management. We need to understand what habitat is there (extent and distribution), if and how it is changing, and preferably also the mechanisms driving its change. Neckles et al. (2012) promote a hierarchical approach to monitoring seagrasses for conservation, and this approach for the Swan-Canning estuary is outlined in Figure 77.

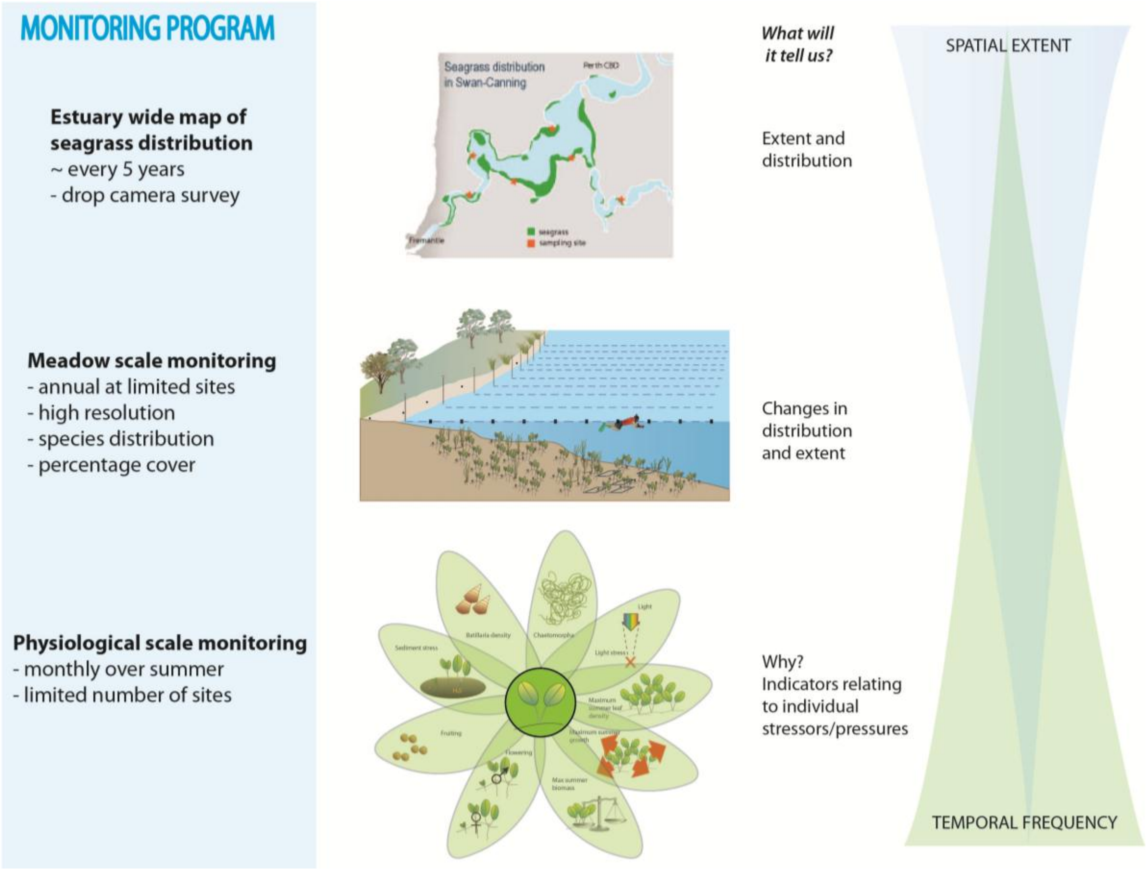


Figure 77 *Hierarchical monitoring framework for understanding seagrass condition in the Swan-Canning estuary*

This study was designed to test mechanistic relationships between the seagrass *H. ovalis* and environmental stressors to allow development of physiological-scale indicators for seagrass condition in the Swan-Canning estuary. These 'process-scale' indicators are designed to work in conjunction with regular seagrass distribution surveys and inform on *why* seagrass cover might be changing. The inclusion of these measures of seagrass condition may enable the detection of change in seagrass performance before large-scale loss of seagrass occurs.

Table 11 outlines our recommendations for what these physiological indicators might look like for an ongoing monitoring program of seagrass condition in the Swan-Canning estuary. Each *indicator* is explained in the text following.

Table 11 *Potential ‘indicators’ and the stress which they indicate*[6]

	Light	Nutrient	Sediment/other
***H. ovalis* productivity**	✓		✓
***Chaetomorpha* survey**	✓		
Seagrass reproduction assessments	✓		
PAR surveys in estuary	✓		
Nutrient sink in leaves		✓	
δ^{15}N in leaves		✓	
Sediment-stress indicator			✓

