TROPHIC DYNAMICS IN AUSTRALIAN SEAGRASS BEDS: UNRAVELLING LINKAGES FROM NUTRIENTS TO FISH

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CERTIFICATE OF AUTHORSHIP/ORIGINALITY

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Paul H. York

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General Abstract

The study of food webs and trophic dynamics has been a major endeavour for biologists for over half a century. The major aim of trophic ecology has been to explain the complex structures and interaction of food webs using simple models and generalities. A key debate in this research field has been the extent to which trophic structures of communities are driven by resource or consumer control. This issue has important implications for the management of marine ecosystems with anthropogenic disturbance rapidly altering bottom-up (e.g. nutrient enrichment) and top-down (e.g. fisheries exploitation) processes. To enhance understanding of bottom up and top-down influences in productive estuaries, this study investigated a prominent four level food chain (epiphytic algae, mesograzers, juvenile fish, and piscivorous fish) in seagrass habitats. Large-scale mensurative field experiments were combined with manipulative experiments to gain an improved understanding of changes in biomass and community structure of trophic levels under different nutrient loading and to determine the type of functional response driving these changes.

In general, epiphyte and grazer biomass were greater in developed catchments with higher nutrient loads during long-term field sampling of systems approaching equilibrium. However, a decoupling from the epiphyte trophic pathway was evident in higher trophic levels. The biomass of carnivorous juvenile fish and large piscivores displayed no significant change across the nutrient gradient of this study. This pattern indicates that a ratio-dependent functional response would be most appropriate for modelling the lower trophic levels and their responses to nutrient enrichment. However, more complex models taking into account other factors such as trophic transfer and subsidy are required for models across all four trophic levels.

Short-term experiments involving epiphytes, grazers and juvenile fish that manipulated nutrient levels and predation rates often behaved in ways that contradicted the patterns described above for long-term studies. In particular, manipulative studies showed strong evidence for top-down control sometimes resulting in lower or similar levels of epiphytes in enriched plots compared to ambient controls.

The structure of fish assemblages was influenced by the nutrient status of the waters they inhabited. Assemblages from developed and undeveloped catchments separated into distinct communities with higher abundances of fish in low nutrient waters. The species that contributed most to these differing assemblages were small pelagic carnivores (*Ambassis jacksoniensis* and *Redigobius macrostoma*). These species appeared to be sensitive to poor water quality making them candidates for bio-indicators of anthropogenic disturbance in catchments.

Enclosure experiments and mensurative field sampling found that growth, survival and condition of juvenile trumpeter (*Pelates sexlineatus*) were greater under ambient nutrient levels despite increased prey items in nutrient enriched sites. This suggests that the positive indirect effects of increased trophic support from nutrient enrichment may have been countered by direct negative effects of toxicity either from the nutrients in their dissolved forms of ammonia; nitrate; or from other anthropogenic pollutants such as metals or hydrocarbons.

Overall, nutrient enrichment of coastal waters in south-eastern Australia promoted epiphyte growth that may compete with seagrasses for light, causing stress and possible declines in seagrass health and distribution. My study, however found greater seagrass biomass at some sites with nutrient enrichment suggesting moderate increases to nitrogen-limited systems may be beneficial to seagrasses. It also appears that the increase in primary productivity associated with nutrient loading did not translate into an increase in biomass of juvenile fish or piscivores in seagrass habitats, providing no benefit to fisheries production. Given the potential for nutrient enrichment to alter trophic structure in coastal waters and to have negative impacts on seagrass health it is recommended that an integrated system of management be implemented. This should include further targeted research for a better understanding of seagrass trophic systems, monitoring of environmental indicators that alert catchment managers to early signs of eutrophication, remediation of existing eutrophic systems through nutrient reduction mechanisms and planning policies that identify and afford greater protection to ecologically-important habitats such as seagrass; and estuaries with high residence times that are more vulnerable to eutrophication.

Chapter 1

INTRODUCTION

FOOD WEBS, TROPHIC CASCADES & BOTTOM-UP FORCING

The study of food webs and trophic dynamics has been a major endeavour of biologists since early last century (eg. Elton 1927; Lindeman 1942; Odum and Odum 1955). Food webs are complex models describing the trophic interactions between organisms within natural systems (Pimm et al. 1991). Ecologists have tried to unravel and explain the complexity of food webs by consigning organisms to trophic levels, examining large-scale processes and interactions that structure communities, and by developing simple models that describe and predict how these processes work in nature (Hairston and Hairston 1997).

Early theories of food web dynamics assumed that populations were controlled from below by resource limitation (e.g. food and habitat). In 1960 a seminal paper known as the Green World Hypothesis (GWH) changed this assumption by suggesting that forces from above (predation and grazing) were equally important in structuring communities (Hairston et al. 1960). The GWH stated that in a tri-trophic system, seen in most terrestrial environments, carnivores, primary producers and decomposers were controlled by competition for resources while herbivores were seldom food limited and were controlled by predation. Later, Fretwell (1977) theorised that as productivity increased so did food chain length. Given that the top predator is always limited by resources, food chains of odd and even lengths alternated between grazer limited and producer limited respectively. This theory was developed and tested (Oksanen et al. 1981) and became known as the Exploitative Ecosystem Hypothesis (EEH).

Since the development of GWH and EEH, a great deal of research has been devoted to the indirect effects of food web dynamics with trophic cascades being a major theme (Polis et al. 2000). Trophic cascades are strong multi-trophic linkages where predator-prey interactions indirectly affect non-adjacent trophic levels, resulting in changes of density or biomass (Persson 1999). They sometimes occur across whole communities, but are more commonly seen among a subset of communities at the level of individual

species (Polis 1999). Although trophic cascades appear to be strongest in aquatic and marine environments (Strong 1992), species-level cascades have recently been identified in many terrestrial environments (Pace et al. 1999; Persson 1999).

In parallel with the development of the concept of trophic cascades there has been much research and debate regarding the major processes driving trophic dynamics (see Carpenter and Kitchell 1992; DeMelo et al. 1992). Trophic cascades were originally developed to describe the influence of predation on lower trophic levels, which was also referred to as top-down control (Carpenter et al. 1985). Concurrent research also determined that nutrient availability affected the productivity of primary producers and this also played a significant part in regulating higher trophic levels (McQueen et al. 1986; McQueen et al. 1989). This became known as the bottom-up effect. In practice, it is difficult to separate the magnitude of top-down and bottom-up forces because in most systems some form of resource and consumer control are operating together and are not independent (Pace et al. 1999; Worm et al. 2002).

PREDATOR-PREY MODELS FOR PREDICTING TROPHIC DYNAMICS

In addition to empirical research, trophic dynamics have also been a focal point for theoretical scientists (Abrams and Ginzburg 2000). Much debate has focused around two simple predator-prey models (ratio-dependent vs resource-dependent), and which one best describes the behaviour of natural systems. These models, based on differential predator-prey equations, determine changes in the population of each trophic level in a food chain exposed to perturbation (Ginzburg and Akcakaya 1992). The models differ only in the functional response that describes the rate at which an individual predator can consume its prey as a function of prey density (Abrams and Ginzburg 2000). Traditional resource-dependent predator-prey models are based on Lotka-Volterra equations where changes in the rate of predation by an average predator are determined solely by abundance of prey (Fussmann et al. 2005). The basis of these models was challenged by the concept of ratio-dependent trophic functions (Arditi and Ginzburg 1989), where predation rates depend on the ratio of predators to prey.

The clearest difference between the two models occurs in predictions of community responses to sustained long-term bottom-up pressure in multi-trophic systems.

Traditional resource-dependent models predict that the top level of the food chain, which has no predatory pressure, will increase proportionally to the bottom-up force. The second-from-top level will remain constant and each successive level below will show an alternating non-linear increase or decrease in biomass (Ginzburg and Akcakaya 1992). The more recently developed ratio-dependent models predict a proportional increase in the biomass of all trophic levels in response to resource enrichment and these are closer to observations within natural systems (Ginzburg and Akcakaya 1992, see Table 4.1).

NUTRIENT ENRICHMENT, EUTROPHICATION AND SECONDARY PRODUCTION

The study of trophic dynamics, bottom-up forcing and how to accurately model and predict them has occurred during a period of escalating anthropogenic disturbance. One of these disturbances is the human induced change to watersheds through deforestation and development of agricultural, urban or industrial land-cover. This leads to changes in the hydrological cycles and allochthonous inorganic and organic inputs of downstream estuarine and coastal systems (Hopkinson and Vallino 1995; Valiela et al. 1997). These anthropogenic inputs, particularly additions of limiting nutrients into these systems, promote increased primary productivity and the process of eutrophication (Paerl 1997; Cloern 2001; Pinckney et al. 2001).

It is generally considered that nitrogen is the primary limiting nutrient in coastal and estuarine systems (Vitousek et al. 1997a; Howarth and Marino 2006). The majority of anthropogenic nitrogen is derived from diffuse sources such as runoff from fertilized and pastured agricultural lands, heavily populated urban areas and atmospheric deposition (Paerl 1997; Carpenter et al. 1998). Many studies have measured or modelled linkages between catchment development and large increases of nutrient loads in coastal and estuarine systems (McClelland and Valiela 1998; Valiela and Bowen 1999; Bowen and Valiela 2001; Gorman et al. 2009).

Nutrient cycling in estuaries and shallow coastal waters are characterised by a tight coupling of benthic and pelagic processes linking sediment pore water with overlying surface waters (Cloern 2001). Increases in water column nutrients can lead to greater primary production of phytoplankton and marine macrophytes (Duarte 1995). As this organic matter is decomposed, buried and mineralised the sediment chemistry is changed

through depletion of oxygen resulting in hypoxic and anoxic conditions and nutrient release (Cloern 2001). The transition of microbial metabolism in sediments from aerobic to anaerobic stimulates sulphide reduction resulting in an increase in hydrogen sulphide in pore waters (Viaroli et al. 2008) and greater fluxes of Phosphate and Ammonia from sediments (Cowan and Boynton 1996). Wind-driven re-suspension and bioturbation of sediments in shallow estuaries can lead to the advection of nutrient rich pore water into the surface waters (Giffin and Corbett 2003). This nutrient regeneration from sediments pore water to surface water can supply a large portion of the nitrogen budget of epiphtyes and macroalgae at particular times of the year (Cowan and Boynton 1996).

It is an accepted paradigm that increases in excessive inputs of nutrients into coastal ecosystems can be detrimental, causing harmful algal blooms, loss of seagrass habitat and long periods of hypoxia and anoxia (Heck and Valentine 2007). These conditions also lead to fish kills and loss of biodiversity. However, it has also been asserted that moderate increases in nutrients can stimulate secondary production (Nixon and Buckley 2002), and that fisheries yields increase with moderate increases of nutrients (Potter et al. 1983; Caddy 2000; Breitburg et al. 2009a). Responses to nutrient enrichment could therefore be positive at low to moderate levels by increasing secondary production, however after reaching a threshold of eutrophication, negative effects such as decline of habitats, loss of productivity and the breakdown of community structure will occur (Caddy 1993). Due to their proximity to coastal development, their high productivity, and the important role they play in facilitating biodiversity, seagrasses are one of the most important ecosystems being influenced by nutrient enrichment.

THE IMPORTANCE OF SEAGRASSES AND SEAGRASS FOOD CHAINS

Seagrasses are a small but taxonomically diverse group (ca. 60 species) of angiosperms that are specially adapted to live exclusively in sub-tidal or inter-tidal marine and estuarine habitats (Kuo and den Hartog 2006). They are distributed in coastal and estuarine waters of all continents except Antarctica and are usually confined to shallow depth due to their high light requirements (den Hartog and Kuo 2006). Seagrasses are a foundation species that engineer habitats through their physical properties (Hughes et al. 2009). They provide a variety of important ecosystem services in the marine environment, estimated over a decade ago to be worth more than US$ 3.8 trillion per

year to the global economy (Costanza et al. 1997). These services include: cycling of nutrients (particularly nitrogen) (McMahon and Walker 1998); regulation and storage of carbon dioxide and conversion to organic forms (Duarte et al. 2005); stabilisation of sediments; the provision of habitat and supply of food for diverse assemblages of organisms from almost every phyla (Hemminga and Duarte 2000); contribution of organic matter to detritus based food webs (Heck et al. 2008); and provision of nursery habitat for commercially and recreationally important fish and invertebrates (Heck et al. 2003).

Seagrasses have among the highest rates of primary production of all marine ecosystems and often produce more energy than the ecosystem requires (Duarte and Cebrian 1996). Much of the excess seagrass biomass decomposes and enters an important detrital food web (Cebrian 1999). The surfaces of seagrass leaves also carry a complex layer of periphyton including diatoms, macroalgae, encrusting algae, bacteria, fungi, sponges and ascidians (Borowitzka et al. 2006). In temperate regions of the world where herbivory is generally low, study of carbon flux suggests that epiphytic algae support a dominant food chain in seagrass beds (Mateo et al. 2006). This food chain consists of small crustaceans and molluscs which graze upon the epiphytic algae (Jernakoff et al. 1996). These are in turn preyed upon by small fish, many of which are juveniles (Edgar and Shaw 1995a). Finally, these small fish become the food resource for larger piscivorous fishes (Hindell et al. 2002). The high productivity of the epiphytic algae supports a large number of economically important species of fish and invertebrates at some stage of their life cycle (Gillanders 2006).

SEAGRASS DECLINE

Over the last century, seagrass beds have been in global decline and the rate of decline is increasing (Waycott et al. 2009). Since 1879, when seagrass areas were first recorded, more than 29% of known areal coverage has disappeared (Waycott et al. 2009). Furthermore, almost 15% of seagrass species and many of their associated fauna are threatened (Hughes et al. 2009). Causes for seagrass decline include natural disturbances such as storms, disease and overgrazing by herbivores (Short and Wyllie-Echeverria 1996). Loss of seagrass has been attributed to many different anthropogenic stresses including direct physical disturbance from coastal development, changes in water quality

due to sedimentation, pollution and nutrient enrichment (Ralph et al. 2006; Short et al. 2007). The future effects of man-made climate change, whether they manifest themselves in sea-level change, heat stress or increased storm activities are also likely to place further stress on seagrass habitat (Orth et al. 2006; Short et al. 2007).

Of this extensive list of anthropogenic stressors, eutrophication is considered to be one of the leading culprits to seagrass decline, particularly in estuarine systems (Short and Wyllie-Echeverria 1996; Kenworthy et al. 2006). Increased nutrient levels lead to increased phytoplankton in the water column and increased epiphytic algal growth resulting in reduced light reaching seagrass leaves (Neverauskas 1987; Dennison et al. 1993). As seagrasses are sensitive to light levels and require high irradiances for photosynthesis, eutrophication generally leads to a decline in seagrass health and loss of habitat (Ralph et al. 2006). A few studies have found a positive correlation between nutrient enrichment and seagrass biomass (Ralph et al. 2006), particularly when ambient nutrient levels are comparatively low by global standards, as seen in south-east Australian waters (Young et al. 1996; Harris 2001). Several recent studies have also found that seagrass losses are reversible with recovery on large scales occurring through both clonal growth and new seedlings if anthropogenic stresses are alleviated or reversed (Walker et al. 2006). This makes temperate seagrass beds an ideal model for testing the bottom-up effect of nutrient enrichment on aquatic food chains.

STUDY SYSTEM

Seagrasses make up a large portion of the submerged aquatic vegetation in Australian estuaries. Australia has the greatest number of seagrass species of all the world's continents with a high degree of endemism (Larkum and den Hartog 1989). In south-eastern Australia there are eight known seagrass species, with eelgrass (*Zostera capricorni*) being the most common seagrass in New South Wales (West and Larkum 1989).

Z. capricorni is found from sheltered marine waters on sandy substrates to non-tidal brackish water on mudflats. It is a relatively fast growing seagrass and has the ability to recolonise bare substrate (West 1983). Leaves are typically around 30 cm long but can range from 2 cm to 50 cm in length. Width varies from 0.5 to 5 mm (Kuo and den

Hartog 2001). *Z. capricorni* is found from sheltered marine waters on sandy substrates to non-tidal brackish water on mudflats.

In developed catchments of south-eastern Australia, *Z. capricorni* beds have declined by as much as 60% since European settlement (Walker and McComb 1992). Much of this has been attributed to anthropogenic inputs of nutrients that are in naturally low concentrations in Australian waters (Scanes et al. 2007). Enrichment of estuarine waters with nutrients, particularly nitrogen can lead to phytoplankton blooms and excess epiphytic algal growth that shade seagrass leaves causing stress (Walker et al. 1999). Excessive increases in nutrients to a system can cause regime shifts from seagrass-dominated to phytoplankton-dominated estuaries (Webster and Harris 2004). More moderate increases however, may be less stressful to seagrasses, but led to changes in the structure of seagrass communities through bottom-up mediation of food webs (Valentine and Duffy 2006; Douglass et al. 2007; Morris et al. 2007; Jaschinski and Sommer 2008; Moksnes et al. 2008).

In temperate regions like south-eastern Australia, there is little direct consumption of seagrass biomass, primarily due to the absence of higher vertebrates that feed on seagrass shoots such as dugong and green turtles and invertebrate grazers like echinoids (Klumpp et al. 1989). It also now appears that the most important and abundant invertebrates in these seagrass beds, crustaceans and gastropods, are detritivores (Collett et al. 1984; Watson et al. 1984) and epiphyte grazers (Jernakoff et al. 1996). Very few fish species in south-eastern Australia feed on seagrasses; the exceptions being some monacanthid species (Bell et al. 1978; Conacher et al. 1979; Wressnig and Booth 2008) and sea garfish (Klumpp and Nichols 1983; Robertson and Klumpp 1983). The available evidence of limited consumption of seagrass has led to the belief that seagrasses in temperate Australia play a limited role in trophic dynamics and act primarily as a substratum for benthic and epiphytic algae. These algae, as well as seagrass detritus, form the trophic base of primary production and dominate the seagrass system (Klumpp et al. 1989).

Seagrass epiphytes are made up of a diverse assemblage of organisms including bacteria, fungi, sponges, bryozoans, hydroids, and ascidians (Borowitzka et al. 2006). The

dominant phyla of seagrasses however are the algae ranging from diatoms and dinoflagellates (Jernakoff and Nielsen 1997) to larger species of green, red and brown algae. In a study of *Z. capricorni* in Botany Bay, Prado and Thibaut (2008) found colonial diatoms, cyanobacteria, fourteen taxa of Rhodophyceae, four taxa of Phaeophyceae and six taxa of Chlorophyceae in the epiphytic assemblages. Epiphytic algae are constantly grazed upon by small molluscs and crustaceans, generally amphipods (Jernakoff et al. 1996). Seagrass beds have been shown to be the most prolific habitat for a great variety of fish and crustaceans including many important juvenile commercial and recreational species because of the food and shelter they provide (Pollard 1984; Bell and Pollard 1989; Humphries et al. 1992; Loneragan et al. 1998; Loneragan et al. 2001). These small fish feed upon the small grazing epifaunal molluscs and crustaceans (Edgar and Shaw 1995b). Finally, large piscivorous fish prey on the smaller fish (Hindell et al. 2000a). This four level food chain (epiphytes, grazers, small fish, and large fish) is generally thought to occur in temperate seagrass meadows in Australia, though few studies have examined the interactions among all trophic links. Interactions between nutrient enrichment from catchment development and trophic dynamics within this food chain will form the basis of the studies within this thesis.

SIGNIFICANCE AND AIMS OF THIS STUDY

Australia has the largest and most diverse seagrass assemblages in the world with more than half of the world's species. Additionally research on these systems generates a considerable proportion of the world's literature (Walker et al. 1999). Even so, a recent strategic review of Australian seagrasses has highlighted a number of knowledge gaps that require research to improve future management of seagrass habitat (Butler and Jernakoff 1999). In coastal south-eastern Australia, where research for this thesis was conducted and where a significant proportion of the population of the country resides, temperate seagrass species are generally confined to estuaries and sheltered embayments (West 1983). These seagrasses are under threat from catchment development and anthropogenically-derived nutrients (Shepherd et al. 1989; Larkum and West 1990). Nutrient enrichment is also likely to be affecting multi-trophic interactions in seagrass food webs. The study of these trophic interactions or cascades is of general scientific interest and has significant implications for the management and conservation of natural systems (Estes and Peterson 2000).

In south-eastern Australia there appears to be very little direct consumption of seagrass biomass (Klumpp et al. 1989). Exceptions to this include several species of fish: e.g. *Meuschenia spp.* (Wressnig and Booth 2008) and *Hyporhamphus melanochis* (Klumpp and Nichols 1983) and isolated outbreaks of grazing sea urchins (Larkum and West 1990). Instead, epiphytic algae form the base of a dominant trophic web of seagrass fauna which supports grazing invertebrates, small fish and larger piscivorous fish.

The effect of nutrient enrichment on this four level trophic cascade depends on whether it responds according to the predictions of resource-dependent or ratio-dependent models. This will have direct implications for seagrass conservation and fisheries management because the functional responses determine how nutrients affect the standing stock of epiphytes, which in turn affects the growth and survivorship of seagrass and juvenile fish. If the trophic interactions follow a traditionally assumed resource-dependent pattern, then moderate increases in nutrients will decrease the standing stock of epiphytes, increase grazer and piscivore biomass, but have negligible influences on the growth and survivorship of juvenile fish (see Table 4.1). Alternatively, if the trophic interactions are ratio-dependent, increased nutrients will proportionally increase the abundance of epiphytes, grazers, and juvenile and piscivorous fish. Moreover, if the tropic cascade responds in any other way, alternative factors or feedbacks must be operating.

While some aspects of trophic cascades emanating from epiphytes on seagrass are well understood, experimental programs linking all trophic steps from seagrass and epiphytes through grazers to fish are extremely rare (but see Heck et al. 2000). Many studies of seagrass food webs have identified trophic responses to bottom-up forcing due to increased nutrients (Gil et al. 2006; Douglass et al. 2007; Morris et al. 2007; Jaschinski and Sommer 2008; Moksnes et al. 2008). These studies however, generally fail to test for long-term trophic dynamic responses to nutrient enrichment across the whole system for one or several reasons. Firstly, experiments are primarily conducted with only two or three trophic levels and often in small enclosures or mesocosms (eg. Jaschinski and Sommer 2008; Jephson et al. 2008; Moksnes et al. 2008). Secondly, studies involve manipulations over short time periods of weeks and months rather than on steady state systems (eg. Gil et al. 2006; Douglass et al. 2007; Jaschinski and Sommer 2008). Finally, many studies look at interactions between changes to top-down and bottom-up

processes in combination, rather than bottom-up forces in isolation, making it difficult to distinguish between producer and consumer effects (eg. Borer et al. 2006; Heck and Valentine 2007). While separating bottom-up and top-down control is difficult to achieve in natural experiments mensurative studies, manipulative field studies can play a key role in isolating the role bottom-up processes.

Overall, this study aims to link the trophic chains that include seagrass epiphytes, grazers, juvenile fish and their piscivorous predators to enable predictions of the effects of nutrient loads on the growth and survivorship of seagrass and some of the economically-important fish that it supports. In contrast to many other empirical studies of trophic cascades, the experimental predictions in this study have a strong theoretical basis. By combining rigorous investigations involving field surveys, manipulative experiments and ecological theory, this study generates a mechanistic understanding of these seagrass systems and provides a basis upon which indirect effects may be assessed. The major aims are to: i) investigate the existence of strong trophic cascades that link epiphyte biomass to the growth and survival of economically-important juvenile fish in temperate Australian seagrass meadows; ii) determine whether the change in community structure due to nutrient enrichment of a seagrass system is best described by resource-dependent or a ratio-dependant models and; iii) evaluate how increased nutrients (bottom-up effects) or variation in predation or recruitment of fish (top-down effects) affect trophic interactions, the productivity of seagrass and the growth of economically-important fish.

THESIS OUTLINE

This thesis consists of eight chapters including the current introduction. Chapters 2 (general methods) and 7 (discussion) are literary summaries and the remaining chapters, 3-6 are data chapters written as individual research papers for publication in peer-reviewed scientific journals. The body of the thesis consists of the following three major themes: i) scientific methods for sampling various trophic levels within seagrass food chains (Chapter 2); ii) mensurative studies to ascertain equilibrium-based observations to long-term perturbation of nutrient enrichment (Chapters 3 & 4); and iii) short-term manipulative experiments to determine the relative strength of bottom-up and top-down forces in seagrass systems (Chapters 5 & 6).

Chapter 2 describes in detail the general methods that have been used throughout many of the experimental chapters. These include: site descriptions; common field sampling methods; taxonomy of invertebrate grazers and fishes; and commonly used statistical analyses. Many of these methods are described again more briefly in following data chapters, however, as these sections are intended as stand-alone publications some redundancy is inevitable. Chapter 3 investigates several different sampling methods to determine the best way to detect changes in the diversity of large predatory fish assemblages in shallow seagrass beds. This is achieved through a pilot study comparing sampling techniques as well as a thorough literature review.

Chapters 3 and 4 describe results from mensurative field studies comparing seagrass food chains in developed catchments (with elevated loads of nutrients) to those in more pristine catchments (with low ambient nutrient levels). Chapter 3 assesses differences in the assemblages of fish and invertebrates in seagrass communities with low ambient nutrient levels and communities that have undergone long-term nutrient enrichment. Chapter 4 uses biomass derived from the abundance data set in chapter 3 to test the predictions of trophic dynamics made by two simple predator-prey models with respect to sustained bottom-up forcing from nutrient enrichment. As the seagrass morphology and nutrient levels at Careel Bay were vastly different from those of the other 3 enriched sites I determined to omit this site from the analysis. To ensure a balanced design Smiths Creek was also omitted from the study and this site was chosen because of its greater spatial separation from the other sites.

Chapter 5 combines both mensurative and manipulative field experiments to investigate the growth and survival of juvenile fish in nutrient enriched seagrass beds through indirect effects of upwardly cascading trophic interactions. Chapter 6 then uses manipulative, caged field experiments to look at the interactions between top-down and bottom-up forcing on lower trophic levels within the seagrass food chain. Finally, the main findings of the thesis are synthesised in the discussion (Chapter 7). This incorporates an overall review of the novel and important findings, areas of interest for further studies and how the study can be used to aid future management and conservation decisions.

Chapter 2

GENERAL METHODS

LOCATION OF STUDY

The study was carried out on the central coast of New South Wales, Australia, c.a. 33°S, 151°E (Figure 2.1). The region receives an average of 1322 mm rainfall per annum with higher rainfall in the first half of the year (January-June) with monthly averages ranging from 118 mm (May) to 152 mm (March) and lower precipitation in the latter half of the year (July-December) ranging from 68 mm (September) to 103 mm (December). Average temperature for the region is 23° C and ranges a monthly average of 17.5° C in July to 27.6° C in January (Australian Bureau of Meteorology).

Field sampling and experiments (Chapters 4 & 5) were carried out at nine different locations in Brisbane Water, Pittwater and tributaries of the Hawkesbury River estuary, 30 to 50 km north of Sydney Australia (Figure 2.1). Brisbane Water is a shallow, wave dominated barrier estuary, while the Hawkesbury River estuary and Pittwater are drowned river valleys (Roy et al. 2001). The entrances of these water bodies converge in Broken Bay. Sites were selected based on their level of catchment development, which was used as a proxy for their status of nutrient loading. Sites where a majority of their catchment consists of residential, industrial or agricultural land-use (Pittwater Council 2002; Olmos and Birch 2008) were considered to be nutrient enriched while sites with a relatively undisturbed catchment consisting of national park were used as reference sites assuming low ambient nutrient levels. All sites were located in marine dominated lower estuaries with semi-diurnal tides and a maximum range of 2.1 m (Hatje et al. 2003). Salinities range from 32-37 PSU.

Brisbane Water has a catchment of ~185 km^2 (Paterson et al. 2003b). Nutrient enriched sites were selected at the mouths of Narara Creek (33°25'56"S, 151°19'57"E), Erina Creek (33°26'26"S, 151°21'41"E) and Kincumber Creek (33°28'20"S, 151°22'54"E). Narara and Kincumber Creek catchments are highly developed residential catchments while the Erina Creek catchment is predominantly agricultural (Olmos and Birch 2008). Also in Brisbane Water, reference sites were selected at Woy Woy Bay (33°28'31"S,

151°18’08”E) and Waterfall Bay (33°28’05”S, 151°18’30”E) where the catchment is undeveloped and consisting mainly of national park (Paterson et al. 2003b).

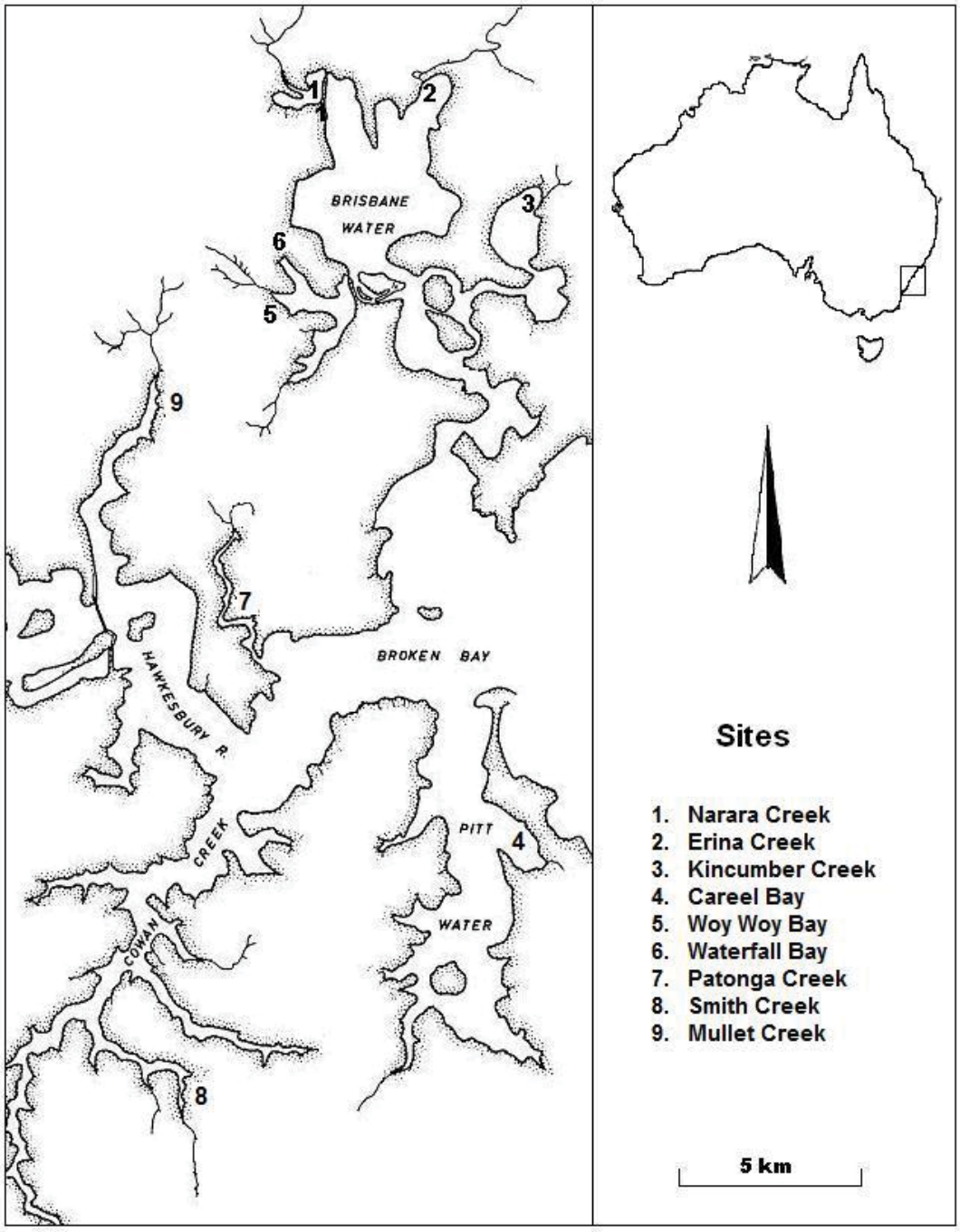

Figure 2.1: Map of Brisbane Water, Pittwater, the lower Hawkesbury River estuary and Broken Bay showing the nine sites used in this study. Field sampling was conducted at sites 1 to 8 while manipulative field experiments were carried out in Mullet Creek (Site 9).

Pittwater is a drowned river valley entering the southern side of Broken Bay. It has a surface area of 18.1 km^2 and a total catchment of ~51 km^2 (Pittwater Council 2002). The catchment to the east consists of heavily developed urban areas while the western side primarily surrounded by Ku-ring-gai Chase National Park. One nutrient enriched site was selected on the eastern side at Careel Bay (33°37'06"S, 151°19'30"E), surrounded by the suburban catchments of Avalon and Whale Beach. Reference sites were selected in tributaries of the lower Hawkesbury River estuary in Patonga Creek (33°32'24"S, 151°15'53"E) with a catchment surrounded by Brisbane Water National Park and at Smith Creek (33°38'49"S, 151°12'38"E) within the undeveloped catchment of Ku-ring-gai Chase National Park.

The manipulative field studies (Chapters 5 & 6) were conducted in a large shallow seagrass bed at the northern end of Mullet Creek (33°29'20"S, 151°15'46"E). Mullet Creek is a tidal creek and tributary of the Hawkesbury River (Figure 2.1) with a catchment predominantly consisting of National Park (Brisbane Water).

NUTRIENT SAMPLES

Field collection

At each of the eight sites in the field study, duplicate water samples were collected from estuarine waters above seagrass beds during each sampling period. Samples were collected in 200 mL PET bottles, taken to the laboratory and frozen at -80 °C before being sent away for analysis of total nitrogen. During the 2 manipulative studies (Chapters 6 & 7) carried out in Mullet Creek, water samples were collected in 200 mL PET bottles at fortnightly intervals. On returning to the laboratory, half the sample was filtered through a 20 µm sterile filter and frozen at -80 °C for analysis of nitrate and nitrite (NO_x). The remainder of the sample was refrigerated for less than 24 hours before analysis of Ammonia (NH_3) was carried out.

