The interactive effect of sandprawn (*Callichirus kraussi*) Stebbing bioturbation and nutrients on macrofaunal communities.

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DECLARATION

This thesis documents original research carried out in the Department of Zoology, University of Cape Town under the supervision of Deena Pillay and George M. Branch. It has not been submitted in whole, or in part for any other degree at any other University. All of the work presented in this thesis is my own, except where otherwise stated in the text. Any other sources are fully acknowledged.

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ABSTRACT

Disturbance and productivity are important factors shaping community structure and diversity, alone or interactively. Bioturbation by the sandprawn *Callichirus kraussi* is a form of disturbance and is known to alter sediment properties, the physico-chemical environment, biofilms, microalgal and microbial levels, and meiobenthic and macrobenthic communities. Similarly, nutrient levels affect productivity, thereby altering diversity and community composition. Nutrients can modify the effect of disturbance and visa versa. Superimposed on this, seasonal fluctuations in ambient nutrient concentrations and temperature can alter metabolic rates of organisms and activities such as feeding, which may directly influence bioturbation rates. My thesis combined an observational study and field and laboratory experiments to investigate how nutrients and bioturbation by *C. kraussi* interact to structure macrofaunal communities and how ambient temperatures influence these factors.

Natural physical and biogeographical gradients exist along the South African coastline: nutrients, productivity and biotic abundance are higher on the upwelling west coast, while water temperatures and diversity are higher on the non-upwelling east coast. I first conducted an observational study to investigate whether *Callichirus kraussi* bioturbation modifies these expected inter-coastal patterns at selected sites, by comparing sand-prawn dominated sandflats in two marine-driven lagoons - Durban Bay on the east coast and Langebaan Lagoon on the west coast. Decisive differences emerged between both sites and seasons, but these did not all correspond with expected differences. Abundance, as anticipated, and diversity, contrary to expectations, were greater in Langebaan Lagoon. Both were unexpectedly greater in winter. Also contrary to hypothesised differences, there were no site or seasonal differences in microphytobenthic biomass. Temperatures were higher in summer than winter, but did not differ significantly between sites. Unexpectedly, nutrient levels were rarely greater in Langebaan than Durban, and no clear patterns of seasonality emerged. Local solar heating and pollution probably explain the deviations from expected trends.

Callichirus kraussi had clear effects on communities that superseded site differences: in both systems, burrowing forms (which are favoured by *C. kraussi*) were dominant, while surface-dwelling species (which are negatively affected by *C. kraussi*) occurred at low abundances. The study supported the idea that *C. kraussi* has a dominant effect on soft-sediment communities, but raised questions about whether its influence is modified by seasonal differences in temperature and nutrients.

To test the idea of an interaction between the effects of *C. kraussi* bioturbation and nutrients on macrofaunal communities, I then conducted a field experiment in the intertidal sandflats of Langebaan Lagoon, in which nutrient levels and sandprawn density were manipulated in cages. This specifically tested the hypothesis that diversity should be highest at intermediate levels of both factors. Employment of control treatments showed that although caging may have had an effect on the outcomes, associated disturbance did not. Nutrient levels were significantly enhanced by experimental enrichment, but in the final samples taken after six months, neither microphytobenthic biomass nor bacterial levels (measured as extra-polymeric substances) responded to manipulations of either nutrient or sandprawn densities. *C. kraussi* bioturbation did have significant effects on macrofaunal communities, enhancing burrowing infauna, but also unexpectedly promoting the surface grazer, *Assiminea globulus*. Bioturbation significantly affected diversity and abundance of macrofauna, producing peaked or positive (logarithmic or power) relationships in many instances, and negative associations in the case of Pielou's evenness index: but in the majority of cases no significant relationships were detected. Nutrient level had no significant effects on either macrofaunal community structure or diversity. I suggest the failure to detect any significant effects of nutrient enrichment was because background nutrient levels were sufficiently high due to upwelling that nutrients were not limiting.

A manipulative laboratory experiment employing luminophores was then used to examine whether temperature and nutrient levels affect bioturbation rate, measured in sediment profiles and as bioadvection (r) and biodiffusion (D_b). An increase in temperature increased the depth and extent to which luminophores were transported down sediment profiles. It also increased bioadvection (r), at least below detrimental temperatures. Nutrients enhanced bioadvection (r) at low (winter-like) temperatures, producing a peak at intermediate nutrient levels, and promoted bioadvection (r) and biodiffusion (D_b) at high (summer-like) temperatures, producing increased values at both intermediate and high levels of enrichment. Temperature therefore moderated the effects of nutrients on *C. kraussi* activity.

I concluded that *C. kraussi* bioturbation is an important determinant of macrofaunal community composition and diversity and that *C. kraussi* has the potential to promote as well as diminish diversity. Physico-chemical factors such as temperature and nutrients can affect *C. kraussi* bioturbation. Thus changes in such factors, whether natural or anthropogenic, or the loss of a bioturbator like *C. kraussi*, could have profound and complex secondary effects for the ecosystems in which *C. kraussi* occurs.

CHAPTER 1

GENERAL INTRODUCTION

1. Background

1.1. The Importance of disturbance and productivity

Disturbance (caused by physical factors or by biological factors such as grazing or bioturbation), and productivity (which is largely regulated by resource supply), are two major forces that shape communities and ultimately ecosystem structure and function. They are often cited as the major determinants of community diversity and stability and of spatial and temporal patterns of species (Connell, 1978; Huston, 1979; Sousa, 1979; Worm *et al*., 2002; Worm and Duffy, 2003; Sipura *et al*., 2005; Canning-Clode *et al*., 2008; Guerry 2008, Wootton *et al*.; 2009). Disturbance, a form of ecological disruption, can be defined as a pulsing variation away from an equilibrium point, or the removal of living biomass from an ecosystem by physical or biological processes (Wootton *et al*., 2009). Productivity can be thought of as the turnover of organic matter in a system, and is often defined in terms of primary productivity or nutrient availability (Canning-Clode *et al*., 2008).

Much empirical evidence supports the importance of disturbance and nutrient availability. For example, nutrient enrichment significantly changes the composition of aquatic fungal, salt-marsh, bacterial and macrofaunal communities (Beukema, 1991, Pennings *et al*., 2002; Sipura *et al*., 2005; Artigas *et al*., 2007), and increases biomass, productivity and abundance of primary producers and consumers in a number of marine and terrestrial systems (Beukema, 1991; Leps, 1999; Sipura *et al.*, 2005; Jara *et al*., 2006; Posey *et al*., 2006; Worm and Lotze, 2006). Disturbance also affects community structure, richness and diversity (Connell 1978; Huston 1978; Sousa, 1979; Sommer, 1993; Valdivia *et al*., 2005; Jara *et al*. 2006; Svensson *et al.* 2007).

Diversity generally displays a unimodal relationship with disturbance and productivity when they are each individually manipulated (Worm *et al*., 2002). This conforms to the expectations of the intermediate disturbance hypothesis (IDH), which specifies that highest diversity will occur at intermediate levels of disturbance, and also to general expectations of the productivity-diversity relationship, in which low levels of nutrients inhibit many species and thus reduce diversity, while high levels of nutrients increase competition and therefore also decrease diversity (Connell, 1978; Huston, 1979; Sousa, 1979). Although there is empirical support for the IDH (Sousa, 1979; Svensson *et al.*, 2007), Worm *et al.* (2002) found, via experimentation and meta-analysis of aquatic communities, that the effects of consumers (a form of disturbance) and nutrient enrichment on diversity were consistently interdependent. Other empirical evidence provides support for this, with both Hillebrand (2003) and Valdivia *et al.* (2008) demonstrating opposing and interactive effects of grazing and nutrient enrichment on diversity.

Models have been developed to try to explain the interactive effect of disturbance and productivity on diversity, including Kondoh's (2001) dynamic equilibrium model, which is an expansion of Huston's (1979) model and generalises about the relationship between diversity, disturbance and nutrient enrichment. There is some empirical support for this model (Widdicombe and Austen, 2001; Jara *et al.* 2006; Guerry 2008), Specifically, the Kondoh model was upheld by Proulx and Mazumder (1998) when they examined the joint effects of nutrient levels and grazing (as a form of disturbance), and became termed the grazer-reversal hypothesis to capture the views that (a) in high-nutrient environments an increase in grazing pressure increases plant species richness by curbing competition, (b) in low-nutrient environments grazing decreases richness by adding to stress, and (c) at intermediate nutrient levels richness peaks at intermediate intensities of grazing. However, the grazer-reversal or Kondoh models do not necessarily apply to all communities, since some studies have found that different communities or even different members of a community respond differently to changes in nutrients and disturbance (Cole *et al.*, 2008; Pfaff *et al.*, 2010). The grazer-reversal hypothesis may also not hold equally for all diversity measures. For example, Hillebrand (2003) found that it held for species evenness, but not species richness. Additionally, other factors may modify the grazer-nutrient interaction, such as light intensity (Liess *et al.*, 2009).

Related to this issue is the question of whether communities will respond similarly to different types of biological disturbance, such as bioturbation, which represents more than a simple removal of primary producers. My thesis tests how bioturbation and nutrient enrichment, will interact to structure macrofaunal communities and whether bioturbation will follow the expectations of the Kondoh model or grazer-reversal hypothesis.

1.2 Bioturbation as a disturbance

Bioturbation, which may be defined as the biological reworking and redistribution of sediment particles and movement of porewater by the activities of (benthic) organisms (Maire *et al.*, 2008), represents a disturbance in the same sense as grazing, in that it alters living biomass in a system. However, the two are quite different in the way that they achieve this. While grazing typically removes or damages primary producers through ingestion or feeding, bioturbation acts in a number of ways, such as burial or smothering of macrofauna via activities that include movement, food collection and burrow construction, depletion of bacteria, or enhancement of faunal emigration (Cadée, 2001). Bioturbation has the capacity to remove organisms, affect production and change community composition and biodiversity via alteration of the physical environment.

Bioturbation is thus an important force structuring benthic soft-sediment communities, altering macrofaunal recruitment, abundance and community structure (Posey *et al*., 1991; Tamaki, 1994; Van Nes *et al.* 2007; Pillay *et al*., 2007a,b, 2008). It also influences the distribution of seagrass beds and negatively affects their growth (Suchanek, 1983; Townsend and Fonseca, 1998; Dumbauld and Wyllie-Echeverria, 2003; Siebert and Branch, 2005a). Although it can stimulate bacterial community growth, it negatively affecting sulphate-producing bacteria (Mermillod-Blondin, 2004), and generally decreases microalgal growth (or surface sediment chlorophyll *a*), bacteria and extracellular polymeric substances produced by microflora (Branch and Pringle, 1987; Webb and Eyre, 2004; Pillay, 2006, Pillay *et al*., 2007c). It influences meiofaunal abundance and diversity (Branch and Pringle, 1987; Kuhnert, 2010), affects the emergence of zooplankton groups from the benthos (Viitasalo *et al*., 2007), and alters deposit-feeding infaunal communities (Volkenborn and Reise, 2006). Bioturbators have also been observed to be involved in regime shifts. For example, Tamaki *et al.* (2008) state that a decline in phytoplankton production, coupled with bioturbation by *Upogebia major* and *Nihonotrypaea japonica*, led to a decline in one of the dominant species, the Manila clam (*Ruditapes philippinarum*) in the Ariake Sound in Japan over two decades.

Callichirus kraussi (Stebbing), commonly known as the sandprawn and until recently placed into the genus *Callianassa*, is a particularly powerful bioturbator, and is the focal organism of my study. This callianassid is found in intertidal and subtidal soft, sandy sediments of sheltered, coastal areas from San Martinho in Mozambique to the Orange River on the west coast of South Africa, and is frequently the dominant macrofaunal organism (Forbes, 1973; Day 1981); incredibly it turns over up to 12.14 kg.m^{-2} sediment per day (Branch and Pringle, 1987). Callianassids may burrow as deep as 3–5 m (Cadée, 2001), depositing sediment from burrow excavation at the surface and altering the geotechnical properties of the sediment.

Callianassids have a large effect on floral and faunal populations of estuaries and lagoons, altering bacterial abundance and biofilms, meiofaunal and seagrass abundance and macrofaunal recruitment and community structure so far as to even cause localised extinctions of some species (Posey, 1986; Branch and Pringle 1987; Tamaki, 1994; Siebert and Branch 2005a,b; Pillay *et al.,* 2007a,b, 2008, Pillay and Branch, 2011). Because of their effects on the physical composition of sediments, some callianassids have been termed "ecosystem engineers" (Berkenbusch and Rowden, 2003; Siebert and Branch 2006, 2007; Pillay and Branch, 2011). The effects of callianassids on macrofauna appear to be specific to particular functional groups, generally being negative for sedentary species, suspension feeders, deposit feeders and surface grazers and positive for burrowing infauna (Posey, 1986; Tamaki, 1988; Pillay *et al*., 2007a, 2008).

Bioturbators, including callianassids such as *Callichirus*, can alter sediment properties by lateral and vertical mixing of sedimentary layers, removal of organic matter or particular grain sizes of sediment, increases in the proportion of small grain sizes of sediment and organic matter content, enhancement of sediment permeability and porewater exchange, and oxygenation of sediments down to a metre or more through increased gas flux with the overlying water column (Suchanek 1983; Waslenchuk *et al*., 1983; Murphy and Kramer, 1992; Hughes *et al*., 2000; Flach and Tamaki, 2001; Webb and Eyre, 2004; Volkenborn *et al.*, 2007a,b; Figueiredo-Barros *et al.*, 2009). *Trypaea australensis* (a callianassid shrimp) has been shown to increase sediment oxygen demand by 81%, with 15% of amount being consumed by the shrimp itself and the other 85% by oxidation reactions and microbial respiration (Webb and Eyre, 2004). Bioturbators also alter nutrient flux in sediments, and callianassids have been found to enhance phosphate, ammonia and sulphide in their burrows relative to the overlying water column (Waslenchuk *et al*., 1983; Kristensen, 1991; Biles *et al*., 2002). They have also been shown to increase denitrification rates and ammonium efflux and may be responsible for as much as 76% of denitrification at sites where they occur, contributing to an overall net efflux of nitrogen from sediment in subtropical estuarine habitats (Webb and Eyre, 2004).

Along with sediment properties and tidal height, bioturbators also modify the effects of nutrient enrichment on communities (O'Brien *et al*., 2009). Additionally, even individual species of bioturbators, such as the urchin *Echinocardium,* have been shown to be pivotal for the functioning of biogeochemical processes in soft-bottom sediments, driving ammonium efflux to the overlying water column at higher urchin densities, and thereby increasing microalgal productivity and decreasing dissolved reactive phosphorous through their oxygenation of sediments and consequent adsorption of reactive phosphate to clay particles (Lohrer *et al.*, 2004). In short, these bioturbators have a major influence on the productivity and elemental budgets of overlying waters wherever they occur, and since 70% of the ocean floor is covered by soft-sediments, the activity of bioturbators has a globally-important influence on the functioning of benthic habitats (Lohrer *et al.*, 2004). Understanding the function of such systems and the influence of bioturbators is therefore important.

Since bioturbators are a significant force disturbing soft-bottom marine communities, they are ideal candidates for testing hypotheses relating to the interactive effects of nutrients and disturbance on communities. Indeed, O'Brien *et al.* (2009) have shown that bioturbation by the lugworm *Arenicola marina* has the potential to influence how nutrient enrichment affects benthic soft-sediment communities. My study further elucidates this interaction, with the abundant and powerful bioturbator *Callichirus kraussi* being the target organism for this investigation.

1.3 Thesis layout

Following this introductory Chapter, Chapter 2 compares the macrofaunal communities of two sites that are both dominated by *Callichirus kraussi* but lie on contrasting coasts of South Africa, and examines whether natural differences in temperature and nutrients between bioregions affect benthic macrofaunal communities. This cross-coastal comparison was made between the intertidal sandflats of marine-dominated lagoons on the upwelling west coast and the non-upwelling east coast of South Africa, in respectively Langebaan Lagoon and Durban Bay. The comparison provides preliminary insights into the extent of an interactive relationship between physical conditions and bioturbation.

Chapter 3 describes experimental tests carried out within Langebaan Lagoon, which examined interactive effects of nutrient enrichment and bioturbation on intertidal macrofaunal communities. This was achieved through a field caging experiment, located in an intertidal sandflat dominated by *C. kraussi* at a relatively pristine and undisturbed site, in which densities of *C. kraussi* (i.e. bioturbation levels) and nutrient levels were manipulated in a cross-factorial, split-plot design.

In Chapter 4, I describe a laboratory experiment to determine whether the rate of bioturbation by *C. kraussi* is modified by nutrient levels and temperature. In an aquarium environment, the interactive effect of a range of temperatures and nutrient levels on bioturbation rates was examined through a manipulative approach. The turnover rate of sediment by *C. kraussi* was quantified through the use of fluorescently-labelled, sediment-like, luminophore particles.

Finally, in Chapter 5 I evaluate the outcomes of the preceding chapters and provide an overall insight into the interactive role of *Callichirus* bioturbation and nutrient levels in terms of their influence on soft-bottom communities, and explain how these factors and the resulting patterns of community structure may be modified by ambient conditions in different biogeographic settings.

CHAPTER 2

FIELD OBSERVATIONS – THE EFFECT OF DIFFERENCES IN AMBIENT CONDITIONS BETWEEN UPWELLING AND NON-UPWELLING COASTS ON COMMUNITIES IN SANDFLATS DOMINATED BY *CALLICHIRUS KRAUSSI*

2.1 Introduction

The South African coastline experiences a gradient of nutrients and productivity, with higher levels on the west coast, which experiences strong upwelling, than on the east coast, where upwelling is absent or relatively weak (Bustamante *et al*., 1995). This cross-coastal difference is generated by the Agulhas and Benguela Currents, which meet on the southern edge of the country. The Agulhas Current moves southwards down the east coast, while the Benguela Current flows northwards along the west coast of South Africa (Shannon, 1985; Bustamante *et al.*, 1995; Bustamante and Branch, 1996).

Upwelling in the Benguela Current system on the southern section of the west coast is seasonal, being concentrated in summer but semi-permanent and subject to short-term variation. It is generated by the predominating south-easterly wind that prevails in September to March (Andrews and Hutchings, 1980), which brings colder, denser, nutrient-rich water to the surface. Conversely, in winter, the flow of water becomes reversed with the predominance of north-westerly winds, although an increased intensity in the South Atlantic Gyre may bring cold, dense water onto the coastal shelf late in the season (Andrews and Hutchings, 1980). Because of this, nutrient levels on the west coast are higher than on the east coast, whereas temperatures are lower there, and may paradoxically be lower in summer than winter.

Bustamante *et al.* (1995) showed that cross-coastal trends extend into the intertidal zone of the open coast, with nutrient (phosphate, nitrate, nitrite and silicate) concentrations being lowest on the east coast and highest in the west, with intermediate levels along the south coast. Primary production of epilithic intertidal algae follows the same trends, being correlated with nutrient levels (Bustamante *et al*., 1995). In addition to regional gradients in nutrients and primary productivity, the coast is divisible into distinct bioregions. There are three major biogeographic provinces, the Namaqua, Agulhas and Natal provinces, on the west, south and east coasts, respectively (Emanuel *et al.*, 1992; Bustamante and Branch, 1996), with an overlap region on the north-easterly extremity that grades into the Indo-West Pacific in Mozambique (Sink *et al*., 2005). Invertebrate diversity is higher on the east coast than the west, with some invertebrate groups showing the highest diversity on the south coast (Awad *et al.,* 2002).

A study in Brazil by De Leo and Pires-Vanin (2006) suggests that differences between upwelling and non-upwelling areas should exist in soft-sediment habitats. They showed that abundance and biomass of coastal-shelf megafauna living in soft-sediments was higher in an area of upwelling than in an area of non-upwelling and suggested that the greater values of density and biomass are responses to (organic) nutrient enrichment in sediments arising from upwelling, and corresponding increases in meiofauna and macrofauna.

Bioturbation may modify cross-coastal patterns of nutrients, primary productivity and associated macrofaunal diversity and community structure in soft-sediment habitats. There is, however, a paucity of cross-coastal studies on bioturbation in South Africa. Generally, studies have shown that bioturbation changes nutrient flux in sediments, and water in callianassid burrows has higher levels of ammonia, phosphate and sulphide than the overlying water column (Waslenchuk *et al*., 1983; Kristensen, 1991). In addition, bioturbators may increase ammonium release from the sediments through their activities (Biles *et al.*, 2002). Along with tidal height and sediment characteristics, bioturbation modifies the effect of nutrients on benthic communities, by altering the release, movement and amounts of porewater nutrients in complex ways (O'Brien *et al*., 2009).

In general, bioturbation has important structuring effects on communities. *Callichirus kraussi*, a powerful bioturbator and the species of central interest in my study, negatively affects the abundance, species richness and diversity of macrofauna through its bioturbative actions, radically altering macrofaunal community structure (Pillay *et al.* 2007a). However, burrowing species are favoured by the activities of *C. kraussi* (Siebert & Branch 2005b, 2007; Pillay *et al*., 2011). Pillay *et al.* (2007a,b,c, 2008) found that *C. kraussi* had negative effects on suspension feeders, deposit feeders and surface grazers, but favoured burrowing infauna through the destabilization of sediment. It also diminished biofilms and thereby influenced juvenile recruitment. *C. kraussi* also has negative effects on microalgae, bacteria and levels of EPS – extracellular polymeric substances produced by microorganisms (Branch and Pringle, 1987; Pillay, 2006). Decreases of 50–70% have been recorded for bacteria and microalgae in the presence of *C. kraussi* (Pillay *et al*., 2007c). This can negatively affect macrofauna that rely on these as a food source or as a biofilm-derived cue for juvenile recruitment (Pillay *et al*., 2007b,c).

Bustamante *et al.* (1995) identified the cross-coastal differences in oceanographic characteristics in South Africa as being ideally suited for comparative ecological studies, and this chapter is concerned with such studies. It seeks to compare macrofaunal abundance, diversity and functional-group composition in soft substrata dominated by *C. kraussi* on the west coast where nutrient levels were expected to be high because of upwelling, and those on the non-upwelling east coast of South Africa where nutrients were expected to be low.

Several hypotheses were tested:

1. Nutrients were predicted to be higher in Langebaan Lagoon than Durban Bay and higher in summer than winter in Langebaan, in line with the established biogeographic trends around the coast.
2. Physico-chemical variables were expected to reflect regional marine conditions and seasonal trends.
    a) Temperature would be higher in Durban Bay than Langebaan Lagoon and higher in summer than winter at both sites.
    b) Salinity and specific conductivity would be higher in Langebaan Lagoon than Durban Bay in summer, but less than Durban Bay in winter, because of summer rainfall in Durban and winter rainfall in Langebaan. Because both systems are marine-driven and evaporation will be greater in summer, salinity and specific conductivity were predicted to be higher in summer than winter in both systems.
3. Microphytobenthic biomass was expected be higher in Langebaan Lagoon than Durban Bay, reflecting a higher concentration of nutrients there., and elevated in summer at both sites due to increased photoperiod and temperature and (in Langebaan) greater nutrient availability with seasonal summer upwelling.

4. Macrofaunal abundance would be higher in Langebaan Lagoon than Durban Bay, while diversity would be higher in Durban Bay, following biogeographic trends previously recognized for the open-coast. Abundance would increase in summer, both in Langebaan Lagoon, where nutrient inputs are at that time elevated by upwelling, and in Durban where temperatures are at that time elevated, leading to an increase of microphytobenthic biomass.

5. Species composition would be different between the two sites due to biogeographic differences and would differ between summer and winter because of differential recruitment.

6. Because both systems are dominated by *Callichirus kraussi* bioturbation, relative proportions of functional groups (community structure) were expected to be similar at both sites and in both seasons, with burrowing deposit-feeders being the predominant functional group among the macrofauna, whereas surface-feeders would be inhibited by bioturbation at both sites.

2.2 Methods and materials

2.2.1 Study sites

Samples were taken in winter 2009 (June/July) and summer 2010 (January), in Durban Bay on the non-upwelling east coast of South Africa, and in Langebaan Lagoon on the upwelling west coast. These sites were chosen to compare soft-sediment communities across coastal gradients of nutrients and ambient temperature conditions because they are comparable marine-driven lagoons in which freshwater input has little effect (Day 1959, 1981). These two systems have a similar tidal range of approximately 1.5 m and both contain sandflat habitats dominated by *C. kraussi*.

Durban Bay is an estuarine embayment that spans 892 ha at high tide and is also the site of a major harbour development, with the majority of the original mangrove (90%) and sandflat habitats (75%) having been destroyed and replaced by docks and quays (Forbes *et al*., 1996; Pillay *et al*., 2008). Small patches of mangrove forest remain (Fig. 2.1); these comprise white mangroves (*Avicennia marina*), black mangroves (*Brugueira gymnorrhiza*) and red mangroves (*Rhizophora mucronata*) (Forbes *et al*., 1996). The sandflats contain *Callichirus* *kraussi* and macrofauna dominated by polychaetes, gastropods and bivalves – together comprising 60% of taxa (Forbes *et al*., 1996). Being the largest harbour in South Africa and located in the middle of a large metropolis with many of the cities storm drains emptying into it, Durban Bay has been and is subject to pollution and human disturbance (Brown, 1987; Leuci, 2000; Newman *et al*., 2007).

Langebaan lagoon (33″10′S:18″5E) is a marine-dominated lagoon that forms part of the West Coast National Park in South Africa (Christie and Moldan, 1977; Siebert and Branch, 2005a). The 14-km-long lagoon has many large intertidal sandflats dominated by *Callichirus kraussi* and *Upogebia africana*, which together comprise 48% of the benthic infaunal biomass of the lagoon, and has the highest diversity of benthic invertebrates of any South African lagoonal or estuarine system (Christie and Moldan, 1977). In addition to sandflats there are also saltmarsh and seagrass bed areas in the intertidal area (Fig. 2.1; Siebert and Branch, 2005a). Currents and wave action decrease from the mouth to the head of the lagoon and there is a gradient from course to fine sediments towards the head of the lagoon and from the West to East sides of the channel (Flemming, 1977); the sites that I examined on the Eastern shore of the lagoon all fall within the areas of fine sediment dominated by sand. These sites were chosen to compare soft-sediment communities because they have equivalent sizes, and are comparable marine-driven lagoons in which freshwater input has little effect (Day 1959, 1981).

Three replicate 'subsites' were sampled within Durban Bay and within Langebaan Lagoon (Fig. 2.1), all of which had intertidal sandflats dominated by *Callichirus kraussi*. Within Langebaan Lagoon, only Klein Oesterwal (KO: 33°06'460"S, 18°02'320"E) is exploited for bait collection, including removal of *C. kraussi*. Oesterwal (O: 33°07'395"S, 18°03'402"E) could be accessed by pedestrians, but collection of bait is prohibited. At Bottelary (B: 33°08'627"S, 18°05'300"E), all public access is prohibited (Nel, 2006). In Durban Bay, all subsites could be accessed by the public and are exploited for bait, including *C. kraussi*. The subsite of Roundbush (RB: 29°52'833"S, 031°00'671"E) in the harbour is less easily accessed and exploited than the Yacht Club (YC: 29°51'836"S, 031°01'655"E) or Wilson's Wharf (WW: 29°52'052"S, 031°01'164"E). Bait collection is prohibited at RB, although some illegal collecting takes place.