- Measurement of ***H. ovalis* productivity** is important to determine how the seagrass are actually growing. We see that productivity is unrelated to biomass, so distribution alone is a poor indicator of growth conditions. While this indicator integrates a range of stressors, it is – at the most basic level – the best indication for how seagrass are faring in the estuary. We saw in 2011–12 that the summer peak in production did not peak in a single month, so it would be important to have measures across multiple months. We propose obtaining productivity measures (using the rhizome-tagging method) during the months December to February. To be able to scale productivity on a shoot level to a per-area scale, measurement of apex density and leaf density is also required.
- Surveys of the nuisance macroalga ***Chaetomorpha*** should be carried out in the spring (and early summer) months. This macroalga is clearly responding to nutrient input from the catchment (and start of the warmer months more favourable for growth), however its position within the estuary is likely to be wind-driven (rather than from a localised source of nutrients). The presence of *Chaetomorpha* significantly reduces light to the seagrass and inhibits its ability to photosynthesise, with notable effects on its reproduction.
- The reproductive success of *H. ovalis* was significantly reduced at sites where light was severely limited during November and December by *Chaetomorpha.* We propose **seagrass reproduction assessments** that would involve assessing flowering and fruiting success of the seagrass at peak times (December to February), as well as the resultant seed bank. There are significant gaps in knowledge about the size of the seagrass seed bank, relationship to flowering or fruiting effort, and the persistence of the resulting seed bank over the course of the year.

---

[6] Note salinity stress was not included in this proposed program since salinity was perceived as an attribute of the system rather than a pressure that required specific management action.

- Undertaking regular **surveys of photosynthetically active radiation (PAR)** would provide a valuable insight into how light is changing throughout the estuary (and seasonally). These data could be linked in a predictive way with the seagrass distribution surveys, and inform on the understanding of *H. ovalis* productivity measurements.
- Nutrients were not believed to be either limiting or inhibiting growth in the estuary during this study. However, determining the annual **nutrient sink** for nitrogen and phosphorus bound to seagrass tissue could be a useful estuarine indicator. To do this, we propose analysing seagrass leaves for their nutrient content in February.
- The **stable nitrogen isotope ratio ($\delta^{15}N$)** of leaves showed spatial variability in the estuary, indicating differing local sources of nitrogen (and potentially nitrogen pollution). At present we do not have enough information to fully explain the possible sources, but they might be due to septics, groundwater nitrates or differing contributions from fauna. Further investigation into potential sources would be required to use this indicator fully, but it shows potential.
- Potential is also shown by the ratio observed in leaves and rhizomes of sulfur stable isotope ratio over the percentage sulfur, and we propose this might be a useful **sediment-stress indicator**. Summer growth of seagrass is reduced lower when seagrass leaves and rhizomes exhibit a lower ratio. As changes in sediment condition are an emerging concern for the estuary, further validation of this potential indicator is recommended.

The above metrics provide an excellent basis for establishing physiological indicators for *H. ovalis* in the Swan-Canning estuary. The refinement of this suite of indicators over several years will be essential given the high inter-annual variability of peak seagrass production previously observed (Section 7.2), which relates strongly to climatic conditions. The seagrass indicators are designed to be implemented in conjunction with regular seagrass distribution surveys and will provide an early warning signal, enabling guidance for the implementation of policies to manage estuary health.

Appendices

Appendix A — Further statistical analysis details

1. Data were square-root transformed and normalised. Resemblance matrix based on Euclidian distance.
2. Restricted permutations of main effects (ANOVA) for sediment data, using R. NS = not significant. Data was not transformed as Levene's test showed equal variance. Unrestricted permutations were used to test for the interaction.

	TN	**TOC**	**TP**	**AVS**	**Moisture**
	p value	*p value*	*p value*	*p value*	*p value*
Site	NS	NS	<0.001	<0.01	NS
Month	<0.001	<0.001	NS	NS	<0.001
Site x month	<0.001	<0.01	<0.005	NS	NS

3. By importing sample data into PRIMER 6 and telling it the samples were variables (i.e. the converse of what is actually the case), the SIMPROF test can be used to determine whether variables are statistically different from each other in the way they behave in multi-variate space (B. Clarke, pers. comm. 2012). SIMPROF analysis on sediment variables suggested that TN and AVS were not different, and TP and moisture were not different.
4. Data for leaf production rate, rhizome extension rate and total production were analysed independently at the individual tagged rhizome level. Data pre-treatment as per Appendix A-1. Univariate two-way ANOVAs were performed (using PERMANOVA with permutations restricted under a reduced model, n=99999). Results are shown below.

	Leaf production rate (new leaves. apex^{-1}d^{-1})		**Rhizome extension rate (mm apex^{-1}d^{-1})**		**Total production rate (mg apex^{-1}d^{-1})**	
	pseudo-F	*p value*	pseudo-F	*p value*	pseudo-F	*p value*
Site	3.6	<0.01	4.1	<0.01	3.7	<0.01
Month	21	<0.00001	31	<0.00001	10	<0.00001
Site x month	2.9	<0.00001	5.9	<0.00001	2.9	<0.00001

5. Using PRIMER 6, production data for each site x month combination (which itself was an average of 8–30 individual shoot measurements) were square-root transformed and normalised, and a resemblance matrix determined using Euclidian distance. Variables included in the multi-variate analysis were: leaf formation rate, rhizome extension rate, above- and below-ground and total production rate. A cluster analysis with SIMPROF test was also carried out and a factor added for the samples determined to be significantly different from each other using the SIMPROF test. This

resemblance matrix was then compared with an environmental variables resemblance matrix using the RELATE function. The environmental variables were normalised before a resemblance matrix determined by Euclidian distance and included: the depth of site in mAHD, the average water depth overlying at low and high tides during the month of sampling, in-air PAR, average peak light, median peak light, percentage surface irradiance as PAR, average daily temperature, minimum daily temperature, maximum daily temperature, hours >I_{sat}, and average temperature x hours >$I_{sat.}$.