Laboratory analyses

Samples from the field experiment were sent to Sydney Water Analytical and Field Services laboratory for analysis of total nitrogen using the method APHA 4500 - PH & NO_3 – I (APHA et al. 2005). This method allowed for a lower detection limit of 0.05 mg/L total nitrogen. Samples collected from the manipulative experiments were

analysed for nitrate and nitrite (NO_x) and ammonia (NH_3) using flow injection analysis with the Lachat Instruments ASX 520 series XYZ Autosampler. NO_x concentrations were determined by the automated cadmium reduction method (QuikChem® Method 31-107-04-1-A). NH_3 levels were determined using the automated Berthelot Reaction method (QuikChem® Method 31-107-06-1-B).

DESCRIPTION OF GENERAL SAMPLING METHODS

Seagrass, epiphyte and grazers

Seagrass, epiphyte and associated epifauna were collected in a specifically designed invertebrate sampler (see O'Neill et al. 2007). This sampling device consisted of a 200 mm diameter galvanised metal tube 600 mm in length. At the base of the tube is a slot for a cutting blade and at the top is a (500 µm) mesh sieve (Figure 2.2). To use the BROMAR sampler the cylinder is placed over the seagrass leaves in shallow water, the blade is then inserted into the slot slicing the seagrass leaves off 10-20 mm above the substrate. The sampler is then inverted and the seagrass, epiphytes and associated epifauna are collected in the sieve and bagged.

Samples were preserved in 7% formalin and then sieved through a 500 µm mesh in the lab. Fauna were enumerated, identified to the lowest possible taxon, dried in an oven at 60 °C for 24 hours and then weighed to determine dry biomass. To determine algal load, 20 individual seagrass blades were removed from each sample and epiphytes were carefully scraped from the leaves with the edge of a microscope slide. The surface area of these leaves was calculated using a leaf area meter and then dried in an oven at 60 °C for 24 hours to determine dry biomass. Epiphytes were also dried and weighed and algal load was expressed as algal biomass per gram of seagrass biomass.

Juvenile fish

Also at each site and sampling period, four 25 m^2 sweeps with a seine net (6 mm mesh size x 12m long x 2m deep) were taken in shallow water (< 2 m at high tide) to collect small and juvenile fishes. Seine nets have been found to be very efficient at sampling assemblages of small and juvenile fish (< 20 mm length) in seagrass beds and the width of this net greatly reduces the chance of escape by fast moving fish species of this size range (Guest et al. 2003). Species that were considered to prey upon the grazing

invertebrates (determined from gut analysis samples and from previous studies) were removed from the net, euthanized in ice slurry and taken back to the laboratory where they were preserved in formalin prior to processing. Measurements of abundance and dry weight biomass were determined per square metre of seagrass bed.

Piscivorous fish

To determine the most effective method of sampling of piscivorous fish, a pilot study was conducted testing the relative performance of five sampling techniques. Two sampling methods used visual techniques; Underwater Visual Census (UVC) and Baited Remote Underwater Video (BRUVS), while the other three; gill nets, baited traps and line fishing were capture techniques. For a detailed description of fishing methods used in this study see Appendix I.

The study was conducted on four separate days in spring 2006 at Smiths Creek (33°38'49"S, 151°12'38"E) in the Hawkesbury River, 30 km north of Sydney (Figure 2.1) over a period of approximately four hours centred on morning high tides. This location has a continuous seagrass bed (approx 8000 m^2) that allowed for the independent deployment of at least four replicates of each sampling method per sampling day. Although time of day/night can influence fish assemblages sampled, all methods were carried out during daylight hours because the strong light was required for visual methods to be effective. The aims of the study were to determine the best method or combination of methods for determining the i) abundance, and ii) species richness of predatory fishes in these habitats using experimental data.

A total of 85 piscivorous fish were sampled during the study from five species. The two visual methods of sampling (UVC and BRUV) recorded higher abundances and species richness, with a total of 52 and 31 individuals sighted respectively from two piscivorous species (Table 2.1). Capture methods proved unsuccessful with only one individual piscivore caught on line and in gill nets and nothing in baited traps (Table 2.1). The most common predatory fish sampled during the study was *Acanthopagrus australis*, followed by *Rhabdosargus sarba.* Three other species were represented by single individuals (*Tylosurus gavialoides, Dinolestes lewini* and *Platycephalus laevigatus*, Table 2.1).

For assemblage of piscivorous fishes, separate methods are recommended to target species of interest according to their biology and behaviour (Willis et al. 2000). On the basis of the results and a review of the benefits costs and limitations of the sample methods trialled (see Appendices II & III) a combination of underwater visual census complemented with line fishing was chosen for sampling large piscivorous fishes that dominate the fourth trophic level in seagrass systems. Unfortunately variation between visibility across sites lead to sampling biases with UVC making comparisons impossible. This method was discarded after the first sampling period and line fishing was used to compare differences in fish assemblages. While gill nets used at night were considered as a more efficient capture method, it was overlooked due to the high mortality of non-targeted species and occupational health and safety issues concerning remote sampling and the use of boats at night.

Table 2.1: Measures of abundance and species richness of piscivorous fish for four different sampling methods used during a pilot study to determine assemblage diversity. No fish were caught in traps during the study.

Species	Common name	BRUV (video) n=16	UVC (visual) n=32	Line fishing n=16	Gill nets n=12
PISCIVOROUS FISH					
Tylosurus gavialoides	Stout longtom	-	1	-	-
Acanthopagrus australis	Yellow-fin bream	30	51	1	-
Dinolestes lewini	Longfin pike	1	-	-	-
Platycephalus laevigatus	Grass flathead	-	-	-	1
Predator abundance	**Total**	**31**	**52**	**1**	**1**
Predator abundance	Mean	1.688	1.625	0.083	0.083
	Standard error	0.237	0.320	0.083	0.083
Predator richness	**Total**	**2**	**2**	**1**	**1**
	Mean	1.000	0.594	0.083	0.083
	Standard error	0.091	0.088	0.083	0.083

On the basis of this, data presented in Chapters 3 & 4 for piscivorous fish were sampled using a 1.8 m fibreglass Jarvis Walker spinning rod (line class 3-4 kg, cast weight 5-20 g) and Eagle Claw spinning reel (gear ratio 5.1:1) and monofilament fishing line (breaking strain 2.7 kg). Each rod and reel combination was equipped with a general running rig consisting of a bean sinker (size 1) above a barrel swivel (size 6) with a 40 cm trace of monofilament line (breaking strain 2.7 kg) and a 1/0 claw suicide hook. Frozen Hawkesbury river prawns were used as bait. As the diet of fish occupying seagrass beds indicate that few fish had predominantly piscivorous diets and target species were also known to consume

prawns (Burchmore et al. 1984; Edgar and Shaw 1995b) it was not considered necessary to use a fish bait. During each sampling period, two such lines were cast haphazardly between 5 and 20 m from an anchored 6 m aluminium boat and tended by individual fishers for a period of 90 minutes at each site. All fish caught were identified, measured (total length) and weighed (wet biomass), fin clipped for future identification, then immediately returned to the water. The use of line fishing had the advantage of targeting carnivorous and piscivorous species that were the focus of the particular trophic studies being undertaken.

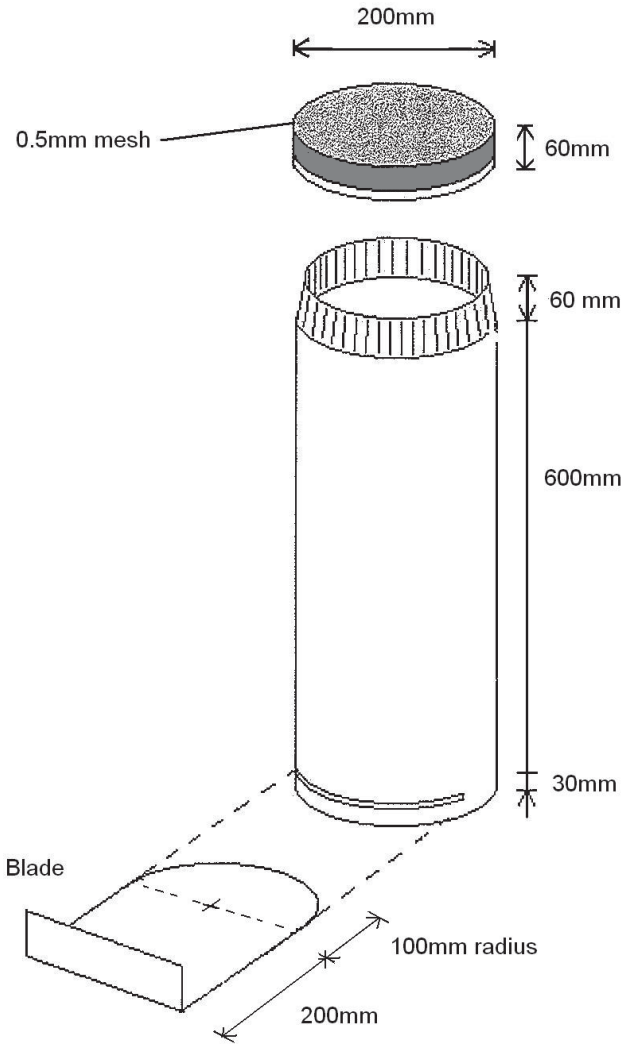

Figure 2.2: BROMAR sampler, specifically designed to collect seagrass, epiphytes and mesograzers in one sample. Source O'Neill et al 2007.

DESCRIPTIONS OF MACROFAUNA AND TAXONOMIC RESOLUTION

Taxonomic resolution for identifying samples varied depending on faunal groups. Fish and some gastropods were identified to species, using an online key in the case of gastropods (Ponder et al. 2000) and guidebook with supplementary key for fishes (Kuiter 2000). Crustaceans, predominantly amphipods and tanaids, are not as well described in Australia and therefore cannot always be reliably identified to species. An online key (Lowry 1999 onwards) from the Australian museum was used to identify these crustaceans to the lowest possible taxon, usually family.

A total 8,529 individuals from 59 taxa of seagrass epifauna grazers were collected and identified to morphospecies during the study. Epifaunal grazers fell into two main faunal groups: gastropods (45 morphospecies with individuals) and crustaceans (14 morphospecies with 5,478 individuals). Nine taxa dominated the epifaunal grazer assemblages contributing to approximately 90% of the total abundance. These included four amphipods from families Maeridae, Amphilochidae Caprellidae and Corophiidae, three gastropods (*Abala sp*, *Diala megapicals* and *Aplysia sp*), a Tanaidae species and a valviferan isopod (Idoteidae *sp*).

Seine net samples captured eight species of decapods with 5,790 individuals. Over 70% were of the caridean shrimp *Macrobranchium intermedium*, while *Penaeus plebejus* and *Hippolyte australis* contributed to the majority of the remaining abundance. A total of 37,799 individuals from 47 fish species (Table 2.2) were also caught during the study. Fish assemblages were dominated by *Ambassis jacksoniensis* and several species of gobids (*Redigobius macrostoma*, *Pandaculus lidwilli*, *Arenigobius frenatus* and *Favonigobius lateralis*). Fish were also classified into feeding guilds based on the major components of their diet, where they predominately fed in the water column, and at the particular life stage they were caught during the study based on dietary studies of fish in seagrass beds in south-eastern Australia (Bell et al. 1984; Burchmore et al. 1984; Edgar and Shaw 1995b; Platell and Freewater 2009). Fish known to have a diet consisting predominately of small crustaceans such as amphipods that fed in the seagrass canopy zone were included in the trophic analysis as secondary consumers in the seagrass epiphyte food chain (Chapter 4).

Table 2.2: List of fish species caught during field sampling in this study

Family	Species	Authority	Feeding zone	Major Diet	Life history stage
Hemiramphidae	*Hyporhamphus australis*	(Steindachner, 1866)	SC	H	A
Atherinidae	*Atherinomorus ogilbyi*	(Whitely, 1930)	UWC	Z	A
Belonidae	*Tylosurus gavialoides*	(Castelnau, 1873)	SC	F	A
Syngnathidae	*Hippocampus whitei*	(Bleeker, 1855)	SC	Z	A
	Stigmatapora argus	(Richardson, 1840)	SC	Z	A
	Stigmatapora nigra	(Kaup, 1856)	SC	Z	A
	Urocampus carinirostris	(Castlenau, 1872)	SC	Z	A
	Vanacampus margaritifer	(Peters, 1869)	SC	Z	A
Scorpaenidae	*Centrogon australis*	(White, 1790)	B	C	J
Tetrapontidae	*Pelates sexlineatus*	(Quoy and Gaimard, 1824)	SC	C	J
Ambassidae	*Ambasis jacksoniensis*	(Macleay, 1881)	UWC	Z	A
Apogonidae	*Siphamia cephalotus*	(Olgilby, 1886)	SC	C	A
Dinolestidae	*Dinolestes lewini*	(Griffith, 1834)	SC	F	A
Sillaginidae	*Sillago maculata*	(Quoy and Gaimard, 1824)	B	C, P	J
Pomatomidae	*Pomotomus saltatrix*	(Linnaeus, 1766)	SC	F	J
Sparidae	*Acanthopagrus australis*	(Munro, 1949)	SC	C, F	J, A
	Rhabdosargus sarba	(Forsskål, 1775)	SC	C	J, A
Gerreidae	*Gerres subfasciatus*	(Cuvier, 1830)	SC	C, P	J
Mullidae	*Upeneus tragula*	(Richardson, 1846)	B	C	J
Girellidae	*Girella tricuspidata*	(Quoy and Gaimard, 1824)	SC	H	J
Scatophagidae	*Selenotoca multifasciata*	(Richardson, 1846)	SC	Z	J
Mugillidae	*Liza argentus*	(Quoy and Gaimard, 1825)	SC	H, D	J
Sphyraenidae	*Sphyraena obtusata*	(Cuvier, 1829)	SC	F	J
Labridae	*Achoerodus viridis*	(Steindachner, 1866)	SC	C	J
Odacidae	*Neodax balteotus*	(Valenciennes, 1840)	SC	C	J
Clinidae	*Cristaceps australis*	(Valenciennes, 1836)	SC	C	J
Blenniidae	*Petrocertes lupus*	(De Vis, 1886)	B	C	A
Gobiidae	*Arenigobius frenatus*	(Günther, 1861)	B	C	A
	Arenigobius bifrenatus	(Kner, 1865)	B	C	A
	Bathygobius kreffti	(Steindachner, 1866)	B	C	A
	Cristagobius goboides	(Olgilby, 1886)	B	C	A
	Favonigobius lateralis	(Macleay, 1881)	B	C	A
	Favonigobius exquisites	(Whitely, 1950)	B	C	A
	Gobiopterus semivestitus	(Munro, 1949)	B	C	A
	Pandaculus lidwilli		B	C, Z	A
	Redigobius macrostoma	(Günther, 1861)	B	C, Z	A
Microdesmidae	*Parioglosus marginalis*	(Rennis and Hoese, 1985)	B	C	A
Eleotrididae	*Philypnodon sp.*	(Krefft, 1864)	B	C	A
Siganidae	*Siganus nebulesus*	(Quoy and Gaimard, 1824)	SC	Z	J
Monacanthidae	*Acanthaluteres spilomelanurus*	(Quoy and Gaimard, 1824)	SC	H	J
	Brachyluteres jacksoniarus	(Quoy and Gaimard, 1824)	SC	H	J
	Meuschenia trachylepis	(Günther, 1870)	SC	H	J, A
	Meuschenia freycineti	(Quoy and Gaimard, 1824)	SC	H	J, A
	Monacanthus chinensis	(Osbck, 1765)	SC	H	J, A
	Nelusatta ayraudi	(Quoy and Gaimard, 1824)	SC	P	J
Tetraodontidae	*Tetractenos glaber*	(Freminville, 1813)	B	C	J
Diodontidae	*Dicotylichthys punctatus*	(Kaup, 1855)	B	C	J

Feeding zone: B = benthic, SC = seagass canopy & UWC = upper water column

Major diet: H = herbivore, Z = zooplankton, C = crustaceans, F = fish, P = polychetes & D = detritus

Life history stage: J = juvenile & A = adult

STATISTICAL ANALYSIS

Univariate

Analysis of variance (ANOVA) and analysis of co-variance (ANCOVA) was used to test univariate hypotheses for significant differences ($\alpha = 0.05$). These ANOVAs included both nested and orthogonal design and were computed using SPSS 11.0 and GMAV5.All analyses were preceded by investigation of homogeneity of variance using Cochran's or Levene's tests. Where these tests indicated unequal variance, transformations were conducted using square root; $\sqrt{(x+1)}$, Natural log; Ln(x+1) or Arcsine; $\sin^{-1}(\sqrt{x})$ where appropriate. When variances remained heterogenous after transformation analyses were proceeded with cautiously only when experiments contained large balanced samples (Underwood 1997). Where appropriate, ANOVAs were followed by *a posteriori* Student–Newman–Keuls (SNK) tests to identify significant differences among means.

Multivariate

To evaluate variation in assemblages of grazing invertebrates and juvenile fish PERMANOVA was used (Anderson 2001). This is a non-parametric method that partitions multivariate variation in data allowing for more complex multifactor designs similar to univariate analysis of variance. The analyses of biological assemblages were based on a Bray-Curtis Similarity Co-efficent (Bray and Curtis 1957). Non-metric multi-dimensional scaling (nMDS) plots were created to graphically present assemblage patterns (Clarke 1993). Where assemblages differed significantly, similarity percentages (SIMPER) were used to determine which species made the largest contribution to the dissimilarities.

To test the potential importance of habitat and environmental variables, BioEnv was used to correlate multivariate species assemblage data with Environmental and habitat variables using Spearman's rank coefficient to determine which variable or which combination of predictors best explains community structure. Data were pre-treated by standardising environmental variables around a mean of zero. Environmental predictor matrices were created using the Euclidean distance coefficient. The null hypothesis of no agreement between multivariate pattern of assemblage structure and environmental predictors was tested using RELATE, a non-parametric version of Mantel test.

Chapter 3

CHANGES IN SEAGRASS FAUNAL ASSEMBLAGES UNDER DIFFERENT NUTRIENT REGIMES AND LEVELS OF ANTHROPOGENIC DISTURBANCE

ABSTRACT

Human alteration to natural landscapes over the last two centuries has resulted in increasing changes at the land-water interface. This manifests as pollution, degraded water quality, and destruction of habitat and leads to loss of ecosystem structure and functioning. Nitrogen levels from terrestrial and atmospheric inputs have greatly increased in receiving waters over this period leading to eutrophication of estuaries and coastal seas. This study looks at how nutrient enrichment in estuaries of south-eastern Australia affected the assemblages of fish and grazing invertebrates associated with important seagrass habitats by comparing developed (nutrient enriched) and undeveloped (low nutrient) catchments. A combination of multivariate analysis using PERMANOVA and PRIMER was used to distinguish between assemblage structure between nutrient treatments and combined with ANOVA to investigate the species richness, total abundance, and abundances of major taxa contributing to differences in assemblage composition. The importance of environmental, habitat and catchment characteristics were analysed using BIOENV to determine the best predictor variables for changes in these assemblages. Nutrient enriched sites were found to have different fish assemblages to low nutrient sites, with higher abundances of *Ambassis jacksoniensis* and *Redigobius macrostoma* contributing to a large amount of the variation. Grazer assemblages showed little overall variation between nutrient enriched and ambient sites with most of the variation in community structure attributed to spatial positioning within estuaries. Turbidity was the best environmental predictor, having a weak correlation with fish assemblage structure, while total nitrogen levels in water column samples were the major driver of grazer community composition. This study highlights the potential utility of using fish assemblages as biological indicators of estuarine health and reinforces the need for implementing carefully planned policies for determining future development of catchments surrounding habitats of high ecological value such as seagrass beds.

INTRODUCTION

A large proportion of the earth's natural systems have been transformed due to human alteration of landscapes since the industrial revolution. This has increased rapidly in the late 20th century, resulting in modification of the carbon, nitrogen and water cycles (Vitousek et al. 1997b). In the case of nitrogen, human activity has at least doubled the level of global fixation through fertilizer production and fossil fuel combustion (Vitousek et al. 1997a). Run-off from agricultural and urban activities (Carpenter et al. 1998) as well as atmospheric deposition (Paerl 1997) have become major sources of nutrient enrichment in aquatic systems leading to eutrophication of coastal and estuarine waters. Eutrophication, along with other anthropogenic disturbances such as degraded water quality, and pollution of water and sediments (Dauer et al. 2000) alter the marine community structure and the functioning of these systems (Vitousek et al. 1997a).

Eutrophication of estuaries from terrestrially-derived nitrogen enrichment increases primary productivity and leads to shifts in the dominant species of flora and fauna (McClelland and Valiela 1998). As estuaries and coastal waters are considered to be nitrogen limited, increase in nitrogen loads stimulates production of phytoplankton and submerged macrophytes as well as epiphytic-algal growth (Howarth and Marino 2006). A secondary consequence of increased phytoplankton growth is degraded water quality and a reduction in the photic zone, limiting light to benthic macrophytes such as seagrass and macro-algae (Rabalais 2002). This results in shifts from benthic to pelagic dominance in plant and algal communities and leads to changes in the structure of higher level consumer assemblages such as invertebrates and fish (Deegan et al. 2002).

Seagrass beds in estuarine habitat are known to be high in faunal diversity and provide a nursery role for juvenile fish and invertebrates (Beck et al. 2001; Heck et al. 2003). Seagrasses are foundation species that facilitate biodiversity through their physical structure (Hughes et al. 2009). Many studies have noted that more complex habitats like seagrass have higher abundances of both invertebrates and small fish because of the increase in shelter from predation and abundance of food (Orth et al. 1984; Hosack et al. 2006). It is well established that alteration from benthic seagrass habitat to phytoplankton-dominated systems will have a dramatic impact on trophic pathways and affect assemblages of epifauna and juvenile fish in estuaries (Mouillot et al. 2005; Gil et

al. 2006; Pihl et al. 2006). Little is known, however, about how moderate enrichment affects community structure of seagrass habitat. Nutrient enrichment may compromise the nursery role of seagrass habitat before the seagrass itself disappears (Deegan 2002).

Eelgrass, *Zostera capricorni* Aschers, is a dominant seagrass in south-eastern Australia. This species is generally found in shallow estuarine waters in sandy to muddy sediment (West et al. 1985). Studies have shown that areas covered by *Z. capricorni* have a consistently higher diversity and abundance of fish when compared to unvegetated areas of bare sand (Ferrell and Bell 1991; Gray et al. 1996; Jenkins and Wheatley 1998). Seagrasses themselves are not heavily grazed in temperate Australian waters (Klumpp et al. 1989), however an important food chain in this habitat is based on the primary productivity of epiphytic algae which colonises their leaves. These epiphytes are grazed on by small crustaceans and molluscs which in turn are a ready food source for small and juvenile fish that ultimately become the prey of larger predators like piscivorous fish. Many of the juvenile fishes found in *Z. capricorni* beds are economically-important species such as yellowfin bream *Acanthopagrus australis,* tarwhine *Rhabdosargus sarba*, luderick *Girella tricuspidata* and the six-striped trumpeter *Pelates sexlineatus* (McNeill et al. 1992; Smith and Suthers 2000; Griffiths 2001) as well as protected species like the eastern blue groper *Achoerodus viridis* (Gillanders 1997). The diet of these species consists in large part of mesograzing invertebrates that consume the epiphytic algae on *Z. capricorni* leaves.

Spatially heterogeneous catchment development in south-eastern Australia has provided the opportunity to study the alteration of seagrass communities exposed to different levels of anthropogenic disturbance. This study explores changes in the assemblages of grazing invertebrates and seagrass-associated fish in *Z. capricorni* beds within developed and undeveloped watershed. Changes in biotic variables such as invertebrate and fish assemblage structure, species richness and abundance of major taxonomic and trophic groups were investigated at sites with low levels of deforestation and compared to sites in catchments with varying degrees of urban and agricultural land use. Environmental, habitat and catchment attributes such as water quality parameters, seagrass characteristics and density and percentage catchment development were explored to determine the best predictors of change in assemblage structure.

METHODS

Study sites

The study was carried out in the Brisbane Water and Hawkesbury River catchment just north of Sydney 33°S 151°E (see Figure 2.1). Eight seagrass meadows were sampled to determine the community and trophic structure of systems with elevated nutrient status. Four seagrass beds (Narara Creek, Kincumber Creek, Erina Creek and Careel Bay) are situated in catchments adjacent to urbanised, industrial and agricultural land use with a demonstrated history of elevated nutrient levels (Paterson et al. 2003a; b). The remaining four seagrass beds are situated in catchments that are surrounded by National Park and are considered to have low ambient nutrient levels (Waterfall Bay, Woy Woy Bay, Patonga Creek and Smith Creek). None of the sites were subject to direct commercial fishing and field observations indicated very low and similar levels of top-down pressure from recreational fishing among sites.

Spatial information used in multivariate analysis was derived from NSW Land and Property Authority using the NSW Spatial Data Infrastructure (SDI). Catchment features were determined using the Spatial Information Exchange (SIX) metadata node which integrates cadastral, topographic and satellite imagery. Polygons were placed around the drainage lines above each sampling site to determine their catchment area using the measuring tool on the Map Viewer interface. Polygons were also placed around parcels of developed land (residential, industrial or agricultural) within each catchment to determine their area and used to determine the percentage of catchment development. These variables were used as environmental predictors in multivariate analyses.

Experimental design

The eight seagrass beds were sampled on three separate sampling occasions (October/November 2005, January/February 2006 & May 2006) to compare assemblages of benthic invertebrates and fishes in the elevated nutrient sites to those in the reference sites. For reasons of brevity sampling periods will hereafter be referred to as spring, summer and autumn sampling. Since there was no replication of season incorporated into the experimental design, no intention is made to infer seasonality effects. In each sampling period, four replicate samples of seagrass epifauna were collected using a BROMAR sampler and sieved through a 500 μm mesh (see Chapter 2). Grazing

invertebrates within the epifaunal communities were counted and identified to lowest possible taxonomic level. Four replicate 25 m^2 sweeps with a seine net (6 mm mesh) were also taken at each site to collect small and juvenile fishes. Fish were also counted and identified to species.

Environmental variables (water temperature, pH, salinity and turbidity) were measured at each site for each sampling occasion using a YSI (650 MDS) multiprobe meter to monitor for temporal and spatial fluctuations likely to affect the abundances of estuarine fauna. Water samples were collected at each time and location of sampling and were sent to Sydney Water Analytical and Field Services laboratory for analysis of total nitrogen using the method APHA 4500 - PH & NO_3 – I (APHA et al. 2005). Seagrass leaves and shoots collected in the BROMAR sampler were dried in the oven and then weighed to determine seagrass biomass/m^2. Leaf lengths and densities were measured at each sampling site and period by counting the number of leaves and measuring the lengths of 20 seagrass blades in four 0.25 m^2 quadrats. Epiphytic algae were scraped off seagrass leaves from each sample and dry weight of epiphytes was used to determine algal load as biomass.

Multivariate analysis

Multivariate analyses were conducted using the software package PRIMER v.6 (Clarke and Gorley 2006) and PERMANOVA. Variation in assemblages of grazing invertebrates and juvenile fish was evaluated using PERMANOVA with a three-factor mixed design with sites (four levels) nested within nutrient status (enriched vs control) sampled over three time periods (Anderson 2001). This is a non-parametric method that partitions multivariate variation in data allowing for more complex multifactor designs equivalent to univariate analysis of variance. The analyses were based on a Bray-Curtis similarity co-efficient using square-root transformed data (Bray and Curtis 1957). No test was possible for the main effect of nutrient status so quasi *F"* ratios were calculated for this effect (Winer 1991). As negative denominators were encountered during the approximation, Cochran's (1951) *F"* was used in preference to Satterthwaite's (1946) *F'* method (see Appendix IV). Non-metric multi-dimensional scaling (nMDS) plots were created to graphically present assemblage patterns (Clarke 1993). Where assemblages

differed significantly, similarity percentages (SIMPER) were used to determine which species made the largest contribution to the dissimilarities.

To test the potential importance of habitat and environmental variables, BioEnv correlated multivariate species assemblage data with Environmental and habitat variables using Spearman's rank coefficient to determine which variable or which combination of predictors best explained community structure. Data were pre-treated by transforming (fourth root) for fish and grazer assemblages and standardising environmental variables around a mean of zero. Assemblage matrices were created using the Bray-Curtis similarity co-efficient while a resemblance matrix based on environmental predictors was created using the Euclidean distance coefficient. The null hypothesis of no agreement between the multivariate pattern of assemblage structure and environmental predictors was tested using RELATE, a non-parametric version of Mantel test.

Univariate analysis

Three-factor ANOVAs with sites (four levels) nested within nutrient status (enriched vs control) over sampling period (three levels) were performed for the following: total abundance and species richness of fish and grazer assemblages; abundance of functional groups (carnivorous and omnivorous juvenile fish) and taxonomic groups (amphipods and gastropods); and dominant species of fish identified in SIMPER analysis (see Appendix IV for model and terms used in Mean Square). The same design was also used to test for differences among habitat attributes (seagrass density, height and biomass and algal load on seagrass leaves). No test was possible for the main effect of nutrient status so quasi *F"* ratios were calculated for this effect (Winer 1991). Separate two-factor ANOVA (site nested in nutrient status) was also performed on water quality parameters (pH, turbidity, salinity, temperature and total nitrogen) to determine the extent of spatial variability among these measures. One-way ANOVAs were conducted on catchment attributes (size and percentage development) with nutrient status as the factor. Prior to each ANOVA, the assumption of homogeneity of variances was examined using Cochran's test. In the event of unequal variances, data were Ln(x+1) transformed. When transformation did nothing to improve heterogeneous variances, analysis was undertaken relying on the balanced experimental design and large sample to make these tests robust to type 1 error (Underwood 1997).

RESULTS

Environmental data

Catchment size ranged from approximately 270-5260 ha around nutrient enriched sites and 250-1026 ha surrounding ambient nutrient sites; however, catchment size was not significantly different between among nutrient statuses. The percentage of deforestation for catchment development was significantly higher in enriched sites 53.2 ± 11.6 % (1 S.E.) compared to ambient sites 5.9 ± 5.3 (ANOVA, $F_{1,6} = 13.77$, P = 0.01, Table 3.1, & Table 3.4).

Habitat attributes varied both spatially and temporally with all four measures having a significant interaction between sampling time and sites nested within nutrient level (Table 3.2). Seagrass densities tended to be higher in the spring sampling period compared to other times. Narara, Erina, Kincumber Creeks, Waterfall Bay and Woy Woy Bay had higher frond densities than the other three sites (Table 3.4). Seagrass canopy height had a general trend of being lowest in the spring and highest in the late autumn period in May 2006. Careel Bay and Kincumber Creek had substantially shorter leaf blades than the other sites at almost all sampling periods (Table 3.4). Seagrass biomass peaked in the late summer sampling period with Careel Bay again having noticably lower biomass than the other sites (Table 3.4). Algal loads on seagrass blades varied across sampling periods but was generally higher in spring and at sites with higher nutrient status, particularly Erina, Kincumber and Narara Creeks (Table 3.4).

Water quality parameters were fairly stable across all sites, showing no significant differences apart from turbidity (ANOVA, $F_{6,16} = 4.15$, $P = 0.01$) and salinity (ANOVA, $F_{6,16} = 3.45$, P = 0.02) levels which exhibited significant variation among sites nested within nutrient status (Table 3.3). Salinity stayed within a narrow range averaging between 35.07 and 35.29 PSU across the nutrient enriched sites. However there was slightly more variation in the ambient sites with Smith Creek averaging 33.76 PSU which was significantly lower (SNK, $P < 0.05$) than the average range (35.17 – 34.40 PSU) among the other three sites. Turbidity fluctuated to a greater extent and among enriched sites. Kincumber Creek (6.0 ± 3.25 NTU) was significantly higher and Smith Creek (0.83 ± 0.78 NTU) was significantly lower than the intermediate levels at Erina and Narara

Table 3.1: Analysis of variance results for catchment attributes upstream of sampling sites. Significant results are highlighted by bold text.

		Catchment size			Percent development		
Source	**DF**	**MS**	**F**	**P**	**MS**	**F**	**P**
Nutrient	1	1.6678	1.28	0.301	4459.929	13.77	**0.010**
Residual	6	1.3004			323.9627		

Table 3.2: Analysis of variance results for habitat characteristics of sites. Quasi-*F*''-ratios were estimated for nutrients and P-values were determined the using degrees of freedom for numerator and denominator shown in subscript. Significant results are highlighted by bold text.

		Seagrass height			Seagrass density			Seagrass biomass			Algal load		
Source	**DF**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**
Nutrient	1	3.79	$3.81_{1,8}$	0.087	200.10	$0.47_{3,8}$	0.173	3.64	$0.24_{6,6}$	0.948	0.130	$17.63_{1,8}$	**0.003**
Site (Nu)	6	0.77	4.35	**0.015**	550.26	4.24	**0.016**	41.37	6.37	**0.003**	0.006	0.78	0.599
Time	2	1.14	6.46	**0.013**	1009.92	7.78	**0.007**	28.98	4.46	**0.036**	0.016	2.00	0.177
Nu x Ti	2	0.27	1.56	0.250	155.06	1.19	0.337	1.11	0.17	0.845	0.002	0.23	0.796
Ti x Si (Nu)	12	0.18	5.49	**<0.001**	129.89	8.45	**<0.001**	6.50	6.5	**<0.001**	0.008	2.80	**0.003**
Residual	72	0.03			15.38			0.99			0.003		

Table 3.3: Analysis of variance results for water quality parameters. Significant results are highlighted by bold text.

