

## **1 General introduction**

### **1.1 Estuarine ecosystems and their value**

Estuaries represent an important ecological link between freshwater, terrestrial and marine habitats. Due to their shallow water and the availability of nutrients, they are highly productive environments and provide numerous ecosystem services such as nutrient recycling and recreational activities. Furthermore, numerous commercially valuable species use estuaries as nursery and feeding habitats (Beck et al., 2001). For these reasons estuaries and associated environments are among the most economically valuable ecosystems in the world, providing over US\$19 trillion of services (e.g. nutrient cycling, production of food and raw materials) every year (Costanza et al., 1997).

Estuaries harbor a great variety of benthic habitats, including unvegetated sandy and muddy sediments, rocky reefs, boulder fields, as well as saltmarshes, seagrass beds and mangrove forests. These habitats support dense and sometimes diverse animal and plant communities. Among estuarine habitats, seagrass beds are one of the most important due to their productivity and the biodiversity they sustain (Hemminga and Duarte, 2000; Almeida et al., 2008). The presence of seagrass structure increases sediment stability (De Falco et al., 2000; Gacia and Duarte, 2001), provides shelter for invertebrate (Battley et al., 2011) and fish species (Beck et al., 2001), and substrate for epiphytes and epifaunal assemblages (Boström and Bonsdorff, 1997; Bologna and Heck, 1999). In addition, seagrass beds provide food resources for herbivores and detritivores that, in turn, are consumed by species at higher trophic levels (Heck et al., 2008). Sand and mud flats are another important habitat where remineralisation of allochthonous and autochthonous detritus and nutrients sustain high rates of both autotrophic and heterotrophic productivity (Hammond et al., 1985). Soft sediment fauna obtain organic matter from detritus and microalgae and pass it on to secondary consumers, and maintain favorable sediment conditions for microorganisms through

bioturbation and biostabilisation (Mermillod-Blondin and Rosenberg, 2006; Nizzoli et al., 2007).

Numerous human activities are, nevertheless, modifying the biodiversity and functioning of estuarine ecosystems (Edgar et al., 2000; Kennish, 2002). On a planetary scale, global climate change (GCC), the ecological effects of which are already apparent in several regions of the world (Parry et al., 2007), is likely to alter the responses of biological systems to local stressors and, possibly, interact synergistically with coastal development (Harley et al., 2006; Mantyka-Pringle et al., 2012). On a local scale, activities deriving from coastal development and the use of marine resources are having major consequences on the estuarine biota. Severe ecological impacts have already been documented where coastal development has modified the physical habitat (Bulleri and Chapman, 2010) or environmental factors such as the availability of nutrient (Cloern, 2001; Conley et al., 2007).

## **1.2 Predicting the impacts of global climate change on estuaries**

### *1.2.1 Potential impacts*

The recent increase in the emission of greenhouse gasses is causing rapid changes in the world's climate. GCC is predicted to severely alter the physico-chemical environment of estuaries (Scavia et al., 2002; Najjar et al., 2010). In addition to warming of sea and air temperatures, major ramifications of GCC include sea level rise and seawater acidification, a direct consequence of increased carbon dioxide concentrations in the atmosphere.

Recent climate warming has already been associated with changes in the biogeochemical cycles of temperate estuaries (Fulweiler and Nixon, 2009) and migration toward the poles of estuarine fish (Nicolas et al., 2011). Sea level rise can increase saltwater intrusion within estuaries and, where artificial structures impede the landward migration of the coastline, can reduce sedimentary intertidal habitat with negative consequences for benthic communities (Fujii and Raffaelli, 2008). Ocean acidification, resulting from

anthropogenic CO<sub>2</sub> emissions, can alter the calcification rates of estuarine organisms such as mussels and oysters with consequent impacts on their populations (Gazeau et al., 2007; Parker et al., 2011). Ocean acidification, however, may be less relevant for estuarine fauna because they are generally adapted to the highly variable pH that characterise these systems (Amaral et al., 2011).

Despite the increasing number of studies on the potential biological effects of climate change, there is still a great deal of uncertainty as to which stressors and what mechanisms will produce the greatest impacts, especially at the estuary and regional spatial scales (Gillanders et al., 2011; Wernberg et al., 2012). Most of the studies dealing with GCC have made use of natural gradients or laboratory experiments to gain knowledge about the responses of biological systems (from species to ecosystems) to GCC (Wernberg et al., 2012). The inherent natural variability of natural systems and the effects of concomitant local changes represent the main challenges to realistic assessments of GCC effects (Sanderson et al., 2002; Halpern et al., 2008).

At the estuary or within estuary scale, urbanisation is increasing the availability of nutrients and substituting natural features of the coastline with artificial, hard structures. It is therefore of particular importance to understand the extent to which nutrient enrichment and the artificial hardening of the coastline are affecting the potential of estuaries to adapt to GCC and continue to provide essential services to human and natural populations.

### *1.2.2 Investigation methods*

Most studies have investigated the effects of climate change by means of *ex situ* experiments run in small aquaria and mesocosms (Wernberg et al., 2012). Despite being a useful tool that, in specific circumstances, can provide valuable insights into GCC (Benton et al., 2007, Van Doorslaer et al., 2010), this type of experiment is unable to recreate the spatio-temporal scales across which GCC is occurring. Testing hypotheses in the controlled

environment of mesocosms also requires the exclusion of random and predictable (e.g. seasonal and yearly) variability of the physico-chemical and biological factors that organisms experience in the natural environment (Wernberg et al., 2012). Moreover, mesocosm experiments can only include a sub-set of species interactions and, consequently, they do not encompass the complexity of real biological systems (e.g. Sanz-Lazaro et al., 2011). As a consequence, the results obtained from mesocosm experiments conducted in highly controlled realms can produce different conclusions to those conducted in more ecologically realistic conditions (Skelly, 2002). Mesocosm studies, however, can be very useful in combination with field experiments when a particular set of variables of interest cannot be manipulated in the field.

Field experiments using natural gradients or localized disturbance regimes that recreate climate change scenarios have previously been employed to forecast the potential impacts of GCC (Schiel et al., 2004). Natural CO<sub>2</sub> springs, for instance, have been extensively used to assess the long-term evolutionary responses of terrestrial plants to increased atmospheric CO<sub>2</sub> concentrations (Onoda et al., 2009; Nakamura et al., 2011). In the marine environment, seawater acidification caused by CO<sub>2</sub> vents has also been used to assess the physiological boundaries of calcifying organisms such as corals (Crook et al., 2012) and bryozoans (Rodolfo-Metalpa et al., 2010).

Another approach to investigate the effects of GCC encompasses the opportunistic use of latitudinal gradients to make predictions about future shifts in species distributions in response to warming (Andrew and Hughes, 2004) and to test interactive effects between temperature and additional, and potentially synergistic stressors (Dunne et al., 2004). Latitudinal gradients have been extensively utilised in terrestrial studies and have been successfully employed in marine research in recent years (e.g. Engle and Summers, 1999). Moreover, the gradient in mean annual temperature associated with latitudinal gradients becomes particularly useful to recreate GCC settings in the field, where interactive effects of

local stressors can be tested in factorial experiments (Menge et al., 2002; Dunne et al., 2004). The planning and interpretation of experiments conducted along latitudinal gradients need, however, to take into account possible confounding factors such as covariance between temperature and other relevant environmental factors including day-length and nutrient availability (Fielding et al., 1999; Oleksyn et al., 2003; Andrew and Hughes, 2007).

### **1.3 Impacts of coastal development on estuarine ecosystems**

Due to the combination of freshwater availability, port facilities and fisheries resources, estuaries and coastal areas have historically been the focus of human settlement and urban centres (Timmerman and White, 1997). About 41 % of the world's population can be found within 100 km from the coast and, within this strip of land, 28 % of the area has already been urbanised or converted to agriculture (Martinez et al., 2007). In some areas of Europe, for example, it is expected that by the year 2025, 75 % of the population will reside within the coastal region (EEA, 2006). Many activities deriving from urbanization negatively affect the ecology of associated estuarine environments (Kennish, 2002; Lotze et al., 2006). Coastal armouring, such as seawalls and bulkheads that protect areas of human settlement from inundation and storm events, can change the natural features of coastal habitats and modify the geophysical properties of the coast (Bulleri and Chapman, 2010). Nutrient, organic and inorganic pollution lead to eutrophication and ecotoxicological problems (Cloern et al., 2001, Bryan and Langston, 1992). Land clearing and changes in the use of river and estuary watershed areas modify the quantity and quality of exogenous material that these systems receive (Eberbach, 2003).

The ecological impacts of human activities on the world's estuarine ecosystems are well documented (Lotze et al., 2006; Airoldi and Beck, 2007). Artificial structures along the coast line can cause habitat loss and changes to the identity and biodiversity of associated benthic and pelagic organisms (Fletcher, 1997; Fujii, 2007; Sobocinski et al., 2010; Chapman

and Underwood, 2011), with important ramification for secondary consumers such as fish (Moreira et al., 2006; Clynick et al., 2008). In estuarine environments, nutrients (primarily nitrogen and phosphorus) are often limiting to primary productivity and, therefore, their increased availability boosts the growth of primary producers, especially of fast growing species, such as phytoplankton and macroalgae (Herbert, 1999). The resultant excess in biomass decomposing on the sediment surface or in the water column can create hypoxic (defined as a level of dissolved oxygen  $< 2$  mg/l) and anoxic conditions (in extreme circumstances), which can have a deleterious impacts on the fauna and, ultimately disrupt the balance between the production and the decomposition of organic matter (Cloern, 2001; Davis and Koop, 2006; Conley et al., 2007).

For these reasons, coastal armouring and artificial fertilisation of coastal areas are regarded as most serious threats for the integrity of estuarine ecosystems (Cloern, 2001; McLusky and Elliott, 2004; Lotze et al., 2006). In general, however, the impacts of these stressors depend on the physical and biological context to the impacted estuary. Seawalls, for instance, can have particularly detrimental impacts on epifaunal communities where they replace rocky shores (Moreira et al., 2006; Bulleri and Chapman, 2010), or facilitate species invasion (Marzinelli et al., 2012), whereas their impacts on mudflat habitats are typically indirect and result from a reduction of intertidal area (Fujii and Raffaelli, 2008). Impacts of nutrient enrichment depend, on the other hand, on the physical features of the receiving basin such as flushing time, sedimentology and primary consumer assemblages (Cloern et al., 2001).

#### **1.4 Climate change adaptation of coastal ecosystems: preparing estuaries for future climate**

Despite our global efforts to significantly reduce greenhouse gas emissions (Hope, 2009), the growth rate in emissions is still positive (Raupach et al., 2007). As a result, continued GCC is inevitable. In order to preserve the ecological value and the functionality of

coastal systems in the face of GCC, management strategies need to be put in place in order to maximise their adaptive potential. It is therefore of key importance to understand how human activities that induce local stresses interact and influence the resilience of estuarine ecosystems to climate change (Scheffer et al., 2001).

The interaction between multiple stressors can be cumulative, synergistic or antagonistic (Folt et al., 1999). Because multiple stressors affect 41 % of marine areas (Halpern et al., 2008), it is of utmost importance to understand how natural systems respond to interacting drivers of change (Crain et al., 2008). The identification of those sets of stressors where synergistic interactions are more likely to be observed, will provide managers with useful indications on how to expend limited resources to best sustain and conserve estuarine ecosystems (Mantyka-Pringle et al., 2012). Moreover, a multi-stressor approach will provide useful information on ecosystem resilience, intended as the capacity of a system to withstand disturbance and return to a stable state without changes in processes and structures (Gunderson, 2000), and how resilience can be preserved or increased (Mantyka-Pringle et al., 2012).

In order to maximize the resilience of coastal systems to GCC, it is important to identify the consequences of human activities on as long (possibly 10s of years) a temporal scale as possible and to understand how they can be modified to accommodate future environmental changes (Morecroft et al., 2012). In the UK, for example, sea defenses have been removed or translocated further inland to allow the coastline to adapt to future increase in the sea level (Dixon et al., 2008). There are also strong theoretical bases suggesting that warming may increase the probability of benthic hypoxic events through increase of stratification and consequent oxygen diffusion to bottom water (Rabalais et al., 2009). This may necessitate more stringent nutrient pollution caps. Further research is needed to test hypotheses about effects of warming on eutrophication and to provide guidelines on nutrient discharge. Developing resilience to climate change thus requires specific knowledge of the

variability of environmental (e.g. hydrology, geomorphology) and biotic factors (e.g. plant, benthic and planktonic communities, trophic interactions) at the bioregion scale (Gillanders et al., 2011).

## **1.5 Interacting effects of coastal development and climate change on southeast**

### **Australian estuaries**

South-eastern Australian estuaries may differ in their response to perturbation as compared to similarly impacted estuaries in Europe and North America, because they are naturally oligotrophic and are of low and highly variable freshwater input. Australian soils are poor in nutrients, and the eastern coastline is not subject to significant upwelling, resulting in nutrient-limited estuaries (Harris, 2001; Scanes et al., 2007). In addition, the low rainfall and low relief that characterize the geography of Australia result in a low and irregular input of freshwater (Harris, 2001; Power et al., 1999). Consequently, it is difficult to apply concepts and extrapolate information from European or North American studies to understand the functioning of Australian estuaries (Davis and Koop, 2006; Young et al., 1996).

Along the southeast (SE) coast of Australia, the overall anthropogenic impact on estuaries is still relatively low compared to other areas of the world. With some exceptions, the fraction of nutrient loading deriving from human activities is still on the lower range compared to estuaries elsewhere, such as in the United States (Scanes et al., 2007). Coastal armouring is, however, a relatively common feature of Australian shorelines, especially near the major cities. In fact, in some estuaries of southeast Australia, artificial structures built in intertidal areas cover up to 45 % of the coastline (Creese et al., 2009).

Despite the fact that disturbance pressure on estuaries from coastal development is likely to grow in the near future, relatively little research has been done on the consequences of an increase in anthropogenic disturbance on Australian estuaries (Davis and Koop, 2006). With the predicted average temperature increase of 1.8 - 4.0 °C (IPCC, 2007) and sea level



rise between 0.48 and 0.94 to occur in Australia over the next century (Horton et al., 2008), it is likely that communities, trophic interactions and functions of estuarine ecosystems will change. Furthermore, the lack of long-term datasets on environmental and biological variables makes predicting potential GCC impacts a difficult endeavour (Gillanders et al., 2011). Most studies have focused on impacts deriving from the establishment of local disturbance, including the effects of artificial structures on epibiota and fish assemblages (e.g. Moreira et al., 2006; Clynick et al., 2008; Chapman and Underwood, 2011; Marzinelli et al., 2012) and point source nutrient inputs on benthic and pelagic food webs (Bishop et al., 2006; York et al., 2012). Despite a general theoretical background (e.g. Gillanders et al., 2011), little is known, to date, on the combined effects of climate change and local stressors on southeast Australian estuaries and there is a paucity of empirical investigations on large spatio-temporal scales that can provide a solid basis for informed management strategies.

## **1.6 This thesis**

Habitat modification and nutrient pollution represent the more important local drivers of change for estuarine ecosystems (Lotze et al., 2006) and the importance of studying their effects is strengthened by ongoing global changes (Rabalais et al., 2009; Wernberg et al., 2012). By using existing gradients in key environmental variables as proxy for future changes, I investigated the relative importance of local and global drivers in influencing the spatial variability of species and processes occurring in seagrass meadows and unvegetated soft sediment habitats.

The first aim of this thesis was to examine some of the potential consequences arising from the combination of coastal armouring and future sea level rise on intertidal and shallow subtidal seagrass habitats. I made use of natural gradients in intertidal elevation to assess how a reduction in intertidal area caused by sea level rise in urbanized estuaries might affect 1) seagrass morphology and decomposition and 2) habitat provision for invertebrates.

The second aim was to examine the effects of recent increases in the nitrogen delivery to SE Australian estuaries, and how climatic warming might exacerbate effects on benthic community assemblages and trophic relations. GCC has the potential to modify the spatial distribution of species, and increase the risk of hypoxia. Moreover, it can modify the nutritional characteristics of primary producers and cause changes in the way they are assimilated by consumers. I assessed how warming can exacerbate the effects of nutrient enrichment on soft sediment habitats by using the latitudinal gradient as proxy for warming, and available data on the nutrient pollution levels of SE Australian estuaries.

Specifically, I addressed the following broad hypotheses:

Chapter 2) Tidal elevation affects the decomposition rate of the seagrass *Zostera muelleri* directly, by determining the decomposition environment, and indirectly through driving changes in the leaf traits.

Chapter 3) Tidal elevation affects the spatial distribution of macroinvertebrates living in *Zostera muelleri* meadows by increasing air exposure-related stresses and, indirectly, by modifying the complexity of seagrass below-ground structures.

Chapter 4) Climate, environmental context and recent increases in the availability of nutrients affect the among-estuary spatial variability of soft sediment macroinvertebrates.

Chapter 5) Climatic context and recent increases in the availability of nutrients influence the quality of primary resources and their assimilation by two important benthic consumers, the gastropod *Pyrazus ebeninus* and the polychaete *Nephtys australiensis*.

## 1.7 References

- Airolidi, L., Beck, M.W., 2007. Loss, status and trends for coastal marine habitats of Europe, in: Gibson, R.N., Atkinson, R.J.A., Gordon, J.D.M. (Eds.), *Oceanography and Marine Biology*, Vol 45. Crc Press-Taylor & Francis Group, Boca Raton, pp. 345-405.
- Almeida, C., Coelho, R., Silva, M., Bentes, L., Monteiro, P., Ribeiro, J., Erzini, K., Gonçalves, J.M.S., 2008. Use of different intertidal habitats by faunal communities in a temperate coastal lagoon. *Estuarine, Coastal and Shelf Science* 80, 357-364.
- Amaral, V., Cabral, H.N., Bishop, M.J., 2011. Resistance among wild invertebrate populations to recurrent estuarine acidification. *Estuarine, Coastal and Shelf Science* 93, 460-467.
- Andrew, N.R., Hughes, L., 2004. Species diversity and structure of phytophagous beetle assemblages along a latitudinal gradient: predicting the potential impacts of climate change. *Ecological Entomology* 29, 527-542.
- Andrew, N.R., Hughes, L., 2007. Potential host colonization by insect herbivores in a warmer climate: a transplant experiment. *Global Change Biology* 13, 1539-1549.
- Battley, P.F., Melville, D.S., Schuckard, R., Ballance, P.F., 2011. *Zostera muelleri* as a structuring agent of benthic communities in a large intertidal sandflat in New Zealand. *Journal of Sea Research* 65, 19-27.
- Beck, M.W., Heck, K.L., Able, K.W., Childers, D.L., Eggleston, D.B., Gillanders, B.M., Halpern, B., Hays, C.G., Hoshino, K., Minello, T.J., Orth, R.J., Sheridan, P.F., Weinstein,

M.R., 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *Bioscience* 51, 633-641.

Benton, T.G., Solan, M., Travis, J.M.J., Sait, S.M., 2007. Microcosm experiments can inform global ecological problems. *Trends in Ecology and Evolution* 22, 516-521.

Bishop, M.J., Kelaher, B.P., Smith, M.P.L., York, P.H., Booth, D.J., 2006. Ratio-dependent response of a temperate Australian estuarine system to sustained nitrogen loading. *Oecologia* 149, 701-708.

Bologna, P.A.X., Heck, K.L., 1999. Macrofaunal associations with seagrass epiphytes - Relative importance of trophic and structural characteristics. *Journal of Experimental Marine Biology and Ecology* 242, 21-39.

Boström, C., Bonsdorff, E., 1997. Community structure and spatial variation of benthic invertebrates associated with *Zostera marina* (L) beds in the northern Baltic Sea. *Journal of Sea Research* 37, 153-166.

Bryan, G.W., Langston, W.J., 1992. Bioavailability, accumulation and effects of heavy-metals in sediments with special reference to united Kingdom estuaries – a review. *Environmental Pollution* 76, 89-131.

Bulleri, F., Chapman, M.G., 2010. The introduction of coastal infrastructure as a driver of change in marine environments. *Journal of Applied Ecology* 47, 26-35.

Chapman, M.G., Underwood, A.J., 2011. Evaluation of ecological engineering of "armoured" shorelines to improve their value as habitat. *Journal of Experimental Marine Biology and Ecology* 400, 302-313.

Cloern, J.E., 2001. Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology-Progress Series* 210, 223-253.

Clynick, B.G., Chapman, M.G., Underwood, A.J., 2008. Fish assemblages associated with urban structures and natural reefs in Sydney, Australia. *Austral Ecology* 33, 140-150.

Conley, D.J., Carstensen, J., Aertebjerg, G., Christensen, P.B., Dalsgaard, T., Hansen, J.L.S., Josefson, A.B., 2007. Long-term changes and impacts of hypoxia in Danish coastal waters. *Ecological Applications* 17, 165-184.

Costanza, R., d'Arge, R., deGroot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., Oneill, R.V., Paruelo, J., Raskin, R.G., Sutton, P., vandenBelt, M., 1997. The value of the world's ecosystem services and natural capital. *Nature* 387, 253-260.

Crain, C.M., Kroeker, K., Halpern, B.S., 2008. Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters* 11, 1304-1315.

Creese, R.G., Glasby, T.M., West, G., Gallen, C., 2009. Mapping the habitats of NSW estuaries - Fisheries Final Report Series No. 113. Department of Industry and Investment, NSW, Australia.

- Crook, E.D., Potts, D., Rebolledo-Vieyra, M., Hernandez, L., Paytan, A., 2012. Calcifying coral abundance near low-pH springs: implications for future ocean acidification. *Coral Reefs* 31, 239-245.
- Davis, J.R., Koop, K., 2006. Eutrophication in Australian rivers, reservoirs and estuaries - a southern hemisphere perspective on the science and its implications. *Hydrobiologia* 559, 23-76.
- De Falco, G., Ferrari, S., Cancemi, G., Baroli, M., 2000. Relationship between sediment distribution and *Posidonia oceanica* seagrass. *Geo-Marine Letters* 20, 50-57.
- Dixon, M., Morris, R.K.A., Scott, C.R., Birchenough, A., Colclough, S., 2008. Managed realignment - lessons from Wallasea, UK. *Proceedings of the Institution of Civil Engineer-Maritime Engineering* 161, 61-71.
- Dunne, J.A., Saleska, S.R., Fischer, M.L., Harte, J., 2004. Integrating experimental and gradient methods in ecological climate change research. *Ecology* 85, 904-916.
- Eberbach, P.L., 2003. The eco-hydrology of partly cleared, native ecosystems in southern Australia: a review. *Plant Soil* 257, 357-369.
- Edgar, G.J., Barrett, N.S., Graddon, D.J., Last, P.R., 2000. The conservation significance of estuaries: a classification of Tasmanian estuaries using ecological, physical and demographic attributes as a case study. *Biol. Conserv.* 92, 383-397.

Engle, V.D., Summers, J.K., 1999. Latitudinal gradients in benthic community composition in Western Atlantic estuaries. *Journal of Biogeography* 26, 1007-1023.

EEA, 2006. The changing faces of Europe's coastal areas 6/2006, EEA Report. European Environment agency, Copenhagen, Denmark.

Fielding, C.A., Whittaker, J.B., Butterfield, J.E.L., Coulson, J.C., 1999. Predicting responses to climate change: the effect of altitude and latitude on the phenology of the Spittlebug *Neophilaenus lineatus*. *Functional Ecology* 13, 65-73.

Fletcher, C.H., Mullane, R.A., Richmond, B.M., 1997. Beach Loss along Armored Shorelines on Oahu, Hawaiian Islands. *J. Coast. Res.* 13, 209-215.

Folt, C.L., Chen, C.Y., Moore, M.V., Burnaford, J., 1999. Synergism and antagonism among multiple stressors. *Limnology and Oceanography* 44, 864-877.

Fujii, T., 2007. Spatial patterns of benthic macrofauna in relation to environmental variables in an intertidal habitat in the Humber estuary, UK: Developing a tool for estuarine shoreline management. *Estuarine, Coastal and Shelf Science* 75, 101-119.

Fujii, T., Raffaelli, D., 2008. Sea-level rise, expected environmental changes, and responses of intertidal benthic macrofauna in the Humber estuary, UK. *Marine Ecology Progress Series* 371, 23-35.

Fulweiler, R.W., Nixon, S.W., 2009. Responses of benthic-pelagic coupling to climate change in a temperate estuary. *Hydrobiologia* 629, 147-156.

- Gacia, E., Duarte, C.M., 2001. Sediment retention by a mediterranean *Posidonia oceanica* meadow: The balance between deposition and resuspension. *Estuarine, Coastal and Shelf Science* 52, 505-514.
- Gazeau, F., Quiblier, C., Jansen, J.M., Gattuso, J.P., Middelburg, J.J., Heip, C.H.R., 2007. Impact of elevated CO<sub>2</sub> on shellfish calcification. *Geophysical Research Letters* 34.
- Gillanders, B.M., Elsdon, T.S., Halliday, I.A., Jenkins, G.P., Robins, J.B., Valesini, F.J., 2011. Potential effects of climate change on Australian estuaries and fish utilising estuaries: a review. *Marine and Freshwater Research* 62, 1115-1131.
- Gunderson, L.H., 2000. Ecological resilience - in theory and application. *Annual Review of Ecology and Systematics* 31, 425-439.
- Halpern, B.S., Walbridge, S., Selkoe, K.A., Kappel, C.V., Micheli, F., D'Agrosa, C., Bruno, J.F., Casey, K.S., Ebert, C., Fox, H.E., Fujita, R., Heinemann, D., Lenihan, H.S., Madin, E.M.P., Perry, M.T., Selig, E.R., Spalding, M., Steneck, R., Watson, R., 2008. A global map of human impact on marine ecosystems. *Science* 319, 948-952.
- Hammond, D.E., Fuller, C., Harmon, D., Hartman, B., Korosec, M., Miller, L.G., Rea, R., Warren, S., Berelson, W., Hager, S.W., 1985. Benthic fluxes in San Francisco Bay. *Hydrobiologia* 129, 69-90.



Harley, C.D.G., Hughes, A.R., Hultgren, K.M., Miner, B.G., Sorte, C.J.B., Thornber, C.S., Rodriguez, L.F., Tomanek, L., Williams, S.L., 2006. The impacts of climate change in coastal marine systems. *Ecology Letters* 9, 228-241.

Harris, G. 2001. A Nutrient Dynamics Model for Australian Waterways: Land Use, Catchment Biogeochemistry and Water Quality in Australian Rivers, Lakes and Estuaries. Australia State of the Environment Second Technical Paper Series (Inland Waters), Department of the Environment and Heritage, Canberra.

Heck, K.L., Carruthers, T.J.B., Duarte, C.M., Hughes, A.R., Kendrick, G., Orth, R.J., Williams, S.W., 2008. Trophic Transfers from Seagrass Meadows Subsidize Diverse Marine and Terrestrial Consumers. *Ecosystems* 11, 1198-1210.

Hemminga, M., Duarte, C.M., 2000. *Seagrass Ecology*. Cambridge University Press, Cambridge, UK.

Herbert, R.A., 1999. Nitrogen cycling in coastal marine ecosystems. *FEMS Microbiology Reviews* 23, 563-590.

Hope, C., 2009. How deep should the deep cuts be? Optimal CO<sub>2</sub> emissions over time under uncertainty. *Climate Policy* 9, 3-8.

Horton, R., Herweijer, C., Rosenzweig, C., Liu, J.P., Gornitz, V., Ruane, A.C., 2008. Sea level rise projections for current generation CGCMs based on the semi-empirical method. *Geophysical Research Letters* 35, 1-5.

Kennish, M.J., 2002. Environmental threats and environmental future of estuaries.

Environmental Conservation 29, 78-107

Lotze, H.K., Lenihan, H.S., Bourque, B.J., Bradbury, R.H., Cooke, R.G., Kay, M.C., Kidwell, S.M., Kirby, M.X., Peterson, C.H., Jackson, J.B.C., 2006. Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science* 312, 1806-1809.

Mantyka-Pringle, C.S., Martin, T.G., Rhodes, J.R., 2012. Interactions between climate and habitat loss effects on biodiversity: a systematic review and meta-analysis. *Global Change Biology* 18, 1239-1252.

Martinez, M.L., Intralawan, A., Vazquez, G., Perez-Maqueo, O., Sutton, P., Landgrave, R., 2007. The coasts of our world: Ecological, economic and social importance. *Ecological Economics* 63, 254-272.

Marzinelli, E.M., Underwood, A.J., Coleman, R.A., 2012. Modified habitats change ecological processes affecting a non-indigenous epibiont. *Marine Ecology Progress Series* 446, 119-129.

McLusky, D.S., Elliott, M., 2004. *The estuarine ecosystem: ecology, threats and management*. Third edition. Oxford University Press, UK.

Menge, B.A., Sanford, E., Daley, B.A., Freidenburg, T.L., Hudson, G., Lubchenco, J., 2002. Inter-hemispheric comparison of bottom-up effects on community structure: Insights revealed using the comparative-experimental approach. *Ecological Research* 17, 1-16.

Mermillod-Blondin, F., Rosenberg, R., 2006. Ecosystem engineering: the impact of bioturbation on biogeochemical processes in marine and freshwater benthic habitats. *Aquatic Science* 68, 434-442.

Morecroft, M.D., Crick, H.Q.P., Duffield, S.J., Macgregor, N.A., 2012. Resilience to climate change: translating principles into practice. *Journal of Applied Ecology* 49, 547-551.

Moreira, J., Chapman, M.G., Underwood, A.J., 2006. Seawalls do not sustain viable populations of limpets. *Marine Ecology-Progress Series* 322, 179-188.

Nakamura, I., Onoda, Y., Matsushima, N., Yokoyama, J., Kawata, M., Hikosaka, K., 2011. Phenotypic and genetic differences in a perennial herb across a natural gradient of CO<sub>2</sub> concentration. *Oecologia* 165, 809-818.

Najjar, R.G., Pyke, C.R., Adams, M.B., Breitburg, D., Hershner, C., Kemp, M., Howarth, R., Mulholland, M.R., Paolisso, M., Secor, D., Sellner, K., Wardrop, D., Wood, R., 2010. Potential climate-change impacts on the Chesapeake Bay. *Estuarine, Coastal and Shelf Science* 86, 1-20.

Nicolas, D., Chaalali, A., Drouineau, H., Lobry, J., Uriarte, A., Borja, A., Boet, P., 2011. Impact of global warming on European tidal estuaries: some evidence of northward migration of estuarine fish species. *Regional Environmental Change* 11, 639-649.

Nizzoli, D., Bartoli, M., Cooper, M., Welsh, D.T., Underwood, G.J.C., Viaroli, P., 2007. Implications for oxygen, nutrient fluxes and denitrification rates during the early stage of

sediment colonisation by the polychaete *Nereis* spp. in four estuaries. *Estuarine, Coastal and Shelf Science* 75, 125-134.

Oleksyn, J., Reich, P.B., Zytowskiak, R., Karolewski, P., Tjoelker, M.G., 2003. Nutrient conservation increases with latitude of origin in European *Pinus sylvestris* populations. *Oecologia* 136, 220-235.

Onoda, Y., Hirose, T., Hikosaka, K., 2009. Does leaf photosynthesis adapt to CO<sub>2</sub>-enriched environments? An experiment on plants originating from three natural CO<sub>2</sub> springs. *New Phytologist* 182, 698-709.

Parker, L.M., Ross, P.M., O'Connor, W.A., 2011. Populations of the Sydney rock oyster, *Saccostrea glomerata*, vary in response to ocean acidification. *Marine Biology* 158, 689-697.

Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden, P.J., Hanson, C.E., 2007. Contribution of working group II to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, UK.

Power, S., Tseitkin, F., Mehta, V., Lavery, B., Torok, S., Holbrook, N., 1999. Decadal climate variability in Australia during the twentieth century. *International Journal of Climatology* 19, 169-184.

Rabalais, N.N., Turner, R.E., Diaz, R.J., Justic, D., 2009. Global change and eutrophication of coastal waters. *Ices Journal of Marine Science* 66, 1528-1537.

Raupach, M.R., Marland, G., Ciais, P., Le Quere, C., Canadell, J.G., Klepper, G., Field, C.B., 2007. Global and regional drivers of accelerating CO<sub>2</sub> emissions. *Proceedings of the National Academy of Sciences of the United States of America* 104, 10288-10293.

Rodolfo-Metalpa, R., Lombardi, C., Cocito, S., Hall-Spencer, J.M., Gambi, M.C., 2010. Effects of ocean acidification and high temperatures on the bryozoan *Myriapora truncata* at natural CO<sub>2</sub> vents. *Marine Ecology-An Evolutionary Perspective* 31, 447-456.

Sanderson, E.W., Jaiteh, M., Levy, M.A., Redford, K.H., Wannebo, A.V., Woolmer, G., 2002. The human footprint and the last of the wild. *Bioscience* 52, 891-904.

Sanz-Lazaro, C., Valdemarsen, T., Marin, A., Holmer, M., 2011. Effect of temperature on biogeochemistry of marine organic-enriched systems: implications in a global warming scenario. *Ecological Applications* 21, 2664-2677.

Scanes, P., Coade, G., Doherty, M., Hill, R., 2007. Evaluation of the utility of water quality based indicators of estuarine lagoon condition in NSW, Australia. *Estuarine, Coastal and Shelf Science* 74, 306-319.

Scavia, D., Field, J.C., Boesch, D.F., Buddemeier, R.W., Burkett, V., Cayan, D.R., Fogarty, M., Harwell, M.A., Howarth, R.W., Mason, C., Reed, D.J., Royer, T.C., Sallenger, A.H., Titus, J.G., 2002. Climate change impacts on US coastal and marine ecosystems. *Estuaries* 25, 149-164.

Scheffer, M., Carpenter, S., Foley, J.A., Folke, C., Walker, B., 2001. Catastrophic shifts in ecosystems. *Nature* 413, 591-596.

Schiel, D.R., Steinbeck, J.R., Foster, M.S., 2004. Ten years of induced ocean warming causes comprehensive changes in marine benthic communities. *Ecology* 85, 1833-1839.

Skelly, D.K., 2002. Experimental venue and estimation of interaction strength. *Ecology* 83, 2097-2101.

Sobocinski, K.L., Cordell, J.R., Simenstad, C.A., 2010. Effects of Shoreline Modifications on Supratidal Macroinvertebrate Fauna on Puget Sound, Washington Beaches. *Estuaries and Coasts* 33, 699-711.

Timmerman, P., White, R., 1997. Megahydropolis: coastal cities in the context of global environmental change. *Global Environmental Change-Human and Policy Dimensions* 7, 205-234.

Van Doorslaer, W., Stoks, R., Swillen, I., Feuchtmayr, H., Atkinson, D., Moss, B., De Meester, L., 2010. Experimental thermal microevolution in community-embedded *Daphnia* populations. *Climate Research* 43, 81-89.

Wernberg, T., Smale, D.A., Thomsen, M.S., 2012. A decade of climate change experiments on marine organisms: procedures, patterns and problems. *Global Change Biology* 18, 1491-1498.

York, P.H., Kelaher, B.P., Booth, D.J., Bishop, M.J., 2012. Trophic responses to nutrient enrichment in a temperate seagrass food chain. *Marine Ecology-Progress Series* 449, 291-296.

Young, W.J., Marston, F.M., Davis, J.R., 1996. Nutrient exports and land use in australian catchments. *Journal of Environmental Management*, 165-183.





## **2 Direct and indirect effects of tidal elevation on eelgrass decomposition**

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# Direct and indirect effects of tidal elevation on eelgrass decomposition

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**ABSTRACT:** In urbanised coastal environments, where shoreline migration is impeded by hard structures, sea-level rise is expected to impact decomposition processes by changing the patterns of tidal inundation. We assessed how tidal elevation directly (by determining the decomposition environment) and indirectly (by determining leaf traits) influences the decay of the south-east Australian seagrass *Zostera muelleri*. First, we assessed how the standing biomass and quality of *Z. muelleri* leaves varied across a tidal elevation gradient. Second, we used a litter-bag experiment to assess how the effects of tidal elevation on leaf traits and the decompositional environment interact to influence the decomposition rate. Surveys of 3 estuaries revealed that the size and stiffness of seagrass blades increased with depth, and the carbon and fibre content of blades was greater at subtidal and low intertidal zones than at high intertidal elevations. The differences in leaf traits among tidal elevations were, however, less important than the differences in aerial exposure for controlling the decomposition rate. The litter bags incubated at subtidal and low intertidal elevations had a faster rate of decomposition than those incubated in the high intertidal, irrespective of litter quality. Across elevations, the decay rates of the intertidal seagrass and the stiffer subtidal *Zostera* were similar. These results suggest that sea-level rise will influence decay processes by changing the decompositional environment rather than by changing tissue quality. Studies investigating differences in litter production among tidal elevations and the capacity of hydrodynamic processes to transport excess litter are now required to facilitate ecosystem-level predictions of the effects of sea-level rise.

**KEY WORDS:** Climate change · Intertidal · Seagrass · Litter bag · Litter quality · Sea-level rise

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## INTRODUCTION

The decomposition of plant material is a fundamental process that regulates the remineralisation of nutrients and the cycling of carbon in ecosystems (Swift et al. 1979). In many ecosystems, a large proportion (>50%) of primary production is not consumed by herbivores but persists in the environment as dead organic matter (Cebrián & Lartigue 2004). Some organic matter is subjected to decomposition and used by decomposers and detritivores, whereas recalcitrant components accumulate, storing carbon (Cebrián 1999). Given the relevance of the decompo-

sition process to carbon cycles, understanding the factors that influence decompositional processes is of critical importance.

The rate at which litter is decomposed or accumulated is determined by the interplay among litter quality, climate and the decomposer community (Aerts 1997). The physico-chemical and material characteristics of litter, such as carbon, nitrogen and fibre content, concentration of phenolics and tissue strength, influence its decomposition rate and palatability to consumers (Enríquez et al. 1993, Cornelissen & Thompson 1997, Hättenschwiler & Vitousek 2000, Pérez-Harguindeguy et al. 2000, Cornwell et al.

2008). Climate can influence decomposition rates directly by modifying the environment for decomposition and indirectly by modifying the physico-chemical traits of leaves (Swift et al. 1979). Many of the biochemical reactions involved in decomposition are temperature-dependent, resulting in a tendency of decomposition rates to decrease with increasing latitude (Zhang et al. 2008). Additionally, moisture can accelerate the decompositional process by facilitating the loss of soluble compounds and influencing the activity of decomposers (Swift et al. 1979, Dick & Osunkoya 2000), possibly explaining the observed general pattern of higher decomposition rates in aquatic systems (Cebrián & Lartigue 2004).

By influencing the decompositional environment and litter traits, human-induced climatic change is predicted to have major impacts on decompositional processes (Davidson & Janssens 2006). Researchers have begun to consider how warming might accelerate decomposition and how the resulting release of carbon may feedback positively to further enhance warming (Davidson & Janssens 2006). In coastal systems, however, major changes in decompositional processes may also arise from altered patterns of tidal inundation. Although coastal ecosystems have previously survived many rises and falls in sea level (Kopp et al. 2009), the urbanisation of estuaries is replacing natural shorelines with sea walls and revetments that undermine the adaptive capacity of coastal ecosystems by impeding the landward migration of the shoreline (Bulleri & Chapman 2010). Where shoreline migration is impeded, eustatic sea-level rise is projected to transform a large proportion of the intertidal habitat into a permanently submerged environment (Galbraith et al. 2002, Fujii & Raffaelli 2008). So-called 'coastal squeeze', already evident in several parts of the world (Doody 2004), might directly alter decompositional processes within estuaries by transforming the intertidal environments in which autochthonous and allochthonous organic matter accumulates and decomposes into permanently submerged environments (Marinucci 1982, Twilley et al. 1986, Robertson 1988). Coastal squeeze may indirectly modify decomposition by altering the growing environment for aquatic primary producers and, by extension, the quantity and quality of autochthonous litter that they produce as well as by influencing the distribution and composition of decomposer communities.

Seagrasses are important sources of detritus in estuarine and coastal ecosystems (Heck et al. 2008). Seagrasses display high rates of primary production ( $1$  to  $18.7 \text{ g C m}^{-2} \text{ d}^{-1}$ ; Stevenson 1988, Dawes 1998)

and in many, but not all, parts of the world (see Valentine & Duffy 2006) are subject to little herbivory (Mateo et al. 2006). Where herbivory is low, a large fraction of seagrass biomass enters the detrital pool during seasonal senescence or during storm events that can break blades and uproot shoots. Depending on the buoyancy of the seagrass, winds and water circulation patterns, 10 to 60% of the leaf production can be exported from seagrass beds (Mateo et al. 2006). Of the exported seagrass, most washes ashore and accumulates on intertidal beaches, forming wrack banks (Kirkman & Kendrick 1997, Ochieng & Erftemeijer 1999, Ince et al. 2007).