2.2.2 Sampling strategy

Nutrient concentrations were determined from single column-water and pore-water samples (450 ml each) taken at each subsite in the mid-intertidal zone approximately 0.75 m above the spring low tide mark, and were sampled when the tide was coming in and had covered the site (column water), or at low tide when no water had yet covered the site (pore water). Water samples were immediately frozen and colourimetrically analysed for nitrates plus nitrites (henceforth termed ‘nitrates’), ammonium and phosphate. Microphytobenthic biomass was measured as chlorophyll *a* content of the sediment: five replicate cores (diameter = 2cm, depth = 1 cm) were taken per subsite from the sediment surface, dissolved in 30 ml of 90% acetone, stored in the dark for 48 hours at -20°C and then analysed on a Turner designs Trilogy fluorimeter with a Chlorophyll-*a* non-acidified module.

Temperature, salinity, oxygen saturation, specific conductivity and pH were measured *in situ* using a YSI 6600 V2 multiparameter water probe in the column water at each sub-site. Prawn densities were estimated for all subsites in summer 2010, by counting prawn holes in five random 0.5 x 0.5 m quadrats per subsite, and assuming that one hole represents one sandprawn (Wynberg and Branch 1994, Nel 2006). Estimates of prawn density could not be made in winter 2009, as prawn holes were covered over and obscured after storm events disturbed sediments.

At each subsite and in each sampling period, three replicates of a triple-core (i.e. three replicates of three pooled cores of diameter = 10 cm, depth = 30 cm) of sediment was taken to sample macrofauna. The data were pooled for presentation and presented ‘per triple-core’, i.e. per 235 cm^2. Each batch of cores was sieved through a 500-µm mesh by the ‘stir-and-pour’ method, whereby sediment is swirled in a bucket and the fauna-containing supernatant (seawater) poured through the sieve a total of five times. Following this, the remaining sediment was washed through a 1000-µm sieve to collect larger, heavy specimens. Samples were then stored in a 70% ethanol solution (diluted with seawater) and identified to the highest possible taxonomic resolution, mostly to species level, and counted under a dissecting microscope. Taxa were then grouped into six functional groups for comparative purposes: surface-feeding predators/scavengers; burrowing predators/scavengers; surface deposit-feeders; burrowing deposit-feeders; surface suspension feeders, and surface grazers. The functional grouping was based on Day (1969) and Branch *et al.* (2010). The advantage of functional grouping is that it allows comparison of different species that share common ecological characteristics (Bustamante and Branch, 1996).

2.2.3 Statistical analysis

Using Statistica 9, all untransformed data were first tested for normality (Kolmogorov-Smirnoff) and equality of variance (Levene's test), with alpha set at 0.05, and where necessary, subjected to transformation to meet the assumptions of parametric tests. In cases where even transformed data violated these assumptions, untransformed data were then subjected to equivalent non-parametric tests.

Nutrient levels, chlorophyll *a*, physico-chemical factors and macrofaunal species abundance and diversity data were subjected to Two-Way factorial ANOVAs with site and season set as fixed factors. Where significant differences occurred ($p < 0.05$), the data were then analysed with Tukey post-hoc tests to determine where the differences lay. Non-parametric data were subjected to one-way Mann-Whitney *U*-tests for season within Langebaan and Durban separately, and for site within winter and summer separately. Nutrient data were also explored with a 3-way MANOVA, with water source (column or pore water), site and season as variables, to determine whether there were differences between pore and column water within seasons and sites. Where significant differences occurred ($p < 0.05$), post-hoc Tukey tests were performed. The density of sandprawns was subjected to a one-way ANOVA by site.

Macrofaunal community data were subjected to a Two-Way PERMANOVA, using PRIMER 6.1.11 with PERMANOVA+ 1.0.1, with site and season as fixed factors. Where significant differences were found, data were analysed with post-hoc "pair-wise" PERMANOVA tests to determine where the differences lay. Species data were also used to produce a Multi-Dimensional Scaling (MDS) ordination plot and a dendrogram via MDS and CLUSTER analyses in PRIMER 6.1.11.

Macrofaunal species-abundance data were used to calculate indices of diversity and abundance in PRIMER 6.1.11, employing the DIVERSE procedure. These values were then subjected to two-way, factorial ANOVA with site and season as fixed factors, and to post-hoc Tukey tests when appropriate. For some indices, parametric assumptions were violated for season, but not for site. In such cases, two one-way ANOVAs between sites were run for winter and summer separately, and two Mann-Whitney *U*-tests between seasons for each site.

Species abundance data were aggregated into functional groups and subjected to a SIMPER analysis in PRIMER 6.1.11 to determine defining functional groups, i.e., those that contributed the majority of the similarity (> 50%) within a site. Individual functional-group abundance data were subjected to ANOVA by site for winter and summer. To meet ANOVA assumptions data were square-root or log+1 transformed where necessary and, in the case of surface suspension feeder data for summer, where assumptions were not met, data were analysed with a Mann-Whitney *U*-test by site.

BioEnV analyses, in PRIMER 6.1.11, were also applied to determine which physical and biological parameters best explain community structure at the species and functional group level, both within individual seasons and for both seasons and sites together. A reduced set of biological and physical parameters was used for the analyses: nutrient levels for pore and column water, temperatures, salinity and microphytobenthic biomass. Data from macrofaunal cores were combined for each subsite when conducting BioEnv analysis, to match the number of cores with the number of replicates of physico-chemical variables.

2.3 Results

2.3.1 Nutrient conditions

In general, nutrient levels were influenced by an interaction between season and site, being consistently higher in Langebaan than Durban in summer, but usually higher in Durban than Langebaan in winter (Fig. 2.2). Averaged across seasons there was no difference in column-water nutrients between Langebaan and Durban, but pore-water nutrients reached higher levels in Langebaan. Seasonally, in all but one case, nutrients reached higher levels in summer than winter in Langebaan, but were almost always higher in winter than summer in Durban. The main exception to these patterns was orthophosphate concentration in porewater, which was less in summer than winter at Langebaan. These trends were not always statistically significant, as outlined below.

Site interacting with season had a significant effect (ANOVA, $F_{1,8} = 6.353$, $p = 0.036$) on ammonia in column water, but not in porewater ($F_{1,8} = 0.082$, $p = 0.782$), with levels being higher in summer than winter in Langebaan for both column and pore water, and higher in winter than summer for column water only at Durban (Fig. 2.2a).

Nitrates in pore-water and column-water were also significantly affected by an interaction of site with season (ANOVA, pore water: $F_{1,8} = 7.031$, $p = 0.029$, column water: $F_{1,8} = 48.091$, $p < 0.001$). Tukey tests revealed significant differences ($p < 0.05$ in all cases) for column water between sites in both summer and winter, with values being higher in Langebaan than Durban in summer, but the reverse in winter. Pore water exhibited the same pattern but the site difference was only significant in summer (Fig. 2.2b). Seasonal differences within sites were significant only for column water.

No significant differences in column-water orthophosphate concentrations were found between sites or seasons. For pore water, season had a significant effect (ANOVA, $F_{1,8} = 8.699$, $p = 0.018$), and site a marginally non-significant effect (ANOVA, $F_{1,8} = 4.723$, $p = 0.062$), with levels being higher in winter than summer at both sites, and values for Langebaan higher than those of Durban in both seasons (Fig. 2.2c).

When comparing pore-water and column-water nutrient concentrations, ammonia levels were not significantly different (3-way MANOVA, df = 1, $p > 0.05$) between water-sources, seasons or site. For nitrates, season and site had a significant interactive effect on pore and column-water levels (three-way MANOVA, $F_{1,16} = 20.209$, $p < 0.001$), but no significant differences were found between column-water and pore-water nitrates for any given season or site (Tukey tests, $p > 0.05$). For orthophosphate levels, effects of site and water-type (i.e. column-water vs. pore-water) as well as the interactive effects of site, season and water-type were significant (3-way MANOVA, df = 1, $p < 0.05$). Tukey tests indicated that the only occasion that a significant difference existed between column-water and pore-water was in

Langebaan during winter, where the orthophosphate concentrations in pore-water were substantially elevated above those in column-water.

2.3.2 Physico-chemical conditions

Temperature was higher in summer than winter at both sites (Fig. 2.3a), but the difference was marginally non-significant (Mann-Whitney *U* tests, Z = -1.746, U = 0, p = 0.081). During winter (but not summer) the water temperature in Durban was significantly higher than in Langebaan (Mann-Whitney *U* test, Z = 1.746, U = 0, p = 0.081).

Salinity (Fig. 2.3b) was significantly different between sites and between seasons (ANOVA, site: $F_{1,8}$ = 16.930, p = 0.003, season: $F_{1,8}$ = 13.810, p = 0.006). The mean value in Langebaan was significantly greater than in Durban during summer (Tukey test, p = 0.042), and marginally non-significantly greater (p = 0.063) in summer than winter in Langebaan (Fig. 2.3b). A similar, but non-significant trend was seen for Durban. Specific conductivity exhibited the same pattern (Fig. 2.3c), being significantly different between sites and seasons (ANOVA, site: $F_{1,8}$ = 11.200, p = 0.010, season: $F_{1,8}$ = 40.970, p < 0.001), being significantly greater in summer than winter at both Langebaan and Durban (Tukey tests, p < 0.05 in all cases). It was also marginally non-significantly greater in Langebaan than Durban during winter only (p = 0.061).

No significant difference (ANOVA, site: $F_{1,8}$ = 2.00, p > 0.05, season: $F_{1,8}$ = 2.32, p > 0.05) was found for pH between sites or seasons (Fig. 2.3d).

2.3.3 Biological conditions

Microphytobenthic biomass (Fig. 2.4) did not differ significantly between sites or seasons (season: ANOVAs, Durban: $F_{1,28}$ = 0.228, p = 0.635, Langebaan: $F_{1,28}$ = 0.173, p = 0.681; site: Mann-Whitney: winter: Z = 1.078, U = 86, p = 0.281, summer: Z = 1.535, U = 75, p = 0.125).

The average density of prawns in Langebaan Lagoon (184 m^{-2}) was slightly greater than that in Durban Bay (153 m^{-2}), but the difference was not significant (ANOVA, $F_{1,28} = 1.200$, $p = 0.283$).

2.3.4 Biological community differences

Species Composition

Analysed in terms of species composition, communities were significantly different between Langebaan and Durban (PERMANOVA, Pseudo-F = 10.477, df = 1, $p < 0.001$) and between the two seasonal periods (PERMANOVA, Pseudo-F = 5.517, df = 1, $p < 0.001$). Site and season also had a significant interactive effect on communities (PERMANOVA, Pseudo-F = 3.296, df = 1, $p < 0.001$).

These seasonal and site differences were clearly evident in ordination and MDS analyses (Fig. 2.5). Durban and Langebaan were only 13% similar in terms of species composition. Within Durban, two major subclusters existed for winter and summer groups, with 18% similarity. Within Langebaan, two subclusters corresponding to the two seasons also existed, although with greater overlap than at Durban.

BioEnV analyses

During winter, communities at both sites were best explained by the combination of pore and column water nitrate levels (correlation coefficient (r) = 0.630). Column water nitrate (r = 0.620) and temperature (r = 0.274) were the two single variables best explaining community composition in both Durban and Langebaan.

In summer, nutrients were again important; communities at both sites were best explained by the combination of pore and column water orthophosphate and column water nitrate (r = 0.726). The single variables best explaining community composition at the two sites were pore water orthophosphate (r = 0.667), salinity (0.464) and column water nitrate (0.409).

When both seasons are considered together, the combination of variables best explaining community structure at different sites were either temperature, salinity and chlorophyll-*a* (r = 301) or pore water orthophosphate, temperature, salinity and chlorophyll-*a* ($r = 0.301$). The most predictive single variables were pore water orthophosphate ($r = 0.293$), chlorophyll-*a* ($r = 0.280$), and temperature ($r = 0.107$).

Species diversity and abundance

Macrofaunal species richness (S; Fig. 2.6a) was significantly greater in Langebaan than Durban during both summer and winter (ANOVA, summer: $F_{1,16} = 19.977$, $p < 0.001$, winter: $F_{1,16} = 5.955$, $p = 0.027$). No significant seasonal difference existed in Langebaan (Mann-Whitney, $Z = 0$, $U = 40$, $p = 1.0$), but species richness was significantly greater in winter than summer in Durban (Mann-Whitney *U* tests, $Z = 3.002$, $U = 6$, $p = 0.003$).

Total abundance (N; Fig. 2.6b) was significantly different between sites and seasons (ANOVA, site: $F_{1,32} = 17.890$, $p < 0.001$, season: $F_{1,32} = 15.719$, $p < 0.001$). Tukey tests revealed that N was significantly greater in winter than summer in Durban ($p = 0.002$), with a similar but non-significant seasonal trend in Langebaan. Durban had significantly lower N values than Langebaan in summer ($p = 0.001$). The difference in winter was non-significant, but the trend was the same.

Species evenness (J′; Fig. 2.6c) was significantly different between seasons (ANOVA, $F_{1,32} = 10.603$, $p = 0.003$) but not sites (ANOVA, $F_{1,32} = 0.371$, $p = 0.547$). Within Durban, J′ was significantly greater in summer than in winter (Tukey-test, $p = 0.008$), but no significant seasonal differences existed in Langebaan ($p = 0.675$).

Margalef's index of species evenness (d′; Fig. 2.6d) was significantly greater in Langebaan than Durban during summer (ANOVA, $F_{1,16} = 22.334$, $p < 0.001$) and marginally non-significantly greater during winter ($F_{1,16} = 4.087$, $p = 0.060$). Within Durban d′ was significantly greater (Mann-Whitney *U* test, $Z = 2.826$, $U = 8$, $p = 0.005$) in winter than summer (Fig. 2.6), but no significant seasonal difference existed at Langebaan (Mann-Whitney *U* test, $Z = -0.883$, $U = 30$, $p = 0.377$).

The Shannon-Weiner diversity index (H′; Fig 2.6e) was significantly greater in Langebaan than Durban in both winter (ANOVA, $F_{1,16} = 6.835$, $p = 0.019$) and summer ($F_{1,16} = 22.899$, $p < 0.001$). Diversity (H′) was significantly greater during winter than summer in Durban (Mann-Whitney *U* test, Z = 2.208, U = 15, p = 0.027), but no significant seasonal differences existed in Langebaan (Mann-Whitney *U* test, Z = -0.530, U = 34, p = 0.596).

Functional Groups

Functional group composition was significantly different between sites (PERMANOVA, Pseudo-F =8.884, df = 1, p < 0.001) and between seasons (Pseudo-F = 10.148, df = 1, p < 0.001). Pair-wise tests revealed significant differences (pair-wise PERMANOVA, p < 0.05) between Durban and Langebaan within both winter and summer, and between the two seasons within each site.

Throughout all seasons and sites, burrowing deposit feeders followed by surface-dwelling deposit feeders were the most important defining groups (Fig. 2.7), contributing 22.35% to 61.09% and 14.77% to 23.29% of within-site similarity, respectively. Surface grazers and surface suspension feeders were generally less abundant, apart from Langebaan in winter where they were an abundant and defining group, constituting 21.57% of within-site similarity.

Almost all functional groups were more abundant in Langebaan than Durban in both seasons (Fig. 2.7). During winter, significantly greater densities of surface suspension feeders (ANOVA, $F_{1,16} = 35.067$, $p < 0.001$) and burrowing predators/scavengers ($F_{1,16} = 6.286$, $p = 0.023$) were present in Langebaan than Durban; these groups made up respectively 27.03% and 15.66% of the dissimilarity between sites during winter. During summer, all functional groups were found at significantly (p < 0.05) greater densities in Langebaan than Durban, apart from surface grazers, which were absent from both sites (Fig. 2.7).

Significant seasonal differences were obvious at both sites and more pronounced at Durban, with higher abundances of almost all functional groups in winter than in summer (Fig. 2.7). In Durban, such differences were significant for burrowing deposit feeders (ANOVA, $F_{1,16} =$

14.087, p = 0.002), surface deposit feeders ($F_{1,16}$ = 8.016, p = 0.012), surface grazers (Mann-Whitney *U*-test, U = 18, Z = 2.459, p = 0.014), surface suspension feeders (U = 21, Z = 2.006, p = 0.045) and surface predators/scavengers (U = 12, Z = 2.577, p = 0.010); only burrowing predators/scavengers did not differ ($F_{1,16}$ = 0.708, p = 0.413). In Langebaan, densities of surface deposit feeders (ANOVA, $F_{1,16}$ = 14.093, p = 0.002) and surface grazers (Mann-Whitney *U*-test, Z = 2.467, U = 18, p = 0.014) were significantly greater in winter than summer.

For the most part, functional groups were present in similar relative proportions at both sites and in both seasons (Fig. 2.7). There was no difference in their proportions between Langebaan and Durban in summer (χ^2 = 2.24, df = 5, p = 0.815), but there was in winter (χ^2 = 13.8, df = 5, p = 0.017), caused by the disproportionately high contribution of surface suspension feeders during summer in Langebaan. Within sites, winter-summer differences were not significant at either Durban (χ^2 = 5.89, df = 5, p = 0.315) or Langebaan (χ^2 = 10.0, df = 5, p = 0.075). Burrowing deposit feeders were consistently the most abundant group, whereas surface grazers were always rare, to the extent of not being recorded in summer.

2.4 Discussion

The main purpose of this chapter was to identify differences in community composition between sandflats that were both dominated by *Callichirus kraussi* in two marine-driven lagoons on the east and west coasts of South Africa, thereby providing an example of a marine-driven lagoon in each of two biogeographic zones. Decisive differences emerged between both sites and seasons, although the latter should be used only for a qualitative inspection of seasonality, since an examination of seasonality should require at least two years of data. Similarly, since only one site in each biogeographic zone was examined, no definitive biogeographic trends in marine-driven lagoons can be elucidated from the data, although the individual systems can be compared against each other and previous studies on other systems in each of the respective biogeographic zones.

Table 2.1 provides a synthesis of both site and seasonal patterns in terms of (1) expectations arising from the hypotheses outlined in the Introduction, (2) whether or not the observations accorded with the hypotheses, and (3) likely explanations. I had two central expectations. The first was that differences in community composition between the two coasts would coincide with established biogeographic patterns: that relative to communities found in Langebaan on the west coast, those in Durban on the east coast would be more diverse but support lower abundances of benthic invertebrates. Associated with this, I also expected temperatures to be higher in Durban Bay, which lies on the warmer east coast, and nutrients higher in Langebaan, which lies on the cooler, upwelling west coast, and that these differences would be most evident in summer when upwelling cools sea temperatures and elevates nutrient levels on the west coast. Secondly, I forecast that as the sandflat communities examined were both dominated by *Callichirus kraussi*, they would both be predominated by burrowers, which are known to be promoted by *C. kraussi*, whereas surface grazers and suspension feeders would be inhibited (Siebert and Branch, 2005b, 2007; Pillay *et al.*, 2007a,c).

Largely, these expectations were met, but there were some interesting exceptions that are amplified below.

2.4.1 Nutrients

Although not always significant, nutrient levels were higher in winter than summer within Durban and higher in the summer than winter within Langebaan (with the exception of orthophosphate). Unexpectedly, nutrients in the water column did not differ significantly between Langebaan and Durban when averaged across seasons, and although porewater values were higher in Langebaan as anticipated, the differences were again not significant (although they approached significance in two instances). In many cases there were significant season $\times$ site interactions because nutrient levels in Durban were lower than Langebaan in summer, but higher than Langebaan in winter. This may be explained by the impermanent summer upwelling, which raises nutrient levels in summer on the west coast where Langebaan is situated (Andrews and Hutchings, 1980; Bustamante *et al.,* 1995). Conversely, Durban lies on the east coast, which experiences very little nutrient-enriching upwelling throughout the year. The higher nutrient levels in Durban during winter may be due to an accumulation of pollution, with storms and freshwater flushing of the lagoon being reduced in winter because that is the dry-season on the east coast. Indeed, Newman *et al.* (2007) indicate that a number of major storm water drains empty into Durban Bay.

Durban Bay is the site of the largest harbour in South Africa and lies in the centre of a densely populated city (Marshall and Rajkumar, 2003). During fieldwork in Durban Bay it was apparent that some pollution was taking place, through yachts dumping sewerage and storm-water drains emptying directly into the bay. Near many of these source points of pollution large green algal mats were observed and although these areas did not coincide with the sampling sites it is likely that the diffuse pollution has an effect throughout the lagoon. Durban also has two offshore sewage outfalls (Bailey, 2000), which although managed could constitute a nutrient addition to the system. Mardon and Stretch (2004) found that water quality standards on Durban beaches were poor when compared with international norms, at least as far as bacterial contamination is concerned. Indeed, a number of different types of pollution have been identified in Durban Bay (Brown, 1987; Leuci, 2000; Marshall and Rajkumar, 2003; Newman *et al*., 2007).

Another potential explanation for elevated levels of nutrients in winter is the occurrence of a semi-permanent eddy in the Agulhas Current 20–40 km off Durban (Pearce *et al*., 1979; Meyer *et al.*, 2002). This eddy has been observed periodically, but although recurrent, it is transient either in time or location (Carter and d'Aubrey, 1988; Lutjeharms, 2006; Lutjeharms *et al.*, 2009). It is possible that some upwelling was occurring around the time of winter sampling, but localised pollution within the harbour seems a much more likely explanation.

Site interacting with season had a significant effect on column-water ammonia levels, but no obvious pattern emerged for this nutrient species for either pore or column water. Also, no significant difference was found between pore and column water ammonia concentrations. Waslenchuk *et al.* (1983) found raised levels of ammonia in callianassid burrows, Murphy and Kremer (1992) showed that callianassids could increase ammonia levels in the overlying water column, and Hughes *et al.* (2000) showed that *C. subterranea* increased ammonium flux between the sediment and the water column. Thus, the lack of difference in ammonia levels between pore and column water could be due to *C. kraussi* raising both pore and column water levels. Further experimental studies are needed to determine how *C. kraussi* affects nutrient fluxes.

Site and season also had important interactive effects on nitrate levels in column water and pore water. Levels in pore water were higher in Langebaan than Durban during summer, which corresponds to the annually averaged higher nitrate and nitrite levels of the west coast relative to the east coast found by Bustamante *et al.* (1995). In column water, nitrate levels were significantly higher in Durban during winter and in Langebaan during summer. Within Langebaan summer values were significantly greater than in winter, and within Durban the reverse was true. The fact that Durban experiences higher winter values than Langebaan has already been discussed above (p. 23, paragraph 3), in the context of potential pollution in Durban, or transient eddies off the coast there. Coupled with this, the west coast where Langebaan is located experiences less upwelling in winter (Andrews and Hutchings, 1980; Bustamante *et al*., 1995), leading to decreased nutrient levels.

No significant differences were found for site or season in column water phosphate levels. This lack of between-site differences in the intertidal column water contrasts with the expectation of differences between sites located in different biogeographic regions, as illustrated by the findings of Bustamante *et al.* (1995), who showed that sites on the upwelling west coast of South Africa have higher levels of phosphate than sites on the non-upwelling east coast. The unexpected absence of any significant difference between sites may be due to elevation of nutrients in Durban Bay by pollution, as discussed before. The lack of seasonality in column-water orthophosphates in Langebaan, where elevated levels of upwelling and therefore nutrients were expected to occur in summer (Andrews and Hutchings, 1980) may be due to consumption of phosphates by primary producers. Downing *et al.* (1999) state that pristine marine habitats tend to be phosphorous-limited. Langebaan is a relatively pristine system and as such it is possible that phosphate-limitation plays some role in primary production. However, Christie (1981) indicates that phytoplankton production in Langebaan is nitrogen-limited. Nevertheless, increases in orthophosphate in the water column during the summer months due to upwelling may be masked by an increased demand by primary producers, which increase in spring and summer with warmer weather and lengthened photo-period (Coma *et al*., 2000). Indeed, Christie (1981) found that phytoplankton (chlorophyll *a*) levels were highest in the lagoon in summer, and decreased from the mouth down the lagoon, similar to the findings of Henry *et al*. (1977) for Langebaan Lagoon and the adjacent Saldanha Bay.

Interestingly, pore water orthophosphate levels on the whole were higher than those in the column water. This is quite possibly due to the action of *C. kraussi*. Waslenchuk *et al.* (1983) observed that callianassid burrows have enriched levels of phosphate relative to the water column. Given the high densities of *C. kraussi* living within the sediment at both sites, it is quite likely that *C. kraussi* influences pore water phosphate concentrations.

2.4.2 Physico-chemical data

Water temperature changed seasonally, summer being warmer than winter at both sites as expected. Unexpectedly, Durban Bay was not significantly warmer than Langebaan Lagoon. Durban experiences hot, wet summers and warm dry winters and lies in the subtropical Natal biogeographic zone, while Langebaan experiences a Mediterranean climate with hot, dry summers and cooler, wet winters and falls in the cool temperate Namaqua biogeographic province (Day 1981, Schulze, 1984, Emanuel *et al.*, 1992; Bustamante and Branch, 1996). Differences in temperature were thus expected. Local solar heating of the relatively shallow lagoons is the likely explanation for the equivalence of temperatures between the two systems. Langebaan is shallow, being on average only 1-2 m deep and 6 m deep at its maximum (Flemming, 1977), and it is worth noting that the average summer water temperature of Langebaan was 26.17^{o}C, which is substantially higher than the average of 8-10°C of the upwelled summer water on the adjacent open coast (Andrews and Hutchings, 1980). This emphasises the role of solar heating in warming the relatively shallow lagoon during the summer months.

Salinity in both Langebaan and Durban was consistently close to values expected of seawater – around 35 psu (Vernberg and Vernberg, 1972), a reflection that both systems are marine-dominated lagoons with relatively little freshwater input (Day 1981). Salinities spanned 32-36 psu, a range that is unlikely to be of biological consequence for benthic infauna (Vernberg and Vernberg, 1972; Day, 1981). A critical salinity boundary of 5-8 psu is recognised as separating marine and fresh water fauna, and coastal marine species tend to be more tolerant to changes in salinity than oceanic species (Vernberg and Vernberg, 1972). Additionally, Day (1981) points out that (in estuaries) salinities of 25-40 psu are tolerated by stenohaline fauna, 5-50 psu by euryhaline fauna and 2-60 psu by a few truly estuarine species. All of these limits lie well outside the salinity range of my study areas. The same was true for conductivity. Both salinity and conductivity were greater in summer than winter, probably because of evaporation (Schulze, 1984), but again the difference was small and unlikely to be biologically significant.

2.4.3 Biological conditions

The density of *C. kraussi* was not significantly different between the two sites, so any differences in the composition of communities between the two systems cannot be attributable to differences in the abundance of *C. kraussi*.

Contrary to expectations, and negating my hypothesis, no significant seasonal or site differences were found for microphytobenthic biomass. This contrasts with the expectation that sites would follow biogeographic trends in line with the findings of Bustamante *et al.* (1995) for open-coast rocky shores, where primary productivity was shown to be substantially higher on the west coast. It is possible that pollution input into Durban Bay has elevated nutrient levels to a point where they do not differ significantly from those in Langebaan Lagoon, and that this has raised primary productivity in Durban to levels similar to those in Langebaan, which is enriched by upwelling (Brown *et al*., 1991; Bustamante *et al*., 1995).

The absence of any significant seasonal difference in microphytobenthic biomass may reflect elevation of bioturbation rates by *C. kraussi* in summer, as observed for other bioturbators across the globe (Teal *et al.* 2008), which may offset any summer increase in primary productivity associated with higher temperatures and greater nutrient input, since bioturbation by *C. kraussi* does have negative effects on microalgae (Branch and Pringle, 1987; Pillay *et al.,* 2007b).