6. Univariate analysis of seagrass chemical analysis (for five sites x eight months) by two-way ANOVA with site and month as factors using R, where restricted permutations used to test for main effects. Data only transformed for %S in root, as Levene's test showed equal variances for all other variables.

	δ^{13}C	**δ^{15}N**	**%C**	**%N**	**%P**	
Site	<0.001	<0.001	NS	<0.001	<0.001	
Month	<0.001	<0.001	<0.001	<0.001	<0.001	
Site x month	<0.001	<0.001	<0.001	<0.001	<0.001	
	δ^{34}S leaf	**δ^{34}S rhizome**	**δ^{34}S root**	**%S leaf**	**%S rhizome**	**%S root**
Site	NS	NS	<0.001	<0.001	NS	NS
Month	<0.001	<0.001	<0.01	<0.01	<0.001	<0.001
Site x month	<0.001	<0.001	NS	<0.001	<0.001	NS

Univariate analysis of seagrass chemical analysis (for six sites x five months) by two-way ANOVA with site and month as factors using R, where restricted permutations used to test for main effects. Data only transformed for %S in root, as Levene's test showed equal variances for all other variables.

	δ^{13}C	**δ^{15}N**	**%C**	**%N**	**%P**	
Site	<0.001	<0.001	<0.001	<0.001	<0.001	
Month	<0.001	<0.001	<0.001	<0.001	<0.001	
Site x month	<0.001	<0.01	<0.001	<0.001	<0.001	
	δ^{34}S leaf	**δ^{34}S rhizome**	**δ^{34}S root**	**%S leaf**	**%S rhizome**	**%S root**
Site	<0.001	<0.001	<0.05	<0.001	NS	<0.05
Month	<0.001	NS	<0.01	<0.001	<0.001	NS
Site x month	<0.001	<0.05	<0.05	<0.01	<0.001	NS

7. Fsulfide data for leaves, roots and rhizomes was square-root transformed, normalised and Euclidian distance resemblance matrix calculated. A PERMANOVA with site and month as fixed factors, showed site: pseudo-F=9.9 $p<0.0001$, month: pseudo-F=10.4 $p<0.0001$ and site x month: pseudo-F= 2.4 $p<0.0001$.

Appendix B — Further detail regarding methods

1. The isotopic $\delta^{34}S$ ratio of the reduced inorganic sulfur within sediment was determined on sulfide extracted using the CRS method (as per the QASSIT Laboratory guidelines). Sulfide gas generated was trapped in a solution containing zinc acetate, with sediment samples repeatedly gassed into the same tube until the solution was milky. Tubes were then centrifuged for 10 minutes at 4000 rpm. 50 mL of Milli-Q water was used to wash the precipitate (centrifuging and decanting supernatant), and rinsing was repeated three times. Precipitate was dried at 105° C until dry.
2. Sulfate within filtered water was precipitated by the addition of a solution containing $BaCl_2$. Once sufficient $BaSO_4$ was obtained, tubes containing precipitate (and trapping solution) were centrifuged for 10 minutes at 4000 rpm. 50 mL of Milli-Q water was used to wash the precipitate (centrifuging and decanting supernatant), and rinsing was repeated three times. Precipitate was dried at 105° C until dry.
3. Precipitates were weighed into tin foil capsules, and analysed by EA-IRMS (Flash Elemental Analyser and Isotope Ratio Mass Spectrometer, Delta V plus, both from Thermo Fisher) using a method based on the recent paper: Fry, B 2007, *Rapid. Com. Mass. Spect.*, 21, 750–756. As quality control, an internal standard (sulfanilamide) was run every 10 samples. The internal standard had previously been analysed against an international standard, Ag_2S (IAEA S2). The precision of the sample measurement was 0.2 ppt.
4. Plant sample was analysed for sulfur, carbon and nitrogen isotope analysis by EA-IRMS (EA from Thermo Scientific and Delta V Advantage Isotope Ratio Mass Spectrometer at the Southern Denmark University. For sulfur analysis, tin capsules containing either reference material or dried, ground sample with a catalyst of vanadium pentoxide are dropped into a furnace at 1020 °C and combusted in the presence of oxygen. Helium is used as a carrier gas stream, and water is removed on a magnesium perchlorate column.