Here, we investigate the direct and indirect effects of tidal inundation on the decomposition of *Zostera muelleri*, a major primary producer along the south-east Australian coast. In estuaries, this species extends from the upper intertidal to the shallow subtidal (2 m water depth). Across this tidal elevation gradient, the leaf length and width of *Z. muelleri* varies, with subtidal plants displaying longer and wider leaves than intertidal plants (Larkum et al. 1984). We hypothesise that the physico-chemical properties of *Z. muelleri* leaves, such as fibre content and C:N ratio, will also follow the tidal elevation gradient and that, consequently, tidal inundation will influence the *Z. muelleri* decay rate not only directly by altering the decompositional environment but also indirectly by modifying the morphology of the litter entering the detrital pool. We focus our work on urbanised Australian estuaries where seawalls are constraining the landward migration of the intertidal zone due to sea-level rise. In addition to surveys of leaf traits across tidal elevations and a litter-bag experiment, we conduct shoreline profiling to assess the area of intertidal habitat that may be lost in such environments under various scenarios of sea-level rise.

## MATERIALS AND METHODS

### Study sites

Assessments of how tidal elevation influences the quality of the detrital resources provided by seagrass beds in urbanised estuaries were conducted at 3 sites, each within the Sydney region (New South Wales, Australia): Careel Bay, Pittwater ( $33^{\circ} 37' 06'' \text{ S}$ ,  $151^{\circ} 19' 36'' \text{ E}$ ); Pretty Beach, Brisbane Water ( $33^{\circ} 31' 36'' \text{ S}$ ,  $151^{\circ} 20' 42'' \text{ E}$ ); and Budgewoi Lake, Tuggerah Lakes ( $33^{\circ} 12' 28'' \text{ S}$ ,  $151^{\circ} 35' 9'' \text{ E}$ ). The sites were selected based on their (1) shoreline armouring (sea-walls) that would prevent shoreward

migration of the intertidal zone in the event of sea-level rise and (2) abundant intertidal and subtidal populations of the eelgrass *Zostera muelleri*. Pittwater is a tide-dominated estuary, while Brisbane Water and Tuggerah Lakes are wave-dominated (Roy et al. 2001). These estuaries ranged in size from 18.4 km<sup>2</sup> (Pittwater) to 28.9 km<sup>2</sup> (Brisbane Water), and each have diurnal tides with a mean range of ~1.3 m.

### Shoreline profiling

In March 2009, we conducted shoreline profiling to estimate the reduction in intertidal area that could be expected under different scenarios of eustatic sea-level rise (ESLR). Although *Zostera muelleri* does not grow beyond 1 m above the mean low water level (MLWL), we considered the loss of the entire intertidal zone because it is an important habitat for the decomposition of seagrass washed in from adjacent beds. At each study site, we measured the width of the intertidal zone (defined as the horizontal distance between the mean high water level [MHWL] and the MLWL) along 5 regularly spaced transects, each separated by tens of meters. Along each transect, we identified the MHWL by locating the obvious water-mark left by the last tidal cycle on the sea walls, ascertaining its elevation from Australian Bureau of Meteorology tidal observations and using a dumpy level (Lufkin X24) to determine the position of the MLWL relative to this known reference point. With the same instrument, we also assessed the position of the MHWL under possible ESLR scenarios for year 2100 of 0.3, 0.5 and 1 m (CSIRO 2007). We calculated the average percentage of reduction in transect length at each site under each scenario of change.

### Variation in *Zostera muelleri* foliar material

To test the hypothesis that the *Zostera muelleri* leaf morphology and quality would vary across a tidal elevation gradient, we compared the leaf morphology, above-ground biomass and mechanical and chemical properties of *Z. muelleri* among 3 elevations at each of our study sites: high intertidal (MLWL + 0.9 m), low intertidal (MLWL + 0.3 m) and shallow subtidal (MLWL – 0.5 m).

We collected entire *Zostera muelleri* plants for measurements of their shoot length, tensile properties and above-ground biomass in March 2009. Ten seagrass-containing cores of sediment, 100 mm in diameter and 200 mm deep, were randomly collected

1 to 10 m apart from each other at each of the tidal elevations at each site. Sediments were removed from the seagrass *in situ* by washing the contents of each core over a 0.5 mm sieve. The seagrasses were transported back to the laboratory in seawater to avoid loss of turgor pressure and held in aerated seawater at 20°C until biomechanical tests could be carried out within 36 h of collection. The maximal shoot length was compared among tidal elevations and sites by randomly selecting 6 undamaged shoots per core and averaging the length ( $\pm 1$  mm) of the longest leaf of the selected shoots from the top of the shoot sheath to the leaf tip. Following the measurements, the material was retained for later assessment of the biomass.

Tensile tests of *Zostera muelleri* leaves (assessing the leaves' resistance to tearing) were carried out using a material testing machine (5542, INSTRON) equipped with pneumatic controlled clamps to hold the leaf specimens at either end during the test. The longest intact leaf of 3 randomly chosen shoots for each replicate was scraped free of epiphytes, and the width ( $w$ ) and thickness ( $d$ ) of the leaves were measured using a precision calliper ( $\pm 0.01$  mm). The leaf was then placed between the clamps, and the distance between the clamps was adjusted so that the length to width ratio of the leaf was between 20 and 25 (as per Aranwela et al. 1999). The specimen was extended at a constant speed of 0.17 mm s<sup>-1</sup>, and the force ( $F$ ) and displacement ( $\delta$ ) were recorded every 0.1 s until the leaf broke. Trials in which the fracture occurred within 5 mm of the clamps were rejected because the clamping compression can cause a local weakening of the foliar tissue, resulting in an underestimation of the tensile stress of the specimen (Aranwela et al. 1999). In the case of an invalid trial, the tensile test was repeated on a leaf from a new randomly selected shoot from the same replicate to maintain a balanced experimental design. To compare the stiffness of seagrass blades among elevations, Young's modulus ( $E$ ) was calculated from the initial slope of the relationship between  $F$  and  $\delta$  using the following equation:

$$E = \frac{F/A}{\delta/l_0} \quad (1)$$

where  $A$  and  $l_0$  are respectively the cross-sectional area of the leaf ( $w \times d$ ) and the initial length of the leaf. Averaged values of the 3 measurements of  $w$ ,  $d$  and  $E$  taken within each core were used for the data analysis. Following testing, the leaves were retained and returned to the samples from which they had come for measurement of the above-ground biomass

(the mass following drying to a constant weight at 60°C).

Foliar material for neutral-detergent fibre (NDF), carbon (C) and nitrogen (N) analyses was collected between 22 and 24 May 2010 from *Zostera muelleri* meadows located at the same sites and positions used for the biomass and biomechanical analyses. Shortly before this sampling, the intermittently open entrance to Tuggerah Lake closed, resulting in a negligible tidal range in Budgewoi Lake, which during our previous sampling had previously been tidal. However, comparisons of other leaf traits from before to after the closure revealed that this environmental change was sufficiently recent that the eelgrass had not yet responded. Five eelgrass samples were collected at each tidal elevation and depth. Following collection, the seagrass was carefully cleaned of epiphytes using a razor blade and rinsed with deionised water to remove all other organisms. The samples were dried to a constant weight at 60°C and then pulverised using a stainless-steel electric mill. A subsample of ~2.5 mg was used for the determination of the total carbon and total nitrogen content using a Leco CHN-900 elemental analyser. Another subsample was used to determine the fibre content of *Z. muelleri* leaves.

The amount of NDF of *Zostera muelleri* leaves was determined following the Van Soest (1994) method with some modifications. A known amount of dried, ground leaf material (~20 mg) was boiled for 1 h with 1.8 ml of neutral detergent solution (ANKOM Technology) in a 2 ml tube. The supernatant was removed after centrifugation at  $1000 \times g$  for 5 min. The pellet was washed with distilled water and centrifuged at  $1750 \times g$ . This rinsing cycle was repeated 5 times with water and lastly with acetone. The samples were desiccated at 60°C until they reached a constant weight and weighed. The NDF content was calculated as the difference between the initial weight and the weight after treatment with the neutral detergent.

### Litter-bag experiment

We tested hypotheses about the interacting effects of the source of the foliar material and the environmental setting on the decay rate of *Zostera muelleri* using a litter-bag experiment (sensu Bockock & Gilbert 1957) at Careel Bay in the Pittwater estuary. The litter bags were 150 × 100 mm in area and constructed of 1 mm nylon mesh to allow colonisation by meiofauna and small macrofauna whilst limiting the loss of fragmented material (Bradford et al. 2002). Although by

excluding larger detritivores, litter bags can modify decomposition rates (Bradford et al. 2002), leaf-shredding crabs of the size that would have been excluded by our litter bags were uncommon at our study site (mean ± 1 standard error [SE] density:  $0.33 \pm 0.19$  per 10 cm diameter × 10 cm deep core,  $n = 12$ ), and the crab abundance did not differ between low intertidal and subtidal elevations ( $t$ -test:  $t_{10} = 0.88$ ,  $p = 0.41$ ; high intertidal zone not sampled). Moreover, the abundance ( $t$ -test:  $t_{10} = 1.26$ ,  $p = 0.24$ ) and species richness ( $t$ -test:  $t_{10} = 0.14$ ,  $p = 0.89$ ) of sediment-dwelling macroinvertebrates (>500 µm diameter) was similar between low intertidal and subtidal elevations, such that any experimental artefact of the litter bags should have been similar across treatments.

The experiment had 2 fully orthogonal factors: (1) tidal elevation with 3 levels: high intertidal (MLWL + 0.9 m; emerged for 6 to 8 h per tidal cycle), low intertidal (MLWL + 0.3 m; emerged for 2 to 4 h per tidal cycle) and shallow subtidal (MLWL – 0.5 m); and (2) litter source with 2 levels representing *Zostera muelleri* leaves collected from the low intertidal or shallow subtidal. A total of 25 litter bags of each litter source were deployed at each tidal elevation.

The foliar material used in the experiment was collected fresh at Careel Bay from the low intertidal and shallow subtidal elevations at which the morphological measurements had previously been collected. *Zostera muelleri* from the high intertidal elevation was not included in this study because it accounts for only 7% of the total biomass of this species within Pittwater and was difficult to collect in sufficient quantities for manipulation. Entire leaves were cleaned of epiphytes, towel dried and weighed into 10 g portions for deployment within litter bags within 24 h of collection. For each of the 2 plant sources, 10 samples, each of 10 g (wet weight), were dried to a constant weight at 60°C and reweighed to determine the dry- to fresh-weight conversion factors.

The litter bags were deployed on 3 September 2009, and 5 replicates for each treatment were retrieved after 5, 12, 25, 47 and 98 d (total  $n = 150$ ). At each depth, the litter bags were anchored to the sediment surface using fishing line tied to plastic posts. After retrieval, the remaining plant material in the litter bags was gently rinsed with water to remove exogenous material, dried to a constant weight at 60°C and weighed.

The decomposition constant  $k$  ( $d^{-1}$ ) for each treatment of the litter-bag experiment was calculated using an exponential model (Olson 1963):

$$X_t = X_0 e^{-kt} \quad (2)$$



where  $x_0$  is the amount of litter at the beginning of the experiment, and  $x_t$  is the amount of litter after time  $t$  (in days). This equation was linearised by plotting  $\ln(x_0/x_t)$  versus  $t$ , and a linear regression with a y-intercept (i.e. dry weight at  $t = 0$ ) of 100% was performed to calculate the decomposition rate,  $k$ . The decomposition constant  $k$  was used to calculate the half-life ( $t_{1/2} = \ln(2)/k$ ) of the *Zostera muelleri* litter (i.e. the time in days required for a reduction of half of its initial mass).

### Data analysis

Differences in the morphological, biomechanical and biochemical traits of *Zostera muelleri* among tidal elevations and across the 3 sites were assessed using a permutational analysis of variance (PERMANOVA, PRIMER v6; Anderson et al. 2007). This non-parametric procedure uses permutations to partition the variation among individual and interaction terms, using a dissimilarity matrix as its data input (the Euclidian distances among samples, in this instance). Consequently, unlike ANOVA, PERMANOVA does not require the data to be normally distributed. The analyses had 2 orthogonal factors: (1) 'site', random with 3 levels (Careel Bay, Pretty Beach and Budgewoi Lake); and (2) 'depth', fixed with 3 levels (high intertidal [H], low intertidal [L] and subtidal [S]). Where significant differences between treatments were detected ( $p_{\text{perm}} < 0.05$ ), we ran pair-wise *a posteriori* tests to identify the sources of the differences.

To test the effect of the litter source and the depositional environment on the decomposition rate of *Zostera muelleri*, we used PERMANOVA with 2 orthogonal factors, depth (H, L or S) and litter source (low intertidal or subtidal), and with time (i.e. Days 0 to 98) as a covariate. We used the raw values of the percentage of dry weight remaining at each sampling time to construct the dissimilarity matrix. Pair-wise tests were run to identify the sources of the differences when significant differences were detected ( $p_{\text{perm}} < 0.05$ ).

## RESULTS

### Shoreline profiling

At Careel Bay, the average ( $\pm 1$  SE) reduction of the intertidal foreshore width was estimated to be  $8 \pm 7\%$  for an ESLR of 0.3 m,  $49 \pm 6\%$  for a 0.5 m rise and  $91 \pm$

5% for a 1.0 m rise. Larger reductions of the intertidal foreshore were predicted for Pretty Beach, where the average beach reduction was  $32 \pm 11\%$ ,  $69 \pm 12\%$  and  $100 \pm 5\%$  for an ESLR of 0.3, 0.5 and 1.0 m respectively. The measurements indicated that Budgewoi Lake would experience no reduction with the occurrence of an ESLR of 0.3 m and reductions of  $11 \pm 5\%$  and  $24 \pm 7\%$  with sea-level rises of 0.5 and 1.0 m respectively.

### Variation in *Zostera muelleri* foliar material

As indicated by the significant interaction term in the PERMANOVA analyses (Table 1), the magnitude of differences among depths in *Zostera muelleri* foliar length, width, biomass and nitrogen and fibre content varied among the sites. The leaf thickness, stiffness and carbon content, by contrast, were mainly driven by depth (Table 1). Nevertheless, within each site, the length and width of seagrass blades generally increased with water depth, as did thickness, which displayed a strong response to depth (Table 1a–c, Fig. 1a–c). Consequently, there was a positive relationship between depth and biomass within each site (Fig. 1d). The material stiffness (measured using Young's modulus) displayed a pattern of increase with depth across all of the sites (Table 1e, Fig. 1e), with statistically significant differences among each of the depths considered at each site (Student-Newman-Keuls tests, main effect of depth). The carbon and NDF content of *Z. muelleri* leaves were consistently lower in the high intertidal zone compared to the other 2 depths (Table 1f,i, Fig. 1f,i). The nitrogen content, by contrast, did not show any consistent pattern among depths across the 3 study sites (Table 1g, Fig. 1g). The C:N ratio was not influenced by depth (Table 1h, Fig. 1h).

### Litter-bag experiment

The decomposition constant ( $k$ ) of *Zostera muelleri* litter followed a negative exponential decay pattern (linear regression:  $p < 0.001$ ; Table 2, Fig. 2), with significant mass loss already evident by the first time of sampling ( $t = 5$  d). The average half-life of *Z. muelleri* litter across all of the treatments was  $53 \pm 3$  d and ranged from  $47 \pm 1$  d in the low intertidal treatment to  $62 \pm 1$  d in the high intertidal treatment (Table 2). The rate at which *Z. muelleri* decomposed over the duration of the experiment was strongly affected by the tidal elevation at which the litter bags were incu-

Table 1. Two-way PERMANOVA testing for spatial variation in leaf traits of *Zostera muelleri* among high intertidal, low intertidal and subtidal depths (Depth, D) and among 3 study sites (Site, S). (a–e):  $n = 10$ ; (f–i):  $n = 5$ . p-values in **bold** are significant at  $\alpha = 0.05$

Source	df	MS	Pseudo- <i>F</i>	$p_{\text{perm}}$
<b>(a) Length</b>				
Site	2	66	7.6	<b>0.002</b>
Depth	2	3206	27.5	<b>0.009</b>
S $\times$ D	4	117	13.5	<b>&lt;0.001</b>
Residual	81	9		
<b>(b) Width</b>				
Site	2	18.4	211.4	<b>&lt;0.001</b>
Depth	2	8.2	9.1	<b>0.040</b>
S $\times$ D	4	0.9	10.4	<b>&lt;0.001</b>
Residual	81	0.1		
<b>(c) Thickness</b>				
Site	2	0.015	23.4	<b>&lt;0.001</b>
Depth	2	0.024	42.8	<b>0.003</b>
S $\times$ D	4	0.001	0.9	0.406
Residual	81	0.001		
<b>(d) Above-ground biomass</b>				
Site	2	107170	30.0	<b>0.001</b>
Depth	2	658920	17.7	<b>0.003</b>
S $\times$ D	4	37221	10.4	<b>0.001</b>
Residual	81	3571		
<b>(e) Young's modulus</b>				
Site	2	14628	20.9	<b>&lt;0.001</b>
Depth	2	31830	36.4	<b>0.003</b>
S $\times$ D	4	873	1.2	0.302
Residual	81	700		
<b>(f) Carbon</b>				
Site	2	13.5	15.6	<b>0.001</b>
Depth	2	23.4	26.9	<b>0.025</b>
S $\times$ D	4	0.9	1.0	0.417
Residual	36	0.9		
<b>(g) Nitrogen</b>				
Site	2	0.7	29.1	<b>0.001</b>
Depth	2	0.1	0.8	0.505
S $\times$ D	4	0.1	4.6	<b>0.005</b>
Residual	36	0.0		
<b>(h) C:N</b>				
Site	2	36.5	13.7	<b>0.001</b>
Depth	2	1.0	0.1	0.874
S $\times$ D	4	6.9	2.6	0.052
Residual	36	2.7		
<b>(i) Neutral fibre</b>				
Site	2	163.2	53.4	<b>0.001</b>
Depth	2	152.8	8.7	<b>0.040</b>
S $\times$ D	4	17.7	5.8	<b>0.001</b>
Residual	36	3.1		

bated (PERMANOVA, pseudo- $F_{2,150} = 26.6$ ,  $p_{\text{perm}} = 0.0001$ ) but not by the source of the litter (PERMANOVA, pseudo- $F_{1,150} = 0.08$ ,  $p_{\text{perm}} = 0.77$ ) or the interaction between the 2 factors (PERMANOVA, pseudo- $F_{2,150} = 2.0$ ,  $p_{\text{perm}} = 0.14$ ; Fig. 2). Among the 3

elevations of incubation, foliar material decomposed more slowly at the high intertidal level than at the low intertidal or subtidal levels (PERMANOVA, pair-wise tests,  $p_{\text{perm}} < 0.01$ ; Fig. 2). However, no difference was detected between the rate at which the litter decomposed at the low intertidal and subtidal depths (PERMANOVA, pair-wise tests,  $p_{\text{perm}} = 0.87$ ; Fig. 2).

## DISCUSSION

Decompositional processes in terrestrial ecosystems are affected by the physical environment, the physico-chemical and mechanical traits of litter material and the decomposer community (Swift et al. 1979, Aerts 1997, Pérez-Harguindeguy et al. 2000, Cornwell et al. 2008). Consequently, we hypothesised that tidal inundation would influence the decomposition of the morphologically plastic seagrass *Zostera muelleri* by (1) altering blade traits and (2) altering the abiotic and biotic conditions in which decomposition occurs. Despite significant morphological variation of the seagrass across a tidal elevation gradient, our experiments failed to detect a dominant effect of within-species trait variability on decomposition rate. Instead, the decomposition rate of low intertidal and subtidal plants was determined by elevation along a tidal gradient.

Within each of the 3 estuaries, we documented distinct changes in the leaf traits of *Zostera muelleri* along a tidal elevation gradient extending from the

Table 2. Decomposition constant ( $k$ ) and half-life ( $t_{1/2}$ ) of *Zostera muelleri* litter collected from low intertidal (Int) and subtidal (Subt) sources and deployed at high intertidal, low intertidal and subtidal depths at Careel Bay. Estimates of  $k$  and  $t_{1/2}$  were derived separately for each treatment using linear regression analysis between time and  $\ln(x_0/x_t)$ , where  $x_0$  is the amount of litter at the beginning of the experiment, and  $x_t$  is the amount of litter after time  $t$  (in days). The y-intercept (i.e. dry weight at  $t = 0$ ) was forced through 100%.

All regressions were significant at  $\alpha = 0.001$ .  $n = 25$

Litter source	$r^2$	$k$ (d <sup>-1</sup> )	SE	$t_{1/2}$ (d)
<b>High intertidal</b>				
Int	0.93	0.0112	0.0006	62
Subt	0.88	0.0112	0.0008	62
<b>Low intertidal</b>				
Int	0.91	0.0152	0.0009	46
Subt	0.90	0.0143	0.0009	48
<b>Subtidal</b>				
Int	0.88	0.0145	0.0010	48
Subt	0.87	0.0132	0.0009	52



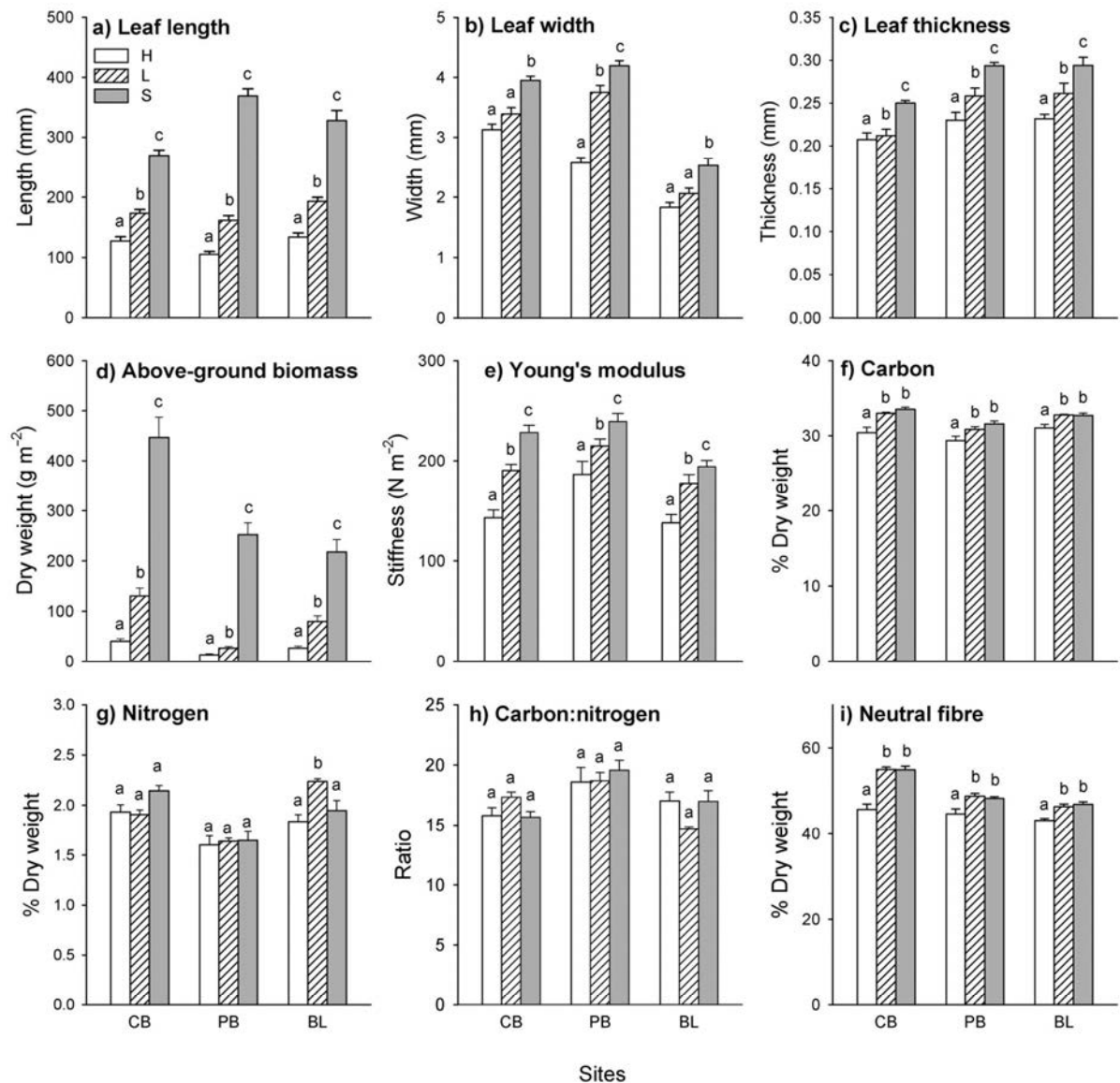


Fig. 1. *Zostera muelleri*. Mean (+1 SE) values of leaf traits within high intertidal (H; white bars), low intertidal (L; striped bars) and subtidal (S; grey bars) elevations at Careel Bay (CB), Pretty Beach (PB) and Budgewoi Lake (BL). Letters denote significant (at  $\alpha = 0.05$ ) within-site differences among depths (post-hoc tests for significant PERMANOVA analyses; Table 1). (a-e):  $n = 10$ ; (f-i):  $n = 5$

high intertidal to the shallow subtidal. The length, width and thickness of seagrass blades each displayed a general trend of decrease with increasing tidal elevation. Additionally, the carbon and fibre contents and the stiffness of seagrass blades were lower for plants collected from the high intertidal zone than from either the low intertidal or subtidal elevations. Previous comparisons of the morphology of this species among subtidal elevations found a

decrease in shoot biomass with depth, as light availability decreased (Larkum et al. 1984). We found that above a depth of  $-0.5$  m, *Z. muelleri* showed a decrease in biomass with increasing elevation, presumably as a result of desiccation stress. Other species of *Zostera* have also shown marked morphological variability with respect to environmental variables, such as wave exposure, sediment properties, light availability and desiccation stress (Silva &

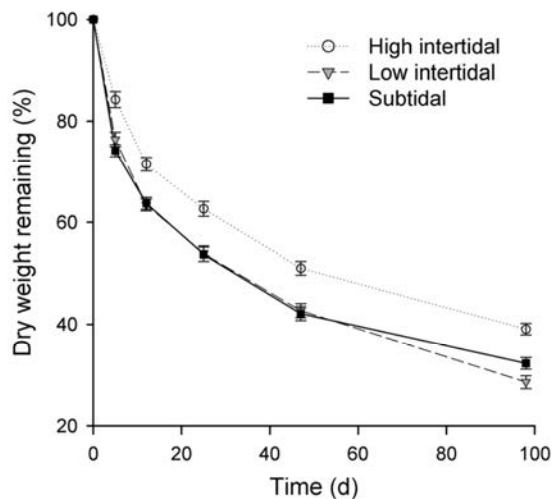


Fig. 2. *Zostera muelleri*. Mean ( $\pm 1$  SE) percentage of the initial litter weight remaining in bags deployed at high intertidal, low intertidal and subtidal elevations on each of 5 sampling occasions. Litter sources were pooled because there was no significant difference in the decomposition of litter sourced from low intertidal or subtidal plants (PERMANOVA,  $p_{\text{perm}} > 0.05$ ).  $n = 10$

Santos 2003, Boese et al. 2005, Cabaço et al. 2009). The high morphological variability of *Zostera* spp. appears to be a general adaptation to tolerate a variable environment.

Despite morphological differences between the low intertidal and the subtidal morphs, their decomposition rates did not differ. The absence of a dominant effect of litter source on decomposition rate might be explained by the similar carbon, nitrogen and fibre contents of the low intertidal and the subtidal morphs used in the decomposition experiments. Among leaf traits, the total nutrient content and C:N ratio are often considered to be the most important determinants of the decomposition rate (Enríquez et al. 1993, Pérez-Harguindeguy et al. 2000, Zhang et al. 2008). According to the hypothesis that the total nutrient content and C:N ratio are the key leaf traits influencing decomposition, the high intertidal morph, which had a lower carbon and fibre content than the other 2 morphs, might be expected to display a higher decomposition rate. The high intertidal morph was not, however, included in litter bag experiments because it did not produce enough biomass to be collected in sufficient quantities for experimentation or to significantly contribute to litter decomposition at the ecosystem level.

Tidal elevation, in contrast to leaf morphology, had a strong effect on the decomposition rate of

*Zostera muelleri*. Consistent with previous observations of *Spartina alterniflora* and mangrove leaf-litter decomposition across tidal elevation gradients (Marinucci 1982, Twilley et al. 1986, Robertson 1988, Dick & Osunkoya 2000), we observed that the rate at which *Z. muelleri* decomposed increased with immersion time. In particular, *Z. muelleri* litter decomposing in the high intertidal had a 27% longer half-life than litter decomposing in the low intertidal and subtidal zones. The greater rate of decomposition at low intertidal and subtidal elevations may reflect a faster initial leaching of nutrients from materials that are immersed. Over the first 5 d of the experiment, the litter deployed in the high intertidal decomposed more slowly than the litter deployed in the low intertidal or subtidal zone (Fig. 2). After this initial period, however, the decomposition rates were similar among the 3 tidal elevations. Alternatively, a faster decomposition rate at the low intertidal and subtidal depths may reflect (1) initial retardation of microbial growth and decomposition at the high intertidal elevation by the environmentally variable conditions (Robertson 1988) or (2) differences between the high intertidal and lower elevations in micro- and meiofaunal communities, which play an important role in decomposition through ingestion and mechanical damage of decomposing plant material (Hunter et al. 2003).

Although at more exposed locations, differences in hydrodynamic conditions among tidal elevations can lead to differences in rates of the fragmentation of litter material (C. Willison pers. comm.), this mechanism is unlikely to have contributed to the differences in mass loss observed among tidal elevations in the present study. First, our experiment was conducted at an extremely sheltered estuarine site. Second, whereas the greatest differences in decomposition rates among elevations were seen early in our study, the effects of fragmentation would be expected to be more pronounced among litter that had been given more time to degrade (Bradford et al. 2002).

In the present study, litter bags were used to track the decomposition of litter material through time. Litter bags may, however, produce unrepresentative decomposition rates by (1) reducing desiccation stress in the high intertidal through enhancement of moisture retention and shading and (2) modifying the complexity of associated faunal communities (e.g. Bradford et al. 2002). At our study site, the macroinvertebrate communities of unvegetated sediments did not significantly differ between the low

intertidal and subtidal elevations (see 'Materials and methods'). Hence, we suspect that any positive or negative artefact of macrofaunal exclusion on decomposition rates would be similar among the treatments. Moisture retention by litter bags will, however, modify environmental conditions more at high intertidal than at lower elevations. Therefore, we suspect that, if anything, our study underestimated differences in decomposition between high intertidal and lower elevations.

The shoreline profiles at each of our study sites allowed us to predict that the sea-level rise projected for the year 2100 will cause major changes in the inundation regimes in these urbanised estuaries where shoreline migration is constrained. Our sampling and experiments indicate that in the most severe cases, where the intertidal zone is severely reduced or even completely lost, a shift towards larger-bladed *Zostera muelleri* and a compositional environment that accelerates decay rate may occur in temperate Australian estuaries. Whereas moderate detrital loads sustain estuarine productivity, large quantities of rapidly decomposing detritus can induce sediment anoxia and negatively affect benthic communities (Pearson & Rosenberg 1978). Whether a shift towards larger-bladed seagrass and rapid decomposition will lead to sediment anoxia will, however, depend on the extent to which shoreline armouring reduces intertidal areas, the productivity of the seagrass, rates of herbivory and transport processes, such as currents and waves, which remove excess production from the system. Furthermore, the net outcome of sea-level rise on decomposition processes will depend on the capacity of *Z. muelleri* to adapt to changing geomorphological conditions.

Overall, this study demonstrates that the direct effects of tidal elevation on a decompositional environment are more important than the indirect effects of intraspecific variation in leaf traits in determining decomposition rates. Hence, without large shifts in litter materials from refractory to labile sources or major changes in litter supply, the main effect of sea-level rise on decomposition processes is likely to come from alteration of the decompositional environment.

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## LITERATURE CITED

- ▶ Aerts R (1997) Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. *Oikos* 79:439–449
- ▶ Anderson MJ, Gorley RN, Clarke KR (2007) PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth
- ▶ Aranwela N, Sanson G, Read J (1999) Methods of assessing leaf-fracture properties. *New Phytol* 144:369–383
- ▶ Bockock KL, Gilbert OJW (1957) The disappearance of leaf litter under different woodland conditions. *Plant Soil* 9: 179–185
- ▶ Boese BL, Robbins BD, Thursby G (2005) Desiccation is a limiting factor for eelgrass (*Zostera marina* L.) distribution in the intertidal zone of a northeastern Pacific (USA) estuary. *Bot Mar* 48:274–283
- ▶ Bradford MA, Tordoff GM, Eggers T, Jones TH, Newington JE (2002) Microbiota, fauna, and mesh size interactions in litter decomposition. *Oikos* 99:317–323
- ▶ Bulleri F, Chapman MG (2010) The introduction of coastal infrastructure as a driver of change in marine environments. *J Appl Ecol* 47:26–35
- ▶ Cabaço S, Machas R, Santos R (2009) Individual and population plasticity of the seagrass *Zostera noltii* along a vertical intertidal gradient. *Estuar Coast Shelf Sci* 82:301–308
- ▶ Cebrián J (1999) Patterns in the fate of production in plant communities. *Am Nat* 154:449–468
- ▶ Cebrián J, Lartigue J (2004) Patterns of herbivory and decomposition in aquatic and terrestrial ecosystems. *Ecol Monogr* 74:237–259
- ▶ Cornelissen JHC, Thompson K (1997) Functional leaf attributes predict litter decomposition rate in herbaceous plants. *New Phytol* 135:109–114
- ▶ Cornwell WK, Cornelissen JHC, Amatangelo K, Dorrepaal E and others (2008) Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecol Lett* 11:1065–1071
- CSIRO (2007) Climate change in Australia. CSIRO Marine and Atmospheric Research, Aspendale
- ▶ Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* 440:165–173
- Dawes CJ (1998) Marine botany, 2nd edn. Wiley & Sons, New York, NY
- ▶ Dick TM, Osunkoya OO (2000) Influence of tidal restriction floodgates on decomposition of mangrove litter. *Aquat Bot* 68:273–280
- ▶ Doody JP (2004) 'Coastal squeeze'—an historical perspective. *J Coast Conserv* 10:129–138
- ▶ Enríquez S, Duarte CM, Sandjensen K (1993) Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. *Oecologia* 94: 457–471
- ▶ Fujii T, Raffaelli D (2008) Sea-level rise, expected environmental changes, and responses of intertidal benthic macrofauna in the Humber estuary, UK. *Mar Ecol Prog Ser* 371:23–35
- ▶ Galbraith H, Jones R, Park R, Clough J, Herrod-Julius S, Harrington B, Page G (2002) Global climate change and sea level rise: potential losses of intertidal habitat for shorebirds. *Waterbirds* 25:173–183
- ▶ Hättenschwiler S, Vitousek PM (2000) The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends Ecol Evol* 15:238–243

- Heck KL, Carruthers TJB, Duarte CM, Hughes AR, Kendrick G, Orth RJ, Williams SW (2008) Trophic transfers from seagrass meadows subsidize diverse marine and terrestrial consumers. *Ecosystems* 11:1198–1210
- Hunter MD, Adl S, Pringle CM, Coleman DC (2003) Relative effects of macroinvertebrates and habitat on the chemistry of litter during decomposition. *Pedobiologia* 47: 101–115
- Ince R, Hyndes GA, Lavery PS, Vanderklift MA (2007) Marine macrophytes directly enhance abundances of sandy beach fauna through provision of food and habitat. *Estuar Coast Shelf Sci* 74:77–86
- Kirkman H, Kendrick G (1997) Ecological significance and commercial harvesting of drifting and beach-cast macroalgae and seagrasses in Australia: a review. *J Appl Phycol* 9:311–326
- Kopp RE, Simons FJ, Mitrovica JX, Maloof AC, Oppenheimer M (2009) Probabilistic assessment of sea level during the last interglacial stage. *Nature* 462:863–867
- Larkum AWD, Collett LC, Williams RJ (1984) The standing stock, growth and shoot production of *Zostera capricorni* Aschers. in Botany Bay, New South Wales, Australia. *Aquat Bot* 19:307–327
- Marinucci AC (1982) Trophic importance of *Spartina alterniflora* production and decomposition to the marsh-estuarine ecosystem. *Biol Conserv* 22:35–58
- Mateo MA, Cebrian J, Dunton KH, Mutchler T (2006) Carbon flux in seagrass ecosystems. In: Larkum AWD, Orth RJ, Duarte CM (eds) *Seagrasses: biology, ecology and conservation*. Springer, Dordrecht, p 159–192
- Ochieng CA, Erftemeijer PLA (1999) Accumulation of seagrass beach cast along the Kenyan coast: a quantitative assessment. *Aquat Bot* 65:221–238
- Olson JS (1963) Energy storage and the balance of producers and decomposers in ecological systems. *Ecology* 44: 322–331
- Pearson TH, Rosenberg R (1978) Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanogr Mar Biol Annu Rev* 16: 229–311
- Pérez-Harguindeguy N, Díaz S, Cornelissen JHC, Vendramini F, Cabido M, Castellanos A (2000) Chemistry and toughness predict leaf litter decomposition rates over a wide spectrum of functional types and taxa in central Argentina. *Plant Soil* 218:21–30
- Robertson AI (1988) Decomposition of mangrove leaf litter in tropical Australia. *J Exp Mar Biol Ecol* 116:235–248
- Roy PS, Williams RJ, Jones AR, Yassini I and others (2001) Structure and function of south-east Australian estuaries. *Estuar Coast Shelf Sci* 53:351–384
- Silva J, Santos R (2003) Daily variation patterns in seagrass photosynthesis along a vertical gradient. *Mar Ecol Prog Ser* 257:37–44
- Stevenson JC (1988) Comparative ecology of submersed grass beds in freshwater, estuarine, and marine environments. *Limnol Oceanogr* 33:867–893
- Swift MJ, Heal OW, Anderson JM (1979) *Decomposition in terrestrial ecosystems*. Blackwell, Oxford
- Twilley RR, Lugo AE, Patterson-Zucca C (1986) Litter production and turnover in basin mangrove forests in south-west Florida. *Ecology* 67:670–683
- Valentine JF, Duffy JE (2006) The central role of grazing in seagrass ecology. In: Larkum AW, Orth RJ, Duarte CM (eds) *Seagrass: biology, ecology and conservation*. Springer, Dordrecht, p 463–501
- Van Soest PJ (1994) *Nutritional ecology of the ruminant*, 2nd edn. Cornell University Press, Ithaca, NY
- Zhang DQ, Hui DF, Luo YQ, Zhou GY (2008) Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. *J Plant Ecol* 1:85–93

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### **3        Effects of tidal inundation on benthic macrofauna associated with the eelgrass**

#### ***Zostera muelleri***

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### 3.1 Abstract

Processes, such as sea level rise, that alter tidal inundation regimes have the potential to modify the structure of seagrasses and their dense and diverse faunal communities. This study tested the hypothesis that seagrass-dwelling invertebrate communities would vary across a tidal inundation gradient as a result of direct effects of tidal inundation and indirect effects, arising from changes in seagrass morphology across this gradient. First, we conducted mensurative sampling across tidal elevation gradients to assess how above- and below-ground seagrass biomass, and epi- and infaunal invertebrate communities co-varied with depth. Second, we conduct a manipulative field experiment, utilizing artificial seagrass rhizomes, to separate out direct effects of tidal elevation on infaunal communities from indirect effects arising from changes in seagrass root morphology. Mensurative sampling revealed that the abundance and taxon richness of seagrass epi- and infauna, and the above- and below- ground biomass of seagrass each increased with depth across a tidal elevation gradient extending from the high intertidal to the shallow subtidal. The manipulative experiment revealed that the extent to which direct effects of tidal inundation and the indirect effects resulting from effects on *Z. muelleri* rhizomes drove patterns in the distribution and abundance of infauna was taxon-specific. In general, however, the facilitative effects of rhizome structure were more evident at the intertidal compared to the subtidal elevation. Our results indicate that changes to tidal inundation regime will affect seagrass-dwelling macroinvertebrates through a combination of direct and indirect effects. Therefore, future changes in tidal inundation regimes should be taken into account in developing conservation plans for protecting seagrasses and the biodiversity they sustain.

**Keywords:** artificial seagrass unit; ecological zonation; habitat; facilitation; sea level changes; urbanization.

### **3.2 Introduction**

Seagrasses are important bioengineers of marine and estuarine shallow water environments that contribute to the productivity and biodiversity of coastal systems (Boström et al., 2006). They facilitate coastal nutrient and carbon cycling, attenuate water-flow, trap and bind sediments, and add hard structure to sedimentary environments (Heck and Orth, 1980; Hemminga and Duarte, 2000; de Boer, 2007). The complex and heterogeneous habitat provided by seagrasses offer substrate, shelter and food for many coastal organisms (Heck and Orth, 1980; Boström et al., 2006). Consequently, compared to adjacent unvegetated substrate, seagrasses frequently sustain a high biomass and species richness of associated flora and fauna (Boström and Bonsdorff, 2000; Lee et al., 2001; Heck et al., 2003).