2.4.4 Biological community differences

Species Composition

In terms of species composition, the communities in Langebaan Lagoon and Durban Bay were significantly distinct, with only 13% similarity. This between-site difference was predictable, given the fact that the two sites lie in different biogeographic regions, the cool temperate Benguela and the subtropical Natal provinces, separated by the warm temperate

Agulhas Province (Brown and Jarman 1978; Emanuel *et al.,* 1992; Bustamante and Branch, 1996; Awad *et al.,* 2002).

Within each site, there were also differences between communities in summer and winter. It is likely that these reflect differences in both breeding cycles and responses to prevailing environmental conditions. Indeed, Puttick (1977) found that there were seasonal fluctuations in the numbers of benthic fauna in Langebaan Lagoon (with numbers being lower in winter than summer), and that different groups of macrofauna underwent recruitment at different times of the year.

BioEnV analyses

For all seasons and sites nutrients appeared to have been the dominant variable explaining macrofaunal community composition. In winter, temperature and pore and column water nitrates were the most important determinants of community composition. In summer, nutrients (pore and column water orthophosphate and column water nitrate) were important determinants and salinity played a smaller role. Given that temperatures were only marginally non-significantly different between sites in winter and salinities were only significantly different between sites in summer, it follows that temperature and salinity were only important determinants of communities among sites within those seasons, respectively. The fact that nutrients were a dominant factor in determining community structure is in agreement with the observations that (1) nitrate levels differed between sites within seasons, and (2) nitrates appear to limit primary production in Langebaan Lagoon (Christie, 1981).

The overall determinants of community structure across sites and seasons were orthophosphates, temperature, chlorophyll *a* and salinity. These all correspond to the factors highlighted in the introduction as being important determinants of intertidal communities between coasts in South Africa.

Species Diversity and Abundance

Marine invertebrate species diversity in South Africa generally increases eastwards, with diversity being greater on the east coast than the west, which is characterised as being poor in diversity at the species level (Bustamante and Branch, 1996; Awad *et al*., 2002). I therefore expected that because Durban lies on the east coast, it would have had higher diversity than Langebaan. However, in my study, invertebrate species richness (S) and most other indices of diversity were greater in Langebaan than Durban in both seasons. This is most probably due to higher levels of pollution and human disturbance in Durban, and reflects the fact that estuaries and lagoons are vulnerable to local effects that may over-ride expected biogeographic trends. Wynberg and Branch (1991) estimated that in collecting a bag limit of 50 sandprawns, each bait collector will disturb approximately 54 g of macrofauna, and Wynberg and Branch (1997) found that trampling associated with sandprawn collection, in addition to the removal of prawns *per se*, had negative effects on macrofaunal abundance. Two of the three subsites sampled in Langebaan fall in zones where harvesting is banned, whereas in Durban Bay all the subsites sampled are regularly harvested (personal observations). It is therefore quite probable that the bait collection by many individuals within the sites at Durban, coupled with other disturbances and pollution, have decreased species richness below levels expected of the east coast. Again, irrespective of the causes, a central message emerging is that in the shallow lagoonal systems examined, local effects appear to over-ride broad-scale biogeographic trends. Further systems need to be analysed to generalise about biogeographic trends in marine-driven lagoons.

Total abundance was greater in Langebaan than Durban (Fig. 2.6), this may be explained by the biogeographic regions in which the sites are found; this pattern conforms to previously established trends of abundance (and biomass) on open-coast rocky shores, which show a progressive decline from the west to the east coast, associated with a parallel decline in primary productivity (Bustamante *et al.*, 1995). The marked reduction of abundance in Durban during summer may be a seasonal effect related to greater physical stresses in summer, but may also reflect an increase in the intensity of bait collecting and associated trampling during the summer holidays.

Functional Groups

Durban Bay and Langebaan Lagoon had communities composed of significantly different functional groups, although their relative proportions differed only in winter, and several trends emerged from their comparison.

First, burrowing deposit feeders were the prevalent group at both sites and in both seasons. I had hypothesized that *C. kraussi* bioturbation, a dominant feature of both sites, would diminish surface feeders while enhancing burrowing infauna, based on previous work (Siebert and Branch 2005b, 2007; Pillay *et al.*, 2007a,c). The abundance of burrowing deposit feeders supports this hypothesis, but is best evaluated by the fact that surface deposit feeders were consistently less abundant than their burrowing counterparts. Burrowing predators, however, achieved roughly the same overall abundance as surface predators, and the numbers of both groups were fairly low, reflecting the fact that predators are typically found in lower abundances than lower trophic levels (Stiling, 2002).

The low abundance of surface grazers at both sites is in agreement with the findings of Pillay *et al.* (2007a) and is likely the result of either the direct effects of smothering or the indirect effects of depressed levels of microalgae on which they feed, both of which are consequences of *C. kraussi* bioturbation and associated sediment destabilization (Branch and Pringle, 1987; Pillay *et al*., 2007b,c).

The second trend was that most functional groups contributed similar proportions at both sites and in both seasons, with both surface deposit feeders and burrowing deposit feeders being shared characteristic groups. Langebaan did differ from Durban in supporting a substantially greater abundance of surface suspension feeders, and in winter this led to a significant difference emerging from a χ^2 analysis of the proportions of functional groups; but, apart from this, the differences were not significant between sites or between seasons. The relatively high proportions of surface suspension feeders in Langebaan was unexpected, as bioturbation generally has negative effects on them by clogging of the filtration apparatus and increasing sediment suspension in the water, hence decreasing filtration rates and feeding (Murphy, 1985; Rhoads & Young, 1970; Ellis *et al*. 2002, Pillay *et al*., 2007c). The lower abundance of suspension feeders in Durban (as compared to Langebaan) may be due to greater human disturbance (trampling, bait collecting, etc.) resulting in unstable sediments. Suspension feeders in soft sediments are known to be susceptible to sediment instability generated by bioturbation (Rhoads & Young, 1970; Pillay and Branch, 2011) and it follows that instability arising from other causes will also be detrimental to these groups.

The third trend was the difference in abundance between seasons. With a single exception, all functional groups were more abundant in winter than summer. One possible explanation could be that activity of *C. kraussi* is lower at cooler temperatures. Langebaan was significantly cooler than Durban during the winter months, and in a global review, Teal (2008) found higher biological sediment turnover rates in summer than winter. Ouellette *et al.* (2004) recorded lower bioturbation rates by the polychaete *Neanthes virens* when temperatures dropped. If *C. kraussi* activity decreases in response to lowered temperature, it is likely that bioturbation levels will also decrease. And since bioturbation has inhibitory effects on many groups (Branch and Pringle, 1987; Pillay *et. al,* 2007c), a winter decrease in bioturbation may result in an increase in abundance. The effects of temperature on bioturbation rate by *C. kraussi* are investigated in Chapter 4 to test this idea. In addition, heightened human disturbance and bait collecting may contribute to the strikingly low abundances of all functional groups in summer at Durban.

2.4.5 Conclusions

Table 2.1 provides a synthesis of the expected patterns of differences between sites and between seasons, and whether observations conformed to these expectations. Two central points emerge. First, although a majority of the biological and physico-chemical characteristics of Langebaan Lagoon and Durban Bay followed expected seasonal and west-east coastal trends, in many cases localised effects within the lagoons such as solar heating or pollution modified parameters so that they diverged from patterns evident on the open coast. This was unexpected, since the systems were chosen for the fact that they are marine-driven, and I expected marine contrasts between the east and west coasts to extend into the lagoons. The hypotheses regarding the effects of *C. kraussi* were largely validated, supporting the idea that it had a powerful influence on functional-group composition at both sites, in spite of the fact that species composition differed substantially between them.

Second, the relative proportions of functional groups remained generally similar across sites and between seasons, regardless of biogeographic differences in species composition, and this consistency accords with the proposal that communities dominated by *C. kraussi* are shaped by bioturbation favouring certain functional groups and disfavouring others, Because this study was observational and correlative, it serves to highlight differences between the two lagoons and to suggest their causes but cannot be used to identify causes and effects. It does not permit comment on general biogeographic patterns in marine-driven lagoons as only one site on each coast was examined. It did, however, point to the possibility that activities of *C. kraussi* may be modified by physico-chemical conditions such as temperature and nutrients. In Chapters 3 and 4, this is pursued through field and laboratory experiments in which these variables are manipulated.

CHAPTER 3

AN EXPERIMENTAL TEST OF INTERACTIVE EFFECTS OF NUTRIENTS AND *CALLICHIRUS* BIOTURBATION ON SOFT-SEDIMENT COMMUNITIES

3.1 Introduction

Disturbance and productivity are important factors shaping community structure and diversity. As outlined in Chapter 1, they may operate individually or interactively. This chapter investigates how nutrient enrichment, which should enhance primary productivity, and disturbance in the form of bioturbation by the sandprawn *Callichirus kraussi*, interact to structure macrofaunal communities.

Disturbance has been proposed to affect diversity in a unimodal way, in what is termed the intermediate disturbance hypothesis (IDH), with the highest diversity being observed at an intermediate level or frequency of disturbance (Connell, 1978; Sousa, 1979; Valdivia *et al*., 2005). Likewise, diversity and productivity are often unimodally related (Waide *et al.*, 1999; Kassen *et al.*, 2000; Worm *et al.*, 2002). However, other trends have been reported: in a study of relationships of disturbance with species richness, diversity and evenness, Mackey and Currie (2001) found that non-significant relationships were most common, and that significant relationships could take the form of positive monotonic, negative monotonic, unimodally peaked or U-shaped relationships. They concluded that disturbance seldom produces peaked relationships with diversity. In a review of 200 relationships of species richness and productivity, Waide *et al*. (1999) found that only 30% were unimodal, while 12% were negatively linear, 26% positively linear, and 32% not significant. They suggested that scale might be important in influencing the nature of the relationship. Other literature indicates that processes such as disturbance, including the action of consumers, may influence the productivity-diversity relationship in an interactive way (Huston, 1979; Proulx and Mazumder, 1998; Kondoh, 2001; Worm *et al*., 2002; Jara *et al*., 2006; Valdivia *et al*., 2008).

Huston (1979) proposed that diversity is maintained through a trade-off between population reductions, which occur through some form of disturbance, and growth rates (productivity) of competitors, which are influenced by such factors as nutrient enrichment. Huston (1979) proposed a hump-shaped relation between diversity and either frequency of population reduction (disturbance) or population growth (productivity). This became known as the Dynamic Equilibrium Model (Huston, 1979).

Kondoh (2001) proposed a modified version of Huston's (1979) Dynamic Equilibrium Model that explains how productivity and disturbance interact to yield an assortment of different relationships with diversity. Kondoh (2001) argues that the relationship between either disturbance or productivity and species richness is generally unimodal, but that the level of either productivity or disturbance needed to achieve maximum diversity is modified by the level of the other factor, i.e. their interactive effects produce different patterns of species richness. Kondoh (2001) predicted that the diversity-disturbance relationship would be negative at low levels of productivity, unimodal at intermediate levels of disturbance and positive at high levels of productivity (Fig. 3.1). These patterns are thought to be the result of (a) increasing disturbance favouring inferior competitors by reducing competitive exclusion and promoting efficient colonizers, and (b) increasing productivity favouring superior competitors. A trade-off between these two processes should therefore produce maximum species richness (Kondoh, 2001). There is some empirical support for the Kondoh's model (Jara *et al*,. 2006; Guerry, 2008), and Valdivia *et al.* (2008) have specifically demonstrated the opposing effects of nutrient enrichment and disturbance on diversity and their interactive effects on communities.

The Kondoh model can be generalized, but was advanced from the specific case of the interaction between productivity and grazing (as a form of disturbance), which was described by Proulx and Mazumder (1998). In a review of a range of different ecosystems, Proulx and Mazumder (1998) found that in all of the 19 nutrient-poor ecosystems examined, high levels of grazing resulted in lower plant species richness than low levels of grazing, while in the majority of cases of nutrient-rich systems (14 out of 25), high grazing levels resulted in greater species richness. There was also more variability in plant species-richness responses to grazing in high-nutrient systems, with nine cases (out of 25) showing no significant response of species richness to grazing and two cases showing a negative response. Proulx and Mazumder (1998) suggested that the negative relationships observed in low-nutrient conditions could be due to the fact that plants are nutrient-limited and therefore unable to regrow after grazing, while the positive relationships in high-nutrient conditions may be the result of a relative increase in abundance of inedible plant species in response to the removal of palatable species at high levels of grazing. Worm *et al.* (2002) also found that the effects of consumers and nutrients on diversity are consistently interdependent and that maximum species diversity and the effects of consumers differ between states of high and low productivity. Hillebrand (2003) recorded that grazing and nutrient enrichment had opposing effects on diversity and evenness, but that neither factor influenced the effects of the other. Other studies have recorded interactive effects, but indicate that grazing cannot override the effects of high levels of eutrophication, suggesting that the positive effect of grazing on diversity under high nutrient conditions has limits (Worm and Lotze, 2006; Guerry, 2008).

The interactive effects of bioturbation, as a form of disturbance, and nutrient enrichment on macrofaunal community structure and diversity have been less studied. As discussed in Chapter 1, bioturbation has strong effects on benthic communities as well as influencing the chemical and physical properties of the sedimentary and overlying aquatic environment. Bioturbation by the sandprawn *Callichirus kraussi* decreases the abundance of surface grazers, suspension feeders (including filter feeders), and deposit feeders, while favouring burrowing infauna (Siebert and Branch, 2005b, 2007; Pillay *et al*, 2007a). Pillay *et al*. (2008) showed that *C. kraussi* causes large changes to macrofaunal communities over time, decreasing the abundance of bivalves by as much as 25-fold. For another callianassid, *Nihonotrypaea harmandi*, large natural increases in its density have led to localised extinctions of a grazer (Tamaki, 1994). Flach and Tamaki (2001) also found that this callianassid has negative effects on tube-building and surface deposit-feeding polychaetes. Macrofauna with restricted mobility, including spionid polychaetes, tanaids, bivalves and filter-feeding gastropods, repeatedly have been shown to be negatively affected by callianassids (Peterson, 1977; Murphy, 1985; Posey, 1986; Berkenbusch *et al.,* 2000; Flach and Tamaki, 2001; Pillay *et al.* 2007a,b,c, 2008). Other studies have shown that, like the case of *C. kraussi*, callianassids positively influence some groups such as burrowing macrofauna and mobile bivalves, ostracods and amphipods (Aller and Dodge, 1974; Posey, 1986; Riddle, 1988; Siebert and Branch, 2005b).

These effects of *C. kraussi*, or other callianassids, on macrofauna may operate through a range of mechanisms, including (1) obstruction of feeding activities by blocking filter-feeding apparatus; (2) burial causing direct mortality or metabolic losses due to decreased feeding time or increased energy expenditure needed to offset burial; (3) destruction of the tubes created by tube-dwelling macrofauna, resulting in smothering or increased metabolic requirements to maintain tubes; (4) emigration and associated increased risk of predation (Cadée 2001; Pillay *et al.* 2007a,b); (5) negative affects on benthic biofilms and, indirectly, the metabolic gain, condition and survival of grazers or filter feeders that feed on biofilms (Ellis *et al.* 2002; Pillay 2006; Pillay *et al.* 2007a); (6) erodability of sediments, which, in addition to clogging the filtration apparatus of filter feeders, can expose macrofauna and increase their vulnerability to predators or to being washed away (Pillay *et al.* 2007a; Pillay and Branch 2011). In the case of suspension feeders, clogging of the filtration apparatus decreases filtration rate and feeding (Murphy 1985; Rhoads & Young 1970; Ellis *et al*. 2002) and leads to a deterioration of the condition of the organisms.

Due to the strong effects bioturbation has on soft-sediment communities, it is of interest to know whether its influence on macrofaunal communities and diversity is modified by an interaction with nutrient levels.

Studies on other types of disturbance in soft-sediment provide some insight into the type of interaction expected for bioturbation and nutrient enrichment. Widdicombe and Austen (2001) and Austen and Widdicombe (2006) examined the effects of (mechanical) disturbance and nutrient-enrichment on meiobenthic and macrobenthic communities in mesocosm experiments, and showed that their interactive effect on meiobenthic nematode diversity was approximately in line with the predictions of Huston's (1979) Dynamic Equilibrium Model (DEM), with diversity being highest at combinations of low levels of physical disturbance and enrichment. Likewise, they documented that macrofaunal communities follow the predictions of the DEM: diversity was higher than expected when disturbance and enrichment were either both low, or both high. However, macrofaunal diversity was lower than expected when disturbance frequency was high and enrichment was low, or when disturbance frequency was low and enrichment level high (Widdicombe and Austen, 2001). In short, they found that macrofaunal community structure was significantly affected by nutrient enrichment, disturbance and their interactive effects.

O'Brien *et al.* (2009), in a study involving both nutrient enrichment and exclusion or inclusion of the lugworm *Arenicola marina*, showed that bioturbation, tidal height and sediment characteristics may have interactive effects on pore-water nutrient discharge (from fertilizer capsules), transformation and movement. They found little effect of nutrient enrichment on benthic fauna overall, although negative effects were observed on a few species: elimination of bioturbation by *A. marina*, in combination with tidal height, had a positive effect on two species of polychaete (*Scoloplos armiger* and *Nereis diversicolor*), both of which increased in high-shore exclusion plots.

My study sought to determine whether bioturbation by *C. kraussi* and nutrient enrichment have interactive effects on macrofaunal community structure. I hypothesized that:

1. Disturbance and nutrient levels will interact in terms of their influence on diversity (Fig. 3.1). At low levels of disturbance, increasing nutrient levels will decrease diversity. At high disturbance levels increasing nutrient levels will offset the negative effects of disturbance and increase diversity. At intermediate disturbance levels, a trade-off will occur, where diversity is highest at intermediate levels of nutrients.
2. Macrofaunal community composition will be significantly affected by nutrient levels and prawn density. Specifically, surface grazers, surface suspension feeders and deposit feeders will be diminished by increasing *C. kraussi* densities, while burrowing infauna will be favoured, and these effects will be muted by increased nutrient levels.

3.2 Methods and materials

3.2.1 Field caging experiment

To test the interactive effects of nutrients and bioturbation on macrofaunal communities, I employed a field experiment in the intertidal sandflats, between 99 cm and 127 cm below the spring high tide mark, at Bottelary (33°08'627"S, 18°05'300"E) in Langebaan Lagoon on the west coast of South Africa, in which I sought to manipulate productivity through nutrient adjustments and to regulate bioturbation intensity by controlling sandprawn densities in cages, on the assumption that density will be proportional to bioturbation intensity. This assumption is based on the grounds that previous studies have demonstrated (a) that one sandprawn produces one hole (Wynberg and Branch, 1997; Nel, 2006), and (b) that at normal maximum densities, *C. kraussi* deposits an approximate 4.5-cm layer of sediment on the surface per week (which amounts to a turnover of 12.14 kg.m^{-2} per day), (c) that sediment turnover increases with prawn density, being almost absent in treatments from which *C. kraussi* were excluded (Branch and Pringle, 1987).

Cages were constructed from large 90-L black plastic crates (45 x 58 x 43 cm), because large-sized cages tend to minimize caging effects (Virnstein, 1978). The sides and bottoms of the crates were cut out, leaving only the cubic frame, which was covered side-and-bottom with fly meshing (mesh diameter 1 mm) to retain *C. kraussi* at particular densities and to avoid ingress by macrofaunal predators or other organisms from the sediment (see Virnstein 1978). The cages lacked mesh lids, since roofs can cause shading (Spivak *et al*., 2009), which could have a significant effect on the microphytobenthos and therefore on infaunal communities.

To install each cage, a large metal frame (1 x 1 m) with handles was pushed into the ground down to about 30 cm. All of the sand inside the frame was then dug out, the cage placed into the hole, and the sand sieved back into the cage using a sieve of 1-mm mesh diameter. All sandprawns and other large (visible) macrofauna were removed from the sand in this way. Once the cage was filled with sand, it was left protruding approximately 10 cm above the sediment surface, and the metal frame was removed, allowing sand to fill in around the cage. Cages were installed from September to October 2009.

Prawn densities and nutrient levels were manipulated as follows. Three levels of nutrient treatment were used (Table 3.1): background (natural) nutrient levels, to which no fertilizer was added, an intermediate level of enrichment, to which 250 g of fertilizer per bag was added, and a high nutrient enrichment treatment, to which 500 g of fertilizer per bag was added. (These levels were determined through a series of field nutrient-enrichment trials in early 2009, which demonstrated that these levels of nutrient additions achieved effective enrichment.) Plantacote-Plus™ 8M (Aglukon) controlled-release fertilizer was used for all treatments as it releases nutrients at a steady rate relative to its weight and has been shown to be an effective means of regulating nutrient levels (Worm *et al*., 2000; Canning-Clode *et al*., 2008). It was weighed into bags made of nylon 1-mm-mesh shade cloth (which was more durable than fly meshing, which was initially employed but proved vulnerable to attack by gulls). Four of these bags were installed on the inside of each cage using cable ties (see inset in Fig. 3.2a). Four prawn density treatments were applied, using intermediate size prawns (4 – 5 cm from head to tail): no prawns (0 prawns per cage), half prawn density (30 prawns), normal prawn density (60 prawns) and double prawn density (120 prawns), based on approximately 250 m^{-2} being the natural density at this site (Siebert and Branch, 2005a; Nel, 2006).

Sandprawns used in caging treatments were collected from the sediment using a bait suction pump, from areas no less than 600 m away from the caging site. These prawns were kept in buckets filled with seawater and then installed into the cages as the tide was coming in on the same day. Care was taken to ensure that all prawns had dug into the sediment before the site was covered by the incoming tide and that no prawns escaped. Any prawns that were damaged or did not dig into the sediment were replaced. The process of collecting and installing the prawns started at the end of November 2009, at least five weeks after installation of the cages, to allow the sediment to become stabilised before introducing the prawns.

Nutrient enrichment bags were simultaneously attached to cages. Cages were numbered to record treatment and GPS information, and were monitored every week for the first month after installation and then every two to three weeks to check for damage and make repairs where necessary.

The set of nutrient treatments (see Table 3.1) resulted in a matrix of twelve treatments, which was replicated four times in a split-plot design of two replicates in each of two plots (A and B). Treatments were randomly interspersed in a plot, each cage being approximately 5 m from other cages to ensure independence. One of the plots is shown in Fig. 3.2a to demonstrate the layout. The two split plots were approximately 100 m apart, Plot A lying at 127 cm and Plot B at 99 cm below the spring high tide mark. The difference in height was unintentional and was detected only when the heights of the plots on the shore were determined at the conclusion of the experiment.

3.2.2 Sampling

Following completion of prawn and nutrient installation (by the first week of December 2009) the experiment ran for six months. At three months a first set of samples was taken. Sampling was carried out around low spring tide when the sandflats were exposed. Microphytobenthic chlorophyll-*a* (n = 3 per cage) and pore-water ammonia, nitrate plus nitrite (henceforth collectively referred to as 'nitrates') and orthophosphate (n = 1 per cage) were sampled and analysed as described in Chapter 2. (Microalgal community–composition and meiofaunal samples were also taken for later independent analysis, but fall outside the scope of this thesis.) To determine N:P Redfield ratios, nitrogen was calculated as the sum of nitrogen molar values of ammonia and nitrate (plus nitrite), and phosphorus as the molar phosphorous value of orthophosphate.

EPS (extracellular polymeric substance) was sampled as follows. Three replicate cores (2 cm diameter, 1 cm depth) were taken from the sediment surface of each cage. These were kept cold and then frozen at -20°C. One gram (wet weight) of each sample was then analysed within two days of collection, using the colorimetric phenol-sulphuric acid assay of Dubois *et al.* (1956) to measure total sediment carbohydrate concentrations (Underwood *et al.*, 1995). Pre-weighed sediment was mixed with 2 ml of distilled water and to this was added 1 ml of 5% aqueous phenol (wt/vol) and 5 ml concentrated sulphuric acid (H_2SO_4). This was then diluted (1 ml solution with 9 ml distilled water), and absorbance measured on a spectrophotometer against a reagent blank at 485 nm. A calibration curve using a glucose standard was used to convert absorbance values to values of grams glucose per gram sediment. This EPS value provided an estimation of the quantity of biofilm in the sediment (Underwood *et al.*, 1995; Pillay *et al.* 2007b).

Macrofaunal samples were collected using a corer (10 cm diameter, 30 cm deep) to extract two samples from opposite corners of the cages. These two cores were combined, and processing and identification followed the procedures outlined in Chapter 2. Data for total abundance are expressed as numbers (N) per 157.2 cm^2.

After six months (May-June 2010), in the space of a fortnight, an identical set of samples was extracted except that during this sampling period macrofaunal cores were taken from the central region of cages to avoid re-sampling areas disturbed during the three-month sampling period.

3.2.3 Controlling for caging artefacts

To determine the effects of caging and disturbance associated with installation of cages, cage controls and disturbance controls were used (Fig. 3.2b). Disturbance controls constituted cages that had no bottom mesh and could be pushed directly into the sediment without disturbing it. Cage controls were areas where no cages were installed. Cages with a background level of prawns (250 m^{-2}) and natural (background) levels of nutrients could be compared against disturbance controls (which contained equivalent background levels of prawns and nutrients) to determine the effects of disturbance, since these treatments differed only in disturbance. The disturbance controls were also compared against the cage control to determine the effect of caging, since the two differed only in the presence or absence of a cage.

Any differences between caged treatments and cage controls could have been due to a combination of caging and disturbance. It should, however, be possible to isolate the individual effects of caging and disturbance by comparing disturbance controls with respectively cage controls and cage treatments.

3.2.4 Statistical analyses

For all statistical analyses, the Kolmogorov-Smirnoff normality test and Levene's test for equality of variance were first performed. Where data conformed to normality and equality of variance, parametric tests were applied. Where data did not conform, even after transformation, corresponding non-parametric Kruskal-Wallis or Mann-Whitney *U* tests were applied, as specified in the results. Where significant differences were found and the data were parametric, Tukey post-hoc tests were employed to determine where the differences lay.

The effect of the time-period of sampling on macrofaunal communities was assessed by a one-way PERMANOVA between time periods. As the communities were found to be significantly different between the two sampling periods (Pseudo-F = 10.838, df = 1, p <

0.001) all subsequent statistical analyses for treatment effects were performed separately for each time period.

Effects of caging

To determine the effects of caging and disturbance due to caging on samples, one-way ANOVAs were run among caging treatments for nutrients, sediment chlorophyll *a* and EPS. Similarly, one-way PERMANOVAs were run among caging treatments for species composition and for functional group composition. Where significant differences occurred, pair-wise PERMANOVAs were used to determine where the differences lay. SIMPER analyses identified which species differed between treatments.

Effects of treatments

To test whether nutrient applications resulted in the intended enrichment, two-way ANOVAs were run with nutrient treatment and prawn density treatment as fixed factors. All nutrient data were log-transformed to conform to normality and equality of variance.

Microphytobenthic biomass and EPS were analysed with two-way ANOVAs, with prawn densities and nutrient levels as fixed factors. All data conformed to normality and equality of variance, except for EPS values after three months.

To determine whether communities differed between the two plots of the split-plot design, PERMANOVA analyses were performed, based on both species composition and functional groups for each sampling period. Associated SIMPER analyses identified the main species and functional groups accounting for differences between plots. Since plot differences were significant, communities were then analysed separately for each plot within each time period. MDS plots based on species composition and cross-factorial PERMANOVA tests were run for each plot within each time period to examine the effects of prawn density and nutrient treatments.

One-way ANOVAs or non-parametric equivalents were run for each plot within each time period to test for the effects of prawn densities on the densities of each functional group. The effects of nutrient were not considered in this way, since nutrient treatments never produced any significant effect on communities.