Shortened forms

DIN	dissolved inorganic nitrogen
LOR	limit of reporting
MDS	multi-dimensional scaling
PAR	photosynthetically active radiation
PCA	principal component analysis
RIS	reduced inorganic sulfur
SRP	soluble reactive phosphorus
TN	total nitrogen
TP	total phophorus

Glossary

Ascidian	A sessile marine invertebrate, such as a sea squirt
Physiological	Relating to the physiology or normal functioning of an organism
Endemic	Native to a local area or county
Ephemeral	Short-lived, transitory
Rhizomes	Horizontal stem within the sediment from which roots are produced below and shoots produced above
Epiphytic	A plant (or algae) which uses another living thing (e.g. plant) as a surface to grow on
Nutrient sink	A store of nutrients
Eutrophication	Water pollution relating to excess supply of nutrients
Phytoplankton	Microscopic plant-like organisms
Macroalgae	Algae which are large enough to see with the naked eye, can grow attached to a substrate, or floating within the water
Hydrology	Science dealing with the water on land (or under the earth’s surface) – its properties, laws, geographical distribution etc.
Biota	The animal or plant life of a region
Morphometric	Measurements of the shape and/or dimensions of an organism
Anthropogenic	Caused by human beings
Deoxygenation	To remove oxygen from
Stratification	The process of forming layers (e.g. horizontal layers of water with different physical properties)
Bottom water	Lowest layer of water
Quadrat	Square measure used to select an area to study the organisms within it
Biomass	The quantity of living matter, usually expressed as weight per unit area
Terrestrial	Pertaining to the land or earth (as distinct from the water)
Isotope	Two or more forms of an element, which have the same number of protons, but different numbers of neutrons, therefore have different atomic weights, but almost identical chemical properties
Isotopic discrimination	Processes which preferentially use one isotope over another
Periphyton	Epiphytic growth but on non-living substrates
Epifaunal	Aquatic animals that live on the bottom surface of a substrate
Stoichiometry	The relative proportions (by atom) in which elements form compounds
Invasive species	Introduced or exotic species

References

Ackerman, JD 2006, Sexual reproduction of seagrasses: pollination in the marine context, in AWD Larkum, RJ Orth & CM Duarte (eds) *Seagrasses: biology, ecology and conservation,* Dordrecht. Springer.

Adams, SM & Bortone, SA 2005, Future directions for estuarine indicator research, in SA Bortone (ed.) *Estuarine Indicators,* Boca Raton, 503-506. CRC Press.

Beechey, D 2005, The seashells of New South Wales, *http://seashellsofnsw.org.au/Batillariidae/Pages/Batillaria_australis.htm*.

Beer, S, Björk, M, Hellblom, F & Axelsson, L 2002, 'Inorganic carbon utilization in marine angiosperms (seagrasses)', *Functional Plant Biology,* vol. 29**,** pp. 349-354.

Benjamin, KJ, Walker, DI, McComb, AJ & Kuo, J 1999, 'Structural response of marine and estuarine plants of *Halophila ovalis* (R. Br.) Hook. f. to long-term hyposalinity', *Aquatic Botany,* vol. 64**,** pp. 1-17.

Bortone, SA 2005, The quest for the "perfect" estuarine indicator: An introduction, in SA Bortone (ed.) *Estuarine indicators,* Boca Ratom, 1-3. CRC Press.

Burkholder, JM, Tomasko, DA & Touchette, BW 2007, 'Seagrasses and eutrophication', *Journal of Experimental Marine Biology and Ecology,* vol. 350**,** pp. 46-72.

Cabaço, S & Santos, R 2007, 'Effects of burial and erosion on the seagrass *Zostera noltii*', *Journal of Experimental Marine Biology and Ecology,* vol. 340**,** pp. 204-212.

Carruthers, TJB, Dennison, WC, Kendrick, GA, Waycott, M, Walker, DI & Cambridge, ML 2007, 'Seagrasses of south-west Australia: A conceptual synthesis of the world's most diverse and extensive seagrass meadows', *Journal of Experimental Marine Biology and Ecology,* vol. 350**,** pp.

Casares, FA & Creed, JC 2008, 'Do small seagrasses enhance density, richness, and diversity of macrofauna?', *Journal of Coastal Research,* vol.**,** pp. 790-797.

Choney, G 2012, The impact of Black swan (*Cygnus atratus*) grazing on the seagrass *Halophila ovalis* in the Lower Swan River estuary, *School of Natural Sciences*, Edith Cowan University.

Cole, ML, Kroeger, KD, McClelland, JW & Valiela, I 2005, Macrophytes as indicators of land-derived wastewater: Application of a δ^{15}N method in aquatic systems, *Water Resources Research*, W01014. doi:10.1029/2004WR003269

Collier, CJ, Waycott, M & McKenzie, LJ 2012, 'Light thresholds derived from seagrass loss in the coastal zone of the northern Great Barrier Reef, Australia', *Ecological Indicators,* vol. 23**,** pp. 211-219.

Connell, EL 1999, Interaction of *Halophila ovalis* (R. Br.) Hook f. meadows with nutrient cycling of the water column and sediment porewater in the shallows of the Swan Canning estuary, *Department of Botany,* Perth, University of Western Australia.