The density and diversity of the communities facilitated by seagrass varies spatially according to the environmental context (Bell and Westoby 1986a, b; Connolly and Hindell, 2006) and the small-scale structural complexity provided by seagrass shoots and roots (Heck and Orth, 2006; Horinouchi 2007). In line with models of community organisation, predicting that positive interactions among organisms will be most prevalent when biotic or abiotic stress is ameliorated (Bertness and Callaway, 1994), the density of nekton in seagrass appears to be at least partially driven by predation pressure (Hindell et al., 2000). At the landscape scale, the availability of larvae (McNeill and Fairweather, 1993) and the size and shape of seagrass patches also play an important role in determining associated faunal communities (Connolly and Hindell, 2006). Within patches, the density and length of shoots determine habitat availability for epifauna, amelioration of predation pressure and the degree to which water motion is attenuated and larvae and organic matter are hence deposited (e.g. Heck and Orth, 1980; Peterson et al., 2004). Consequently, processes that alter the structural complexity of seagrass and its environmental context have the potential to have large impacts on the communities supported by seagrasses.

Increasingly, seagrass ecosystems are being modified by the combined effects of global climate change and anthropogenic stressors (Orth et al. 2006; Waycott et al. 2009). Already, there are reports of negative effects of increasing global temperature on seagrass survival (Ehlers et al., 2008; Massa et al., 2009; Marbà and Duarte 2010). Additionally, sea level rise represents a significant threat for seagrass systems (Nicastro et al., 2012). Sea level rise can modify depth, salinity and tidal inundation regimes (Short and Neckles, 1999), particularly in urbanised areas where armouring of the coastline by artificial structures prevents landward migration of intertidal and shallow subtidal species (Bulleri and Chapman, 2010). A reduction of the intertidal area through ‘coastal squeeze’ has already been seen in several human-modified coastal areas around the world (Doody, 2004).

A reduction in intertidal area might modify communities of seagrass-dwelling invertebrates directly, via alteration of the environmental context, and indirectly via altering the complexity of seagrass habitats. Tidal inundation influences patterns of food delivery, light and desiccation stress, and predator access to benthic communities. Hence, there may be effects of tidal inundation on infaunal and epifaunal species that occur whether seagrass is present or not (Edgar and Barrett, 2002; Micheli et al. 2008; Mills and Berkenbusch, 2009). Coastal squeeze may, however, also indirectly modify seagrass-dwelling fauna by changing the morphology of their seagrass habitat. Previous studies have documented morphological changes in seagrasses that occur across tidal elevation gradients (e.g. Larkum et al. 1984; Cabaço et al. 2009; Nicastro et al., in press). It is unclear how these direct and indirect effects of changing tidal inundation might interact to influence seagrass communities.

In this study, we make use of naturally occurring *Zostera muelleri* seagrass beds that span tidal elevation gradients from the high intertidal to the shallow subtidal (2 m) to assess how inundation directly and indirectly, via changes in seagrass morphology, influences associated invertebrate fauna. *Z. muelleri* is a dominant seagrass species of the south-eastern Australian coast that shows morphological variation across tidal elevation gradients, with



intertidal plants taking on a stunted form (Larkum et al., 1984; Nicastro et al., 2012). First, we conduct mensurative sampling across tidal elevation gradients to assess how above- and below-ground seagrass biomass, and epi- and infaunal invertebrate communities co-vary with depth. Second, we conduct a manipulative field experiment, utilizing artificial seagrass rhizomes that we have developed, to separate out direct effects of tidal elevation on infaunal communities from indirect effects arising from changes in seagrass root morphology. Although artificial seagrass units have been used extensively to investigate the effect of structural traits of seagrass shoots on associated fauna (e.g. Hovel and Lipcius, 2001; Lee et al., 2001; Micheli et al. 2008), our study is the first, to our knowledge, to use structural mimics to investigate effects of root morphology. Overall, this study will help to predict how future changes in the tidal inundation regime due to sea level will affect the abundance and biodiversity of macrofaunal communities.

### **3.3 Materials and methods**

#### *3.3.1. Field surveys*

To assess how spatial variability in seagrass-dwelling macrofaunal communities correlates to spatial variability in the morphology of seagrass across a tidal elevation gradient, we conducted sampling at three estuarine sites within the greater Sydney region, New South Wales, Australia. The sites, each sampled in March 2009 (late summer), were: Careel Bay, Pittwater estuary (33° 37' 06" S, 151° 19' 36" E); Pretty Beach, Brisbane Waters (33° 31' 36" S, 151° 20' 42" E) and Budgewoi, Tuggerah Lakes (33° 12' 28" S, 151° 35' 9" E). The sites contained continuous, monospecific *Z. muelleri* meadows, extending from the high intertidal zone to a depth of about 2 m. Sites had a semi-diurnal tidal regime with a mean spring tidal range of ~1.3 m.

At each of the sites, we collected samples of seagrass and macrofauna along three transects (about 200 m long) running parallel to the shoreline. Two of the transects, situated at

0.9 ± 0.1 m and 0.3 ± 0.1 m above the mean low water level (MLWL), were intertidal and were emersed for 6-8 h and 2-4 h per tidal cycle respectively. The third, which was 0.5 ± 0.1 m below the MLWL, was subtidal and constantly submersed. Along each transect, ten samples of *Z. muelleri* and associated infauna were randomly collected from the centre of seagrass patches of at least 2 m in diameter using a 10-cm diameter core, inserted into the sediment to a depth of 15 cm. Sampling of macrofauna was carried out during the day, 1-2 hours before spring low tide. Upon collection, samples were refrigerated, sieved through a 0.5 mm mesh within 48 hours of collection to remove sediments, and fixed in formaldehyde solution (7%) prepared with seawater. Macrofauna were removed from seagrass roots and shoots under a dissecting microscope (10x magnification) and transferred to 70% ethanol. *Z. muelleri* biomass was divided into above- and below-ground components, dried to constant weight at 60°C, and weighed. Each animal was classified as either epi- or infauna accordingly to its position on the seagrass and its biology. Animals were identified to the lowest practical taxonomic level, which was species for bivalves and gastropods, order for crustaceans, family for polychaetes, class for oligochaetes and phylum for nemerteans and sipunculids. Use of a mixed taxonomic resolution was necessary due to the poorly documented taxonomy of much of Australia's invertebrate fauna. This approach does not compromise the detection of spatial patterns of invertebrates across environmental gradients (Clarke and Warwick 2001; Dethier and Schoch 2006).

Differences among depths and study sites in *Z. muelleri* above- and below-ground biomass were analysed by two-way analysis of variance (ANOVA). The factor 'site' was random. Depth was a fixed factor with three levels: high intertidal, low intertidal and subtidal. Cochran's test was run prior each ANOVA to test the assumption of homogeneity of variances. Where the test was significant ( $p < 0.05$ ), a  $\ln(x + c)$  transformation was applied to biomass and count data, respectively, in order to remove the heterogeneity of variance. The value of  $c$  was chosen in order to maximize the symmetry of the distribution of the residuals

for each variable (Berry, 1987). Where ANOVA indicated significant effect of a main factor and/or an interaction term, Student-Newman-Keuls (SNK) tests were used for pair-wise comparisons.

Distance-based, permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) tested for differences in (a) epi- and (b) infaunal communities among depths and study sites. The two-factor orthogonal analyses used Bray-Curtis similarities calculated from square root transformed data after the addition of one dummy variable ( $x = 1$ ) to the data set. A dummy variable was added to avoid undefined values in the similarity matrix that would have otherwise resulted from several high intertidal samples containing no macrofauna (Clarke et al. 2006). Unrestricted permutation of raw data was used as the permutation method. Where factors were significant at  $p_{\text{perm}} < 0.05$ , PERMANOVAs were followed by pair-wise post-hoc tests to ascertain sources of differences. Distances among treatment centroids were calculated and represented graphically with non-metric multi-dimensional scaling (MDS) plots. PERMANOVAs were accompanied by two-way ANOVAs testing for spatial differences in epi- and infaunal abundance and number of taxa per sample.

Where PERMANOVA detected spatial differences in macroinvertebrate community structure, we used the SIMPER routine (Clarke and Warwick 2001) to identify the taxa that best discriminated among levels of significant factors. Only taxa with a ratio of the mean Bray-Curtis dissimilarity to the standard deviation of greater than or equal to 1 were considered good discriminators (Clarke and Warwick, 20001). Differences in abundance of good discriminator taxa were tested using the ANOVA design described above.

To assess whether there was a relationship between plant and macrofauna biomass across the depth gradient, we conducted Pearson's correlation between *Z. muelleri* above-ground biomass and epifauna abundance, and *Z. muelleri* below-ground biomass and infauna abundance. Seagrass biomasses were log-transformed prior to analysis.

### 3.3.2 Field experiment

To test the relative influence on seagrass infaunal communities of (1) the direct effects of depth and (2) the indirect effect of depth arising from differences in the physical structure provided by the *Z. muelleri* root system among elevations, we conducted a manipulative experiment using artificial *Zostera* rhizomes (AZR). The experiment was done in Careel Bay (one of the three sites surveyed), in a 200 m–long site containing patches of intertidal and subtidal *Z. muelleri* separated by unvegetated sediment. The experiment had two orthogonal factors: (1) position on the shore with two fixed levels, intertidal and subtidal (MLWL +0.3 [ $\pm$  0.1] m and -0.5 [ $\pm$  0.1 m], respectively), and (2) ‘habitat’ with four fixed levels, unvegetated sediment, natural *Z. muelleri*, low density AZR and high density AZR. The low density AZR mimicked the morphology of the low intertidal *Zostera*, while the high density AZR mimicked the morphology of the subtidal *Zostera*. The high intertidal elevation and morphology was excluded because of the negligible *Z. muelleri* biomass (about 7% of the total biomass in Careel Bay) and the scantness of associated macroinvertebrates. Within each of the two positions on the shore, we established six 0.5 x 0.5 m experimental plots of each ‘habitat’. Plots assigned to the two AZR treatments and the unvegetated control were randomly established on bare sediment. Plots of natural *Z. muelleri* were randomly established in the centre of natural seagrass patches of at least 2 m diameter.

AZR were constructed by tying short strands of polyethylene string to the twisted strands of laid polyethylene rope (Zenith poly rope mini coil). The rope was 3-mm in diameter, roughly matching the average rhizome diameter of  $2.4 \pm [1 \text{ S. E.}] 0.3$  mm of *Z. muelleri*, which did not differ between intertidal and subtidal elevations of this site. The main structural difference between intertidal and subtidal *Z. muelleri* was the rhizome length, so the two AZR treatments (low density, high density) differed only in the length of rope they received. The 0.25 m<sup>2</sup> plots assigned to the low density AZR plots received a 1.63 m length of rope, corresponding to the mean rhizome length of  $6.5 [\pm 0.7]$  m per m<sup>2</sup> at intertidal

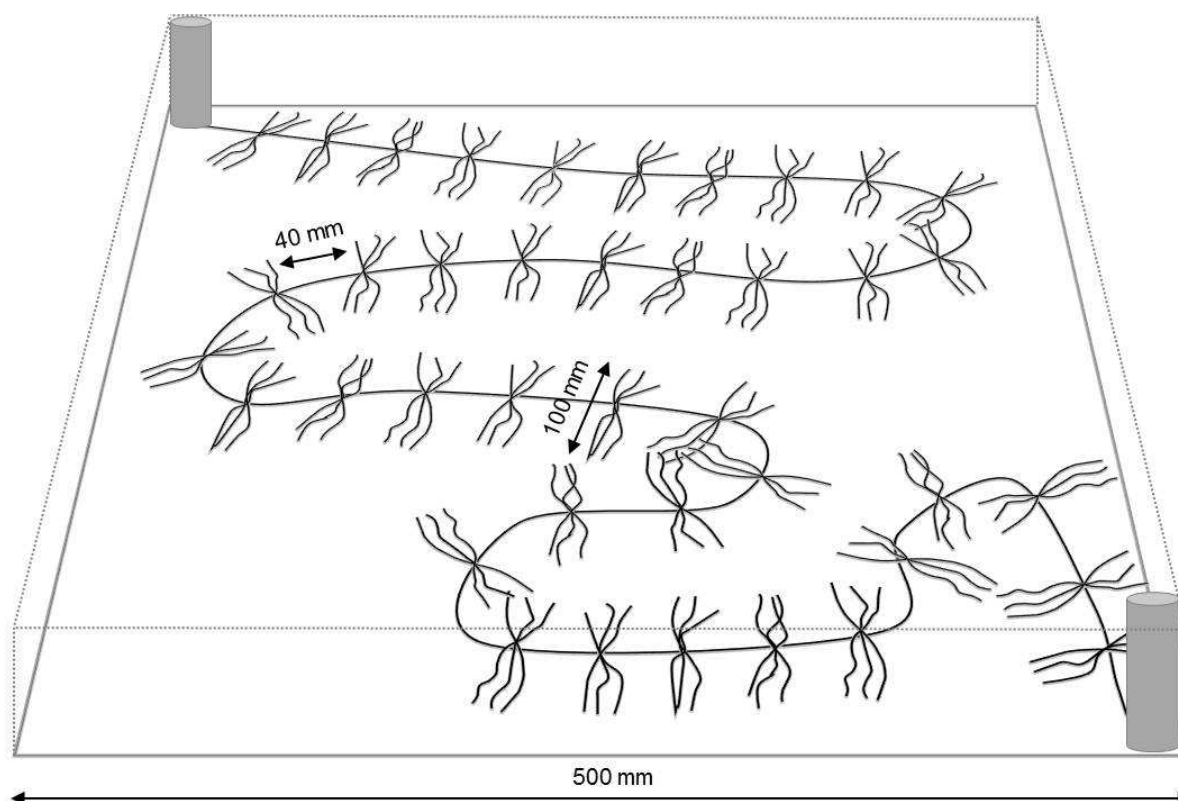
elevations. The plots assigned to the high density AZR treatment received a 3.08 m length (mean rhizome length at subtidal elevations,  $12.3 \pm [0.8 \text{ m}] \text{ per m}^2$ ). Polyethylene strings, 1 mm in diameter and 100 mm in length were tied at 40 mm interval along each artificial rhizome to mimic the branching roots departing from the rhizome nodes. The interval between strings roughly corresponded to the mean internode length of  $34 [\pm 5] \text{ mm}$  at intertidal and subtidal elevations ( $n=10$ ). Polyethylene is resistant to weathering, such that we did not expect any chemical interference of the AZR with the sediment over the time scale of the experiment.

In the field, each end of the AZR was tied to a 0.5-m long PVC stake and the two stakes were inserted into the sediment at diagonally opposite corners of the assigned  $0.5 \times 0.5 \text{ m}$  square plot (Fig. 3.1). The AZR was uniformly laid over the plot area and pushed manually into the sediment to a depth of about 3-5 cm, because approximately 90% of the *Z. muelleri* below-ground biomass is found within these surface sediments (Hansen et al. 2000). Plots assigned to control replicates (bare sediment) received a similar sediment perturbation, and these and the undisturbed seagrass plots were marked with two PVC stakes at diagonally opposite corners, as per the plots receiving AZRs.

We assessed differences among experimental treatments in the organic matter content and macrofaunal communities of sediments in October 2010, the middle of the Austral spring and 3 mo after the start of the experiment. This experimental duration of 3 mo was considered long enough to allow recolonization by infaunal invertebrates, both through migration from adjacent seagrass beds and recruitment (Guerra-García and García-Gómez, 2006). We hypothesised that not only would the AZU influence macrofaunal communities through provision of habitat structure but also by facilitating accumulation of organic matter.

A 10-cm deep sediment core of 3 cm inner diameter was taken from each experimental plot for assessment of sediment organic matter (OM) content using the loss on ignition method. Specifically, samples were homogenised and subsamples of about 3 g wet

weight were dried at 105°C for 48 h and then combusted at 550°C for 4 h. Organic matter was calculated as the percent difference between the two measurements.



**Fig. 3.1** Diagram of artificial *Zostera* rhizomes. The length of the main strand was 1.63 m for the low and 3.08 m for the high density artificial *Zostera* rhizomes. The rope was laid in a horizontal plane 30-50 mm below the surface of the sediment.

We sampled macrofauna by collecting a single 15-cm deep and 10 cm-diameter sediment core from the centre of each plot. For plots receiving an AZR, the rope falling within the core was gently removed and placed in the same bag as the sediment. In the laboratory, AZR, *Z. muelleri* rhizomes and sediment were carefully rinsed over a 0.5 mm mesh sieve and the retained material was fixed in 7% formalin. Shoots and associated fauna were removed from the cores containing natural *Z. muelleri* and disposed of because this experiment was only concerned with the below-ground structure of seagrass and how it influences associated infauna. The remaining core of below-ground material was gently washed over the sieve to

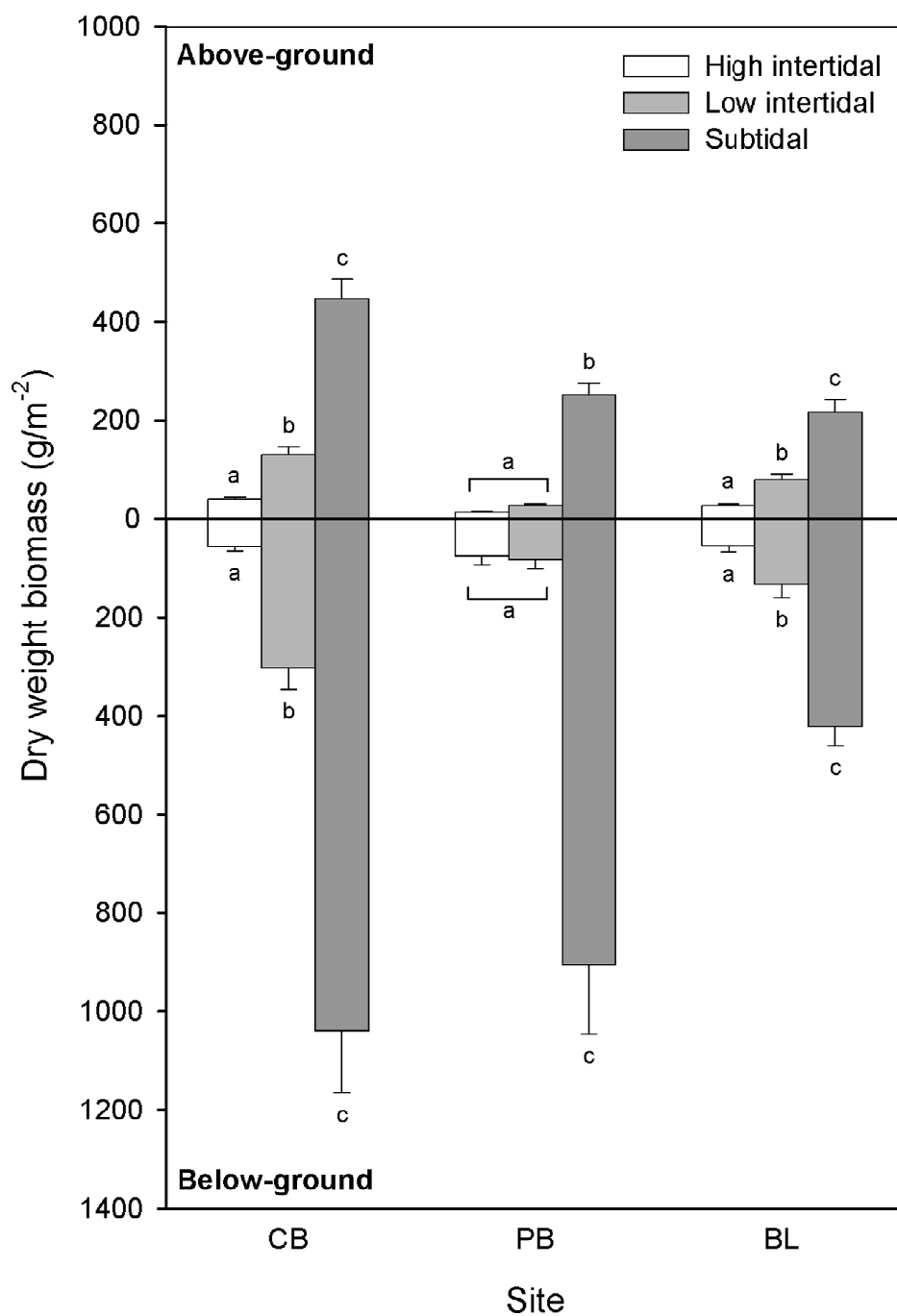
separate roots from fauna and sediment. Macroinvertebrate samples were processed as described in the field sampling section above.

Two-way PERMANOVAs tested for significant effects on infaunal communities of ‘depth’ (2 levels: intertidal, subtidal) and ‘habitat’ (4 levels: control, low density AZR, high density AZR, natural *Z. muelleri*). A 4th root transformation was applied before multivariate analyses to reduce the contribution of the numerically abundant bivalve *Mysella* sp. on the Bray-Curtis similarities (Clarke and Warwick 2001). PERMANOVAs were followed with analogous ANOVAs on macrofaunal abundance, taxon richness, taxa that were identified by SIMPER to be important contributors to multivariate differences among treatments, as well as sediment organic matter content.

### 3.4 Results

#### 3.4.1. *Zostera muelleri* biomass and associated macrofaunal community

Both the above- and below-ground biomass of *Zostera muelleri* increased with water depth (Table 3.1, Fig. 3.2). Although the effect size varied among sites (significant site x depth interaction, Table 3.1), each seagrass component was consistently of greater biomass at subtidal than intertidal depth (Fig. 3.2). Within the intertidal, the above- and below-ground components of *Z. muelleri* were of greater biomass lower than higher on the shore, at two of the three sites (Fig. 3.2). The exception was Pretty Beach, where high intertidal and low intertidal plants did not differ significantly.



**Fig. 3.2** Mean (+ 1 SE) dry weight of *Zostera muelleri* (per m<sup>2</sup>), at high intertidal, low intertidal and subtidal elevations of three study sites (CB, Careel Bay; PB, Pretty Beach; BL, Budgewoi Lake).  $n = 10$ . Letters indicate significant differences between depths, within sites (SNK tests,  $p < 0.05$ ).



**Table 3.1** Analyses (ANOVAs for univariate data; PERMANOVAs for multivariate community data) testing for differences in above- and below-ground *Zostera muelleri* biomass, epi- and infauna assemblage structure, epi- and infauna abundance and taxon richness, abundance of discriminating macrobenthic species among sites (3 levels, random) and depths (3 levels, fixed: subtidal, low intertidal, high intertidal). n = 10. The results of SNK tests are presented in Fig. 3.2 for *Z. muelleri* biomass, in Fig. 3.4 for the epi- and infaunal abundance and taxon richness and in Fig. 3.5 for discriminating species.

Source	df	Above-ground biomass <sup>a</sup>			Below-ground biomass <sup>a</sup>			Epifauna community <sup>b</sup>			Infauna community <sup>b</sup>		
		MS	F	p	MS	F	p	MS	pseudo-F	p <sub>perm</sub>	MS	pseudo-F	p <sub>perm</sub>
Site	2	9.4	47.3	≤0.001	3.4	10.0	≤0.001	9898	12.2	≤0.001	9458	6.7	≤0.001
Depth	2	48.7	42.0	0.002	51.9	23.6	0.006	11643	4.9	≤0.001	22304	10.8	≤0.001
Si × De	4	1.2	5.8	≤0.001	2.2	6.4	≤0.001	2357	2.9	≤0.001	2071	1.5	0.077
Residual	81	0.2			0.3			813			1414		
Source	df	Epifauna abundance <sup>c</sup>			Epifauna taxon richness			Infauna abundance <sup>c</sup>			Infauna taxon richness		
		MS	F	p	MS	F	p	MS	F	P	MS	F	p
Site	2	1.3	3.7	0.029	2.6	3.7	0.028	0.4	1.8	0.172	7.8	4.5	0.014
Depth	2	13.4	29.9	0.004	20.2	13.3	0.017	19.5	25.8	0.005	115.3	12.8	0.018
Si × De	4	0.4	1.2	0.301	1.5	2.1	0.082	0.7	3.1	0.020	9.0	5.2	≤0.001
Residual	81	0.4			0.7			0.2			1.7		
Source	df	<i>Nassarius burchardi</i> <sup>d</sup>			<i>Opheliidae</i> <sup>d</sup>			<i>Notomastus</i> sp. <sup>d</sup>					
		MS	F	p	MS	F	p	MS	F	P			
Site	2	16.7	11.1	≤0.001	0.3	0.2	0.797	2.19	1.1	0.335			
Depth	2	22.2	6.9	0.050	33.6	10.5	≤0.001	12.82	26.0	0.005			
Si × De	4	3.2	2.1	0.084	0.0	0.0	0.999	0.49	0.3	0.909			
Residual	81	1.5			1.4			2.0					

<sup>a</sup> data ln (x) transformed

<sup>b</sup> data square root transformed

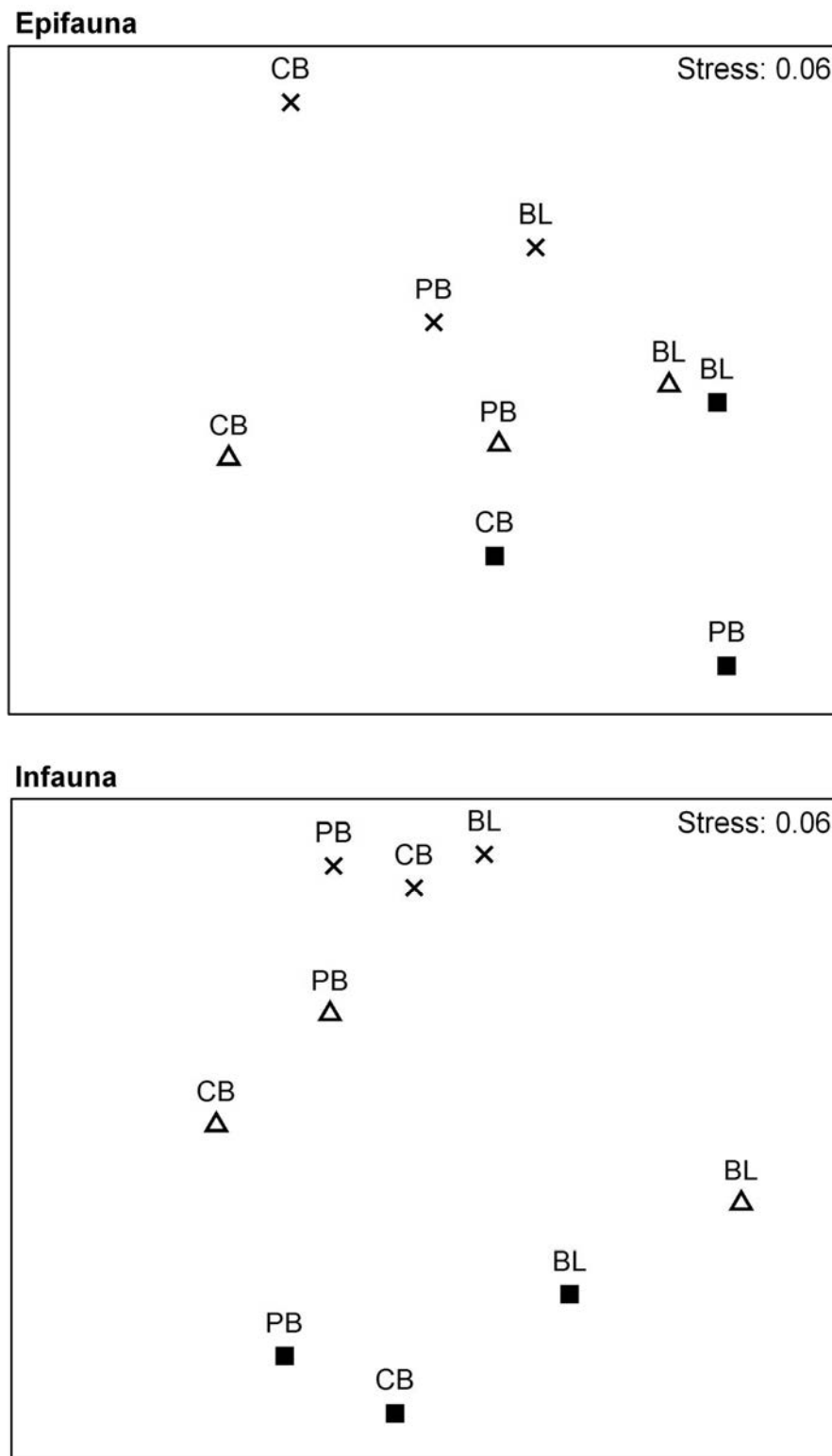
<sup>c</sup> data ln (x + 1) transformed

<sup>d</sup> data ln (x + 0.1) transformed



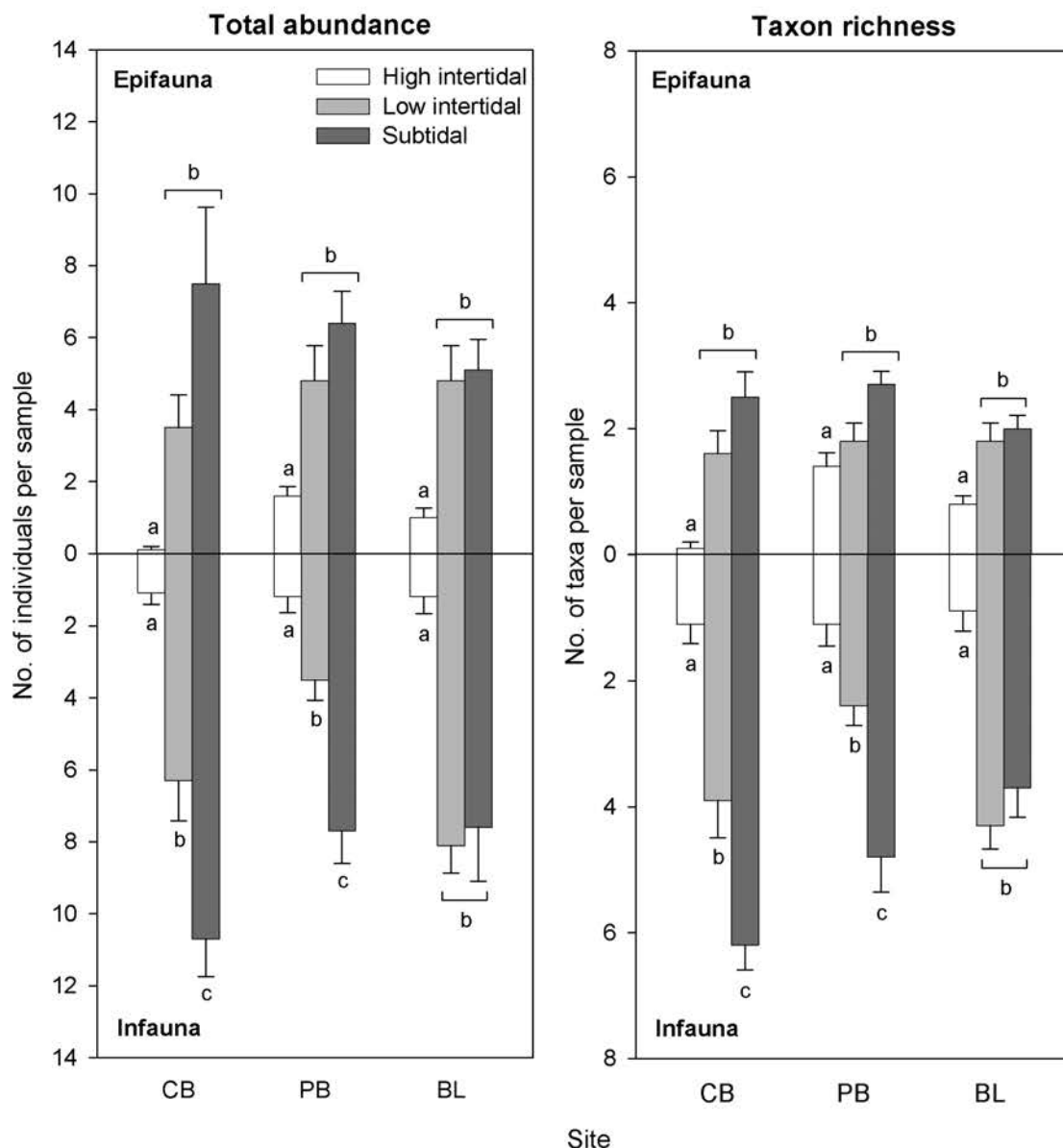
Across all three sites, a total of 12 epifaunal and 25 infaunal taxa were found in *Z. muelleri* cores. The most abundant groups were polychaetes (37%), gastropods (34%), bivalves (15%) and crustaceans (13%).

Multivariate analysis revealed a strong effect of depth on epifaunal assemblages that varied among study sites (Table 3.1). At Careel Bay, epifaunal assemblages differed among each of the three depths (Fig. 3.3). At Pretty Beach, epifaunal assemblages differed between the subtidal and higher elevations, but not between the two intertidal elevations (Fig. 3.3). In Budgewoi Lake, the high intertidal community was significantly different from the low intertidal and subtidal communities, but no significant differences were found between the latter two depths (Fig. 3.3). Infaunal assemblages, by contrast, varied similarly among depths across all sites (Table 3.1). Distinct assemblages of infauna were seen at each of the three depths (Fig. 3.3).



**Fig. 3.3** Non-metric MDS ordinations showing variation in epifaunal and infaunal communities among high intertidal (crosses), low intertidal (triangles) and subtidal (squares) elevations of three study sites (CB, Careel Bay; PB, Pretty Beach; BL, Budgewoi Lake). Points represent centroids for the average community present at each depth of each site.  $n = 10$ .

Multivariate differences in assemblages of epi- and infauna were driven by increases in both the abundance and species richness with depth (Fig. 3.4, Table 3.1). The high intertidal *Z. muelleri* had a significantly lower abundance and richness of both epifauna and infauna compared with the low intertidal and subtidal elevations (Fig. 3.4). Between the latter two elevations, however, differences in the abundance and richness of epifaunal and infaunal taxa were not always apparent (Fig. 3.4).

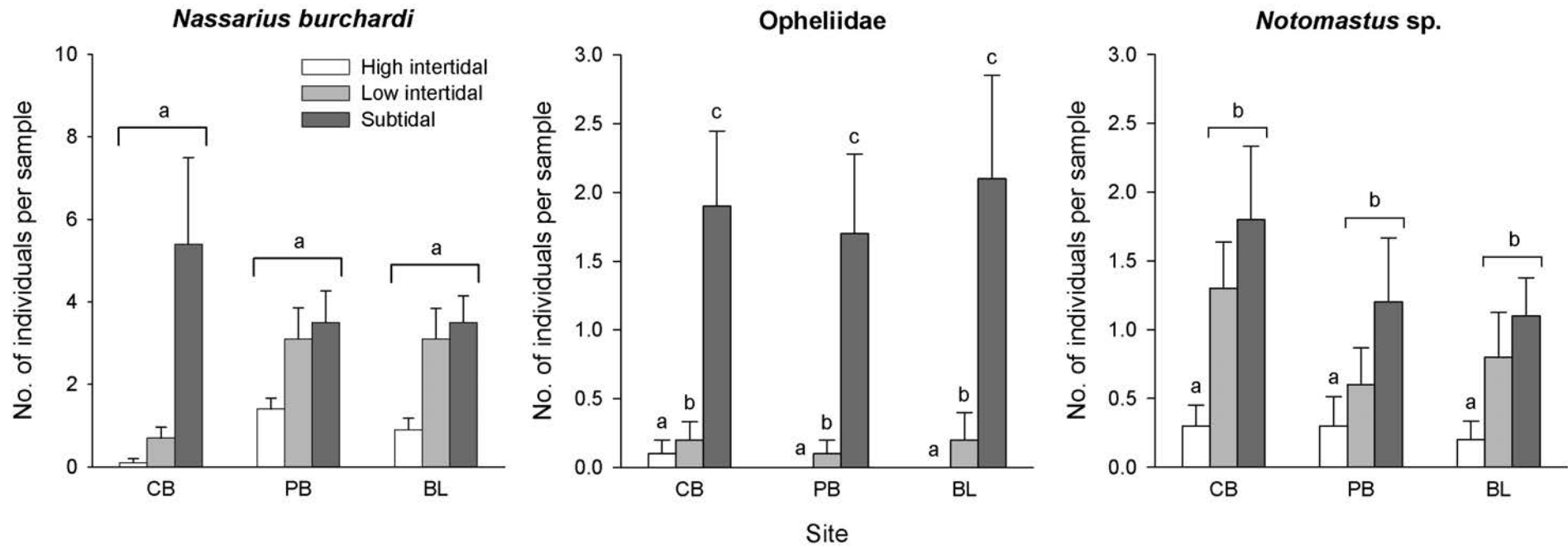


**Fig. 3.4** Mean (+ 1 SE) total abundance and taxon richness of epi- and infauna within *Zostera muelleri* beds at high intertidal, low intertidal and subtidal elevations of three study sites (CB, Careel Bay; PB, Pretty Beach; BL, Budgewoi Lake).  $n = 10$ . Letters indicate significant differences between depths, within sites (SNK tests,  $p < 0.05$ ).

The gastropod *Nassarius burchardi* was, consistently among sites, the best discriminator of epifaunal assemblages among depths ( $36.2 \pm 3.3$  % mean  $\pm$  SE] contribution to dissimilarity, SIMPER). Yet, the abundance of this taxon did not significantly differ among depths and instead displayed significant variation among sites (Table 3.1, Fig. 3.5). Species of infauna that were good discriminators among depths were opheliids and *Notomastus* sp.

polychaetes ( $10.1 \pm 2.8$  % and  $12.2 \pm 1.3$  % mean [ $\pm$ SE] contribution to dissimilarity respectively, SIMPER). Across all sites, the abundance of opheliids significantly differed among each of the tidal elevations sampled, increasing with depth (SNK tests,  $p < 0.05$ ; Fig. 3.5). The capitellid *Notomastus* sp. was significantly less abundant in the high intertidal compared with the other two depths, between which their abundance did not differ (SNK tests,  $p < 0.05$ ; Fig. 3.5).

Correlations indicated significant positive relationships between the above-ground biomass of seagrass and epifaunal abundance ( $r = 0.51$ ,  $df = 88$ ,  $p < 0.001$ ) and richness ( $r = 0.46$ ,  $df = 88$ ,  $p < 0.001$ ), and the below-ground biomass of seagrass and infaunal abundance ( $r = 0.64$ ,  $df = 88$ ,  $p < 0.001$ ) and richness ( $r = 0.67$ ,  $df = 88$ ,  $p < 0.001$ ) across the depths sampled.



**Fig. 3.5** Mean (+ 1 SE) abundance of discriminating epi- (*Nassarius burchardii* gastropod) and infaunal (opheliids and *Notomastus* sp. polychaetes) macrobenthic taxa collected in *Zostera muelleri* at three fixed depths.  $n = 10$ . Letters indicate significant difference between depths, within sites (SNK tests,  $p < 0.05$ ).



#### 3.4.2 Effect of root structure of *Z. muelleri* on the infauna along the shore gradient

A total of 31 taxa were identified in samples collected from the manipulative experiment. The most abundant group was bivalves (47%), followed by polychaetes (32%) and crustaceans (17%).

Macrofaunal communities displayed depth-dependent responses to habitat (Table 3.2). At the intertidal elevation, assemblages differed among natural *Z. muelleri*, control and AZR plots, but assemblages in low density and high density AZR plots were statistically indistinguishable (Fig. 3.6). At the subtidal elevation, assemblages within natural *Z. muelleri* plots differed from those in the other three treatments (Fig. 3.6), which supported similar assemblages to one another. At each depth, the dissimilarity between natural *Z. muelleri* and control plots was greater than between the natural *Z. muelleri* and AZR plots or between the AZR and the control plots.

**Table 3.2** Analyses (ANOVAs for univariate data; PERMANOVAs for multivariate community data) testing for differences in assemblage structure, macrofauna abundance and taxon richness, sediment organic matter content, and abundance of discriminating macrobenthic species among depths (2 levels, fixed: intertidal and subtidal) and habitat treatments (4 levels, fixed: bare sediment, low and high density artificial *Z. muelleri* units, and natural *Z. muelleri*) at the end of the manipulative experiment. n = 6. The results of SNK tests are presented in Fig. 3.7 for macrofaunal abundance and taxon richness, in Fig. 3.8 for discriminating species and in Fig. 3.9 for sediment organic matter content.