To determine if macrofaunal diversity and abundance measures differed between plots within each time period, ANOVAs (or non-parametric equivalents) of nutrients and prawns nested within plot were run for 3-month and 6-month sampling periods, for which most data met parametric requirements. Diversity was measured as Total species richness (S), Margalef's index of diversity (d′), Pielou's index of evenness J′ and the Shannon-Wiener index H′. Abundance was quantified as numbers per 157.08 cm^2 (twin-cores).

Due to significant between-plot differences (except for S and d′ at six months), diversity measures for each plot (within each time period) were separately subjected to two-way ANOVAs with prawn densities and nutrients as fixed factors. Data that violated parametric assumptions for nutrients were subjected to one-way ANOVAs for prawn-density treatment and Kruskal-Wallis tests for nutrient treatment. Data for S and d′ at six months were analysed with Kruskal-Wallis for both plots together, although the graphs are plotted separately for the two plots to allow comparison with other measures.

The effects of (a) different levels of nutrients and (b) prawn densities on all diversity indices were investigated by a range of regressions (linear, second-order polynomial, log and power) separately for each plot and time period.

Software

All MDS, SIMPER and PERMANOVA analyses were executed with PRIMER 6.1.11 with PERMANOVA+ 1.0.1, and ANOVAs, non-parametric tests and regressions with Statistica 9.

3.3 Results

3.3.1 Effects of caging and disturbance

One-way ANOVAs among caging treatments for physical and biological variables (Table 3.2) showed that nutrients were affected by caging only in one instance: after three months ammonia levels were significantly higher in the caged treatments, 0.425 mg/L ±0.048 (SE), than in the cage control, 0.225 mg/L ±0.025 ($p < 0.05$), but not different from the disturbance control, 0.325 mg/L ±0.025, indicating that the effect could have been due to caging, but not due to disturbance.

Chlorophyll-*a* (microphytobenthic biomass) never differed among controls. EPS was significantly lower in the disturbance control (averages = 7.427±0.617 and 8.797±0.863 $g_{glucose}/g_{sediment}$) and caged treatments (7.534±0.502 and 8.050±0.767 $g_{glucose}/g_{sediment}$), than in the cage control (11.254±0.852 and 12.880±0.820 $g_{glucose}/g_{sediment}$). This indicates that the effect of caging on EPS was due to the presence of a cage and not disturbance, since disturbance and cage treatments do not differ.

Macrofaunal species composition differed between controls and treatments only after six months, when there were significant differences between the disturbance control and cage control and between the caging treatment and cage control. The polychaetes *Notomastus latericeus* and *Orbinia angrapequensis* and the mysid *Gastrosaccus psammodytes* collectively accounted for 26.9% of the difference between the disturbance and cage controls and 24.9% of the difference between caged treatments and cage controls, being found at higher abundances in the cage controls. The difference between caged treatments and cage controls for macrofaunal species can be attributed to caging, since the disturbance control (undisturbed, caged) differed from the cage control (undisturbed, uncaged), but not from the caged treatment (disturbed, caged). After three months, functional group composition differed significantly between the disturbance control and cage control, and between the cage and cage control, again in a manner reflecting caging effects but no disturbance effects.

3.3.2 Effects of treatments

Effects on nutrient levels

After both three and six months, ammonia levels were significantly different among nutrient treatments (ANOVA, three months: $F_{2, 36} = 14.022$, $p < 0.001$, six months: $F_{2, 36} = 7.072$, $p < 0.01$), but marginally non-significantly or not significantly different among prawn density treatments at three and six months, respectively (three months: $F_{3, 36} = 2.443$, $p = 0.08$, six months: $F_{3, 36} = 0.640$, $p = 0.595$). There was also no significant interaction effect ($p > 0.05$). Tukey tests revealed that ammonia concentrations were significantly greater ($p < 0.01$) in the 250-g and 500-g nutrient treatment than background levels (Fig. 3.3a). However, there was no significant difference ($p > 0.05$) between 250-g and 500-g nutrient treatments.

Nitrate levels (Fig. 3.3b) were significantly different among nutrient treatments after three months (ANOVA, $F_{2, 36} = 12.122$, $p < 0.001$) and after six months (Kruskal-Wallis: $H_{(2, N=48)} = 19.509$, $p < 0.001$). No significant differences existed among prawn density treatments ($p > 0.05$) except for the fact that after six months, prawn densities had a significant effect in the highly-enriched (500-g) treatment (Kruskal-Wallis: $H_{(3, N=16)} = 7.996$, $p = 0.046$). There was no significant interaction effect ($p > 0.05$). At both time periods, nitrates were significantly greater in both enrichment treatments than at natural, background levels (Tukey tests, $p < 0.01$), but no differences existed between the two enrichment treatments ($p > 0.05$).

Nutrient treatment had no significant effect on phosphate levels after three months (ANOVA, $p > 0.05$), but after six months the effect was significant (Kruskal-Wallis: $H_{(2, N=48)} = 7.290$, $p = 0.026$), being higher in the two enrichment treatments than background levels (Fig. 3.3c). Prawn density treatments within individual nutrient treatments had no significant effect after either three months (ANOVA, $F_{3, 36} = 0.550$, df = 3, $p > 0.05$) or six months (Kruskal-Wallis tests, N: $H_{(3, N=16)} = 2.391$, $p = 0.495$; 2N: $H_{(3, N=16)} = 0.249$, $p = 0.969$; 4N: $H_{(3, N=16)} = 0.403$, $p = 0.940$).

The ratio of N:P in the pore water ranged from 0.94 to 4.87 after three months, and from 1.45 to 3.64 after six months (Table 3.3).

Effects on EPS and microphytobenthos

After three months, microphytobenthic biomass (Fig. 3.4) was significantly different among nutrient treatments (ANOVA, $F_{2, 132} = 11.944$, $p < 0.001$), but not among prawn density treatments ($F_{3, 132} = 1.216$, $p = 0.306$), and there was no significant interaction effect ($F_{6, 132} = 0.738$, $p = 0.620$). The biomass was significantly greater in the 500-g nutrient enrichment treatment than at natural background levels (Tukey test, $p < 0.001$) or in the 250-g treatment (Tukey test, $p = 0.005$). No significant difference was found between the background and 250 g nutrient treatments ($p = 0.204$).

After six months, microphytobenthic biomass did not differ significantly among nutrient treatments (ANOVA, $F_{2, 132} = 1.961$, $p = 0.145$), or prawn density treatments ($F_{3, 132} = 0.958$, $p = 0.415$).

EPS levels (Fig. 3.5) failed to differ significantly among nutrient treatments (Kruskal-Wallis test: $H_{(2, N=144)} = 4.594$, $p = 0.101$) or among prawn density treatments after three months (Kruskal-Wallis tests: $p > 0.155$ for all nutrient levels), and the same was true after six months (ANOVA, nutrient levels: $F_{2, 132} = 0.229$, $p = 0.795$; prawn levels: $F_{3, 132} = 1.816$, $p = 0.147$).

Effects on macrofaunal community composition

Macrofaunal species composition differed significantly between plots at three months (PERMANOVA, Pseudo-F = 16.690, df = 1, $p < 0.001$), but not among prawn or nutrient treatments nested within plot ($p > 0.05$). At six months macrofaunal communities were still significantly different between plots (Pseudo-F = 5.20, df = 1, $p < 0.001$), and prawn density nested within plot had by then developed a significant effect (Pseudo-F = 2.125, df = 6, $p = 0.003$). The effect of nutrient treatments was once again non-significant ($p > 0.05$), and the interaction of prawn and nutrient treatments with plot had no significant effect at either stage ($p > 0.05$).

After three months SIMPER analysis showed that Plots A and B were 78.40% different. The three main species that differed between plots were the gastropod *Assiminea globulus*, the polychaete *Orbinia angrapequensis* and the amphipod *Urothoe grimaldii*, which respectively accounted for 48.15%, 5.85% and 5.22% of the difference. All three occurred at higher densities in Plot B than Plot A. By six months Plots A and B were 60.58% different. The top three species accounting for this difference remained the same, respectively accounting for 18.72%, 8.77% and 8.21% of the difference, and were again more abundant in Plot B.

Tighter clustering of samples reflected greater within-group similarity after three months than after six months (Fig. 3.6). After three months, macrofaunal communities were not significantly different among any treatments in Plot A ($p > 0.05$), but in Plot B they differed significantly among prawn density treatments (Table 3.4). PERMANOVA pair-wise tests revealed that a significant difference lay only between zero prawn and double prawn density treatments ($p = 0.031$, $t = 1.696$). After six months, prawn density effects on macrofaunal communities were significant within Plot A and marginally non-significant in Plot B (Table 3.4). Pair-wise tests showed significant differences in Plot A lay between zero prawn and normal prawn density ($p = 0.008$, $t = 1.985$) and a marginally non-significant difference between zero prawn and half prawn density treatments ($p = 0.055$, $t = 1.526$). For all plots and sampling periods nutrient treatments had no significant effect on macrofaunal communities (Table 3.4).

Significant differences between Plots A and B also existed for macrofaunal functional groups in both sampling periods (PERMANOVA, $p < 0.001$). SIMPER analysis showed that after three months the main functional groups contributing to differences between plots were surface grazers (dominated almost entirely by *Assiminea globulus*), which accounted for 74.47% of the difference and occurred at much higher densities in Plot B (Fig. 3.7). After six months the difference was still accounted for mainly by surface grazers, accounting for 39.79% of the difference and again being present at substantially higher densities in Plot B (Fig. 3.8).

Prawn density nested within plot had a significant effect on macrofaunal functional groups (PERMANOVA, three months: Pseudo-F = 2.261, df = 6, $p = 0.017$, six months: Pseudo-F = 2.834, df = 6, $p = 0.004$), but nutrient treatments had no significant effect ($p > 0.05$). The prawn effect was, however, significant only in Plot B after three months, but in both Plots A and B after six months (PERMANOVA, $p < 0.05$).

At both time periods, burrowing deposit feeders were an important element, and after three and six months differed among prawn densities in Plot A (three months: ANOVA $F_{3,\ 20} = 3.023$, $p = 0.05$, six months: Kruskal-Wallis $H_{(3,\ N=24)} = 12.258$, $p = 0.007$). Consistently, their lowest densities were associated with zero prawn densities, with peak values variously at double, normal or half densities (Figs 3.7, 3.8).

Surface grazers were also significantly affected by prawn densities, but only in Plot B where they were abundant (three months: ANOVA, $F_{3,\ 20} = 134.58$, $p < 0.0001$; six months: $F_{3,\ 20} = 3.725$, $p = 0.028$) At both time periods, they increased with prawn densities (Figs 3.7, 3.8). Surface deposit feeders were consistently scarce, and although they were significantly affected by prawn densities, this was evident only in Plot B (Kruskal-Wallis, three months: $H_{(3,\ N=24)} = 9.119$, $p = 0.028$, six months: $H_{(3,\ N=24)} = 13.549$, $p = 0.004$), and their numbers were so low that they followed no clear pattern in relation to prawn densities.

Diversity indices

At three months all diversity indices were significantly different between plots (nested ANOVA, $p < 0.05$, df = 1). At six months diversity indices for Pielou's evenness (J′), Shannon-Wiener diversity (H′) and total abundance (N) were significantly different between plots (nested ANOVA, $p < 0.05$, df = 1), while those for species richness (S) and Margalef's diversity (d′) were not (Mann-Whitney *U*-tests, $p > 0.05$).

Two-way ANOVAs or Kruskal-Wallis tests for the effects of prawn density and nutrient treatments on indices of diversity and abundance (Table 3.5) showed that neither nutrients nor their interaction with prawn density ever yielded significant effects. Significant prawn-density effects were frequent, especially after six months, and only the Shannon-Wiener index H′ failed to respond significantly to prawn density.

Total species richness (S; Fig. 3.9)) showed a hump-shaped or positive (logarithmic or power) relationship against prawn density in some cases, with highest values at either half or normal densities, although regressions of S against prawn density were significant ($p < 0.05$) only for Plot A at 500-g nutrient enrichment at three and six months and for plot B at 250-g nutrient enrichment at six months. After three months, Margalef's index (d′; Fig. 3.10) often displayed a domed or positive relationship as prawn densities rose, but by six months relationships were less obvious, being significant ($p < 0.05$) only for plot B at 250-g enrichment. Pielou's species evenness (J′; Fig. 3.11) appeared generally unaffected by density at three months, but significant ($p < 0.05$) negative (logarithmic) relationships with prawn density emerged for 250-g and 500-g enrichment treatments after six months. The Shannon-Wiener index (H′; Fig. 3.12) was generally not significantly affected by prawn density, barring two marginally non-significant ($p < 0.1$) peaked relationships at six months at 250-g and 500-g enrichments and one significant ($p < 0.05$) positive logarithmic relationship at three months for the 250-g enrichment treatments.

For total abundance (N; Fig.3.13), density had significant or marginally non-significant effects in the majority of cases. In plot B the relationships were positively logarithmic, while in Plot A abundance either increased with prawn density or produced a peaked relationship.

3.4 Discussion

3.4.1 Effects of caging

The effects of caging and associated disturbance on nutrient levels were minor: only one of the three nutrients (ammonia) was affected, and then at only one of the two sampling times. Consequently, I concluded that any direct effects of caging and disturbance on nutrients were negligible and could be disregarded.

The presence of a cage, but not disturbance, did have a clear negative effect on EPS levels at both three and six months, reducing EPS levels by about 33% to 34% in cages relative to uncaged field samples (cage controls). This may be due to shading. Spivak *et al.* (2009) found that caging can reduce light penetration by up to 66%, leading to a decrease in primary producer biomass. Shading is likely to have been much less severe in my study than that of Spivak *et al*. (2009), since no roof was used; however, some shading by the sides of the cages was possible, and may have inhibited micro-autotrophs that contribute to EPS. A decline in microbes and their products could potentially affect consumers. However, there was no detectable affect of either caging or disturbance on the level of chlorophyll-*a* (microphytobenthic biomass).

Caging and associated disturbance had a significant effect on macrofaunal functional-group composition at three months but not six months, but by six months differences appeared in species composition. In both cases, the effects could be ascribed to caging, with disturbance having no significant effects. In terms of species composition, *Notomastus latericeus* and *Orbinia angrapequensis*, both burrowing deposit feeders, and *Gastrosaccus psammodytes*, a surface deposit feeder, accounted for much of the difference, being present at higher abundances in the uncaged areas. Thus caging experiments may underestimate the importance of deposit feeders, especially burrowing ones, and overestimate the importance of grazers or predators in macrofaunal communities.

Caging effects on EPS and community composition were thus demonstrable, and need to be taken into account in interpreting the results; but as their influence would have applied uniformly across all treatments, they do not nullify the effects of prawn density and nutrient manipulations.

3.4.2 Effects of treatments

Effects on nutrient levels

Nutrient enrichment treatments significantly enhanced ammonia and nitrate at three and six months and orthophosphate at six months above ambient levels, although the two enrichment treatments (250 g vs. 500 g fertilizer) did not produce significantly different nutrient levels. Worm *et al.* (2000) found that controlled release fertilizers can enrich sediment phosphate levels by up to 150% and ammonia levels up to 50% without these differences necessarily being statistically significant. Canning-Clode *et al.* (2008) found that experimental addition of nutrients led to variable nutrient enrichment over the course of their experimental period, and attributed this to variability in water movement. It may be that the variability of enrichment I observed for the two levels of nutrient supply, and therefore the lack of significant difference between them, were due to the small-scale heterogeneity of the soft-sediment environment, which caused pooling of water in some areas but not others, and probably slight differences in water movement. Such small-scale topographic effects are likely to change over tidal-cycle time scale. Canning-Clode *et al.* (2008) have argued that doubling the quantity of Plantacote® Plus fertilizer should result in a 100% increase in nutrient concentration. I therefore considered that although differences in observed pore-water values caused by heterogeneity of water-pooling and sand-level might have blurred differences between 250-g and 500-g enrichments, it was likely that nutrient enrichment treatments did raise nutrient levels in proportion to their weight. In my analyses of nutrient enrichment, I therefore assumed three levels of enrichment: zero, 250-g and 500-g.

Prawn density had no statistically significant effect on nutrient levels, with one exception: nitrates in the 500-g enrichment at six months. In view of the fact that this was one isolated case out of 18, I concluded that *C. kraussi* had no effect on nutrient levels. Other studies have shown that callianassids, among other bioturbators, increase flushing and permeability in burrows (Bird *et al.*, 2000), and increase ammonium and phosphate flux, or their concentrations in burrow waters or the overlying water column (Waslenchuk *et al*., 1983; Murphy and Kremer, 1992; Hughes *et al*., 2000; Biles *et al.*, 2002). Increasing densities of the bioturbator *Corophium volutator* led to increased ammonium flux rates (Pelegrí and Blackburn, 1994). Biles *et al.* (2002) also suggested that the increase in eroded sediment in the water column caused by callianassid bioturbation might enhance microbial activity, thereby increasing nutrient cycling. However, my results indicate that *C. kraussi* did not influence nutrient levels. This may be because background nutrient levels in the system are high, fuelled by marine inputs from the nutrient-rich Benguela (Shannon, 1985), so that nutrient inputs by *C. kraussi* may be trivial in comparison.

Effects on microflora

Microphytobenthos showed some response to nutrient enrichment after three months, as expected. This is likely due to nutrient enrichment raising primary productivity levels by the alleviation of limiting nutrients. Canning-Clode *et al.* (2008) also found that addition of nutrients promoted microalgal communities on rocky shores. In the present case, it is likely that nitrogen rather than phosphorus is the liming factor, as the ratios of N:P recorded in the porewater at all levels of enrichment fell well short of the value of 16:1 proposed as the 'Redfield ratio', above which phosphorus is likely to be limiting, and below which nitrogen is limiting. This is in line with the findings of Christie (1981), who argued that phytoplankton in Langebaan Lagoon is likely to be nitrate-limited. In relation to the Redfield ratio, substantial reservations have been raised about accepting a value of 16:1 as a cut-off point (Geider and La Roche, 2002), but the range of ratios I recorded (0.94-4.15) were all sufficiently far below this value as to make it unlikely the phosphorus would have been limiting. As both nutrients were added in our enrichment treatments, it is, however, not possible to comment on which of the two may have enhanced microphytobenthic biomass.

Although there was no statistically significant effect of nutrient enrichment treatment on microphytobenthic biomass after six months, the pattern was the same as after three months, with higher chlorophyll-*a* values in the enriched treatments. It is possible that after six months some factor other than nutrients was the limiting variable for microalgal growth. O'Brien *et al.* (2009) recorded similar findings for a sandflat in the Wadden Sea and attributed limitation of algal growth to factors such as light availability, sediment characteristics, emersion period or water speed. I speculate that as the 6-month sample was taken during winter, temperature or light may have been a limiting factor. Indeed, as discussed in Chapter 2, lower primary productivity has been reported during winter in Langebaan (Christie, 1981).

Prawn density treatments had no effect on microbenthic algal levels. This is in contrast to other studies on *C. kraussi*, indicating that it has significant negative effects on surface microalgae (Wynberg and Branch, 1994; Pillay *et al*. 2007b). However, it is in line with a study by Branch and Pringle (1987) in Langebaan Lagoon, which also failed to detect any effects of *C. kraussi* on microalgal levels. Branch and Pringle (1987) suggested small cage size as a reason for the lack of response to manipulations of sandprawn densities. However, I used larger cages, and employed controls that revealed no effect of either caging or associated disturbance on microphytobenthic biomass levels, as discussed earlier (p. 49: Effects of Caging). This poses an interesting question as to why microalgal levels respond to *C. kraussi* treatments in Durban Bay (Pillay *et al.* 2007a), but not in Langebaan Lagoon.

There were also no obvious patterns in EPS levels among nutrient or prawn density treatments on either sampling occasion. Thus, it appears that nutrient enrichment did not enhance bacterial growth. Contrary to expectations and findings in other studies (Branch and Pringle, 1987; Pillay *et al*., 2007b), increases in sandprawns did not affect surface bacterial abundance. It is possible that the discrepancy between my results and those of Branch and Pringle (1987), which were performed in the same habitat, may be due to the employment of different methods, as they used a direct bacterial count while I used measures of EPS as a proxy for bacteria. Additionally, the fact that caging effects on EPS existed in my study may have muted any expected influence of prawn density on EPS.

Effects on macrofaunal community composition

(a) Differences between plots

Between-plot differences were obvious and may have masked the effects of prawn and nutrient treatments. Three species were major contributors to inter-plot differences. Of these, the grazing gastropod *Assiminea globulus* was found at particularly high densities in Plot B, especially after three months. The other two species, *Orbinia angrapequensis* and *Urothoe grimaldii*, are both burrowing deposit feeders (Branch *et al*. 2010), and they also occurred in higher densities in Plot B. *A. globulus* is usually found in the high-shore above sandflats dominated by *C. kraussi* (Angel *et al.*, 2006; Pillay *et al*., 2009). However, in Plot B it occurred at very high densities, frequently above the average of 200 m^{-2} reported for Langebaan Lagoon (Pillay *et al*., 2009), with as many as a few hundred being found in a single core in some cages. This may have had important consequences for the community associated with Plot B, since *A. globulus* decreases benthic microalgal abundance, promotes bacterial abundance and alters meiofaunal community structure (Pillay *et al*., 2009).

It is likely that the major differences in community composition between plots were due to the 32-cm difference in tidal height between plots A and B, which would have produced differences in the duration of submergence and exposure of the plots. O'Brien *et al*. (2009) found that tidal height and sediment type had important modifying effects on how nutrient enrichment affects benthic community structure.

The large numbers of *A. globulus* at Plot B may reflect a caging artefact. Indeed, higher abundances of grazers were found in cages than in the surrounding sandlflats (cage controls), as discussed earlier. Caging can lead to increased densities of infauna within and adjacent to cages, and may be species-specific (Virnstein, 1978; Hulberg and Oliver, 1980; Steele, 1996). Caging can alter physical attributes of the environment such as current velocity and hydrodynamics, provide habitat heterogeneity or act as a refuge (Virnstein, 1978; Hulberg and Oliver, 1980), and may have caused *A. globulus* to settle in an area typically below its zone of occurrence. Pillay *et al.* (2007c) found that experimental field cages had atypical effects on the gastropod *Nassarius kraussianus*, as it fed on algae growing on experimental field cages. If cages did influence the abundance of *A globulus*, they could only have done so in conjunction with shore height: Plot A, which was lower on the shore, had much lower densities of *A. globulus*.

The overall divergence of within-community similarity at the macrofaunal species level between Plots A and B over time (Fig. 3.6) could reflect successional changes. Initially, all cages would have been populated by macrofauna that are good colonizers and responded quickly after the disturbance of cage installation, while other species gradually colonized over time, leading to divergence. Wootton *et al.* (2009) consider that following disturbances, marine benthic communities may be shaped by events such as unpredictable recruitment from the plankton, which can cause a degree of stochasticity in succession. Thus, although communities in different treatments were relatively similar initially, they would not necessarily have changed in the same direction if different species colonized them.

At a functional group level, macrofaunal communities also differed between Plots A and B. For the most part this reflects results at the species level. The surface grazer that accounted for the major difference in functional groups between plots was *Assiminea globulus*, which occurred at higher densities in Plot B for potential reasons described above.

(b) Nutrient effects

Nutrients had no effects on macrofaunal communities at the species level, or any interactive effects with prawn density treatments, so they did not modify the pattern produced by prawn density treatments. This likely reflects the fact that nutrient enrichment treatments had no significant effects on bacteria at any time or on microalgae at six months. Usually nutrients affect macrofaunal communities by enhancing food sources for consumers, so an absence of enhancement of primary production (at least at six months) and for bacterial EPS (at all times) may nullify any effects of nutrients on the species composition. Posey *et al.* (2002, 2006) discuss the fact that nutrient enrichment has varying effects on macrofaunal community structure, influencing it only in some cases, usually in instances where macrofauna are food-limited.

Nutrient treatments, nested within plot, had no effect on macrofaunal communities at the functional group level either. Possibly nutrients do not influence the abundance of macrofauna in Langebaan Lagoon because ambient levels are high. Conceivably, nutrient enrichment would have greater effects in systems with lower ambient concentrations.

In an enrichment experiment involving two systems, Posey *et al.* (2006) found that macrofauna responded to enrichment in one of them, which had low nutrient concentrations, but not in the other, which was a high-nutrient system. Langebaan may be just such a high-nutrient case, given that its waters are derived from the nutrient-rich Benguela Upwelling Ecosystem (Shannon, 1985). O'Brien *et al.* (2009) found that tidal height and sedimentary characteristics, along with bioturbation, were important factors in modifying the effects of nutrient enrichment on benthic communities. Thus, some caution needs to be applied when generalising the importance and effect of any single factor across systems. Pfaff *et al.* (2010) also state concern over generalising relationships between productivity and disturbance, and in support of their view they note the diverse results obtained in a number of similar studies (Jara *et al*., 2006, Svensson *et al.*, 2007, Canning-Clode *et al*., 2008, Sugden *et al*., 2008, Valdivia *et al*., 2008).

Further studies on *C. kraussi* bioturbation interacting with nutrient enrichment (and/or other environmental variables) in different systems, such as on the oligotrophic east coast of South Africa, may shed light on whether environmental factors such as nutrient enrichment or disturbance differ in their importance and effects in different soft-sediment systems.

(c) Effects of prawn densities

Prawn density had important effects on the macrofaunal communities in both plots in at least one of the sampling periods. Influences of *C. kraussi* on macrofaunal communities have previously been observed in both observational and experimental studies (Wynberg and Branch, 1994; Siebert and Branch, 2005b, 2007; Pillay *et al*., 2007a,b,c, 2008). In my experiment, significant differences in communities were mainly between the prawn exclusion treatments (zero prawns) and the prawn inclusion treatments.

Prawn density also had significant effects on functional groups in both plots. Thus, the effect of varying bioturbation intensity by *C. kraussi* appeared to operate on macrofaunal communities at the functional group level regardless of differences in species composition between plots. It is likely that this effect of *C. kraussi* also holds for other areas and conclusions can be generalised, since studies in totally different parts of the coastline have produced similar results (Pillay *et al*., 2007a,b,c, 2008). This supports the view that functional groups allow comparison of taxonomically unrelated organisms that share common ecological properties (Steneck and Watling, 1982; Steneck and Dethier, 1994; Bustamante and Branch, 1996; and see Chapter 2).

In both plots, burrowing deposit feeders reached their lowest densities in the absence of *C. kraussi* and either increased with sandprawn densities or peaked at 'normal' densities. This outcome conforms to previous studies showing that *C. kraussi* has positive effects on burrowing infauna (Siebert and Branch, 2005b, 2007). Pillay *et al.* (2007a) have proposed that destabilization of the sediment by *C. kraussi* facilitates burrowing and therefore promotes other species of burrowers. However, the lowest densities of all surface-dwelling groups were also associated with 'zero' density of sandprawns. This trend can be disregarded for most groups because their numbers were low and statistical differences among *C. kraussi* densities non-significant, but for surface grazers it cannot be ignored. In Plot A, they were too scarce to permit comment, but in Plot B, where their numbers were very high, they increased as the density of *C. kraussi* rose. This is in direct contrast to what would be expected (even given possible caging artefacts), as *C. kraussi* has previously been found to have negative effects on surface grazers due to sediment destabilization (Pillay *et al*., 2007a,b,c).