Connell, EL, Colmer, TD & Walker, DI 1999, 'Radial oxygen loss from intact roots of *Halophila ovalis* as a function of distance behind the root tip and shoot illumination', *Aquatic Botany,* vol. 63**,** pp. 219-228.

Connell, EL & Walker, DI 2001, 'Nutrient cycling associated with the seagrass *Halophila ovalis* in the Swan-Canning Estuary based on seasonal variations in biomass and tissue nutrients', *Hydrological Processes,* vol. 15**,** pp. 2401-2409.

Cruz-Palacios, V & van Tussenbroek, BI 2005, 'Simulation of hurricane-like disturbances on a Caribbean seagrass bed', *Journal of Experimental Marine Biology and Ecology,* vol. 324**,** pp. 44-60.

Dale, VH & Beyeler, SC 2001, 'Challenges in the development and use of ecological indicators', *Ecological Indicators,* vol. 1**,** pp. 3-10.

Dawson, LA & Dennison, WC 1996, 'Effects of ultraviolet and photosynthetically active radiation on five seagrass species', *Marine Biology,* vol. 125**,** pp. 629-638.

De Villèle, X & Verlaque, M 1995, 'Changes and degradation in a *Posidonia oceanica* bed invaded by the introduced tropical alga *Caulerpa taxifolia* in the north western Mediterranean', *Botanica Marina,* vol. 38**,** pp. 79-87.

den Hartog, C & Kuo, J 2006, Taxonomy and biogeography of seagrasses, in AWD Larkum, RJ Orth & CM Duarte (eds) *Seagrasses: Biology, Ecology and Conservation,* Dordrecht, 1-23. Springer.

Dennison, WC & Alberte, RS 1985, 'Role of daily light period in the depth distribution of *Zostera marina* (eelgrass)', *Marine Ecology Progress Series,* vol. 25**,** pp. 51-61.

Devereux, R, Yates, DF, Aukamp, J, Quarles, RL, Jordan, SJ, Stanley, RS & Eldridge, PM 2011, 'Interactions of *Thalassia testudinum* and sediment biogeochemistry in Santa Rosa Sound, NW Florida', *Marine Biology Research,* vol. 7**,** pp. 317-331.

Duarte, CM 1991, 'Seagrass depth limits', *Aquatic Botany,* vol. 40**,** pp. 363-377.

Eklöf, JS, McMahon, K & Lavery, PS 2009, 'Effects of multiple disturbances in seagrass meadows: shading decreases resilience to grazing.', *Marine and Freshwater Research,* vol. 60**,** pp. 1317-1327.

Folke, C, Carpenter, S, Walker, B, Scheffer, M, Elmqvist, T, Gunderson, L & Holling, C 2004, 'Regime shifts, resilience, and biodiversity in ecosystem management', *Annual Review of Ecology, Evolution, and Systematics,* vol.**,** pp. 557-581.

Fourqurean, JW, Duarte, CM, Kennedy, H, Marbà, N, Holmer, M, Mateo, MA, Apostolaki, ET, Kendrick, GA, Krause-Jensen, D & McGlathery, KJ 2012a, 'Seagrass ecosystems as a globally significant carbon stock', *Nature Geoscience,* vol. 5**,** pp. 505-509.

Fourqurean, JW, Kendrick, GA, Collins, LS, Chambers, RM & Vanderklift, MA 2012b, 'Carbon, nitrogen and phosphorus storage in subtropical seagrass meadows: examples from Florida Bay and Shark Bay', *Marine and Freshwater Research,* vol. 63**,** pp. 967-983.

Fourqurean, JW, Moore, TO, Fry, B & Hollibaugh, JT 1997, 'Spatial and temporal variation in C:N:P ratios, d15N, and d13C of eelgrass *Zostera marina* as indicators of ecosystem processes, Tomales Bay, California, USA', *Marine Ecology Progress Series,* vol. 157**,** pp. 147-157.

Frederiksen, MS, Holmer, M, Borum, J & Kennedy, H 2006, 'Temporal and spatial variation of sulfide invasion in eelgrass (*Zostera marina*) as reflected by its sulfur isotope composition', *Limnology and Oceanography,* vol. 51**,** pp. 2308-2318.

Frederiksen, MS, Holmer, M, Pérez, M, Invers, O, Ruiz, JM & Knudsen, B 2008, 'Effect of increased sediment sulfide concentrations on the composition of stable sulfur isotopes (δ^{34}S) and sulfur accumulation in the seagrasses *Zostera marina* and *Posidonia oceanica*', *Journal of Experimental Marine Biology and Ecology,* vol. 358**,** pp. 98-109.

Fry, B 2006, Using stable isotope tracers, *Stable isotope ecology,* New York, 40-75. Springer

Gavin, NM & Durako, MJ 2011, 'Localization and antioxidant capacity of flavonoids from intertidal and subtidal *Halohpila johnsonii* and *Halophila decipiens*', *Aquatic Botany,* vol. 95**,** pp. 242-247.

Grice, AM, Loneragan, NR & Dennison, WC 1996, 'Light intensity and the interactions between physiology, morphology and stable isotope ratios in five species of seagrass', *Journal of Experimental Marine Biology and Ecology,* vol. 195**,** pp. 91-110.