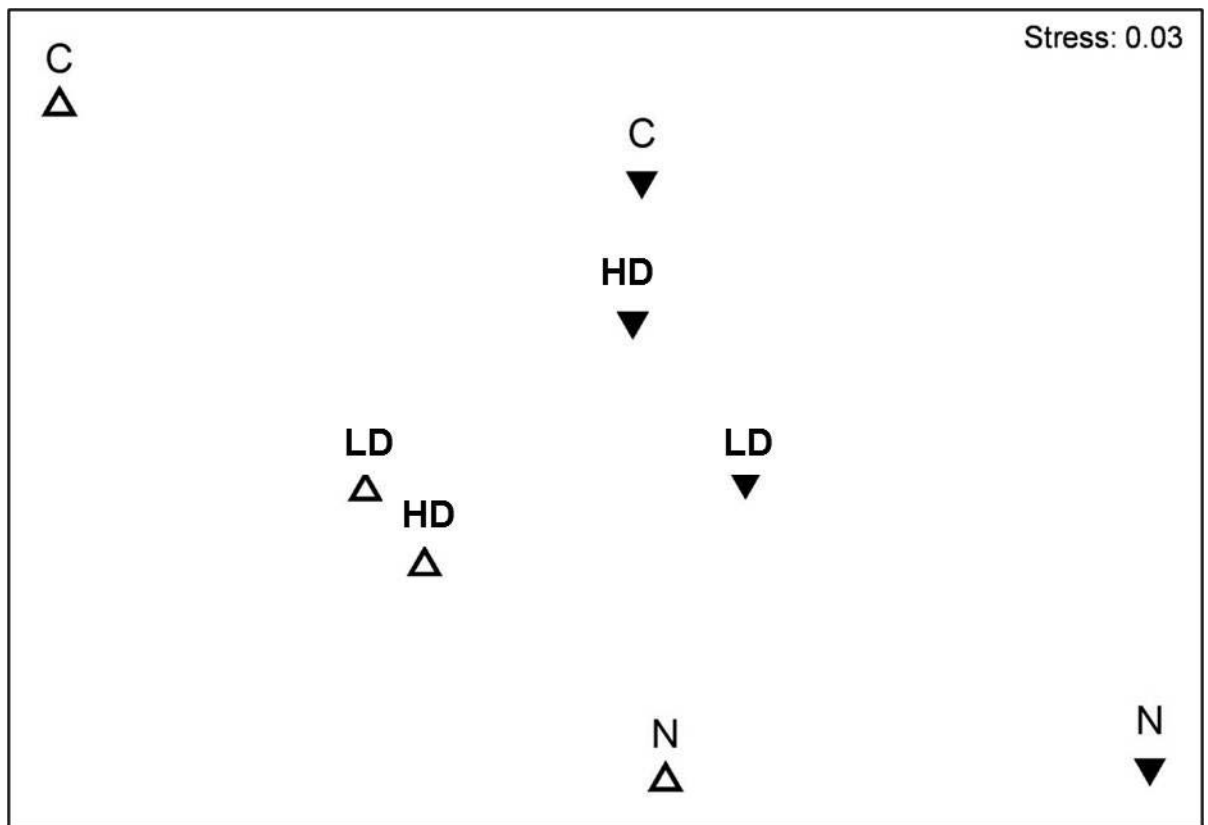
Source	df	Macrofauna community <sup>a</sup>			Macrofauna abundance			Macrofauna taxon richness			<i>Mysella</i> sp. <sup>b</sup>		
		MS	<i>F</i>	<i>p</i> <sub>perm</sub>	MS	<i>F</i>	<i>p</i>	MS	<i>F</i>	<i>p</i>	MS	<i>F</i>	<i>p</i>
Depth	1	7031	6.2	≤0.001	972	13.9	≤0.001	16.3	5.7	0.0222	8.6	5.0	0.030
Habitat	3	6190	5.2	≤0.001	242	3.5	0.025	31.7	11.0	≤0.001	20.1	11.7	≤0.001
De × Ha	3	3105	2.9	≤0.001	747	10.7	≤0.001	19.7	6.8	≤0.001	1.4	0.8	0.481
Residual	40	975			70			2.9			1.7		
<hr/>													
Source	df	Gammaridae <sup>c</sup>			Callianassidae			Notomastus sp. <sup>c</sup>			Nephtyidae <sup>c</sup>		
		MS	<i>F</i>	<i>p</i>	MS	<i>F</i>	<i>p</i>	MS	<i>F</i>	<i>p</i>	MS	<i>F</i>	<i>p</i>
Depth	1	0.4	0.9	0.350	0.0	0.0	0.880	1.6	6.9	0.012	2.9	11.2	0.002
Habitat	3	3.1	6.2	0.002	2.2	2.4	0.082	2.5	11.2	≤0.001	0.4	1.6	0.198
De × Ha	3	0.6	1.2	0.307	0.6	0.6	0.599	0.6	2.8	0.050	0.1	0.5	0.678
Residual	40	0.5			0.9			0.2			0.3		
<hr/>													
Source	df	Magelonidae <sup>c</sup>			Oligochaeta <sup>c</sup>			Organic matter					
		MS	<i>F</i>	<i>p</i>	MS	<i>F</i>	<i>p</i>	MS	<i>F</i>	<i>p</i>			
Depth	1	0.9	6.1	0.018	0.2	0.6	0.444	0.0	0.1	0.765			
Habitat	3	0.7	5.0	0.005	1.0	2.5	0.070	3.3	54.2	≤0.001			
De × Ha	3	0.3	2.4	0.080	0.3	0.8	0.519	0.9	15.5	≤0.001			
Residual	40	0.1			0.4			0.1					

<sup>a</sup> data fourth root transformed

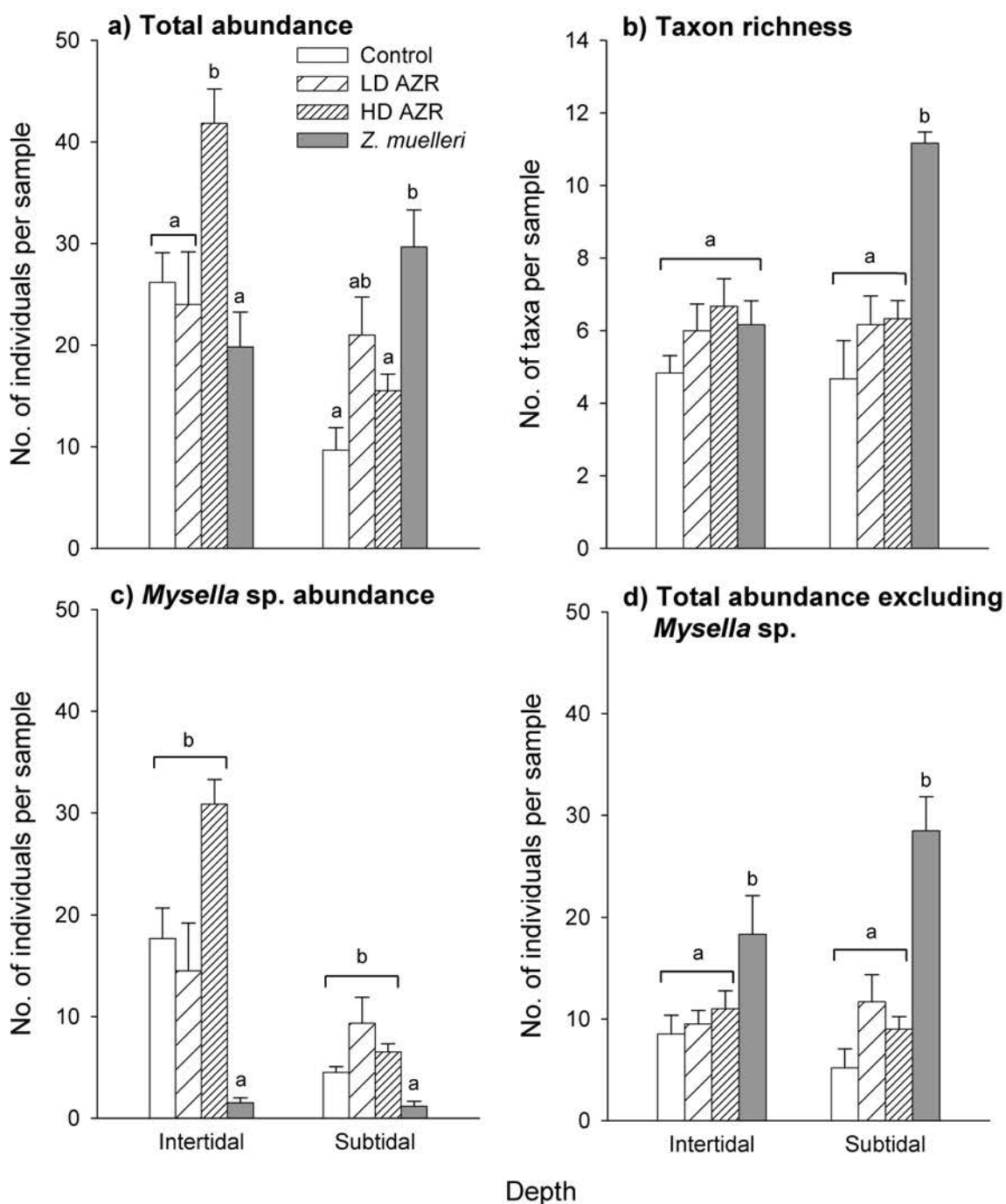
<sup>b</sup> data ln (x + 0.1) transformed

<sup>c</sup> data ln (x + 1) transformed

The total abundance and the species richness of macrofauna were affected by position on the shore, habitat, and an interaction between the two factors (Table 3.2; Fig. 3.7). At the intertidal elevation, plots with high density AZR contained more macrofauna than control, low density AZR or natural *Z. muelleri* plots (Fig. 3.7). At the subtidal depth, by contrast, plots with natural *Z. muelleri* contained more macrofauna than high density AZR or bare plots and, on average, had a similar macrofaunal abundance to the low density AZR plots (Fig. 3.7). Spatial patterns in total abundance were, however, highly modified by the bivalve *Myssella* sp., which accounted for 46% of all macrofauna ( $17.2 \pm 2.8$  % mean [ $\pm$ SE] contribution to dissimilarity, SIMPER). At each of the depths, *Myssella* sp. was much less abundant in natural *Z. muelleri* plots than in all other habitats (Table 3.2, Fig. 3.7). Excluding *Myssella* sp. from analyses of total abundance substantially modified their outcome. The cumulative abundance of all other taxa was much greater in natural *Z. muelleri* plots than in the other treatments at each of the depths (Table 3.2, Fig. 3.6). The taxon richness of infauna was greater in the natural subtidal *Z. muelleri* plots than in any of the other three treatments, irrespective of depth (Fig. 3.6). By contrast, at each depth, the AZR treatments supported a similar number of taxa to the control plots (Fig. 3.6).

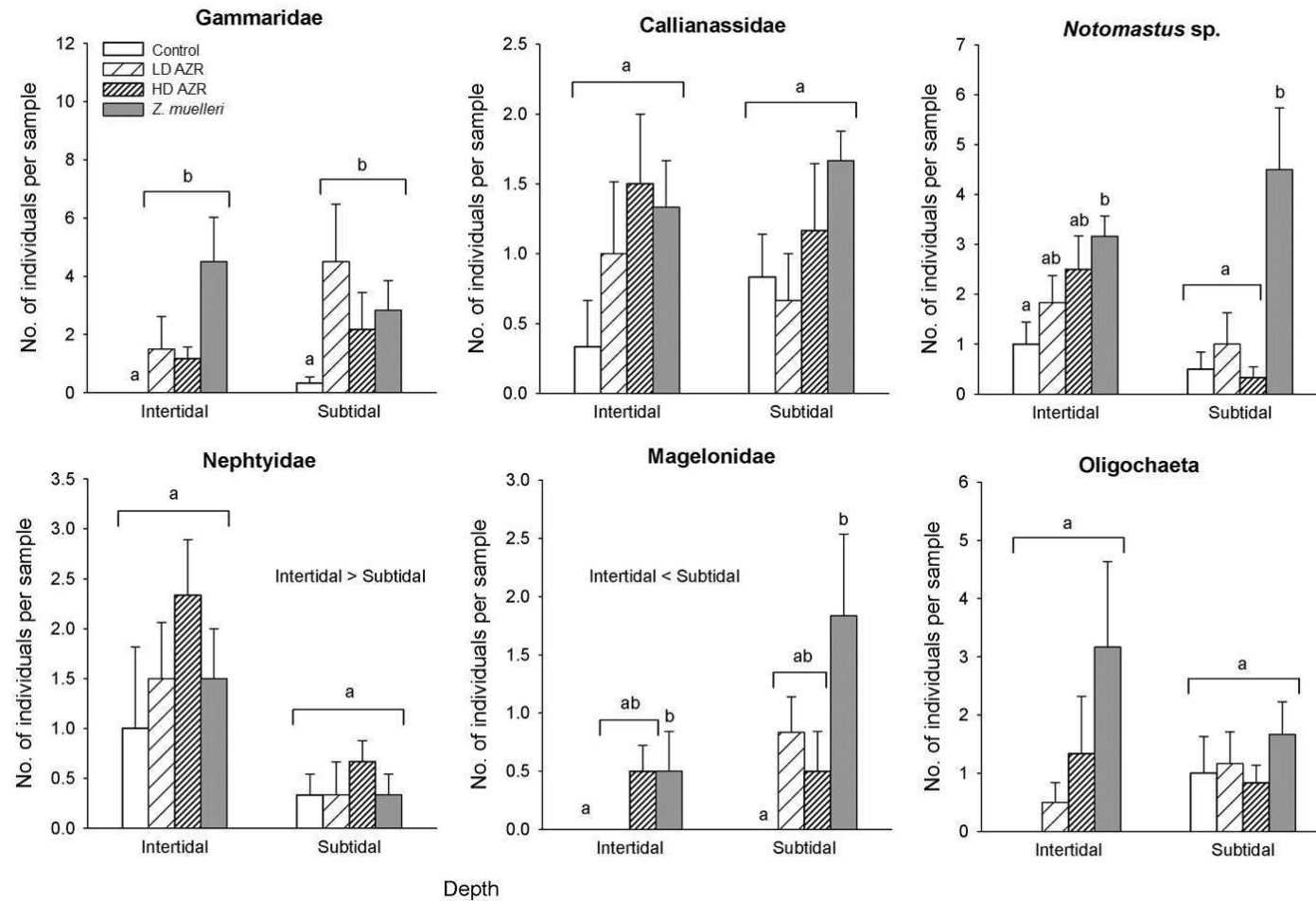


**Fig. 3.6** Non-metric MDS ordination of macrofaunal assemblages sampled in control (C), low density (LD) and high density (HD) artificial *Zostera* and natural *Zostera muelleri* plots (N) at low intertidal (empty triangle) and subtidal (solid triangle) depths at the end of the manipulative experiment. Points represent treatment centroids.  $n = 6$ .



**Fig. 3.7** Mean (+ 1 SE) (a) total abundance and (b) taxon richness of macrofauna (including *Mysella* sp.), (c) number of *Mysella* sp. and (d) total abundance of macrofauna excluding *Mysella* sp. in plots of bare sediment (control), low density (LD) and high density (HD) artificial *Zostera* rhizomes (AZR) and natural *Zostera muelleri*, at intertidal and subtidal depths, at the end of the manipulative experiment.  $n = 6$ . Letters indicate significant differences among treatments within depths (SNK tests,  $p < 0.05$ ).

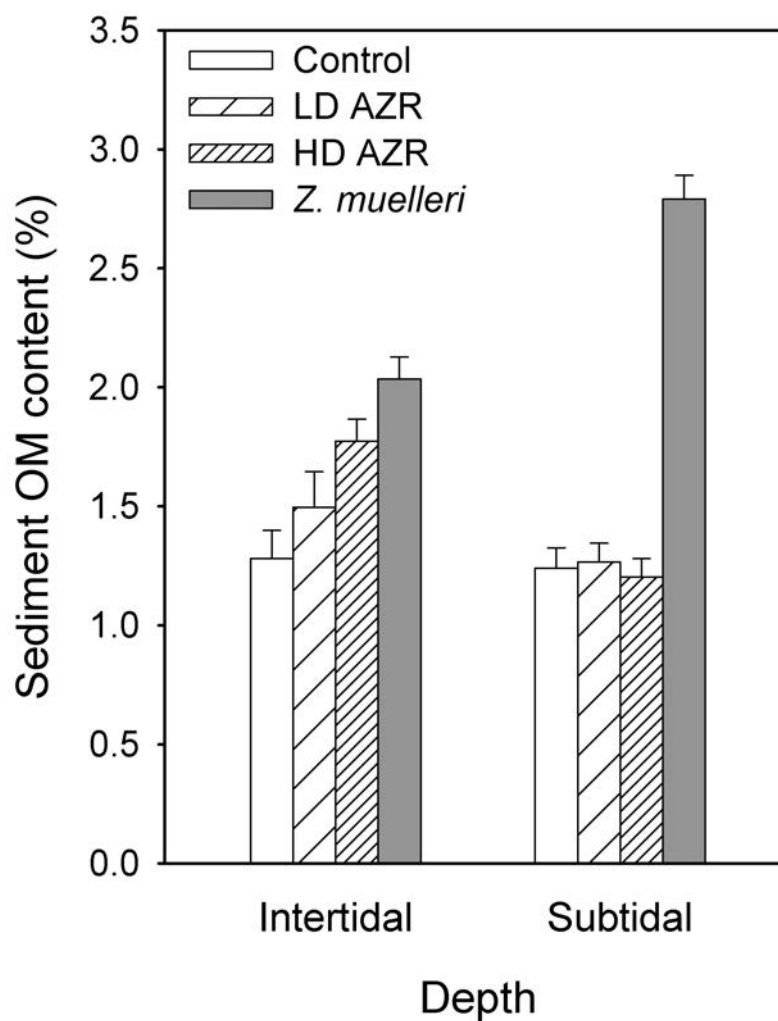
In addition to the bivalve *Mysella* sp., gammarids ( $9.7 \pm 1.1$  % mean [ $\pm$ SE] contribution to dissimilarity), callianassid shrimp ( $5.8 \pm 0.5$  %), *Notomastus* sp. ( $7.4 \pm 0.6$  %), nephtyid ( $5.9 \pm 1.0$  %) and magelonid ( $5.1 \pm 0.6$  %) polychaetes and oligochaetes ( $6.7 \pm 0.9$  %) were the main contributors to multivariate differences among habitats, within each depth (dissimilarity to standard deviation ratio  $> 1$ , SIMPER). Further investigation of the spatial distribution of these groups through two-way ANOVA revealed that the abundances of some of the discriminating species were affected by habitat (Gammaridae) or depth (Nephtyidae) alone, but others were influenced by a combination of both factors (*Mysella* sp., *Notomastus* sp. Magelonidae) (Table 3.2; Fig 3.8 for SNK tests). Two of the discriminating taxa, callianassids and oligochaetes, were not affected by either depth or habitat (Table 3.2).



**Fig. 3.8** Mean (+ 1 SE) abundance of discriminating macrobenthic taxa collected from plots of bare sediment (control), low density (LD) and high density (HD) artificial *Zostera* rhizomes (AZR) and natural *Zostera muelleri*, at intertidal and subtidal depths, at the end of the manipulative experiment.  $n = 6$ . Letters indicate significant differences among treatments within depths (SNK tests,  $p < 0.05$ ).

Habitat had depth-dependent effects on sediment organic matter content (Table 3.2, Fig. 3.9). At the intertidal elevation, low and high AZR contained similar amount of organic matter compared with natural *Z. muelleri* plots. Between the two artificial *Zostera* treatments, the high density AZR accumulated the greater amount and its mean sediment organic matter content was significantly higher than control plots (Fig. 3.9). However, in the subtidal, where the organic matter content of natural *Z. muelleri* was greater than in the intertidal, the presence of AZR did not have any effect on the sediment organic matter content. Sediment in natural *Z. muelleri* plots had significantly higher organic matter content than in any of the other treatments (Fig. 3.7).





**Fig. 3.9** Mean (+ 1 SE) sediment organic matter content (%) at the end of the manipulative experiment in plots of bare sediment (control), low density (LD) and high density (HD) artificial *Zostera* rhizome (AZR) and natural *Zostera muelleri*, at low intertidal and subtidal depths. Letters indicate significant differences among treatments within depths (SNK tests,  $p < 0.05$ ).

### 3.5 Discussion

It is well established that tidal inundation is a major driver of spatial variation in the benthic invertebrate communities of unvegetated sediments (Ysebaert and Herman, 2002; Edgar and Barrett, 2002). Nevertheless, it had not previously been determined how co-variation in the morphology of seagrass across such gradients might modify the response of seagrass-dwelling invertebrates to depth. In this study, we found that tidal inundation affected spatial patterning of seagrass-dwelling epi- and infauna, but this was strongly correlated to spatial variation in the above-and below-ground biomass of *Z. muelleri* across the tidal elevation gradient. A subsequent experiment partitioned effects of tidal elevation on seagrass-dwelling infauna into effects arising from changes in seagrass root morphology and environmental context. This experiment revealed that neither depth nor the structure of roots alone could explain tidal elevation gradients in invertebrate community composition, although certain taxa responded to one factor more strongly than the other.

Our assessments of spatial variation in the above- and below-ground biomass of *Z. muelleri* revealed that as for other *Zostera* spp. (e.g. Boese et al., 2005; Cabaço et al., 2009), the Australasian species displayed a strong pattern of decreasing biomass with increasing elevation from the shallow subtidal to the high intertidal (see also Nicastro et al., 2012). Previous studies have implicated photo-inhibition at high light intensities and desiccation stress in contributing to this pattern (Silva and Santos, 2003; Boese et al., 2005). In addition, other environmental parameters such as sediment grain size, hydrodynamic disturbance, and temperature, each of which also vary with elevation, might contribute to this pattern. Consistent with the strong tidal elevation gradient in seagrass above-and below-ground biomass, both epifauna and infauna showed marked differences in community composition and an overall pattern of increasing abundance and taxon richness with increasing depth.

In the manipulative experiment, focussing specifically on infauna, we found, however, that not one taxon responded differentially to artificial *Zostera* rhizomes mimicking the

structure of subtidal and intertidal *Zostera*. Instead, where responses to structure were seen, they occurred independently of the amount of structure provided and were often specific to either intertidal or subtidal elevations. In the intertidal, the addition of structure to unvegetated sediments resulted in infaunal assemblages becoming more similar to those within natural intertidal seagrass, but in the subtidal, the addition of structure had little effect on community composition. In the intertidal, the effect of structure on infaunal assemblages was driven by an enhancement of taxon richness, rather than an enhancement of abundance. Differences in the response of infaunal communities to structure between intertidal and subtidal elevations reinforces the dependence of facilitative interactions on the abiotic and biotic context (Bertness and Callaway, 1994). The provision of the structure may be more important in the intertidal due to its role in reducing desiccation stress or in reducing foraging efficiency of shorebirds that forage at low tide.

Taxa could be divided into those that responded to 1) differences in environmental and biotic conditions within each habitat, 2) tidal inundation or 3) a combination of the two factors. In line with previous observations of effects of vegetation on their abundance (Orth et al., 1984; Rossi and Underwood, 2002), gammarid amphipods were among the taxa to respond to structure. At each of the tidal elevations, greater abundances of gammarids were found in structured than unstructured habitat, irrespective of whether the structure came from AZRs or natural seagrass. Seagrass is known to provide food, in the way of live and dead seagrass and epiphytic algae to gammarids (Bolam et al. 2000). In our study, an increase in the stability of the sediment, possibly enhancing the growth of benthic microalgae and deposition of detrital material, may have facilitated high abundances of gammarids in the AZRs and plots of natural seagrass alike.

Abundances of nephtyid polychaetes, by contrast, were primarily determined by tidal elevation. Nephtyids are known to tolerate a wide range of environmental conditions (Beukema, 1987; Arndt and Schiedek, 1997) and inhabit both unvegetated and seagrass

habitats (Hutchings and Recher, 1974). A higher abundance in the intertidal may be the result of a higher prey availability for this predatory taxon, although total abundance of invertebrates was less in the intertidal than the subtidal, or a stronger predation pressure by infaunal or epibenthic predators in the subtidal. Their greater abundance in this study in the intertidal than at the subtidal elevation is consistent with previous observations that nephtyids were the most abundant polychaetes in the intertidal *Z. muelleri* habitat of Careel Bay (Hutchings and Recher, 1974).

The capitellid *Notomastus* sp., magelonid polychaetes and the bivalve *Mysella* sp., were among the taxa that displayed patterns of abundance that reflected both effects of depth and *Zostera* sp. root structure. In the case of the deposit-feeding *Notomastus* sp., there was an interaction between the two factors, habitat and depth, which reflected the differing efficacy of the AZRs in trapping organic matter between intertidal and subtidal elevations. Across all treatments, the abundance of *Notomastus* sp. was closely correlated to organic matter content of the sediments, with the AZR accumulating more organic matter in the intertidal than in the subtidal. The abundance of magelonids increased with depth and was greater in natural *Zostera* than AZRs, and more abundant in AZRs than in unvegetated sediments. The presence of AZR may have facilitated the burrowing and feeding activity of surface deposit feeding magelonids (Fauchald and Jumars, 1979) by increasing the stability of the sediment and the deposition of food particles (see Omena and Creed, 2004). An overall higher density of magelonids in the subtidal may be the result of reduced desiccation stress for these surface-dwelling species. The bivalve *Mysella* sp. was much more abundant in bare sand and AZR than in natural *Z. muelleri*, and in the intertidal than the subtidal. In other studies, the abundance of *Mysella* sp. has been negatively correlated with the content of silt and clay in the sediment (Macfarlane and Booth, 2000) and positively correlated with the redox potential (Kerr and Corfield, 1998). Although we did not analyse sediment characteristics other than organic matter content, we suspect that these patterns may reflect differences in sediment

grain size and redox potential among treatments. We expect that the mean grain size and redox potential of sediments would be lower in plots with than without root structure to trap and bind small particles. Similarly, we expect that mean grain size and redox potential would be less in the subtidal than the intertidal due to the greater reworking of sediments by waves at shallower depth.

The failure of the AZR to fully replicate the effects of live seagrass on macrobenthic fauna may reflect differences in the structure of our AZRs as compared to natural seagrass, the duration of the experiment, the low mobility of adult infaunal organisms or important biological functions of live seagrasses that were not reproduced. First, the AZRs mimicked only the below-ground physical structure of *Z. muelleri*, not the above ground structure. Previous studies have shown that artificial seagrass units (ASUs) manipulating only the above-ground structure of seagrasses can also influence sediment properties through the trapping of organic matter and fine particles (Almasi et al., 1987; Boström and Bonsdorff, 2000). It is possible that if we had simultaneously manipulated above-ground structure, stronger effects on infauna may have been seen. Second, the 3 mo duration of our experiment may not have been adequate for some taxa to recruit and the full effects of post-settlement mortality to be seen in others. Third, areas of bare sand separating natural and artificial *Zostera* plots may have impeded the movement and colonisation of low mobile species. In fact, gammarids, one of the most mobile taxon found in here, did respond to the structuring effect of AZR. Fourth, in manipulating only the structure of roots, we did not reproduce other important features of seagrass habitats, such as detritus production, associated epiphytes, and excretion of organic compounds and oxygen, which can have a considerable influence on the biodiversity of the associated fauna. Nevertheless, results from this study suggest that interactions between rhizome structure and depth do make a contribution to the differences in macrofaunal assemblage structure between intertidal and subtidal elevations. Future crossed-design experiments that simultaneously manipulate root structure, using AZRs, and shoot

structure, using ASUs, may lead to major advances in how the above- and below-ground structure of seagrasses interact to influence associated invertebrate communities.

Overall, our study indicates that processes, such as coastal squeeze and eutrophication, that alter the depth at which seagrasses can grow will have large impacts on the structure of associated faunal communities. We have shown that tidal inundation strongly affects the spatial distribution of macroinvertebrates associated with *Z. muelleri*, both directly and indirectly via a depth-dependence of the role of seagrass root structure. Interestingly, we did not find evidence of taxa that were limited to the intertidal habitat, which is predicted to be lost from some highly urbanised estuaries under sea-level rise. Nevertheless, we do not exclude a possible reduction under sea level rise of the biodiversity of macroinvertebrates occupying *Z. muelleri* habitats. Not only may artificial seawalls, by obstructing the inland migration of the coastline, reduce the area of intertidal habitat available to *Z. muelleri*, but they might also lead to loss of subtidal *Z. muelleri* meadows where increasing water depth is coupled with a decline in water clarity. Any loss of *Z. muelleri* and associated fauna will have important consequences for higher trophic levels. Therefore, future changes in tidal inundation regimes should be taken into account in developing conservation plans for protecting seagrasses and their important associated biodiversity.

### 3.6 References

- Almasi, M.N., Hoskin, C.M., Reed, J.K., Milo, J., 1987. Effects of natural and artificial *Thalassia* on rates of sedimentation. *Journal of Sedimentary Petrology* 57, 901-906.
- Anderson, M. J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26, 32-46.
- Arndt, C., Schiedek, D., 1997. *Nephtys hombergii*, a free-living predator in marine sediments: energy production under environmental stress. *Marine Biology* 129, 643-650.
- Bell, J.D., Westoby, M., 1986a. Abundance of macrofauna in dense seagrass is due to habitat preference, not predation. *Oecologia* 68, 205-209.
- Bell, J.D., Westoby, M., 1986b. Variation in seagrass height and density over a wide spatial scale: Effects on common fish and decapods. *Journal of Experimental Marine Biology and Ecology* 104, 275-295.
- Berry, D.A., 1987. Logarithmic Transformations in ANOVA. *Biometrics* 43, 439-456.
- Bertness, M.D., Callaway, R., 1994. Positive interactions in communities. *Trends in Ecology and Evolution* 9, 191-193.
- Beukema, J.J., 1987. Influence of the predatory polychaete *Nephtys hombergii* on the abundance of other polychaetes. *Journal of Experimental Marine Biology and Ecology* 40, 95-101.

- Boese, B.L., Robbins, B.D., Thursby, G., 2005. Desiccation is a limiting factor for eelgrass (*Zostera marina* L.) distribution in the intertidal zone of a northeastern Pacific (USA) estuary. *Botanica Marina* 48, 274-283.
- Bolam, S.G., Fernandes, T.F., Read, P., Raffaelli, D., 2000. Effects of macroalgal mats on intertidal sandflats: an experimental study. *Journal of Experimental Marine Biology and Ecology* 249, 123-137.
- Boström, C., Bonsdorff, E., 2000. Zoobenthic community establishment and habitat complexity - the importance of seagrass shoot-density, morphology and physical disturbance for faunal recruitment. *Marine Ecology-Progress Series* 205, 123-138.
- Boström, C., Jackson, E.L., Simenstad, C.A., 2006. Seagrass landscapes and their effects on associated fauna: A review. *Estuarine, Coastal and Shelf Science* 68, 383-403.
- Bulleri, F., Chapman, M.G., 2010. The introduction of coastal infrastructure as a driver of change in marine environments. *Journal of Applied Ecology* 47, 26-35.
- Cabaço, S., Machas, R., Santos, R., 2009. Individual and population plasticity of the seagrass *Zostera noltii* along a vertical intertidal gradient. *Estuarine, Coastal and Shelf Science* 82, 301-308.
- Clarke, K. R., Warwick R. M., 2001. Changes in marine communities: an approach to statistical analysis and interpretation. 2nd edition. PRIMER-E Ltd, Plymouth.



- Clarke, K.R., Somerfield, P.J., Chapman, M.G., 2006. On resemblance measures for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray-Curtis coefficient for denuded assemblages. *Journal of Experimental Marine Biology and Ecology* 330, 55-80.
- Connolly, R.M., Hindell, J.S., 2006. Review of nekton patterns and ecological processes in seagrass landscapes. *Estuarine, Coastal and Shelf Science* 68, 433-444.
- de Boer, W.F., 2007. Seagrass-sediment interactions, positive feedbacks and critical thresholds for occurrence: a review. *Hydrobiologia* 591, 5-24.
- Dethier, M.N., Schoch, G.C., 2006. Taxonomic sufficiency in distinguishing natural spatial patterns on an estuarine shoreline. *Marine Ecology-Progress Series* 306, 41-49.
- Doody, J.P., 2004. 'Coastal squeeze' - an historical perspective. *Journal of Coastal Conservation* 10, 129-138.
- Edgar G.J., Barrett N.S., 2002. Benthic macrofauna in Tasmanian estuaries: scales of distribution and relationships with environmental variables. *Journal of Experimental Marine Biology and Ecology* 270, 1-24.
- Ehlers, A., Worm, B., Reusch, T.B.H., 2008. Importance of genetic diversity in eelgrass *Zostera marina* for its resilience to global warming. *Marine Ecology-Progress Series* 355, 1-7.
- Fauchald, K., Jumars, B.A., 1979. The diet of worms: a study of polychaete feeding guilds. *Oceanography and Marine Biology Annual Review* 17, 193-284.

Guerra-García J.M., García-Gómez, J.C., 2006. Recolonization of defaunated sediments: fine versus gross sand and dredging versus experimental trays. *Estuarine, Coastal and Shelf Science* 68, 328-342.

Hansen, J.W., Udy, J.W., Perry, C.J., Dennison, W.C., Lomstein, B.A., 2000. Effect of the seagrass *Zostera capricorni* on sediment microbial processes. *Marine Ecology-Progress Series* 199, 83-96.

Heck, K.L., Orth, R.J., 1980. Seagrass habitats: the roles of habitat complexity, competition and predation in structuring associated fish and motile macroinvertebrate assemblages. Academic Press, New York.

Heck, K.L., Hays, G., Orth, R.J., 2003. Critical evaluation of the nursery role hypothesis for seagrass meadows. *Marine Ecology-Progress Series* 253, 123-136.

Hemminga, M., Duarte, C.M., 2000. *Seagrass Ecology*. Cambridge University Press, Cambridge, UK.

Hindell, J.S., Jenkins, G.P., Keough, M.J., 2000. Evaluating the impact of predation by fish on the assemblage structure of fishes associated with seagrass (*Heterozostera tasmanica*) (Martens ex Ascherson) den Hartog, and unvegetated sand habitats. *Journal of Experimental Marine Biology and Ecology* 255, 153-174.

Horinouchi, M., 2007. Review of the effects of within-patch scale structural complexity on seagrass fishes. *Journal of Experimental Marine Biology and Ecology* 350, 111-129.

Hovel, K.A., Lipcius, R.N., 2001. Habitat fragmentation in a seagrass landscape: Patch size and complexity control blue crab survival. *Ecology* 82, 1814-1829.

Hutchings, P.A., Recher, H.F., 1974. The Fauna of Careel Bay with comments on the ecology of mangrove and sea-grass communities. *Australian Zoologist* 18, 99-128.

Kerr, G., Corfield, J., 1998. Association between the ghost shrimp *Trypaea australiensis* Dana 1852 (Crustacea: Decapoda) and a small deposit-feeding bivalve *Mysella vitrea* Laserson 1956 (Mollusca: Leptonidae). *Marine and Freshwater Research* 49, 801-806.

Larkum, A.W.D., Collett, L.C., Williams, R.J., 1984. The standing stock, growth and shoot production of *Zostera capricorni* Aschers. in Botany Bay, New South Wales, Australia. *Aquatic Botany* 19, 307-327.

Lee, S.Y., Fong, C.W., Wu, R.S.S., 2001. The effects of seagrass (*Zostera japonica*) canopy structure on associated fauna: a study using artificial seagrass units and sampling of natural beds. *Journal of Experimental Marine Biology and Ecology* 259, 23-50.

MacFarlane, G.R., Booth, D.J., 2001. Estuarine macrobenthic community structure in the Hawkesbury River, Australia: relationships with sediment physicochemical and anthropogenic parameters. *Environmental Monitoring and Assessment* 72, 51-78.

Marbà, N., Duarte, C.M., 2010. Mediterranean warming triggers seagrass (*Posidonia oceanica*) shoot mortality. *Global Change Biology* 16, 2366-2375.

- Massa, S., Arnaud-Haond, S., Pearson, G., Serrão, E., 2009. Temperature tolerance and survival of intertidal populations of the seagrass *Zostera noltii* (Hornemann) in Southern Europe (Ria Formosa, Portugal). *Hydrobiologia* 619, 195-201.
- McNeill, S.E., Fairweather, P.G., 1993. Single large or several small marine reserves? An experimental approach with seagrass fauna. *Journal of Biogeography* 20, 429-440.
- Micheli, F., Bishop, M.J., Peterson, C.H., Rivera, J., 2008. Alteration of seagrass species composition and function over two decades. *Ecological Monographs* 78, 225-244.
- Mills, V.S., Berkenbusch, K., 2009. Seagrass (*Zostera muelleri*) patch size and spatial location influence infaunal macroinvertebrate assemblages. *Estuarine, Coastal and Shelf Science* 81, 123-129.
- Nicastro, A., Onoda, Y., Bishop, M.J., 2012. Direct and indirect effects of tidal inundation on eelgrass decomposition. *Marine Ecology-Progress Series. Marine Ecology Progress Series* 456, 53-62.
- Omena, E., Creed, J.C., 2004. Polychaete fauna of seagrass beds (*Halodule wrightii* Ascherson) along the coast of Rio de Janeiro (Southeast Brazil). *Marine Ecology* 25, 273-288.
- Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., Short, F.T., Waycott, M., Williams, S.L., 2006. A global crisis for seagrass ecosystems. *Bioscience* 56, 987-996.

Peterson, C.H., Luetlich, R.A.Jr, Micheli, F., Skilleter, G.A., 2004. Attenuation of water flow inside seagrass canopies of differing structure. *Marine Ecology-Progress Series* 268, 81-92.

Rossi, F., Underwood, A.J., 2002. Small-scale disturbance and increased nutrients as influences on intertidal macrobenthic assemblages: experimental burial of wrack in different intertidal environments. *Marine Ecology-Progress Series* 241, 29-39.

Short, F.T., Neckles, H.A., 1999. The effects of global climate change on seagrasses. *Aquatic Botany* 63, 169-196.

Silva, J., Santos, R., 2003. Daily variation patterns in seagrass photosynthesis along a vertical gradient. *Marine Ecology-Progress Series* 257, 37-44.

Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., Williams, S.L., 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences* 106, 12377-12381.

Ysebaert, T., Herman, P.M.J., 2002. Spatial and temporal variation in benthic macrofauna and relationships with environmental variables in an estuarine, intertidal soft-sediment environment. *Marine Ecology Progress Series* 244, 105-124.



**4 Sources of spatial variation in estuarine macrofaunal communities across a latitudinal gradient**

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#### 4.1 Abstract

Estuaries are highly productive environments and provide essential ecosystem services. Estuarine biodiversity and the goods and services it provides are, however, increasingly under threat from a combination of local and global stressors. In order to understand how future climate warming in combination with nutrient enrichment might impact estuarine soft sediment invertebrate communities, we examined how: (1) intertidal macrofaunal assemblages varied among temperate south-east Australian estuaries in relation to their latitude and nitrogen enrichment and (2) how these relationships were modified by local environmental context. To test these hypotheses, we sampled invertebrates from intertidal sediments in sixteen estuaries along the temperate coast of New South Wales, Australia, spanning a latitudinal gradient of 7° (or ~ 4°C difference in sea surface temperature), and experiencing varying degrees of diffuse nutrient loading. Multivariate multiple regression revealed that sediment grain size was the best predictors of macrofaunal assemblage composition. When samples were stratified according to median grain size, relationships between faunal communities and nitrogen loading and latitude emerged, but only among estuaries with sandier sediments. In these estuaries, capitellid and nereid polychaetes and chironomid larvae were the taxa that showed stronger correlations with nutrient loading. Low levels of nutrient enrichment and natural oligotrophic conditions characteristic of southeast Australian estuaries may explain the low predicting power of nitrogen loading in explaining macroinvertebrate communities. The weak correlation between climatic context and macrofaunal assemblages may reflect the adaptation of macrofaunal species to variable environmental conditions that characterize estuarine habitats. Despite the weak and idiosyncratic correlations observed between macrobenthic communities and nutrient enrichment and climatic context, the possibility of future impacts of coastal development and warming remains. Future research should be aimed at determining threshold levels of nutrient loading at which ecological effects start to be seen.



**Keywords:** climate warming; distLM; eutrophication; intertidal; spatial variation; pollution; urbanisation.

## **4.2 Introduction**

Estuaries are important transition zones between terrestrial, freshwater and marine ecosystems, and support high productivity (Wall et al., 2001; Gray, 2002). They are, however, also the sites of human population centres, and are consequently under pressure from multiple stressors introduced by coastal development. These stressors may act independently or interactively to deleteriously affect estuarine biodiversity and functioning. They might also modify the response of coastal systems or their resilience to anthropogenic climate change (Crain et al., 2008; Przeslawski et al., 2009; Nicastro et al., 2012).