		Temperature			pH			Salinity			Turbidity			Total nitrogen		
Source	**DF**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**
Nutrient	1	0.277	0.13	0.736	0.000	0.01	0.914	2.458	4.58	0.076	0.874	1.05	0.344	0.0002	0.06	0.8104
Site (Nu)	6	2.213	0.10	0.996	0.016	0.36	0.892	0.537	4.15	**0.011**	0.829	3.45	**0.022**	0.0024	1.98	0.1285
Residual	16	23.155			0.044			0.129			0.240			0.0012		

Table 3.4: Water quality, habitat and catchment attributes of each site. Means ± 1 S.E are presented. (n = 3 sampling periods)

	Enriched nutrient sites								Low nutrient sites							
	Narara		Erina		Kincumber		Careel Bay		Waterfall Bay		Woy Woy		Patonga		Smiths	
Catchment area (ha)	5259.0		3530.0		567.5		271.4		253.3		291.9		977.0		1026.0	
% development	49.6		47.1		30.4		85.5		0.0		0.0		2.0		21.7	
Total nitrogen (ug/L)	0.23	± 0.02	0.27	± 0.02	0.27	± 0.02	0.20	± 0.01	0.27	± 0.01	0.26	± 0.02	0.24	± 0.03	0.22	± 0.01
Temperature (°C)	21.24	± 2.86	20.52	± 2.92	21.67	± 3.15	22.49	± 2.32	21.57	± 2.93	22.64	± 3.23	20.53	± 2.40	22.04	± 2.23
pH	8.04	± 0.14	8.03	± 0.16	8.05	± 0.12	8.08	± 0.09	8.17	± 0.04	8.05	± 0.15	8.03	± 0.11	7.92	± 0.11
Turbidity (NTU)	2.6	± 0.7	2.5	± 0.7	6.00	± 3.25	0.83	± 0.78	1.03	± 0.09	0.97	± 0.46	3.70	± 0.97	0.27	± 0.09
Salinity (PSU)	35.09	± 0.08	35.07	± 0.11	35.09	± 0.42	35.29	± 0.20	35.17	± 0.08	34.65	± 0.15	34.40	± 0.14	33.76	± 0.25

Table 3.5: Seagrass attributesfor each site at each sampling periods. Means ± 1 S.E are presented. (n = 3 sampling periods)

	Enriched nutrient sites								Low nutrient sites							
	Narara		Erina		Kincumber		Careel Bay		Waterfall Bay		Woy Woy		Patonga		Smiths	
SG density (Nov 05)	28.4	± 1.2	38.8	± 1.6	25.9	± 2.2	11.0	± 1.7	30.9	± 2.3	20.6	± 0.9	8.6	± 1.3	14.6	± 2.1
SG density (Feb 06)	10.6	± 1.2	29.7	± 3.1	21.5	± 3.7	7.0	± 0.6	20.3	± 1.9	21.6	± 3.9	7.8	± 0.3	8.2	± 0.9
SG density (May 06)	11.8	± 1.4	8.7	± 0.3	14.7	± 1.4	6.5	± 0.6	12.6	± 3.7	14.1	± 1.4	8.1	± 1.1	12.6	± 1.7
SG height (Nov 05)	215.2	± 25.7	123.8	± 16.6	114.9	± 6.2	106.2	± 4.7	171.6	± 19.3	325.3	± 30.5	244.8	± 11.4	263.4	± 11.0
SG height (Feb 06)	269.1	± 24.3	296.4	± 28.7	224.9	± 27.1	130.9	± 7.8	237.1	± 10.1	330.6	± 47.5	384.2	± 20.4	312.2	± 10.4
SG height (May 06)	272.4	± 32.4	314.1	± 22.0	159.8	± 15.9	139.0	± 6.6	281.8	± 31.5	371.4	± 19.9	232.6	± 15.8	207.5	± 21.1
SG biomass (Nov 05)	79.8	± 8.4	35.7	± 9.0	17.5	± 2.0	6.3	± 1.6	22.9	± 2.5	19.8	± 3.2	28.5	± 4.0	46.3	± 1.7
SG biomass (Feb 06)	115.0	± 9.3	65.4	± 2.6	40.2	± 15.2	9.2	± 0.6	43.0	± 2.4	60.7	± 8.8	48.4	± 7.8	82.0	± 11.3
SG biomass (May 06)	74.0	± 3.4	48.0	± 9.4	28.4	± 10.5	15.7	± 2.5	68.7	± 7.5	60.5	± 7.4	49.5	± 7.0	16.7	± 2.7
Algal load (Nov 05)	0.11	± 0.03	0.14	± 0.03	0.18	± 0.11	0.07	± 0.02	0.04	± 0.01	0.02	± 0.01	0.08	± 0.01	0.04	± 0.01
Algal load (Feb 06)	0.21	± 0.02	0.22	± 0.01	0.13	± 0.02	0.02	± 0.00	0.07	± 0.02	0.07	± 0.00	0.03	± 0.01	0.00	± 0.00
Algal load (May 06)	0.06	± 0.02	0.04	± 0.01	0.12	± 0.08	0.11	± 0.03	0.01	± 0.00	0.01	± 0.00	0.02	± 0.00	0.02	± 0.01

Creeks (SNK, $P < 0.05$). In the ambient sites Patonga (3.70 ± 0.97 NTU) was significantly higher and Smith Creek (0.27 ± 0.09 NTU) was significantly lower (SNK, $P < 0.05$) than the other two intermediate sites (Table 3.4).

Patterns in fish and macro-crustacean assemblages

Throughout the study a total of 43,589 individual fish were caught in seine net samples representing 39 fish species and four shrimp species. Assemblage structure did not differ significantly between catchments of different nutrient status (Table 3.6), however there was a significant interaction between sampling time and sites nested within nutrients (PERMANOVA, $F_{12, 72} = 5.714$, $P < 0.01$,Table 3.6) and pairwise *a posteriori* comparisons found that assemblages differed between enriched and ambient sites at all three sampling times ($P < 0.05$). Non-metric MDS plots for each sampling period showed a fairly consistent separation of sites with enriched and ambient nutrient status although this was more pronounced in summer and autumn. Enriched sites were generally more dispersed than sites in low nutrient catchments, particularly in spring and summer indicating greater variability in assemblage composition at these sites (Table 3.1).

SIMPER analysis was carried out to determine the major taxa contributing to differences between nutrient status at the three different sampling periods. Average dissimilarity among sampling times was consistent (62-68%) after square-root transformation of the data (Table 3.7). Glass perchlets (*Ambassis jacksoniensis*), and gobies, *Redigobius macrostoma*, and *Pandaculus lidwilli* consistently contributed the most to dissimilarities between nutrient status with higher abundances in low nutrient sites (Table 3.7). *Arenigobius frenatus, Gobiopterus semivestitus* and *Penaeus plebejus* were in consistently greater abundances in enriched sites (Table 3.7). *Favonigobius lateralis,* and juvenile recruits of trumpeter (*Peletes sexlineatus*) and silver biddy (*Gerres subfasciatus*) were more abundant in enriched sites in spring and autumn however, abundances were greater in undisturbed sites in summer (Table 3.7). *Macrobranchium intermedium* were caught in much greater numbers in summer in low nutrient sites but showed little difference in abundance between nutrient status in spring an autumn (Table 3.7).

Abundance and richness of major fish taxa

Abundances and species richness of fish including the abundance of all major taxa varied both spatially and temporally with a significant interaction between sampling time and sites nested within nutrients. Significant main effects for differences in abundances between sites in nutrient enriched and low nutrient catchments were detected for juvenile omnivorous fish, as well as *Ambassis jacksoniensis* and *Arenigobius frenatus* (ANOVAs, Table 3.8). Total abundance of fishes was generally higher at sites with low ambient nutrient levels but was variable among sites and sampling periods, while species richness was also highly variable across sampling times (Figure 3.2).

Juvenile recruits with a carnivorous diet (*Acanthopagrus australis*, *Rhabdosargus sarba*, *Pelates sexlineatus* and *Gerres subfasciatus*) had high autumn abundances at Narara and Erina Creeks (nutrient enriched) and Smith Creeks (low ambient nutrients) in the autumn sampling period but were otherwise distributed fairly evenly among sites (Figure 3.3). In contrast omnivorous fishes (*Girella tricuspidata*, *Meuschenia trachylepis*, *M. freycineti* and *Acanthaluteres spilomelanurus*) were significantly higher in abundance at ambient sites, particularly at Waterfall Bay and Woy Woy during the spring sampling period (Figure 3.3, Table 3.8). The glass perchlet (*Ambassis jacksoniensis*), a small pelagic zooplanktivore, was the most abundant species caught throughout the study and was significantly more abundant in low ambient nutrient sites, though varying in densities among sampling times (Figure 3.3, Table 3.8).

Fish from the family Gobidae were also very abundant during the study. Three species (*Arenigobius frenatus*, *Redigobius macrostoma* and *Pandaculus lidwilli*) dominated the catch though showed different distribution patterns. *A. frenatus* was found in significantly higher densities at enriched sites and also in similar abundances at Waterfall Bay and Woy Woy while *R. macrostoma* was not significantly different in abundances (Figure 3.4). *P. lidwilli* had a variable distribution but again was generally more abundant in ambient sites with the exception of high densities at an enriched site (Kincumber) and low densities at Patonga, an ambient site (Figure 3.4).

Table 3.6: PERMANOVA results for differences in fish assemblages in sites nested within nutrient status at different times. Quasi-*F*'-ratios were estimated for nutrients and P-values were determined the using degrees of freedom for numerator and denominator shown in subscript. Data were square-root transformed and analysed based on Bray-Curtis similarities.

Source	df	SS	MS	F	P
Nu	1	26290.2	26290.2	$2.595_{1,7}$	0.151
Si (Nu)	6	56404.0	9400.7	2.506	<0.001
Ti	2	22269.988	11134.9	2.969	<0.001
Nu x Ti	2	4350.1	2175.0	0.580	0.934
Si (Nu) x Ti	12	45012.2	3751.0	5.714	<0.001
Residual	72	47266.2	656.5		
Total	95	201592.5			

Table 3.7: SIMPER analysis of differences between fish assemblages in ambient and nutrient enriched sites for each sampling period. Data were square-root transformed prior to analysis.

	Spring			Summer			Autumn		
	Average dissimilarity = 67.63			Average dissimilarity = 65.63			Average dissimilarity = 62.05		
	Enriched	**Ambient**		**Enriched**	**Ambient**		**Enriched**	**Ambient**	
Species	Average Abundance	Average Abundance	Contrib. %	Average Abundance	Average Abundance	Contrib. %	Average Abundance	Average Abundance	Contrib. %
Ambassis jacksoniensis	2.38	11.33	14.08	2.67	11.83	15.78	2.36	14.79	17.7
Pandaculus lidwilli	4.67	6.5	10.52	6.18	8.96	15.18	3.47	8.2	10.69
Redigobius macrostoma	3.83	9.11	10.94	2.53	9.74	12.22	4.12	9.5	9.4
Macrobranchium intermedium	4.33	4.39	7.46	1.93	5.06	7.36	8.68	8.23	9.35
Arenigobius frenatus	4.51	1.84	5.88	4.89	2.25	6.88	4.02	1.41	4.63
Favonigobius lateralis	5.12	4.1	5.62	3.16	3.49	4.56	6.11	2.84	4.96
Gerres subfasciatus	2.51	2.29	4.94	1.07	2.02	3.57	5.82	5.16	8.02
Pelates sexlineatus	3.45	2.96	4.78	2.16	2.59	3.35	6.42	4.67	5.33
Gobiopterus semivestitus	3.14	0.64	4.22	2.47	0.66	3.34	3.22	1.27	4.04
Hippolyte australicasis	1.94	1.29	3.33	0.79	1.08	2.39	2.85	2.28	4.25
Peneaus plebejus	2.58	0.42	3.46	2.4	0.34	3.17	3.33	0.32	4.12
Girella tricuspidata	1.27	1.5	2.83	1.12	1.19	2.57	0.78	0.43	1.08
Rhabdosargus sarba	1.55	0.47	2.45	0.54	1.04	1.73	2.02	0.48	2.75
Monacanthus chinensis	0.61	1.53	2.25	0.88	2.5	3.25	0.79	1.5	1.58
Meuschenia trachylepis	0.54	1.17	2	0.54	1.6	2.64	0	0.76	1.15

Table 3.8: Analysis of variance results for fish abundance, species richness and major taxonomic groups. Quasi-*F*'-ratios were estimated for nutrients and P-values were determined the using degrees of freedom for numerator and denominator shown in subscript. Significant results are highlighted by bold text.

		Total abundance			**Species richness**			**Carnivores**			**Omnivores**		
Source	**DF**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**
Nutrients	1	11.33	$2.72_{1,5}$	0.160	0.01	$0.97_{12,5}$	0.557	1.71	$0.37_{6,7}$	0.887	20.72	$8.26_{1,8}$	**0.021**
Site (Nu)	6	2.07	0.58	0.742	17.57	0.47	0.817	11.20	3.70	**0.026**	1.96	0.52	0.780
Time	2	1.44	0.40	0.678	34.57	0.93	0.423	23.22	7.66	**0.007**	12.88	3.45	0.065
Nu x Ti	2	3.41	0.95	0.413	20.95	0.56	0.585	1.45	0.48	0.631	1.01	0.27	0.768
Ti x Si (Nu)	12	3.59	6.05	**<0.001**	37.32	8.51	**<0.001**	3.03	5.86	**<0.001**	3.73	8.00	**<0.001**
Residual	72	0.59			4.39			0.52			0.47		
		Ambassis			**Arenigobius**			**Redigobius**			**Pandanculus**		
Nutrients	1	196.20	$11.90_{1,7}$	0.011	34.20	$6.90_{1,8}$	0.030	143.69	$5.71_{1,6}$	0.054	18.85	$0.62_{3,6}$	0.627
Site (Nu)	6	16.38	1.91	0.159	4.51	1.48	0.265	25.24	8.48	**0.001**	48.83	4.23	**0.016**
Time	2	2.37	0.28	0.763	5.74	1.89	0.194	5.81	1.95	0.185	0.71	0.06	0.941
Nu x Ti	2	0.82	0.10	0.909	0.89	0.29	0.751	0.45	0.15	0.860	0.51	0.04	0.957
Ti x Si (Nu)	12	8.56	3.50	**<0.001**	3.05	2.46	**0.010**	2.98	5.94	**<0.001**	11.54	11.06	**<0.001**
Residual	72	2.44			1.24			0.50			1.04		

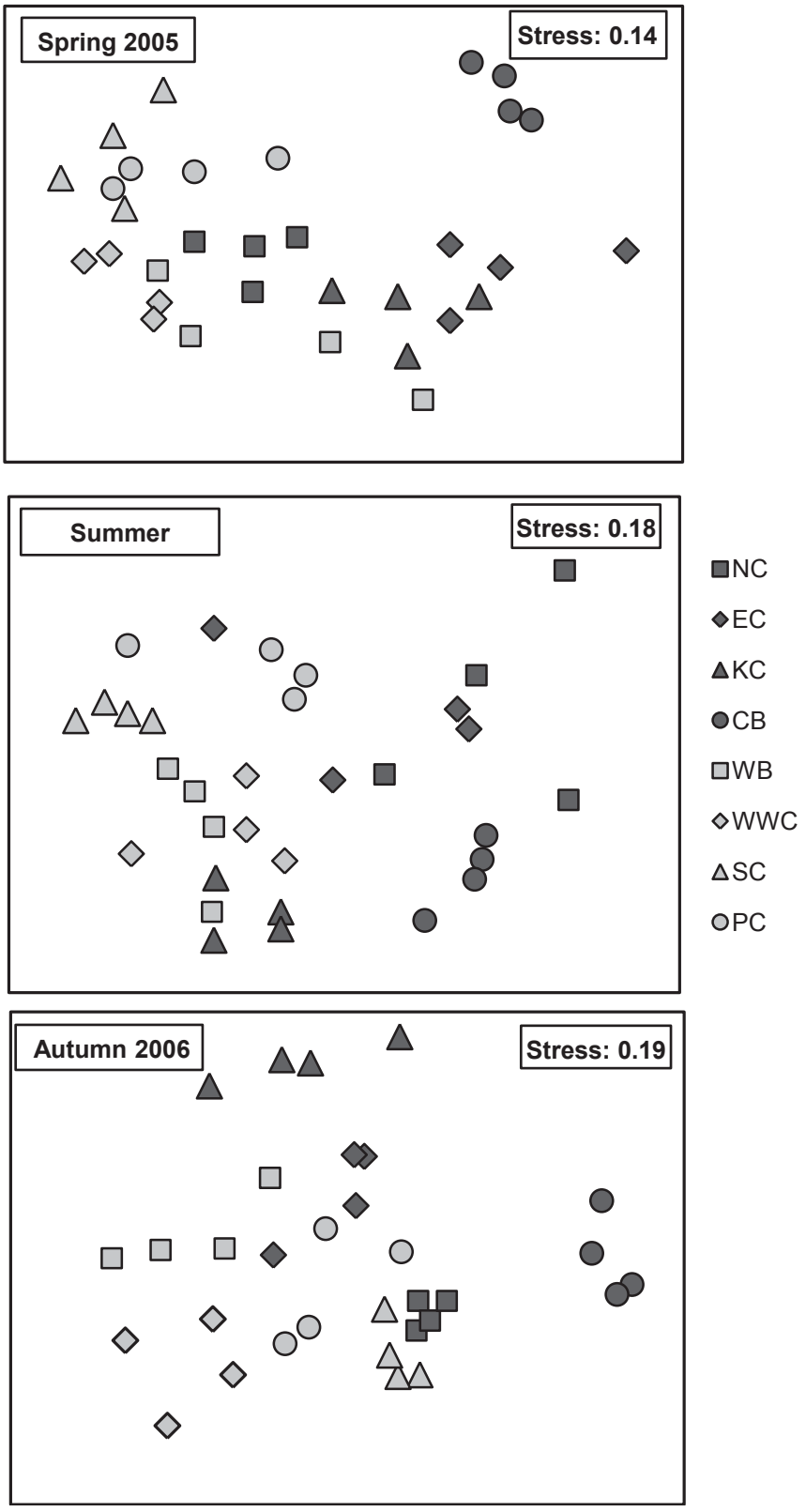

Figure 3.1: MDS plots of fish assemblages at sites with ambient nutrients (light grey) nutrient enriched sites (dark grey) at the three different sampling periods.

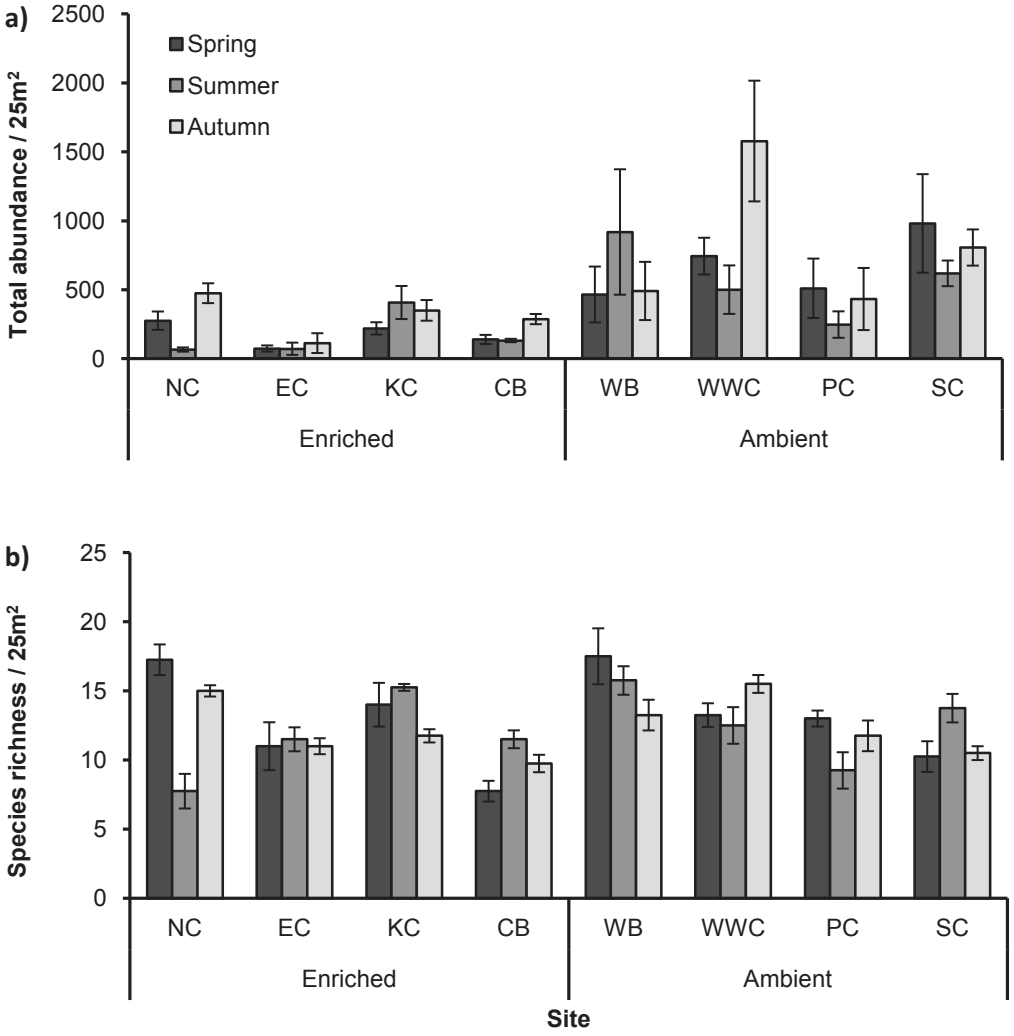

Figure 3.2: Mean values ± 1 S.E. for; a) Total abundance and b) species richness of juvenile fish at all sites over three sampling periods. NC = Narara Creek, EC = Erina Creek, KC = Kincumber Creek, CB = Careel Bay, WB = Waterfall Bay, WWC = Woy Woy Creek, PC = Patonga Creek, SC = Smith Creek.

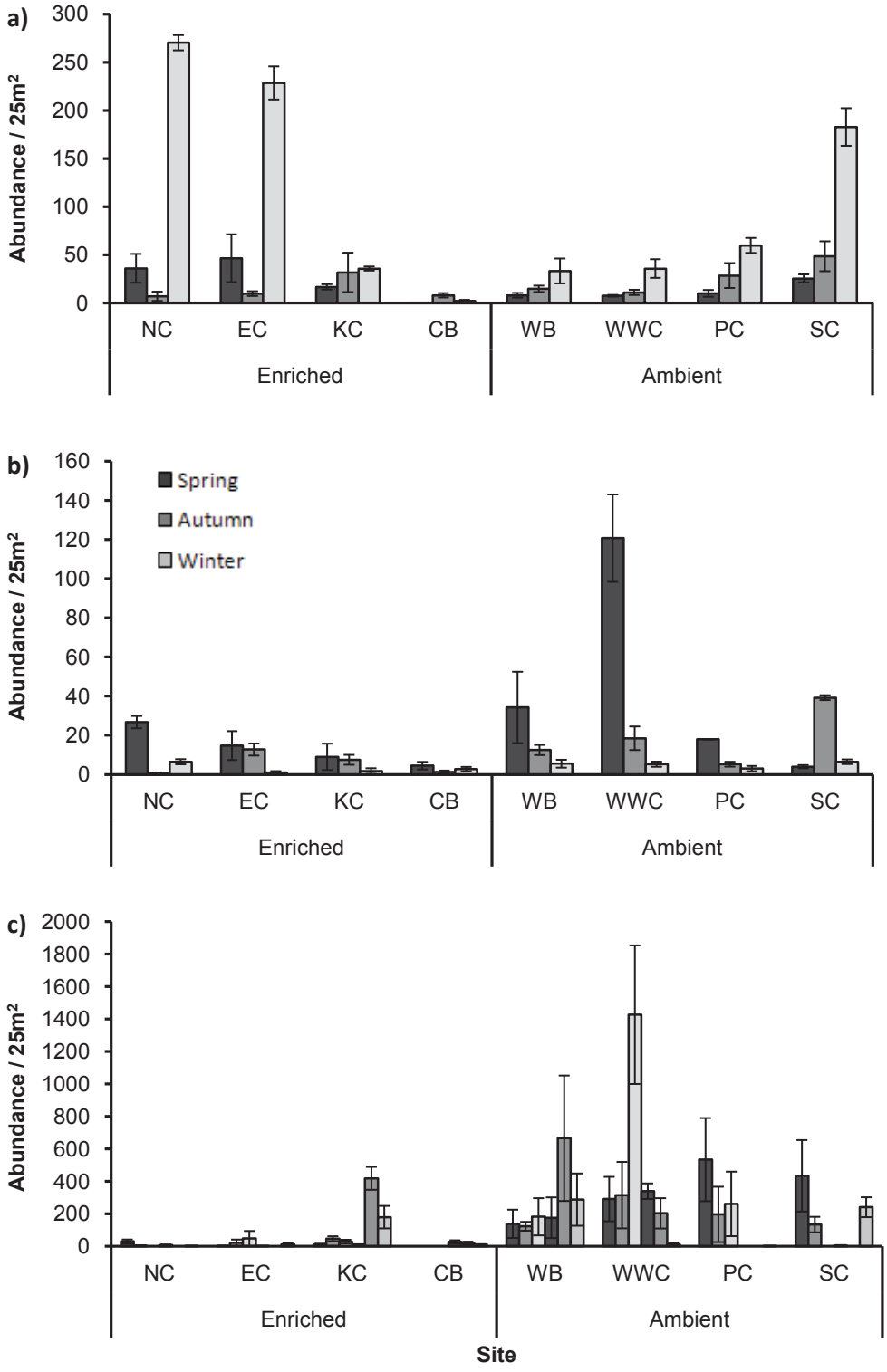

Figure 3.3: Mean values ± 1 S.E. for abundance of a) carnivorous juvenile fish, b) omnivorous juvenile fish and c) *Ambassis jacksoniensis* at all sites over three sampling periods. NC = Narara Creek, EC = Erina Creek, KC = Kincumber Creek, CB = Careel Bay, WB = Waterfall Bay, WWC = Woy Woy Creek, PC = Patonga Creek, SC = Smith Creek.

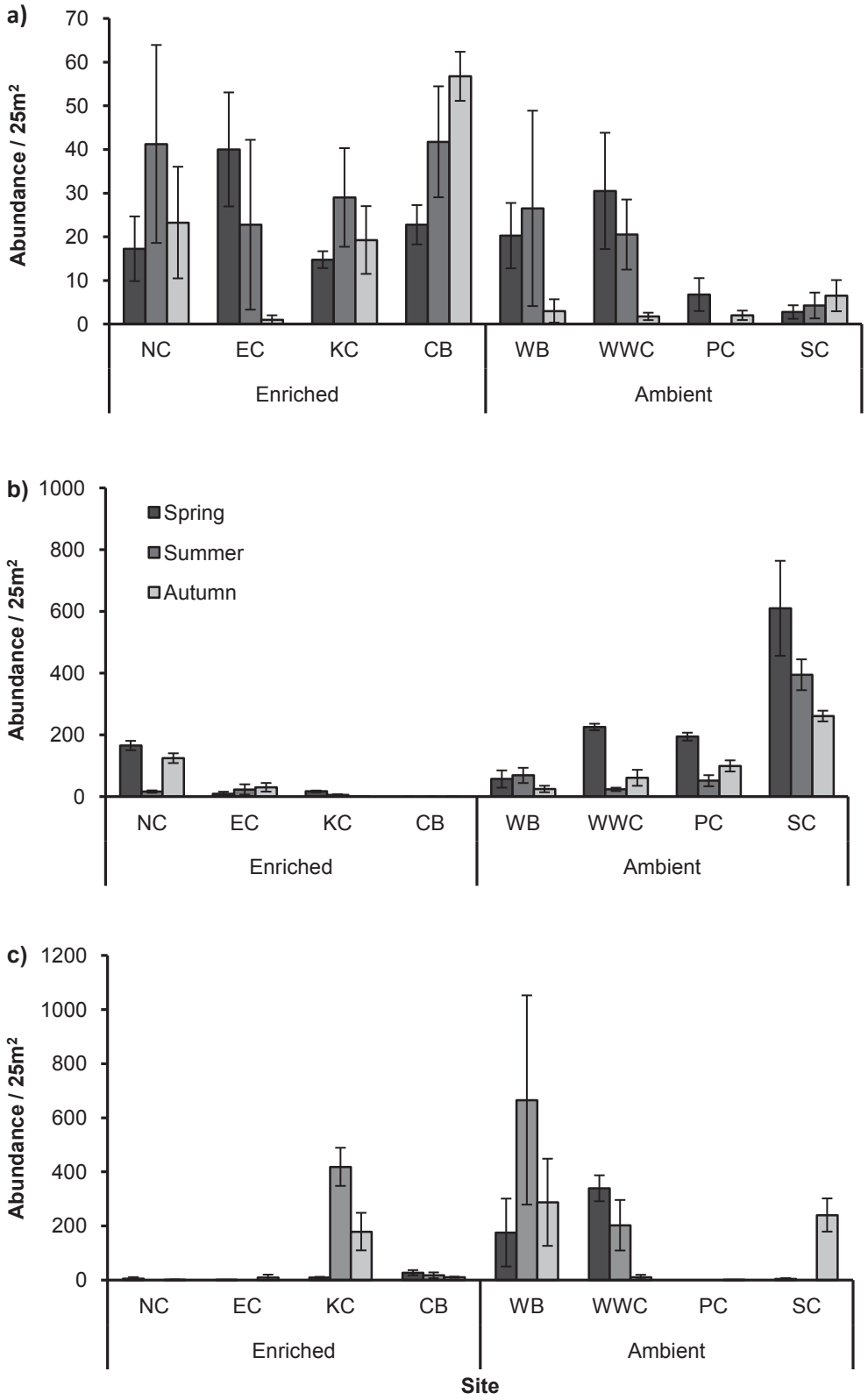

Figure 3.4: Mean values ± 1 S.E. for; abundance of a) *Arenigobius frenatus* b) *Redigobius macrostoma* and c) *Pandaculus lidwilli* at all sites over three sampling periods. NC = Narara Creek, EC = Erina Creek, KC = Kincumber Creek, CB = Careel Bay, WB = Waterfall Bay, WWC = Woy Woy Creek, PC = Patonga Creek, SC = Smith Creek.

Patterns in grazer assemblages

Assemblages of mesograzers also showed a significant interaction between sampling time and sites nested within nutrients (PERMANOVA, $F_{12, 72} = 5.413$, $P < 0.02$), however, significant differences between undisturbed and nutrient enriched catchments were not detected (Table 3.9). When investigating the patterns at each sampling period, no distinct separation was found in samples between nutrient levels, rather the sites are separated along spatial grounds with the spatial proximity apparently influencing the similarity between assemblages (Figure 3.5). The contribution of major species to differences between assemblages at sites with ambient and enriched nitrogen levels was determined using SIMPER analysis. Amphipod morphospecies Maeridae *sp* and Amphilochidae *sp* contributed to 20% of the difference between nutrient groups, while a Tanaidae morphospecies contributed 10% and three major gastropod species (*Diala megapicalis Alaba sp*, and Aplysiidae *sp*.) contributed a combined total of 26%.

Abundance and richness of major grazer taxa

The interaction term for sampling time and sites nested within nutrients total abundance and species richness of grazing invertebrates was significant (ANOVA, Table 3.11). Total abundance was extremely high in enriched sites at Narara, Erina and Kincumber Creeks in summer and at intermediate levels at two ambient sites (Waterfall Bay and Patonga Creek). Levels were much lower at the summer and autumn sampling periods and at Careel Bay and Smith Creek at all times (Figure 3.6). Species richness was at its highest at Narara, Erina and Kincumber Creeks, intermediate at Waterfall Bay, Woy Woy Bay and Patonga Creek and low at Careel Bay and Smith Creek. Generally, species richness was highest during the spring sampling period and lowest during summer (Figure 3.6).

The abundance of the two main taxonomic groups of grazers (Amphipoda and Gastropoda) also displayed significant interactions between sampling time and sites nested within nutrients (ANOVA, Table 3.11). Amphipods were dominated by large abundances in spring at Narara and higher abundances in Erina Creek compared to Kincumber and Patonga Creeks. These abundances dwarfed all other sites and sampling times (Figure 3.7). Gastropod abundances were generally higher in enriched sites with

the highest levels at Narara and Erina in spring and summer and at Kincumber and Waterfall Bay in spring alone (Figure 3.7).

Relating fish and grazer assemblages to environmental characteristics

Fish assemblages and environmental similarity matrices were significantly correlated however the degree of correlation was relatively low (RELATE, $\rho = 0.102$, $P = 0.021$). BioEnv identified turbidity as the variable with the greatest correlation for predicting fish assemblage composition, while the percentage of developed catchment was the best correlated catchment variable and seagrass canopy height was the best predictor from the habitat attributes (Table 3.12). The inclusion of extra variables to turbidity did not improve the correlation (Table 3.12). Grazer assemblages were also significantly correlated with rank-order similarity, though again with a relatively low correlation (RELATE, $\rho = 0.119$, $P = 0.008$). In water quality samples, total nitrogen was identified as the best single predictor of grazer assemblage structure using BioEnv, followed by pH. Seagrass density was the most highly correlated habitat attribute and percentage development the best catchment predictor (Table 3.12). Including extra variables improved correlation with the best combination of four variables being total nitrogen, pH, seagrass density, and seagrass height (Table 3.12).

Table 3.9: PERMANOVA results for differences in grazer assemblages in sites nested within nutrient status at different times. Quasi-*F*'-ratios were estimated for nutrients and P-values were determined the using degrees of freedom for numerator and denominator shown in subscript. Data were square-root transformed and analysed based on Bray-Curtis similarities.

Source	df	SS	MS	F	P
Nu	1	7562.6	7562.6	$0.867_{3,7}$	0.502
Si (Nu)	6	52067.2	8677.9	1.731	**0.008**
Ti	2	53741.6	26870.8	5.361	**0.000**
Nu x Ti	2	11656.7	5828.3	1.163	0.286
Si (Nu) x Ti	12	60147.6	5012.3	5.413	**<0.001**
Residual	72	66669.7	926.0		
Total	95	251845.4			

Table 3.10: SIMPER analysis of differences between grazer assemblages in ambient and nutrient enriched sites for each sampling period. Data were square-root transformed prior to analysis.