Hemminga, MA 1998, 'The root/rhizome system of seagrasses: an asset and a burden', *Journal of Sea Research,* vol. 39**,** pp. 183-196.

Hemminga, MA, Harrison, PG & van Lent, F 1991, 'The balance of nutrient losses and gains in seagrass meadows', *Marine Ecology Progress Series,* vol. 71**,** pp. 85-96.

Hemminga, MA, Marbà, N & Stapel, J 1999, 'Leaf nutrient resorption, leaf lifespan and the retention of nutrients in seagrass systems', *Aquatic Botany,* vol. 65**,** pp. 141-158.

Hemminga, MA & Mateo, MA 1996, 'Stable carbon isotopes in seagrasses: variability in ratios and use in ecological studies', *Marine Ecology Progress Series,* vol. 140**,** pp. 285-298.

Hillman, K 1985, The production ecology of the seagrass *Halophila ovalis* (R. Br.) Hook, in the Swan/Canning Estuary, Western Australia, University of Western Australia.

Hillman, K, McComb, AJ & Walker, DI 1995, 'The distribution, biomass and primary production of the seagrass *Halophila ovalis* in the Swan/Canning Estuary, Western Australia', *Aquatic Botany,* vol. 51**,** pp. 1-54.

Holmer, M, Duarte, CM & Marbà, N 2003, 'Sulfur cycling and seagrass (*Posidonia oceanica*) status in carbonate sediments', *Biogeochemistry,* vol. 66**,** pp. 223-239.

Holmer, M, Duarte, CM & Marbà, N 2005a, 'Iron additions reduce sulfate reduction rates and improve seagrass growth on organic-enriched carbonate sediments', *Ecosystems* vol. 8**,** pp. 721-730.

Holmer, M, Frederiksen, MS & Møllegaard, H 2005b, 'Sulfur accumulation in eelgrass (*Zostera marina*) and effect of sulfur on eelgrass growth', *Aquatic Botany,* vol. 81**,** pp. 367-379.

Holmer, M & Kendrick, GA 2013, 'High sulfide intrusion in five temperate seagrasses growing under contrasting sediment conditions', *Estuaries and Coasts,* vol. 36**,** pp. 116-126.

Jacobs, SWL, Les, DH & Moody, ML 2006, 'New combinations in Australasian *Zostera* (Zosteraceae)', *Telopea,* vol. 11**,** pp. 127-128.

Jensen, SI, Kühl, M, Glud, RN, Jørgensen, LB & Priemé, A 2005, 'Oxic microzones and radial oxygen loss from roots of *Zostera marina*', *Marine Ecology Progress Series,* vol. 293**,** pp. 49-58.

Jordan, SJ & Smith, LM 2005, Indicators of ecosystem integrity for estuaries, in SA Bortone (ed.) *Estuarine Indicators,* Roca Baton, 467-480. CRC Press.

Kilminster, K, Forbes, V & Holmer, M 2014, 'Development of a ‘sediment-stress’ functional-level indicator for the seagrass *Halophila ovalis*', *Ecological Indicators,* vol. 36**,** pp. 280-289.

Kilminster, KL, Walker, DI, Thompson, PA & Raven, JA 2006, 'Limited nutritional benefit of the seagrass *Halophila ovalis*, in culture, following sediment organic matter enrichment', *Estuarine, Coastal and Shelf Science,* vol. 68**,** pp. 675-685.

Kilminster, KL, Walker, DI, Thompson, PA & Raven, JA 2008, 'Changes in growth, internode distance and nutrient concentrations of the seagrass *Halophila ovalis* with exposure to sediment sulphide', *Marine Ecology Progress Series,* vol. 361**,** pp. 83-91.

Kirk, JTO 1983, *Light and photosynthesis in aquatic ecosystems*, Cambridge University Press, Cambridge.

Koch, MS, Schopmeyer, SA, Kyhn-Hansen, C & Madden, CJ 2007, 'Synergistic effects of high temperature and sulfide on tropical seagrass', *Journal of Experimental Marine Biology and Ecology,* vol. 341**,** pp. 91-101.

Kuo, J & Kirkman, H 1992, 'Fruits, seeds and germination in the seagrass *Halophila ovalis* (Hydrocharitaceae) ', *Botanica Marina,* vol. 35**,** pp. 197-204.

Lee, K-S, Park, SR & Kim, YK 2007, 'Effects of irradiance, temperature and nutrients on growth dynamics of seagrasses: A review', *Journal of Experimental Marine Biology and Ecology,* vol. 350**,** pp. 144-175.

Lee, K-S, Short, FT & Burdick, DM 2004, 'Development of a nutrient pollution indicator using the seagrass, *Zostera marina*, along nutrient gradients in three New England estuaries', *Aquatic Botany,* vol. 78**,** pp. 197-216.

Longstaff, BJ & Dennison, WC 1999, 'Seagrass survival during pulsed turbidity events: the effects of light deprivation on the seagrasses *Halodule pinifolia* and *Halophila ovalis*', *Aquatic Botany,* vol. 65**,** pp. 105-121.