Predicting the effect of multiple stressors on the ecological integrity of estuaries is critical for sustainable management, yet difficult for several reasons. First, stressors act on a variety of spatial and temporal scales (Crain et al., 2008). Second, the combined effects of multiple stressors can be highly context-dependent, varying across habitats according to their resident taxa (Jones, 1992; Lindegarth and Hoskin, 2000; Thrush et al. 2008). Since an estuarine ecosystem usually comprises a variety of habitats (e.g. sand and mud flats, mangroves, seagrass), studies aimed at predicting impacts on these systems should account for differences among the communities and the intensity of the stressor that different habitats receive (Lindegarth and Hoskin, 2000). Most previous assessments of how stressors interact to influence estuarine species, communities or ecosystem functions have, however, been done in controlled laboratory conditions or at a small scale (Wernberg et al., 2012). Consequently, their ability to make realistic predictions about when and where multiple stressors will interact to produce ecosystem-level effects is limited (Wernberg et al., 2012). Field studies quantifying spatial and temporal variation in ecological communities across environmental

gradients of stressors have the advantage of examining changes at ecologically relevant scales.

Among the two greatest contemporary threats to estuarine ecosystems are nutrient enrichment and climate warming. In many estuaries and coastal lakes nitrogen (N) and/or phosphorous (P) limits primary production (Nixon 1995). Urbanization, deforestation, and agriculture, can lead to diffuse, catchment-scale enhancement of nutrient loading by adding nutrients to the system or by removing biotic nutrient stores (Rothenberger et al., 2009; Dugan et al., 2011). Point source discharges, such as from sewage treatment plants, can locally enhance nutrient availability. Point sources provide a continuous and localized source of nutrients, whereas diffuse sources are affected by the freshwater input and rainfall (Gabric and Bell, 1993; Bilkovic et al., 2006; Davis and Koop, 2006). Moderate enhancement of nutrient supply to nitrogen- or phosphorous-limited waters can stimulate the growth of planktonic and benthic plants and, in turn, the productivity of higher trophic levels (Pearson and Rosenberg, 1978; Bishop et al., 2006; York et al., 2012). High loadings of nutrients can, however, lead to excess organic matter production (eutrophication), hypoxia or anoxia of bottom sediments (Conley et al., 2007) and death of benthic organisms (Diaz, 2001; Rabalais et al., 2002).

A warmer climate may produce major changes in estuarine ecosystem structure and function, especially where coupled with nutrient enrichment. Temperature alters the physiology of many organisms and, consequently, can affect their geographic distribution (Pearson and Dawson, 2003; Diez et al., 2012) and their interactions (e.g. predation, competition) with other organisms (Genner et al., 2004; Walther 2010). In addition, climate warming may affect estuarine ecosystems indirectly by altering other aspects of the estuarine physico-chemical environment. Nutrient flux from the sediment to the water column, for instance, can be negatively affected by an increase in temperature through the modification of infaunal species behavior (Bulling et al., 2010). Moreover, warming can exacerbate the risk of

eutrophication by stimulating growth of algae in nutrient-rich waters (Rabalais et al., 2009). Warming of surface waters can lead to estuarine stratification, inhibiting oxygenation of bottom-waters and, in eutrophied areas, leading to the establishment of an anoxic or hypoxic ‘dead zone’ (Rabalais et al., 2009).

Estuarine soft sediment macrofauna represent an important element of estuarine ecosystems. They recycle organic material and nutrients (Hutchings, 1998; Penniford and Davis, 2001; Biles et al., 2002; Nizzoli et al., 2007) and serve as an important trophic linkage between primary producers and higher trophic levels, such as fish and birds (Amara et al., 2001; Platell et al., 2006; Rosa et al., 2008). A plethora of studies from the USA and Europe have documented the response of macrofauna to organic enrichment resulting from nutrient pollution such that they can be used as biotic indicators (Diaz and Rosenberg, 1995; Bilkovic et al., 2006; Cardoso et al., 2007). Initially, as nutrient pollution increases organic matter the density of the benthic macroinvertebrates increases, while the number of species decreases (Pearson and Rosenberg, 1978; Gray et al., 2002). This is because crustaceans and molluscs are negatively affected but opportunistic polychaetes positively impacted by moderate organic inputs (Warwick and Clarke, 1993; Wildsmith et al., 2009). Above a certain threshold level of enrichment, however, both abundance and richness decrease as a consequence of anoxic conditions (Pearson and Rosenberg, 1978; Gray et al., 2002). Biotic indicators of nutrient pollution may be particularly important for oligotrophic south-eastern Australian estuaries in which detecting nutrient enrichment by monitoring of water quality can be problematic because nutrients are rapidly taken up by the biota (Davis and Koop, 2006, Scanes et al., 2007).

Here we test the hypotheses that: (1) intertidal macrofaunal assemblages will differ in composition among temperate south-east Australian estuaries according to their degree of nitrogen enrichment and (2) the strength of the relationship between nutrient enrichment and macrofauna will be greater at warmer than cooler latitudes and will be modified by local

environmental context. Sampling across a latitudinal gradient is a useful tool for examining effects of climate on communities and ecosystem processes (Andrew and Hughes, 2004, 2005) as it allows examination of ecological processes at environmentally realistic scales.

### **4.3 Materials and methods**

#### *4.3.1. Study sites and sampling design*

To assess how latitude, nutrient loading and local environmental context interact to determine the abundance and diversity of macrobenthic invertebrates, we sampled 16 estuaries along the coast of New South Wales, Australia (Table 4.1, Fig. 4.1). Estuaries were chosen along a stretch of coast spanning 7° in latitude, corresponding to a difference in mean annual temperature of about 4°C. The stretch of coast was subdivided into four regions of similar size, within each of which we randomly selected two replicate estuaries receiving a total nitrogen (TN) loading similar to the undisturbed, pre-European settlement levels (ratio of TN loading pre-European settlement to present, < 2) and two estuaries that had been subjected to significant anthropogenic nutrient loading (ratio of TN loading pre-European settlement to present, > 2.5). The ratios of present day to pre-European TN loading were obtained from the NSW Office of Environment and Heritage (Roper et al. 2011). The pre-European TN loading was modeled based on the present TN loading, the spatial extent and typology of human activities that the estuary catchment is presently undergoing and the geomorphological attributes of each estuary (e.g. estuary and catchment area, flushing time; Roper et al. 2011).

#### *4.3.2 Macrofauna and sediment properties methods*

Within each of the estuaries, we collected samples of macrofauna and sediment from three 100 m<sup>2</sup> intertidal sites (~1.3 m mean tidal range), situated 50-100 metres apart. All sites were fully marine (salinity ranging from 30 to 35 ‰) and sampling was carried out within 14 days during low tides in late spring (November 2009). Seven replicate sediment cores (10 cm

in diameter and 15 cm deep) were randomly collected from unvegetated sediment at each site for faunal analysis. Four sediment cores (3 cm in diameter and 10 cm deep) were collected for analysis of organic matter content and sediment size composition.

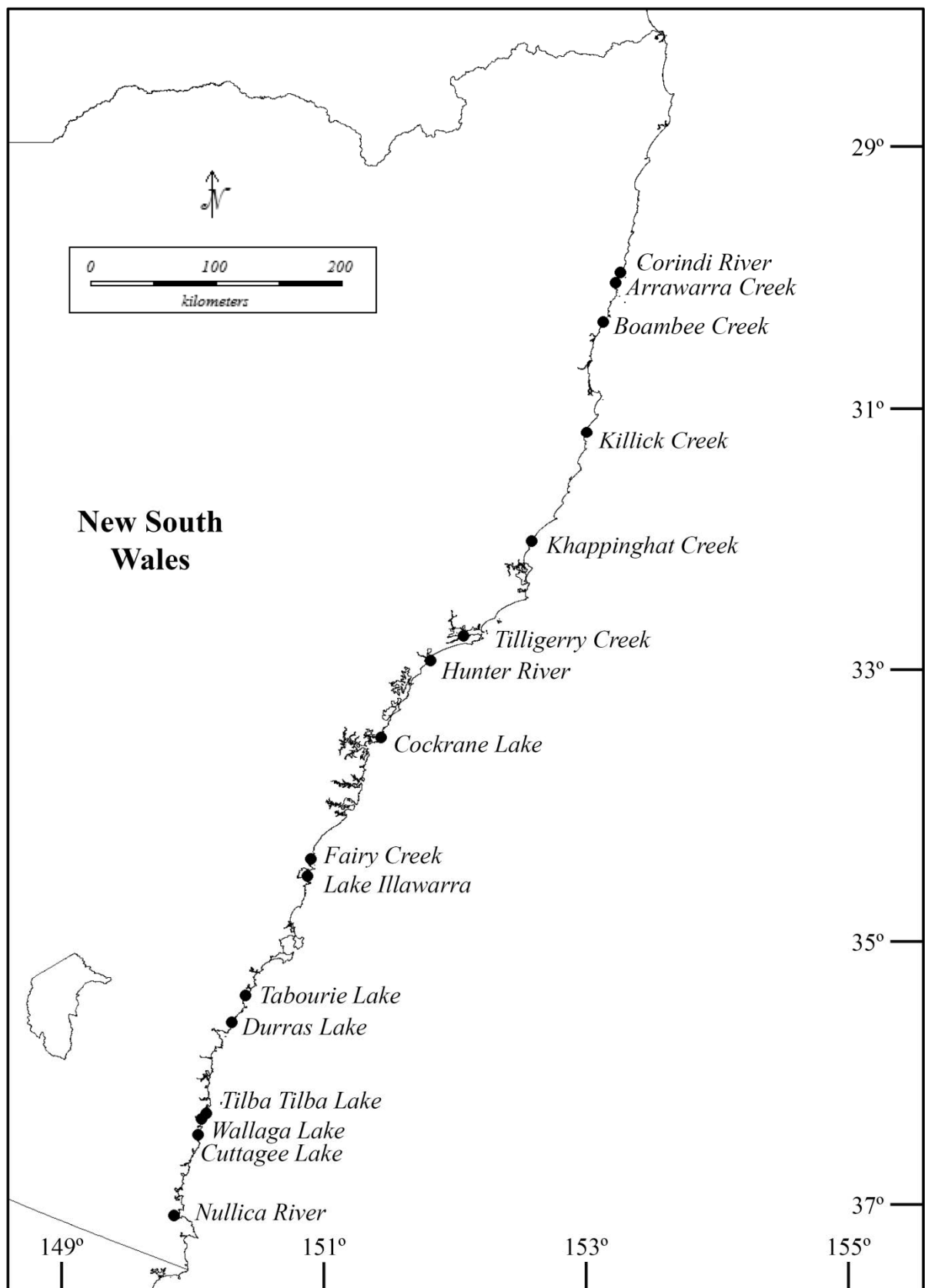
**Table 4.1** Physical and chemical attributes and mean values of sediment characteristics (mean  $\pm$  SE, n = 6 for median grain size, sorting and silt/clay content, n = 12 for organic matter [OM] content) for the 16 estuaries surveyed. Abbreviations: TN flux = flux of total nitrogen, TN ratio = ratio of total nitrogen loading pre-European settlement to present.

Estuary	Lat	Long	Estuary area (km <sup>2</sup> ) <sup>a</sup>	Catchment area (km <sup>2</sup> ) <sup>a</sup>	% disturbed catchment area <sup>a</sup>	Actual TN flux (mg m <sup>-2</sup> d <sup>-1</sup> ) <sup>a</sup>	Flushing time (d <sup>-1</sup> ) <sup>a</sup>	TN ratio <sup>a</sup>	Median grain size (μm)	Sediment sorting (μm)	% Silt/Clay	% OM
Corindi River	29° 59'	153° 14'	1.9	148.3	19.3	53	5.0	1.5	151.4 $\pm$ 17.7	310.1 $\pm$ 79.2	21.5 $\pm$ 6.1	2.1 $\pm$ 0.4
Arrawarra Creek	30° 04'	153° 12'	0.1	18.0	14.9	117	3.6	2.5	171.5 $\pm$ 1.8	68.5 $\pm$ 6.0	1.0 $\pm$ 0.2	1.3 $\pm$ 0.2
Boambee Creek	30° 21'	153° 06'	1.0	62.2	49.5	237	2.4	10.5	172.6 $\pm$ 0.7	210.2 $\pm$ 96.3	5.7 $\pm$ 2.8	1.5 $\pm$ 0.1
Killick Creek	31° 11'	152° 59'	0.3	8.2	30.7	15	56.1	1.9	200.9 $\pm$ 6.4	97.2 $\pm$ 10.6	0.9 $\pm$ 0.2	0.7 $\pm$ 0.1
Khappinghat Creek	32° 01'	152° 34'	1.2	91.9	28.4	47	12.2	1.9	252.7 $\pm$ 12.6	414.9 $\pm$ 75.2	4.5 $\pm$ 0.5	3.6 $\pm$ 0.5
Tilligerry Creek	32° 44'	152° 03'	134.4	135.2	30.4	1	36.2	3.8	244.0 $\pm$ 5.6	119.7 $\pm$ 5.0	1.6 $\pm$ 0.2	0.6 $\pm$ 0.1
Hunter River	32° 53'	151° 48'	47.0	21414.0	61.2	171	5.1	2.6	179.6 $\pm$ 70.4	301.0 $\pm$ 98.8	30.2 $\pm$ 11.6	4.3 $\pm$ 1.0
Cockrane Lake	33° 29'	151° 26'	0.3	7.2	38.6	13	31.5	2.0	113.9 $\pm$ 72.1	301.4 $\pm$ 45.5	46.9 $\pm$ 13.8	10.2 $\pm$ 4.0
Fairy Creek	34° 24'	150° 54'	0.1	20.8	75.1	487	1.2	3.2	329.0 $\pm$ 4.3	140.8 $\pm$ 15.1	1.6 $\pm$ 0.5	1.1 $\pm$ 0.3
Lake Illawarra	34° 32'	150° 53'	35.8	274.3	59.3	8	126.7	3.2	291.8 $\pm$ 18.0	212.4 $\pm$ 24.0	2.7 $\pm$ 1.1	1.3 $\pm$ 0.1
Tabourie Lake	35° 27'	150° 25'	1.5	47.6	15.4	11	13.3	1.4	314.8 $\pm$ 1.0	112.8 $\pm$ 4.5	0.9 $\pm$ 0.1	0.6 $\pm$ 0.1
Durras Lake	35° 39'	150° 18'	3.8	62.2	6.2	4	102.8	1.3	263.9 $\pm$ 12.6	218.9 $\pm$ 55.4	2.7 $\pm$ 0.7	2.2 $\pm$ 0.3
Tilba Tilba Lake	36° 20'	150° 07'	1.2	18.3	72.4	9	69.6	2.5	325.0 $\pm$ 11.0	117.3 $\pm$ 7.1	1.2 $\pm$ 0.3	0.5 $\pm$ 0.1
Wallaga Lake	36° 22'	150° 05'	9.3	273.1	37.4	21	97.4	2.3	392.6 $\pm$ 3.7	405.0 $\pm$ 124.0	4.1 $\pm$ 1.8	2.7 $\pm$ 1.6
Cuttagee Lake	36° 29'	150° 03'	1.4	54.5	4.8	17	40.0	1.3	336.2 $\pm$ 8.0	126.6 $\pm$ 6.4	1.4 $\pm$ 0.1	0.8 $\pm$ 0.1
Nullica River	37° 06'	149° 52'	0.3	55.1	4.7	40	8.3	1.1	205.6 $\pm$ 28.5	135.1 $\pm$ 40.0	3.4 $\pm$ 1.6	2.3 $\pm$ 0.7

<sup>a</sup> Data from Roper et al. (2011).

Upon collection, samples for faunal analysis were refrigerated and sieved through a 0.5 mm mesh within 72 hours to remove fine sediment. Animals were fixed in formaldehyde solution (5%) prepared with seawater and buffered with sodium borate to prevent the dissolution of calcified structures and facilitate faunal identification. Macrofauna were sorted under a dissecting microscope (10x magnification) and transferred to 70% ethanol. Most specimens were identified to species, except for polychaetes and crustacea, which were identified to morphospecies and family respectively, and nemerteans and sipunculids, which were grouped by phylum. Use of a mixed taxonomic resolution was necessary because of the scarce documentation available for Australia's invertebrate fauna. This approach does not compromise the detection of spatial patterns of macroinvertebrates (Clarke and Warwick 2001; Dethier and Schoch 2006).

To assess how the relationship between nutrient enrichment and macrofaunal communities is influenced by the local environmental context, we quantified sediment organic content and grain size. To assess sediment organic content, a subsample of about 4 g was taken from each of the four small sediment samples after homogenization. The sub-samples were dried at 105 °C for 48 h and weighed prior to combustion at 550 °C for 4 h hours. The organic content was calculated as the percentage difference in weight from before to after combustion. Sediment grain size was determined for two randomly selected replicates at each site. The silt/clay fraction was determined by wet sieving through a 63 µm screen. The remaining sediment was dry sieved through a stack of sieves of decreasing mesh size (2000, 1000, 500, 250 and 125 µm) and the weight of each fraction was measured. Median grain size and sorting were calculated using the software GRADISTAT 4.0 (Blott and Pye, 2001).



**Figure 4.1** Map showing the location of surveyed estuaries along the New South Wales (Australia) coastline.



#### 4.3.3 Statistical analysis

To test the hypotheses that nutrient enrichment would (1) be correlated to macrofaunal community structure and (2) the strength of this relationship would be determined by the climatic and local environmental context, we collated a matrix of environmental variables. This included: 1) site averages of environmental data collected during this study (sediment organic matter and silt/clay content, median grain size and sorting), and 2) estuary physical and chemical attributes (see Table 4.1 for complete list) from Roper et al. (2011). The distribution of each environmental variable across sites was visually inspected and an appropriate transformation was applied to minimise skewness. Environmental variables were normalized and principal component analysis (PCA) was used to outline and visualise the relationships between variables.

To assess the contribution of the environmental variables to the variation observed in the macrofaunal community structure we carried out a multivariate regression using distance-based redundancy analysis (db-RDA) (McArdle and Anderson, 2001). Multivariate multiple regression (DistLM routine) tested the significance of these contributions by fitting a linear model based on Bray-Curtis dissimilarities from  $\log(x + 1)$  transformed abundance data using permutations. Abundances of macrofaunal taxa were summed such that sites became replicates. First, we assessed the contribution of each environmental variable to the variation in the macrofauna community structure. Then we used AICc as selection criterion (Burnham and Anderson, 2004) and BEST selection procedure in order to find a reduced model that retained only variables with good explanatory power. The reduced model was visualized with a db-RDA plot. In order to identify which taxa showed the highest correlation to the environmental variables (multiple correlation coefficient  $> 0.3$ ), we superimposed vectors. Linear regressions tested for relationships between each of the faunal variables, macrofaunal abundance, taxon richness and key discriminating taxa, with latitude, TN ratio and the environmental variables with the highest correlation with macrofaunal assemblages in the db-

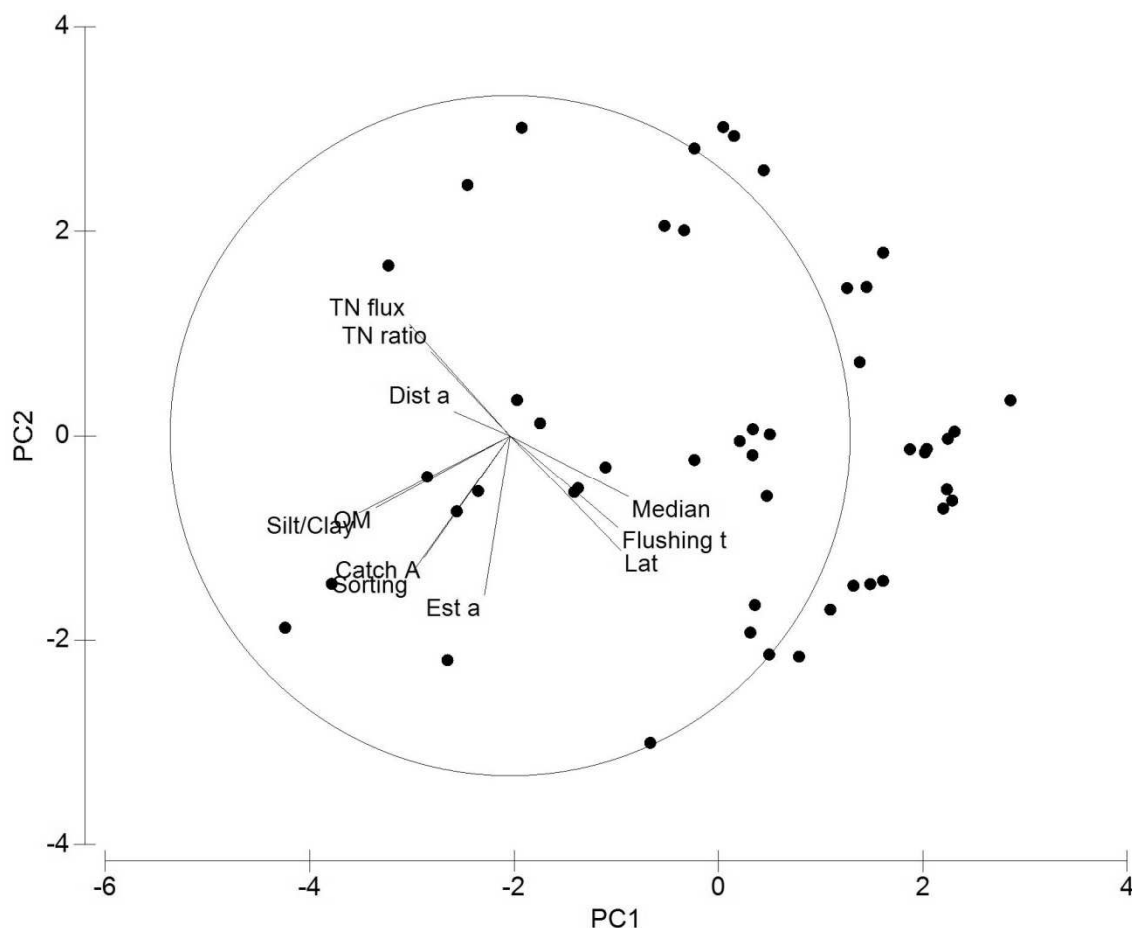
RDA analysis. We used the SIMPER routine to identify the taxa that best discriminated between low and high median grain size estuaries. Only taxa with a ratio of the mean to Bray-Curtis dissimilarity to the standard deviation of greater than or equal to 1 were considered.

All multivariate procedures were carried out with PRIMER v6 (Clarke and Gorley 2006) and PERMANOVA+ (Anderson et al. 2008) statistical package. Univariate analyses were performed using Minitab 16.

#### **4.4 Results**

Principal component analysis revealed a high degree of interrelatedness among environmental variables. As a result, the first two principal component axes explained approximately 52 % of the total variation. The two-dimensional PCA plot revealed two groups of interrelated variables (Fig. 4.2). Latitude, flushing time and median grain size were negatively correlated with total nitrogen (TN) flux, TN ratio and the percentage of disturbed catchment area. The second group, roughly orthogonal to the first, consisted of sediment silt/clay and organic matter content, sediment sorting and catchment and estuary area.

A total of 70 taxa and 18510 macrofaunal individuals were found. The most abundant group was the polychaete worms, followed by bivalves, chironomid larvae, crustaceans and gastropods. Macrofaunal community structure was weakly correlated with individual environmental variables (Table 4.2a). Those abiotic variables most strongly correlated to macrofaunal assemblages were latitude, median sediment grain size (MGS), flushing time and sediment silt/clay content (Table 4.2a; Fig. 4.3). The combination of environmental variables that was most closely correlated to the macrofaunal data, explaining 30 % of the variability, included the 5 variables, MGS and silt/clay, TN ratio, TN flux and % disturbed area (BEST procedure PRIMER, Table 4.2a; Fig. 4.3a). *Mictyris sp.*, capitellid and nereid polychaetes, chironomids and lysianassids were the taxa that mostly correlated with abiotic variables (Fig. 4.3b).



**Figure 4.2** PCA (Principal component analysis) plot of environmental the 11 environmental variables, excluding longitude, of Table 1 (transformed and normalised). Dots represent sites within each of estuaries. Vectors show the two-dimensional (PC1 and PC2) correlation structure among the environmental variables (% of variance explained = 52.4). Abbreviations: catch a = catchment area, dist a = % of disturbed area of the catchment, est a = estuary area, Flushing t = flushing time, lat = latitude, median = median sediment grain size, OM = sediment organic matter, silt/clay = % sediment silt/clay content, sorting = sediment sorting, TN flux = flux of total nitrogen, TN ratio = ratio of total nitrogen loading pre-European settlement to present.

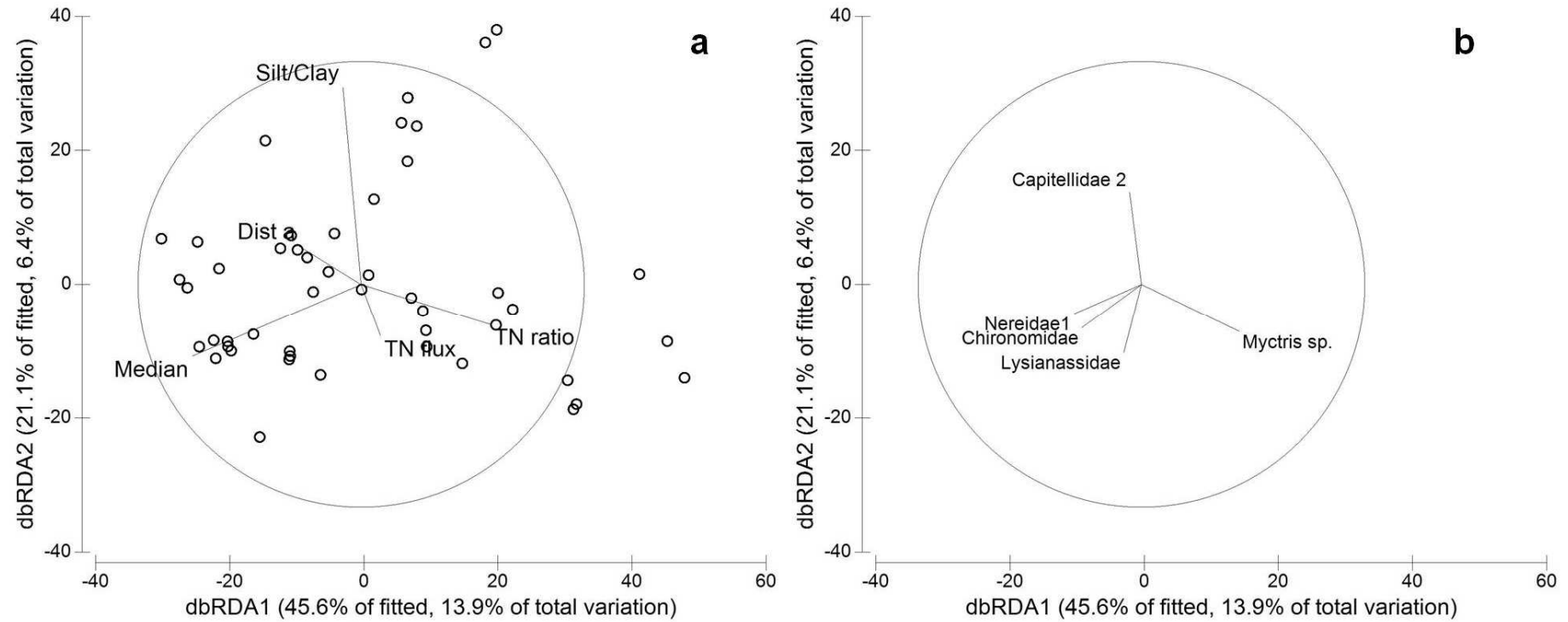
Overall, each of MGS and latitude were strongly correlated to macrofaunal abundance and taxon richness (MGS: Pearson correlation coefficient ( $r$ ) = 0.61 and  $r$  = 0.33, latitude:  $r$  = - 0.55 and  $r$  = 0.40 for abundance and taxon richness respectively,  $p$  < 0.05), but no relationship was found between TN ratio with either of the faunal variables ( $r$  = - 0.25,  $r$  = - 0.21, for abundance and taxon richness respectively,  $p$  > 0.05). Latitude and MGS were, however, highly correlated ( $r$  = -0.61,  $p$  < 0.001) such that it was impossible to separate their influence.

**Table 4.2 (following page)** Results of multivariate multiple regression analyses (distLM) run using data from: (a) all estuaries, (b) estuaries with a median sediment grain size of  $< 250 \mu\text{m}$  and (c) estuaries with a median grain size of  $> 250 \mu\text{m}$ . Prop. = the proportion of variance in the macrofaunal community structure explained by each environmental variable. Prop. BEST = the proportion of variance explained by the variables selected as the key environmental drivers using the BEST procedure (AICc selection criterion). Significant ( $p < 0.05$ ) predictor variables are in bold. Macrofaunal data were  $\log(x + 1)$  transformed prior to analysis. Abbreviations: TN flux = flux of total nitrogen, TN ratio = ratio of total nitrogen loading pre-European settlement to present.

**Table 4.2**

<i>Environmental variables</i>	a) All estuaries			
	Pseudo-F	$p_{\text{perm}}$	Prop.	Prop. BEST
Latitude	5.31	<b>0.000</b>	0.10	
Sediment median grain size	5.28	<b>0.000</b>	0.10	0.10
Flushing time <sup>a</sup>	3.49	<b>0.001</b>	0.07	
Sediment Silt/Caly <sup>b</sup>	3.07	<b>0.002</b>	0.06	0.05
Sediment OM <sup>b</sup>	2.46	<b>0.009</b>	0.05	
TN ratio <sup>b</sup>	2.40	<b>0.010</b>	0.05	0.07
Sediment sorting <sup>b</sup>	2.22	0.015	0.05	
TN flux <sup>c</sup>	1.99	0.030	0.04	0.05
Catchment area <sup>b</sup>	1.56	0.102	0.03	
Disturbed area	1.54	0.106	0.03	0.03
Estuary area <sup>b</sup>	1.24	0.233	0.03	
$R^2$ BEST solution				0.30
<i>Environmental variables</i>	b) Low median grain size			
	Pseudo-F	$p_{\text{perm}}$	Prop.	Prop. BEST
Sediment Silt/Caly <sup>b</sup>	3.3	<b>0.001</b>	0.13	0.13
Sediment OM <sup>b</sup>	2.8	<b>0.002</b>	0.11	
Sediment sorting <sup>b</sup>	2.2	<b>0.018</b>	0.09	
Catchment area <sup>b</sup>	2.1	<b>0.021</b>	0.09	0.09
Flushing time <sup>a</sup>	2.0	<b>0.029</b>	0.08	0.10
Estuary area <sup>b</sup>	1.8	<b>0.048</b>	0.08	
TN ratio <sup>b</sup>	1.3	0.216	0.06	
TN flux <sup>c</sup>	1.2	0.269	0.05	
Disturbed area	1.2	0.293	0.05	
Latitude	1.2	0.293	0.05	
Sediment median grain size	1.1	0.342	0.05	
$R^2$ BEST solution				0.32
<i>Environmental variables</i>	c) High median grain size			
	Pseudo-F	$p_{\text{perm}}$	Prop.	Prop. BEST
Latitude	4.7	<b>0.000</b>	0.18	0.18
Sediment sorting <sup>b</sup>	4.5	<b>0.000</b>	0.17	
Sediment OM <sup>b</sup>	3.8	<b>0.000</b>	0.15	
Sediment Silt/Caly <sup>b</sup>	3.8	<b>0.000</b>	0.15	0.09
Flushing time <sup>a</sup>	3.5	<b>0.000</b>	0.14	
Catchment area <sup>b</sup>	3.2	<b>0.001</b>	0.13	0.12
TN flux <sup>c</sup>	2.8	<b>0.005</b>	0.11	
Estuary area <sup>b</sup>	2.8	<b>0.004</b>	0.11	
TN ratio <sup>b</sup>	2.7	<b>0.004</b>	0.11	0.13
Disturbed area	2.3	<b>0.016</b>	0.09	0.10
Sediment median grain size	1.9	0.052	0.08	0.07
$R^2$ BEST solution				0.69

<sup>a</sup> data square root transformed<sup>b</sup> data log transformed<sup>c</sup> data 4<sup>th</sup> root transformed

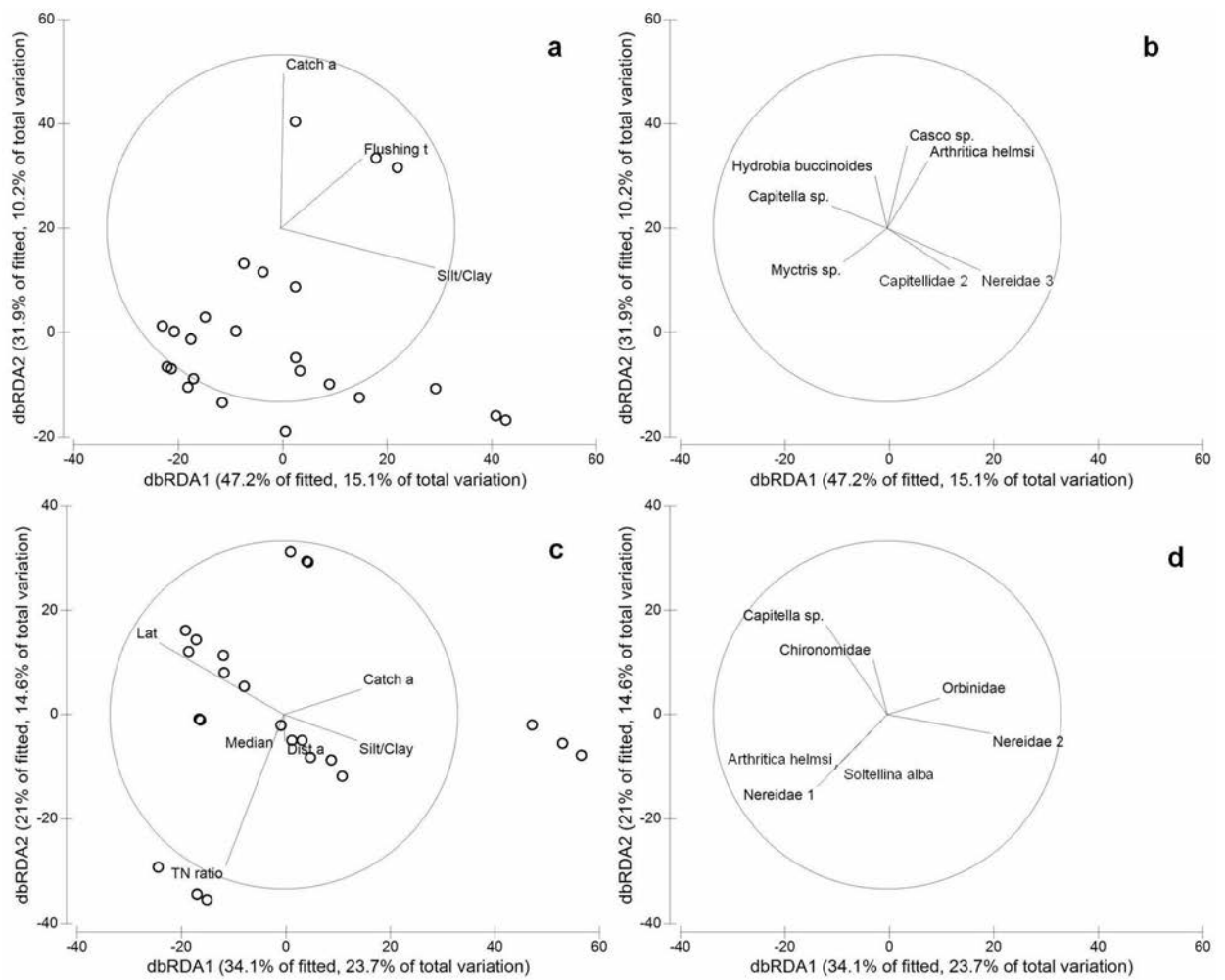


**Figure 4.3** dbRDA plots representing the reduced model of the macrofaunal community structure grouped by taxa and its relationship to a) environmental variables and b) taxa significantly correlated with db-RDA axes (multiple correlation > 0.30). Points omitted in plot b for clarity. See Fig. 2 for abbreviations.

Visual analysis of the MGS distribution revealed that this variable had a bimodal distribution with two peaks at about 170 and 330  $\mu\text{m}$  and median value of 255  $\mu\text{m}$ . Therefore, in order to minimize the correlation between latitude and MGS, we separated the dataset into two groups of eight estuaries, based on their MGS value (low MGS < 250  $\mu\text{m}$ , high MGS > 250  $\mu\text{m}$ ), and ran the same multivariate analyses for each subgroup as for the complete data set. Multiple regression analysis on low MGS estuaries indicated that sediment variables other than MGS explained most of the variability in macrobenthic assemblages (Table 4.2b). These were silt/clay content, organic matter content, and sediment sorting. Several other physical variables (catchment area, flushing time, estuary area) were more weakly but significantly correlated with macrofaunal community structure (Table 4.2b). The combination of environmental variables best explaining overall variation (as indicated by BEST analysis, PRIMER), silt/clay content, catchment area, and flushing time, explained 32 % of the variability (Table 4.2b; Fig. 4.4a). Nereid and capitellid polychaetes, the crustaceans *Casco* sp. and *Myctris* sp. and the gastropod *Hydrobia buccinoides* were the taxa that mostly correlated with the environmental variables of the BEST model (Fig. 4.4b).







**Figure 4.4** dbRDA plots representing the reduced model of the macrofaunal community structure of estuaries with a median sediment grain size of  $< 250 \mu\text{m}$  (a, b) and estuaries with a median grain size of  $> 250 \mu\text{m}$  (c, d) and its relationship with environmental variables (a, c) and taxa significantly correlated with db-RDA axes (b, d) (multiple correlation  $> 0.30$ ). Points omitted in plot b and d for clarity. See figure 2 for abbreviations.

Across the high MGS estuaries, macrofaunal assemblages were significantly correlated with each of the environmental variables included in the model, except MGS (Table 4.2c). Latitude was the individual variable most strongly correlated to macrofaunal communities, explaining 18 % of the variability, and along with sediment silt/clay content, catchment area, TN ratio, disturbance area and MGS, was among the group of variables chosen by the BEST procedure as being most correlated to the fauna (Table 4.2c). Together, the sub-group explained 69 % of the total variation (Table 4.2c; Fig. 4.4c).

Low MGS estuaries had a lower abundance of macrofauna (mean  $15.9 \pm 17.8$  and  $97.4 \pm 71.6$  individuals per core for low and high MGS, respectively;  $t_{45} = 6.81$ ,  $p < 0.001$ ) and lower number of taxa (mean  $1.5 \pm 0.7$  and  $2.0 \pm 0.5$  individuals per core for low and high MGS, respectively;  $t_{45} = 2.86$ ,  $p < 0.01$ ) per core than high MGS estuaries. However, macrofauna abundance and species richness did not show any relationship with latitude, TN ratio or sediment silt/clay content in either low or high MGS estuaries (Table 4.3, Fig. 4.5). Among the low MGS estuaries, nereid and capitellid polychaetes were the taxa that were most correlated with abiotic variables (Fig. 4.4b). However, regression analysis showed that the relationships between the abundance of these taxa and the environmental variables were not significant (Table 4.4, Fig. 4.5), except for nereidae 3 which was weakly but significantly correlated with sediment silt/clay content ( $R^2 = 0.21$ ). Among the high MGS estuaries, spatial variation in macrofaunal assemblages were driven by variation in *Capitella* sp. and nereid polychaetes, chironomid larvae and the bivalves *Arthritica helmsi* and *Soltellina alba* (Fig. 4.4d). Analyses of the abundance of these taxa in high MGS estuaries showed that the abundance of nereidae 3 was significantly correlated with sediment silt/clay content ( $R^2 = 0.36$ ), *Capitella* sp. abundance was significantly correlated with latitude and TN ratio ( $R^2 = 0.58$  and  $R^2 = 0.28$ , respectively), nereidae 1 abundance was significantly correlated to TN ratio ( $R^2 = 0.18$ ) and chironomid larvae abundance was significantly correlated with TN ratio and sediment silt/clay content ( $R^2 = 0.45$ ,  $R^2 = 0.24$ , respectively) (Table 4.4, Fig. 4.6).