Possibly *C. kraussi* has a combination of promotive and negative effects on surface feeders in Langebaan. The negative effects are likely an increase in sediment turnover with increasing density of *C. kraussi*, since sediment destabilization is thought to negatively affect microalgae on which the surface fauna feed, as well as clogging their filtration mechanisms or burying them directly (Pillay *et al.*, 2007a,b,c). The promotive effect may be linked to the enhancement of bacteria by *C. kraussi* in Langebaan and, potentially, an increase in microalgal concentrations below the sediment surface (Branch and Pringle, 1987). However, empirical validation of this speculation is needed.

The differences between the negative effects of *C. kraussi* on surface grazers at Durban (Pillay *et al.*, 2007a,b,c) and its positive effects on them at Langebaan (present data) may, however, simply reflect the fact that different species of grazers are involved: *Nassarius kraussianus* and *Assiminea globulus* respectively.

Overall, the most striking result was that burrowing deposit feeders were consistently abundant and consistently promoted by sandprawns, conforming to my hypothesis that burrowing infauna will be favoured by *C. kraussi*.

Effects on macrofaunal diversity and abundance

Diversity generally differed between plots. This suggests that there were variables external to the manipulated factors of sandprawn density and nutrient enrichment that were affecting diversity, the most likely of which is the 32-cm difference in shore height between the two plots.

Nutrients never had any significant effect on either the various macrofaunal diversity indices or on total abundance. The apparent lack of importance of nutrients may reflect the fact that enrichment had no effect on microalgae (at six months) or EPS (at either time period), but more likely it distils to the fact that the system is nutrient-rich and consequently somewhat unreceptive to nutrient enrichment (Posey *et al*., 2006). In a review of 200 studies of relationships between diversity and productivity, Waide *et al.* (1999) reported, that in 32% of cases, non-significant relationships were recorded. Thus, it is not surprising that almost none of the diversity indices showed any significant relationship with nutrient enrichment.

However, prawn density treatment had significant (or marginally non-significant) effects on the majority of diversity indices, as was expected from other studies on *C. kraussi* (Pillay *et al*., 2007a,b, 2008). Interestingly, though, and contrary to expectations and the findings of most other studies, increasing *C. kraussi* density did not appear to have a negative effect on most of the indices of diversity, abundance, richness or evenness. Rather, in most cases, the indices significantly or qualitatively increased as densities of *C. kraussi* rose, or they reached some peak at intermediate densities of *C. kraussi*. This reinforces the idea that in Langebaan *C. kraussi* has some promotive effects on diversity, evenness and richness (Siebert and Branch, 2005b, 2007).

In some cases, significant positive logarithmic or unimodal humped relationships of diversity indices were found in relation to sandprawn density (and therefore intensity of bioturbation). The majority of these occurred in enriched treatments. Humped relationships are characteristic of the IDH, but Kondoh (2001) predicted that they would occur only at intermediate nutrient concentrations, and that under conditions of high nutrient concentrations, diversity would rise with disturbance. This suggests that although no statistically significant interactions between prawn density and nutrients were found, nutrients may have subtly modified the effects of prawns on macrofaunal diversity. Presumably these unimodal or positive patterns reflect an intermediate to high concentration of nutrients present in the Lagoon.

The scarcity of negative effects of *C. kraussi* density on diversity and abundance indices may reflect the fact that nutrient concentrations were never sufficiently low for a negative relationship between diversity and disturbance to materialise. The exception to this was the evenness index J′, which showed a negative logarithmic relationship for three cases of enrichment after six months. Langebaan Lagoon lies on an upwelling coast, as discussed in Chapter 2, and as such may not experience sufficiently low nutrient concentrations to allow a negative relationship between diversity and prawn density (bioturbation intensity) to develop at background concentrations. Rather, the high ambient concentrations of nutrients in combination with prawn densities may have produced the positive or domed relationships. Alternatively, the domed or positive relationships of prawn-density with diversity may reflect the balance between promotive and inhibitory effects as prawn density rises.

However, the difficulty with interpreting these patterns is that an objective measure of what can be classed as 'intermediate' or 'high' nutrient concentrations cannot be based on the resultant shape of the diversity-disturbance relationship without the argument quickly becoming circular.

Importantly, in 36 out of 60 possible comparisons, the density of *C. kraussi* had no significant effect on the five measures of diversity and abundance: an absence of any response was thus more frequent than significant responses of any type.

Conclusions

The two hypotheses I advanced in the Introduction centred around the effects of nutrient enrichment, and bioturbation by *C. kraussi* on macrofaunal communities. My hypotheses were partially validated, but there were interesting departures from them.

Firstly, *C. kraussi* bioturbation did have significant effects on macrofaunal communities in both Plots in at least one time period. Burrowing fauna were favoured, as hypothesized, increasing with densities of *C. kraussi* to a peak at 'normal' densities ($250m^{-2}$), likely due to sediment destabilization by *C. kraussi*. However, contrary to the second hypothesis and other studies (Pillay *et al*., 2007a,c) surface grazers were found at lowest densities in the absence of *C. kraussi* and increased with increasing prawn densities, suggesting a promotive effect of *C. kraussi*. This can be distilled to the promotive effect of *C. kraussi* on *Assiminea globulus*, since this species was the predominant grazer, especially in Plot B. This promotive effect might be linked to the positive effects of *C. kraussi* on sub-surface microalgae and bacteria previously observed in Langebaan (Branch and Pringle, 1987). This effect warrants further examination through laboratory studies.

Nutrients, unlike *C. kraussi*, had no significant effect on macrofaunal communities, as found by Posey *et al.* (2002, 2006) for systems that are nutrient-rich, and had no significant interactive effect modifying *C. kraussi* bioturbation. Presumably, the general lack of nutrient effects on microalgae and bacteria, a major food source for consumers, could translate to a negligible effect of nutrient enrichment on macrofaunal species composition, but the ultimate cause is likely to be that Langebaan is not nutrient-limited.

Secondly, my hypothesis about the effects of *C. kraussi* and nutrients on diversity was only partially validated. Nutrients had no effects on diversity. A lack of nutrient effect is not all that surprising and has been found in 32% of studies (Waide *et al.*, 1999). Conversely, prawn density often significantly affected diversity indices and abundance or had marginally non-significant effects, and produced positive or peaked relationships in several instances. These positive and peaked relationships correspond to those predicted by Kondoh (2001) for intermediate to high nutrient concentrations. Presumably, background concentrations in Langebaan were not sufficiently low to have produced the expected negative relationship of bioturbation on diversity at low nutrient concentrations. However, these relationships could also be the result of the balance between promotive and negative effects of *C. kraussi* changing as its density rises.

My results indicate that it is important not to generalize the effects and significance of any single factor across systems. While *C. kraussi* has negative effects on surface grazers such as *Nassarius kraussianus* in Durban Bay (Pillay *et al*., 2007a,c), it appears to promote another surface grazer, *Assiminea globulus*, in Langebaan Lagoon. Similarly, while nutrients have been shown to influence diversity or community composition in many studies, in my case they had no significant effects.

Finally, as pointed out in other studies, several factors, such as tidal height, sediment characteristics, bioturbation and nutrients may interact to determine macrofaunal diversity, abundance and community structure. Although no significant interactive effect of bioturbation and nutrients on macrofaunal diversity or community structure was demonstrable in my study, it appears that some other factor, most likely shore height, is modifying the relationship between *C. kraussi* bioturbation levels and macrofaunal diversity or abundance. It seems that in different systems and at different times the relative importance and effects of productivity, disturbance and other factors may be different.

CHAPTER 4

A LABORATORY EXPERIMENT ON THE EFFECTS OF TEMPERATURE AND NUTRIENT CONCENTRATIONS ON BIOTURBATION BY *CALLICHIRUS KRAUSSI*

4.1. Introduction

Nutrients are an important factor in determining productivity, diversity and community structure in coastal habitats, and have negative effects on some macrofaunal species at high concentrations (O'Brien *et al*., 2009). Thus, in any study examining the interactive effects of nutrients and bioturbation on communities, the question arises whether an increase in nutrient concentrations affects bioturbation rates by species such as *Callichirus kraussi*. Whereas a rich literature explores how bioturbators modify nutrient flux (Aller *et al*., 1983; Waslenchuk *et al.*, 1983; Kristensen, 1991; Murphy and Kremer, 1992; Biles *et al*., 2002; O'Brien *et al*., 2009) the reverse effect of nutrient concentrations on bioturbators has received little attention. Some conjecture on a connection between callianassid sediment turnover and nutrient concentrations was made by Suchanek *et al.* (1986), who suggested that low nutrient concentrations necessitated increased processing of sediment by callianassids, and Nickell *et al*. (1995) demonstrated that *Callianassa subterranea* produced smaller mounds (implying less sediment turnover) in nutrient-enriched sediments.

Since Chapters 2 and 3 both involve studies that occurred over different seasons, across which nutrients and temperature are likely to vary, the joint effects of temperature and nutrients on bioturbation by *C. kraussi* warrant investigation. Other studies have shown or suggested that bioturbation changes seasonally, being lowest in the winter months, highest in summer and extending to the greatest depth in autumn. These seasonal differences have in part been linked to temperature or nutrient effects on bioturbators (Posey, 1986; Rowden *et al.*, 1998; Berkenbusch and Rowden, 1999; Ouellette *et al.*, 2004; Teal *et al*., 2008). *Callianassa filholi* and *C. subterranea* have both been shown to have higher sediment expulsion rates at higher (summer) temperatures (Rowden *et al.*, 1998; Berkenbusch and Rowden, 1999). A relationship also exists between seawater temperature and expulsion activity of *Neotrypaea californiensis*, a temperate thalassinidean (Swinbanks and Luternauer, 1987). Changes in bioturbation intensity could have important consequences for the associated communities and physico-chemical characteristics of sediments, as pointed out in Chapter 1.

In Chapter 2, temperatures varied seasonally at both Durban Bay and Langebaan Lagoon. Globally, temperature is known to be an important variable influencing marine species and their distribution (Bhaud *et al*., 1995; Fritzsche and von Oertzen, 1995; Turpie *et al*., 2000; Pörtner, 2002). Temperature variations affect metabolism and other physiological processes, growth, reproduction, the duration of the planktonic larval phase, and the duration and rate of development of larvae (Neuhoff, 1979; Thessalou-Legaki, 1990; Bhaud *et al.,* 1995; Pörtner, 2002). At an organismal level, temperature alters movement, feeding and digestion rates, including ventilation and bioturbation rates by thalassinideans, which could lead to changes in bioturbation kinetics (Thessalou-Legaki, 1990; Hymel and Plante, 2000; Ouellette *et al.*, 2004; Stanzel and Finelli, 2004).

In this chapter I determine whether temperature and nutrient concentrations that correspond to winter and summer conditions influence bioturbation by the sandprawn *Callichirus kraussi*. Bioturbation kinetics can be measured in terms of biodiffusion and bioadvection. The biodiffusion coefficient (D_b) is a measure of the diffusive-like transport of sediment, including organic and inorganic material, which occurs in the surface layers through the activity of organisms, while the bioadvection coefficient (r), also referred to as biotransport or non-local transport, measures non-local mixing processes that occur below the surface and are connected to biologically-generated transport of particles from one point to another in a discontinuous way, e.g., the transport of sediment from the upper to lower layers (Boudreau, 1986a,b; Gerino *et al*., 1998; Francois *et al*., 2002; Meysman *et al*., 2003).

Two (opposing) hypotheses can be advanced for the effects of temperature on bioturbation rate and depth (and hence bioadvection and biodiffusion). The first is that bioturbation will increase with temperature until temperature begins to have detrimental effects. The alternate hypothesis is that as temperatures rise, microalgal (and bacterial) abundance will increase, potentially reducing feeding rate and hence diminishing bioadvection and biodiffusion coefficients at higher temperatures (Fig. 4.1). In relation to nutrient effects, I hypothesized that at any given temperature, increases in nutrients will result in a decrease in bioadvection and biodiffusion, because of feeding rate can be reduced with an increase in the availability of microalgal and bacterial food.

The overall approach I adopted in addressing these hypotheses was a laboratory experiment in which temperature and nutrients concentrations were manipulated, and the bioturbation rates of *C. kraussi* measured by the transport rate of luminophore particles introduced on the surface of the sediment.

4.2. Methods and materials

4.2.1. Aquaria

The experiment was carried out in the University of Cape Town research aquarium. Laboratory housings were created for *C. kraussi* using tubes of PVC piping (length 60 cm, diameter 10 cm) covered at the base with a PVC cap and filled to about 10 cm from the top with defaunated sediment collected from the intertidal zone at Oesterwal in Langebaan Lagoon, following procedures outlined by Ouellette *et al*. (2004). Defaunation was achieved by sieving of all sediment through a 2-mm sieve and hand-sorting. Four tubes were randomly installed into each of eight 220-L buckets that were filled with seawater and fitted with air-bubblers and thermostatically-controlled heaters set to the desired temperature (Fig. 4.2). One of the four tubes constituted a control that lacked *C. kraussi*, while the other three were replicate treatments each containing two individuals of *C. kraussi*, simulating the natural density of ~250 m^{-2} in the field at Langebaan (Siebert and Branch, 2005a; Nel, 2006). The buckets themselves were housed in an aquarium at ~13.6°C with a 12:12 dark-light cycle. Salinity was monitored with a salinometer and maintained at 35 psu through additions of fresh water to compensate for evaporation.

4.2.2. Effects of temperature

Water temperatures were monitored with data loggers and were maintained at 13.4 ±0.41SE, 18.7 ±0.01, 23.8 ±1.09 and 27.0 ±0.03°C in the four different buckets.

After the PVC tubes had been filled with sediment and the water and heaters installed, the tubes were allowed to stand for five days to settle the sediment and stabilise water temperatures. *C. kraussi* was then collected from Langebaan Lagoon and two prawns installed into each treatment tube. Tubes were then covered with a 5-mm mesh to prevent prawns escaping. Prawns that died or did not dig were replaced over a period of a week. From the day of the last prawn installation, a week was allowed to pass before measurement started; this allowed sufficient time for prawns to acclimate to laboratory conditions and complete burrow construction before measurement of bioturbation rate started. Seven grams of pink Partrac Ltd luminophores (125 - 350μm) were soaked in a small quantity of seawater and then evenly applied above the sediment surface of each PVC tube (Fig. 4.2 inset). The experiment was then left to run undisturbed for 28 days after which the cores were removed from the water and sectioned to determine the distribution of luminophores (see below).

4.2.3. Effects of nutrients

To assess the effects of nutrients, a similar experimental set-up was established, except that nutrient concentrations were manipulated under two temperature regimes – aquarium temperature (±13.4°C) as a simulation of winter, and 23.8°C as a simulation of summer. At these temperatures the seawater in each tube was either left untreated (0N), or treated with 250 g Plantacote Plus 8M fertilizer (1N) or 500 g of the fertilizer (2N) in a single nylon bag attached inside the 220-L bucket, to achieve three levels of enrichment. As for the temperature experiment, three tubes contained two prawns each and a control tube contained no prawns. The experiment was again run for 28 days, after which nutrient concentrations in the water column were sent to CSIR (Council for Scientific and Industrial Research) for colorimetric analysis of ammonia, nitrate plus nitrite and orthophosphate, to test whether the treatments had achieved the desired enrichment.

4.2.4. Analysis

After each experiment was terminated, cores were removed from the water and sectioned into 0.5-cm layers for the upper 2 cm and then every 2 cm below this (2-48 cm). Each section was homogenized and weighed. The layers of each core were then subsampled into 24-well microplates and read on a microplate reader (Biotek Synergy Mx) at 598 nm after excitation at 539 nm, following the method of Lagauzere *et al.* (in press). Fluorescence values were then corrected for layer weight. The vertical distributions of luminophores were analysed in Matlab® (version R2007a) using a code developed by Gilbert *et al*. (2007), which is based on the gallery-diffuser model of Francois *et al*. (2002).

For each core, this model allows calculation of the biodiffusion coefficient (D_b – the diffusive-like mixing of sediment particles in the region of *C. kraussi* activity near the sediment surface) and biotransport or bioadvection coefficient (r – the transfer or biotransport of particles by bioturbation from the surface to lower regions of the sediment).

4.2.5. Statistical analysis

Kolmogorov-Smirnoff tests for normality and Levene's test for homogeneity of variance were applied for all data, and the data were transformed to meet these assumptions when necessary. To determine whether bioturbation by *C. kraussi* had a significant effect on bioturbation kinetics a t-test was run comparing control r and log D_b values with those of experimental treatments (i.e. cores containing *C. kraussi*). To determine whether temperature had an effect, a Kruskal-Wallis test was run among temperatures. A range of regression tests, including unimodal, linear, logarithmic and power functions were also run for r and D_b against temperature. Similarly, to determine the effects of nutrients on bioturbation kinetics, an ANOVA of nutrient treatment nested within temperature was run, with nutrient level and temperature as fixed factors, using untransformed values of D_b and square-root-transformed values of r, followed by a range of regressions of D_b and r against nutrient levels. All statistical tests were performed using Statistica 9.

4.3. Results

Nutrient enrichment was effectively achieved, with the 1N and 2N treatments being proportionally greater than the zero enrichment treatment (Fig. 4.3). However, nutrient concentrations were unexpectedly higher at 23.8°C than at 13.4°C, despite equal applications of nutrient supplementation at the two temperatures.

4.3.1. Profiles

In the control cores, almost all of the luminophores (~95%) remained restricted to the upper 0.5 cm and the rest were limited the upper 2 cm, indicating virtually no redistribution of sediments in the absence of *C. kraussi*. This pattern is illustrated in Fig. 4.4 for 23.8°C, but was virtually identical for all temperatures. In the sediment profiles of the treatment cores, the relative abundance of luminophores declined exponentially with depth, and extended to the bottom of each core (Figs 4.5 and 4.6).

Overall, there was an increasing relative abundance of luminophores at greater depths and an increased depth of luminophore penetration with increasing temperature (Fig. 4.5). At 13.4°C there was notably less redistribution of luminophores.

Nutrient enrichment initially had little effect and then a positive effect on luminophore penetration at high nutrient levels at the winter-like temperature of 13.4°C, and a negative effect on luminophore penetration at the summer-like temperature of 23.8°C (Fig. 4.6). At 13.4°C there was little difference between the no-nutrient and low nutrient treatments, the latter decreasing in luminophores slightly more rapidly with depth than the former. However, the high nutrient treatment yielded higher relative abundances of luminophores below 3 cm than did the other two treatments. Conversely, at 23.8°C the no-nutrient treatment shows a greater depth of luminophore penetration and a much greater relative abundance of luminophores below 3 cm than the 1N and 2N nutrient treatments (Fig. 4.6). The least penetration of luminophores occurred in the 2N high-nutrient treatment.

4.3.2. Bioturbation kinetics

Cores containing *C. kraussi* (at all temperatures combined) had significantly greater r and D_b values relative to controls (t-test for r: $t_{12} = 2.548$, $p = 0.026$; for log D_b: t12 =7.879, $p < 0.001$), indicating that greater turnover of sediment took place in the experimental cores that experienced bioturbation than in the control cores.

No significant differences among temperatures were recorded for D_b (Kruskal-Wallis test; $H_{(3, N=14)} = 5.656$, $p = 0.130$) or for r (Kruskal-Wallis test; $H_{(3, N=14)} = 2.757$, $p = 0.431$). No significant regression relationships were found that were biologically meaningful. There was a high range of variability between replicates, indicated by the high standard error in Fig. 4.7. Although non-significant, the apparent relationship for bioadvection was a unimodal, peaked one (Fig. 4.7), as it appeared to increase with temperature to a peak at 23.8°C and then decrease again by 27.0°C.

Although there was a marginally non-significant difference in r-values between 23.8°C and 13.4°C ($F_{1,12}$ = 4.35 p = 0.059), nutrients, nested within temperature, did not significantly affect bioturbation kinetics (Nested ANOVAs: p > 0.05). At 13.4°C nutrients were marginally non-significantly related to the bioadvection coefficient (r) in a unimodal relationship ($F_{1,6}$ = 4.5, p = 0.08; Fig. 4.8a). No significant relationships between nutrients and bioturbation kinetics were found at 23.8°C, but the highest mean value was recorded at intermediate (1N) nutrient enrichment, as was the case at 13.4°C, and the apparent, although non-significant, relationship at 23.8°C was an increase of D_b and r from 0N to 1N, followed by stabilization between 1N to 2N (Fig. 4.8b).

4.4. Discussion

The experimental design isolated the effects of temperature and nutrients, and their interactive effects, as all other factors were held constant among treatments. The negligible mixing observed in the control core profile and the significant difference between control and experimental cores confirmed that *C. kraussi* alone was responsible for any significant sediment mixing or bioturbation. The minor mixing in the control cores (less than 5% of luminophores to 2 cm only) was likely due to density differences between luminophores and sediment, which Ouellette *et al*., (2004) have proposed will cause some downward particle displacement. Ouellette *et al.* (2004) also suggested that meiofaunal communities can be responsible for the movement of luminophores near the sediment surface, since meiofauna destabilize sediment near the surface through the construction of microstructures (Nehring *et al*., 1990). However, control cores showed near-identical profiles across all temperatures and nutrient levels, so I concluded that meiofauna had little or no effect, since I would have expected some response in their activities to changes in temperature.

4.4.1. Effects of temperature

Temperature treatments fell approximately within the ambient range of seawater temperatures observed in Langebaan Lagoon in winter and summer (see Chapter 2). Increases in the depth and amount of luminophore redistribution in the sediment profiles (both indicators of increased bioturbation) occurred as temperature increased. The increases in bioturbation depth and the proportions of bioturbation at different depths were similar to results found by Ouellette *et al*. (2004) for *Nereis virens*, and correspond to findings of increased sediment turnover at higher temperatures that have been recorded in seasonal studies of other thalassinideans (Swinbanks and Luternauer, 1987; Rowden *et al*., 1998; Berkenbusch and Rowden, 1999; Stanzel and Finelli, 2004). This pattern is likely due to increased metabolic activity of *C. kraussi* at higher temperatures, together with associated increases in feeding and activity, resulting in greater movement of sediment and organic matter from the burrow surface to lower reaches of the burrow, and associated excavation in the burrow. This conforms to the first of the two alternative hypotheses I advanced: that bioturbation activity of *C. kraussi* would increase with temperature.

This effect of temperature on bioturbation profiles did not completely extend to bioturbation kinetics. There was no significant effect of temperature on either the biodiffusion (D_b) or bioadvection (r) coefficients, despite (1) an apparent increase in r from zero at 13.4°C to a peak of 4.1 $cm^2\ y^{-1}$ at 23.8°C, a pattern that conforms to the hypothesised elevation of *C. kraussi* activity with a rise in temperature, and (2) the higher values of bioadvection (r) recorded at 23.8°C compared with those at 13.4°C in the nutrient study. This suggests that the temperature study did not include sufficient replication to detect statistical differences because of the high variability in the data. It is also possible that the gallery-diffusor model, despite its apparent applicability to different bioturbation functional groups (Gilbert *et al.* 2007), may not be valid for the specific bioturbatory activity of *C. kraussi*, and may require refinement.

In any event, the results suggest that seasonal differences in temperature between winter and summer do affect at least the non-local, discontinuous bioadvection (r) of particles from upper to lower layers, manifested as an increase in the depth and relative concentration of surface particles that were shifted downwards, as evidenced by the profiles. Ouellette *et al.* (2004) found that summer temperatures produced higher bioadvection rates than winter temperatures for *Nereis virens*, and Rowden *et al.* (1998) and Berkenbusch and Rowden (1999) showed higher temperatures increased callianassid bioturbation rates until temperatures became detrimental, similar to my findings. However, Ouellette *et al.* (2004) also found that for *N. virens*, higher temperatures increased biodiffusion to a point and then decreased it, whereas for *C. kraussi* temperature appeared to have no significant effect on biodiffusive mixing.

4.4.2. Interactive effects of nutrients and temperature

My second hypothesis, that enrichment of nutrients would reduce bioturbation, was refuted. At 13.4°C, the sediment profiles indicated that high levels of enrichment enhanced bioturbation, but at 23.8°C, enrichment diminished bioturbation. Biodiffusion ($D_{b)}$ and bioadvection (r) were, however, not significantly affected by nutrient level. Thus, nutrient level did have some effect on bioturbation depth, but this effect was modulated in part by temperature. Their interactive effects are somewhat confounded, since higher temperatures were associated with higher nutrient concentrations in the enriched treatments, presumably caused by increasing diffusion of nutrients from controlled-release pellets into the water column. It is also possible that increases in *C. kraussi* metabolism and activity at higher temperatures resulted in an increase in nutrient concentrations, but this is unlikely, given that in un-enriched treatments nutrient concentrations remained low. Thus the effect of high temperature is a combination of the effects of increased temperature *per se*, and associated increased nutrient concentrations.

At a winter-like temperature of 13.4°C, tracer profiles suggested that high nutrient levels (2N) produced a greater transport of surface particles to depths below 3 cm than at low (0N) nutrient levels. Bar plots of bioadvection (r) show this increase as a peak in activity at intermediate nutrient levels (1N). This peak in bioadvection (r) was tentatively supported by a marginally non-significant unimodal relationship between nutrient levels and bioadvection (r). No relationship between biodiffusion at the surface and nutrients was, however, apparent.

At the summer-like temperature of 23.8°C, increasing nutrient levels reduced transport of particles to lower reaches of the core and decreased depth of bioturbation, as was evident in the sediment profiles. Bioadvection (r) values again appeared to peak slightly at the low enrichment (1N) treatment, stabilizing with only a slight decrease from 1N to 2N. Biodiffusion (D_b) values showed a similar trend. However, no significant relationships or effect of nutrient levels was found for biodiffusion (D_b) or bioadvection (r) coefficients. The lower amounts of transport at high nutrient levels that were apparent in the profiles at 23.8°C could possibly be due to negative effects of high concentrations of nutrients on *C. kraussi*. Indeed, Widdicombe and Austen (2001) observed the lowest abundances of most species of large macrofauna at their highest experimental levels of nutrients and suggested that different species experience negative effects at different nutrient concentrations.

Lastly, there was evident conflict between the conclusions derived from interpreting sediment profiles and those arising from bioadvection and biodiffusion coefficients, and this may be due to the gallery-diffuser model being insufficiently refined for *C. kraussi* bioturbation, which extends to considerably greater depths than is the case for other species so far explored with this model.

4.4.3. Conclusions

Higher temperatures were associated with high bioadvection (r) values and greater depth and amounts of penetration of particles from the sediment surface, and when the effects of nutrients were compared at 13.4°C and 23.8°C, higher r-values were recorded at the summer-like higher temperature. Similarly, higher bioturbation rates during summer (associated with higher temperatures) have been observed in other studies (Swinbanks and Luternauer, 1987; Rowden *et al*., 1998; Berkenbusch and Rowden, 1999; Ouellette *et al*., 2004; Teal *et al.*, 2008). This provides support for the first of my hypotheses, that higher temperatures will produce greater bioturbation, and hence higher bioadvection coefficients (r) and greater depth of bioturbation, at least below temperatures that become detrimental to *C. kraussi*. The alternate hypothesis, that bioturbation would decline at higher temperatures because microbial food sources might increase with temperature (and thus diminish feeding rate) was rejected.

Under a 'winter' temperature of 13.4°C, the peaked relationship of bioadvection relative to nutrients, and the greater depth of bioturbation evident in profiles as enrichment increased, refute the hypothesis that increasing nutrients will decrease bioturbation intensity. It appears that the hypothesis also did not fully hold for high temperatures: the biokinetic coefficients (D_b and r) were highest at intermediate to high nutrient levels, and profiles demonstrated that highest rates of sediment transfer took place at the lowest nutrient level. Perhaps when nutrients exceed certain concentrations, possibly in combination with high temperatures, they start to become detrimental to *C. kraussi*.