Longstaff, BJ, Loneragan, NR, O'Donohue, MJ & Dennison, WC 1999, 'Effects of light deprivation on the survival and recovery of the seagrass *Halophila ovalis* (R. Br) Hook', *Journal of Experimental Marine Biology and Ecology,* vol. 234**,** pp. 1-27.

Marbà, N, Holmer, M, Gacia, E & Barrón, C 2006, Seagrass beds and coastal biogeochemistry, in AWD Larkum, RJ Orth & CM Duarte (eds) *Seagrasses: biology, ecology and conservation,* Dordrecht, 135-157. Springer.

Martinez-Crego, B, Verges, A, Alcoverro, T & Romero, J 2008, 'Selection of multiple seagrass indicators for environmental monitoring', *Marine Ecology Progress Series,* vol. 361**,** pp. 93-109.

Mascaró, O, Valdemarsen, T, Holmer, M, Perez, M & Romero, J 2009, 'Experimental manipulation of sediment organic content and water column aeration reduces *Zostera marina* (eelgrass) growth and survival', *Journal of Experimental Marine Biology and Ecology,* vol. 373**,** pp. 26-34.

Mateo, MA, Cebrián, J, Dunton, K & Mutchler, T 2006, Carbon flux in seagrass ecosystems, in AWD Larkum, RJ Orth & CM Duarte (eds) *Seagrasses: Biology, Ecology and Conservation,* Dordrecht, 159-192. Springer.

McClelland, JW, Valiela, I & Michener, RH 1997, 'Nitrogen-stable isotope signatures in estuarine food webs: A record of increasing urbanization in coastal watersheds', *Limnology and Oceanography,* vol. 42**,** pp. 930-937.

McMahon, K, Collier, CJ & Lavery, PS 2013, 'Identifying robust bioindicators of light stress in seagrasses: A meta-analysis', *Ecological Indicators,* vol. 30**,** pp. 7-15.

McMillan, C 1983, 'Morphological diversity under controlled conditions for the *Halophila ovalis-H. minor* complex and the *Halodule univervis* complex from Shark Bay, Western Australia', *Aquatic Botany,* vol. 17**,** pp. 29-42.

Neckles, HA, Kopp, BS, Peterson, BJ & Pooler, PS 2012, 'Integrating scales of seagrass monitoring to meet conservation needs', *Estuaries and Coasts,* vol. 35**,** pp. 23-46.

Nice, HE 2009, *A baseline study of contaminants in the sediments of the Swan and Canning estuaries, Water science technical series, Report No. 6,*, Department of Water, Western Australia.

Novak, AB & Short, FT 2011, 'UV-B induces leaf reddening and supports photosynthesis in the seagrass *Thalassia testudinum*', *Journal of Experimental Marine Biology and Ecology,* vol. 409**,** pp. 136-142.

Ooi, JLS, Kendrick, GA & Van Niel, KP 2011, 'Effects of sediment burial on tropical ruderal seagrasses are moderated by clonal integration', *Continental Shelf Research,* vol. 31**,** pp. 1945-1954.

Orth, RJ, Carruthers, TJB, Dennison, WC, Duarte, CM, Fourqurean, JW, Heck, JR, Hughes, AR, Kendrick, GA, Kenworthy, WJ, Olyarnik, S, Short, FT, Waycott, M & Williams, SL 2006a, 'A global crisis for seagrasses', *Bioscience* vol. 56**,** pp. 897-996.

Orth, RJ, Harwell, MC & Inglis, GJ 2006b, Ecology of seagrass seeds and seagrass dispersal processes, in AWD Larkum, RJ Orth & CM Duarte (eds) *Seagrasses: Biology, Ecology and Conservation,* Dordrecht, 111-133. Springer.

Orth, RJ, Heck, JR & van Montfrans, J 1984, 'Faunal communities in seagrass beds: A review of the influence of plant structure and prey characteristics on predator-prey relationships', *Estuaries,* vol. 7**,** pp. 339-350.

Pedersen, O, Binzer, T & Borum, J 2004, 'Sulphide intrusion in eelgrass (*Zostera marina* L.)', *Plant, Cell and Environment,* vol. 27**,** pp. 595-602.

Pérez, M, Invers, O, Ruiz, JM, Frederiksen, MS & Holmer, M 2007, 'Physiological responses of the seagrass *Posidonia oceanica* to elevated organic matter content in sediments: An experimental assessment', *Journal of Experimental Marine Biology and Ecology,* vol. 344**,** pp. 149-160.

Prange, JA & Dennison, WC 2000, 'Physiological responses of five seagrass species to trace metals', *Marine Pollution Bulletin,* vol. 41**,** pp. 7-12.

Ralph, PJ, Durako, MJ, Enriquez, S, Collier, CJ & Doblin, MA 2007, 'Impact of light limitation on seagrasses', *Journal of Experimental Marine Biology and Ecology,* vol. 350**,** pp. 176-193.