The taxa that mostly contributed to differences between sandier and finer sediment estuaries were *Capitella* sp., chironomid larvae, *Arthritica helmsi* and orbinids (SIMPER routine, PRIMER), which were all more abundant in sandier sediment estuaries (t-test,  $p < 0.05$ ). Assemblages in finer sediments of  $< 250 \mu\text{m}$  grain size were dominated by *Capitella* sp. and nereid polychaetes, chironomid larvae and the bivalve *Arthritica helmsi*. Assemblages in sandier sediments, of  $> 250 \mu\text{m}$  grain size, were characterised by nereids and *Nephtys* sp. polychaetes, the bivalve *Mysella vitrea* and the decapods *Mictyris* sp..

**Table 4.3**  $R^2$  values of the regression analyses between predictor environmental variables and univariate community descriptors for estuaries with a median sediment grain size of  $< 250 \mu\text{m}$  (low MGS) and estuaries with a median grain size of  $> 250 \mu\text{m}$  (high MGS). Significant ( $p < 0.05$ ) predictor variables are in bold. TN ratio = ratio of total nitrogen loading pre-European settlement to present.

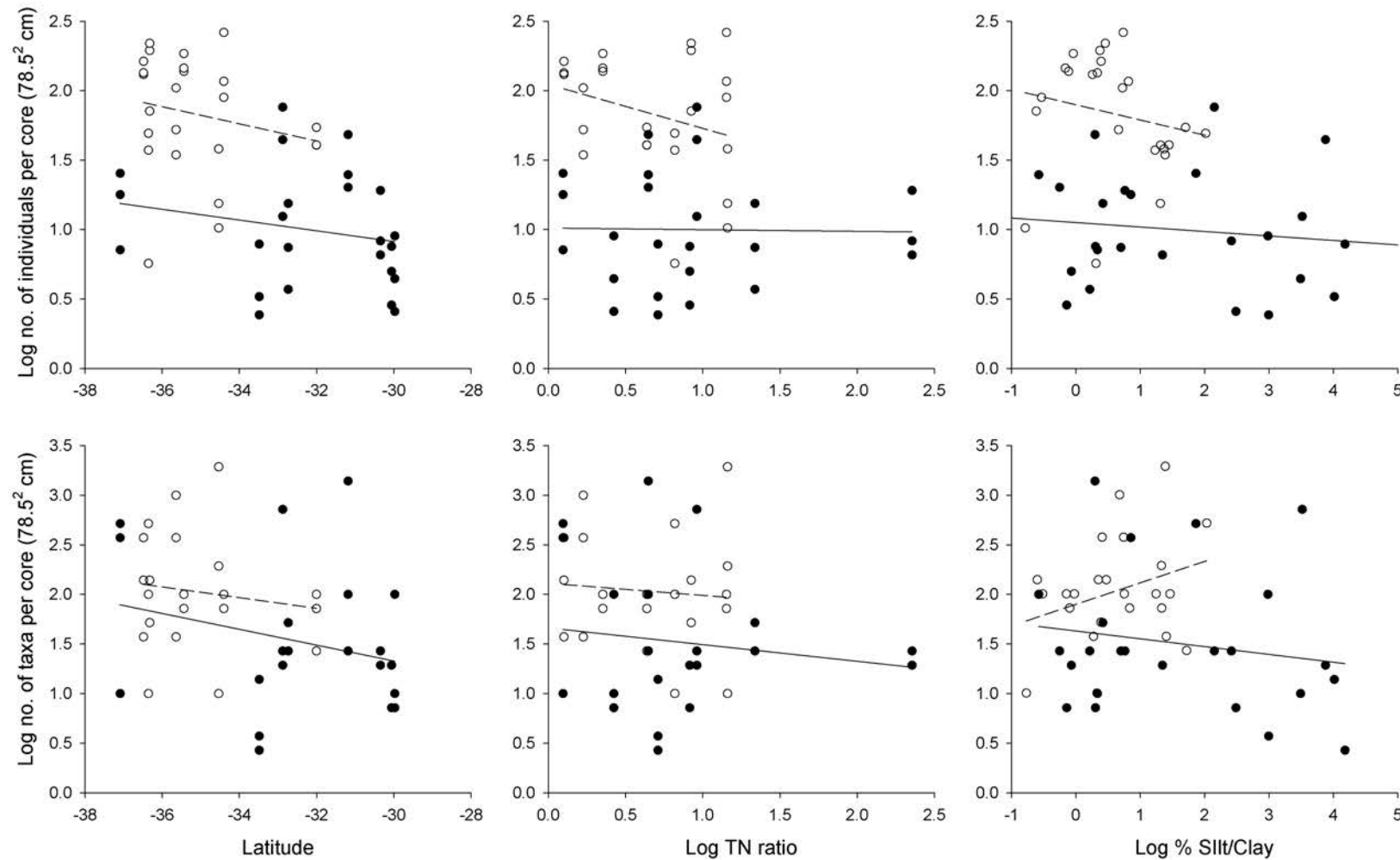
	No. of individuals <sup>a</sup>		No. of taxa	
	Low MGS	High MGS	Low MGS	High MGS
Latitude	0.05	0.04	0.07	0.02
TN ratio <sup>a</sup>	0.01	0.08	0.04	0.01
% Silt/Clay <sup>a</sup>	0.01	0.04	0.03	0.09

<sup>a</sup> data log transformed

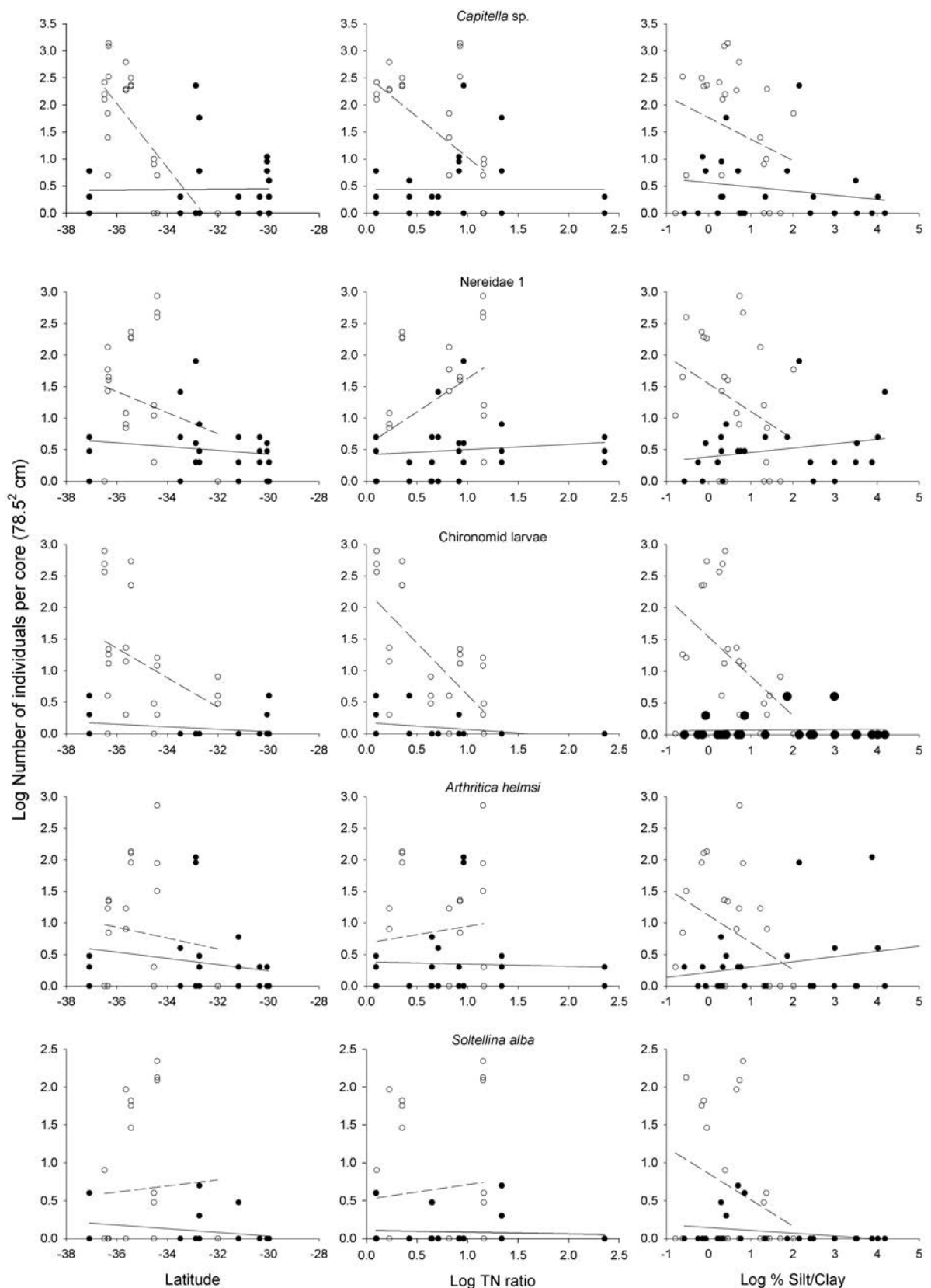
**Table 4.4**  $R^2$  values of the regression analyses between abundance of macrofaunal taxa and predictor environmental variables for estuaries with a median sediment grain size of  $< 250 \mu\text{m}$  (low MGS) and estuaries with a median grain size of  $> 250 \mu\text{m}$  (high MGS). Significant ( $p < 0.05$ ) predictor variables are in bold. TN ratio = ratio of total nitrogen loading pre-European settlement to present.

	Latitude		TN ratio <sup>a</sup>		% Silt/Clay <sup>a</sup>	
	Low MGS	High MGS	Low MGS	High MGS	Low MGS	High MGS
<i>Capitella</i> sp.	0.01	<b>0.58</b>	0.01	<b>0.28</b>	0.04	0.07
Nereidae 1	0.02	0.06	0.02	<b>0.18</b>	0.06	0.12
Chironomid larvae	0.06	0.12	0.15	<b>0.45</b>	0.01	<b>0.24</b>
<i>Arthritica helmsi</i>	0.04	0.02	0.01	0.01	0.05	0.13
<i>Soltellina alba</i>	0.07	0.01	0.01	0.01	0.08	0.09

<sup>a</sup> data log transformed



**Figure 4.5** Linear regression between latitude, total nitrogen (TN) ratio (i.e. ratio of total nitrogen loading pre-European settlement to present), % of sediment silt/clay content and the mean number of individuals and taxa per core in each site. Empty and full circles represents low and high median sediment grain size estuaries, respectively.



**Figure 4.6** Linear regression analyses between latitude, total nitrogen (TN) ratio (i.e. ratio of total nitrogen loading pre-European settlement to present), % of sediment silt/clay content and the mean number of individuals of the taxa that mostly correlated with the variability in environmental variables (see Fig. 4.4).

## 4.5 Discussion

Of the site-specific and estuarine-scale environmental variables considered by this study, we found that sediment grain size was the best predictor of macrofaunal assemblage composition. Only when samples were stratified according to median grain size did relationships between macrofauna and latitude and the nitrogen loading of estuaries emerge, and even then, only among sites with sandy sediments. This supports a rich literature on the important role of sediment granulometry in structuring sediment-dwelling communities (Gray, 1974; Snelgrove and Butman, 1994; Chapman and Tolhurst, 2007).

A stronger relationship between nutrient enrichment on macrofaunal communities of sandy than muddy sediments has previously been reported by Lindegarth and Hoskin (2001), also assessing impacts of diffuse runoff within south-east Australia. In comparing the structure of macrofaunal communities between sections of a south-eastern Australian estuary that drained urbanized and forested land, they found that impacts of coastal development were confined to sites with sandy sediments. Among sites with muddy sediment, there was no relationship between adjacent land use and macrofaunal assemblage structure (Lindegarth and Hoskin 2001). Our study, which sampled across the larger scale of estuaries, rather than sites within an estuary, found a similar pattern at the community-scale. Nevertheless, the specific taxa responding most strongly to disturbance differed between the two studies (Lindegarth and Hoskin, 2001). This suggests that this pattern is not the result of a set of specific taxa, but, rather, a generalised response of macrofauna living in sandy sediments (Lindegarth and Hoskin, 2001).

Differences in the effect of TN loading on macrofaunal communities between estuaries with sandy and muddy sediments may be due to (1) differences in the effective intensity of nutrient disturbances between these sediment types, (2) differences in the background organic enrichment of these habitats and/or (3) differences in their assemblage composition which influences susceptibility to nutrient pollution. First, it is possible that

macrofaunal taxa responded indirectly to sediment properties because these were correlated with hydrological characteristics (e.g. flushing time, tidal inundation) that would affect the fate and utilization of N in the system (Davis and Koop, 2006). Second, the differing response of macrofauna to nutrient disturbance between the two sediment types may have arisen from intrinsic differences between sandy and muddy sediments in sediment organic content. Previous studies directly manipulating nutrient loading through experimental additions have shown that effects can vary markedly according to background organic enrichment (e.g. Morris and Keough 2003). Third, differences in the assemblage composition of sandy and muddy sediments may be responsible for the differing relationships between nutrient enrichment and macrofaunal community composition between these habitat types (Lindegarth and Hoskin, 2001). Crustaceans were more abundant in sandy than muddy sediments and chironomid larvae showed the opposite pattern. Furthermore, overall sandy sediments contained more taxa than muddy sediments. Crustaceans are regarded as being very sensitive to environmental perturbations compared to polychaetes (Warwick and Clarke, 1993; Wildsmith et al., 2009, 2011). They did not, however, display a strong relationship to nutrient enrichment in this study, perhaps because their identification to family level prevented the detection of changes in species composition. A higher mean number of species in the sandy sediment means that bias, associated with the greater probability of the community including a sensitive taxon, may have also contributed to in the stronger relationship between macrofaunal communities and TN loading in sediment samples.

In contrast to studies done in North America or Europe (Kemp et al., 2005; Conley et al., 2007), the relationship between TN loading and macrofaunal assemblage composition found in this study, even in sandy sediments, was weak. This may be attributed to (1) the relatively low loadings of nitrogen that southeast Australian estuaries receive relative to other parts of the world and/or (2) the oligotrophic starting condition of southeast Australian estuaries. Even the most modified of south-east Australian estuaries contain equal or lower



TN loading than the most pristine estuaries in the US (Scanes et al., 2007). Whereas many of the estuaries that are most severely degraded by nutrient pollution in Europe (Conley et al. 2007), the USA (Kemp et al., 2005) and Western Australia (Chan et al. 2002) are naturally eutrophic systems, of high organic load, south-east Australian estuaries are oligotrophic (Scanes et al., 2007; Young et al., 1996). Therefore, the recent anthropogenic increase in nitrogen loading that some NSW estuaries are experiencing may not sufficiently enhance organic load so as to impact benthic faunal communities (Conley et al., 2007). Indeed, recent studies in south-eastern Australian estuaries have shown that nutrient added by either point-source or diffuse runoff is rapidly assimilated by the biota (Scanes et al. 2007), enhancing productivity of all trophic levels in estuarine ecosystems (Bishop et al. 2006, York et al. 2012). Further, studies experimentally fertilizing sediments have found minimal impacts of enrichment on macrofaunal assemblages of south-east Australian estuaries (O'Brien et al., 2009, 2010).

Despite our study spanning 7° of latitude and five meso-scale bioregions (Commonwealth of Australia, 2006), we found only weak latitudinal gradients in the abundance of several common and widespread taxa (*Capitella* sp. and chironomid larvae), and only in sandy estuaries. Further, we found no relationship between latitude and taxon richness. This contrasts previous studies in aquatic and terrestrial systems, including some on macrofauna, documenting strong latitudinal gradients in species richness and abundance (e.g. Engle and Summers, 1999; Andrew et al., 2003; Bairstow et al., 2010). In a study spanning 17° of latitude, Engle and Summers (1999) found that whereas at small spatial scales (of up to 1° of latitude), macrofauna assemblages are mostly driven by local-scale environmental variables (e.g. salinity, sediment features), over larger spatial scales, temperature became the dominant driver. Although it is possible that had we sampled across a larger latitudinal gradient, stronger spatial patterns may have been found, our study nevertheless suggests that

the benthic bioregions developed by the Commonwealth of Australia (2006), based on fish populations and geophysical data, may not be appropriate for benthic macrofaunal groups.

In conclusion, this study failed to provide evidence of differential effects of nutrient enrichment on benthic macrofauna across a gradient of 7° of latitude and 4°C temperature. Instead, the relationship between nutrient loading and benthic macrofaunal communities was dependent on sediment grain-size and was, on the whole, weak. Nevertheless, as human population growth continues to place increasing pressure on southeast Australian estuaries, and climate warming continues, further studies examining when and where nutrient loading will lead to significant changes in estuarine community structure are needed.

## 4.6 References

- Amara, R., Laffargue, P., Dewarumez, J.M., Maryniak, C., Lagardere, F., Luczac, C., 2001. Feeding ecology and growth of O-group flatfish (sole, dab and plaice) on a nursery ground (Southern Bight of the North Sea). *Journal of Fish Biology* 58, 788-803.
- Anderson, M. J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26, 32-46.
- Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E Ltd, Plymouth, UK.
- Andrew, N.R., Rodgerson, L., Dunlop, M., 2003. Variation in invertebrate-bryophyte community structure at different spatial scales along altitudinal gradients. *Journal of Biogeography* 30, 731-746.
- Andrew, N.R., Hughes, L., 2004. Species diversity and structure of phytophagous beetle assemblages along a latitudinal gradient: predicting the potential impacts of climate change. *Ecological Entomology* 29, 527-542.
- Andrew, N.R., Hughes, L., 2005. Arthropod community structure along a latitudinal gradient: Implications for future impacts of climate change. *Austral Ecology* 30, 281-297.
- Bairstow, K.A., Clarke, K.L., McGeoch, M.A., Andrew, N.R., 2010. Leaf miner and plant galler species richness on *Acacia*: relative importance of plant traits and climate. *Oecologia* 163, 437-448.

- Biles, C.L., Paterson, D.M., Ford, R.B., Solan, M., Raffaelli, D.G., 2002. Bioturbation, ecosystem functioning and community structure. *Hydrology and Earth System Sciences* 6, 999-1005.
- Bilkovic, D.M., Roggero, M., Hershner, C.H., Havens, K.H., 2006. Influence of land use on macrobenthic communities in nearshore estuarine habitats. *Estuaries and Coasts* 29, 1185-1195.
- Bishop, M.J., Kelaher, B.P., Smith, M.P.L., York, P.H., Booth, D.J., 2006. Ratio-dependent response of a temperate Australian estuarine system to sustained nitrogen loading. *Oecologia* 149, 701-708.
- Blott, S.J., Pye, K., 2001. GRADISTAT: a grain size distribution and statistics package for the analysis of unconsolidated sediments. *Earth Surface Processes and Landforms* 26, 1237-1248.
- Bulling, M.T., Hicks, N., Murray, L., Paterson, D.M., Raffaelli, D., White, P.C.L., Solan, M., 2010. Marine biodiversity-ecosystem functions under uncertain environmental futures. *Philosophical Transactions of the Royal Society B-Biological Sciences* 365, 2107-2116.
- Burnham, K.P., Anderson, D.R., 2004. Multimodel inference - understanding AIC and BIC in model selection. *Sociological Methods and Research* 33, 261-304.
- Cardoso, P.G., Bankovic, M., Raffaelli, D., Pardal, M.A., 2007. Polychaete assemblages as indicators of habitat recovery in a temperate estuary under eutrophication. *Estuarine, Coastal and Shelf Science* 71, 301-308.

Chan, T.U., Hamilton, D.P., Robson, B.J., Hodges, B.R., Dallimore, C., 2002. Impacts of hydrological changes on phytoplankton succession in the Swan River, Western Australia. *Estuaries* 25, 1406-1415.

Chapman, M.G., Tolhurst, T.J., 2007. Relationships between benthic macrofauna and biogeochemical properties of sediments at different spatial scales and among different habitats in mangrove forests. *Journal of Experimental Marine Biology and Ecology* 343, 96-109.

Clarke, K.R., Warwick, R.M., 2001. Changes in marine communities: an approach to statistical analysis and interpretation. 2nd edition. PRIMER-E Ltd, Plymouth, UK.

Clarke, K. R., Gorley, R.N., 2006. PRIMER v6. PRIMER-E Ltd, Plymouth, UK.

Commonwealth of Australia, 2006. Map 2 IMCRA 4.0: Meso-scale Bioregions. ERIN, Department of the environment and Heritage, Australian Government.

Conley, D.J., Carstensen, J., Aertebjerg, G., Christensen, P.B., Dalsgaard, T., Hansen, J.L.S., Josefson, A.B., 2007. Long-term changes and impacts of hypoxia in Danish coastal waters. *Ecological Applications* 17, S165-S184.

Crain, C.M., Kroeker, K., Halpern, B.S., 2008. Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters* 11, 1304-1315.

- Davis, J.R., Koop, K., 2006. Eutrophication in Australian rivers, reservoirs and estuaries - a southern hemisphere perspective on the science and its implications. *Hydrobiologia* 559, 23-76.
- Dethier, M.N., Schoch, G.C., 2006. Taxonomic sufficiency in distinguishing natural spatial patterns on an estuarine shoreline. *Marine Ecology-Progress Series* 306, 41-49.
- Diaz, R.J., Rosenberg, R., 1995. Marine benthic hypoxia: A review of its ecological effects and the behavioural responses of benthic macrofauna, in: Ansell, A.D.G.R.N.B.M. (Ed.), *Oceanography and Marine Biology - an Annual Review*, Vol 33, pp. 245-303.
- Diaz, R.J., 2001. Overview of hypoxia around the world. *Journal of Environmental Quality* 30, 275-281.
- Diez, I., Muguerza, N., Santolaria, A., Ganzedo, U., Gorostiaga, J.M., 2012. Seaweed assemblage changes in the eastern Cantabrian Sea and their potential relationship to climate change. *Estuarine, Coastal and Shelf Science* 99, 108-120.
- Dugan, J.E., Hubbard, D.M., Page, H.M., Schimel, J.P., 2011. Marine Macrophyte Wrack Inputs and Dissolved Nutrients in Beach Sands. *Estuaries and Coasts* 34, 839-850.
- Engle, V.D., Summers, J.K., 1999. Latitudinal gradients in benthic community composition in Western Atlantic estuaries. *Journal of Biogeography* 26, 1007-1023.
- Gabric, A.J., Bell, P.R.F., 1993. Review of the effects of non-point nutrient loading on coastal ecosystems. *Australian Journal of Marine and Freshwater Research* 44, 261-283.

Genner, M.J., Sims, D.W., Wearmouth, V.J., Southall, E.J., Southward, A.J., Henderson, P.A., Hawkins, S.J., 2004. Regional climatic warming drives long-term community changes of British marine fish. *Proceedings of the Royal Society of London Series B-Biological Sciences* 271, 655-661.

Gray, J.S., 1974. Animal-sediment relationship. *Oceanography and Marine Biology* 12, 223-261.

Gray, J.S., Wu, R.S.S., Or, Y.Y., 2002. Effects of hypoxia and organic enrichment on the coastal marine environment. *Marine Ecology-Progress Series* 238, 249-279.

Hutchings, P., 1998. Biodiversity and functioning of polychaetes in benthic sediments. *Biodiversity and Conservation* 7, 1133-1145.

Jones, J.B., 1992. Environmental impact of trawling on the seabed: A review. *New Zealand Journal of Marine and Freshwater Research* 26, 59-67.

Kemp, W.M., Boynton, W.R., Adolf, J.E., Boesch, D.F., Boicourt, W.C., Brush, G., Cornwell, J.C., Fisher, T.R., Glibert, P.M., Hagy, J.D., Harding, L.W., Houde, E.D., Kimmel, D.G., Miller, W.D., Newell, R.I.E., Roman, M.R., Smith, E.M., Stevenson, J.C., 2005. Eutrophication of Chesapeake Bay: historical trends and ecological interactions. *Marine Ecology-Progress Series* 303, 1-29.

Lindgarth, M., Hoskin, M., 2001. Patterns of Distribution of Macro-fauna in Different Types of Estuarine, Soft Sediment Habitats Adjacent to Urban and Non-urban Areas. *Estuarine, Coastal and Shelf Science* 52, 237-247.

McArdle, B.H., Anderson, M.J., 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology* 82, 290-297.

Morris, L., Keough, M.J., 2003. Testing the effects of nutrient additions on mudflat macroinfaunal assemblages in the presence and absence of shorebird predators. *Marine and Freshwater Research* 54, 859-874.

Nicastro, A., Onoda, Y., Bishop, M.J., 2012. Direct and indirect effects of tidal elevation on eelgrass decomposition. *Marine Ecology-Progress Series* 456, 53-62.

Nixon, S.W., 1995. Coastal marine eutrophication – a definition, social causes, and future concerns. *Ophelia* 41, 199-219.

Nizzoli, D., Bartoli, M., Cooper, M., Welsh, D.T., Underwood, G.J.C., Viaroli, P., 2007. Implications for oxygen, nutrient fluxes and denitrification rates during the early stage of sediment colonisation by the polychaete *Nereis* spp. in four estuaries. *Estuarine, Coastal and Shelf Science* 75, 125-134.

O'Brien, A.L., Volkenborn, N., van Beusekom, J., Morris, L., Keough, M.J., 2009. Interactive effects of porewater nutrient enrichment, bioturbation and sediment characteristics on benthic assemblages in sandy sediments. *Journal of Experimental Marine Biology and Ecology* 371, 51-59.



O'Brien, A.L., Morris, L., Keough, M.J., 2010. Multiple sources of nutrients add to the complexities of predicting marine benthic community responses to enrichment. *Marine and Freshwater Research* 61, 1388-1398.

Pearson, T.H., Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanography and Marine Biology - An Annual Review* 16, 229-311.

Pearson, R.G., Dawson, T.P., 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecology and Biogeography* 12, 361-371.

Penniford, M., Davis, J., 2001. Macrofauna and nutrient cycling in the Swan River Estuary, Western Australia: experimental results. *Hydrological Processes* 15, 2537-2553.

Platell, M.E., Orr, P.A., Potter, I.C., 2006. Inter- and intraspecific partitioning of food resources by six large and abundant fish species in a seasonally open estuary. *Journal of Fish Biology* 69, 243-262.

Przeslawski, R., Zhu, Q., Aller, R., 2009. Effects of abiotic stressors on infaunal burrowing and associated sediment characteristics. *Marine Ecology-Progress Series* 392, 33-42.

Rabalais, N.N., Turner, R.E., Wiseman, W.J., 2002. Gulf of Mexico hypoxia, aka "The dead zone". *Annual Review of Ecology and Systematics* 33, 235-263.

Rabalais, N.N., Turner, R.E., Diaz, R.J., Justic, D., 2009. Global change and eutrophication of coastal waters. *Ices Journal of Marine Science* 66, 1528-1537.

Roper, T., Creese, B., Scanes, P., Stephens, K., Williams, R., Dela-Cruz, J., Coade, G., Coates, B., & Fraser, M., 2011. Assessing the condition of estuaries and coastal lake ecosystems in NSW, Monitoring, evaluation and reporting program, Technical report series, Office of Environment and Heritage, Sydney.

Rosa, S., Granadeiro, J.P., Vinagre, C., Franca, S., Cabral, H.N., Palmeirim, J.M., 2008. Impact of predation on the polychaete *Hediste diversicolor* in estuarine intertidal flats. *Estuarine, Coastal and Shelf Science* 78, 655-664.

Rothenberger, M.B., Burkholder, J.M., Brownie, C., 2009. Long-Term Effects of Changing Land Use Practices on Surface Water Quality in a Coastal River and Lagoonal Estuary. *Environmental Management* 44, 505-523.

Scanes, P., Coade, G., Doherty, M., Hill, R., 2007. Evaluation of the utility of water quality based indicators of estuarine lagoon condition in NSW, Australia. *Estuarine, Coastal and Shelf Science* 74, 306-319.

Snelgrove, P.V.R., Butman, C.A., 1994. Animal sediment relationships revisited - Cause versus effect, in: Ansell, A.D., Gibson, R.N., Barnes, M. (Eds.), *Oceanography and Marine Biology*, Vol 32: An Annual Review. UCL Press Ltd, London, pp. 111-177.

Thrush, S.F., Hewitt, J.E., Hickey, C.W., Kelly, S., 2008. Multiple stressor effects identified from species abundance distributions: Interactions between urban contaminants and species habitat relationships. *Journal of Experimental Marine Biology and Ecology* 366, 160-168.

Wall, D.H., Palmer, M.A., Snelgrove, P.V.R., 2001. Biodiversity in critical transition zones between terrestrial, freshwater, and marine soils and sediments: Processes, linkages, and management implications. *Ecosystems* 4, 418-420.

Warwick, R.M., Clarke, K.R., 1993. Comparing the severity of disturbance – a meta analysis of marine macrobenthic community data. *Marine Ecology-Progress Series* 92, 221-231.

Wernberg, T., Smale, D.A., Thomsen, M.S., 2012. A decade of climate change experiments on marine organisms: procedures, patterns and problems. *Global Change Biology* 18, 1491-1498.

Wildsmith, M.D., Rose, T.H., Potter, I.C., Warwick, R.M., Clarke, K.R., Valesini, F.J., 2009. Changes in the benthic macroinvertebrate fauna of a large microtidal estuary following extreme modifications aimed at reducing eutrophication. *Marine Pollution Bulletin* 58, 1250-1262.

Wildsmith, M.D., Rose, T.H., Potter, I.C., Warwick, R.M., Clarke, K.R., 2011. Benthic macroinvertebrates as indicators of environmental deterioration in a large microtidal estuary. *Marine Pollution Bulletin* 62, 525-538.

York, P.H., Kelaher, B.P., Booth, D.J., Bishop, M.J., 2012. Trophic responses to nutrient enrichment in a temperate seagrass food chain. *Marine Ecology-Progress Series* 449, 291-296.

Young, W.J., Marston, F.M., Davis, J.R., 1996. Nutrient exports and land use in australian catchments. *Journal of Environmental Management*, 165-183.

**5        Effects of increased nutrient loading on the assimilation of organic matter by two  
          benthic consumers across a latitudinal gradient**

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## 5.1 Abstract

Coastal development and climate change are modifying the physico-chemical characteristics of primary producers and the species composition of aquatic plant communities. The effects of these changes on the utilization of plant food resources and the flow of organic matter and energy through food webs are, however, poorly understood. This study assessed 1) how the C:N stoichiometry of two key primary producers, the seagrass *Zostera muelleri*, and the mangrove *Avicennia marina*, vary spatially as a function of climatic setting (latitude) and diffuse nutrient enrichment, 2) whether the  $\delta^{15}\text{N}$  signature of *A. marina* and *Z. muelleri* was correlated with diffuse anthropogenic N loading of catchments and, 3) using stable isotope methods, how spatial variability in the quality and supply of mangrove, seagrass, seston and microphytobenthos influence the ultimate organic matter sources of the detritivorous mud whelk, *Pyrazus ebeninus* and the predatory polychaete, *Nephtys australiensis*. We sampled primary producers and consumers in twelve estuaries of New South Wales Australia along a 7° latitudinal gradient spanning a difference in mean annual temperature of ~ 4°C, and representing a gradient of anthropogenic nutrient disturbance. In agreement with observations of terrestrial plants, the nitrogen content of *A. marina* and *Z. muelleri* leaves was positively correlated with latitude. Nutrient loading, by contrast, positively correlated to the nitrogen content of seagrass but not mangrove leaves. The C and N isotopic signature of consumers did not indicate a shift in their sources of organic matter caused by nutrient enrichment. Instead, the benthic detritivore *P. ebeninus* displayed latitudinal variation in its assimilation of producers, reflecting spatial variation in the stoichiometry of *A. marina* and *Z. muelleri*. Conversely, *N. australiensis* did not display predictable spatial variation in its utilization of the four organic matter sources. These results suggest that future warming may alter the stoichiometry of important benthic producers and, consequently, their assimilation by detritivore consumers. By contrast, present nutrient discharge to the south east Australian estuaries studied seems insufficient to produce major changes in the quality of producers that

influence the flow of organic matter in estuarine food webs. The relatively low  $\delta^{15}\text{N}$  values of macrophytes support this conclusion. Yet, as coastal development continues, the possibility for future impacts of nutrient enrichment remains. Research is required to ascertain the tipping point at which nutrient loading in combination with climatic change leads to changes in estuarine food web structure and function.

**Keywords:** carbon cycling; eutrophication; leaf traits; mixing model; producer palatability; trophodynamics.

## **5.2 Introduction**

As fixers of carbon and assimilators of nutrients, plants are pivotal sources of energy and matter to food webs. Most plant material is not consumed by herbivores, but instead enters the detrital pool, fuelling detritivores and decomposers (Moore 2004). These first order consumers, in turn, transfer energy to higher trophic levels (Moore 2004). The nutritional value of plant material (i.e. carbon to nutrient ratio) is one of the main factors controlling the portion of plant production consumed by herbivores and the portion that enters the detrital pathway (Cebrián and Lartigue, 2004).

The nutrient stoichiometry (carbon [C] to nitrogen [N] to phosphorous [P] ratio) of primary producers can vary within and among species (Gusewell, 2004; Reich and Oleksyn, 2004; Ågren, 2008). Among aquatic primary producers, algae tend to have lower C:N ratios than angiosperms, such as seagrasses and mangroves (Baird and Middleton, 2004). Labile resources of low carbon to nitrogen ratio (C:N) are rapidly decomposed and consumed (Cebrian, 1999; Cebrian and Lartigue, 2004). Refractory sources, of higher C:N, are less readily decomposed and may serve as carbon sinks (Cebrian, 1999).

Coastal development and climate change are modifying the physico-chemical characteristics of primary producers (Touchette et al., 2003; Lee, 2004; Apostolaki et al., 2009; Christianen et al., 2012) and the species composition of aquatic plant communities (McGlathery, 2001; Hauxwell et al., 2003; Schiel et al., 2004; Diez et al., 2012; Hoffle et al., 2012). Coastal settlements input nutrients into waterways as a consequence of industry, agriculture and human waste (Pitt et al., 2009; Petrone, 2010). Changes in the N:P of estuarine waters, from nutrient loading or its management, can result in changes in the C:N:P ratios of primary producers, which rapidly assimilate excess nutrients (Christianen et al., 2012; Lee, 2004; Touchette et al., 2003). Further, nutrient enrichment, in combination with warming temperatures, can stimulate the growth of algal blooms, which in turn overgrow and shade seagrasses, leading to changes in the species composition of primary producers (Short



et al., 1995; Short et al., 1999; Touchette et al., 2003; Orth et al., 2006; Waycott et al., 2009). Mangroves, another important element of coastal vegetation, are in some parts of the world expanding and encroaching onto saltmarsh as sea levels rise (Saintilan and Wilton, 2001). In other areas, mangroves are contracting as artificial hard structures obstruct their landward migration (Gilman et al., 2007). Additionally, because plants' physiological stoichiometry (in particular C:N:P) is affected by climatic context (Reich and Oleksyn, 2004; Kerkhoff et al., 2005), warming has the potential to modify the physico-chemical properties of primary producers. In cooler climates, the shorter growing season of angiosperms, such as mangroves, can result in higher nutrient contents as a consequence of seasonally high rates of growth (Lovelock et al., 2007).

Changes in the productivity and quality of primary producers may influence the flow of organic matter and energy through food webs (Deegan and Garritt, 1997). Generalist consumers are able to modify their diet according to the availability and the palatability of food resources (Fox et al., 2009). Although there is a large body of literature investigating changes in the chemical composition and productivity of primary producers in response to disturbance, such as nutrient pollution (Morand and Briand, 1996; Hauxwell et al., 2003; Touchette et al., 2003; Xia and Wan, 2008) and climate change (Reich and Oleksyn, 2004), the effects of such changes on the utilization of plant food resources are poorly understood. Studies to date suggest that impacts of changing detrital resource availability may be significant for food webs. For example, seagrass loss negatively affected the recycling of nutrients through estuarine food webs (Olsen et al., 2011; McClelland and Valiela, 1998). Changes in the composition of macroalgal assemblages alter decomposer communities (Bishop et al., 2010; Rossi et al., 2011).

Nitrogen and carbon stable isotope signatures of producers and consumers can help elucidate trophic relationships and, hence, the food resources used by consumers (Tewfik et al., 2005; McClelland and Valiela, 1998). This is because producers have a recognizable

isotopic signature that can be traced in the tissue of primary consumers (Zieman et al., 1984). The isotopic signature of consumers is determined by the weighted average of the isotopic signature of the food ingested (Gannes et al., 1997). As C and N flow across trophic levels, consumers' isotopic values tend to increase due to the selectivity of metabolic reactions (Peterson and Fry, 1987). This phenomenon, fractionation, must be taken into account when interpreting stable isotope data in food web studies (Dubois et al., 2007; Fry, 2006).

N stable isotopic signature of primary producers can be used as an indicator of changes in the sources of N to an estuary (McClelland and Valiela, 1998; Olsen et al., 2011). The isotopic signature of N transported from the watershed to the estuary is characteristic of the land uses on the estuary catchment (McClelland et al., 1997; McClelland and Valiela, 1998). The major N sources (in the form of nitrate) in urban and undisturbed watersheds are atmosphere, soil, fertilizer, manure and sewage. Nitrate derived from the atmosphere and in soil has a N isotopic signature ranging from  $-7$  to  $8$  ‰ (Kreitler and Jones, 1975; Nestler et al., 2011),  $\delta^{15}\text{N}$  values of nitrate in fertilizer ranges from  $-4$  to  $5$  ‰ (Kellman and Hillaire-Marcel, 2003; Nestler et al., 2011), nitrates from manure and sewage have generally a heavier  $\delta^{15}\text{N}$  signature ranging from  $7$  to  $25$  ‰ (Nestler et al., 2011). Therefore, higher  $\delta^{15}\text{N}$  values of primary producers have been interpreted as a consequence of increases in the N load from wastewater and farming (McClelland et al., 1997; Martinetto et al., 2006; Olsen et al., 2011).

The mud whelk, *Pyrazus ebeninus*, and the polychaete, *Nephtys australiensis*, are broadly distributed consumers along the east and south coast of Australia, inhabiting seagrass and unvegetated habitats of estuaries. *P. ebeninus* is a detritivore, consuming particulate organic matter (Bishop et al., 2007). *N. australiensis* is a predator that derives its nutrition from sediment-dwelling organisms, many of which are detritivores (Connolly et al. 2005). Here we assess 1) how the C:N stoichiometry of two key primary producers, the seagrass *Zostera muelleri*, and the mangrove, *Avicennia marina*, vary spatially as a function of climatic setting (latitude) and diffuse nutrient loading and 2) how spatial variability in the

quality and supply of these primary producers influences the ultimate organic matter sources of *P. ebeninus* and *N. australiensis*. We predict that N content of each of the two producers will be negatively correlated with latitude and positively correlated with nutrient enrichment. We hypothesize that the diet of each of the two consumers will vary spatially, as a consequence of spatial variation in the quality and supply of detrital resources that correlates to latitude and nutrient input. Effects of spatial variation in climate on key ecological variables may help us to understand impacts of future climate change (Andrews and Hughes 2004, 2005).