The discrepancy between sediment profiles and biokinetic coefficients indicates that the gallery-diffuser model may need refinement for use with *C. kraussi*, despite the fact that it is generally regarded as being applicable to different functional bioturbation groups (Gilbert *et al*., 2007). This refinement falls outside the scope of this thesis.

My findings suggest that nutrient enrichment of systems by human activity can have varied and complex effects on organisms, including bioturbators, which may be modified by other environmental parameters. Therefore, in addition to their direct effects on communities, nutrients may have complex secondary effects, through their influence on bioturbation. The potential increase of temperatures associated with global climate change could also alter bioturbation parameters and modify the effects of nutrients on bioturbators. For a species such as *C. kraussi*, which is known to be an ecosystem engineer (Siebert and Branch, 2005b), changes in its activities and ultimately bioturbation parameters could have important consequences for the communities that it helps to shape.

CHAPTER 5

GENERAL CONCLUSION – BIOTURBATION, NUTRIENT ENRICHMENT AND SOFT SEDIMENT COMMUNITY STRUCTURE: LESSONS LEARNED

Callichirus kraussi, an ecosystem engineer and powerful bioturbator that turns over as much as 12.14 $kg.m^{-2}$ of sediment per day (Branch and Pringle, 1987) is widely spread along the South African coastline (Forbes, 1973; Day 1981; Siebert and Branch, 2006, 2007; Branch *et al*., 2010), In my study, summarised in Fig. 5.1, I investigated how nutrient levels and bioturbation by *C. kraussi* interact to influence soft-sediment communities, with special emphasis on macrofauna. After an overview establishing the background and defining the scope of my thesis in Chapter 1, I initiated research in Chapter 2 with an observational and correlative investigation of differences in nutrients, microbiota, physico-chemical variables and macrofaunal composition between sites on the east and west coasts, with a focus on intertidal sandflats dominated by *C. kraussi*, to highlight any potential modification of expected patterns by *C. kraussi* and suggest their causes. I then tested interactions between bioturbation and nutrient concentrations in a field experiment in Langebaan Lagoon (Chapter 3) and a laboratory experiment (Chapter 4).

Shortcomings of the study

With hindsight, I recognized a number of short-comings in my study. In the field observations (Chapter 2) it would have been an improvement to have a longer and more complete time-series of data, so as to infer seasonal changes more fully. There were, however, time-constraints on the research programme, which were recognized at the onset of the study. It would also have been useful to measure the activity of *C. kraussi* in the field at Durban and Langebaan, and at different times of the year. There are, however, practical difficulties to doing this. Other authors have inferred activity from the rate of sediment transfer to the openings of sandprawn burrows (e.g., Berkenbusch and Rowden, 1999), and I did attempt to do the same, but was thwarted by storm-disruption of the sediment surface. I also attempted to use luminophores in the field to measure activity, but tidal washing dissipated them too rapidly for measurements of turnover. Additionally, had there been more marine-driven lagoons on the west and east coasts it would have been productive to examine these to be able to comment on biogeographic differences between such systems. However, no other marine – driven lagoons exist, apart from perhaps Knysna on the south coast.

In the field-based experimental study (Chapter 3) it would have been better to have used a single plot design with increased replication, to avoid the confounding effects of split-plot differences that emerged. Ideally, greater replication at two or more plots would have strengthened the design, since this would have improved both the chances of detecting statistical differences among treatments and allowed generalisation of the findings among sites. However, time- and resource-limitations made it impossible to include more replication at both plots. The most important limitation of the findings arising from the field experiment was that inter-plot differences were substantial. The two plots were originally selected as being equivalent, and it was only after the experiment was completed that the differences in tidal height became evident.

It would have been an improvement to have had more replication in the laboratory study of nutrient and temperature effects (Chapter 4), since *C. kraussi* displayed considerable variability in its bioturbatory activities. The gallery-diffuser model used in Chapter 4 probably needs refinement of the model parameters for an organism such as *C. kraussi* that digs to substantially greater depths than other species for which the model has been applied.

Despite these shortcomings, several clear conclusions emerged from the study, as outlined below.

Synopsis of findings

As discussed in Chapter 2, because Langebaan lies on the upwelling west coast, whereas Durban lies on the east coast where upwelling is limited, and because of biogeographic trends around the coast of southern Africa, I expected that nutrients, primary productivity and macrofaunal abundance would be higher in the intertidal sandflats of Langebaan Lagoon, while temperatures and macrofaunal diversity would be higher in Durban Bay. Since *C. kraussi* dominates these intertidal sandflats and is a powerful bioturbator with known effects on macrofaunal communities I also expected that at both sites macrofaunal communities would be dominated by burrowers, whereas surface grazers and suspension feeders would be less abundant.

Local effects within both lagoons contradicted patterns expected on the basis of the biogeography of the individual sites for many variables. Langebaan Lagoon was only cooler than Durban Bay in winter, and nutrient levels were rarely significantly greater in Langebaan than in Durban. Microphytobenthic biomass was never significantly different between sites. Macrofaunal abundance generally conformed to the expectation that it would be greater in Langebaan Lagoon than in Durban Bay, but macrofaunal diversity ran counter to expectations, being consistently higher in Langebaan. Localised effects such as pollution, human disturbance and solar heating of water in the lagoons were proposed to account for these divergences from expectations.

An emergent possibility was that factors such as macrofaunal diversity and microphytobenthic biomass might be influenced by seasonal changes in *C. kraussi* activity related to differences in temperature and nutrient concentrations.

Further motivation for this idea was the finding that bioturbation by *C. kraussi* is an important force structuring soft-sediment communities wherever it occurs, as evidenced by previous work and my findings. This was also implied by the relative constancy of the proportions of functional groups between sites and seasons, suggesting that communities dominated by *C. kraussi* are functionally similar, irrespective of site.

The observational study in Chapter 2 identified further research questions. (a) How does *C. kraussi* bioturbation influence soft-sediment macrofaunal communities in Langebaan Lagoon, given that it is a marine-driven lagoon on an upwelling-coast? b) Are *C. kraussi* activities and effects on macrofaunal communities modified by physico-chemical conditions, such as temperature (which might elevate its activities) and nutrients (which might increase microalgal standing stocks and offset the effects of *C. kraussi* on them). These ideas were subsequently explored empirically in two manipulative studies, the first field-based and the second laboratory-based (Chapters 3 and 4).

Both questions were explored in Chapter 3 through a field experiment manipulating nutrient concentrations and bioturbation intensity (*C. kraussi* densities) in Langebaan Lagoon. I expected to find from this experiment that bioturbation would act as a disturbance and be influenced by nutrient concentrations to produce negative, peaked and positive relationships with diversity at low, intermediate and high nutrient levels as hypothesised by Kondoh (2001). I also expected that densities of burrowers would increase while surface-dwelling grazers, filter feeders and deposit feeders would decrease with increasing *C. kraussi* densities, and that these effects would be muted by increased nutrient concentrations.

The anticipated interactive effects of nutrient levels with *C. kraussi* bioturbation on macrofaunal diversity were not realised, since nutrient additions never had any effects on surface bacteria, did not influence microalgae (at least at the final sampling period), and failed to affect macrofaunal diversity. This suggests that nutrients were not limiting in Langebaan, most likely as a consequence of tidal exchange supplying the system with nutrient-rich upwelled water. However, in many cases prawn density alone significantly affected diversity, irrespective of nutrient level and, interestingly, producing positive or peaked relationships with diversity indices in about a third of the cases (10 out of 36). Kondoh's (2001) model predicts that peaked and positive relationships between disturbance and diversity should emerge at respectively intermediate and high levels of nutrients. The general absence of any negative relationships between bioturbation level and indices of diversity may be a further indication that the system never has sufficiently low nutrient concentrations for negative relationships to emerge. Whether this is true or not cannot simply be inferred from the shape of the bioturbation-diversity curves without entering into a circular argument. Thus, other explanations for the shape of the curves, such as additional physico-chemical variables or a balance between promotive and inhibitory effects of *C. kraussi* on macrofauna could not be ruled out.

Bioturbation by *C. kraussi* had significant effects on macrofaunal communities. Burrowing fauna increased with densities of *C. kraussi* to a peak at some intermediate level of densities, as expected. However, contrary to expectations based on the patterns inferred from Chapter 2 and other studies (Pillay *et al*., 2007a,c), surface grazers were found at lowest densities in the absence of *C. kraussi* and increased as its densities rose. This further suggests a promotive effect of *C. kraussi*. This grazer effect can be distilled to an effect on *Assiminea globulus*, since that species was the predominant grazer, and might be linked to the positive effects of *C. kraussi* on sub-surface microalgae and bacteria previously observed in Langebaan (Branch and Pringle, 1987). It does, however, raise the possibility that the effects of *C. kraussi* on grazers may be species-specific, as earlier studies demonstrating that grazers are negatively influenced by *C. kraussi* were also based on a single species of grazer, the gastropod *Nassarius kraussianus* (Pillay *et al.*, 2007c).

In summary, this experiment showed that the effects or importance of any single factor should only be generalised with caution. In particular, many studies have shown nutrients to be an important factor modifying the disturbance-diversity relationship (Huston, 1979; Proulx and Mazumder, 1998; Kondoh, 2001; Worm *et al*., 2002; Jara *et al*., 2006; Valdivia *et al*., 2008), but nutrients were not important in Langebaan Lagoon, presumably because the system is naturally rich in nutrients. This warrants further investigation by implementation of a similar field experiment manipulating nutrients and *C. kraussi* density in a nutrient-limited system.

Nevertheless, my study did support previous work (Siebert and Branch, 2005a,b, 2006, 2007, Pillay *et al*., 2007a,b,c) indicating that *C. kraussi* is an important agent structuring soft-sediment communities wherever it occurs, so that this remains a robust generalised conclusion even if the precise mechanisms of the effects of *C. kraussi* may differ from place to place or among shore heights.

In Chapter 4, a laboratory experiment was carried out to test whether nutrients and temperature affect *C. kraussi* bioturbation rate, an idea conceptualised in Chapter 2. The laboratory environment allowed these two variables to be manipulated in isolation. It was expected that increasing temperature would increase the transport of sediments by *C. kraussi*, as evidenced by sediment profiles and measures of bioadvection (r) and biodiffusion (D_b), but that increasing nutrients would decrease r and D_b. Temperature did affect *C. kraussi* bioturbation in the predicted manner. This is in agreement with other studies that have found seasonal or temperature effects on bioturbation rate for a variety of bioturbators (Swinbanks and Luternauer, 1987; Rowden *et al*., 1998; Berkenbusch and Rowden, 1999; Ouellette *et al*., 2004; Teal *et al.,* 2008). To the best of my knowledge, my study is the first study to quantify the effect of nutrients on bioturbation. The results were ambivalent because neither D_b nor r responded significantly to nutrient levels, and examination of the sediment profiles revealed different patterns at the two temperatures examined: at the summer-like temperature of 23.8°C, bioturbation was greatest in the absence of nutrient enrichment, whereas at 13.7°C it was greatest at high levels of enrichment. This points to two things: first, that nutrient effects may be temperature-dependent; second, that there were obvious conflicts between the interpretations based on the sediment profiles versus the values of D_b and r derived from the gallery-diffuser model of Francois *et al*. (2002). The latter may need refinement to capture the dynamics of a deep-burrowing species such as *C. kraussi*.

Temperature increased *C. kraussi* bioadvection (r) and therefore, the depth of penetration of particles from the sediment surface, probably because of its direct effects on metabolism and activity, but possibly also indirectly through increased food availability (bacterial or microalgal growth) and associated feeding rate at higher temperatures. This effect will obviously only operate below temperatures that are detrimental to *C. kraussi*.

These findings suggest that biogeographic differences in soft-sediment communities may be moderated or superseded by the activities of *C. kraussi*, which will affect diversity and community structure. As temperature and, to a lesser extent, nutrient concentrations both influence bioturbation, alteration of these factors associated with global change and human activities may have complex secondary effects, in addition to direct effects on benthic communities, via their influence on bioturbation rates of *C. kraussi,* a known ecosystem engineer (Siebert and Branch, 2005b).

In conclusion, *C. kraussi* bioturbation has been upheld as an important determinant of macrofaunal communities and diversity through both natural observations in Langebaan and Durban and experiments in Langebaan. Although it has widely been reported to have negative effects on a range of functional groups (Siebert and Branch, 2005b, 2007; Pillay *et al.*, 2007a,b,c; Pillay and Branch, 2011), it does have some promotive effects on macrofauna by, for example, facilitating burrowers because of its destabilization of the sediment. As a result, increases in its density were never negatively associated with richness or diversity, as measured by species richness, Margalef's index and the Shannon-Wiener index. In the majority of instances (72.2%; n = 36) no significant relationship existed, but in the remaining 27.8% of cases explored, the relationship was either positive or domed. Pielou's index of evenness did decline with prawn density, but only in 25% of cases, with the balance of the cases exhibiting no significant relationship. Total abundance responded more clearly, with either a positive or domed relationship in the majority of cases (66.7%). Temperature and nutrients were confirmed in the laboratory to influence the turnover of sediment by *C. kraussi* bioturbation, although the effects of nutrients were temperature dependent.

The idea that global environmental change and human-induced nutrient enrichment of systems could influence *C. kraussi* bioturbation rate through elevation of temperature and nutrient concentrations is significant, given the importance of bioturbation, which powerfully alters sediment characteristics and the abundance, recruitment, diversity and structure of macrofaunal and meiofaunal communities, in addition to effects on microalgal and bacterial growth (Branch and Pringle, 1987; Posey *et al*., 1991; Tamaki, 1994; Mermillod-Blondin, 2004; Pillay, 2006; Volkenborn and Reise, 2006; Pillay *et al*., 2007a,b,c, 2008; Van Nes *et al.* 2007; Kuhnert, 2010). It can also alter ecosystem function by producing and stimulating the release of nutrients from the sediment (Ieno *et al*., 2006). The importance of individual species of bioturbators in influencing biogeochemical cycles and productivity was highlighted by Lohrer *et al.* (2004). To complicate matters, Solan *et al.* (2008) indicate that biodiversity of the benthic community itself has implications for the ecosystem-level effects of bioturbation, such as nutrient flux. They argue that different bioturbators provide different functions: some may be more important for nutrient generation and others for sediment turnover – and that different activities, e.g. feeding versus burrow irrigation, will have different consequences for the environment. My study indicates that *C. kraussi* potentially has both negative and promotive effects on different elements of the community. Thus, changes in the physical environment, or the loss of a bioturbator like *C. kraussi*, may have profound and complex secondary effects on the ecosystem.

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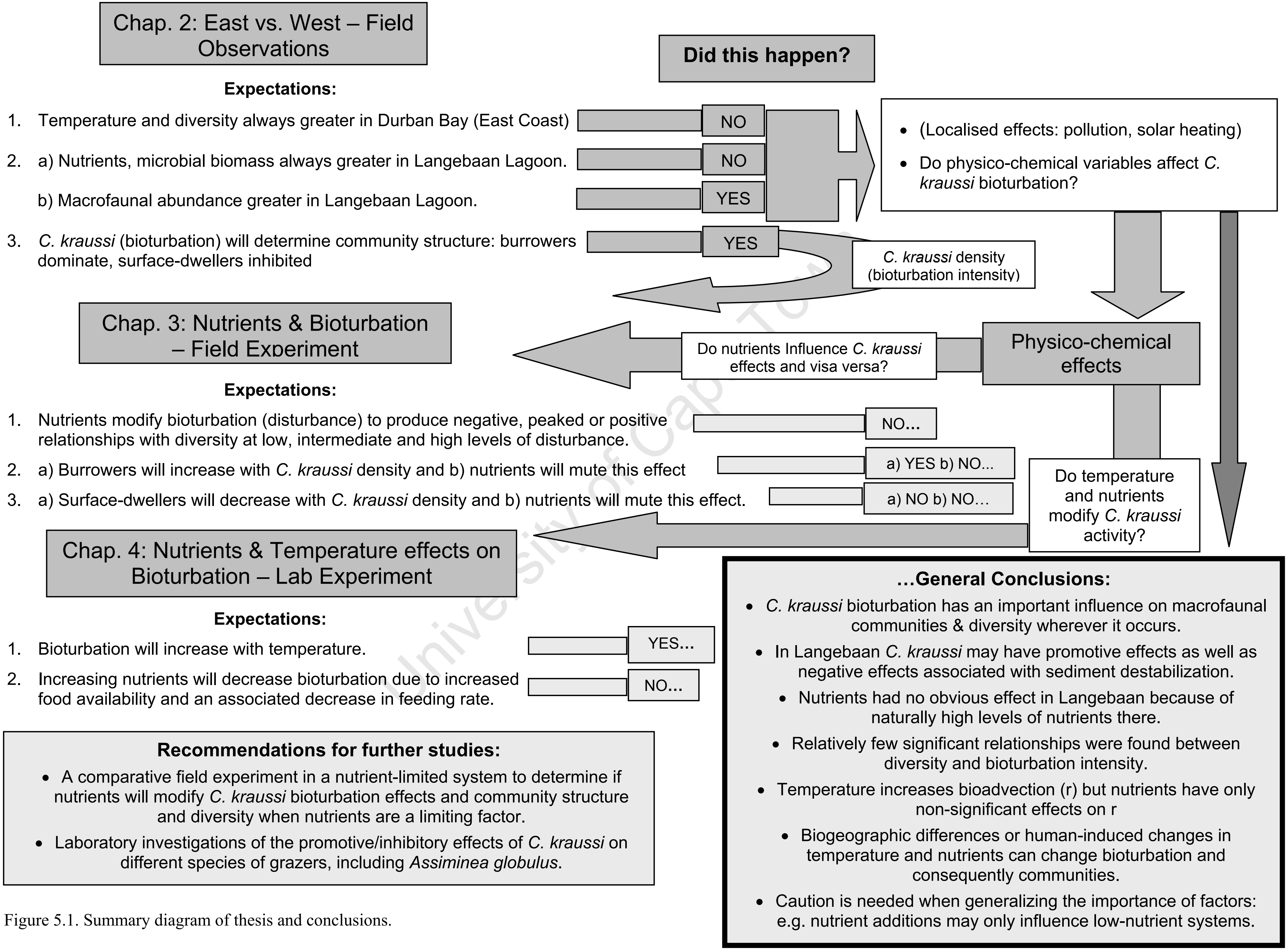


Figure 5.1. Summary diagram of thesis and conclusions.

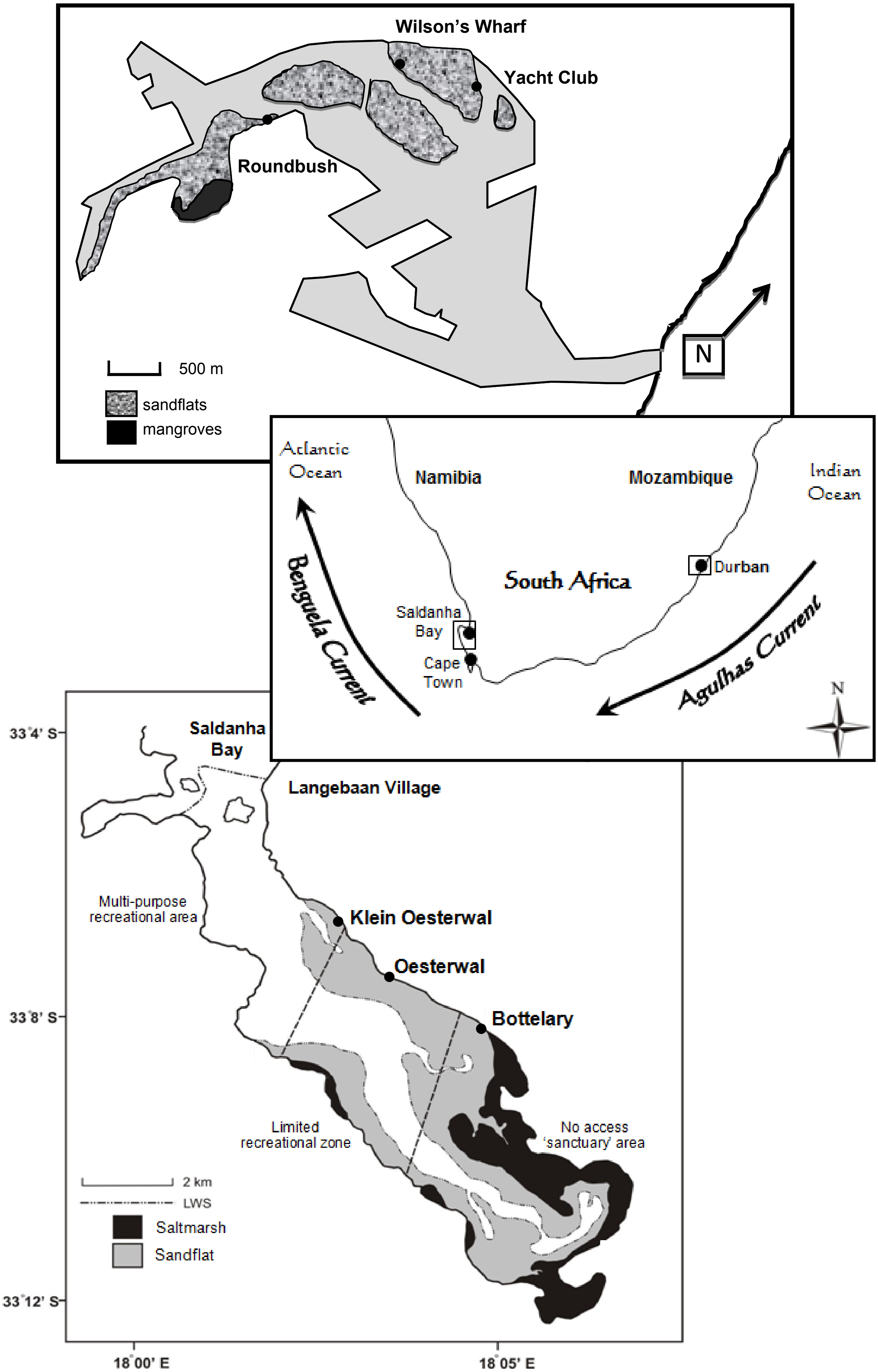


Figure 2.1. Site map showing Durban Bay (top), adapted from Pillay *et al.* (2008), and Langebaan Lagoon (bottom) with subsites and their locations on the South African coastline (inset - right).

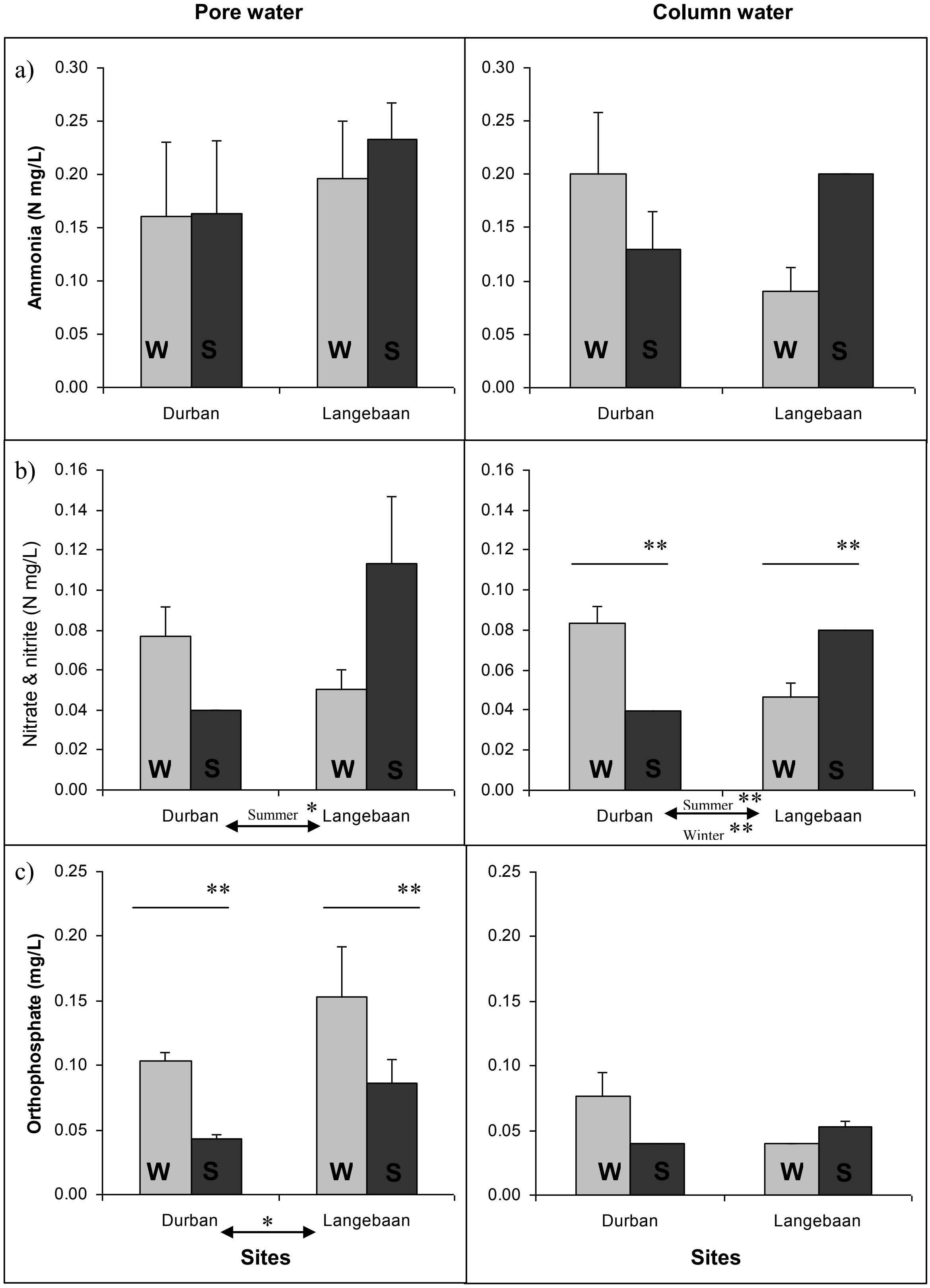


Figure 2.2. Mean (+1SE) pore and column water nutrient (ammonia, nitrate plus nitrite, and orthophosphate) levels taken from Durban Bay and Langebaan Lagoon in winter 2009 (W) and summer 2010 (S). Statistical seasonal differences are indicated by arrows with * ($p < 0.10$) or ** ($p < 0.05$). Bars with ** show significant differences between seasons within a site.