Ralph, PJ, Tomasko, D, Moore, KA, Seddon, S & Macinnis-Ng, CMO 2006, Human impacts on seagrasses: Eutrophication, sedimentation and contamination, in AWD Larkum, RJ Orth & CM Duarte (eds) *Seagrasses: Biology, Ecology and Conservation,* Dordrecht 567-593. Springer.

Rasheed, MA 2004, 'Recovery and succession in a multi-species tropical seagrass meadow following experimental disturbance: the role of sexual and asexual reproduction', *Journal of Experimental Marine Biology and Ecology,* vol. 310**,** pp. 13-45.

Raven, JA & Scrimgeour, CM 1997, 'The influence of anoxia on plants of saline habitats with special reference to the sulphur cycle', *Annals of Botany,* vol. 79 (Supplement A)**,** pp. 79-86.

Rollón, RN, de Ruyter van Steveninck, ED & van Vierssen, W 2003, 'Spatio-temporal variation in sexual reporduction of the tropical seagrass *Enhalus acoroides* (L. f.) Royle in Cape Bolinao, NW Philippines', *Aquatic Botany,* vol. 76**,** pp. 339-354.

Shepherd, SA, McComb, AJ, Bulthuis, DA, Neverauskas, V, Steffensen, DA & West, R 1989, Decline of Seagrasses, in AWD Larkum, AJ McComb & SA Shepherd (eds) *Biology of Seagrasses,* Amsterdam, 346-356. Elsevier.

Short, FT & Duarte, CM 2001, Methods for the measurement of seagrass growth and production, in FT Short & RG Coles (eds) *Global seagrass research methods,* Amsterdam. Elsevier Science B.V.

Smale, DA, Wernberg, T, Peck, LS & A., BDK 2011, 'Turning on the heat: Ecological response to simulated warming in the sea', *PLoS One,* vol. 6**,** pp. e16050.

Somerfield, PJ & Clarke, KR 2013, 'Inverse analysis in non-parametric multivariate analyses: distinguishing groups of associated species which covary coherently across samples', *Journal of Experimental Marine Biology and Ecology,* vol. 449, pp. 261-273.

Sterner, RW & Elser, JJ 2002, Ecological stoichiometry, Princeton, USA, 80-134. Princeton University Press.

Terrados, J, Duarte, CM, Kamp-Nielsen, L, Agawin, NSR, Gacia, E, Lacap, D, Fortes, MD, Borum, J, Lubanski, M & Greve, T 1999, 'Are seagrass growth and survival constrained by the reducing conditions of the sediment?', *Aquatic Botany,* vol. 65, pp. 175-197.

Thomsen, MS & Wernberg, T 2009, *Drift algae, invasive snails and seagrass health in the Swan River: patterns, impacts and linkages*, Perth, Center for Marine Ecosystems Research, Edith Cowan University.

Trust, BA & Fry, B 1992, 'Stable sulfur isotopes in plants: a review', *Plant, Cell & Environment,* vol. 15, pp. 1105-1110.

Valentine, JF & Duffy, JE 2006, The central role of grazing in seagrass ecology, in AWD Larkum, RJ Orth & CM Duarte (eds) *Seagrasses: Biology, Ecology and Conservation,* Dordrecht, 463-501. Springer.

Walker, DI 1999, The Marine Angiosperms, *Flora of Australia,* 2nd ed ed. Canberra, 420-435. Australian Biological Resources Study CSIRO.

Walker, DI, Dennison, W & Edgar, G 1999, Status of Australian seagrass research and knowledge, in A Butler & P Jernakoff (eds) *Seagrass in Australia: strategic review and development of a R & D plan,* Collingwood, Victoria, Australia, 1-24. CSIRO Publishing.

Walker, DI & McComb, AJ 1992, 'Seagrass degradation in Australian coastal waters', *Marine Pollution Bulletin,* vol. 25, pp. 191-195.

Welsh, DT, Bourguès, S, de Wit, R & Herbert, RA 1996, 'Seasonal variations in nitrogen-fixation (acetylene reduction) and sulphate-reduction rates in the rhizosphere of *Zostera noltii*: nitrogen fixation by sulphate-reducing bacteria', *Marine Biology,* vol. 125, pp. 619-628.

Williams, S 2007, 'Introduced species in seagrass ecosystems: Status and concerns', *Journal of Experimental Marine Biology and Ecology,* vol. 350, pp. 89-110.

Yaakub, SM, Chen, E, Bouma, TJ, Erftemeijer, PLA & Todd, PA 'Chronic light reduction reduces overall resilience to additional shading stress in the seagrass Halophila ovalis', *Marine Pollution Bulletin,* vol., pp.

Yamamuro, M, Kayanne, H & Yamano, H 2003, 'δ^{15}N of seagrass leaves for monitoring anthropogenic nutrient increases in coral reef ecosystems', *Marine Pollution Bulletin,* vol. 46, pp. 452-458.

WaterScience
technical series

Department of Water
168 St Georges Terrace, Perth, Western Australia
PO Box K822 Perth Western Australia 6842
Phone: (08) 6364 7600
Fax: (08) 6364 7601
www.water.wa.gov.au

9082 30 1014