	Spring			**Summer**			**Autumn**		
	Average dissimilarity = 64.33			Average dissimilarity = 71.24			Average dissimilarity = 62.14		
	Enriched	**Ambient**		**Enriched**	**Ambient**		**Enriched**	**Ambient**	
Species	Average Abundance	Average Abundance	Contrib. %	Average Abundance	Average Abundance	Contrib. %	Average Abundance	Average Abundance	Contrib. %
Maeridae *spp.*	10.63	3.63	18.85	1.42	1.33	8.68	1.58	1.29	9.75
Tanaidae spp.	3.59	2.22	8.90	1.70	0.83	10.56	0.88	1.51	12.43
Diala megapicalis	5.83	3.20	14.26	1.32	0.06	8.08	0.50	0.50	6.15
Aplysiiadae spp.	0.00	0.00	0.00	2.67	1.21	13.92	0.38	0.71	6.36
Amphilochidae *sp.*	5.61	2.12	11.59	0.00	0.00	0.00	1.16	0.13	9.18
Alaba sp.	3.25	1.97	6.90	2.03	0.77	11.00	1.62	2.48	10.95
Caltholotia fragrum	0.58	0.36	1.79	1.13	0.06	6.84	1.20	0.15	9.68

Table 3.11: Analysis of variance results for grazer abundance and species richness and abundance of major taxonomic groups. Quasi-*F*'-ratios were estimated for nutrients and P-values were determined the using degrees of freedom for numerator and denominator shown in subscript. Significant results are highlighted by bold text.

		Total abundance			**Species richness**			**Amphipods**			**Gastropods**		
Source	**DF**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**
Nutrients	1	11.66	$1.93_{1,8}$	0.202	100.04	$3.01_{1,7}$	0.126	12.37	$1.24_{1,7}$	0.302	14.21	$1.10_{1,6}$	0.335
Site (Nu)	6	4.67	3.67	**0.026**	31.91	4.88	**0.010**	7.37	2.84	0.059	7.67	5.52	**0.006**
Time	2	36.04	28.32	**<0.001**	141.78	21.70	**<0.001**	45.96	17.71	**<0.001**	19.03	13.70	**<0.001**
Nu x Ti	2	2.04	1.61	0.241	3.51	0.54	0.598	4.67	1.80	0.207	6.57	4.73	**0.031**
Ti x Si (Nu)	12	1.27	5.89	**<0.001**	6.53	1.90	**0.048**	2.60	5.83	**<0.001**	1.39	4.86	**<0.001**
Residual	72	0.22			3.44			0.45			0.29		

Table 3.12: Correlation coefficients of BIO-ENV analysis to test for relationships between fish and grazer assemblage and environmental and habitat variables.

Fish assemblages			Grazer assemblages		
# Var	*r*	Selections	# Var	*r*	Selections
1	0.214	TURB	1	0.235	TN
1	0.148	%DEV	1	0.167	SGD
1	0.099	SGH	1	0.100	%DEV
2	0.194	TURB, ALG	2	0.261	TN, pH
2	0.182	TURB, %DEV	2	0.248	TN, SGD
3	0.190	TURB, SGH, ALG	3	0.272	TN, pH, SGD
4	0.194	TURB, SGH, ALG, %DEV	4	0.276	TN, pH, SGD, SGH

For brevity, only the best single environmental, habitat and catchment variables and the best combinations are presented for any given number of variables. TURB = turbidity, ALG= algal load, SGH = seagrass height, SGD = seagrass density, %DEV = percentage catchment development, TN = total nitrogen.

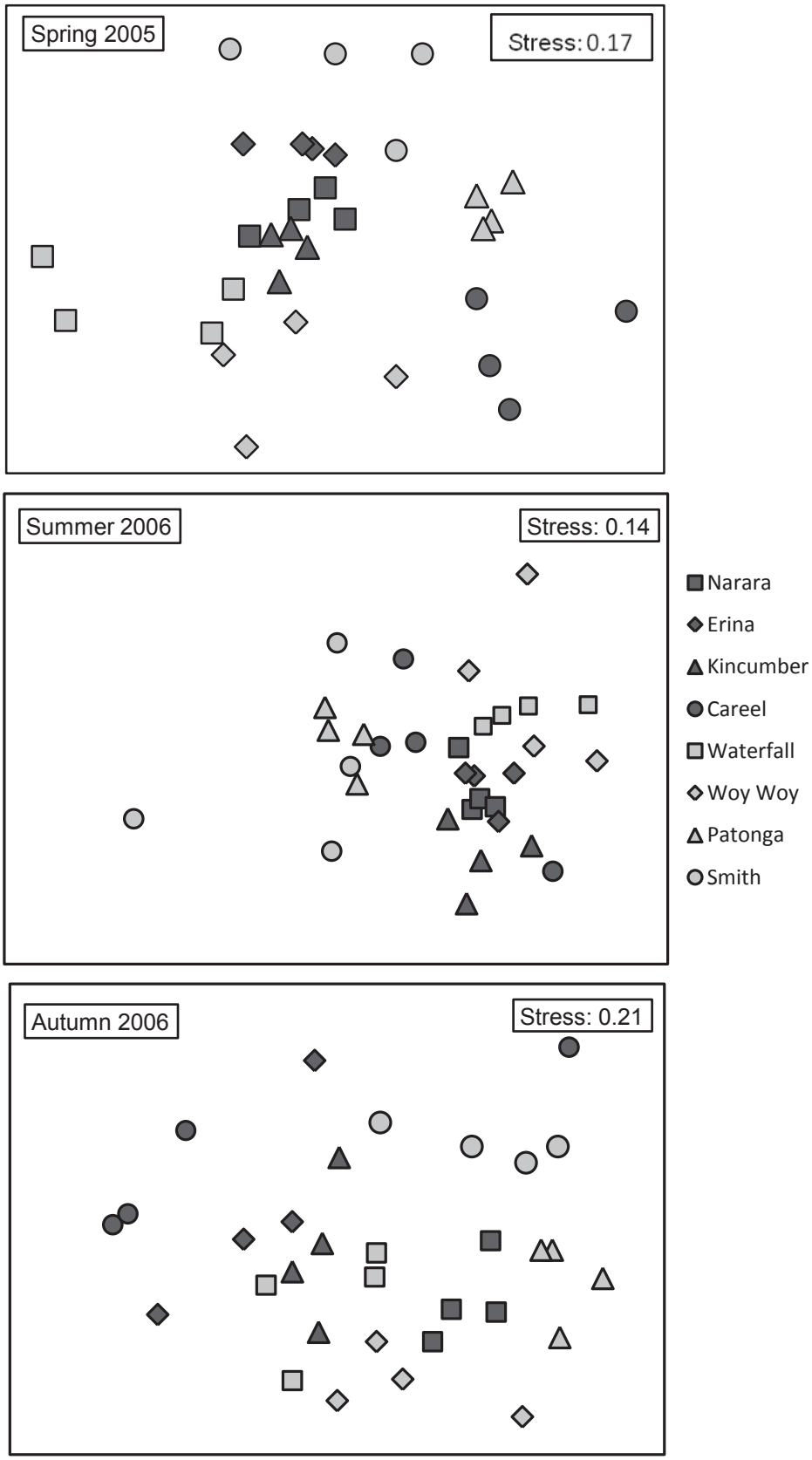

Figure 3.5: MDS plots of grazer assemblages at sites with ambient nutrients (light grey) nutrient enriched sites (dark grey) at the three different sampling periods.

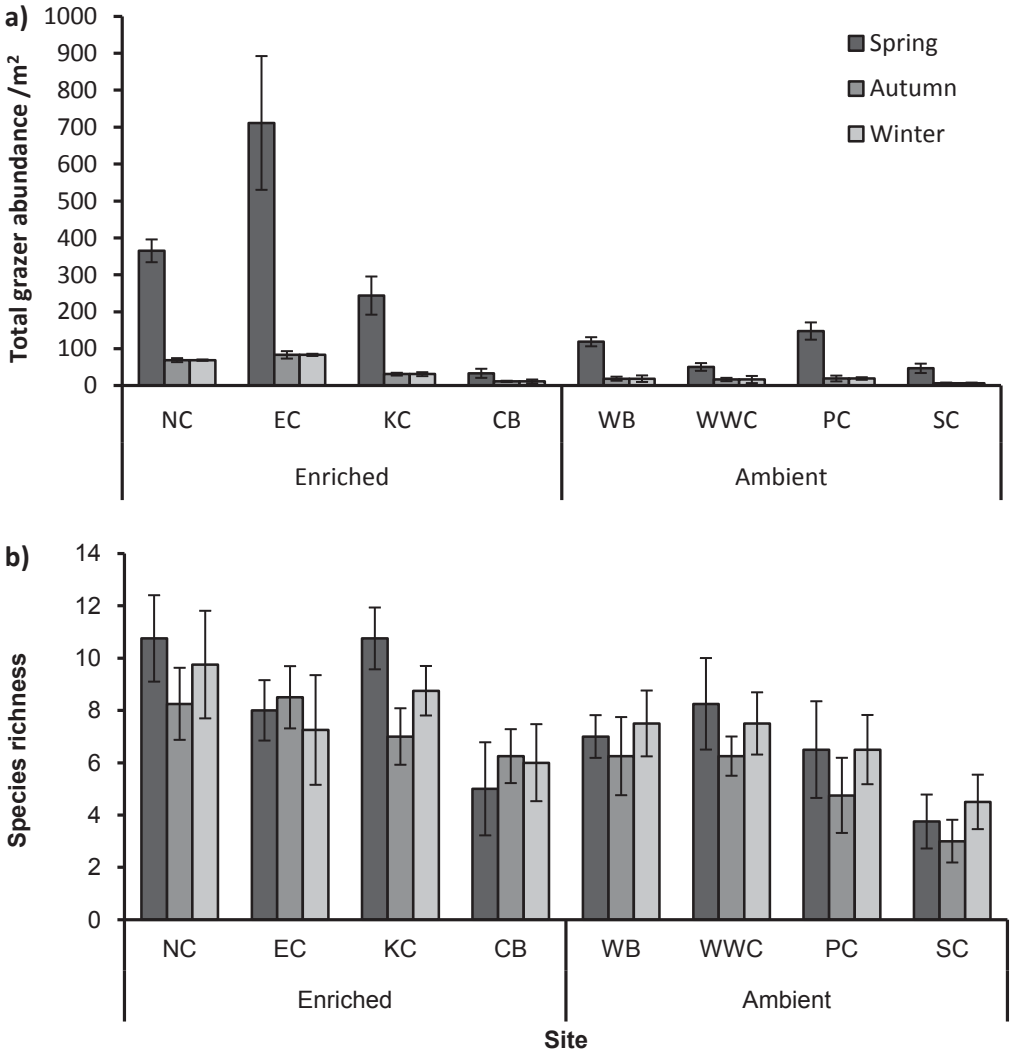

Figure 3.6: Mean values ± 1 S.E. for a) total abundance and b) species richness of mesograzer assemblages at all sites over three sampling periods. NC = Narara Creek, EC = Erina Creek, KC = Kincumber Creek, CB = Careel Bay, WB = Waterfall Bay, WWC = Woy Woy Creek, PC = Patonga Creek, SC = Smith Creek.

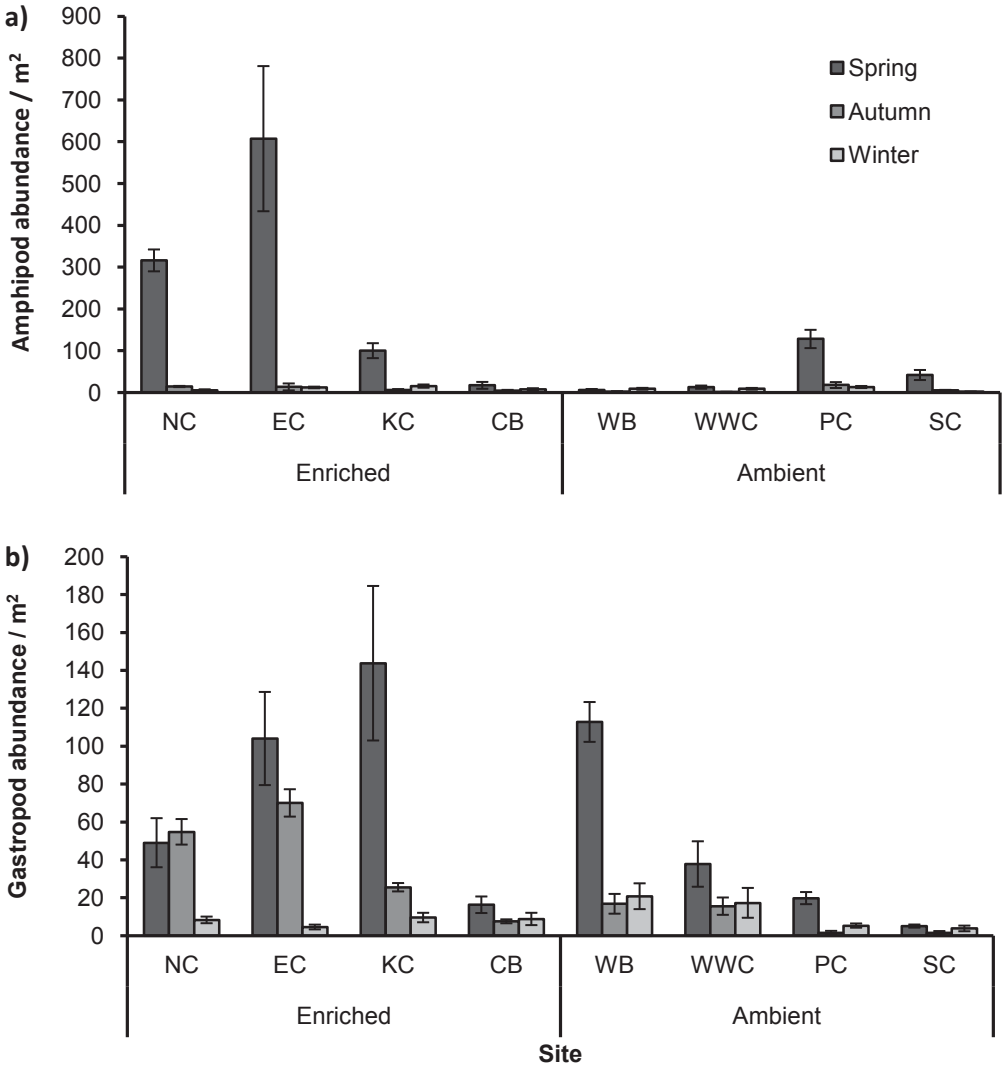

Figure 3.7: Mean values ± 1 S.E. for abundance of a) amphipods and b) gastropods at all sites over three sampling periods. NC = Narara Creek, EC = Erina Creek, KC = Kincumber Creek, CB = Careel Bay, WB = Waterfall Bay, WWC = Woy Woy Creek, PC = Patonga Creek, SC = Smith Creek.

DISCUSSION

This study compared differences in fish and grazer assemblage structure at sites with forested catchments and low ambient nutrients against those at developed catchments linked to elevated nutrients. Fish assemblages differed significantly among developed and undeveloped catchments. Patterns in grazer assemblages between catchment types were less well defined due to similarities in assemblages within estuaries dominating variation among communities. Environmental variables such as turbidity (for fish) and total nitrogen (for grazers) were found to have the greatest influence on the variation of these assemblages.

Fish assemblages

Fish assemblages in seagrasses from developed and undeveloped catchments showed a clear separation in community structure even allowing for the inherent spatial and temporal variability known to exist within these systems. Overall, the abundance of fishes was higher in undeveloped catchments and species richness was also slightly higher, though more variable across sites and sampling periods.

The percentage of catchment development was weakly correlated with the structure of fish assemblages. Although the link between deforestation and nutrient enrichment is well established (Hopkinson and Vallino 1995; Wahl et al. 1997), land-use type may be a key factor to incorporate into estuarine models. Run-off from urbanised catchments into estuaries can be up to an order of magnitude greater than that from agricultural land (Gorman et al. 2009). Water quality, particularly turbidity, was the best predictor of fish community structure. Previous studies have linked turbidity level to the structure of fish assemblages with species distributed along turbidity gradients (Cyrus and Blaber 1987; Cyrus and Blaber 1992). Increased turbidity has been positively correlated with recruitment of some juvenile fish species in a tropical estuary, with suggestions that it may act as a sensory cue for finding nursery habitat or reduce predation risk from visual predators (Blaber and Blaber 1980).

Increased nutrient loads in developed watersheds are known to diminish habitat complexity (seagrass biomass and leaf density) and is associated with a reduction in fish

abundance, species richness and biomass (Wyda et al. 2002). In the present study, fish assemblages were only very weakly correlated with habitat characteristics, with seagrass canopy height being the best predictor of assemblage structure.

A difference in trophic pathways across nutrient gradients was detected in juvenile fish communities with omnivorous species more abundant in low nutrient, undeveloped catchments and carnivorous recruits generally more abundant in enriched, developed catchments. Allochthonous input of nutrients into food webs are capable of altering trophic dynamics by either stabilising or destabilising population densities depending on the amount or type of inputs and the properties of the system (Faria and Costa 2010). In oligotrophic and mesotrophic systems with both periphyton and phytoplankton contributing to primary production, food webs are generally stable but become unstable as greater inputs of nutrients send the systems eutrophic (Jefferies 2000; Vadeboncoeur et al. 2005). Slight increases in the average trophic level of fish communities of an oligotrophic bay have been detected in areas undergoing nutrient enrichment from fish farming (Machias et al. 2004).

A large proportion of structural differences in assemblages were due to the abundances of several fish species, the glass perchlet (*A. jacksoniensis*) and two gobiid species, *R. macrostoma* and *P. lidwilli* reaching higher densities in undeveloped catchments. The glass perchlet (*A. jacksoniensis*) and large-mouth goby have similar life traits and their distributions are often closely associated (Jones and West 2005). Both species are pelagic planktivores feeding dominantly on crab zoeae from adjacent saltmarsh habitat (Melville and Connolly 2005; Mazumder et al. 2006; Platell and Freewater 2009). They are also a common forage fish in the diet of piscivores (Taylor et al. 2006). These species often dominate the abundances of seagrass fish assemblages in south-eastern Australia (Ferrell and Bell 1991; Gray et al. 1996; Rotherham and West 2002) and are also closely associated with saltmarsh and mangrove habitats (Jelbart et al. 2007; Saintilan et al. 2007). Very little is known about the biology of *P. lidwilli*.

A. jacksoniensis have been shown to be sensitive to wastewater effluent with increased nitrogen loads. This was manifested in the form of lower health measured as pathological

tissue change (Schlacher et al. 2007). The adverse health effects in individual fish are likely to lead to higher mortality and reduced recruitment which becomes discernable at higher ecological levels as reduced abundances of this species (Schlacher et al. 2007).

In contrast, the half bridled goby (*A. frenatus*) was more abundant at sites within developed catchments. *A. frenatus* is a benthic carnivore (Edgar and Shaw 1995c) and has been shown to be tolerant of contaminated sites (Roach et al. 2008). This species is also a forage fish for benthic piscivores (Edgar and Shaw 1995b). Abundances may increase under nutrient enrichment due to increased food resources from either greater abundances of epiphyte grazer or trophic subsidy from benthic invertebrates in detrital food webs (Moore et al. 2004).

Grazer assemblages

Grazer assemblages varied both spatially and temporally with no clear pattern of differences between assemblages in developed and undeveloped catchments. Sites tended to loosely group together based on the estuary they were situated in (i.e. Brisbane Water and Hawkesbury). Within estuaries, there was separation between developed and developing catchments.

Abundances varied substantially in space and time with spikes of amphipods at Erina and Narara in spring and high abundances of gastropods at Erina, Kincumber and Waterfall Bay in summer. These spikes in grazer numbers were weakly correlated with total nitrogen levels indicating that nutrient levels were probably driving an increase in food resources by increasing epiphytic algal productivity (Gil et al. 2006). These results also highlight the need to investigate interactions between elevated human-induced nutrient subsidies at finer spatial scales of tens to hundreds of metres rather than kilometres as was the case in this study. Studies of urban and agricultural increases in nutrients have found that they can dilute rapidly with distance from their source over these smaller scales (Gorman et al. 2009).

Indicators of estuarine health

The differences in small and juvenile fish assemblage structure in developed and undeveloped catchments provide the potential for using this measure as a biological

indicator of seagrass ecosystem health. Biological indices such as fish indices are being used increasingly to determine the ecological health of coastal and estuarine waters (Whitfield and Elliott 2002; Harrison and Whitfield 2004; Borja et al. 2009; Pinto et al. 2009). Recent studies have identified them as robust and useful tools for the assessment of estuarine nursery habitat (Courrat et al. 2009). With further investigation, fish such as *A. jacksoniensis* and *R. macrostoma* that appeared to be sensitive to anthropogenic disturbance may serve as indicator species for disturbance in seagrass beds in south-eastern Australia. These indicators would be most effective when used in conjunction with catchment and estuary traits, water quality parameters and habitat characteristics to give a more complete and robust picture of estuarine condition (Burkholder et al. 2007).

This study provides valuable information for the assessment of the health of estuarine seagrass beds that may be subjected to anthropogenic disturbance from development. Careful planning of future development should consider the ecological value of surrounding estuaries and the presence of important habitat and such as seagrasses. Estuarine properties such as tidal flushing and water residence times and the potential effects of increased nitrogen loads should also be considered in planning (Kennish et al. 2007) and measures to reduce impacts such as riparian buffer zones and treatment of wastewater should be implemented to reduce nitrogen loading and the human impacts on these valuable systems.

Chapter 4

TROPHIC DYNAMICS IN TEMPERATE SEAGRASS FOOD CHAINS

ABSTRACT

Simple ecological models that predict trophic responses to bottom-up forcing have the potential to be an invaluable tool for ecosystem managers. Traditionally models using resource-dependent functional responses have been used to predict trophic dynamics of food webs and how each trophic level will respond to perturbations within the system. More recently, the introduction of ratio-dependent functional responses have challenged this way of thinking and resulted in debate regarding the merits of the two types of models and which is best represented in natural systems. This study compares field observations of a four level estuarine seagrass food chain (epiphytic algae, grazing invertebrates, juvenile fish, piscivorous fish) in sites with relatively undisturbed catchments areas and low ambient nutrients to sites in developed catchments that have been subjected to prolonged nutrient enrichment to test the impact of bottom-up forcing. Nutrient enriched sites had significantly greater loads of both epiphytic algae and grazing invertebrates as predicted by ratio-dependent models, however the bottom-up forcing of nutrients is attenuated in higher trophic levels of juvenile and piscivorous fish where biomass did not differ significantly between low nutrient and enriched sites. Reasons for this disconnect in the upward cascade of energy may be due to factors such as diversity, omnivory, trophic subsidy from other sources, or the openness of the system at higher levels being driven by recruitment of juveniles or the transience of predators. These results have major implications for catchment and fisheries management because they indicate that long-term nutrient enrichment increases epiphyte loads and places seagrasses under stress, while providing no benefits to secondary production in juvenile or adult fish that use these areas as a nursery or feeding grounds.

INTRODUCTION

A key goal of theoretical ecology is to explain complex ecosystem processes with straightforward mathematical models (Bascompte and Melián 2005). A classic example of this has been the modelling of trophic dynamics; the movement of energy in food chains through the relationships between consumers and their resources. These models are often based on differential predator-prey equations that determine the population of each trophic level in a food chain. The output of these equations depends on i) a numerical response, which describes the per capita growth rate of a population taking into account all factors that may influence it and; ii) a functional response that describes the rate at which an individual predator can consume its prey as a function of prey density (Abrams and Ginzburg 2000).

The earliest predator-prey models were developed by Lotka (1925) and Volterra (1926). The functional responses used in these early models and almost universally accepted in later advancements (Holling 1959; Rosenzweig and Macarthur 1963; Abrams 1982) are based on resource dependence, where changes in the rate of predation by an average predator is determined by the abundance of prey (Fussmann et al. 2005). The basis of these models was challenged by the concept of ratio-dependent trophic functions (Arditi and Ginzburg 1989), where predation rates depend on the ratio of predators to prey. A vigorous debate has since followed (Hanski 1991; Ginzburg and Akcakaya 1992; Oksanen et al. 1992; Abrams 1994; Akcakaya et al. 1995; Fussmann et al. 2005) with proponents on both sides claiming that their model's predictions best mirror natural systems. There is, however, also general consensus that empirical evidence supporting each case is lacking (Abram and Ginzburg 2000).

Thus far, field tests of these predictions have predominantly concentrated on small aquatic lakes or terrestrial systems with three trophic levels (but see Bishop et al. 2006). Systems with three trophic levels do not, however, provide the best test of the models as their predictions for these simple systems are relatively similar (Ginzburg and Akcakaya 1992). The clearest difference between the two models occurs in predictions of community responses to sustained long-term bottom-up pressure, such as a press perturbation like nutrient enrichment from catchment development (Bender et al. 1984),

in a system with four trophic levels (Table 4.1). In this situation, resource-dependent models predict that the top level of the food chain, which has no predatory pressure, will increase proportionally to the bottom-up force. The next-to-last level will remain constant and each successive level below will show an alternating non-linear increase or decrease in biomass. In a four level trophic system the biomass of producers will decrease while primary producers will increase (Table 4.1). Under the same conditions, however, ratio dependence leads to a proportional increase in the biomass of all trophic levels irrespective of the length of the food chain (Arditi and Ginzburg 1989).

For several reasons, coastal systems, such as estuaries and seagrass beds, provide an ideal platform to conduct field tests correlating trophic level biomass and nutrient loading in different ecosystems. Large aquatic systems generally have four trophic levels whereas terrestrial systems have three (Fretwell 1977; Oksanen et al. 1981). Trophic cascades are also more common in aquatic systems (Strong 1992). Further, in recent decades, coastal aquatic systems have increased in nutrient load, and transformed from oligotrophic to more mesotrophic and eutrophic conditions (Paerl 1997). Generally, the availability of limiting nutrients is the driving bottom-up force controlling primary productivity in biological systems (Cloern 2001).

Table 4.1: Predicted responses to trophic levels when exposed to sustained bottom-up forcing in trophic cascades with four levels (source Ginzburg & Akcakaya 1992).

Trophic level	Ratio-dependent models	Resource-dependent models	Seagrass food chain
1	∞⇑	⇓	Epiphytic algae
2	∞⇑	⇑	Mesograzers
3	∞⇑	=	Juvenile fish
4	∞⇑	∞⇑	Piscivores

∞ indicates a proportional response, = indicates biomass remains constant

Temperate seagrass beds are an important coastal habitat which are increasingly affected by nutrient enrichment due to anthropogenic increases from nearby catchments (Short and Wyllie-Echeverria 1996). Excessive increases in nutrients in seagrass systems can lead to phytoplankton blooms and increased growth of epiphytic algae resulting in shading of seagrass leaves and ultimately habitat collapse (Dennison et al. 1993). The

addition of moderate amounts of nutrients may not be as devastating to the seagrasses themselves, but instead lead to changes in the community structure of the food webs supported by these habitats (Gil et al. 2006; Jorgensen et al. 2007).

This study considers the effects of long-term nutrient enrichment on a four level aquatic food chain. This chain is based on epiphytic algae growing on seagrass blades, through invertebrate mesograzers, juvenile fishes with piscivorous fishes as the top predator. As seagrass tissue is low in nutrients and not palatable to most grazers, epiphytes contribute a large portion of the trophic support in temperate seagrass ecosystems (Moncreiff and Sullivan 2001; Connolly et al. 2005a). The biomass of each trophic level within the system was compared using results from two types of sites: nutrient enriched sites adjacent to catchments containing urban development; and reference sites in catchments surrounded by National Parks with low ambient nutrient levels. It was then determined whether sustained bottom-up pressure produced a trophic cascade upwards through the food chain. If so, did the response of each trophic level support: i) resource-dependent theory, which predicts a non-linear decrease in epiphytic algal biomass, a non-linear increase in the biomass of mesograzers, no change in the biomass of juvenile fish and an increase in piscivorous fish proportional to the increase in nutrient loading; ii) ratio-dependent theory, which predicts an increase in all trophic levels proportional to the increase in nutrient loading; or iii) neither of the two models, suggesting that either an alternate model needs to be developed or something other than nutrients are driving the system. In a valuable estuarine habitat such as seagrass beds, simple models which show predictions of upward cascading and predictable trophic responses, as demonstrated in this study, will have major implications for catchment, conservation and fisheries management.

METHODS

Study sites

The study was carried out in the Brisbane Water and Hawkesbury River catchment just north of Sydney 33°S 151°E (see Figure 2.1) using biomass data from abundance samples collected and presented in Chapter 3. Six seagrass meadows were sampled to determine the trophic dynamics of systems with elevated nutrient status. Three seagrass

beds (Narara Creek, Kincumber Creek and Erina Creek) are situated in sub-catchments of Brisbane Water surrounded by urbanised, industrial and agricultural land use. The remaining three seagrass beds are situated in catchments that are surrounded by National Park and are considered to have low ambient nutrient levels. Two of these are sub-catchments of Brisbane Water (Waterfall Bay, and Woy Woy Bay), while the third (Patonga Creek) is a tributary of Broken Bay and 8-14 km distant from the other sites. All sites were located in lower estuaries with salinity of approximately 35 PSU and *Zostera capricorni* densities varying among sites and time of year (see Table 3.5). A comprehensive investigation of water quality using flow injection analysis (Paterson et al. 2003b) found significant increases of NH_3 (6.6 – 8.48 fold) and NO_x (4.6 -14.2 fold) in developed catchments (Narara Creek and Erina Creek) compared to undeveloped reference sites (Waterfall and Woy Woy Bay). None of the sites were subject to direct commercial fishing and all are considered to have similar levels of top-down pressure from recreational fishing.

Experimental design

The six sites were sampled on three separate sampling occasions (October/November 2005, January/February 2006 & May 2006) to compare the biomass of each trophic level in the elevated nutrient sites to those in the reference sites within a seagrass food chain (epiphytic algae, grazing invertebrates, juvenile fishes, piscivorous fishes).

On each occasion, four replicate samples of seagrass, epiphytes and associated epifauna were taken using a BROMAR sampler (see methods chapter). Samples were preserved in formalin until they were processed. Each sample was sieved through a 500 µm mesh to retain the dominant epiphyte grazers (amphipods and gastropods) (Jernakoff et al. 1996; Luczkovich et al. 2002; Valentine and Duffy 2006) and these grazers were identified and weighed (dry biomass). Epiphytic algae were scraped off 20 haphazardly selected seagrass leaves from each sample with a microscope slide. Dry weight of epiphytes was used to determine algal load as biomass.

Also at each sampling period, four replicate 25 m^2 sweeps with a seine net (12 m length, 2 m drop, 6 mm mesh) were taken at each site to collect small and juvenile fishes.

Species that were considered to prey upon the grazing invertebrates, determined from gut analysis samples (Edgar and Shaw 1995b) were enumerated and measured (standard length, wet weight). Larger piscivorous fish known to prey upon juvenile fish were caught by hook and line (see Chapter 2). During each sampling period, two lines were set and tended for a period of 90 minutes at each site. All piscivorous fish caught were identified, measured (total length, wet weight) giving catch per unit effort (CPUE) then immediately returned to the water.

Water samples were collected from estuarine waters above seagrass beds during each sampling period and were sent to Sydney Water Analytical and Field Services laboratory for analysis of total nitrogen using the method APHA 4500 - PH & NO_3 - I (APHA et al. 2005).

Statistical analyses

Hypotheses about the effect of bottom-up forcing on the biomass of each trophic level were tested to determine if changes matched the predictions of either resource- or ratio-dependent models or neither. It was predicted that if the system responded to nutrient enrichment in a ratio-dependent manner, all four trophic levels should display greater abundance at the enriched sites than the control locations. If, alternatively, the system displayed resource dependence, epiphytic algae should be less abundant, mesograzers and piscivorous fishes more abundant and juvenile fishes similarly abundant at enriched sites compared to reference sites.

As the predictions of the models are based on steady-state systems, data were collected across three different time periods to allow for the long-term relationship among trophic groups to be used as an approximation of ecosystem equilibrium (Arditi et al. 1991; Arditi and Saiah 1992; Ginzburg and Akcakaya 1992). Prior to analysis, data were averaged across the three times of sampling for each site. This was done to reduce the influence of small-scale spatio-temporal variation when this is not the focus of the hypotheses under evaluation (Keough et al. 2007). Analysis of average data was done by a t-test comparing enriched vs. controls with n = 3 sites. A separate t-test was also performed on total nitrogen levels in the water column. Prior to each test, graphical

examination of the data using boxplots and Levenes test indicated that data for different treatments were not highly skewed or substantially different in variance.

In addition to predicting that the biomass of each trophic group will increase, ratio-dependent models predict that this increase will be proportionate to the nutrient loading. To assess this second prediction of proportionality, a single one-way ANOVA was conducted on the ratio of nutrient enriched sites to reference sites among the five groups (nutrients, epiphytes, mesograzers, juvenile fishes and piscivorous fishes). In the event that trophic groups respond proportionately to nutrient input, no significant difference in ratios among the five groups should be seen. All ANOVAs were followed by *a posteriori* Student–Newman–Keuls (SNK) tests to identify any group means that differed significantly at $\alpha = 0.05$.

RESULTS

A diverse array of invertebrates, juvenile and larger fish were collected during the sampling period. Of the invertebrates collected, a total of 42 taxa known to graze on algae were included in the analysis. Dominant mesograzers included gammarid amphipods (Families Maeridae, Amphilochidae and Corophiidae) and small gastropods. Of the 50 small fishes species (< 80 mm TL) encountered during the study, four (*Acanthopagrus australis*, *Rhabdosargus sarba*, *Pelates sexlineatus* and *Gerres subfasciatus*) were included in the analysis as the dominant species consuming mesograzers on seagrass leaves. Juvenile fish with a more omnivorous diet (*Girella tricuspidata*, *Meuschenia spp*.) were omitted from this trophic guild. Large piscivorous fish were low in abundance and patchily distributed in space and time. Dominant species included large Yellow-fin Bream (*Acanthopagrus australis*) and Long Toms (*Strongylura leiura*).