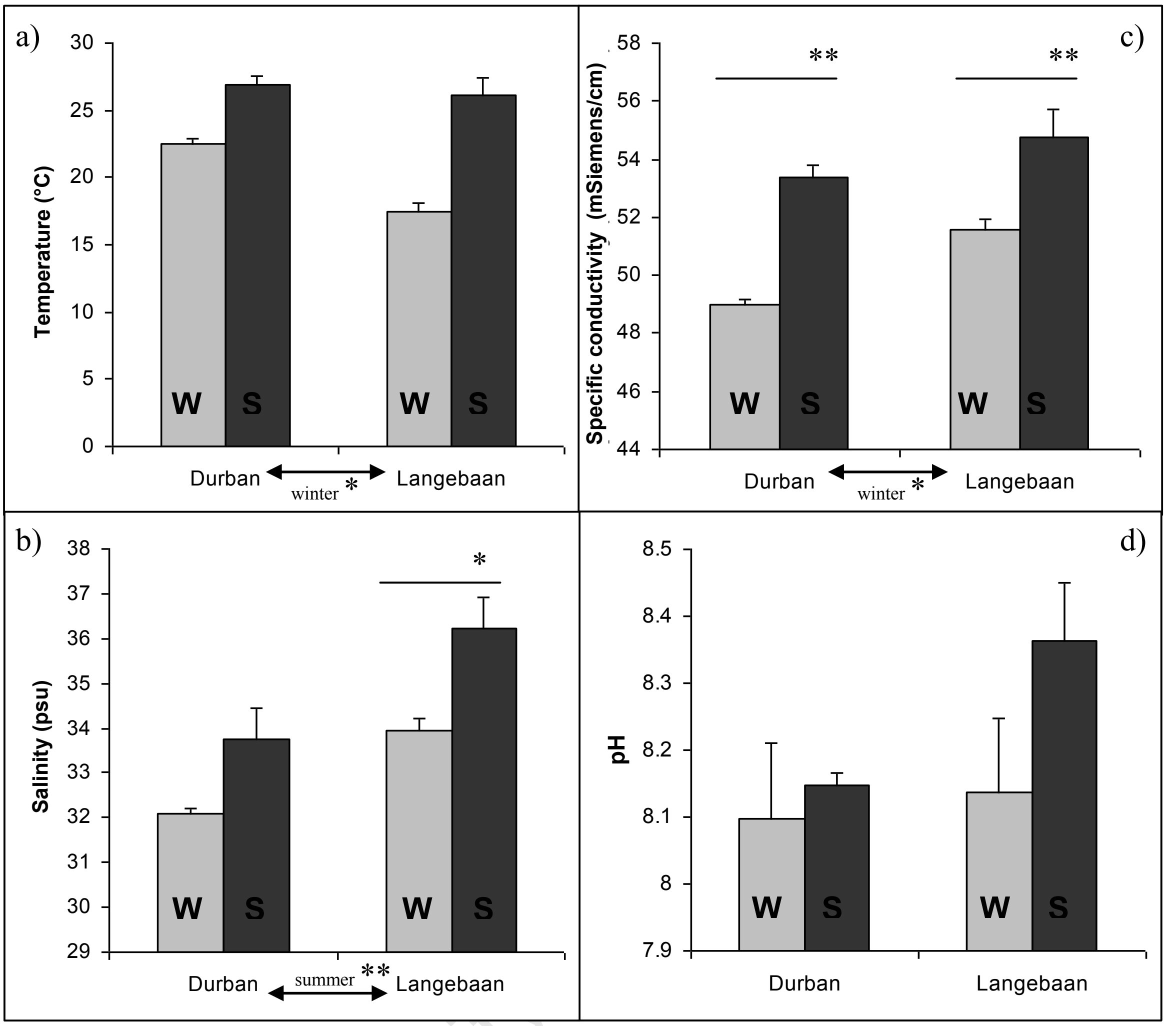


Figure 2.3. Average physico-chemical values (+1SE) in Durban Bay and Langebaan Lagoon in winter 2009 (W) and summer 2010 (S). Statistical seasonal differences are indicated by an arrow with * ($p < 0.10$) or ** ($p < 0.05$). Significant differences between seasons within a site are indicated by a bar and ** (Tukey test, $p < 0.05$) or * ($p < 0.10$).

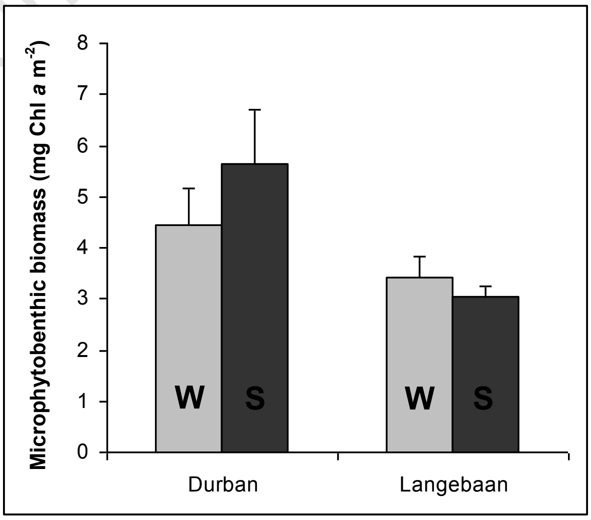
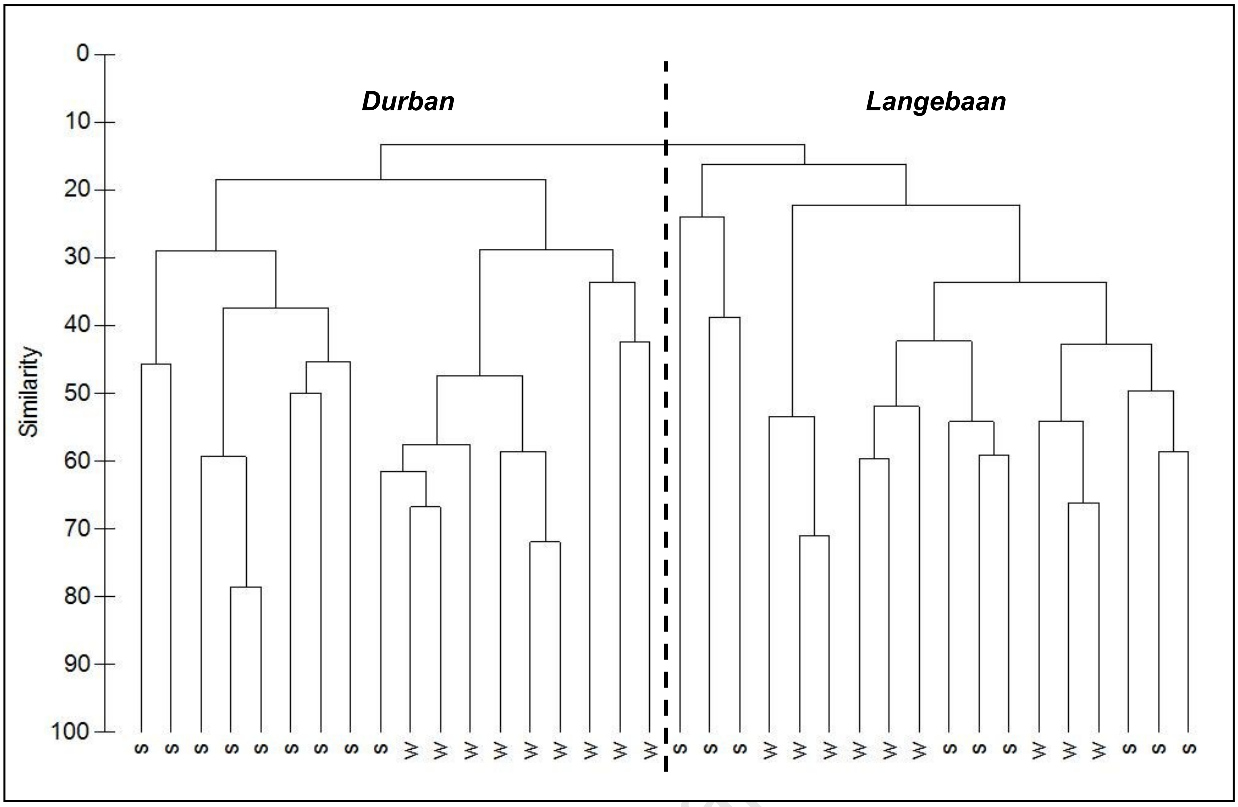


Figure 2.4. Average microphytobenthos biomass (+1SE) in Durban Bay and Langebaan Lagoon in winter 2009 (W) and summer 2010 (S). No statistically significant seasonal or site differences occurred.

a)



b)

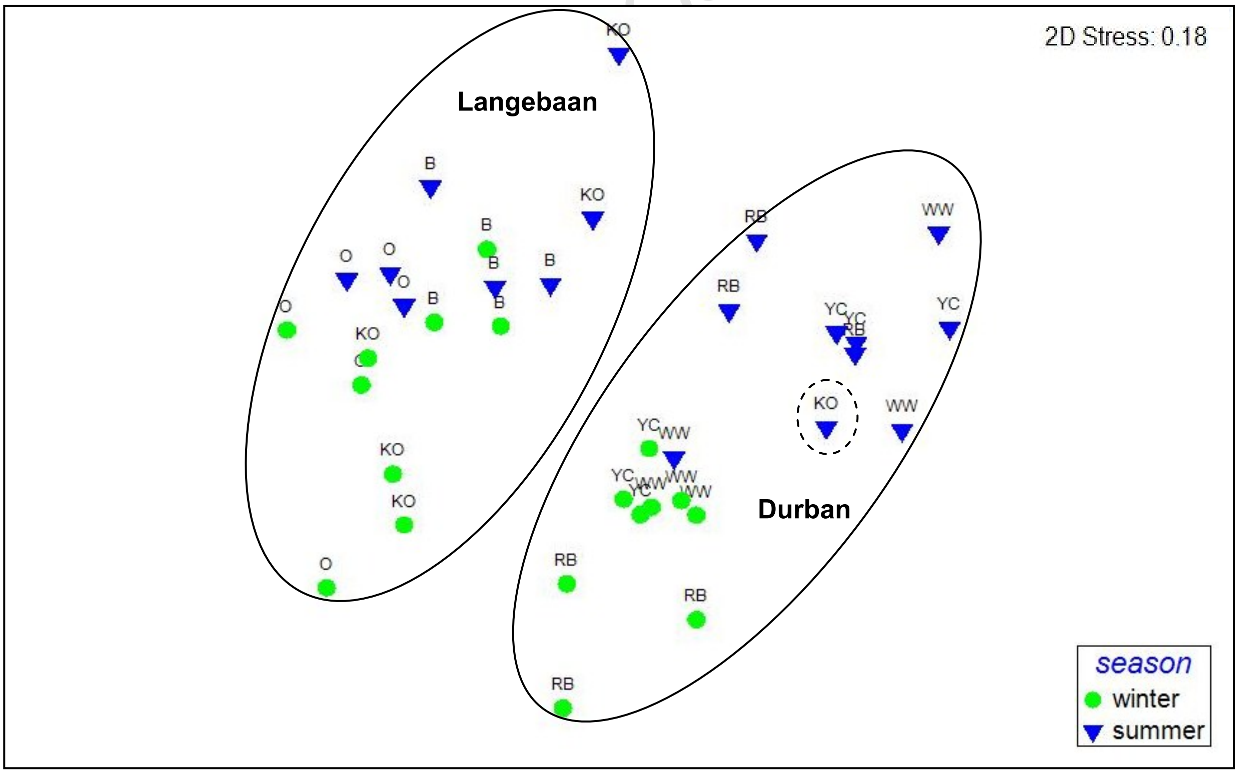


Figure 2.5. a) Dendogram indicating grouping of macrofaunal communities (based on a species comparison) by site and season (W – winter 2009 and S – summer 2010). b) MDS plot of the macrofaunal communities at subsites KO (Klein Oesterwal), O (Oesterwal) and B (Bottelary) within Langebaan Lagoon and RB (Roundbush), YC (Yachtclub) and WW (Wilson's Wharf) within Durban Bay for winter 2009 (W) and summer 2010 (S). The single outlying Langebaan subsite (summer KO) is surrounded by a dotted line.

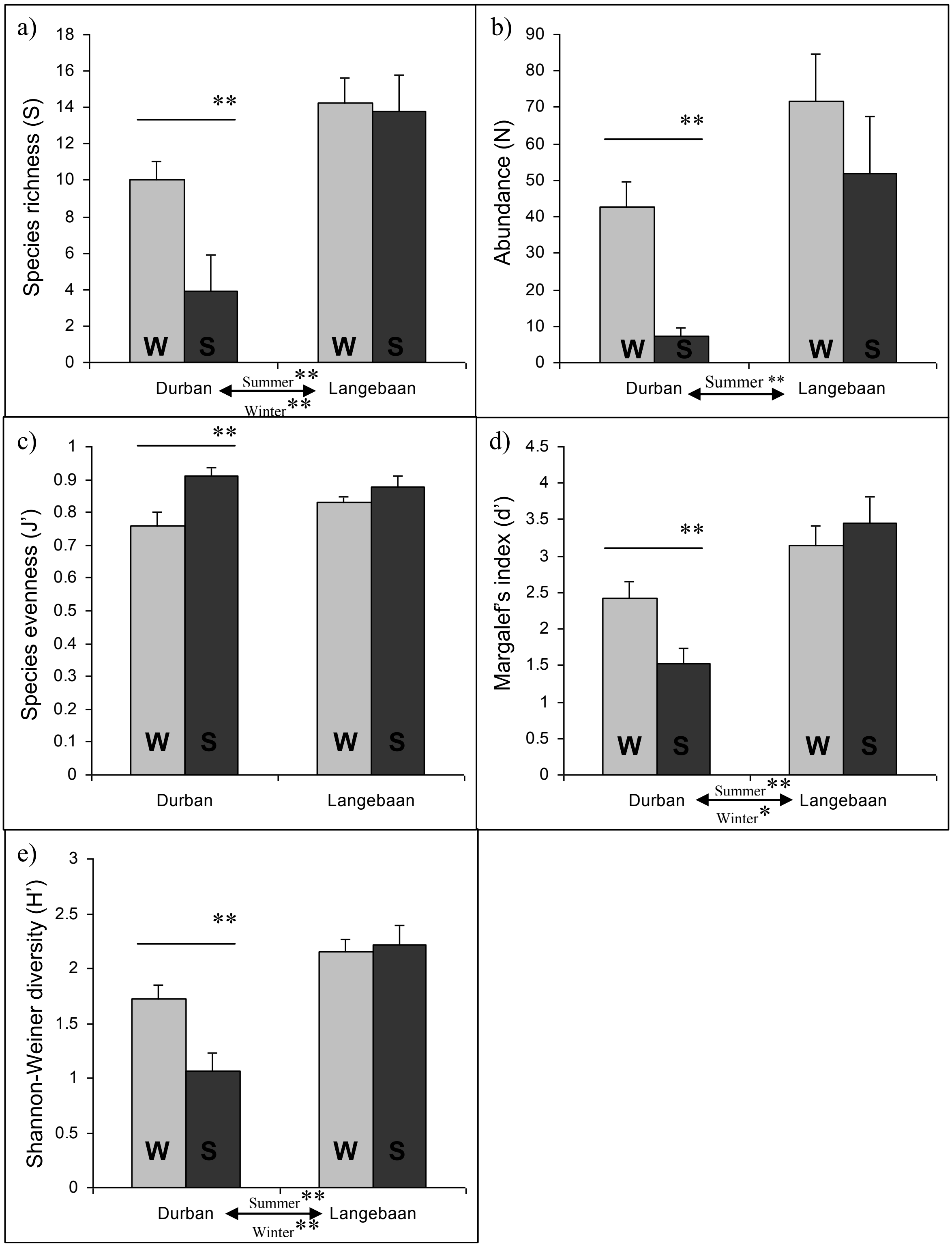


Figure 2.6. Measures of macrofaunal diversity (with standard error) for winter 2009 (W) and summer 2010 (S) in Durban Bay and Langebaan Lagoon. Statistical differences between sites within a specified season are indicated by an arrow with * ($p < 0.10$) or ** ($p < 0.05$). Significant differences between seasons within a site are indicated by a bar and ** (Tukey test, $p < 0.05$).

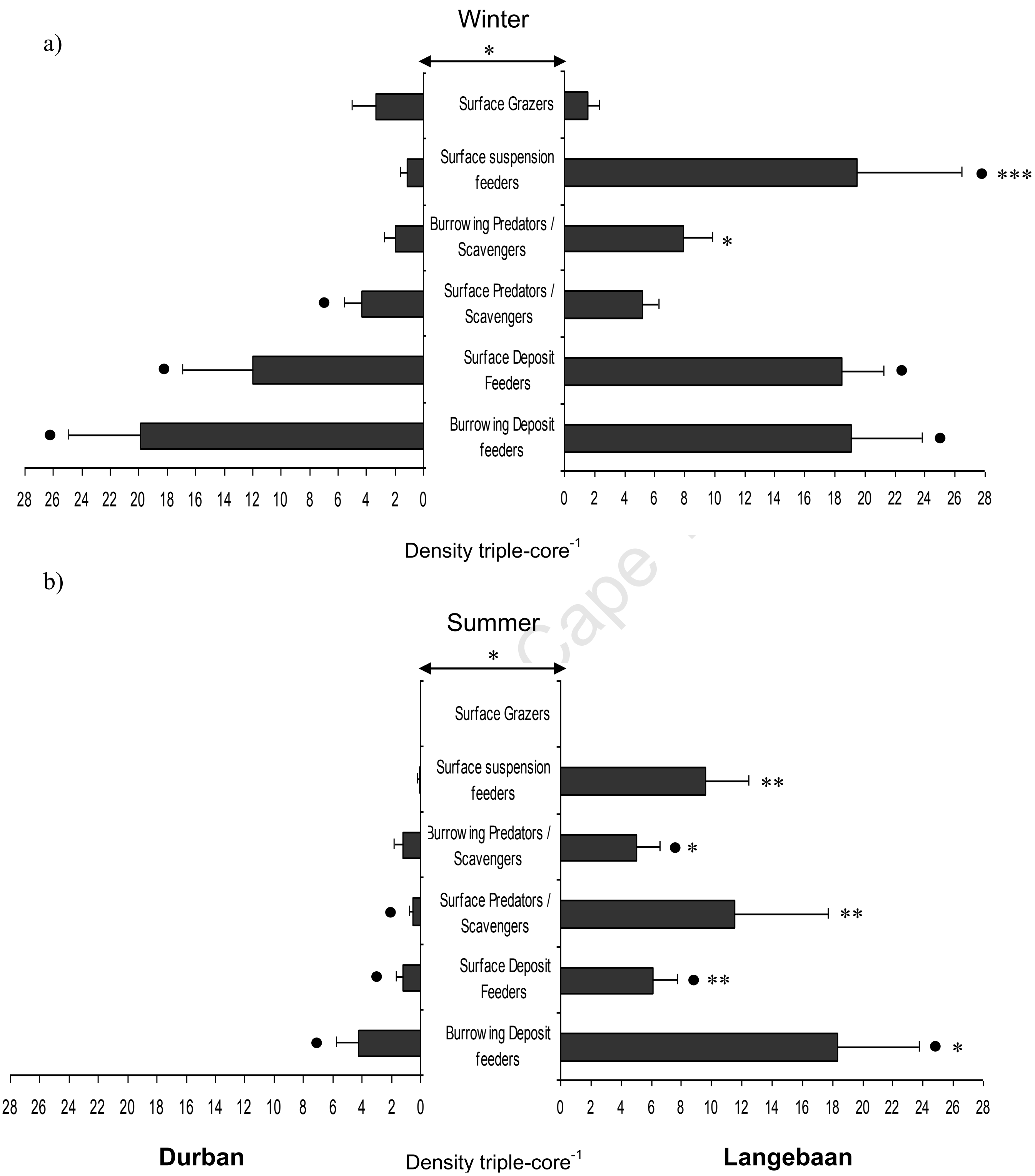


Figure 2.7. Average (+1 SE) density per triple-core (235.6 cm^2) of functional groups of macrofauna present in Durban Bay and Langebaan Lagoon in a) winter 2009 and b) summer 2010. A significant difference in functional group community composition between sites is represented by an arrow with * (PERMANOVA, $p < 0.05$). Significant differences in individual functional groups between sites (ANOVA or Mann-Whitney *U*-tests) are represented by * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$). Defining functional groups that contributed most to similarity (SIMPER) of a particular site and season are represented as ●.

Table 2.1. A summary of findings and brief explanations from the discussion and conclusion. See introduction for hypotheses corresponding to numbers 1 to 6 and discussion for detailed explanations (L = Langebaan, D = Durban, S = summer and W = winter). Where observations corresponded with hypothesized trends they are marked with a √ and where they did not they are indicated by ×.

		Site effects			**Seasonal effects**			
Hypotheses	**Variables**	**expectations**	**observations**		**expectations**	**observations**		**Explanations**
1	**Nutrients**	L > D	L > D in summer	√	LS > LW	LS > LW	√	- Local effects (e.g. pollution in Durban) override expected coastal trends.
			L < D in winter	×	None	DS < DW		
2a	**Temperature**	L < D	L = D in summer	×	S > W	S > W	√	- Local solar heating of lagoons overrides expected coastal trend.
			L < D in winter	√				
2b	**Salinity & specific conductivity**	L > D in summer L < D on winter	L > D in summer L > D in winter	√ ×	S > W	S > W	√	- Values close to those of seawater, but evaporation in lagoons greater in summer and dilution greater in wet season.
3	**Microphyto-benthic biomass**	L > D	L = D	×	S > W	S = W	×	- Local nutrient increases in Durban may override the expectation of greater microphytobenthic biomass in Langebaan. - *C. kraussi* may be more active in summer, lowering micrphytobenthic biomass through bioturbation. (**Chapter 4**).
4	**Macrofaunal diversity (S, J', d' H')**	L < D	L > D	×	None	DS = DW		- Localised effects such as heating, shelter and food supply could increase diversity in Langebaan, overriding expected coastal trend.
				×	None	DS < DW		- Localised effects such as pollution and human disturbance, especially in Durban summer, could decrease diversity in Durban harbour below expected trends for open-coastal systems.
4	**Macrofaunal abundance (N)**	L > D	L > D	√	S > W	S < W	×	- Upwelling enrichment in Langebaan translates to increased food availability. - Human disturbance may have seasonal component, increasing in summer (holidays). - *C. kraussi* activity may increase in summer as temperature and nutrients rise, leading to decreases in macrofaunal diversity and abundance (**Chapter 4**).
5	**Macrofaunal community structure**	L ≠ D	L ≠ D	√	S ≠ W	S ≠ W	√	- Cross-coastal biogeographic variations in species composition along with seasonality of breeding cycles account for taxonomic differences between sites and seasons.
6	**Relative proportions of functional groups**	L = D	L ≈ D	√	S = W	S ≈ W	√	- Localised effects of bioturbation by *C. kraussi* dictates functional-group proportions, favouring burrowers over surface dwellers (**Chapter 3**).

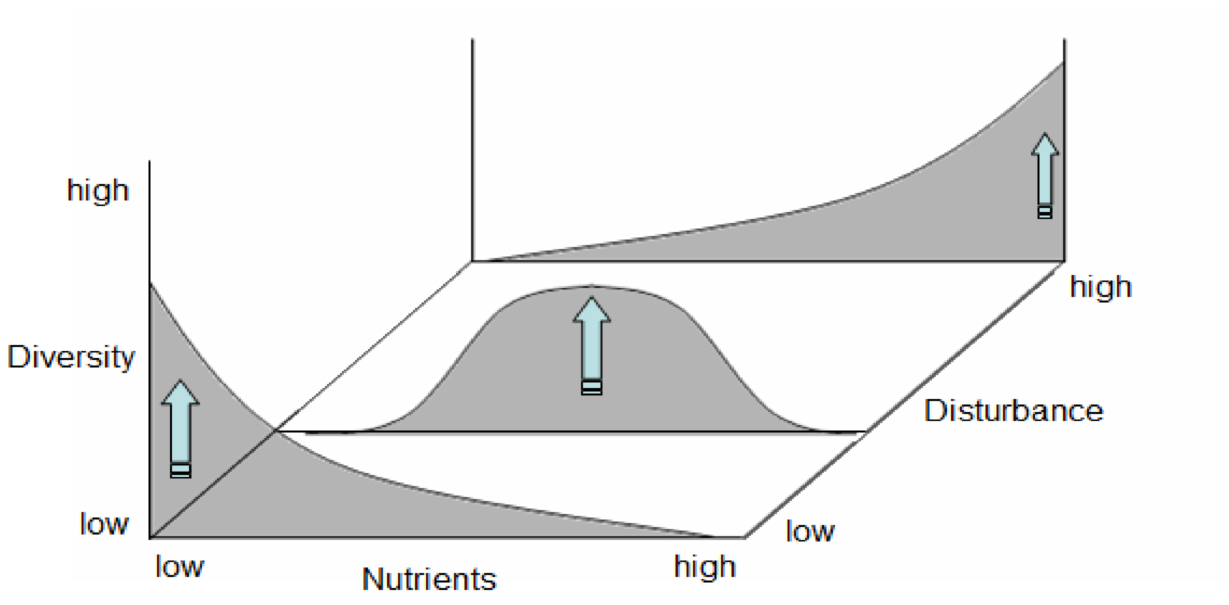


Figure 3.1. The hypothesized interacting effects of nutrients (x-axis) and disturbance (z axis) on macrofaunal diversity (y axis), based on Kondoh (2001).

Table 3.1. Manipulated prawn densities and nutrient treatments forming a series of 12 treatments in a three by four matrix.

	No prawns (0p)	Half Prawn Density (p)	Normal Prawn Density (2p)	Double Prawn Density (4p)
Background Nutrients (N)	0p ; N	p ; N	2p ; N	4p ; N
250 g added nutrients (2N)	0p ; 2N	p ; 2N	2p ; 2N	4p ; 2N
500 g added nutrients (4N)	0p ; 4N	p ; 4N	2p ; 4N	4p ; 4N

a)

b)

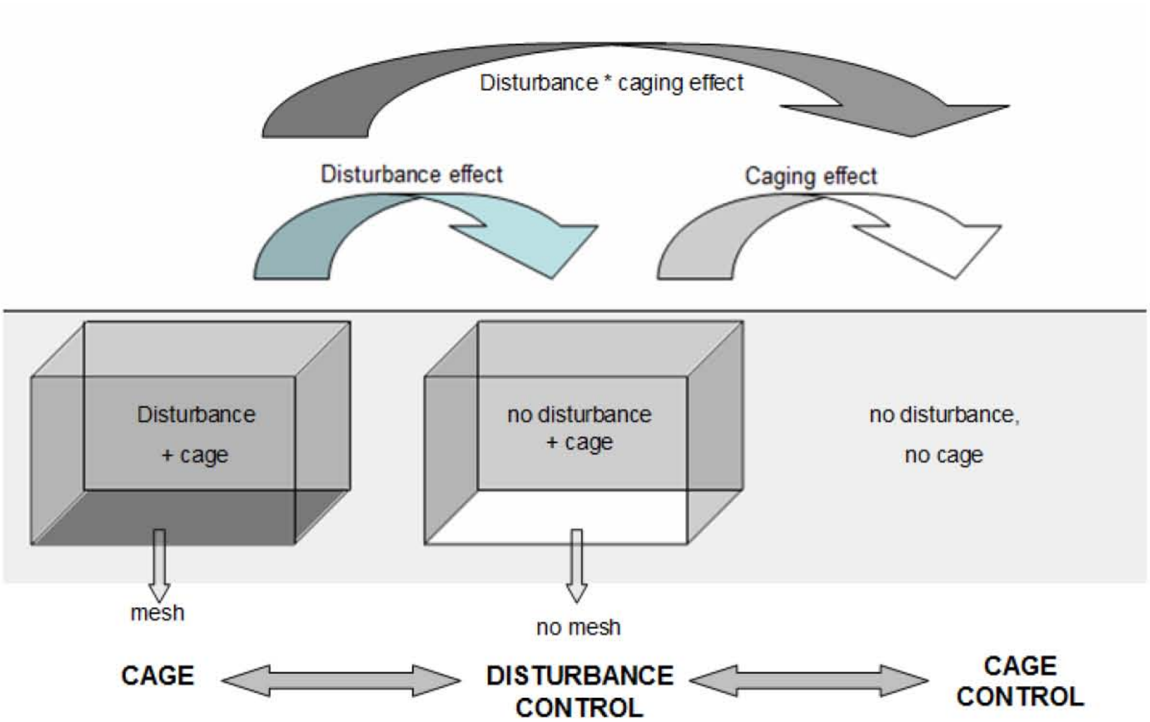


Figure 3.2. (a) Cages installed in one of the plots with an inset of a nutrient-treated cage and a *C. kraussi* individual. (b) Diagram of how the effects of disturbance and caging were estimated. The cage had mesh on all sides and the bottom, while the disturbance control lacked a mesh bottom, and the caging control lacked a cage. All caging controls were carried out in the intertidal sandflats at Bottelary with background levels of *C. kraussi* density, or simulations thereof (250 m^{-2}), and natural, (fluctuating) background levels of nutrients.