### 5.3 Materials and methods

#### 5.3.1. Study sites and sampling design

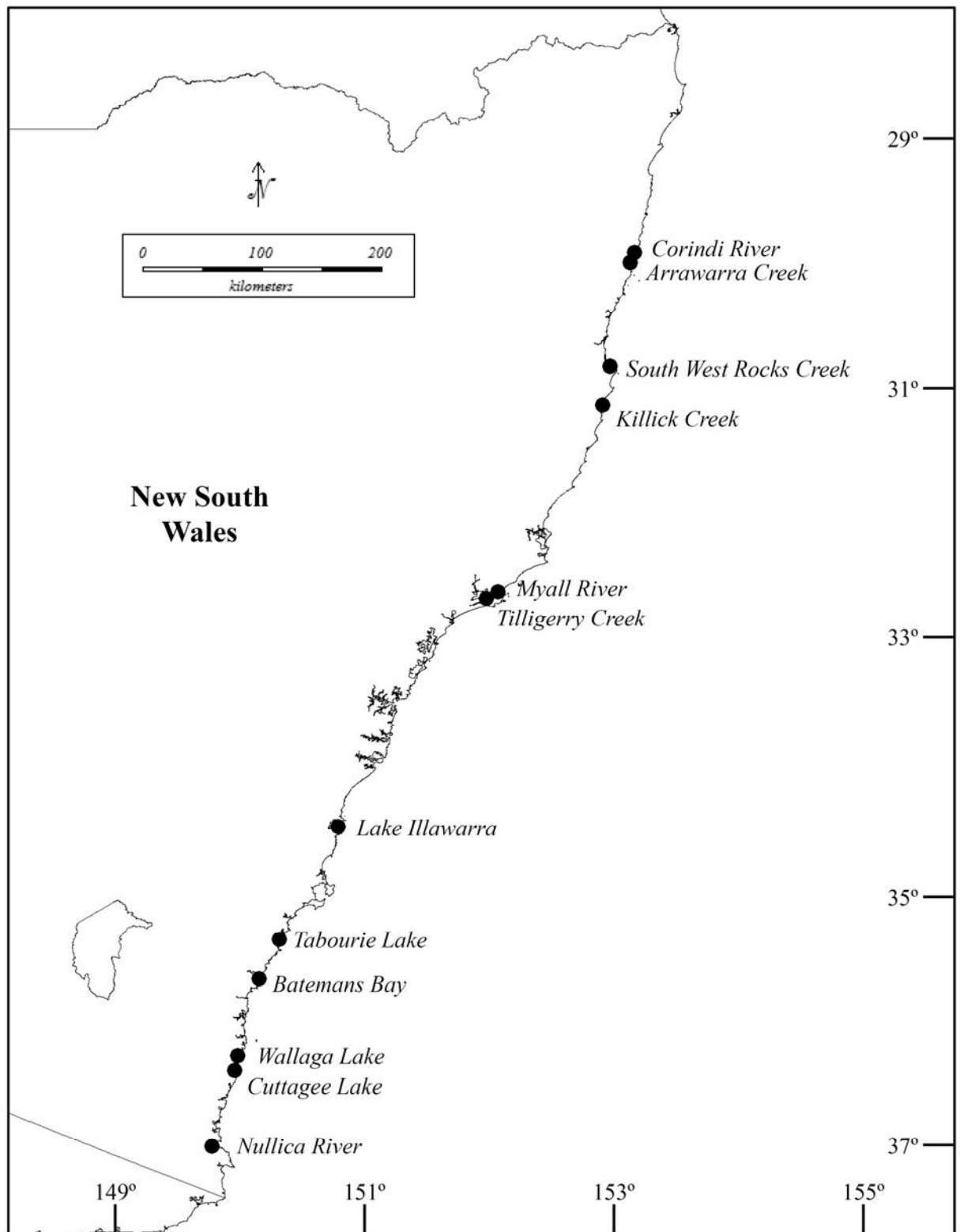
To assess how resource uptake by *Pyrazus ebeninus* and *Nephtys australiensis* varies spatially with respect to diffuse nitrogen enrichment, latitude, and the availability of key primary producers or their detritus, we sampled within 12 estuaries along the temperate coast of New South Wales (NSW), Australia (Table 5.1, Fig. 5.1). Estuaries were selected along a stretch of coast covering 7° in latitude, and spanning a gradient in mean annual temperature of about 4°C. The stretch of coast was subdivided into three sections of similar length. Within each section we randomly selected two replicate estuaries receiving a total nitrogen (TN) loading similar to the undisturbed, pre-European settlement levels (ratio of TN pre-European settlement to present, < 1.9) and two estuaries that had been subjected to a significant increase in nutrient loading due to human activities (ratio of TN pre-European settlement to present, > 2.3). This design ensured that both enriched and unenriched estuaries were represented across the full latitudinal range. The ratios of present day to pre-European TN were obtained from the NSW Office of Environment and Heritage (Roper et al. 2011). The pre-European TN loading was modeled based on the present TN loading, the spatial extent and typology of

human activities that the estuary catchment is presently undergoing and the geomorphological attributes of each estuary (e.g. estuary and catchment area, flushing time; Roper et al. 2011).

**Table 5.1** Physical and chemical attributes, mean chlorophyll-*a* and sediment organic matter [OM] content (mean  $\pm$  SE,  $n = 5$ ) for the 12 estuaries surveyed. Abbreviations: TN flux = flux of total nitrogen, TN ratio = ratio of total nitrogen loading pre-European settlement to present, Chl-*a* = sediment chlorophyll-*a* content, pres = present and sampled, NF = plant not found at the time of sampling.

Estuary	Lat	Long	Estuary area (km <sup>2</sup> ) <sup>a</sup>	% disturbed catch. area <sup>a</sup>	TN flux (mg m <sup>-2</sup> d <sup>-1</sup> ) <sup>a</sup>	Flushing time (d <sup>-1</sup> ) <sup>a</sup>	TN ratio <sup>a</sup>	Mangrove area (dm <sup>-2</sup> ) <sup>a</sup>	Seagrass area (dm <sup>-2</sup> ) <sup>a</sup>	Chl- <i>a</i> (mg m <sup>-2</sup> )	Sediment OM (%)	<i>Avicennia marina</i>	<i>Zostera muelleri</i>
Corindi River	29° 59'	153° 14'	1.9	19	53	5	1.5	37.1	29.3	64.2 $\pm$ 10.5	1.23 $\pm$ 0.24	pres	NF
Arrawarra Creek	30° 04'	153° 12'	0.1	15	117	4	2.5	1.0	0.1	89.6 $\pm$ 27.9	1.25 $\pm$ 0.05	pres	NF
South West Rocks Creek	30° 53'	153° 02'	0.9	27	3	1	2.6	64.8	0.2	193.8 $\pm$ 29.6	0.97 $\pm$ 0.37	pres	pres
Killick Creek	31° 11'	152° 59'	0.3	31	15	56	1.9	4.5	0.0	80.2 $\pm$ 11.3	0.43 $\pm$ 0.01	pres	NF
Myall River	32° 40'	152° 09'	115.2	19	3	16	1.4	302.8	217.3	342.1 $\pm$ 17.7	0.95 $\pm$ 0.48	pres	pres
Tilligerry Creek	32° 44'	152° 03'	134.4	30	1	36	3.8	0.0	275.1	525.2 $\pm$ 22.0	0.47 $\pm$ 0.01	pres	pres
Lake Illawarra	34° 32'	150° 53'	35.8	59	8	127	3.2	0.0	796.6	228.5 $\pm$ 19.1	0.94 $\pm$ 0.08	pres	pres
Tabourie Lake	35° 27'	150° 25'	1.5	15	11	13	1.4	0.0	21.9	1410.0 $\pm$ 252.5	0.48 $\pm$ 0.05	NF	pres
Batemans Bay	35° 45'	150° 15'	34.5	40	0	38	2.7	0.4	18.9	94.9 $\pm$ 8.8	0.40 $\pm$ 0.02	pres	pres
Wallaga Lake	36° 22'	150° 05'	9.3	37	21	97	2.3	0.0	108.5	543.2 $\pm$ 83.3	0.76 $\pm$ 0.15	NF	pres
Cuttagee Lake	36° 29'	150° 03'	1.4	5	17	40	1.3	0.0	38.5	916.8 $\pm$ 238.7	0.47 $\pm$ 0.01	NF	pres
Nullica River	37° 06'	149° 52'	0.3	5	40	8	1.1	0.8	1.2	93.6 $\pm$ 9.9	2.45 $\pm$ 0.10	pres	pres

<sup>a</sup> data from Roper et al. (2011)



**Fig. 5.1** Map showing the location of surveyed estuaries sampled along the New South Wales coast, Australia.

5.3.2. Sampling and processing of food sources and invertebrates for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and N content analyses

Within each of the estuaries, we collected samples of primary producers and consumers for assessment of C:N ratio,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures from a single site, 300 m<sup>2</sup> in area, during low tides in late summer (March 2011). The consumers were the two invertebrates, *N. australiensis* and *P. ebeninus*, and the producers, microphytobenthos (MPB), seston, mangrove and seagrass leaves. Consumers and producers were collected from within close proximity because several studies in east Australian estuaries have demonstrated that invertebrates obtain the majority of their organic matter from immediately adjacent habitats (Guest et al. 2004, Guest and Connolly 2004; but see Connolly et al. 2005 of an example of where this was not the case). All sites were fully marine (salinity ranging from 30 to 35 ‰), had a tidal range of ~1.3 m and where possible encompassed (1) *Avicennia marina* mangrove forests, (2) *Zostera muelleri* eelgrass meadows, and (3) intertidal sand flat.

Invertebrates, *N. australiensis* and *P. ebeninus* and MPB were collected from an area of sandflat roughly equidistant (approximately 20 m away) from mangroves and seagrass. *N. australiensis* (n = 10 per site) were obtained from 10 cm deep cores of sediment, collected 1-2 m apart, and sieved through a 1 mm sieve. *P. ebeninus* (n = 10 per site) were hand collected from the sediment surface. Invertebrates were individually placed in bags of seawater for 6 h to clear their guts. Each individual was treated as a sample and analysed separately.

Sediment samples (n = 10) for extraction of MPB were scraped from the top 1 cm layer of 400 cm<sup>2</sup> unvegetated sediment plots. Samples were kept in the dark and refrigerated with ice for a maximum of 6 hrs before being frozen. Samples of green and intact *A. marina* and *Z. muelleri* (1 and 3-4 leaves for *A. marina* and *Z. muelleri* respectively) were collected from ten randomly chosen plants per site, at least 5 m apart. Five water samples, each ~1 L in volume, for seston extraction were collected from the top 50 cm of waters adjacent to our study sites. Care was taken to avoid collection of water samples from areas of sediment

disturbance. Water samples were filtered in the field through a pre-combusted (490°C) glass fiber filter (GF/F Whatman). A filter was placed at the bottom of a funnel and the sample was gently poured and left decanting until all the water passed through the filter. Filters were folded using tweezers to avoid contamination, individually put in sealable bags and kept refrigerate in the dark. All samples for stable isotope analyses were frozen within six hours of collection and stored at -20°C.

In the laboratory, *P. ebeninus* specimens were extracted from their shells and a ~2 g wet weight sample of the foot was taken for processing. Mangrove and seagrass leaves were rinsed to remove sediments and detritus, and *Z. muelleri* leaves were gently scraped with a razor blade to remove epiphytes. MPB was separated from defrosted sediments by density gradient centrifugation in colloidal silica (Ludox AM-30, Sigma-Aldrich, density = 1.21 g L<sup>-1</sup>). Sediment samples were resuspended in a plastic tray and the suspended material was sieved through a 53 µm sieve to remove infaunal organisms. Material that passed through the mesh was washed through a 5 µm mesh to eliminate fine sediment particles and microorganisms. Material retained on this mesh was centrifuged in colloidal silica until a band became visible at the top of the sample. The supernatant was rinsed again through a 5 µm mesh to remove silica. Visual inspection of the band confirmed it was composed of diatoms. Filters containing seston were acid digested with HCl in order to remove carbonates.

Processed samples were dried until constant weight at 60°C, ground to a fine powder and transferred into tin capsules for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and N analysis. Measurement of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , C and N contents of samples was carried out with an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer at the UC Davis Stable Isotope Facility (University of California, Davis, CA, USA). The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotope ratios for each of the samples were calculated according to conventional international standards (atmospheric nitrogen for  $\delta^{15}\text{N}$  and Pee Dee Belemnite for  $\delta^{13}\text{C}$ ):



$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = \left[ \frac{R_{\text{Sample}}}{R_{\text{Standard}}} - 1 \right] \times 1000\text{‰}$$

where  $R_{\text{standard}} = 0.112372$  and  $R_{\text{sample}} = {}^{13}\text{C}:{}^{12}\text{C}$  and  ${}^{15}\text{N}:{}^{14}\text{N}$  of the sample.

### 5.3.3 Sediment chlorophyll-*a* and organic matter content

To assess how variation among sites in the availability of MPB and organic matter influenced the ultimate organic matter sources of the two invertebrates, we quantified chlorophyll-*a* and organic matter content of the sediment at each intertidal sandflats from which we collected the consumers. Five 2.2 cm cores of sediment (10 cm deep) were collected from each site for Chl-*a* measurements. Upon collection, these were placed immediately into lightproof vials and were frozen until further analysis. Chl-*a* concentration was determined using the method proposed by Lorenzen (1967). Briefly, each sample received 10 ml of acetone and two drops of carbonate solution. After 24 h samples were centrifuged (4500 rpm, 10 min) and the absorbance of the supernatant was measured with a spectrophotometer at 750 and 665 nm, before and after acidification with 1 mol HCl. The Lorenzen (1967) equation was used to calculate the concentration of Chl-*a*. To assess sediment organic content, five sediment cores (2 cm in diameter and 10 cm deep) were taken at each site and preserved as per Chl-*a* samples. After homogenization, a subsample of ~4 g (wet weight) from each sample was dried at 105 °C for 48 h and weighed prior to combustion at 550 °C for 4 h. The organic content was calculated as the percentage difference in weight from before to after combustion.

Data on the areal coverage of *A. marina* and total seagrass within each estuary were obtained from Roper et al. (2011). Although estimates of seagrass cover included not only *Z. muelleri* but also *Posidonia australis*, and *Holophila* spp, *Z. muelleri* was the dominant species. Hence we assumed that total seagrass cover and *Z. muelleri* cover would be strongly correlated.

#### 5.3.4 Statistical analysis

To assess the variability of *A. marina* and *Z. muelleri* N and  $\delta^{15}\text{N}$  content in relation to latitude and present to historic TN ratio, we used linear regression. Kolmogorov-Smirnov tests assessed whether the variables fitted normal distributions and, where necessary, data were log or square-root transformed to achieve normality.

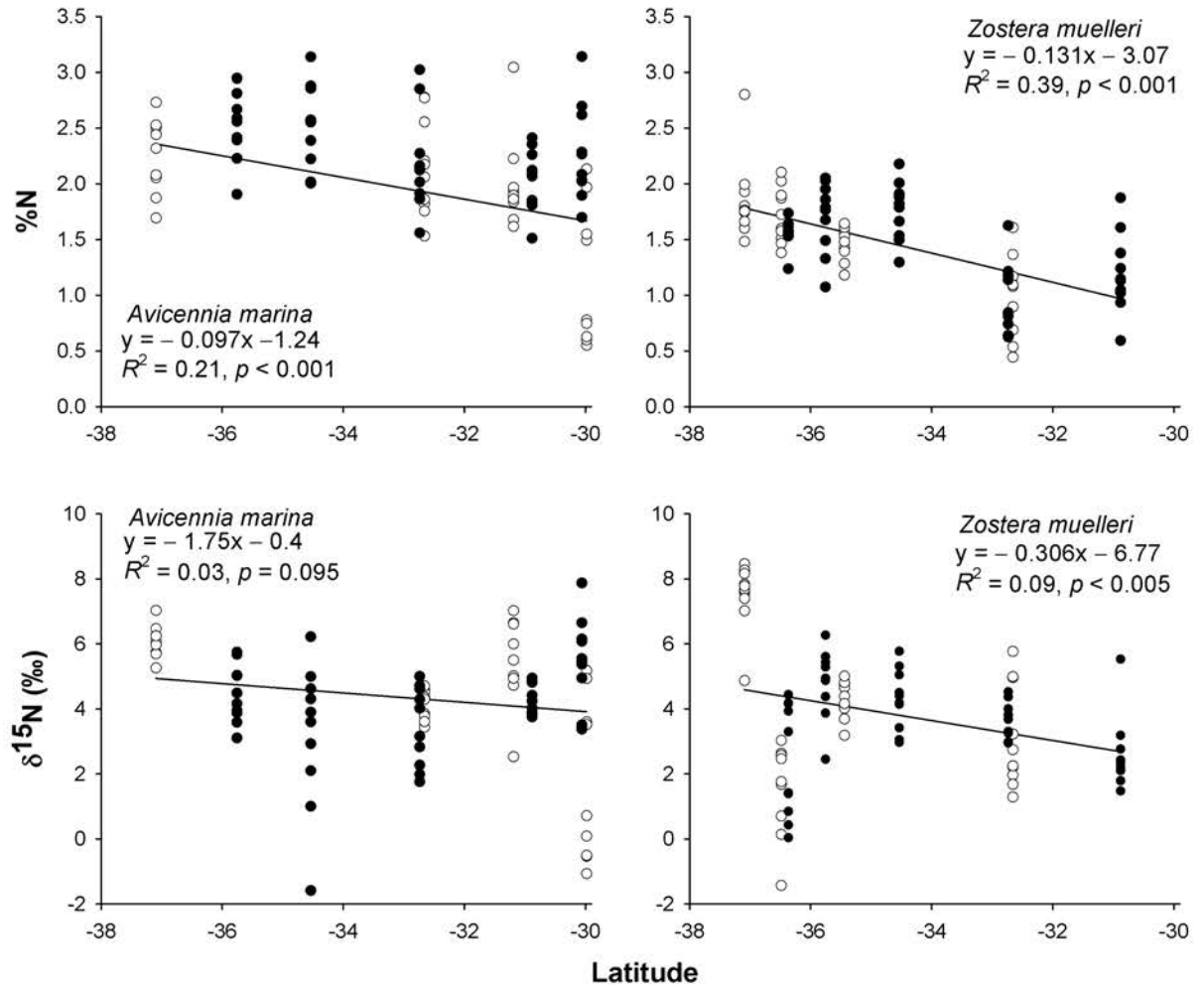
To calculate the average relative contribution of producers to the resources assimilated by consumers we used the Isosource model (Phillips and Gregg, 2003). Before running the Isosource model, we corrected the consumers  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for fractionation that occurs as these elements are transferred along the food chain. We subtracted 0.8 ‰ from  $\delta^{13}\text{C}$  and 3.4 ‰ from  $\delta^{15}\text{N}$  values of *P. ebeninus* and *N. australiensis*. Since no specific values for *P. ebeninus* and *N. australiensis* are available to date, we used average values for a broad range of aquatic consumers (Minagawa and Wada, 1984; Zanden and Rasmussen, 2001). By examining all the possible combinations of each dietary source contribution to the consumer diet in small (1%) increments, this model calculates the feasible combinations of food sources explaining the isotope signature of the consumer within a small tolerance (0.1 in this study). The average percent contribution of each food source calculated by the Isosource model was used for further multivariate analyses. The influence of each of the predictor variables (latitude, TN ratio and loading, sediment OM, Chl-*a* and *A. marina* and *Z. muelleri* area), on the diets of *P. ebeninus* and *N. australiensis* were visualized using distance-based redundancy analysis (db-RDA) (McArdle and Anderson, 2001). This was followed by multivariate regression (DistLM routine) testing the significance of these correlations by fitting a linear model based on Euclidean distance dissimilarities data using permutations. Predictor variables were linearised by applying appropriate transformation prior to analyses.

The software PRIMER v6 (Clarke and Gorley 2006) with the add-on PERMANOVA+ (Anderson et al. 2008) was used to perform multivariate analyses. Univariate analyses were performed using Minitab v16 statistical package.

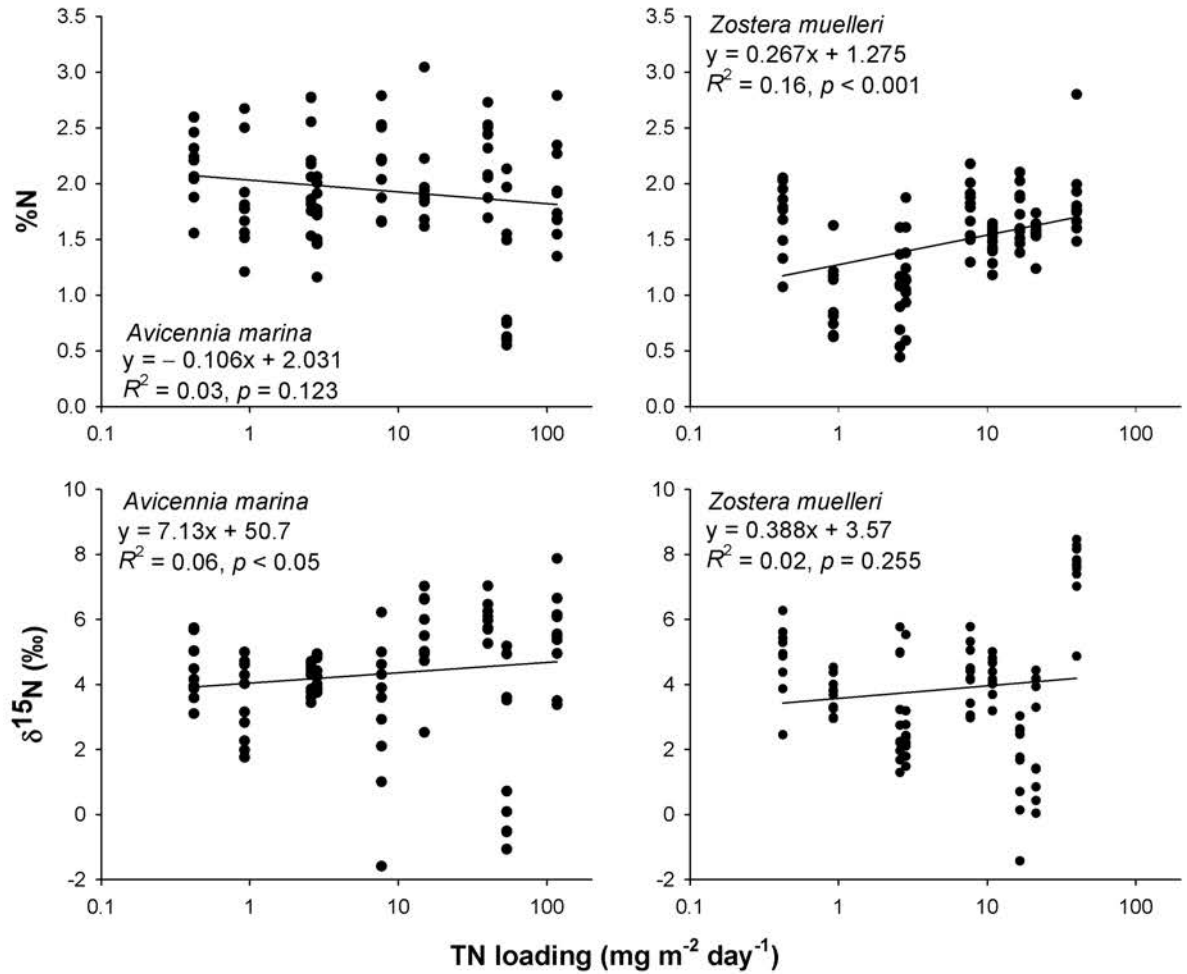
## 5.4 Results

### 5.3.1 N content and $\delta^{15}\text{N}$ signature of mangrove and eelgrass in relation to nutrient enrichment and availability and latitude

Plants of *A. marina* and *Z. muelleri* were found in nine out of twelve estuaries sampled (Table 5.1). The N content and C:N of plants were highly correlated (Pearson's correlation coefficients: 0.95 and 0.93 for *A. marina* and *Z. muelleri* respectively) therefore analyses were run on the N content. The N content of their leaves ranged from 0.55 % to 3.05 % (mean  $1.94 \pm 0.05$  %,  $n = 90$ ) for *A. marina* and from 0.44 % to 2.80 % (mean  $1.47 \pm 0.05$  %,  $n = 90$ ) for *Z. muelleri*. Regression analysis revealed that latitude was a good predictor of the variability of N content of both species (Fig. 5.2). Specifically, N content of *A. marina* and *Z. muelleri* decreased with increasing latitude (Fig. 5.2). The correlation between N content and each of TN ratio and TN loading was, by contrast, species specific. The N content of *A. marina* did not vary spatially in relation to TN ratio ( $df_{1, 88}$ ,  $R^2 = 0.00$ ,  $p = 0.923$ ) or TN loading (Fig. 5.3), whereas the N content of *Zostera muelleri* was significantly and positively related with both TN ratio ( $df_{1, 88}$ ,  $R^2 = 0.07$ ,  $p < 0.05$ ) and TN loading (Fig 5.3).



**Fig. 5.2** Linear regression between latitude and the %N content and the  $\delta^{15}\text{N}$  signature of *Zostera muelleri* and *Avicennia marina* leaves. Empty and full circles represent low and high total nitrogen to estuary respectively.



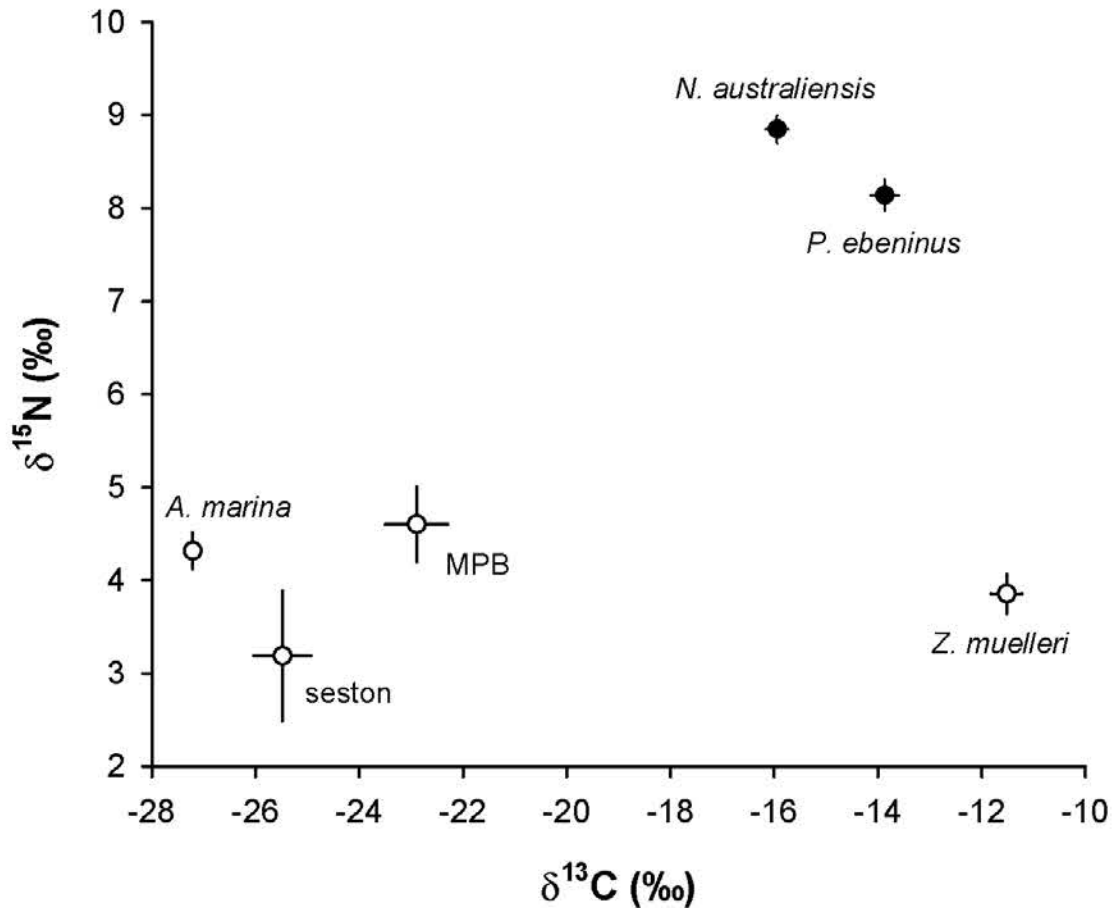
**Fig. 5.3.** Linear regression between total nitrogen (TN) loading to estuary and the %N content and the δ<sup>15</sup>N signature of *Zostera muelleri* and *Avicennia marina* leaves. Note that TN loading is plotted on a log scale.

The δ<sup>15</sup>N signature of *A. marina* ranged from – 1.6 ‰ to 7.9 ‰ (mean  $4.3 \pm 0.2$  ‰,  $n = 90$ ) and *Z. muelleri* had δ<sup>15</sup>N values ranging from – 1.4 ‰ to 8.5 ‰ (mean  $3.9 \pm 0.2$  ‰,  $n = 90$ ) (Fig. 5.4). The N isotopic signature of *A. marina* did not show any significant relationship with latitude (Fig. 5.2). *Z. muelleri* δ<sup>15</sup>N signature, instead, decreased significantly with increasing latitude but the relationship was weak (Fig. 5.2). The variability of *A. marina* δ<sup>15</sup>N signature was weakly explained by TN ratio ( $df_{1,88}$ ,  $R^2 = 0.06$ ,  $p < 0.05$ ) and TN loading (Fig. 5.3), whereas *Z. muelleri* δ<sup>15</sup>N signature did not vary with varying TN ratio ( $df_{1,88}$ ,  $R^2 = 0.02$ ,  $p = 0.150$ ) nor TN loading (Fig. 5.3).

#### 5.4.2 Spatial variability in the sources of organic matter assimilated by invertebrates

*P. ebeninus* was found in all estuaries except Killick Creek and its isotope values ranged from  $-24.7\text{‰}$  to  $-9.2\text{‰}$  (mean  $-13.9 \pm 0.3\text{‰}$ ,  $n = 110$ ) for  $\delta^{13}\text{C}$  and from  $4.7\text{‰}$  to  $12.1\text{‰}$  (mean  $8.1 \pm 0.2\text{‰}$ ,  $n = 110$ ) for  $\delta^{15}\text{N}$  (Fig. 5.4). *N. australiensis* was found in all estuaries and stable isotope values ranged from  $-22.3\text{‰}$  to  $-9.8\text{‰}$  (mean  $-15.9 \pm 0.2\text{‰}$ ,  $n = 120$ ) for  $\delta^{13}\text{C}$  and from  $5.3\text{‰}$  to  $12.7\text{‰}$  (mean  $8.8 \pm 0.2\text{‰}$ ,  $n = 120$ ) for  $\delta^{15}\text{N}$  (Fig. 5.4).

Some seston and microphytobenthos samples collected for the characterization of invertebrate diets did not yield sufficient quantity of material for reliable  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analysis. Consequently, only data from 8 and 9 sites, for *P. ebeninus* and *N. australiensis* respectively, had sufficient replication (minimum  $n = 4$  for MPB and  $n = 3$  for seston) and were analysed with Isosource. Seston isotopic values ranged from  $-29.5\text{‰}$  to  $-20.3\text{‰}$  (mean  $-25.5 \pm 0.6\text{‰}$ ,  $n = 27$ ) for  $\delta^{13}\text{C}$  and from  $0.2\text{‰}$  to  $12.5\text{‰}$  (mean  $3.2 \pm 0.7\text{‰}$ ,  $n = 27$ ) for  $\delta^{15}\text{N}$  (Fig. 5.4). MPB  $\delta^{13}\text{C}$  values ranged from  $-27.4\text{‰}$  to  $-3.4\text{‰}$  (mean  $-22.9 \pm 6\text{‰}$ ,  $n = 62$ ) and  $\delta^{15}\text{N}$  values from  $0.1\text{‰}$  to  $14.9\text{‰}$  (mean  $4.6 \pm 0.4\text{‰}$ ,  $n = 62$ ) (Fig. 5.4).



**Fig. 5.4**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (mean  $\pm$  SE) of potential organic matter sources (seston, microphytobenthos [MPB], *Avicennia marina* and *Zostera muelleri*) and benthic consumers *Pyrazus ebeninus* and *Nephtys australiensis* in all the estuaries. Values shown are not corrected for fractionation.

The Isosource model indicated that of the four C and N sources considered, *P. ebeninus* and *N. australiensis* relied mostly on *Z. muelleri* (mean contributions of  $66 \pm 3$  % and  $60 \pm 5$  % for *P. ebeninus* and *N. australiensis* respectively). A secondary food source was represented by MPB for *N. australiensis* (mean contribution  $26 \pm 9$  %), whereas *P. ebeninus* relied, in similar measure, on organic material derived from seston, MPB and *A. marina* (mean contribution  $13 \pm 2$  %,  $12 \pm 1$  % and  $10 \pm 2$  %, respectively). Similarly to *P. ebeninus*, the contribution of *A. marina* and seston to the diet of *N. australiensis* was  $11 \pm 2$  % and  $11 \pm 2$  %, respectively. *N. australiensis* sampled at Killick Creek, where *Z. muelleri* was not found, relied almost exclusively (90 %) on organic matter derived from MPB. At Lake Tabourie,

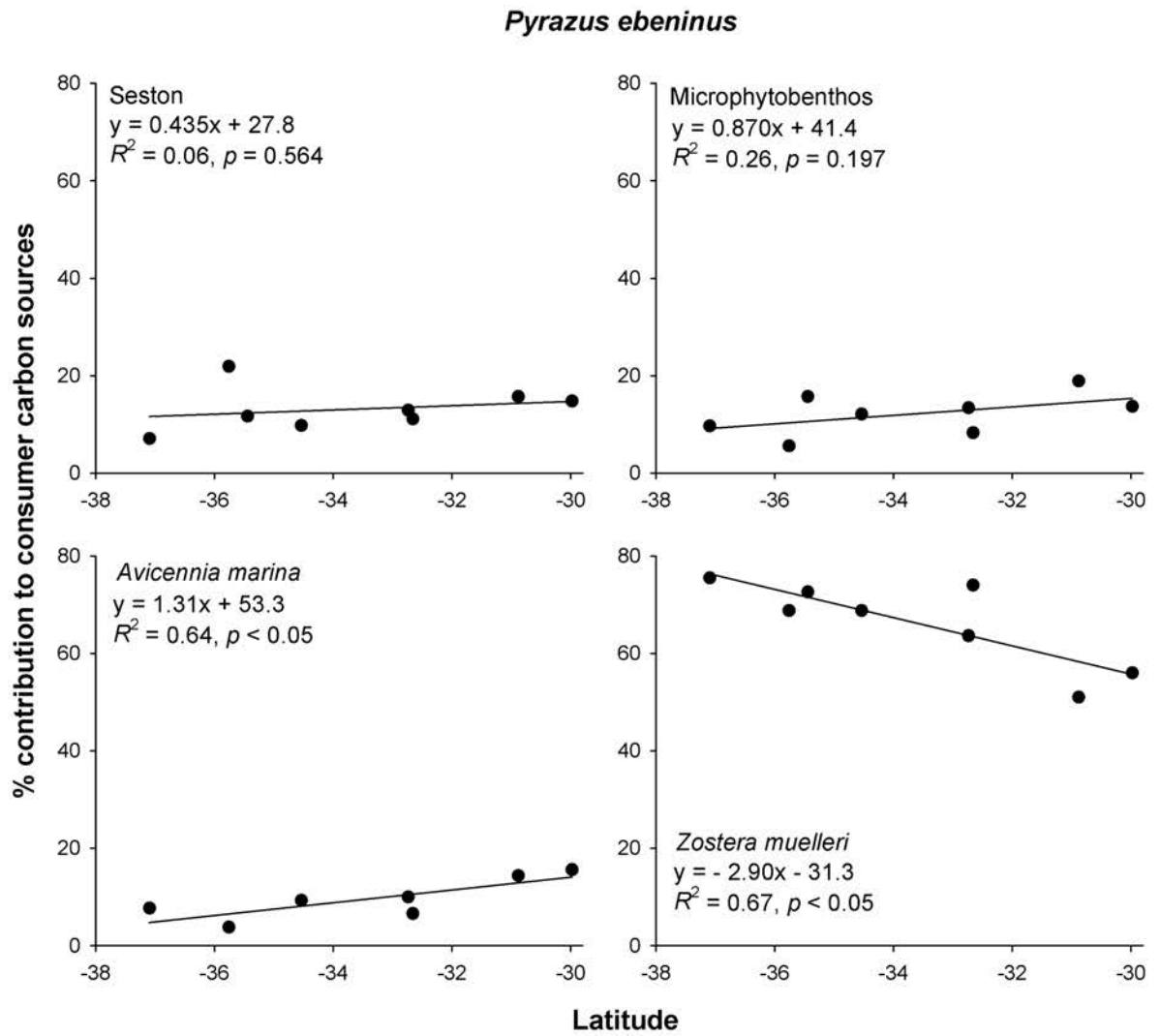
where *A. marina* was not found, both consumers derived the majority of their organic matter from *Z. muelleri* (72 % and 63 % for *P. ebeninus* and *N. australiensis* respectively).

Multivariate analyses indicated that the spatial variability of *P. ebeninus* and *N. australiensis* C sources were not correlated with latitude, TN ratio or loading, or the availability of the main food sources, except for the variability of *P. ebeninus* C sources, which was significantly correlated with latitude (Table 5.2). Linear regression revealed that the contribution of *A. marina* to the C passed on through the trophic chain to *P. ebeninus* increased with latitude, whereas the contribution of *Z. muelleri* decreased with latitude (Fig. 5.5). Nevertheless, the contribution of seston and MPB to *P. ebeninus* C signature were not correlated with latitude. Linear regressions between latitude and the percent contribution of each of the four food sources, *A. marina*, *Z. muelleri*, seston and MPB to the C passed on to *N. australiensis* were not significant (Fig. 5.5 and Fig.5.6).

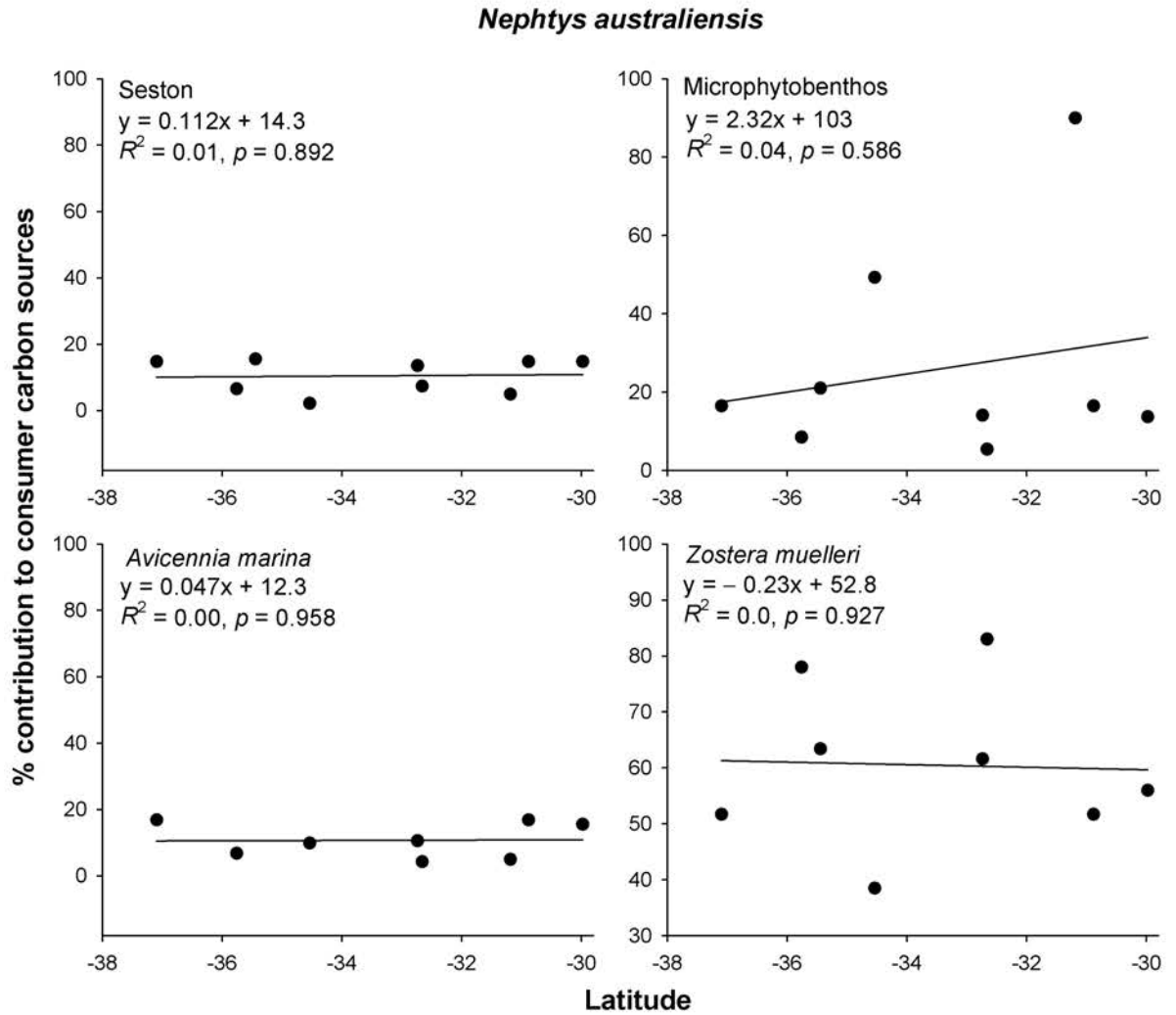
**Table 5.2** Results of multivariate multiple regression analyses (DistLM) testing for correlation between changes in the average relative contribution of producers to the resources assimilated by consumers and each of the predictor variables. Prop. = the proportion of variance explained by each variable. TN ratio = ratio of total nitrogen loading pre-European settlement to present. Significant ( $p < 0.05$ ) predictor variables are in bold.

Variable	<i>Pyrazus ebeninus</i>			<i>Nephtys australiensis</i>		
	Pseudo-F	$p_{\text{perm}}$	Prop.	Pseudo-F	$p_{\text{perm}}$	Prop.
Latitude	<b>6.4</b>	<b>0.016</b>	<b>0.52</b>	0.5	0.586	0.06
TN ratio	0.6	0.545	0.09	0.1	0.917	0.01
TN loading	0.3	0.804	0.05	0.5	0.561	0.06
Seagrass area	1.3	0.290	0.18	0.1	0.897	0.01
Mangrove area	1.3	0.298	0.17	1.0	0.304	0.13
Chlorophyll- <i>a</i>	1.0	0.419	0.14	0.6	0.433	0.08
Sediment organic matter	0.6	0.632	0.09	0.4	0.538	0.05





**Fig. 5.5** Linear regressions between latitude and the % contribution of potential organic matter sources to the diet of *Pyrazus ebeninus*, as indicated by IsoSource.