Epiphytic algal load ($t_{1,4} = 14.17$, $P < 0.001$), and mesograzer biomass ($t_{1,4} = 6.49$, $P = 0.003$) were significantly higher in nutrient enriched sites compared to control sites, however juvenile fish biomass ($t_{1,4} = 0.13$, $P = 0.902$), predatory fish biomass ($t_{1,4} = -0.49$, $P = 0.647$) and total nitrogen levels in water samples ($t_{1,4} = 1.13$, $P = 0.321$) showed no significant differences between treatments or across sites (Figure 4.1). In

ambient to enriched nutrient catchments, the ratios of nutrient levels and biomass of each trophic level of showed a significant difference ($F_{4, 66} = 13.233$, $P < 0.01$). This indicates that the proportional increases across all trophic levels that describe ratio-dependent systems do not hold in this study. Ratios for epiphytes and grazers were statistically similar due to the significant increases in biomass for these trophic groups however ratios for juvenile and piscivorous fish were significantly lower (SNK post hoc tests).

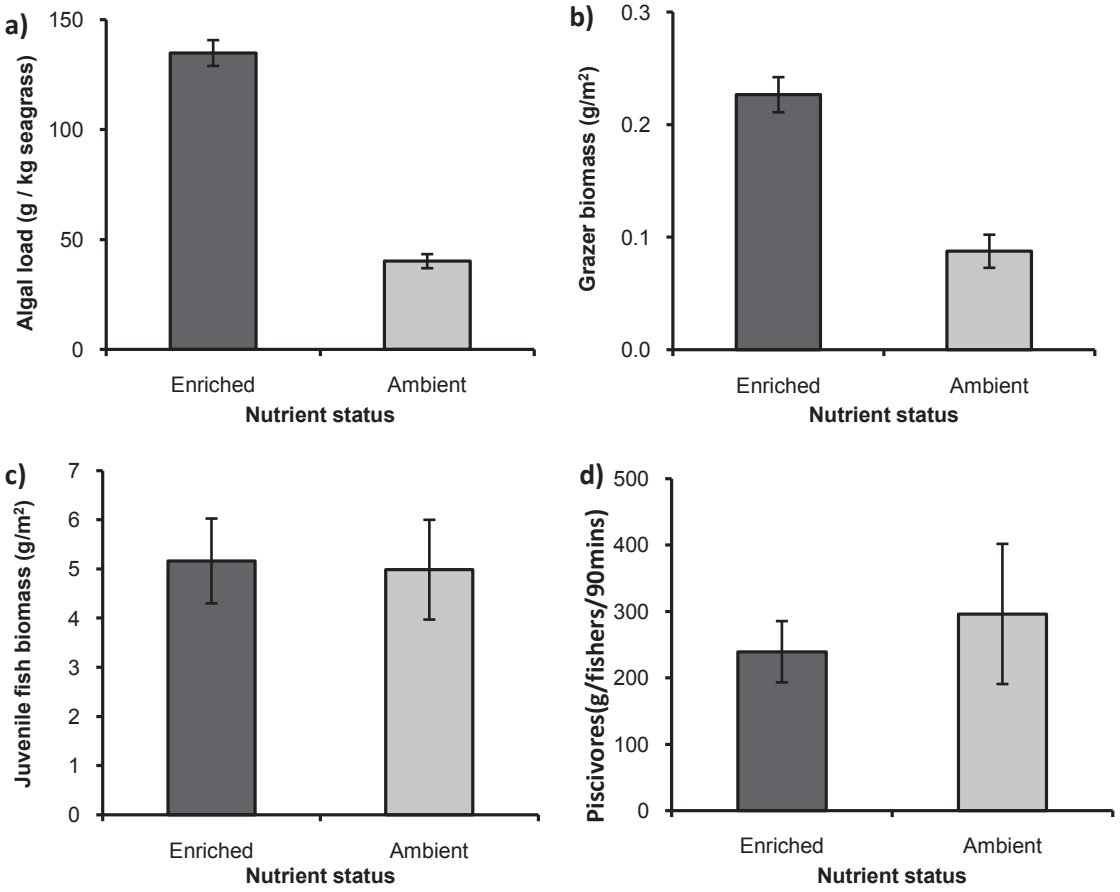

Figure 4.1: Means ± 1 S.E. for biomass of a) algae, b) grazers, c) juvenile fish, and d) piscivorous fish in low ambient nutrient levels and nutrient enriched sites. Data from the three sites within nutrient treatments were pooled even if significant differences were found within treatment variation. The was done to demonstrate differences in the main effects of interest (n = 3 sites).

DISCUSSION

This study compared reference sites of low ambient nutrients to sites that have undergone decades of nutrient enrichment due to catchment development in a four trophic level food chain (epiphytic algae, grazing invertebrates, small fishes, piscivorous fishes), providing an appropriate experimental design for discriminating between these two models. The response to nutrient enrichment was greatest at the lowest levels of the food chain with a consistently higher biomass of epiphytic algae (see Table 3.5) and grazer biomass (see Figure 3.6 for grazer abundances). As seen in many studies (sensu Micheli 1999), the upward flow of energy through the food chain was attenuated at each level. Both juvenile (see Figure 3.3 for abundances for carnivores) and piscivorous fish remained constant at both nutrient enriched and ambient nutrient sites. These findings fail to fully verify the predictions of either ratio-dependent or resource-dependent predator-prey models. Where trophic changes did occur in the bottom two trophic levels, however, they support the predictions of ratio-dependent models. These models predict increases in biomass for both epiphytic algae and grazing invertebrates under sustained bottom-up forcing. Alternately, resource-dependent models predict that the first trophic level (epiphytic algae) in a four level food chain would decrease in biomass.

Attenuation of the upward trophic cascade has been demonstrated in many terrestrial, aquatic and marine systems (Borer et al. 2006) including seagrasses (Moksnes et al. 2008). The dampening of this bottom-up cascade may be due to a variety of reasons, which are either properties of the trophic system or external forces that lead to a decoupling of the food web. It can be demonstrated experimentally in simple, well-understood systems that changes in the density of one trophic level do not necessarily cascade through other trophic levels due to complex direct and indirect effects (Wootton 1994). Properties of food webs that make them resistant to cascading effects include high species diversity, a high degree of omnivory, competition and intra-guild predation (Williams and Martinez 2004; Duffy 2006). A large number of juvenile fishes with omnivorous diets (*Girella tricuspidata* and Monacanthid species) inhabit seagrass beds in south-eastern Australia and were at relatively high abundances during this study. Another possible explanation is a subsidy of juvenile fish diet with invertebrates from the detrital food web in reference sites (Attayde and Ripa 2008).

Alternately, a decoupling from the food chain by non-trophic factors could also lead to a dampening of the bottom-up forcing at higher trophic levels. For example, the openness of the system at higher trophic levels is a feature of seagrass food webs. It is believed that juvenile fish recruit to seagrasses based on the degree of habitat complexity and variation in laval supply rather than likely food availability (Jenkins et al. 1998; Duffy 2006). Recruitment can be variable both spatially and temporally and recruitment pulses that coincide with sampling could mask productivity effects between sites. Large piscivorous fish are also partially removed from the system as they are only transient visitors to seagrass beds and their abundances are highly variable in space and time. Many predators will move into shallow seagrass flats from adjacent habitats on a rising tide, particularly at night when it is thought that a majority of predation occurs (Hindell et al. 2000b). Methodological difficulties and daytime sampling made quantifying biomass of the fourth trophic level difficult (see Chapter 3) and may have led to an underestimation of piscivore abundances.

Although no significant differences in nutrient concentrations were detected between developed and undeveloped catchments, previous studies have demonstrated that water column nutrients are not always indicative of nutrient loads due to the fact that nitrogen is rapidly taken up by algae, seagrass and sediments or flushed away by tides and currents (Lee et al. 2004; Scanes et al. 2007). This means that over-enrichment of coastal ecosystems can rarely be detected by direct measurements of water column nitrogen concentrations. Typically urbanised catchments have more than double the nutrient loads of forested watersheds (Wahl et al. 1997) and more thorough, long-term analysis of nutrient levels have confirmed this in many of the sample sites (Paterson et al. 2003a; b). Measurement of sediment pore water nutrient levels (Fourqurean et al. 1992; McGlathery et al. 2001) or modelling of nitrogen loads from catchments (Hauxwell and Valiela 2004) may prove more effective in determining nutrients levels available to seagrass and epiphyte growth in future studies.

This study's demonstration of trophic responses in seagrass beds exposed to long-term nutrient enrichment builds on the results of Bishop et al. (2006) who demonstrated the presence of ratio dependence in a four level planktivorous food chain at a single site

enriched by a point source of nutrients from a sewage treatment plant. The current study investigated enrichment from more diffuse sources using a more spatially comprehensive sampling design in a benthic habitat based on epiphytic algae. While both studies demonstrated increased primary production and algal loads with nutrient enrichment, secondary production was attenuated at the level of primary consumers in the current study and did not cascade through all trophic levels. This difference in the strength of the upward trophic cascade between these two studies may be due to the higher levels of enrichment in the planktivorous system.

The results of this study also have implications for catchment management. Lower trophic levels and most importantly, epiphytic algal loads, show an increase in biomass with sustained anthropogenic enrichment as predicted by ratio-dependent models. This may have a more detrimental effect on seagrass productivity than responses predicted by resource-dependent models at higher nutrient concentrations. Results throughout this study however, indicate that seagrass biomass is greater in some of the developed catchments. This suggests that moderate levels of enrichment similar to those investigated during this study may improve seagrass growth in systems that are nitrogen-limited. It has also been hypothesised that low increases in nutrient loads can be beneficial to secondary production (Caddy 2000). Our study found little evidence of this. When larger increases of nutrient occur and a threshold is reached, enrichment becomes detrimental and leads to hypoxia and declines in seagrass habitat. Defining this threshold is difficult as it depends on an increase in nutrient load as well as characteristics of the receiving waters such as estuary size, flushing rates and retention times (Pinckney et al. 2001). These factors will vary greatly between regions and estuary types and should be taken into account when assessing the likely change in ecosystem functioning due to nutrient enrichment.

Chapter 5

GROWTH AND SURVIVAL OF JUVENILE FISH IN SEAGRASS UNDER DIFFERENT NUTRIENT REGIMES

ABSTRACT

Habitat quality plays an important role in structuring fish communities by influencing the processes of reproduction, growth, migration and survival. Seagrass in estuaries is considered ecologically-important habitat that provides a nursery area for juvenile fish offering increased shelter from predation and food resources compared to unvegetated areas. Development of catchments increases the nutrient loads in waterways which may be either beneficial or detrimental to fish. Estuaries with higher nutrient loads may have greater fisheries production from increased bottom-up forcing via trophic pathways. Simultaneously, increased toxicity from these same nutrients and other pollutants such as heavy metals, hydrocarbons and chemicals from agriculture and industry may have direct negative impacts on fish health and higher mortality manifesting as lower abundance and biomass of juvenile fish. This study investigated the effects of increased nutrients on the condition, growth and survival of a juvenile fish species (*Pelates sexlineatus*) in 1) field comparisons in developed and undeveloped catchments and 2) enclosure experiments with manipulated nutrient levels. Elevated nitrogen levels were detected in water quality sampling for treatments with nutrient addition in enclosures and developed catchments in field experiments. In enclosure experiments, there was greater survival of fish in ambient vs. enriched treatments although this wasn't statistically significant. This led to a greater biomass of fish in ambient enclosures and what appeared to be a trophic cascade that saw lower grazer abundances and significantly higher algal loads in these treatments as well. No significant differences were found in lipid content (used as a proxy for fish condition) or growth in surviving fish at the termination of the experiment. Field comparisons however, showed significantly higher lipid content in fish from undeveloped catchments despite considerably lower abundances of epiphytic algae and grazing invertebrates compared to developed catchments. These results indicate that the direct negative impacts of toxicity from ammonia and nitrate as well as other contaminants play a greater role in determining the condition and survival of juvenile fish than the indirect benefits of enrichment of greater available food ration.

INTRODUCTION

Quality of habitat plays a vital role in structuring fish assemblages through enhancing reproduction, growth, migration and survival (Levin and Stunz 2005; Fonseca and Cabral 2007). These processes vary in importance to fish at different life stages. In juveniles, growth and survival are key factors in determining the success of adult recruitment (Bailey 1994; Rooker et al. 1999; Stewart and Scharf 2008). Estuarine seagrass beds are habitats that attract and support elevated densities of many species of juvenile fish and invertebrates and are often referred to as nursery areas (Beck et al. 2001). The nursery role of seagrasses includes enhanced survival of juveniles by providing shelter from predation and faster growth due to higher levels of food resources (Heck et al. 2003). Growth and survival of juvenile fish are also influenced by many environmental factors (e.g. temperature) and ecological (e.g. competition & predation) processes (Bailey 1994; Gibson 1994; Phelan et al. 2000; Hixon and Jones 2005) which are controlled by both natural (e.g. storms) and anthropogenic (e.g. pollution) processes (Meng et al. 2001; Amara et al. 2007; Vasconcelos et al. 2009).

Catchment development for agricultural, industrial and residential land-uses in coastal watersheds as well as atmospheric deposition has led to an increase in the anthropogenic nutrient loads to estuarine and coastal waters. This led to some nutrient limited (oligotrophic) near-shore marine environments becoming over-enriched (eutrophic) systems (Paerl 1997; Cloern 2001). Many studies on nutrient enrichment have focused on the negative consequences of increased primary productivity such as hypoxia and anoxia, toxic algal blooms, fish kills, loss of biodiversity, and increased turbidity (Carpenter et al. 1998; Heck and Valentine 2007; Breitburg et al. 2009b). Increased phytoplankton and epiphytic growth from nutrient enrichment can also lead to shading of submerged aquatic vegetation such as seagrass, resulting in large-scale habitat degradation (Dennison et al. 1993). Sometimes overlooked, is the positive effect of nutrient enrichment on secondary production (Nixon and Buckley 2002). It has been suggested in theory (Caddy 1993) and supported by evidence (Caddy 2000; Breitburg et al. 2009a) that fisheries yields increase with moderate increases of nutrients. However, after reaching a threshold of eutrophication productivity begins to decline as habitats degrade.

As moderate nutrient increases lead to increased primary and secondary production, as predicted by some predator-prey models (Akcakaya et al. 1995 - see Chapter 4), greater food supply for juvenile fish is expected at enriched sites and therefore higher condition for these fish. Studies have successfully used condition indices such as percentage lipid content in fish tissue as a proxy for fitness of juvenile and larval fish. Aquaria experiments have shown the fish fed a higher ration have a higher total lipid content (Booth and Hixon 1999). Higher lipid content at larval and juvenile life history stages can increase the growth rate and survival and survival from predation (Booth and Alquezar 2002; Booth and Beretta 2004; Amara et al. 2007; Figueira et al. 2008).

Field enclosures have been used successfully to measure increases in growth and survival by restricting juvenile fish to specific habitats (Stunz et al. 2002). Increased prey densities in enclosure experiments have resulted in elevated growth rates in juvenile fish (Duffy et al. 1996). Furthermore, field surveys have been used to determine growth and condition in different habitats and in areas with different anthropogenic stresses (Meng et al. 2001; Amara et al. 2007; Shervette and Gelwick 2007; Vasconcelos et al. 2009).

Six-lined trumpeter (*Pelates sexlineatus*) are economically-important species caught by both recreational and commercial fishers and distributed along the south-eastern coast of Australia (Gray et al. 1996; Smith and Suthers 2000). Adults occur in shallow shelf waters and are believed to spawn outside estuary mouths (Smith and Suthers 2000). Larvae recruit to seagrass beds in winter and spring (Smith and Suthers 2000) and juveniles are among the most abundant fish species occurring in Australian seagrass beds (Pollard 1984). Juvenile *P. sexlineatus* have a varied diet mainly consisting of crustaceans (harpacticoid copepods, gammarid amphipods, ostracods and tanaids) (Sanchez-Jerez et al. 2002).

This study investigates how moderate increases in nutrients will affect the growth and survival of a juvenile fish species (*P. sexlineatus*) using both manipulative experiments and mensurative sampling of habitat. Firstly, it was predicted that juvenile fish in field enclosures treated with elevated nutrients will have greater growth, condition and survival than fish held in enclosures with low ambient nutrients. This is because of

greater ration from enhanced primary and secondary production of algal epiphytes and mesograzing invertebrates. Secondly, it was predicted that fish captured in seagrass beds within developed catchments with elevated nutrient loads will have greater condition (measured as lipid content) than fish captured in undeveloped catchments with low nutrient loads, again due to higher food ration through bottom-up trophic cascades.

METHODS

Location of Study

To compare the growth, condition and survival of juvenile fish in different nutrient regimes, a mensurative field study and a manipulative enclosure experiment were conducted in shallow intertidal seagrass beds in June and July 2008. The manipulative study was carried out in a low nutrient, large shallow seagrass bed at Mullet Creek (33°29'20"S, 151°15'46"E), part of the Hawkesbury River catchment just north of Sydney (see Figure 2.1). Field sampling was conducted in Brisbane Water at nutrient enriched sites in developed catchments at the mouths of Narara Creek (33°25'56"S, 151°19'57"E) and Erina Creek (33°26'26"S, 151°21'41"E) and in low nutrient sites in undeveloped catchments at Woy Woy Bay (33°28'31"S, 151°18'08"E) and Waterfall Bay (33°28'05"S, 151°18'30"E) see Figure 2.1.

Figure 5.1: Field enclosures at Mullet Creek.

Field study

Juvenile *P. sexlineatus* were collected using a 6 mm seine net in shallow eelgrass beds (*Zostera capricorni*) at each of the four sites (two nutrient enriched and two low ambient nutrient) in November 2008. Four replicate samples of seagrass, epiphytes and

associated epifauna were collected using a BROMAR sampler (see Chapter 2) from each site. These were used to determine the biomass of seagrass, epiphyte and ultimately grazer biomass to give an indication of food availability. Nitrate and nitrite (NO_x) and ammonia (NH_3) levels were analysed using flow injection analysis with the Lachat Instruments ASX 520 series XYZ Autosampler (see Chapter 2) and water temperature, salinity, pH and dissolved oxygen levels were recorded at all sites on the day of collection.

Field Enclosure Experiment

To compare the growth, condition and survival of juvenile *P. sexlineatus* in different nutrient regimes, enclosures were set up in shallow intertidal eelgrass (*Zostera capricorni*) beds. Twelve field enclosures were constructed using high-density polyethylene (HDPE) plastic drums (220 L capacity, 0.58 m diameter x 0.9 m height) with tops and bottoms removed. The drums were pushed into the substrate to a depth of 30 cm and anchored from the outside with plastic star pickets. Drums were placed approximately 10 m apart in *Z. capricorni* beds and nutrient treatments were haphazardly assigned to enclosures to ensure spatial heterogeneity within treatments (Figure 5.1). Similar enclosures have been used to measure short-term growth of juvenile fish in other estuarine habitats (Stunz et al. 2002; Burfeind and Stunz 2007).

Nutrient dispensers made of PVC pipe (50 mm diameter x 300 mm long), capped at both ends and with 22 x 4 mm holes drilled in each were suspended on PVC pickets in each enclosure. In nutrient enriched treatments, 300 g of slow release fertiliser (Osmocote^{TM}) was enclosed in a nylon stocking and placed into nutrient dispensers, while low nutrient treatment dispensers were left empty. This amount of nitrogen has been shown to affect both primary and secondary production in epiphytic algal food chains (Kelaher et al. Unpublished data). Dispensers were not replenished during the study as experiments on nutrient dissolution rates have shown that release rates remain steady after an initial burst for approximately six weeks (Heck et al. 2000). Water column nitrate and nitrite (NO_x) and ammonia (NH_3) levels were analysed using flow injection analysis with the Lachat Instruments ASX 520 series XYZ Autosampler (see Chapter 2) at the mid (3 week) and end (6 week) points of the experiment. At the end of the experiment, Osmocote^{TM} was

retrieved, dried in an oven and weighed to determine the loading of nutrients for each enclosure.

Each enclosure was swept with a dip net (1 mm mesh) to remove any fish before the experiment commenced and 6 mm monofilament netting was cable-tied to the top of each enclosure to prevent the escape of experimental fish and the entry of non-experimental fishes or bird predators. The solid walled enclosures were positioned in an intertidal area where they were submerged at all times except at low tide when water was retained in the drums but receded from adjacent seagrass beds. This enabled the experiment to be set up and dismantled at low tide, however, it also allows for accumulation of nutrients and changes in temperature at low tide. Temperature loggers were placed inside and outside two enclosures for a nine day period during the experiment to determine any difference between the enclosures and surrounding water temperature at low tide. Juvenile six-lined trumpeter *P. sexlineatus* were captured in adjacent seagrass beds at Mullet Creek using a 6 mm seine net. Fish were measured to standard length (SL) and wet weight (WW) then ranked into four size hierarchies and one fish from each class was assigned to an enclosure. Individual fish were tagged subdermally with visible implant elastomer (North West Technologies) for identification at recapture. In trials of tagged fish kept in tanks, no deleterious effects of tagging were observed.

At the completion of the experiment, surviving fish were captured using a dip net and euthanized in an ice slurry for analysis. Enclosures were drained at low tide through a 500 μm mesh and all seagrass shoots were removed to determine biomass (dry weight). Six replicate samples of seagrass, epiphytes and associated epifauna were haphazardly collected using a BROMAR sampler from adjacent seagrass beds outside of the enclosures. Epiphytic algae were scraped off 20 seagrass leaves haphazardly selected from each sample. The dry weight of epiphytes was used to determine algal load as biomass. Invertebrate grazers were counted, and weighed (dry biomass). Comparison of biomass of trophic levels in no-addition enclosure treatments to adjacent natural levels gave an indication of the extent to which experimental artefacts of shading and reduced water flow affected the seagrass system through the duration of the study.

Fish condition

Fish condition was determined using percentage change in lipid content. Previous studies have indicated whole body lipids are a good measure for discerning phenotypic variability in condition among juvenile fish (Figueira et al. 2008). Individual fish standard length (SL) and wet weight (WW) were measured at the commencement (t_0) and the culmination of the experiment (t_{end}), then placed in a freeze drier to determine dry weight (DW). Fish were then ground into a homogenous powder using a mortar and pestle and a subsample of approximately 0.025g was analysed gravimetrically using a modified chloroform-methanol extraction method (Bligh and Dyer 1959; Mann and Gallagher 1985). Extracted lipids are expressed as a percentage of dry body weight of fish.

Statistical analysis

In field studies, two-factor ANOVAs with sites (two levels) nested within nutrient status (enriched vs ambient) were used to investigate the effect of nutrient levels on biomass of seagrass, epiphytic algae and mesograzers, nitrogen levels in water column samples and lipid content of fish. One-way ANOVA was used in the manipulative experiment to test the effects of nutrient enrichment among treatments (enriched enclosures, no addition enclosures and ambient controls) on biomass of seagrass, epiphytic algae and mesograzers, nitrogen levels in water column samples and survival, condition and growth of fish. Prior to each ANOVA, the assumption of homogeneity of variances was examined using Cochran's test. When transformation didn't improve heterogeneous variances, analyses were conducted on raw data as the balanced experimental design made these tests robust to type 1 error (Underwood 1997). When significant, ANOVAs were followed by *a poseriori* Student–Newman–Keuls (SNK) tests to identify differences among levels of treatments.

To test hypotheses about percentage lipid content and growth of fish, one-factor and nested analysis of covariance ANCOVA were performed for the enclosure and field studies respectively. Standard length of fish was used as the covariate to account for allometry. The assumption of homogenous linear regression was tested and met using a customised ANCOVA model containing the pooled covariate-by-factor interaction. The

assumption of homogenous variances was examined using Levenes test. Pair-wise comparisons using Tukeys' test after Bonferroni correction, were used to identify any groups that differed significantly.

Nutrient levels were analysed using repeated measures multiple analysis of variance (RM) MANOVA using the Wilks' Lambda F statistic with nutrient treatment as the independent variable and time as the dependent variable. This analysis was chosen because the degrees of freedom for the hypothesis test were equal to one, and it is not dependent on the assumption of sphericity. Data were transformed (square root) to satisfy the assumptions of homogeneity of covariance matrices (Box's test) and multivariate normality.

RESULTS

Field enclosures

Enclosures with nutrient treatments showed greatly elevated levels of ammonia (NH_3) and oxidised nitrogen (NO_x) in the middle but not at the end of the experiment (NH3: $F_{1,10} = 0.319$, $P = 0.001$ & NO_x: $F_{1,10} = 0.115$, $P < 0.001$, Table 5.1, Figure 5.2). June samples were taken at the end of low tide when water had been pooled in enclosures, while July samples were taken as the tide dropped after an extended period of tidal flushing. Elevated levels of ammonia (116.9 ± (1 S.E) 16.9 μg/L) and oxidised nitrogen (221.6 ± (1 S.E) 31.5 μg/L) in June were many times greater than the default trigger guidelines for slightly disturbed estuaries in south-eastern Australia (ANZECC 2000). Temperatures ranged between 13.5 °C and 18 °C and varied little between the external environment and the two treatment enclosures with data loggers showing all four environments tracking within 0.5°C of each other for the majority of the time (Figure 5.3).

Seagrass biomass and algal load were both significantly reduced inside the enclosures compared to external conditions (Seagrass: $F_{2,15} = 4528.2$, $P < 0.001$ & NO_x: $F_{2,15} = 896.6$, $P = 0.038$, Table 5.2, Figure 5.4) with enriched mesocosms having lower but not significantly different levels than ambient treatments (SNK tests). Differences in grazer biomass between enclosures and adjacent seagrass beds were not significant but were

Table 5.1: Repeated-measures MANOVA results for differences in nutrient levels in ambient and enriched fish enclosures. n = 2.

	Factors	Wilks' Λ value	df for F	Error df	F	Sig
Nitrate/Nitrite	Time $_{(RM)}$	0.131	1	10	66.193	<0.001
	Time x treatment	0.115	1	10	77.025	<0.001
Ammonia	Time $_{(RM)}$	0.51	1	10	9.614	0.011
	Time x treatment	0.319	1	10	21.384	0.001

Table 5.2: Analyses of variance for seagrass, algae, grazer and fish biomass between treatments in the enclosure experiment.

	Factors	df for F	MS	F	Sig
Seagrass biomass	Treatment	2	4528.192	15.407	<0.001
	Residual	15	293.908		
Algal load	Treatment	2	896.588	4.08	0 .038
	Residual	15	219.773		
Grazer biomass	Treatment	2	767.841	1.097	0.359
	Residual	15	699.686		
Fish biomass	Treatment	1	4.626	3.358	0.097
	Residual	10	1.378		
Survivorship	Treatment	1	0.255	4.623	0.057
	Residual	10	0.055		

Table 5.3: Analysis of covariance for lipid content and fish growth between treatments in the enclosure experiment. Standard length is the covariate (cv). Enriched n=15, Ambient n=22.

	Factors	df for F	MS	F	Sig
Lipid content	Standard length $_{(cv)}$	1	7.861	1.255	0.270
	Nutrient treatment	1	0.726	0.116	0.736
	Residual	34	6.263		
Length growth	Standard length $_{(cv)}$	1	0.001	0.733	0.398
	Nutrient treatment	1	0.001	1.012	0.321
	Residual	34	0.001		

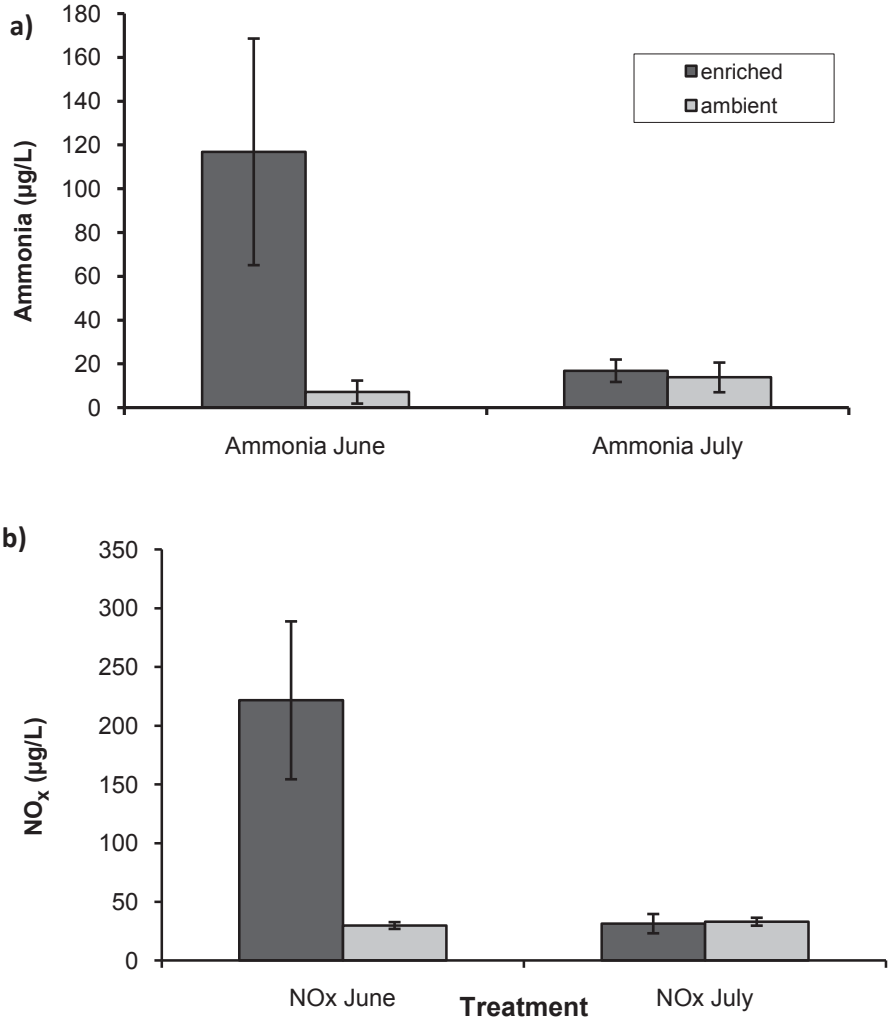

Figure 5.2: Mean ± 1 S. E. for nutrient levels for enriched and ambient enclosures at the middle (June) and end (July) of the manipulative experiment showing a) ammonia and b) oxidised nitrogen.

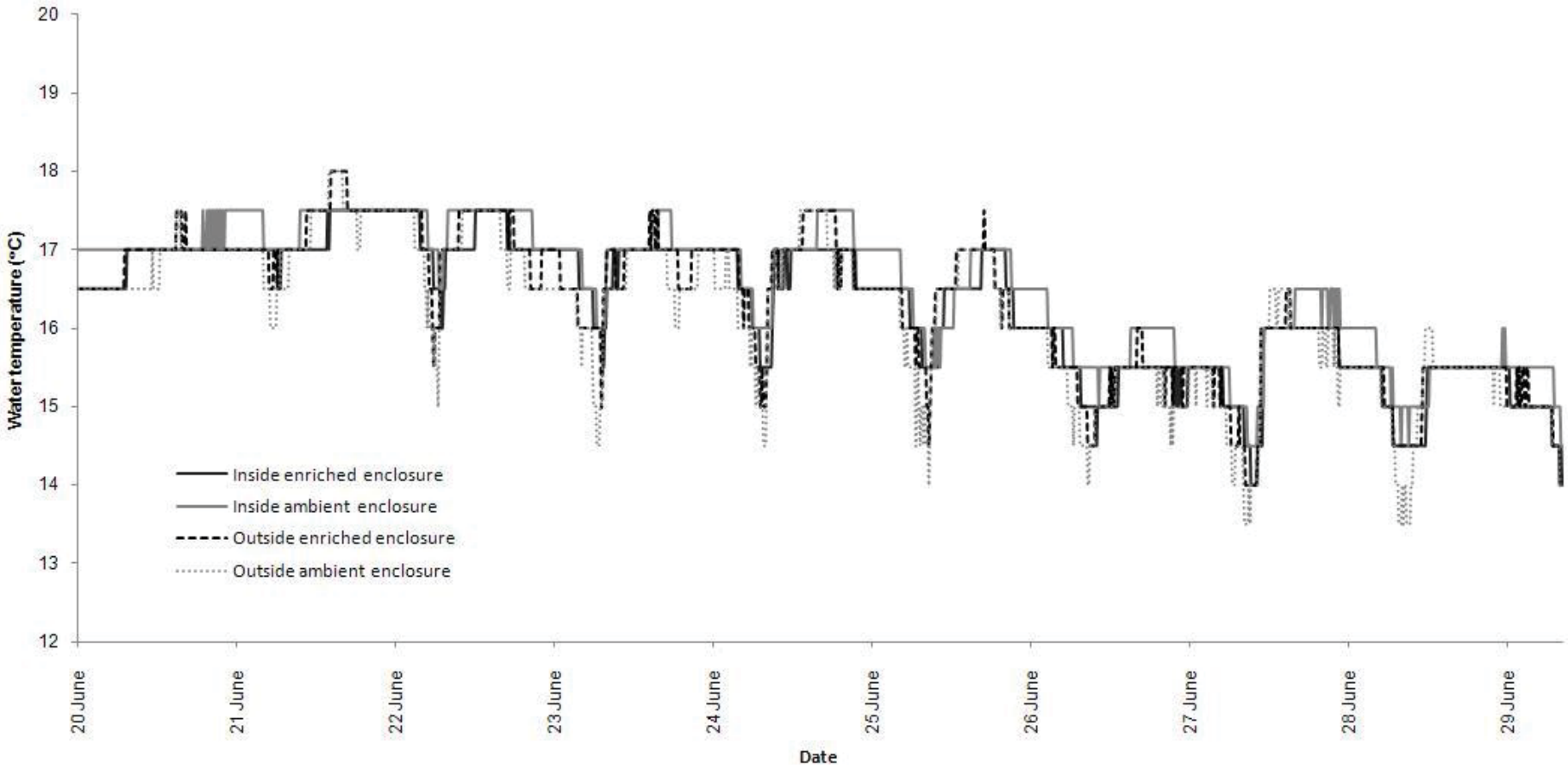

Figure 5.3: Water temperature from data loggers placed inside and outside of enclosures. Temperature data was collected at 10 minute intervals over 9 days during the experiment.