**Fig. 5.6** Relationship between latitude and the indirect % contribution of potential organic matter sources to the nutrition of *Nephtys australiensis*, a benthic predator.

## 5.5 Discussion

An increase in nutrient availability has the potential to affect the nutrient stoichiometry and supply of aquatic primary producers with flow-on effects to consumer communities (McClelland and Valiela, 1998). In this study we assessed how latitudinal and nutrient enrichment gradients influenced producer stoichiometry and how this, in turn, influenced the relative importance of these organic matter sources to key consumers. As expected, the nitrogen content of mangrove (*Avicennia marina*) and seagrass (*Zostera muelleri*) leaves was positively correlated with latitude south. Nutrient loading, by contrast, positively correlated to the nitrogen content of seagrass but not mangrove leaves. The C and N isotopic signature of consumers did not indicate a shift in their sources of organic matter caused by nutrient enrichment. Instead, the latitudinal variation in the stoichiometry of producers was related to the spatial variation in the utilization of two organic matter sources, *A. marina* and *Z. muelleri*, by the benthic detritivore *Pyrazus ebeninus*. Nevertheless, the benthic predator *Nephtys australiensis* did not display predictable spatial variation in its utilization of the four organic matter sources, *A. marina*, *Z. muelleri*, microphytobenthos and seston.

The N content of *A. marina* and *Z. muelleri* leaves was negatively correlated with latitude. Although this trend has previously been observed in terrestrial plants (Sternner and Elser, 2002; Reich and Oleksyn, 2004; Kerkhoff et al., 2005; Lovelock et al., 2007), our study is the first, to our knowledge, to show it also extends to aquatic angiosperms. It has been proposed that a higher N leaf concentration in colder climates may be the result of a decrease in the efficiency of N-rich enzymes with decreasing temperature (Weih and Karlsson, 2001; Woods et al., 2003; Reich and Oleksyn, 2004). Consistent with this hypothesis, Touchette and Burkholder (2007) found that the activity of sucrose synthase and sucrose-P synthase in *Zostera marina* was temperature dependent. Therefore, it is possible

that projected anthropogenic climate warming could decrease the N demand and content of seagrasses.

The availability of nutrients had a positive effect on the N content of *Z. muelleri* but not *A. marina*. Previous studies have demonstrated a correlation between the availability of nutrients and their uptake by seagrasses of the genus *Zostera* (Touchette and Burkholder, 2000). In addition, Udy and Dennison (1997) found similar results in an *in situ* nutrient enrichment experiment with *Zostera muelleri* (ex *Z. capricorni*), supporting the hypothesis that seagrasses living in oligotrophic conditions such as Australian estuaries are generally nutrient limited. The lack of response of *A. marina* N leaf content to nutrient availability may reflect differences in the mechanisms of N assimilation between the two plants. In addition to nutrient uptake through roots and rhizomes, seagrasses are able to absorb nitrate from the water column through their leaves (Short and McRoy, 1984; Terrados and Williams, 1997) and they can also directly utilize N contained in small organic molecules, such as amino acids and urea (McRoy and Goering, 1974). Mangroves, by contrast, are unable to absorb nutrients dissolved in seawater through their leaves, which are generally held above the high-tide line.

Analyses of the  $\delta^{15}\text{N}$  signatures of *A. marina* and *Z. muelleri* indicated that the spatial variability at the within estuary scale was very high compared to the variability across estuaries. Moreover, the N isotopic signature was not correlated with our measures of nutrient loading and enrichment. This may suggest that the nutrient discharge to Australian estuaries is, at the present time, insufficient to produce a detectable  $\delta^{15}\text{N}$  signal in the primary producers and/or that the recent increase in the nutrient loading (i.e. TN ratio) is the result of activities such as agriculture and change in land use that, in contrast to N coming from wastewater, do not produce a detectable enrichment of  $\delta^{15}\text{N}$  (Cole et al., 2006). Similar conclusions were drawn by Scanes et al. (2007) after failing to find a significant correlation between  $\delta^{15}\text{N}$  values of *Z. muelleri* and TN loading of several southeast Australian estuaries.

A higher availability of nutrients could stimulate both planktonic and benthic algal growth that, directly or indirectly through prey-predator relationships, could cause changes in the way C flows through the food web (Olsen et al., 2011). In this study we did not detect spatial variation in the ultimate source of organic matter of two abundant benthic consumers, with respect to nutrient enrichment or the availability of key primary producers. The contribution of *A. marina* and *Z. muelleri* to the C and N passed on to *P. ebeninus* through the trophic chain, however, was correlated to latitude. The rate of decrease in N content with latitude was quite similar for both species of primary producer (Fig. 5.2). *A. marina* leaves, however, had on average a higher content of N compared to *Z. muelleri* across the latitudinal gradient investigated (t-test,  $t_{173} = 6.8$ ,  $p < 0.001$ ). It may be possible that lower N contents at warmer latitudes decreased the flow of *Z. muelleri* organic material through the trophic chain, and that, concomitantly, the availability of organic matter derived from *A. marina* to *P. ebeninus* increased. Yet, future research is needed to elucidate the mechanisms underlying the observed changes in the utilization of organic matter in relation to latitude.

Independent of these factors, *Z. muelleri* was, of the organic matter sources considered by this study, consistently the main source of organic matter for both the detritivore *Pyrazus ebeninus* and the predator *Nephtys australiensis*. This contrasts with observations of changes in the dietary composition of benthic invertebrates following nutrient input to eutrophied estuaries (Fox et al., 2009; McClelland and Valiela, 1998). In these studies, nutrient enrichment caused extensive and prolonged sediment hypoxia (Fox et al., 2009) or a drastic reduction of *Zostera marina* population (McClelland and Valiela, 1998). The failure of our study to detect differences in the organic matter sources of consumers between enriched and unenriched estuaries suggests that in oligotrophic southeast Australian estuaries, nutrient enrichment is not yet severe enough to produce major impacts to estuarine food webs. Here, however, we did not consider more labile macroalgae material that may also enter benthic food webs (Alafaro et al., 2006; Crawley et al., 2009). Nevertheless, Australian estuaries are

not prone to the sizeable *Ulva* sp. and *Enteromorpha* sp. blooms as observed in European estuaries (Scanes et al., 2007).

In this study, we did not consider other sources of organic matter for the consumer *N. australiensis* such as particulate organic matter or prey of this species. Similarly, for neither taxa were terrestrial sources of organic matter considered. Hence, it is possible that taxa other than those considered by this study are more important sources of organic matter.

In conclusion, this study found that spatial variation in the C and N sources of an abundant detritivore gastropod was correlated with changes in the nutritional level of primary producers along a latitudinal gradient. Spatial variation in the organic matter sources of consumers, however, was not correlated with the nutrient enrichment caused by recent urbanization. Nevertheless, nutrient enrichment was correlated with the N content of an important seagrass species, *Z. muelleri* – a pattern that may extend to other seagrass species. As coastal development continues to enhance nutrient enrichment to estuarine systems, the possibility for future impacts remains. Research is required to ascertain the tipping point at which nutrient loading in combination with climatic change leads to changes in estuarine food web structure and function.

## **5.6 References**

- Ågren, G.I., 2008. Stoichiometry and Nutrition of Plant Growth in Natural Communities, *Annual Review of Ecology Evolution and Systematics*, pp. 153-170.
- Andrew, N.R., Hughes, L., 2004. Species diversity and structure of phytophagous beetle assemblages along a latitudinal gradient: predicting the potential impacts of climate change. *Ecological Entomology* 29, 527-542.
- Andrew, N.R., Hughes, L., 2005. Diversity and assemblage structure of phytophagous Hemiptera along a latitudinal gradient: predicting the potential impacts of climate change. *Global Ecology and Biogeography* 14, 249-262.
- Alfaro, A.C., Thomas, F., Sargent, L., Duxbury, M., 2006. Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. *Estuarine, Coastal and Shelf Science* 70, 271-286.
- Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E Ltd, Plymouth, UK.
- Apostolaki, E.T., Marba, N., Holmer, M., Karakassis, I., 2009. Fish farming impact on decomposition of *Posidonia oceanica* litter. *Journal of Experimental Marine Biology and Ecology* 369, 58-64.
- Baird, M.E., Middleton, J.H., 2004. On relating physical limits to the carbon: nitrogen ratio of unicellular algae and benthic plants. *Journal of Marine Systems* 49, 169-175.

Bishop, M.J., Kelaher, B.P., Alquezar, R., York, P.H., Ralph, P.J., Skilbeck, C.G., 2007.

Trophic cul-de-sac, *Pyrazus ebeninus*, limits trophic transfer through an estuarine detritus-based food web. *Oikos* 116, 427-438.

Bishop, M., Coleman, M., Kelaher, B., 2010. Cross-habitat impacts of species decline: response of estuarine sediment communities to changing detrital resources. *Oecologia* 163, 517-525.

Cebrian, J., 1999. Patterns in the fate of production in plant communities. *American Naturalist* 154, 449-468.

Cebrian, J., Lartigue, J., 2004. Patterns of herbivory and decomposition in aquatic and terrestrial ecosystems. *Ecological Monographs* 74, 237-259.

Christianen, M.J.A., Govers, L.L., Bouma, T.J., Kiswara, W., Roelofs, J.G.M., Lamers, L.P.M., van Katwijk, M.M., 2012. Marine megaherbivore grazing may increase seagrass tolerance to high nutrient loads. *Journal of Ecology* 100, 546-560.

Clarke, K. R., Gorley, R.N., 2006. PRIMER v6. PRIMER-E Ltd, Plymouth, UK.

Cole, M.L., Kroeger, K.D., McClelland, J.W., Valiela, I., 2006. Effects of watershed land use on nitrogen concentrations and  $\delta^{15}\text{N}$  in groundwater. *Biogeochemistry* 77, 199-215.

Connolly, R.M., Gorman, D., Guest, M.A., 2005. Movement of carbon among estuarine habitats and its assimilation by invertebrates. *Oecologia* 144: 684-691.



Crawley, K.R., Hyndes, G.A., Vanderklift, M.A., Revill, A.T., Nichols, P.D., 2009.

Allochthonous brown algae are the primary food source for consumers in a temperate, coastal environment. *Marine Ecology-Progress Series* 376, 33-44.

Deegan, L.A., Garritt, R.H., 1997. Evidence for spatial variability in estuarine food webs. *Marine Ecology-Progress Series* 147, 31-47.

Diez, I., Muguerza, N., Santolaria, A., Ganzedo, U., Gorostiaga, J.M., 2012. Seaweed assemblage changes in the eastern Cantabrian Sea and their potential relationship to climate change. *Estuarine Coastal and Shelf Science* 99, 108-120.

Dubois, S., Jean-Louis, B., Bertrand, B., Lefebvre, S., 2007. Isotope trophic-step fractionation of suspension-feeding species: Implications for food partitioning in coastal ecosystems. *Journal of Experimental Marine Biology and Ecology* 351, 121-128.

Fox, S.E., Teichberg, M., Olsen, Y.S., Heffner, L., Valiela, I., 2009. Restructuring of benthic communities in eutrophic estuaries: lower abundance of prey leads to trophic shifts from omnivory to grazing. *Marine Ecology-Progress Series* 380, 43-57.

Fry, B., 2006. *Stable Isotope Ecology*. Springer, New York.

Gannes, L.Z., Obrien, D.M., delRio, C.M., 1997. Stable isotopes in animal ecology: Assumptions, caveats, and a call for more laboratory experiments. *Ecology* 78, 1271-1276.

Gilman, E., Ellison, J., Coleman, R., 2007. Assessment of mangrove response to projected relative sea-level rise and recent historical reconstruction of shoreline position. *Environmental Monitoring and Assessment* 124, 105-130.

Guest, M.A., Connolly, R.M., Loneragan, N.R., 2004. Carbon movement and assimilation by invertebrates in estuarine habitats at a scale of metres. *Marine Ecology-Progress Series* 278, 27-34.

Gusewell, S., 2004. N : P ratios in terrestrial plants: variation and functional significance. *New Phytologist* 164, 243-266.

Hauxwell, J., Cebrian, J., Valiela, I., 2003. Eelgrass *Zostera marina* loss in temperate estuaries: relationship to land-derived nitrogen loads and effect of light limitation imposed by algae. *Marine Ecology-Progress Series* 247, 59-73.

Hoffle, H., Wernberg, T., Thomsen, M.S., Holmer, M., 2012. Drift algae, an invasive snail and elevated temperature reduce ecological performance of a warm-temperate seagrass, through additive effects. *Marine Ecology-Progress Series* 450, 67-85.

Kellman, L.M., Hillaire-Marcel, C., 2003. Evaluation of nitrogen isotopes as indicators of nitrate contamination sources in an agricultural watershed. *Agriculture, Ecosystems and Environment* 95, 87-102.

Kerkhoff, A.J., Enquist, B.J., Elser, J.J., Fagan, W.F., 2005. Plant allometry, stoichiometry and the temperature-dependence of primary productivity. *Global Ecology and Biogeography* 14, 585-598.

Kreitler, C.W., Jones, D.C., 1975. Natural Soil Nitrate: The Cause of the Nitrate Contamination of Ground Water in Runnels County, Texas. *Ground Water* 13, 53-62.

Lee, K.S., 2004. Development of indicator for coastal and estuarine Eutrophication using morphological characteristics and tissue N content of eelgrass, *Zostera marina*. *Algae* 19, 129-137.

Lorenzen, C.J., 1967. Determination of chlorophyll and phaeo-pigments: spectrophotometric equations. *Limnology and Oceanography* 12, 343-346.

Lovelock, C.E., Feller, I.C., Ball, M.C., Ellis, J., Sorrell, B., 2007. Testing the growth rate vs. geochemical hypothesis for latitudinal variation in plant nutrients. *Ecology Letters* 10, 1154-1163.

Martinetto, P., Teichberg, M., Valiela, I., 2006. Coupling of estuarine benthic and pelagic food webs to land-derived nitrogen sources in Walquoit Bay, Massachusetts, USA. *Marine Ecology Progress Series* 307, 37-48.

McArdle, B.H., Anderson, M.J., 2001. Fitting Multivariate Models to Community Data: A Comment on Distance-Based Redundancy Analysis. *Ecology* 82, 290-297.

McClelland, J.W., Valiela, I., Michener, R.H., 1997. Nitrogen-Stable Isotope Signatures in Estuarine Food Webs: A Record of Increasing Urbanization in Coastal Watersheds. *Limnology and Oceanography* 42, 930-937.

- McCelland, J.W., Valiela, I., 1998. Linking nitrogen in estuarine producers to land-derived sources. *Limnology and Oceanography* 43, 577-585.
- McGlathery, K.J., 2001. Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched coastal waters. *Journal of Phycology* 37, 453-456.
- McRoy, C.P., Goering, J.J., 1974. The influence of ice on the primary productivity of the Bering Sea. *The Oceanography of the Bearing Sea*, 403-421. Institute of Marine Science, University of Alaska, Alaska, USA.
- Minagawa, M., Wada, E., 1984. Stepwise enrichment of  $^{15}\text{N}$  along food chains: Further evidence and the relation between  $\delta^{15}\text{N}$  and animal age. *Geochimica et Cosmochimica Acta* 48, 1135-1140.
- Moore, J.C., Eric, L.B., David, C.C., Peter, C.R., Quan, D., Alan, H., Nancy Collins, J., Kevin, S.M., Kim, M., Peter, J.M., Knute, N., Amy, D.R., David, M.P., John, L.S., Kate, M.S., Michael, J.V., Diana, H.W., 2004. Detritus, trophic dynamics and biodiversity. *Ecology Letters* 7, 584-600.
- Morand, P., Briand, X., 1996. Excessive growth of macroalgae: A symptom of environmental disturbance. *Botanica Marina* 39, 491-516.
- Nestler, A., Berglund, M., Accoe, F., Duta, S., Xue, D.M., Boeckx, P., Taylor, P., 2011. Isotopes for improved management of nitrate pollution in aqueous resources: review of surface water field studies. *Environmental Science and Pollution Research* 18, 519-533.

- Olsen, Y.S., Fox, S.E., Teichberg, M., Otter, M., Valiela, I., 2011.  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  reveal differences in carbon flow through estuarine benthic food webs in response to the relative availability of macroalgae and eelgrass. *Marine Ecology-Progress Series* 421, 83-96.
- Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., Short, F.T., Waycott, M., Williams, S.L., 2006. A global crisis for seagrass ecosystems. *Bioscience* 56, 987-996.
- Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18, 293-320.
- Petrone, K.C., 2010. Catchment export of carbon, nitrogen, and phosphorus across an agro-urban land use gradient, Swan-Canning River system, southwestern Australia. *J. Geophys. Res.-Biogeosci.* 115.
- Phillips, D.L., Gregg, J.W., 2003. Source partitioning using stable isotopes: coping with too many sources. *Oecologia* 136, 261-269.
- Pitt, K.A., Connolly, R.M., Maxwell, P., 2009. Redistribution of sewage-nitrogen in estuarine food webs following sewage treatment upgrades. *Marine Pollution Bulletin* 58, 573-580.
- Reich, P.B., Oleksyn, J., 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences of the United States of America* 101, 11001-11006.

- Roper, T., Creese, B., Scanes, P., Stephens, K., Williams, R., Dela-Cruz, J., Coade, G., Coates, B., & Fraser, M., 2011. Assessing the condition of estuaries and coastal lake ecosystems in NSW, Monitoring, evaluation and reporting program, Technical report series, Office of Environment and Heritage, Sydney.
- Rossi, F., Incera, M., Callier, M., Olabarria, C., 2011. Effects of detrital non-native and native macroalgae on the nitrogen and carbon cycling in intertidal sediments. *Marine Biology* 158, 2705-2715.
- Saintilan, N., Wilton, K., 2001. Changes in the distribution of mangroves and saltmarshes in Jervis Bay, Australia. *Wetlands Ecology and Management* 9, 409-420.
- Scanes, P., Coade, G., Doherty, M., Hill, R., 2007. Evaluation of the utility of water quality based indicators of estuarine lagoon condition in NSW, Australia. *Estuarine Coastal and Shelf Science* 74, 306-319.
- Schiel, D.R., Steinbeck, J.R., Foster, M.S., 2004. Ten years of induced ocean warming causes comprehensive changes in marine benthic communities. *Ecology* 85, 1833-1839.
- Short, F.T., McRoy, C.P., 1984. Nitrogen uptake by leaves and roots of the seagrass *Zostera marina* L.. *Botanica Marina* 27, 547-556.
- Short, F.T., Burdick, D.M., Kaldy, J.E., 1995. Mesocosm experiments quantify the effects of eutrophication on eelgrass, *Zostera marina*. *Limnology and Oceanography* 40, 740-749.

Short, F.T., Neckles, H.A., 1999. The effects of global climate change on seagrasses. *Aquatic Botany* 63, 169-196.

Terrados, J., Williams, S.L., 1997. Leaf versus root nitrogen uptake by the surfgrass *Phyllospadix torreyi*. *Marine Ecology Progress Series* 149, 267-277

Tewfik, A., Rasmussen, J., McCann, K.S., 2005. Anthropogenic enrichment alters a marine benthic food web. *Ecology* 86, 2726-2736.

Touchette, B.W., Burkholder, J.M., 2000. Review of nitrogen and phosphorus metabolism in seagrasses. *Journal of Experimental Marine Biology and Ecology* 250, 133-167.

Touchette, B.W., Burkholder, J.M., Glasgow, H.B., 2003. Variations in eelgrass (*Zostera marina* L.) morphology and internal nutrient composition as influenced by increased temperature and water column nitrate. *Estuaries* 26, 142-155.

Touchette, B.W., Burkholder, J.M., 2007. Carbon and nitrogen metabolism in the seagrass, *Zostera marina* L.: Environmental control of enzymes involved in carbon allocation and nitrogen assimilation. *Journal of Experimental Marine Biology and Ecology* 350, 216-233.

Udy, J.W., Dennison, W.C., 1997. Growth and physiological responses of three seagrass species to elevated sediment nutrients in Moreton Bay, Australia. *Journal of Experimental Marine Biology and Ecology* 217, 253-277.

Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., Williams, S.L., 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences* 106, 12377-12381.

Weih, M., Karlsson, P.S., 2001. Growth response of Mountain birch to air and soil temperature: is increasing leaf-nitrogen content an acclimation to lower air temperature? *New Phytologist* 150, 147-155.

Woods, H.A., Makino, W., Cotner, J.B., Hobbie, S.E., Harrison, J.F., Acharya, K., Elser, J.J., 2003. Temperature and the chemical composition of poikilothermic organisms. *Functional Ecology* 17, 237-245.

Xia, J., Wan, S., 2008. Global response patterns of terrestrial plant species to nitrogen addition. *New Phytologist* 179, 428-439.

Vander Zanden, M.J., Rasmussen, J.B., 2001. Variation in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography* 46, 2061-2066.

Zieman, J.C., Macko, S.A., Mills, A.L., 1984. Role of Seagrasses and Mangroves in Estuarine Food Webs: Temporal and Spatial Changes in Stable Isotope Composition and Amino Acid Content During Decomposition. *Bulletin of Marine Science* 35, 380-392.



## **6 General conclusions**

### **6.1 Impacts of local and global stressors**

This study examined the ecological impact of two of the most important ramifications of global climate change (GCC) to the coastal environment: sea level rise and climate warming (Kennish, 2002). Sea level rise has the potential to exacerbate the negative effects of coastal armouring on alteration and loss of intertidal habitats (Fujii and Raffaelli, 2008; Bulleri and Chapman, 2010). Warming can affect a wide range of biological processes, from the species to the community levels (Walther et al., 2002). It can result in shifts in species distribution ranges (Engle and Summers, 1999), alter species interactions (Wiklund et al., 2009) and consequently, change community composition (Andrew and Hughes, 2005). The magnitude of GCC effects will depend on the environmental characteristics and on the ecological resilience of the impacted habitat. Yet, the extent to which these two global changes will drive environmental change in estuarine ecosystems is still not clear (Kennish, 2002; Gillanders et al., 2011).

Coastal armoring and nutrient enrichment are the human activities with possibly the greatest ecological impact on estuaries (Kennish, 2002; Lotze et al., 2006; Airolidi and Beck, 2007). Artificial structures are a common feature that dominate a considerable part of the coastline in urbanised estuaries around the world (Bulleri and Chapman, 2010) and that can have deleterious ecological impacts (Fletcher, 1997; Fujii, 2007; Sobocinski et al., 2010; Chapman and Underwood, 2011). Artificial nutrient fertilisation of estuaries is a common and widespread consequence of urbanization that has substantially contributed to the degradation of estuarine ecosystems (Cloern et al., 2001; Lotze et al., 2006). In addition to the already documented impacts, these local stressors can lower the capacity of estuarine ecosystems to adapt to GCC, and exacerbate GCC impacts by lowering the ecological resilience of estuarine

systems (Wernberg et al., 2011). Thus, management of local stressors represents a valuable tool for preserving and preparing coastal systems in the face of GCC (Kennish, 2002).

This study showed that sea level rise can have major consequences for intertidal and shallow subtidal seagrass habitats where coastal armouring reduces the intertidal area, through so-called coastal squeeze (chapter 2 and 3). Furthermore, this study found that climatic context, used as surrogate for climate change, affects intertidal infaunal assemblages in unvegetated habitats and the nutritional qualities of producers and their assimilation by benthic consumers (chapter 4 and 5). Nonetheless, the current levels of nutrient discharge experienced by southeast Australian estuaries did not have strong disruptive effects on benthic communities and there was no evidence of possible synergistic effects with climate.

In addition to providing indications of the probable interactive effects of local and global stressors, this study is also significant in contributing to our knowledge of southeast (SE) Australian benthic ecology. Long-term observational data and specific base knowledge of the estuaries in this region is of fundamental importance to detect future impacts and to understand the mechanisms underlining the effects of human activities (Gillanders et al., 2011; Kingsford and Watson, 2011).

## **6.2 Potential impacts of sea level rise on seagrass systems**

The eelgrass *Zostera muelleri* is the most abundant seagrass species in southeast Australia and represents an important element of intertidal and shallow subtidal estuarine areas. Given its spatial distribution in relation to the shoreline, it is of primary importance to understand how changes in the geomorphology of the coast (i.e. coastal squeeze) can affect this habitat. By assessing spatial variability in the physico-chemical features of the seagrass *Z. muelleri* and its associated macrofaunal communities across natural gradients in intertidal

elevation, this study found that coastal squeeze may alter 1) plant morphology, 2) leaf chemical characteristics and 3) abundance and composition of macrofaunal assemblages.

Mensurative sampling revealed strong gradients in *Z. muelleri* leaf morphology and tensile strength across tidal elevation gradients (chapter 2). This suggested that a reduction in intertidal area as a consequence of coastal squeeze might alter the trait distribution of this seagrass, with possible flow on effects to consumers. Manipulative experiments investigated the consequences of the observed spatial variability in *Z. muelleri* morphology for the processing of seagrass detritus and the associated faunal community. A litter bag experiment showed that decomposition can be affected by changed environmental conditions along the intertidal gradient. Conversely, changes in some of the leaf traits caused by adaptations of *Z. muelleri* to the intertidal environment were less important in influencing decomposition, such that it is unlikely that they could compensate for an increased decomposition rate in permanently submerged conditions. These findings can have direct consequences for the cycling of nutrients and the turnover of organic matter (Cebrián, 1999).

Even though the manipulative experiment using artificial zostera rhizomes (AZR) was not directly aimed at predicting the effects of coastal squeeze, it highlighted the positive effects that *Z. muelleri* below-ground structures have on the abundance of some taxa (chapter 3). Moreover, while artificial units mimicking seagrass canopy structure have been employed by numerous studies, this experiment represented one of the first to mimic below-ground rhizome structure to assess the mechanisms through which spatial variation in below-ground seagrass structure supports higher numbers of infaunal species and individuals.

The effects that coastal squeeze can have on the decomposition rate of *Z. muelleri* can have important ramifications for nutrient cycling and benthic macrofauna. Nutrient enrichment and reduction of light penetration due to increased water turbidity and epiphytes can have negative effects on seagrasses (Walker and McComb, 1992; Short et al., 1995).

Faster decomposition rates, corresponding to a faster nutrient release, can reduce the nutrient retention of seagrass systems (Hemminga et al., 1999). Together with anthropogenic nutrient enrichment, faster decomposition can make more nutrients available for fast growing macroalgae and phytoplankton that, in turn, can affect seagrass growth by reducing light penetration and shading (Short et al., 1995). Coastal squeeze, thus, can potentially exacerbate the consequences of nutrient pollution for seagrass.

Overall, these results suggest that, in urbanised areas, sea level rise may represent a substantial problem for the conservation of seagrass habitats and their ecological functions. To further understand the risk associated with coastal squeeze, future research is needed to assess the effects of sea level rise on the geomorphology of estuaries and the adaptive potential of *Z. muelleri*, an important producer of southeast Australia estuaries. Moreover, this study emphasizes the importance of undertaking future studies to understand mechanisms that regulate the transport of seagrass detritus and that determine its fate in the ecosystem.

### **6.3 Context dependent effects of nutrient pollution and climate setting on estuarine macrofauna**

The spatial variability of macrofaunal assemblages across estuaries was, overall, weakly correlated with latitude (i.e. climate setting) and nutrient enrichment (chapter 4). Nonetheless, by considering the importance of other abiotic factors in modulating the response to disturbance, this study suggested that the effects of GCC and diffuse nutrient enrichment on macrofaunal communities may depend on the sediment grain size.

Estuaries are complex and extremely variable ecosystems. They can be described as a mosaic of interconnected habitats, each one with different geomorphology, sedimentology, salinity, tidal characteristics and vegetation. As a result, communities are substantially different among these habitats and their response to disturbance can vary considerably

(Lindegarth and Hoskin, 2001; Franca et al., 2012). Differences in the response of communities to disturbance can also arise from differing capacity of habitats to buffer or exacerbate the effects of a stressor (Godbold et al., 2011).

Future studies using natural gradients as surrogates of GCC should carefully control for variability in environmental factors that can alter community responses. In addition, future investigations assessing anthropogenic impacts on estuarine benthic communities should encompass the variety of habitats present in a given estuary. This approach will allow resilience to disturbance to be compared among habitat types, and in addition, it will provide information on how to choose the more sensitive and reliable indicators of anthropogenic disturbance (e.g. Dafforn et al., 2012).

#### **6.4 Utility of correlative studies using natural gradients: advantages and limitations**

This study represents an example of how large-scale observational studies, in combination with manipulative experiments across natural environmental gradients, might be used to predict and identify the extent of GCC impacts on ecosystems and their interactions with local anthropogenic stressors. Results of correlative investigations support the development of scientific theory, modelling and testable hypotheses that can determine causative relationships and further deepen the understanding of GCC impacts (Brown et al., 2011). Moreover, the data collected in this thesis provide an important baseline against which track the responses of soft sediment communities to climate change.

The two manipulative experiments (i.e. the litter bag and the artificial *Zostera* rhizome experiments; chapter 2 and 3) are examples of how manipulative experiments coupled with natural gradients can inform on future effects of GCC. Natural environmental gradients have been extensively used in observational studies to predict effects of GCC (Wernberg et al., 2012), however, few studies have employed them for manipulative experiments to test the

influence of other factors (Dunne et al., 2004). Future studies, therefore, should take advantage of this approach to test the interactive effects between GCC and additional factors. This approach may be further strengthened by also coupling it with carefully controlled mesocosm studies, which on their own are of limited predictive value.

One of the problems associated with observational data, apart from their inability to establish causative relationships, is that there can be a high level of autocorrelation among explanatory environmental variables that may confound the interpretation of results (Legendre et al., 2002). In the survey of macrofaunal community (Chapter 4), latitude and median grain size were correlated and therefore we ran the statistical analysis on two groups of estuaries. This allowed to partially reduce the autocorrelation between the two variables but, on the other hand, halved the number of replicates and, as a result, reduced the statistical power of the test. In order to reduce the likelihood of autocorrelation between climatic and non-climatic predictor variables, future observational studies should account for spatial patterning of predictor variables when selecting sampling locations (Legendre et al., 2002). Previous surveys or pilot studies should be able to provide the background knowledge to optimize the sampling design.

In addition to the confounding effects of environmental variables, at least two factors are ignored when using natural gradients to make inference on GCC impacts: 1) the adaptation potential of species and 2) the occurrence of compensatory mechanisms at various levels of biological organisation. Firstly, despite the already observed changes in species distribution and the importance of climate in shaping species ranges (Parmesan, 2006), it is also widely recognised that species can adapt to varying environmental conditions through phenotypic plasticity (i.e. the capacity of an organism to change its phenotype in response to environmental changes) and evolution (Parmesan, 2006; Gienapp et al., 2008). The question is whether adaptation and evolution will be fast enough to keep-up with the rate of GCC

(Visser, 2008; Chevin et al., 2010). Secondly, it is still unclear how changes at the species level (e.g. phenotypic adaptation, microevolution and range shift) observed in latitudinal studies will translate at the community and ecosystem levels and how it will affect species interactions and ecosystem functioning (Peck et al., 2009).

## **6.5 Preparing Australian estuaries for climate change**

One of the biggest challenges that managers are facing is to sustain coastal ecosystems and the services they provide and, concurrently, allow for coastal urbanisation (Panayotou, 2009). Management should also aim at preserving the natural resilience of coastal environments and taking measures to increase the adaptive potential of these environments to future changes (Morecroft et al., 2012). Effective management should be based on quantitative predictions that scientific research can deliver through observations and experimentation. This study suggested that coastal armouring is probably a more pressing problem for the conservation of estuarine systems of southeast Australia when compared to the impacts of warming and nutrient enrichment. Therefore, when taking decisions on the construction of structures that modify the capacity of the coast line to adapt to sea level rise, managers will have to take into account the consequences that coastal squeeze can have on intertidal seagrass ecosystems.

No effect of the recent increase in diffuse nutrient loading was found on soft-sediment macroinvertebrate communities (chapter 4 and 5). The natural oligotrophic conditions of SE Australian estuaries and the moderate level of human disturbance that they receive are the most likely explanation for these results. Given the meagre state of knowledge of Australian systems, however, the potential for significant future effects of diffuse nutrient enrichment on the biodiversity and functioning of estuarine fauna remains a key concern, especially considering that the human population of Australia is projected to increase from 21 million to

between 30 and 45 million in the next 50 years (Australian Bureau of Statistics, 2008), and this growth will mostly occur in coastal cities. Future research is required in order to establish the capacity of these estuaries to withstand further nutrient enrichment in the face of GCC and to assess thresholds at which deleterious nutrient impacts to estuarine ecosystems occur.

Long-term and comprehensive datasets are needed in order to allow for early interventions and to correct harmful practices that may damage the health of estuarine ecosystems of southeast Australia. Due to the recent European colonisation, levels of knowledge concerning the biology and ecology of estuarine species are still low. The risk of species becoming extinct before they are even studied is still high. In addition, the numerous ecological idiosyncrasies, which make Australian estuaries unique, call for the development and implementation of *ad hoc* indicators of ecological status. Ultimately, any understanding of the functioning, processes and trophodynamics of estuaries is not sufficient in itself to predict the impacts increasing human activities will have on these environments. As such, this area warrants much further investigation.



## 6.6 References

Airoidi, L., Beck, M.W., 2007. Loss, status and trends for coastal marine habitats of Europe, in: Gibson, R.N., Atkinson, R.J.A., Gordon, J.D.M. (Eds.), *Oceanography and Marine Biology*, Vol 45. Crc Press-Taylor & Francis Group, Boca Raton, 345-405.

Andrew, N.R., Hughes, L., 2005. Arthropod community structure along a latitudinal gradient: Implications for future impacts of climate change. *Austral Ecology* 30, 281-297.

Australian Bureau of Statistics, 2008. 3222.0 – Population Projections, Australia, 2006 to 2101. Canberra, Australia. [www.abs.gov.au](http://www.abs.gov.au).

Brown, C.J., Schoeman, D.S., Sydeman, W.J., Brander, K., Buckley, L.B., Burrows, M., Duarte, C.M., Moore, P.J., Pandolfi, J.M., Poloczanska, E., Venables, W., Richardson, A.J., 2011. Quantitative approaches in climate change ecology. *Global Change Biology* 17, 3697-3713.

Bulleri, F., Chapman, M.G., 2010. The introduction of coastal infrastructure as a driver of change in marine environments. *Journal of Applied Ecology* 47, 26-35.

Cebrián, J., 1999. Patterns in the fate of production in plant communities. *American Naturalist* 154, 449-468.

Chapman, M.G., Underwood, A.J., 2011. Evaluation of ecological engineering of "armoured" shorelines to improve their value as habitat. *Journal of Experimental Marine Biology and Ecology* 400, 302-313.

Chevin, L.M., Lande, R., Mace, G.M., 2010. Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biology* 8, e1000357.

Cloern, J.E., 2001. Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology-Progress Series* 210, 223-253.

Dafforn, K.A., Simpson, S.L., Kelaher, B.P., Clark, G.F., Komyakova, V., Wong, C.K.C., Johnston, E.L., 2012. The challenge of choosing environmental indicators of anthropogenic impacts in estuaries. *Environmental Pollution* 163, 207-217.

Dunne, J.A., Saleska, S.R., Fischer, M.L., Harte, J., 2004. Integrating experimental and gradient methods in ecological climate change research. *Ecology* 85, 904-916.

Engle, V.D., Summers, J.K., 1999. Latitudinal gradients in benthic community composition in Western Atlantic estuaries. *Journal of Biogeography* 26, 1007-1023.

Fletcher, C.H., Mullane, R.A., Richmond, B.M., 1997. Beach loss along armored shorelines on Oahu, Hawaiian Islands. *Journal of Coastal Research* 13, 209-215.

Franca, S., Vasconcelos, R.P., Reis-Santos, P., Fonseca, V.F., Costa, M.J., Cabral, H.N., 2012. Vulnerability of Portuguese estuarine habitats to human impacts and relationship with structural and functional properties of the fish community. *Ecological Indicators* 18, 11-19.

Fujii, T., Raffaelli, D., 2008. Sea-level rise, expected environmental changes, and responses of intertidal benthic macrofauna in the Humber estuary, UK. *Marine Ecology-Progress Series* 371, 23-35.

Gienapp, P., Teplitsky, C., Alho, J.S., Mills, J.A., Merila, J., 2008. Climate change and evolution: disentangling environmental and genetic responses. *Molecular Ecology* 17, 167-178.

Gillanders, B.M., Elsdon, T.S., Halliday, I.A., Jenkins, G.P., Robins, J.B., Valesini, F.J., 2011. Potential effects of climate change on Australian estuaries and fish utilising estuaries: a review. *Marine and Freshwater Research* 62, 1115-1131.

Godbold, J.A., Bulling, M.T., Solan, M., 2011. Habitat structure mediates biodiversity effects on ecosystem properties. *Proc. R. Soc. B-Biol. Sci.* 278, 2510-2518.

Hemminga, M.A., Marba, N., Stapel, J., 1999. Leaf nutrient resorption, leaf lifespan and the retention of nutrients in seagrass systems. *Aquatic Botany* 65, 141-158.

Kennish, M.J., 2002. Environmental threats and environmental future of estuaries. *Environmental Conservation* 29, 78-107.

Kingsford, R.T., Watson, J.E.M., 2011. Climate Change in Oceania - A synthesis of biodiversity impacts and adaptations. *Pacific Conservation Biology* 17, 270-284.

Legendre, P., Dale, M.R.T., Fortin, M.J., Gurevitch, J., Hohn, M., Myers, D., 2002. The consequences of spatial structure for the design and analysis of ecological field surveys. *Ecography* 25, 601-615.

Lindgarth, M., Hoskin, M., 2001. Patterns of Distribution of Macro-fauna in Different Types of Estuarine, Soft Sediment Habitats Adjacent to Urban and Non-urban Areas. *Estuarine, Coastal and Shelf Science* 52, 237-247.

Lotze, H.K., Lenihan, H.S., Bourque, B.J., Bradbury, R.H., Cooke, R.G., Kay, M.C., Kidwell, S.M., Kirby, M.X., Peterson, C.H., Jackson, J.B.C., 2006. Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science* 312, 1806-1809.

Morecroft, M.D., Crick, H.Q.P., Duffield, S.J., Macgregor, N.A., 2012. Resilience to climate change: translating principles into practice. *Journal of Applied Ecology* 49, 547-551.

Panayotou, K., 2009. Coastal Management and Climate Change: an Australian Perspective. *Journal of Coastal Research* 56, 742-746.

Parnesan, C., 2006. Ecological and evolutionary responses to recent climate change, *Annual Review of Ecology Evolution and Systematics*, 637-669.

Peck, L.S., Clark, M.S., Morley, S.A., Massey, A., Rossetti, H., 2009. Animal temperature limits and ecological relevance: effects of size, activity and rates of change. *Functional Ecology* 23, 248-256.

Short, F.T., Burdick, D.M., Kaldy, J.E., 1995. Mesocosm experiments quantify the effects of eutrophication on eelgrass *Zostera marina*. *Limnology and Oceanography* 40, 740-749.

Sobocinski, K.L., Cordell, J.R., Simenstad, C.A., 2010. Effects of Shoreline Modifications on Supratidal Macroinvertebrate Fauna on Puget Sound, Washington Beaches. *Estuaries Coasts* 33, 699-711.

Visser, M.E., 2008. Keeping up with a warming world; assessing the rate of adaptation to climate change. *Proceedings of the Royal Society B-Biological Sciences* 275, 649-659.

Walker, D.I., McComb, A.J., 1992. Seagrass degradation in Australian coastal waters. *Marine Pollution Bulletin* 25, 191-195.

Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J.M., Hoegh-Guldberg, O., Bairlein, F., 2002. Ecological responses to recent climate change. *Nature* 416, 389-395.

Wernberg, T., Russell, B.D., Moore, P.J., Ling, S.D., Smale, D.A., Campbell, A., Coleman, M.A., Steinberg, P.D., Kendrick, G.A., Connell, S.D., 2011. Impacts of climate change in a global hotspot for temperate marine biodiversity and ocean warming. *Journal of Experimental Marine Biology and Ecology* 400, 7-16.

Wernberg, T., Smale, D.A., Thomsen, M.S., 2012. A decade of climate change experiments on marine organisms: procedures, patterns and problems. *Global Change Biology* 18, 1491-1498.

Wiklund, A.K.E., Dahlgren, K., Sundelin, B., Andersson, A., 2009. Effects of warming and shifts of pelagic food web structure on benthic productivity in a coastal marine system.

Marine Ecology-Progress Series 396, 13-25.