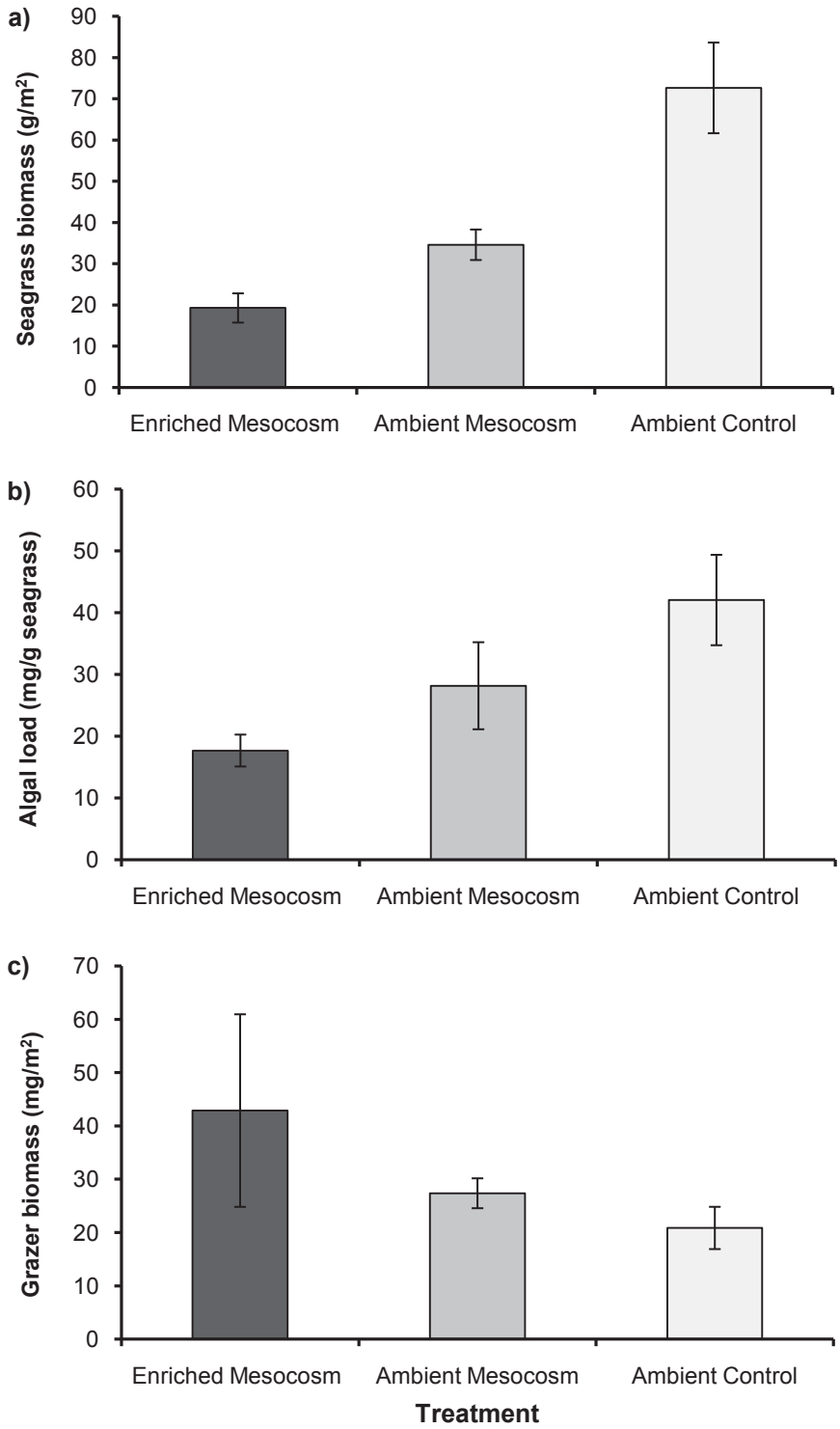

Figure 5.4: Mean ± 1 S. E. for biomass of a) seagrass biomass, b) algal load and c) mesograzer biomass for enriched and ambient nutrient treated enclosures and adjacent seagrass beds at the termination of the experiment.

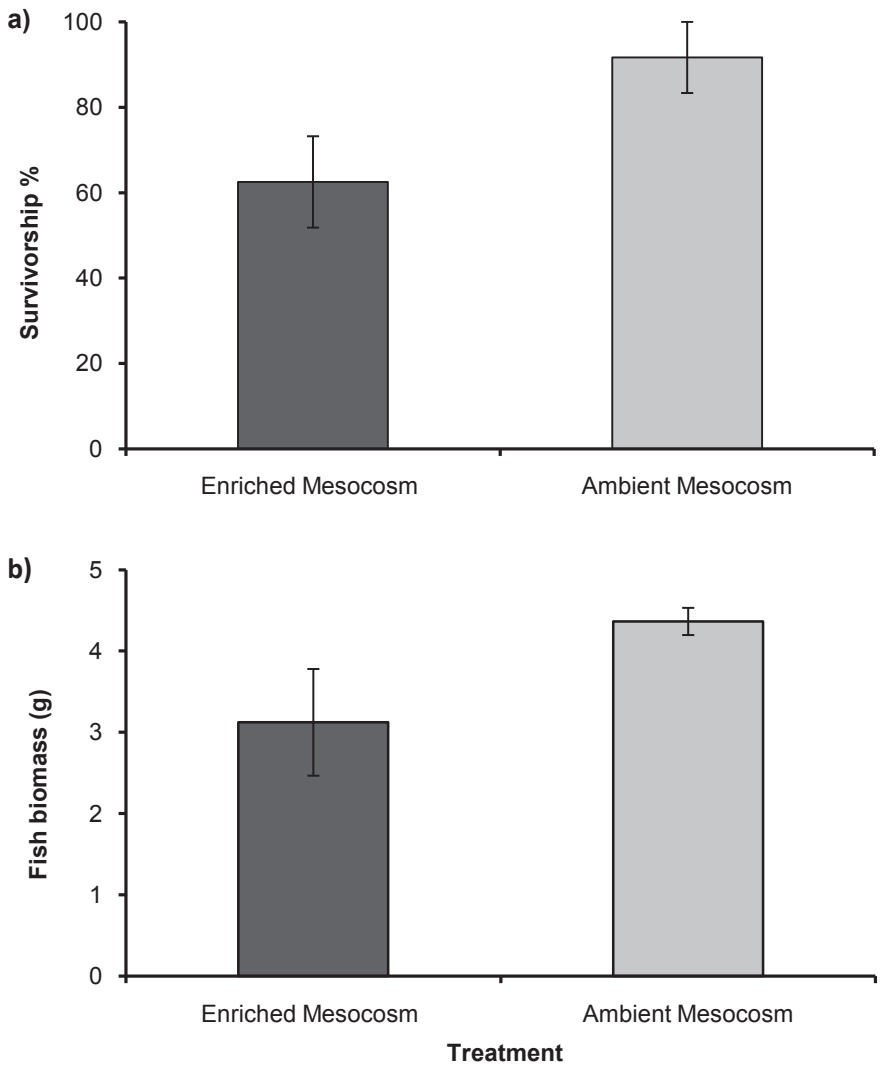

Figure 5.5: Mean ± 1 S. E. for a) fish survivorship; b) and biomass for enriched and ambient mesocosms.

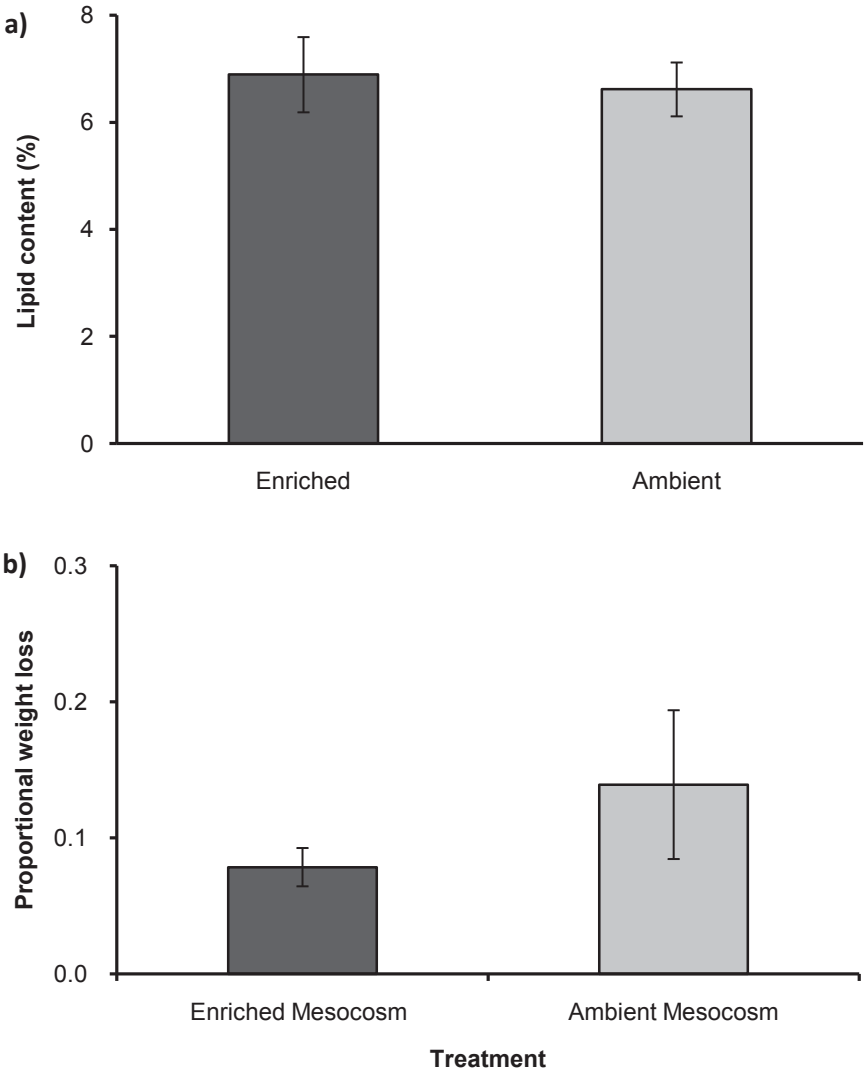

Figure 5.6: Mean ± 1 S. E. for a) fish lipid content & b) proportional weight loss for enriched and ambient mesocosms.

greatest in the enriched mesocosms (Table 5.2, Figure 5.4). Although not statistically significant, both survivorship of fish and the biomass of remaining fish in enclosures was greater in ambient than enriched enclosures (Table 5.2, Figure 5.5). Fish condition measured as lipid content and percentage weight loss during the experiment showed no significant trend between treatments (Table 5.3, Figure 5.6).

Field Comparisons

Environmental variables measured at sampling times varied slightly among sites. Water temperature ranged from 20.6 °C at Narara Creek to 22.3 °C at Waterfall Bay, however, this may have been due to the time of day that sampling occurred (mid-morning and mid-afternoon respectively). Dissolved oxygen (92.5 – 117.6 % saturation) and pH (7.9 – 8.1) were within normal ranges and fairly stable among sites. Long-term salinity levels for these sites averaged around 35 μS/cm (see Chapter 5). Ammonia and oxidised nitrogen levels varied among sites within treatment (NH_3: $F_{2,4} = 230.6$, $P < 0.001$ & NO_x: $F_{2,4} = 105.9$, $P < 0.001$, Table 5.4, Figure 5.7) as well as between nutrient treatments, with elevated ammonia levels at Erina Creek and elevated oxidised nitrogen levels at both Narara and Erina Creeks compared to low nutrient reference locations (NH3: $F_{1,4} = 315.6$, $P < 0.001$ & NO_x: $F_{1,4} = 552.7$, $P < 0.001$, Table 5.4, Figure 5.7).

Seagrass biomass did not differ significantly among sites however algal loads varied among sites within treatments with elevated biomass at Narara Creek ($F_{2,12} = 11.1$, $P = 0.002$, Table 5.4, Figure 5.8) and grazer biomass significantly higher at nutrient enriched sites $F_{1,12} = 7.797$, $P = 0.016$, Table 5.4 Figure 5.8). Fish condition measured as lipid content was significantly higher at low ambient nutrient sites ($F_{1,12} = 46.025$, $P < 0.001$, Table 5.5, Figure 5.9)

Table 5.4: Analysis of variance for nutrient levels and seagrass, algae and grazer biomass, among sites with nutrient enrichment and low ambient nutrient levels. (NH_3 & NO_x n = 2, biomass n = 4).

	Factors	df for F	MS	F	Sig
Ammonia	Nutrient status	1	2148.40	315.54	<0.001
	Site (nutrient status)	2	1570.16	230.61	<0.001
	Residual	4	6.809		
No_x	Nutrient status	1	2027.25	552.69	<0.001
	Site (nutrient status)	2	388.410	105.89	<0.001
	Residual	4	3.668		
Seagrass biomass	Nutrient status	1	1.749	0.75	0.403
	Site (nutrient status)	2	4.137	1.78	0.211
	Residual	12	2.327		
Algal load	Nutrient status	1	0.015	17.69	0.001
	Site (nutrient status)	2	0.009	11.06	0.002
	Residual	12	0.001		
Grazer biomass	Nutrient status	1	0.060	7.80	0.016
	Site (nutrient status)	2	0.026	3.42	0.067
	Residual	12	0.008		

Table 5.5: Analysis of covariance for lipid content among sites with nutrient enrichment and low ambient nutrient levels. Standard length is the covariate (cv), (n = 9).

	Factors	df for F	MS	F	Sig
Lipid content	Standard $length_{(cv)}$	1	0.657	0.558	0.461
	Nutrient status	1	54.133	46.025	<0.001
	Location (nutrients)	2	0.435	0.370	0.694
	Residual	31	1.176		

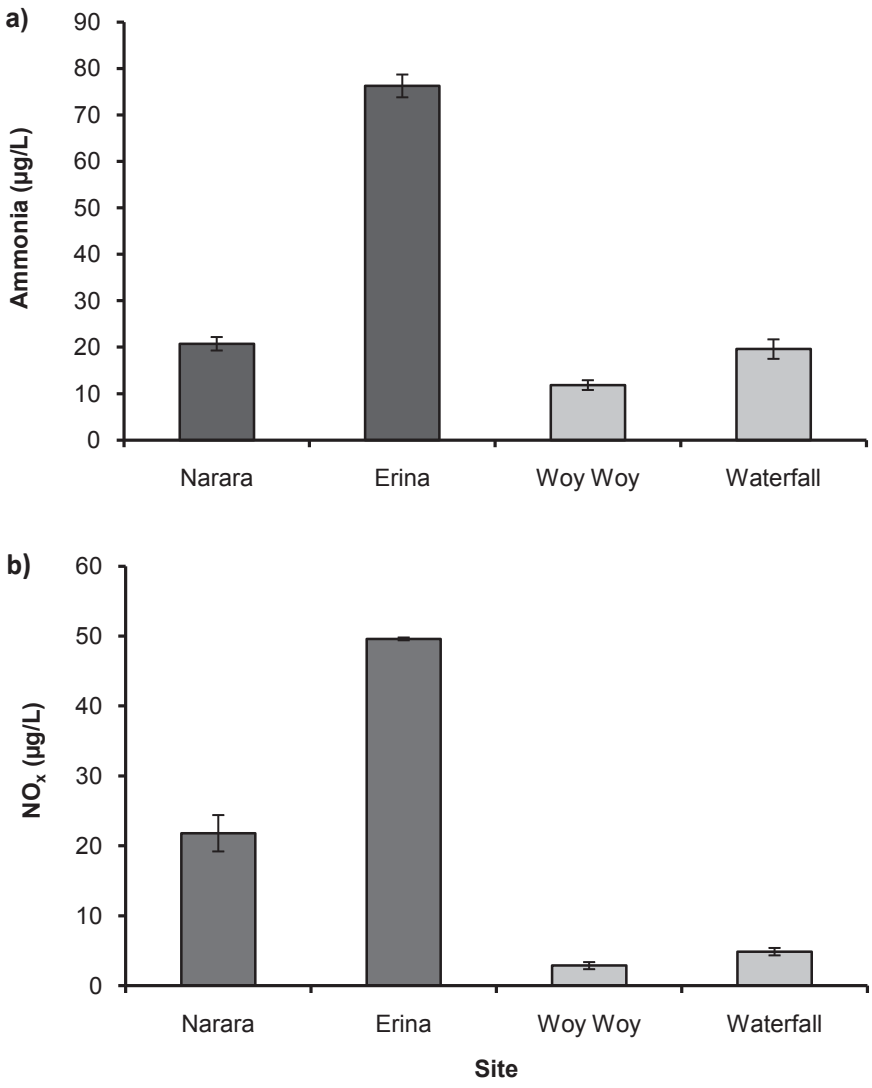

Figure 5.7: Mean ± 1 S. E. for a) ammonia and b) nitrate/nitrite levels for nutrient enriched sites (dark columns) and ambient nutrient sites (light columns).

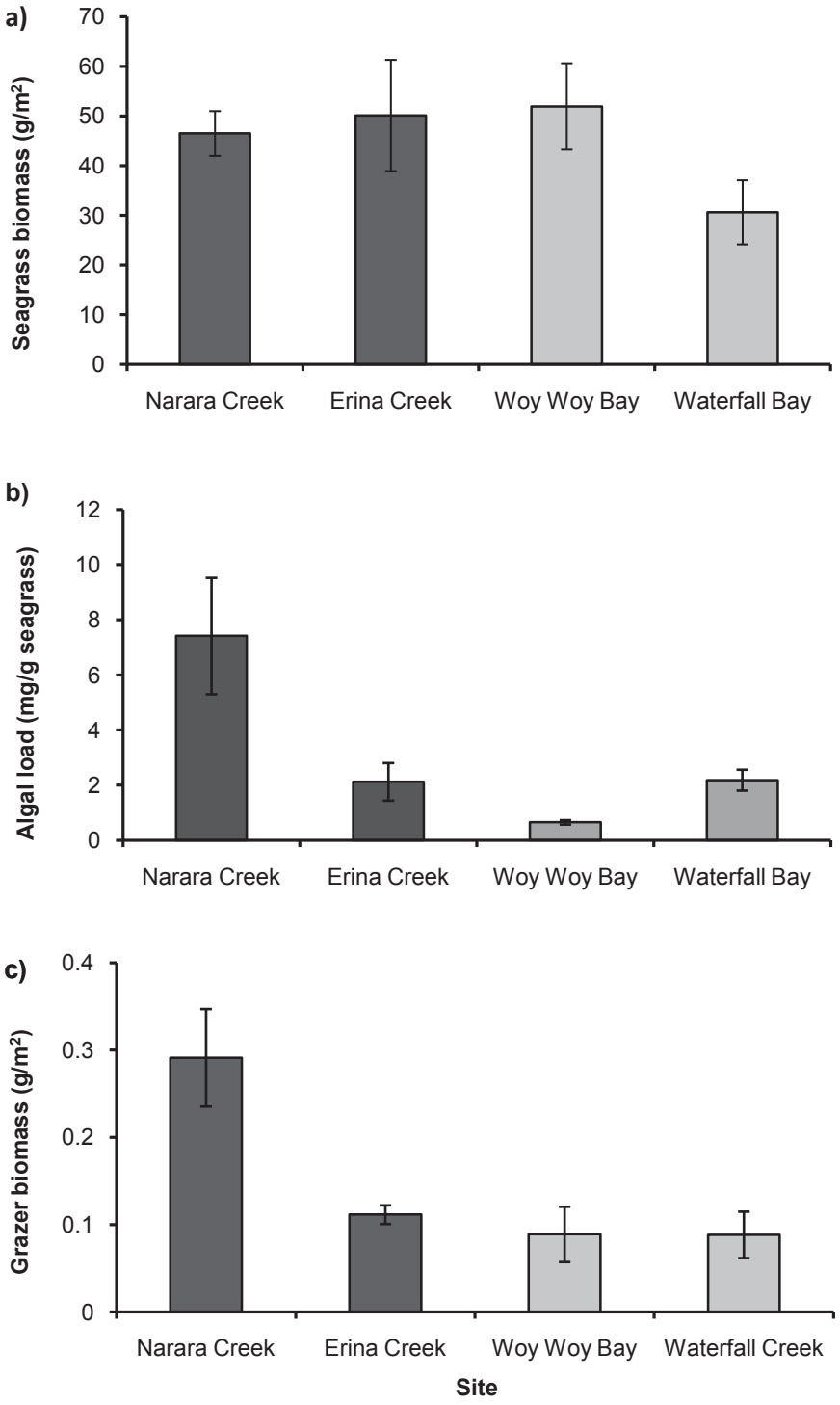

Figure 5.8: Mean ± 1 S. E. for a) seagrass biomass; b) algal load and; c) mesograzer biomass for nutrient enriched sites (dark columns) and ambient nutrient sites (light columns).

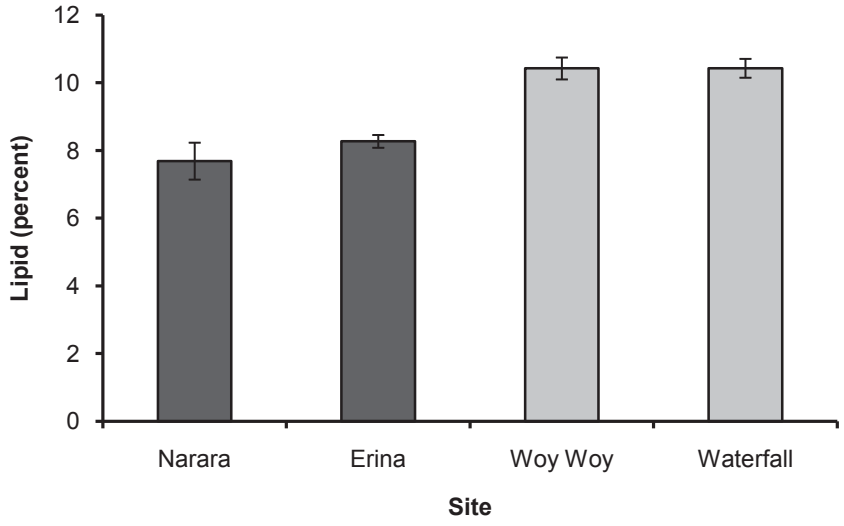

Figure 5.9: Mean ± 1 S. E. for fish condition measured as a) lipid content of fish for nutrient enriched sites (dark columns) and; b) ambient nutrient sites (light columns).

DISCUSSION

The purpose of this study was to determine whether the growth and survival of juvenile fish in seagrass beds was enhanced through trophic support from bottom-up nutrient enrichment. Contrary to expectations, fish condition, as measured by lipid content, in field sites with elevated nutrients was significantly lower than at ambient sites. Survivorship in nutrient enriched enclosures was also lower than those in low ambient nutrient environments, however, this was marginally non-significant ($\alpha = 0.05$). This may be due to lack of power (observed $\beta = 0.493$) as five of six ambient cages had no mortality and 91.67 ± (1 S.E.) 0.08% overall survivorship. This is compared to only one of the six enriched cages having no mortality and 62.5 ± (1 S.E.) 0.11% survivorship. At the completion of the enclosure experiment all juvenile fish had low condition compared to wild caught fish and had lost weight over the course of the study. The timing of the manipulative experiment (winter) may explain the weight loss in fish as low temperatures can curtail the growth rates of juvenile fish (Fowler and Jennings 2003; Meise et al. 2003; Smith and Sinerchia 2004). Another possible explanation could be cage or handling effects (Peterson and Black 1994; Dahlgren and Eggleston 2000; Wilson and

Osenberg 2002).There was no significant difference between treatments in either of these measures and the trend of slightly better condition and higher weight retention in nutrient enriched enclosures was confounded by the density effect caused by higher mortality in these enclosures.

In both experiments nutrient enrichment was validated through testing of components of dissolved inorganic nitrogen levels in the water column. The nutrients in enriched enclosures in June, measured after a period of exposure at low tide, showed high ammonia and oxidised nitrogen levels, whereas July measurements, taken as the tide was dropping, were similar to controls. This indicates that nutrients spiked in enriched treatments when the tide dropped below the top of the enclosures, but when drums were underwater tidal mixing resulted in diluting the nutrients to ambient levels. Another possibility is that the fertiliser in the nutrient dispensers had run out by the end of the experiment. Examination of percentage dissolution of Osmocote^{TM} using laboratory and field trials indicate that nutrients continue to be released for periods much greater than the four week time period in this experiment at similar water temperatures (Heck et al. 2000; Morris et al. 2007). In the mensurative experiment, the two developed catchments had elevated levels of oxidised nitrogen compared to undeveloped catchments. Erina Creek also had highly elevated levels of ammonia. This is consistent with land use in the two developed catchments, predominantly agricultural at Erina Creek and mixed industrial and residential in Narara Creek. It is also consistent with results from other studies at the sites (Paterson et al. 2003a; b). Sampling was undertaken after a period of heavy rainfall (82.4 mm in the previous two days - Commonwealth of Australia Bureau of Meteorology, Gosford) so values are likely to be higher than average. Measurement of sediment pore-water nutrient levels in both the manipulative and field studies could have been off benefit by highlighting differences in nutrient regimes among sites and treatments (McGlathery et al. 2001).

Both the enclosure and field studies were designed to observe the effects of nutrient enrichment on secondary productivity in juvenile fish through bottom-up forcing acting on the epiphytic algae-mesograzers trophic linkage. In the field comparison, algal loads were significantly higher in nutrient enriched sites, particularly Narara Creek, indicating that elevated nitrogen levels increased primary productivity as expected. Algal biomass

provided both food and habitat for mesograzers, which were similarly higher in biomass at enriched sites. Grazing invertebrates are the chief food source of juvenile *P. sexlineatus* and therefore increased the food resources for fish growth, survival and condition in developed catchments.

Although a similar pattern of resource enrichment was predicted in enclosures treated with extra nutrients, higher mortality of fish in enriched enclosures resulted in uneven top-down control in the form of predation. Nutrient enrichment of the epiphytic food web was negated by the top-down pressure from the higher abundance and biomass of predatory fish in ambient enclosures. Growth of fish may have been influenced by density during enclosure experiments (Duffy and Epifanio 1994). As described in many short-term enclosure and mesocosm experiments on seagrass epiphyte food chains (Heck et al. 2000; Hays 2005; Jorgensen et al. 2007; Moksnes et al. 2008), top-down processes dominated, leading to a downward trophic cascade through grazer biomass (not significant) to result in higher algal loads in low nutrient treatments.

A shortcoming of this experiment was that it was conducted in winter when low temperature limits the growth of epiphytic algae. In temperate areas such as south-eastrn Australia epiphytic algal loads peak in summer with warmer temperatures and are at their minimum in winter when temperatures are low (Kendrick and Burt 1997; Hasegawa et al. 2007; Peterson et al. 2007). In hindsight, the manipulative study may have been more effective if carried out in late summer when epiphyte growth is at its peak and more defined differences in growth rates and condition of fish would be expected due to higher water temperatures.

During both the enclosure experiment and field study, levels of ammonia and oxidised nitrogen well above national guidelines for slightly disturbed ecosystems were recorded (ANZECC 2000). Although these levels were below recommended trigger values (460 μg/L) for ammonia toxicity in marine waters (Batley and Simpson 2009) it is possible these levels were exceeded in the rainfall event prior to field sampling or in early stages of the enclosure experiment when initial bursts of nutrient concentrations from Osmocote^{TM} are higher (Heck et al. 2000). In marine and estuarine systems, ammonia in both non-ionised and ionised forms can be toxic to fish (Eddy 2005) by many different

modes including damage to gills (Cardoso et al. 1996). Fish in estuarine systems are more susceptible to ammonia toxicity due to changing temperature, pH and salinity, particularly after rainfall events when pulses occur (Eddy 2005). Fish exposed to short-term pulses of ammonia are able to recover depending on the duration and the magnitude of these events and length of intervals between them (Milne et al. 2000; Diamond et al. 2006). *Pelates sexlineatus* have been observed to be sensitive to ammonia levels in laboratory tanks with regular water changes required to maintain health (personal observation). It is possible that direct negative effects of ammonia toxicity had a greater effect than indirect positive effects on increased food through resource enrichment and bottom-up trophic cascades. Other pollutants such as heavy metals (Cu, Pb and Zn) have been identified in sediments in Narara Creek at levels above recommended toxicity guidelines, however availability levels of these metals were moderate (Olmos and Birch 2008). This may also be a factor in reducing condition in juvenile fish species at this site (Alquezar et al. 2006; Gilliers et al. 2006; Amara et al. 2007).

Field enclosures have been shown to be effective at determining conditional responses of fish to different habitats and food resources (Duffy et al. 1996; Stunz et al. 2002; Burfeind and Stunz 2007; Shervette and Gelwick 2007). Field enclosures can simulate the natural environment but can also produce experimental artefacts such as shading (Keuskamp 2004) and reduced water flow (Miller and Gaylord 2007). These artefacts may lead to a significant reduction of seagrass biomass in enclosures compared to the external environment over the course of the experiment. Enclosure size may also be an artefact affecting prey consumption rates and results should be interpreted comparatively rather than reflecting natural conditions (MacNally 1997). Results of lipid content analysis also varied between field and manipulative enclosure experiments. While this may be due to a lack of control for factors in field studies such as other pollutants discussed above, it also highlights the limitations of mesocosm experiments restricted in both small temporal and spatial scales (Briggs and Borer 2005; Stachowicz et al. 2008). To attempt to determine the effects of food ration and ammonia levels on fish condition, and exclude other anthropogenic effects, further manipulative experiments in the laboratory are recommended. Experiments that include a greater number of economically-important test species are also recommended. This will allow for greater

certainty in management decisions regarding catchment development and pollution abatement in NSW estuarine and coastal watersheds.

Overall, the results from the field component of this study demonstrated that long-term nutrient enrichment of seagrass beds increased productivity and biomass of algal epiphytes and this cascaded upwards through the food chain as increased secondary production of grazing invertebrates. Contrary to expectations, the increase in the food resources for juvenile fish in these habitats did not equate to positive health benefits. Indeed, fish in enriched sites showed poorer body condition than those inhabiting more pristine seagrass beds with low ambient nutrients. Short-term manipulative studies also indicated negative effects of enrichment on fish health, exhibited as a reduction of overall biomass due to lower survivorship. This may be due to the direct negative effects of enrichment (such as toxicity to ammonia and other associated pollutants) being greater than the positive indirect effects of greater food supply through an upwardly driven trophic cascade.

Chapter 6

TOP-DOWN AND BOTTOM-UP FORCING IN TEMPERATE AUSTRALIAN SEAGRASS BEDS

ABSTRACT

A central theme of trophic ecology has been the attempt to identify the relative strengths of consumer and resource control in different ecological systems. In coastal systems this research is also motivated by the need for a greater understanding of the mechanisms driving seagrass decline. For many years conventional wisdom asserted that nutrient enrichment was primarily responsible for seagrass decline. This has recently been challenged by the hypothesis that the overharvesting of large consumers changes ecosystem structure indirectly in a similar way to eutrophication and is the major driver of these changes. In this study I simultaneously manipulated nutrient loads and predation pressure in a shallow seagrass habitat to determine the relative stengths of top-down and bottom-up control in a full-factorial experiment. The biomass of mesograzers was greater in caged treatments where predation from small fishes was prevented, suggesting top-down regulation at higher trophic levels. This increased grazing pressure failed to reduce algal loads on seagrass leaves which were significantly greater in caged plots. This may be due to artefacts of reduced water flow in cages allowing greater settlement of epiphyte propogules. No effects of nutrient enrichment were detected across any trophic level. The higher flow dynamics of the study estuary compared to other studies may have led to greater dissipation of nutrients and reduced the effective increase in nitrogen load into enriched plots. Other factors affecting the experiments were an increase in canopy growth in caged and partially caged plots, possibly due to increased shading, and an increase in seagrass biomass in caged treaments. Due to partial cage effects, further investigation into the strength of top-down and bottom-up forces in temperate Australian seagrass beds is required. The use of artificial seagrasses, finer mesh in cage construction, experimentation in a low energy system and a greater range of nutrient treatments are recommended to reduce cage artefacts and improve the capacity of experiments to detect biological changes.

INTRODUCTION

A central theme of trophic ecology for several decades has been whether consumer (top-down) or resource (bottom-up) control is the dominant force in structuring food webs (Power 1992; Hairston and Hairston 1993; Shurin et al. 2002; Borer et al. 2006; Hillebrand et al. 2007). The relative strength of these opposing forces has been found to vary across ecosystems (Shurin et al. 2002; Hillebrand et al. 2007). Reviews of empirical evidence using meta-analysis have shown that while resource control is pervasive across all ecosystem types (Gruner et al. 2008), consumer control tends to be greater in aquatic than terrestrial systems (Strong 1992; Halaj and Wise 2001; Shurin et al. 2006; Gruner et al. 2008).

Circumstances in nature dictate that the levels of resource supply and consumer control vary both spatially and temporally and it is often difficult to seperate the two as they operate simultaneously and are inter-dependent (Pace et al. 1999). In complex natural food webs these processes are also complicated by increased diversity and heterogeneity of trophic systems including factors such as omnivory, intra-guild predation and plant defences (Strong 1992; Halaj and Wise 2001). The natural processes in marine environments that drive trophic dynamics have been drastically altered over the last century due to anthropogenic impacts such as eutrophication and overfishing (Jackson et al. 2001; Breitburg et al. 2009b; Nixon 2009).

Seagrasses are one of the most productive, species rich habitats in coastal marine systems (Hemminga and Duarte 2000). Seagrasses the world over are in decline, particularly along developed coasts (Orth et al. 2006; Waycott et al. 2009). Seagrass habitat loss has often been attributed to the bottom-up effects of nutrient enrichment which leads to epiphyte and photoplankton blooms that reduce the light levels able to penetrate to seageass leaf surfaces (Short and Wyllie-Echeverria 1996; Ralph et al. 2006). Nonetheless, the concensus that eutrophication is the primary factor driving seagrass habitat loss has recently been challenged. A new theory postulates that the alteration of coastal food chains through the removal of apex predators (piscivorous fish) can be primarily responsible for seagrass decline through the indirect effects of top-down trophic cascades (Valentine and Duffy 2006; Heck and Valentine 2007).

Many studies have attempted to ascertin the relative strengths of top-down and bottom-up forcing in seagrass beds with varying results. A synthesis of this data and meta-analysis from 35 published studies, which manipulated either nutrient enrichment or grazing pressure on epiphytes, concluded that both forces were comparable in magnitude and it was difficult to separate eutrophication (bottom-up) or overfishing (top-down) as the predominant cause of seagrass loss (Hughes et al. 2004). Studies that have manipulated both top-down and bottom-up processes simultaneously have been rare until recently and have generally only tested interactions between grazers and epiphytes. The outcome of these experiments has varied. Some show systems having stronger bottom-up regulation, where epiphtic algal biomass increases with nutrient enrichment even in the presence of increased grazer abundances (Peterson et al. 2007; Jaschinski and Sommer 2008). Others showed top-down regulation, where increased epiphyte productivity can be controlled by increased abundances of small herbivores (Gil et al. 2006). In the few studies that have included higher trophic levels, strong top-down control of grazers by small fish predation generally led to increased aggregate biomass of epiphtyes (Jorgensen et al. 2007; Moksnes et al. 2008). An exception was when juvenile pinfish displayed an omnivorous diet and reduced both the levels of grazers and algae (Heck et al. 2000). Seasonality (Jaschinski and Sommer 2008), the degree of nutrient limitation (Gil et al. 2006), and the composition and diversity of assemblages (Hays 2005; Duffy 2006; Douglass et al. 2007) can all affect how bottom-up and top-down forces interact.

The aim of this experiment was to investigate the strength of both top-down and bottom-up forcing and their interaction on trophic dynamics in temperate Australian seagrass food chains. The experimental design allowed for simultaneous control of consumer pressure with a series of caged and uncaged treatments altering predation on grazing invertebrates and resource supply by enriching some treatment plots with excess nutrients. If strong top-down forces are driving trophic structure, caged experiments would have a greater biomass of grazers and a reduced epiphtic algal load than uncaged or partially-caged plots. If bottom-up supply is dominant in structuring food webs either an increase in grazers and a decrease in epiphytes or a increase in both grazers and epiphytes will be evident in nutrient enriched plots depending on whether a ratio or resource-dependent functional response is in operation (see Chapter 3). An interaction between both top-down and bottom-up forcing would see more pronounced changes in

biomass for nutrient enriched treatments but in the same direction as experienced in ambient treatments.

METHODS

Study site and system

To test for the effects of top-down and bottom-up forcing in shallow estuarine seagrass beds a manipulative study was carried out in a large shallow seagrass bed at Mullet Creek (33°29'20"S, 151°15'46"E), part of the Hawkesbury River catchment just north of Sydney (see Figure 2.1). The beds consisted of *Zostera capricorni* meadows that support a food chain based on epiphytic algae and sustaining grazing invertebrates (typically gastropods and amphipods) and in turn small and juvenile fishes and ultimately large piscivorous fishes. The study was conducted over a period of six weeks in spring 2007; a period of high epiphyte growth.

Cage and nutrient treatments

Experimental cages were constructed by enclosing (1 m^2) plots using 5 mm plastic mesh secured in each corner to a plastic star picket with cable ties. The mesh allowed access for propagules of algal epiphytes and small grazing animals but excluded small predatory fish from entering the caged plots. Cages stood 1 m high and the bottom of the mesh was buried in the sediment to a depth of 5 cm. Each enclosure was swept with a dip net (1 mm mesh) to remove any fish before the experiment commenced and 6 mm monofilament netting was cable-tied to the top of each cage to prevent predation by birds or the entry of predatory fish at high tide. Uncaged treatments were also employed to allow for natural predation rates of invertebrate feeding fishes to be compared to caged plots to assess top-down forcing. Partial cages identical to the full cages but with 50 x 50 cm panels removed from each side to allow entry of small predatory fish were constructed to control for caging artefacts.

The caged treatments were combined with two different nutrient treatments (ambient and enriched) to test for bottom-up effects of nutrient enrichment. Nutrient dispensers made of PVC pipe (50 mm diameter x 300 mm long), capped at both ends and with 22 x 4 mm holes drilled in each were suspended on PVC pickets in each experimental plot. In nutrient enriched treatments, 300 g of slow release fertiliser (Osmocote^{TM}) were enclosed

in nylon stockings and placed into nutrient dispensers (as recommended by Worm et al. 2000), while ambient nutrient treatment dispensers were left empty. This amount of nitrogen has been shown to affect both primary and secondary production in epiphytic algal food chains in the Hawkesbury estuary (Kelaher et al. In prep). Dispensers were not replenished during the study as experiments on nutrient dissolution rates have shown that release rates remain steady after an initial burst for approximately six weeks (Heck et al. 2000). Treatment plots were haphazardly assigned to areas within the seagrass bed approximately 10 m apart. Four replicates of all six orthogonal treatments were deployed.

Sampling

Seagrass canopy height was estimated at each plot at the commencement and completion of the experiment by randomly selecting 20 seagrass leaves from each plot and measuring their length. Netting was removed from the top of cages and three BROMAR samples of seagrass and associated epiphytes and epifauna were collected from each experimental plot and combined to form a replicate sample. From each replicate, epiphytic algae were scraped off 20 haphazardly-selected seagrass leaves and both epiphytes and seagrass were dried in an oven at 60 °C and weighed to determine epiphyte load. The remaining sample was rinsed through a 500 μm mesh and invertebrate grazers (gastropods and amphipods) were identified, and both grazers and seagrass were dried in an oven at 60 °C and weighed to determine biomass (see Chapter 2 for more detailed methods of BROMAR sampling).

Statistical analysis

Hypotheses were tested using fully orthogonal two-way ANOVA with cages (fixed, three levels) and nutrient (fixed, two levels) as factors and nutrient levels, change in seagrass canopy length, epiphyte and grazer biomass, grazer abundance and grazer species richness as response variables. As seagrass biomass was affected by the presence of cages and was significantly different among treatments at the end of the experiment, seagrass biomass was included as a co-variate in the analysis of grazer biomass, abundance and species richness and Analysis of covariance ANCOVA was conducted. Prior to each ANOVA and ANCOVA, the assumption of homogeneity of variances was examined using Cochran's C-test. Data were log-transformed when necessary to meet this assumption. When appropriate, ANOVAs were followed by *a posteriori* Student–

Newman–Keuls (SNK) tests to identify differences among levels. The assumption of homogenous linear regression in ANCOVA was tested and met using a customised model containing the pooled covariate-by-factor interaction.

Multivariate analysis was conducted using PRIMER package (v.6 Plymouth Marine Laboratory, Plymouth UK) on the assemblage of grazing invertebrates to determine differences between caged and nutrient treatments. A Bray-Curtis dissimilarity co-efficient was used on untransformed data (Clarke and Warick 2001). The results were graphically represented using multi-dimensional scaling (MDS) plots. Analysis of similarity (ANOSIM) was conducted to test hypotheses about differences among methods. SIMPER was used to determine the relative contribution of species to differences between methods.

RESULTS

Although nutrient levels were not significantly different between treatments, Ammonia levels were slightly higher in the enriched treatments (12.93 μg/L) than those of ambient treatments (11.83 μg/L;) and oxidised nitrogen levels showed a similar trend with enriched levels of 4.11 μg/L and ambient levels of 3.99 μg/L.

The presence of cages around experimental plots influenced the growth of seagrass leaves during the experiment. The change in canopy height was significantly greater in both caged and partially caged plots (ANOVA, $F_{2, 18} = 14.86$, $P < 0.001$, Table 6.1, SNK < 0.005) with almost a five-fold increase in both caged treatments compared to the uncaged control (Figure 6.1a). Seagrass biomass from BROMAR samples also differed significantly ($F_{2, 18} = 3.82$, $P = 0.041$, Table 6.1, SNK < 0.005) among caged treatments with fully caged plots (9.244 ± 1.025 g/m^2) having significantly greater biomass than those with partial cages (5.638 ± 0.786 g/m^2). Caged seagrass also had a greater biomass, though not significantly so, compared to uncaged plots (6.390 ± 0.947 g/m^2, Figure 6.1b).

Epiphytic algal load on seagrass leaves was significantly different among cage treatments ($F_{2, 18} = 3.65$, $P = 0.047$, Table 6.1). Caged plots (97.80 ± 26.81 g/kg seagrass) had significantly greater algal load than partially caged (41.49 ± 5.93 g/kg seagrass) and uncaged plots (38.44 ± 8.57 g/kg seagrass, Figure 6.2a, SNK < 0.005). Grazer biomass

had a similar pattern among caged treatments (ANCOVA $F_{2, 17} = 5.23$, P = 0.017, Table 6.1) with caged treatments (2.17 ± 0.43 g/kg seagrass) significantly greater than uncaged (1.19 ± 0.18 g/kg seagrass) and partially caged plots (1.17 ± 0.16 g/kg seagrass) (Figure 6.2b, SNK < 0.005).

Grazer assemblages showed no difference among nutrient treatments (ANOSIM, P = 0.932) although caged treatments were significantly different to partial and uncaged treatments (ANOSIM, P = 0.002, pairwise tests, Figure 6.3). SIMPER analysis showed that the same taxa (Maeridae, Amphilochidae and Corophiidae amphipods and Aplysiidae gastropods) were contributing to the differences between these treatments through greater abundances in the caged treatments. This was supported by analysis of grazer abundance, showing no effect of nutrient treatments but significantly greater numbers in caged treatments compared to partially caged and uncaged treatments (ANCOVA $F_{2, 17} = 13.24$, P < 0.001, SNK < 0.005, Table 6.1, Figure 6.4). Grazer biomass can be significantly predicted by seagrass biomass (ANCOVA $F_{1, 17} = 26.55$, P < 0.001, Table 6.1, Figure 6.4). Species richness was not significantly different between nutrient treatments or among caged treatments (Table 6.1, Figure 6.4).

Table 6.1: Analysis of variance table for differences in seagrass algae and grazer attributes.

Source of variation	df	MS	F	p
Seagrass canopy growth				
Nutrient status	1	1872	0.74	0.401
Cage treatments	2	37528	14.86	<0.001
Nutrients x cage	2	1145	0.45	0.643
Residual	18	2525		
Seagrass biomass				
Nutrient status	1	3.06	0.4	0.533
Cage treatments	2	28.954	3.82	0.041
Nutrients x cage	2	2.054	0.27	0.766
Residual	18	7.577		
Algal load				
Nutrient status	1	284.7	0.12	0.737
Cage treatments	2	8938.3	3.65	0.047
Nutrients x cage	2	965.5	0.39	0.68
Residual	18	2451.3		
Grazer biomass				
Seagrass $biomass_{cv}$	1	0.003	0.02	0.902
Nutrient status	1	0.394	2.18	0.158
Cage treatments	2	0.945	5.23	0.017
Nutrients x cage	2	0.059	0.33	0.726
Residual	17	0.181		
Grazer abundance				
Seagrass $biomass_{cv}$	1	3.855	26.55	<0.001
Nutrient status	1	0.003	0.02	0.879
Cage treatments	2	1.922	13.24	<0.001
Nutrients x cage	2	0.059	0.41	0.672
Residual	17	0.145		
Grazer species richness				
Seagrass $biomass_{cv}$	1	3.31	1.15	0.299
Nutrient status	1	1.308	0.45	0.509
Cage treatments	2	2.194	0.76	0.482
Nutrients x cage	2	0.588	0.2	0.817
Residual	17	2.879		

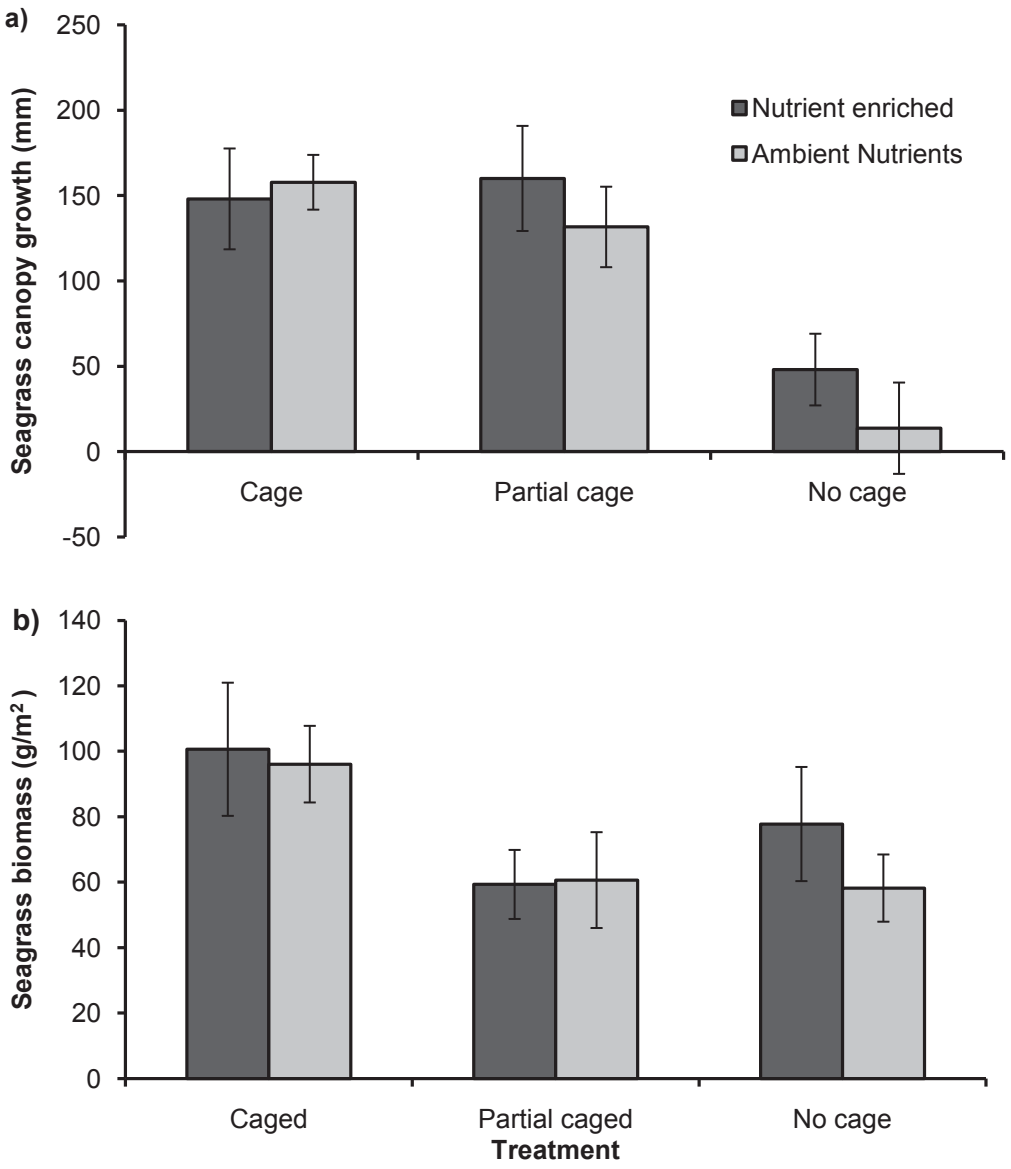

Figure 6.1: Means ± 1 S.E. for (a) seagrass canopy growth and (b) seagrass biomass for all treatments at the conclusion of the experiment. Dark bars represent nutrient enriched treatments and light bars represent ambient nutrient treatments.

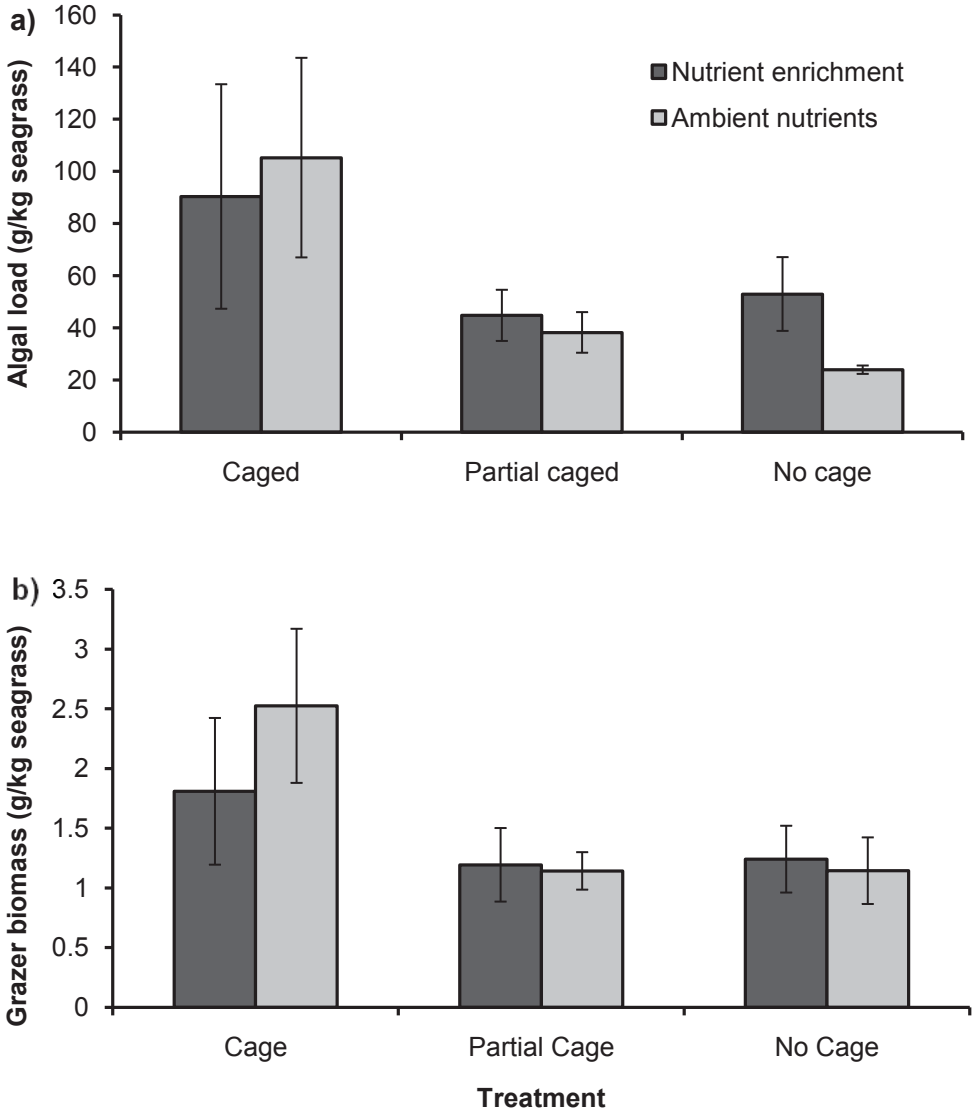

Figure 6.2: Means ± 1 S.E. of (a); algal load on seagrass leaves and (b) grazer biomass standardised to seagrass biomass) at the conclusion of the experiment for all treatments. Dark bars represent nutrient enriched treatments and light bars represent ambient nutrient treatments.

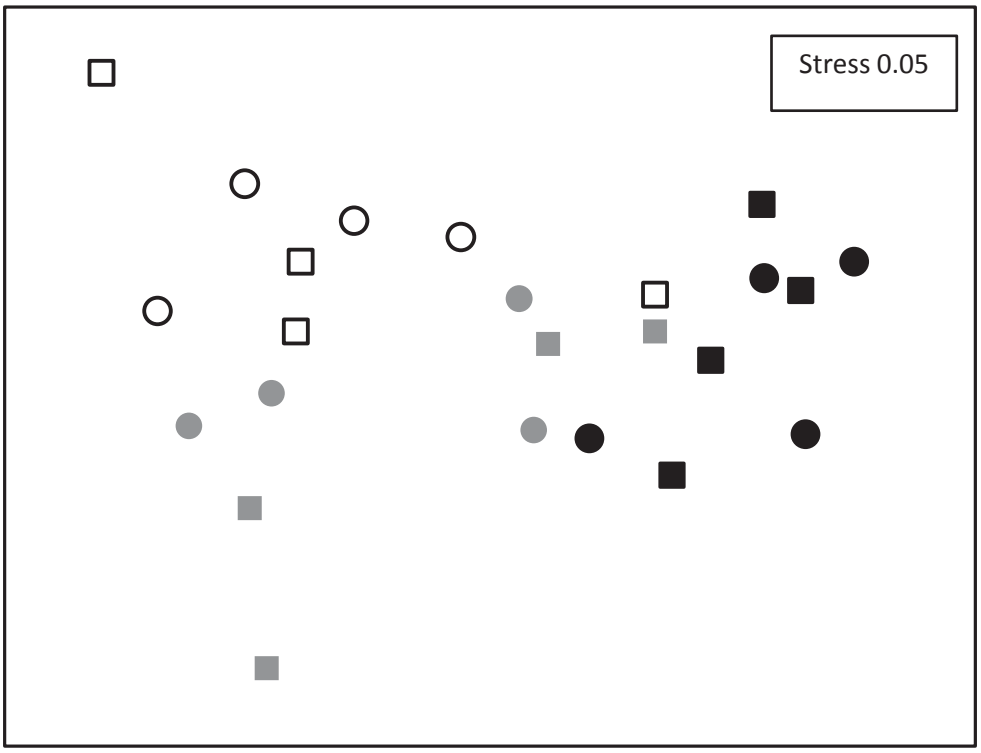

Figure 6.3: Multi-dimensional scaling plot of epiphyte grazer assemblages (amphipods and gastropods) showing caged treatments (black), partially caged treatments (grey) and uncaged treatments (white). Nutrient enriched treatments are represented by squares and ambient nutrient treatments are represented by circles).

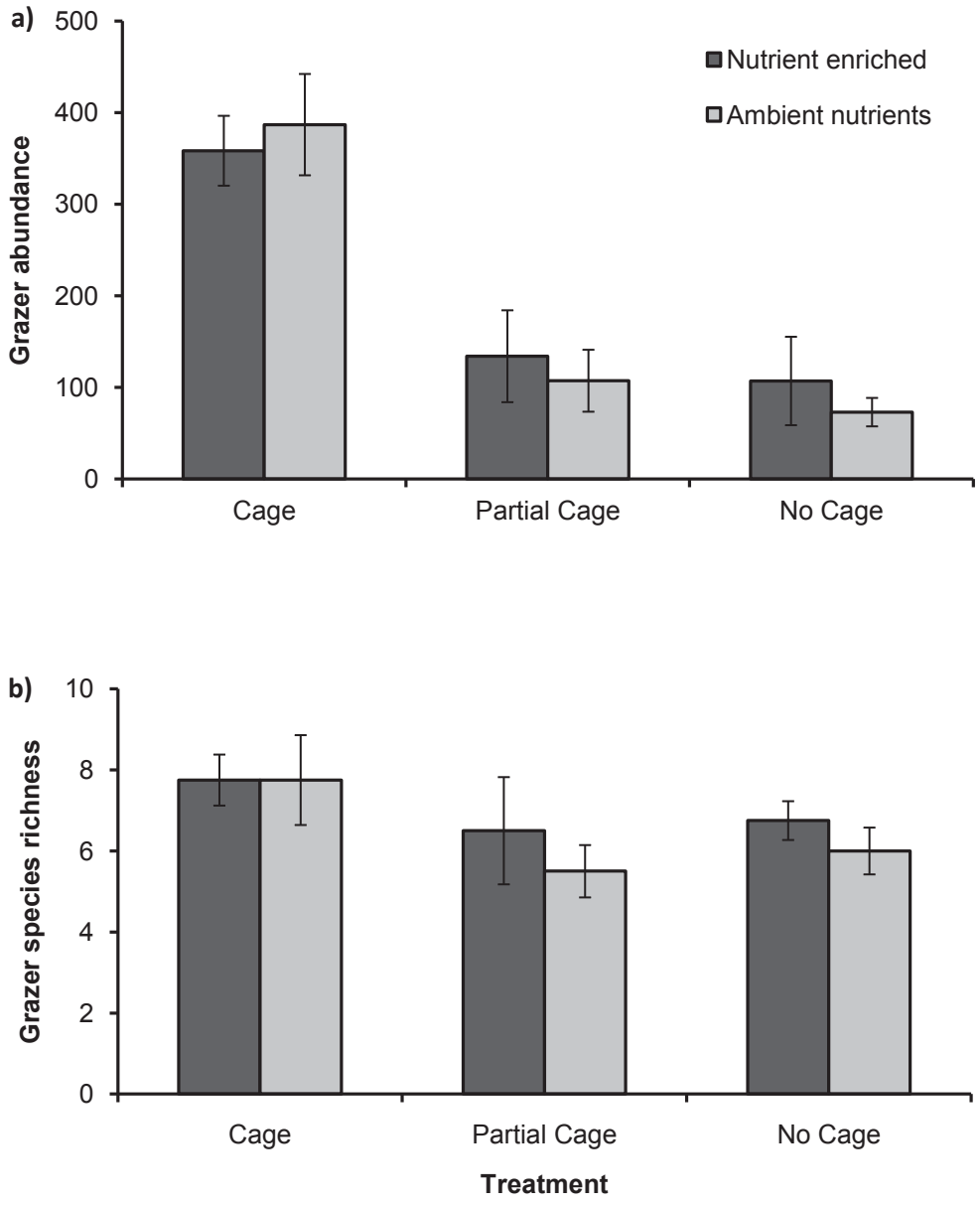

Figure 6.4: Means ± 1 S.E. for (a) abundance and (b) species richness of grazer assemblages at the conclusion of the experiment for all treatments. Dark bars represent nutrient enriched treatments and light bars represent ambient nutrient treatments.

DISCUSSION

The results of this study mirror those of a similar nutrient enrichment experiment in seagrass beds (Morris et al. 2007) that found no linkage between nutrient enrichment and epiphytic algal growth. These results are contrary to many other studies that have found algal loads positively correlated to nutrient levels (see Hughes et al. 2004). It is evident that this study did not effectively demonstrate bottom-up forcing of nutrient enrichment. While the nutrient loading levels in this experiment were similar to, or moderately lower than other studies (Heck et al. 2000; Morris et al. 2007; Moksnes et al. 2008) it is still possible that these levels were insufficient to stimulate changes in seagrass food chains at this site. Mullet Creek is subject to a greater tidal range (≈1.3 m mean tidal range) compared to estuaries in other experimental systems (eg. Heck et al. 2000; Moksnes et al. 2008) and this may have lead to greater dissipation of nutrients out of the experimental plots and reduced the effective load (Morris et al. 2007).

There was, however, a significant experimental effect due to caging. Fully caged treatments that excluded small predatory fish had significantly higher abundances and biomass of grazing invertebrates compared to partially caged and uncaged treatments with predator access. Predator control of grazer populations in seagrass systems has been demonstrated previously in caging experiments that exclude or manipulate the abundance of small predatory fish (Heck et al. 2000; Moksnes et al. 2008) and in field experiments with natural differences in the abundance of these fish among sites (Jorgensen et al. 2007).

The increase in grazer numbers in caged treatments did not cascade down and cause a decrease in epiphytic algal load as predicted by our model. Top-down control of epiphytic algae on seagrass leaves by grazing invertebrates has been demonstrated in many different seagrass studies and is widely accepted (reviews by Hughes et al. 2004; Valentine and Duffy 2006). However, the manipulation of grazer abundance does not always lead to changes in epiphyte loads, particularly in oligotrophic systems (Keuskamp 2004). In field surveys (Chapters 4 & 5), enriched sites with greater algal loads also had greater abundances of invertebrate assemblages. This could suggest that either bottom-up trophic control is greater than top-down consumption or that the presence of epiphytic growth on seagrass leaves is providing enhanced habitat as well as additional trophic

support and increasing invertebrates through non-trophic means. Another possibility is that important functional grazers capable of controlling epiphytes biomass may be missing from the invertebrate assemblages in this study system (Moksnes et al. 2008).

Similarly, given an increase in epiphyte biomass in caged plots, current reasoning would predict a detrimental effect on seagrass biomass (Duffy et al. 2003; Hughes et al. 2004; Hays 2005). In fact, the reverse was demonstrated in the current experiment with greater biomass and canopy growth in caged treatments having higher algal loads. Multivariate analysis also showed significant differences in grazer assemblages in treatments where predatory fishes were excluded. Further investigation into these differences showed that neither the composition of species, nor their order of importance to assemblages, changed significantly among treatments, but rather increases in abundances were driving these differences. There was no significant difference in species richness among treatments although there was a non-significant trend of higher richness in caged treatments with higher resource availability and less predation pressure.

These contradictory findings of high grazer abundances, algal loads and seagrass growth suggest that experimental artefacts may be affecting the lower trophic levels with caged treatments promoting both seagrass and algal biomass. Two of the most discussed artefacts in caging experiments within seagrass systems are the effects of increased shading and reduced water flows (Moksnes et al. 2008). As shading was a factor for both caged and partially caged plots, and seagrass leaves grew at a greater rate during the experiment in these treatments, lower light levels may be a possible cause of this effect. Studies have found that reduced light will generally have a negative effect on seagrass growth, although few studies have looked at the effects of small reductions in light intensity (Short et al. 1995; Leoni et al. 2008). A study of *Zostera noltii* at different irradiances, however, suggested optimal growth may occur at intermediate light levels (Peralta et al. 2002). The structure of experimental cages can also reduce water flow by baffling currents. Hydrodynamics, particularly water flow, have been shown to affect seagrasses and their associated algal and grazer assemblages. Low water flow can lead to a increase in seagrass biomass and changes in leaf morphology (Schanz and Asmus 2003) and an increase in epiphytic algal assemblages due to higher propagule settlement (Lavery et al. 2007) in more sheltered environments. Both of these traits were present in caged plots at the conclusion of the experiment.

The fact that caging artefacts mimicked a bottom-up effect of nutrient enrichment by increasing epiphyte settlement and that nutrient effects from enriched treatments were not significant have made it difficult to separate the top-down effect of predation from the bottom-up effect of increased resources. Further experimentation would benefit from using artificial seagrass units to reduce artefacts of increased seagrass biomass. Use of monofilament netting instead of more rigid plastic mesh for cage construction and selection of a site with a less dynamic lower tidal range would reduce differences in leaf movement and thus also algal load between caged and uncaged controls. Use of more nutrient treatments with higher concentrations would be beneficial to ensure a measurable impact on resource enhancement through enrichment.

Overall this study has supported the current consensus that predation by small predatory fish may affect the biomass of invertebrate grazers in estuarine seagrass beds. As there was no effect from nutrient enrichment in this study it was not possible to ascertain the relative strengths of top-down and bottom-up forces in structuring the food chain. My, results suggest, however, that increased abundances of grazing invertebrates may not be effective at reducing epiphytic algal loads on seagrass leaves and alleviating the stress to seagrasses by nutrient enrichment.

Chapter 7

GENERAL DISCUSSION

Natural systems are characterised by a series of extremely complex interactions that contribute to their dynamic and adaptive properties (Levin 1998). Coupling these systems with human disturbance leads to even greater complexity (Liu et al. 2007). One of the major properties of natural ecosystems is their trophic structure, which is generally controlled by top-down and bottom-up regulation of populations and communities (Leibold et al. 1997). The increasing magnitude of human activity is, however, altering both top-down and bottom-up processes in coastal and marine environments through the exploitation of resources (Pauly et al. 1998; Jackson et al. 2001) and the enrichment of coastal waters with nutrients (Vitousek et al. 1997a). A central focus of ecological research for many decades has been to provide simplified models that best represent these complex trophic interactions (Williams and Martinez 2000; Bascompte and Melián 2005). This study investigated the trophic dynamics of a prominent food chain within ecologically-important seagrass habitat subjected to increased bottom-up forcing due to anthropogenic development of surrounding catchments.

The study was carried out in south-eastern Australia where large declines in seagrass distribution have coincided with residential and industrial development of surrounding catchments (Larkum and West 1990). It focused on a simple four level food chain that plays an important role in structuring seagrass ecosystems (epiphytic algae, grazing invertebrates, small and juvenile fish, and piscivorous fish). Field monitoring to compare developed catchments with elevated nutrient loads against forested catchments with low ambient nutrient levels were conducted to determine long-term shifts in the biomasses of different trophic levels. This field sampling was combined with manipulative field experiments to gain a better understanding of the interactions between trophic levels and the mechanisms structuring these communities.

This chapter synthesises the major findings from individual experiments and discusses trends in the four trophic levels in the study when exposed to bottom-up and top-down forces. The chapter then focuses on the functional response of trophic interactions, indicators that may be useful in assessing changes in estuarine health due to nutrient

enrichment and important information required for the modelling of estuarine processes to determine the effects of eutrophication. Recommendations are also given for management of estuaries and seagrass beds, particularly in south-eastern Australia, and several possibilities for future research arising from this study are outlined.

CHANGES IN BIOMASS AND COMMUNITY STRUCTURE WITH NUTRIENT ENRICHMENT

Epiphytic algae

Epiphytic algal loads were found to be consistently related to the nutrient status of estuaries throughout this study. Comparisons among sites exposed to long-term nutrient enrichment in developed catchments with sites containing low ambient nutrients found a significant, almost threefold increase in the biomass of algae on seagrass leaves. This increase in biomass coincided with increases in the abundance and biomass of grazing invertebrates indicating that the structure and trophic subsidy of epiphytes facilitated higher trophic levels. It also suggests that grazers could not completely control algal biomass through cropping (Chapter 4).

Short-term experiments where seagrass enclosures were manipulated by adding nutrients through slow release fertilisers showed the opposite effect with lower epiphyte loads in enriched plots compared to ambient enclosures and controls (Chapter 5). This pattern was associated with higher grazer biomass in enriched treatments, pointing to top-down regulation of algae over short time periods. The timing of this manipulative experiment in winter may also have minimised the effect of nutrients on algal epiphytes as this coincided with the period of lowest epiphyte growth (Kendrick and Burt 1997). Nutrient enrichment in caged experiments (Chapter 6) did not have any effect on epiphyte load, possibly because the large tidal range resulted in rapid dissipation of nutrients out of the experimental plots and reduced the effective load. This study also highlighted through experimental artefacts that factors other than nutrients, such as rate of water flow and shading, can also have large effects on the variation in epiphyte growth.

Productivity of epiphytic algae on seagrass leaves is enhanced by increased nutrient levels in the water column (Wear et al. 1999). The link between nutrient enrichment and increases in the algal loads on seagrass leaves has long been suspected of being a major

cause of seagrass decline. Localised loss of seagrass beds have been attributed to high levels of nutrient enrichment around point sources (Cambridge et al. 1986; Neverauskas 1987). More moderate increases of nutrients from diffuse sources of nutrients and at greater distances from point sources as experienced during this study may not have as pronounced an impact on epiphyte loads and seagrass health (Frankovich and Fourqurean 1997). In south-eastern Australia where nutrient levels are low by global standards (Young et al. 1996; Harris 2001), seagrasses have been seen to benefit from increased nutrient levels due to the alleviation of nitrogen limitations that can restrict their growth (Ralph et al. 2006). This appears to be the case in this study with higher seagrass biomass in some developed catchments.

Stable isotope analyses indicate that epiphytic algae makes an important contribution to the trophic support of higher trophic levels, including economically-important fish species, in seagrass systems and adjacent systems (Moncreiff and Sullivan 2001; Connolly et al. 2005b). In particular, the epiphytes support significant epifaunal grazing communities on seagrass leaves (Bologna and Heck 1999).

Although it was not investigated in this study, both grazing pressure (Duffy and Harvilicz 2001; Prado et al. 2007) and to a lesser extent nutrient availability (Frankovich et al. 2009) can also be important factors in structuring the community of epiphyte assemblages. The findings from this present study suggest that bottom-up forcing from nutrients seem to be stronger over long time periods in systems approaching equilibrium, however, top-down control from consumers can dominate over smaller spatial and temporal scales.

Grazers

Grazer biomass and abundance increased by similar levels to epiphyte loads in developed, nutrient enriched catchments compared to forested watersheds (Chapter 4). Although community structure separated more among estuaries than on catchment characteristics (Chapter 3), the higher abundances coinciding with increases in algal loads together with a weak correlation between assemblage structure and total nitrogen

levels in water samples suggests strong bottom-up regulation of grazer populations (Chapters 3 and 4).

Nonetheless, short-term manipulative experiments again had different results to longer-term field studies. In enclosure experiments, grazer abundances were higher in enriched treatments however this coincided with lower algal loads and levels of fish biomass. The reverse trend of low grazer biomass and high algal loads and fish biomass in ambient treatments was evident. This suggests a top-down trophic cascade was structuring community populations with fish predators controlling grazer abundances and grazers controlling algal biomass (Chapter 5). Caged experiments also indicated strong top-down control of grazers with higher abundances in plots excluding small predatory fish than uncaged or partially caged controls (Chapter 6).

Grazer densities are often positively correlated with the presence of epiphytic algae (Gil et al. 2006; Peterson et al. 2007; Armitage and Fourqurean 2009). While the added structural complexity of epiphyte habitat plays some part in providing refuge for grazers, their primary role in enhancing abundances is through trophic support (Bologna and Heck 1999). On the other hand, many small-scale manipulative studies report the control of epiphyte abundance by grazing amphipods and gastropods (Howard 1982; Moksnes et al. 2008). The experimental evidence for both bottom-up and top-down processes in structuring grazer communities found here is supported by field studies suggesting an interaction of both (Baden et al. 2010). This was also supported by meta-analysis finding a roughly equal role of resource and consumer control (Hughes et al. 2004). The field sampling component of this study indicated a dominance of resource control with the abundance of grazers increasing in the same direction as epiphyte loads. Nevertheless, strong consumer control which regulated grazer and epiphyte communities was evident over shorter time scales of weeks.

Juvenile and small fish

There was no change in the biomass of juvenile fish that fed on epiphyte grazers between developed, nutrient-enriched catchments and undeveloped catchments with low ambient nutrients (Chapter 4). When examining the structure of juvenile fish assemblages more

thoroughly there was evidence of a shift in dominance from omnivorous to carnivorous species. There were also distinct differences in the abundances and structure of the entire fish community in seagrass beds among catchment types. This was driven primarily by a greater abundance of several pelagic carnivores in undeveloped catchments (Chapter 3).

Several reasons for these patterns have been discussed throughout this thesis. Firstly, marine systems are more open at higher trophic levels where movement of consumers and resources among adjacent habitats can lead to a partial disconnect from the epiphyte-grazer trophic pathway (Duffy 2006). Spatial and temporal variation in the arrival of larval recruits can also play a large role in structuring these populations (Bell and Westoby 1986). Another alternative is that the increased trophic support through greater food supply in enriched catchments was countered by the negative effects of pollution and toxicity. This is supported by results from the enclosure and field experiment (Chapter 6), which showed lower survival and condition of juvenile *Pelates sexlineatus* under nutrient enriched conditions and in developed catchments. This is also supported by evidence showing poorer health in *Ambassis jacksoniensis* at nutrient enriched sites (Schlacher et al. 2007) that is likely to manifest as higher mortality and therefore lower abundances of this species in developed catchments as found in this study.

Piscivorous fish

The effect of nutrient enrichment on the abundance and biomass of piscivorous fish was difficult to assess due to the transient use of seagrass habitat by these species and difficulties with sampling techniques used to measure spatial and temporal differences between catchments (see methods pilot study, Chapter 2). While visual methods; Baited Remote Underwater Video (BRUV) and Underwater Visual Census (UVC), proved by far the most effective in the pilot study in a relatively undisturbed site, higher turbidity in enriched catchments reduced visibility, leading to bias between catchment types using these techniques.

Piscivorous fish in seagrasses have been shown to structure the assemblages of lower trophic levels (Hindell et al. 2000a; Hindell et al. 2000b; Hindell 2006), and increased nutrients are linked to more productive fisheries (Nixon and Buckley 2002; Oczkowski

and Nixon 2008). This study did not effectively determine the strength of links between nutrient enrichment and the abundance or biomass of large piscivorous fish nor the effect that these fish have on structuring the communities of small fish, grazers or epiphytes in seagrass systems. Improved technologies in sampling such as sonar devices, stable isotope analyses tracking carbon from nutrient run-off, gut content analyses and tethering experiments to ascertain relative predation rates on small fish prey could be used in the future to give further insight into these relationships (eg. Edgar and Shaw 1995b; Boswell et al. 2008; Armitage and Fourqurean 2009; Hammerschlag et al. 2010).

Problems of scale

An issue with attempting to conduct experiments to determine the ecosystem-wide effects of press perturbations is the large spatial scale and long time periods required for the system to reach its new equilibrium (Bender et al. 1984). Short-term and small-scale mesocosm and manipulative field studies are useful for their ability to reduce natural variation and allow for replication to increase statistical power and determine cause and effect (Petersen et al. 2003). These experiments, however, suffer from a reduction in realism and generality as they are not easily translated to ecosystem-wide scales (Schindler 1998).

A disconnect has been demonstrated between both the timescales of short-term experiments and equilibrium models (Briggs and Borer 2005), as well as the scale difference between mesocosm and manipulative plots and whole ecosystems (Carpenter 1996). The discrepancies and contradictory findings in this study between the catchment-wide, long-term mensurative studies and short-term manipulative experiments conducted in 1 m^2 plots (described above for algae and grazers) highlight the utility of mensurative field experiments in determining the effects of ecological perturbations at an system-wide level (Underwood 1996).

MODELLING AND MONITORING SEAGRASS ECOSYSTEMS

Trophic dynamics

Long-term studies point to a ratio-dependent functional response at lower trophic levels with an increase in epiphytic algae and mesograzers under nutrient enrichment. This

supports Bishop et al. (2006), who found ratio dependence in a similar study of a detrital food chain in a nutrient enriched estuary. The trophic links between grazers, small and juvenile fish seems decoupled to a large extent from the epiphytic algal pathway due to the generalist nature of the diets of many small fishes (Burchmore et al. 1984; Edgar and Shaw 1995b) and the subsidy provided from the detrital food chain in undeveloped catchments (Moore et al. 2004). The openness of marine and estuarine systems, particularly at higher trophic levels, means that the movement of both consumers and resources among adjacent habitats can also attenuate the tightness of the relationship with lower levels in the food chain (Duffy 2006). This implies that simple ratio-dependent functional response models may be useful for predicting changes in lower trophic levels of algae and grazers under nutrient enrichment. However a more advanced, complex model taking into account these other properties of the food web is needed to explain community structure and biomass for higher trophic levels. Overall, these patterns of trophic structure suggest that stresses on seagrasses from increased epiphyte loads in enriched systems are not balanced by an increase in productivity of economically important fish species in this habitat.

Indicators of nutrient enrichment and of seagrass systems

Environmental indicators are generally physical, chemical or biological measurements that provide an insight into the broader condition of the environment (Burkholder et al. 2007; Niemeijer and de Groot 2008b). They are becoming more common for the monitoring of ecosystem health, often being a legal requirement in Environmental Impact Assessments and State of the Environment reporting. Their selection, however, can be arbitrary and without a rigorous scientific basis (Niemeijer and de Groot 2008b). Individual indicators viewed in isolation can often paint contradictory pictures of the health of a specific environment. Because of this, the use of a network of inter-related indicators is often required for a more comprehensive summary (Niemeijer and de Groot 2008a).

Water quality analysis and particularly levels of nitrogen (e.g. total Kjeldahl nitrogen Ammonia: NH_4, Nitrate: NO_2 and Nitrite: NO_3) are regularly used as indicators of eutrophication in Australian estuaries by local governments and state government departments or authorities. This continues despite mounting evidence that they are not a

reliable predictor of nutrient loads due to either rapid use in primary production by phytoplankton, algal epiphytes and seagrasses or binding to sediments (eg. Capriulo et al. 2002; Lee et al. 2004; Scanes et al. 2007). Research during this study highlighted the inadequacy of water column nutrient levels by failing to detect elevated levels in either developed catchments or enriched experimental plots on most occasions throughout the study. There was however a weak correlation between water-column levels of total nitrogen and grazer assemblage structure. The use of sediment pore-water nutrients may be a more effective way of measuring and monitoring longer-term nutrient loads into estuaries. Nutrient regeneration from this source can supply a large portion of the nitrogen budget of epiphytes at certain times of the year (Cowan and Boynton 1996). Sediment pore water nutrient levels have been correlated to increases in catchment nutrient loads (Cabaco et al. 2008), however they have also been identified as a poor indicator of nutrient availability (McGlathery et al. 2001).

A comprehensive study of environmental indicators in south-eastern Australia recommended turbidity as the best water quality predictor of nutrient loading (Scanes et al. 2007). This supports the findings from our field study (Chapter 3) that found turbidity most highly correlated with differences in fish assemblages between developed and developing catchments, although this study did not investigate any link between turbidity and nutrient loads.

Many biological indicators have also been suggested for monitoring the health of estuaries and seagrass beds including chlorophyll *a* values as a proxy for phytoplankton production (Kennish et al. 2007; Scanes et al. 2007), the presence of harmful algal blooms (Kennish et al. 2007; Bricker et al. 2008), macro-algae (Scanes et al. 2007), seagrass (Kirkman 1996; Madden et al. 2009), microbial activity (Paerl et al. 2003), and fish assemblages (Meador and Goldstein 2003). A recent validation of seagrass indicators found that a majority were ineffective over the relevant spatial scales or across environmental gradients needed to detect eutrophication (Martinez-Crego et al. 2008). While this study found a relationship of higher epiphytic algal loads in developed catchments, these loads were not considered good predictors of assemblages of higher trophic levels in BIO-ENV analysis. Similarly seagrass measures of leaf density, canopy height and seagrass biomass did not discriminate well between catchments with enriched

and ambient nutrient loads. Seagrass health and density is more likely to be an effective indicator of eutrophication under higher nutrient loadings (Scanes et al. 2007). Fish assemblages proved to be a more reliable indicator of differences between catchments under different nutrient loads, particularly several highly abundant species (*A. jacksoniensis*, and *R. macrostoma*) that appeared to be sensitive to waters in disturbed catchments. This may have either been a trophic response to increased nutrient loads or due to other factors such as increased pollution or turbidity.

MANAGEMENT IMPLICATIONS

Eutrophication of coastal waterways through catchment development is a widespread and increasing concern around the globe leading to changes in nutrient cycling, loss of biodiversity, degraded water quality, and shifts in fisheries production (Nixon 1995; Vitousek et al. 1997a). It is important that scientists, natural resource managers and governments work together to develop new and effective ways to detect, understand, manage, reverse, and prevent current and future human-induced impacts of nutrient enrichment on estuarine and coastal systems. This can be achieved through integrated and co-ordinated programs of research and monitoring, remediation, restoration and planning.

Research and Monitoring

Further research into the impacts of anthropogenic enrichment of estuaries and the effect on the health of seagrass ecosystems is required. Broad reviews have suggested the need for a greater understanding of the implications that changing land use patterns, physical circulation of coastal waters and a changing climate will have on the eutrophication of coastal waters (Kennish 2002; Bricker et al. 2007).

Effective monitoring systems should be able to distinguish anthropogenic impacts from natural cycles, disturbance, and spatial and temporal variability (Kennish 2002). A recent review of the eutrophication problem in the USA called for a co-ordinated response that re-evaluated environmental indicators and refined standards leading to the implementation of the same monitoring protocols for estuaries and seagrasses across geographic regions (Bricker et al. 2007). Given that many environmental monitoring programs rely heavily on water-column nutrient levels, and that indicators suitable for

use in higher nutrient waters of the northern hemisphere may not be effective in our naturally oligotrophic waters, a similar review is required in Australia. Water quality guidelines (ANZECC 2000) for monitoring aquatic ecosystems go some way to providing this, but these only recommend thresholds of stressor values and the outcomes of these are often incorrectly inferred by managers (Scanes et al. 2007).

Remediation and restoration

Restriction of nutrient loading is seen as essential to ameliorating the effects of eutrophication in estuaries (Smith et al. 1999). The study of systems where nutrient loads have been reduced over time from enriched to more oligotrophic showed relatively clear and direct recoveries and reduced hypoxia in many cases, however, complex non-linear responses and sometimes further dergradation including regime shifts can also result (Kemp et al. 2009). Nutrient reductions largely occur through improved management practices in the catchment and at the land and water interface such as improved sewage and wastewater treatment, reductions of fertiliser use, and construction and restoration of wetlands (Greenway and Simpson 1996; Boesch 2002; Bricker et al. 2008). Reduction of NO_x from cars and industry through reduced burning of fossil fuel and reforestation of cleared lands will also reduce loads from atmospheric deposition (Boesch 2002).

Once removal of the nutrient stressors has occured, ecosystems that recover will rarely return to the natural condition experienced before eutrophication occured and there is usually greater recovery success in bays and estuaries than open coasts and marine habitats (Elliott et al. 2007; Duarte et al. 2009). In cases where seagrasses have disappeared from a catchment or subcatctchment and not returned after reduction of nutrient stressors it may be desirable to facilitate their reintroduction. Attempts have been made to restore seagrass, particularly *Zostera* species with varying success using both manual transplanting (Fonseca et al. 1996) and seeding (Orth et al. 2003), although these can be costly and labour intensive (Fonseca et al. 2000; Lee and Park 2008).

Planning

Careful planning of future development in catchments is required to prevent or reduce eutrophication of estuaries. Areas in estuaries with high ecological value containing

large areas of important habitats like saltmarsh, mangroves, and seagrass should be identified, particularly where receiving waters have low levels of flushing and high residence times and are therefore more likely to undergo eutrophic conditions. These areas should be afforded higher levels of protection from development and stricter guidelines for the reduction of nutrient run-off through land use practices. Buffer zones of riparian vegetation and wetlands around rivers, streams and estuaries should also be maintained to act as a sink for nutrient retention and reduce enrichment of waterways (Boesch 2002). Given the difficulty of separating the top-down and bottom-up processes in seagrass reduction, the careful management of fisheries exploitation from coastal estuaries should be considered when selecting tools to counter eutrophication (Heck and Valentine 2007). A holistic approach to catchment management is desirable to reduce the effects of nutrient enrichment by tackling both increased nutrient loads and over-fishing simultaneously.

FUTURE RESEARCH

This study has provided important knowledge regarding the interactions between trophic levels in seagrass beds of south-eastern Australia and at the same time has highlighted gaps in current knowledge that would benefit from future targeted research. It is apparent that nutrient enrichment, even at the moderate levels experienced in this study, has an impact on the trophic dynamics of seagrass ecosystems.

Recent shifts in the consensus view that nutrients are the major cause of seagrass decline in anthropogenic disturbed catchments challenge this thinking by promoting the idea that declines in top predators from fisheries exploitation are actually driving these changes (Heck and Valentine 2007). Information on the impact of piscivorous fish and other top predators such as birds on the structuring of lower trophic levels is deficient and should be a priority of future studies. With independent data for abundances of large fish difficult to obtain (see Chapter 2), fisheries dependent data as Catch Per Unit Effort (CPUE) may be a useful tool for exploring this relationship further. Future studies are required that investigate the relative impacts of nutrients and fishing on seagrass systems independently and also the interaction between the two as they often occur simultaneously (Caddy 2000; Breitburg et al. 2009b) and may produce non-linear responses that are greater than the additive effects combined. The recent establishment

of no-take Marine Protected Areas in estuarine systems provides a potential natural experiment where orthogonal designs of these two factors can be tested in the field.

More information is also required for determing nutrient thresholds. Increased nitrogen loading may be beneficial to seagrass growth and secondary production at lower levels of enrichment, but detrimental at higher levels, ultimately leading to regime shifts from seagrass to plankton dominated estuaries (Caddy 2000; Breitburg et al. 2009a). These thresholds, however, are likely to be fairly specific to individual catchments as hydrological and ecological characterstics can differ greatly between estuaries.

Further manipulative studies investigating the role of top-down and bottom-up forces in seagrasses across wide spatial scales are also required. Many of the studies to date have only investigated the interaction between lower trophic levels, and the incorporation of juvenile fish into larger-scale experiments would add more realism to understanding the mechanisms at play at the wider ecosystem level (eg. Heck et al. 2000; Moksnes et al. 2008). As already highlighted in Chapter 6, the use of artificial seagrass units in small scale and short term studies is recommended to reduce artefacts of caging and enclosure or mesocosms when assessing the health of the seagrass itself is not an objective of the study.

Studies looking at the growth, condition and survival of juvenile fish under different nutrient regimes in mesocosm and field enclosures are required to better understand the effects of nutrients on secondary production. Finally, both acute and chronic toxicity testing of important ecological (e.g. *A. jacksoniensis* and *R. macrostoma*), and economic (e.g. *P. sexlineatus*, *A. australis* and *R. sarba*) species would give a better understanding of the sensitivities of these species to the direct effects of anthropogenic pollutants, including nitrate and ammonia.

CONCLUSION

Overall, nutrient enrichment of coastal waters in south-eastern Australia is a potential threat to the health of seagrass systems. Enrichment produced greater epiphyte biomass on seagrass leaves that can reduce photosynthetic capacity of seagrasses. Notwithstanding this, the low to moderate levels of nutrient levels encountered in this

study and typical in Australian waters had not resulted in seagrass declines in catchments that had been urbanised and deforested for decades. The dynamic tidal energy in the study estuaries, relative to Intermittently Closing and Opening Lakes and Lagoons (ICOLLs) that are common features of the south-eastern Australian coast, doubtless reduced the vulnerability of seagrass to eutrophication.

Increased epiphyte growth in enriched sites promoted secondary production in primary consumers (grazing invertebrates). Trophic transfer to higher order consumers, however, was attenuated at the third trophic level (juvenile fish). This negated any benefit to fisheries production that may have eventuated from bottom-up forcing.

Fish assemblages in seagrass beds differed between developed and undeveloped catchments. These differences where characterised by higher abundances in undeveloped catchments driven by pelagic carnivores and also a higher proportion of omnivorous juvenile fish at these low nutrient sites. Juvenile fish were found to have reduced survival and condition in nutrient enriched sites. Whether these differences were caused by trophic interactions or other factors such as sensitivity to pollutants remains to be determined.

Given the potential for nutrient enrichment to alter trophic structure in coastal waters and to have negative impacts on seagrass health it is recommended that an integrated system of management be implemented. This should include further targeted research for a better understanding of seagrass trophic systems, monitoring of environmental indicators that alert catchment managers to early signs of eutrophication, remediation of existing eutrophic systems through nutrient reduction mechanisms and planning policies that identify and afford greater protection to ecologically-important habitats such as seagrass in estuaries with high residence times that are more vulnerable to eutrophication.

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APPENDICES

APPENDIX I: METHODS FOR PILOT STUDY ON SAMPLING PISCIVOROUS FISH IN SHALLOW SEAGRASS BEDS

The study was carried out on four separate days in spring 2006 at Smiths Creek (33°38'49"S, 151°12'38"E) in the Hawkesbury River, 30 km north of Sydney (Figure 2.1) over a period of approximately four hours centred on morning high tides. This location has a continuous seagrass bed (approx 8000 m^2) that allowed for the independent deployment of at least four replicates of each sampling method per sampling day. Although time of day/night can influence fish assemblages sampled, all methods were carried out during daylight hours because the strong light was required for visual methods to be effective.

Fish Traps

Fish traps were designed in a semi-cylindrical shape. The base of each trap was 900 x 500 mm with a dome height of 350 mm. The body of the traps was constructed from 50 x 750 mm wire mesh. Entrances at both ends were constructed from 50 x 50 mm flexible plastic mesh protruding and tapering inwards to an opening of 150 mm x 100 mm.

Each trap had a bait cage (150 x 150 x 70 mm) attached at the centre of the base and constructed of 20 mm plastic mesh. Each trap was baited before deployment with a combination of a 400 g sachet of Katchem pilchards in pure fish oil and additional Western Australian pilchards (*Sardinops neopilchardus*) to make a total of 1 kg.

On each of the four days, four traps were deployed haphazardly within the seagrass bed for a soak time of 0.5 h. Fish caught in traps were identified and measured (TL) and then released. Used bait was then discarded and replaced and the traps were redeployed in a different position for another 0.5 h giving a total of eight replicates per day.

Line Fishing

Line fishing was conducted using a 1.8 m fibreglass Jarvis Walker spinning rod (line class 3-4 kg, cast weight 5-20 g) and Eagle Claw spinning reel (gear ratio 5.1:1) and

monofilament fishing line (breaking strain 2.7 kg). Each rod and reel combination was equipped with a general running rig consisting of a bean sinker (size 1) above a barrel swivel (size 6) with a 40 cm trace of monofilament line (breaking strain 2.7 kg) and a 1/0 claw suicide hook. Frozen Hawkesbury river prawns were used as bait. Four separate fishers each with their own rod/reel combination fished for a period of 1.5 hours each from an anchored 6 m aluminium boat above the seagrass bed. Lines were cast haphazardly between 5 and 20 m from the boat and all fish caught by each fisher were identified, measured to the nearest centimetre and then released.

Gill Netting

Three multi-mesh gill nets were constructed of monofilament netting. Nets were 15 m in length and constructed from 5 m panels of three separate mesh sizes with stretched openings of 50, 70 and 90 mm. These mesh sizes were selected in order to catch the range of predatory estuary fish known to inhabit seagrass beds and regional estuaries (Gray et al. 1990; Gray et al. 2005; Rotherham et al. 2006). Each gill net was constructed differently so that each combination of panel orders was utilised. All nets were attached to head-ropes with plastic floats and foot-ropes with lead weights with a stretch depth of 2 m.

On each of the four sampling days the three gill nets were haphazardly placed on the bottom of the seagrass beds perpendicular to the current. After 1 h soak time nets were retrieved and fish were identified, counted according to mesh size, and measured to the nearest 1 cm (TL).

Baited Remote Underwater Video Stations (BRUVs)

Baited remote underwater videos stations (BRUVs) were used to estimate the size and relative abundance of fishes in seagrass beds. BRUVs consisted of a Sony Mini-DV HandiCam (model DCR HC48E) placed in a PVC underwater housing with a perspex front and mounted on a galvanised, trestle shaped frame. A removable plastic arm (20 mm plastic conduit) with a 1 L plastic mesh canister containing a mixture of 1 kg of felafel mix and 500 ml of tuna oil was attached in the direction of the camera's viewing area. Attached to each BRUV were 8 mm polypropylene ropes and polystyrene marker boys for hand retrieval of BRUVs into the boat (see Cappo et al. 2007). A graduated

ruler of 10 cm length was placed at the end of the bait arm perpendicular to the camera lens for estimating fish lengths.

Four replicate single-camera baited underwater videos (BRUVs) were deployed on four days with 30 minutes sampling windows each. Analysis commenced when the camera settled on the bottom of the seagrass bed and continued for 30 mins. For each species the maximum number of species seen together in one frame of the tape (MaxN) and the time at which MaxN occurred were recorded from reviewing the video tape (see (Cappo et al. 2004). Coarse measurements of total lengths of different species were made if fish could be viewed perpendicular and immediately next to the graduated ruler. Harvey et al. (2002) demonstrated that these estimates can be severely compromised if the fish is not viewed at a right angle, in the middle of the field of view or in front of or behind the scale reference.

Underwater Visual Census (UVC)

A pilot study of Underwater Visual Census techniques was conducted to ascertain the most effective method for sampling large fishes in shallow seagrass beds. Six methods in all were assessed (pre-marked transects under snorkel and from a canoe, unmarked roving transects with GPS tracking under snorkel and from a canoe and point counts and area searches under snorkel). Marked transects were discarded as they were more labour intensive and installation caused fish to leave the study area; point counts were ineffective due to low visibility even in comparatively clear water (approx 2m). This also reduced the effectiveness of area searches leading to recounts or unsurveyed areas due to difficulty in knowing exact location. A more extensive trial comparing canoe and snorkel roving transects with GPS tracking was carried out. Surveys under snorkel were considered more efficient because wind disturbance on the water's surface reduced visibility for canoe surveys.

For the present study, fish abundances were recorded on slates along an unmarked underwater visual transect under snorkel with a hand held GPS unit floated behind the observer. At the beginning of each census the observer sighted a reference point in the distance in the direction of travel, logged a GPS waypoint, activated a stopwatch and began swimming, recording the number and size of all fish species greater than 80 mm in

length. The observer looked up at the reference point every 15 seconds to remain swimming in a straight line. Before the study, swimming speed was calculated by the number of fin-beats per minute that allowed for a distance of 50 m to be covered in three minutes. One observer was used for all UVC surveys to reduce biases in the identification and estimation of length of fishes, as well as swimming speed (Lincoln-Smith 1988). After three minutes the observer stopped and recorded a finishing waypoint on the GPS unit.

APPENDIX II: Measures of abundance and species richness of piscivores and other guilds of fish for the five different sampling methods used to determine assemblage diversity (a & b indicate significantly different groupings SNK test $P < 0.05$)

Species	Common name	**BRUV (video) n=16**	**UVC (visual) n=32**	**Line fishing n=16**	**Gill nets n=12**	**Baited traps n=16**
PISCIVOROUS FISH						
Tylosurus gavialoides	Stout longtom	-	1	-	-	-
Acanthopagrus australis	Yellow-fin bream	30	51	1	-	-
Dinolestes lewini	Longfin pike	1	-	-	-	-
Platycephalus laevigatus	Grass flathead	-	-	-	1	-
Predator abundance	**Total**	**31**	**52**	**1**	**1**	**0**
Predator abundance	Mean	1.688_{a}	1.625_{a}	0.083_{b}	0.083_{b}	0.000
	Standard error	0.237	0.320	0.083	0.083	0.000
Predator richness	**Total**	**2**	**2**	**1**	**1**	**0**
	Mean	1.000_{a}	0.594_{a}	0.083_{b}	0.083_{b}	0.000
	Standard error	0.091	0.088	0.083	0.083	0.000
OTHER SPECIES						
Dasyatis thetidis	Black stingray	-	3	-	-	-
Neoodax balteatus	Little rock whiting	1	1	-	-	-
Silago ciliata	Sand whiting	-	1	-	-	-
Pelates sexlineatus	Six-lined trumpeter	96	76	4	-	-
Gerres subfasciatus	Silver biddy	2	2	-	-	-
Girella tricuspidata	Luderick	1	166	-	-	-
Liza argentea	Flat-tail mullet	1	40	-	3	-
Rhabdosargus sarba	Tarwhine	9	2	1	-	-
Monacanthus. chinensis	Fan-belly leatherjacket	2	-	-	-	-
Meuschenia freycineti	Six-spined leatherjacket	-	1	-	-	-
Meuschenia trachylepis	Yellow-finned leatherjacket	9	-	-	-	-
Acanthaluteres spilomelanurus	Bridled leatherjacket	3	1	-	-	-
Total abundance	**Total**	**155**	**345**	**6**	**4**	**0**
	Mean	9.688_{a}	10.688_{a}	0.375_{b}	0.417_{b}	0.000
	Standard error	0.943	1.7189	0.155	0.336	0.000
Species richness	**Total**	**9**	**12**	**3**	**2**	**0**
	Mean	3.438_{a}	2.188_{a}	0.333_{b}	0.167_{b}	0.000
	Standard error	0.341	0.1875	0.142	0.112	0.000

APPENDIX III: SUMMARY OF THE COSTS, BENEFITS AND LIMITATIONS OF THE FIVE SAMPLING METHODS EMPLOYED DURING THE STUDY.

	Observation Methods		*Capture Methods*		
	BRUVs	**UVC**	**Line fishing**	**Gill netting**	**Baited traps**
Description of Method	▪ Static sampling. Camera is stationary and fish are attracted by bait	▪ Dynamic sampling with observer swimming along transect	▪ Either static (stationary lines) or dynamic (moving lures or bait)	▪ Static sampling method. Nets are set for a nominated time period	▪ Static sampling method. Traps are baited and set for a nominated time period
Attributes Measured	▪ Comparative measure of abundance (Max N) but cannot determine density ▪ Poor estimations of fish size classes	▪ Quantitative measure of abundance and density ▪ Estimation of fish size classes (varies with observer experience)	▪ Comparative measure of abundance (CPUE) ▪ Direct measure of fish length and weight)	▪ Comparative measure of abundance (CPUE) ▪ Direct measure of fish length and weight	▪ Comparative measure of abundance (CPUE) ▪ Direct measure of fish length and weight
Benefits	▪ Non destructive to fish and habitat ▪ Provides a permanent record for identification ▪ Safer for operators not in water	▪ Non destructive to fish and habitat ▪ Minimal expense ▪ Time efficient (All data collected in the field)	▪ Relatively cheap capital outlay ▪ Allows greater certainty for identifying of fish ▪ Can target piscivorous fishes ▪ Effective in turbid waters	▪ Relatively cheap capital outlay ▪ Non destructive to habitat ▪ Easily set and retrieved ▪ Allows greater certainty for identifying of fish ▪ Effective in turbid waters	▪ Relatively cheap capital outlay ▪ Allows greater certainty for identifying of fish ▪ Non destructive to habitat ▪ Easy to set and retrieve ▪ Effective in turbid waters
Limitations	▪ Selective to species depending on bait used. ▪ Poor estimations of size classes unless stereo video is used ▪ Sampling can only be conducted in daylight in waters with low turbidity ▪ Large initial expense on equipment and software ▪ Additional time watching videos in lab	▪ Observer bias depending on expertise ▪ May overestimate diver friendly species ▪ May underestimate diver averse and cryptic species ▪ Sampling can only be conducted in daylight in waters with low turbidity ▪ Extra divers needed for SCUBA	▪ Selective to species depending on bait used. ▪ Slightly destructive to fish due to capture mortality ▪ Potentially time and labour intensive to collect substantial data	▪ Potentially destructive to fish (high mortality of non-target species) ▪ Difficult to obtain permits from Fisheries Departments, Marine Parks and animal ethics committees ▪ Are more efficient for night time sampling	▪ Selective to species depending on bait used, size of mesh and soak time ▪ Slightly destructive to fish due to capture mortality

APPENDIX IV: EXPERIMENTAL DESIGN FOR 3 FACTOR ANOVA

Factor 1 is Nutrient status has 2 levels is orthogonal and fixed
Factor 2 is Site has 4 levels is nested in Nutrient status and random
Factor 3 is Time has 3 levels is orthogonal and random
Number of replicates: 4

The model for this analysis is:
X = MEAN + Nu + Si(Nu) + Ti + NuXTi + TiXSi(Nu) + RES

Source	DF	Terms included in EMS	F versus
Nutrient status	1	RES + Si(Nu)XTi + NuXTi + Si(Nu) + Nu	F"
Site(Nu)	8	RES + Si(Nu)XTi + Si(Nu)	TiXSi(Nu)
Time	2	RES + Si(Nu)XTi + Ti	TiXSi(Nu)
Nu X Ti	2	RES + Si(Nu)XTi + NuXTi	TiXSi(Nu)
Ti X Si(Nu)	12	RES + Si(Nu)XTi	RES
RESIDUAL (RES)	72	RES	
TOTAL	95		

Denominator calculated for F" is:
[Nu + TiXSi(Nu)] / [Si(Nu) + NuXTi]

* Degrees of Freedom recalculated for F" are:
Numerator = $[Nu + TiXSi(Nu)]^2 / [[Nu/df_{Nu}] + [TiXSi(Nu) / df_{TiXSi(Nu)}]]$
Denominator = $[Si(Nu) + NuXTi]^2 / [[Si(Nu) / [df_{Si(Nu)}] + [Nu X Ti / df_{NuXTi}]]$