Table 3.2
One-way ANOVAs, PERMANOVAs and Tukey Tests on the effect of caging treatments (cage; disturbance control = dc; cage control = cc) on a number of physical and biological variables. Significant values are indicated by * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$). EPS refers to extra-polymeric substances and functional group and species refer to macrofaunal communities.

		March (3 Months)			June (6 months)		
Variables	**Test**	F/ Pseudo-F	df	p	F/ Pseudo-F	df	p
Ammonia	ANOVA	8.471	2	***0.009	0.354	2	0.711
	Tukey						-
	dc vs. cage:			0.154			
	dc vs. cc:		9	0.154			
	cage vs. cc			**0.007			
Nitrate	ANOVA	0.141	2	0.870		†	-
Orthophosphate	ANOVA	1.794	2	0.221	2.439	2	0.142
Chlorophyll *a*	ANOVA	0.171	2	0.843	1.778	2	0.185
EPS	ANOVA	10.498	2	***<0.001	10.113	2	***<0.001
	Tukey						
	dc vs. cage:			0.993			0.993
	dc vs. cc:		33	***0.001		33	**0.004
	cage vs. cc			***0.001			***<0.001
Species	PERMANOVA	1.671	2	0.067	1.851	2	*0.021
	PERMANOVA "pair-wise"						
	dc vs. cage:			0.286			0.513
	dc vs. cc:		6	0.057		6	*0.029
	cage vs. cc			0.142			*0.029
Functional groups	PERMANOVA	2.882	2	*0.015	1.571	2	0.163
	PERMANOVA "pair-wise"						-
	dc vs. cage:			0.198			
	dc vs. cc:		6	*0.028			
	cage vs. cc			*0.028			

† - no variance was found therefore no ANOVA could be performed

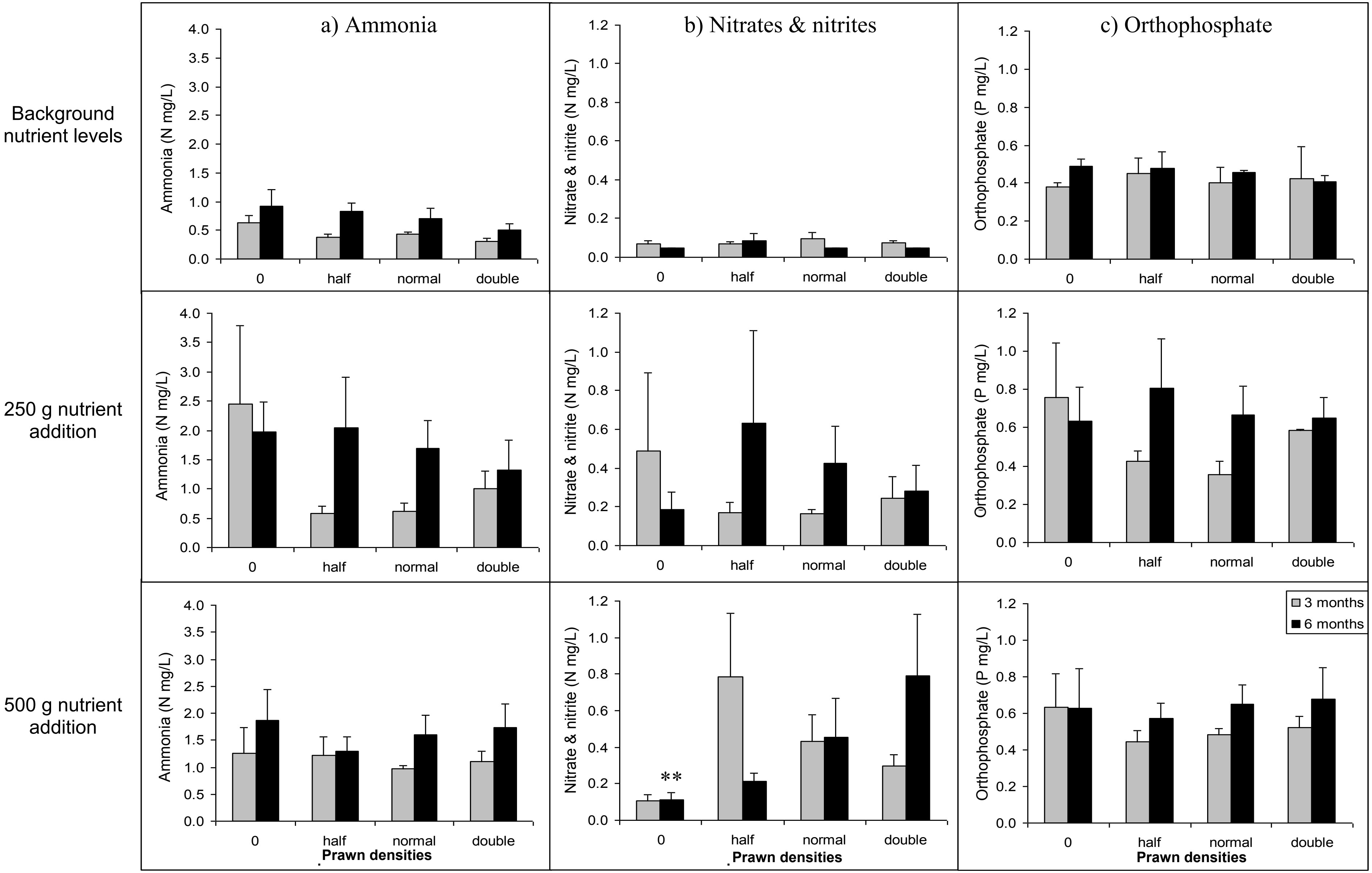


Fig. 3.3. Mean nutrient levels (+1 SE) for different nutrient and prawn-density treatments after 3 and 6 months. Note that ammonia is represented on a different scale to the other nutrients. No significant differences in nutrient levels at different prawn densities were found, except for nitrates with nitrites after 6 months, indicated by ** ($p < 0.05$).

Table 3.3. Ratios of N:P calculated from background and enrichment nutrient levels at all levels of prawn density for three and six months.

		Prawn densities			
	Nutrient levels	**0**	**Half**	**Normal**	**Double**
3 months	**Background**	1.95	1.06	1.38	0.94
	250 g	4.15	1.88	2.39	2.28
	500g	2.30	4.87	3.10	2.88
6 months	**Background**	2.14	2.04	1.77	1.45
	250 g	3.64	3.57	3.41	2.65
	500 g	3.39	2.82	3.40	3.96

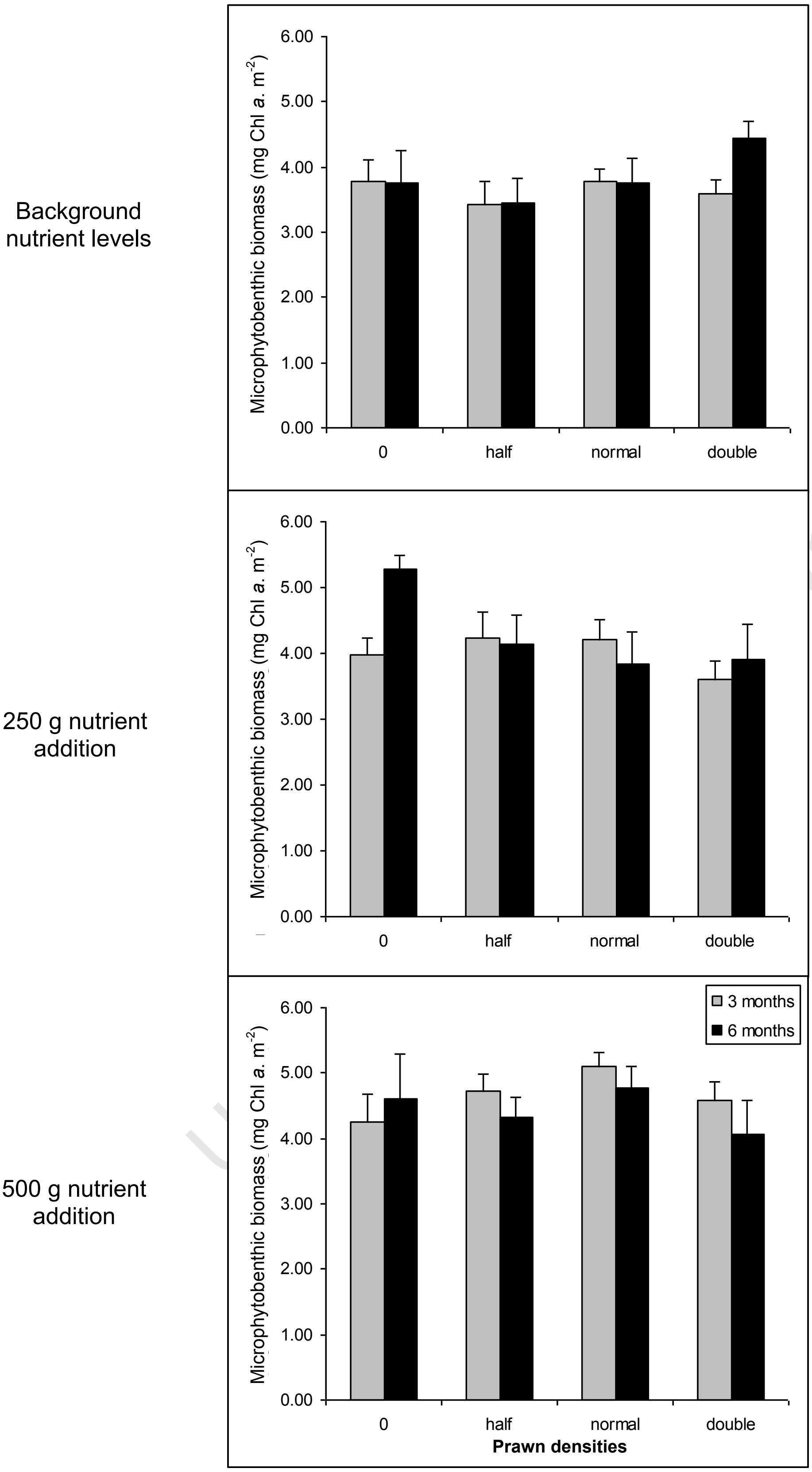


Fig. 3.4. Mean levels of microphytobenthic biomass (+1 SE), measured as chlorophyll *a*, observed for different nutrient and prawn-density treatments at the three month and six month sampling periods.

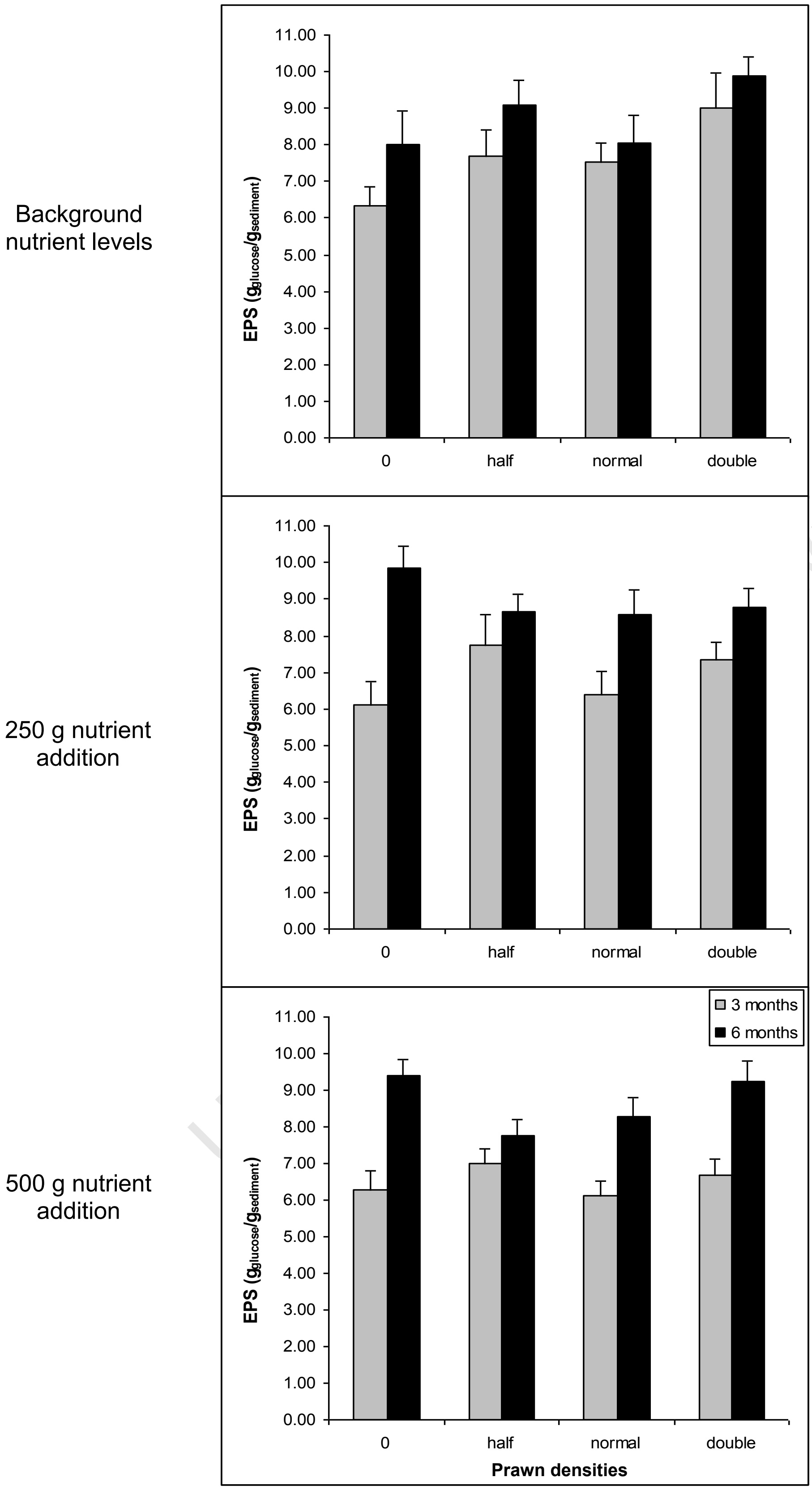
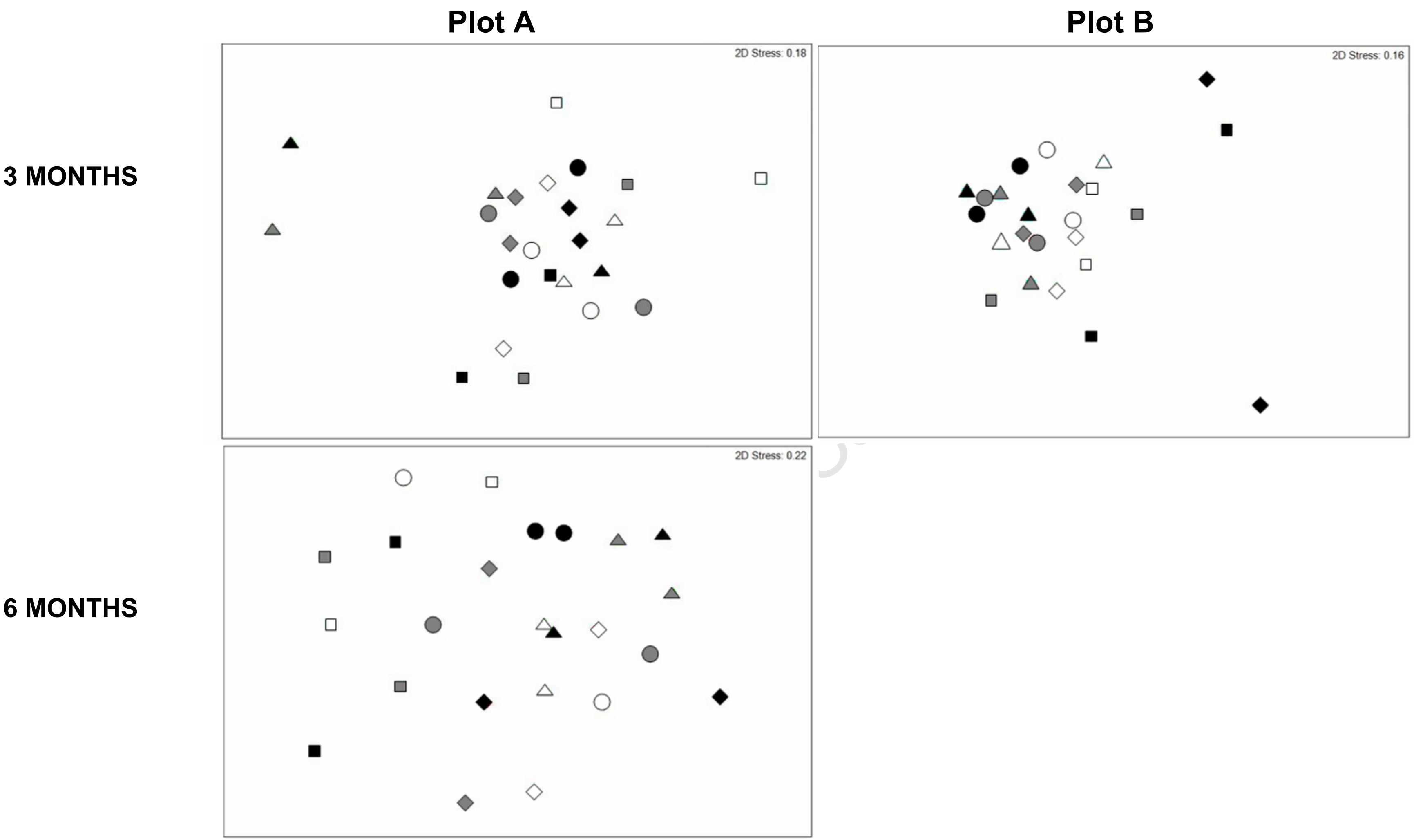


Fig. 3.5. Mean levels of EPS (extra polymeric substance), with standard error, observed for different nutrient and prawn density treatments at the three month and six month sampling periods.



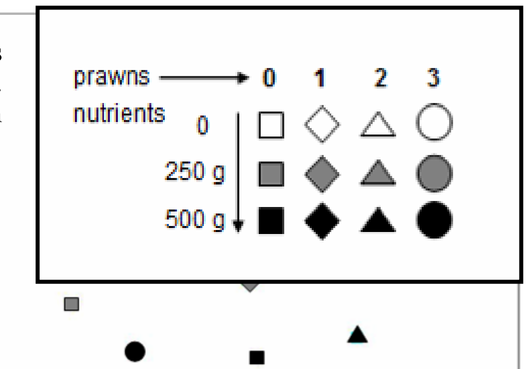


Fig. 3.6. MDS plots of macrofaunal communities analysed in terms of species composition for plots A and B at the three-month and six-month sampling periods. Symbols for prawn density treatments and shading for nutrient treatments are shown in the key (right).

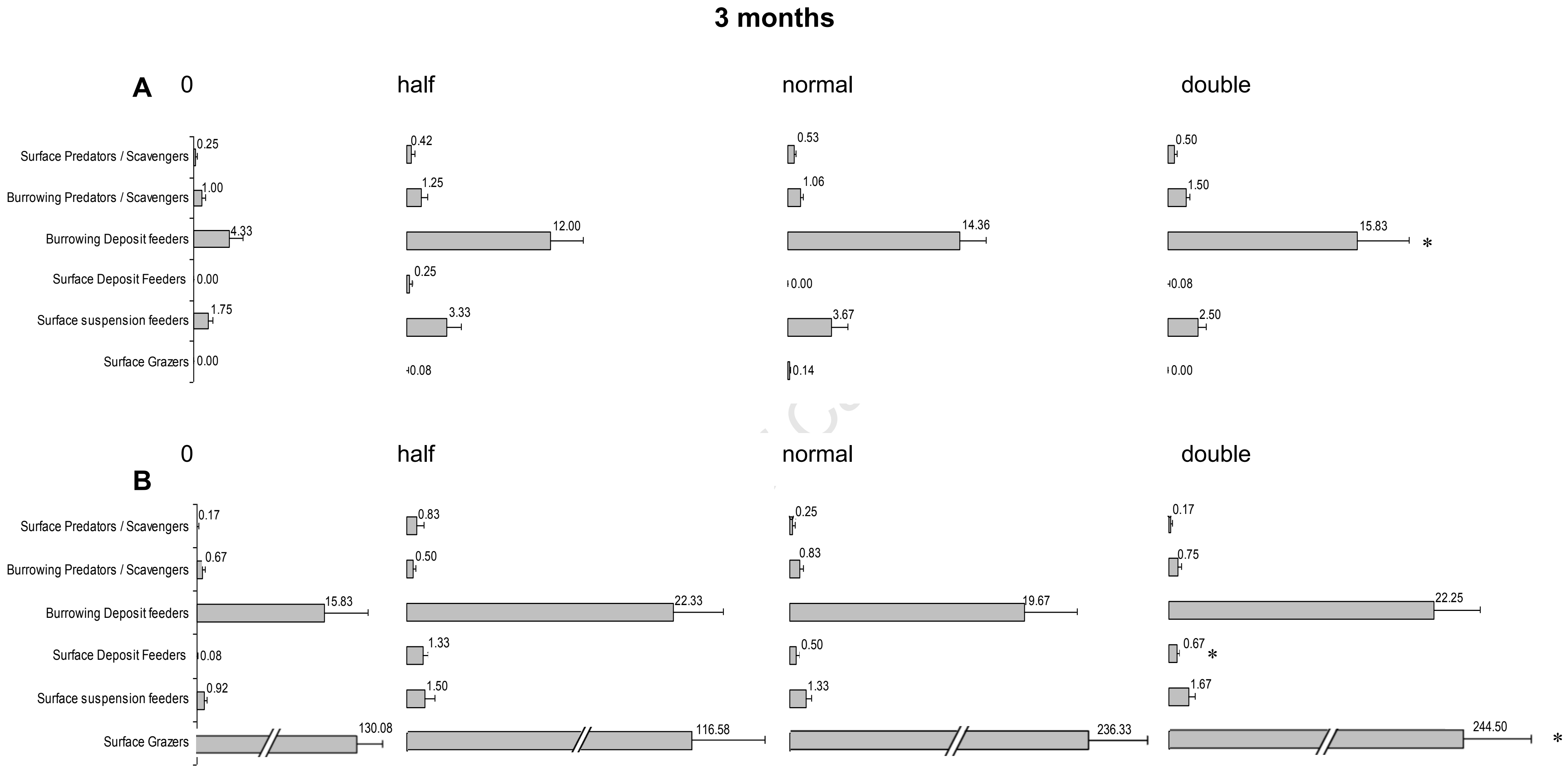


Fig. 3.7. Mean densities of functional groups (per core) with standard error are shown for zero, half, normal and double prawn density treatments for plots A and B at the three month sampling period. Where a functional group as a whole is significantly different in terms of density among prawn density treatments the significance is shown with a * ($p < 0.05$). Note that the data for surface grazers are truncated.

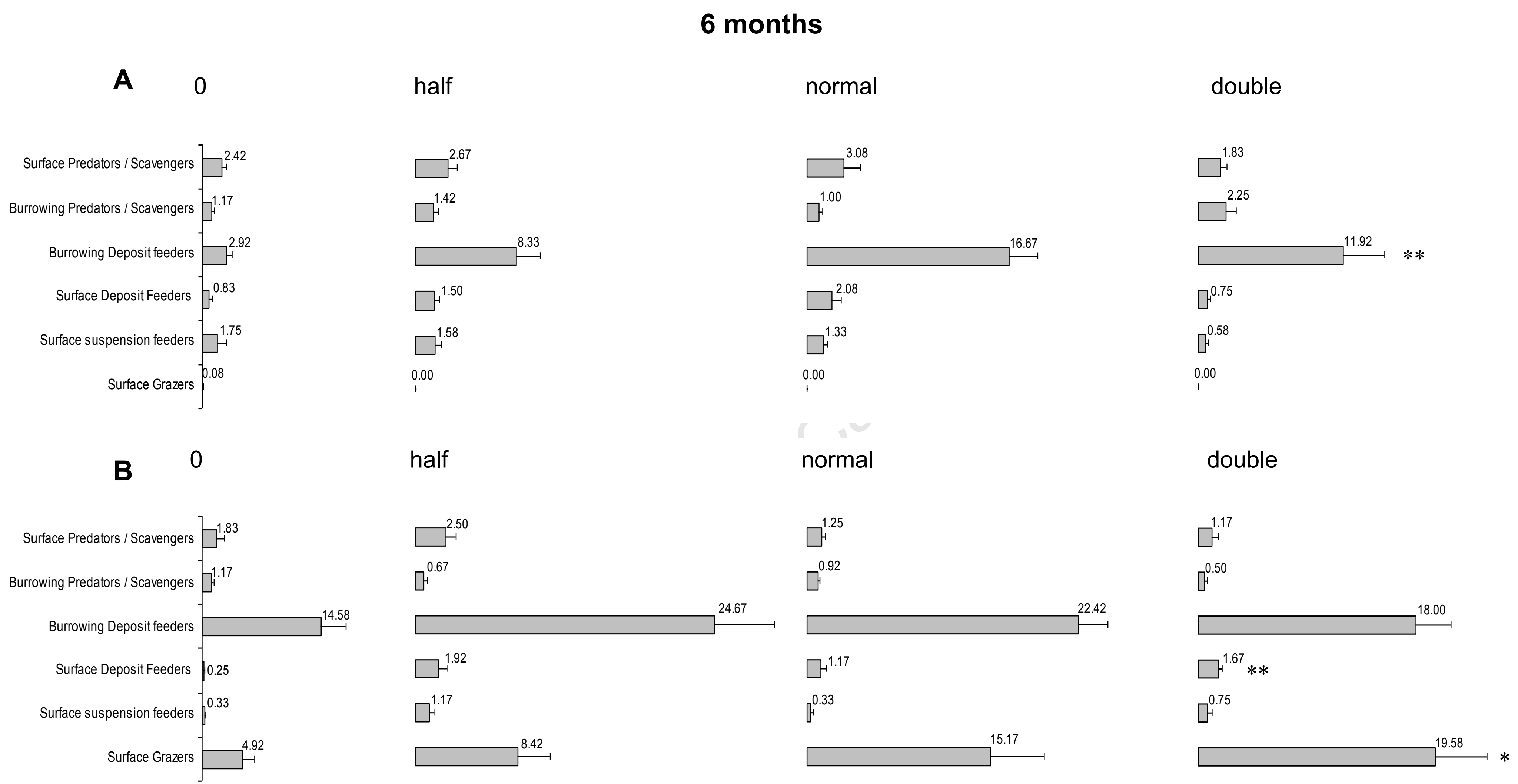


Fig. 3.8. Mean densities of functional groups (per core) with standard error are shown for zero, half, normal and double prawn density treatments for plots A and B at the six month sampling period. Where a functional group as a whole is significantly different in terms of density between prawn density treatments the significance is shown with a * ($p < 0.05$) or ** ($p < 0.01$).

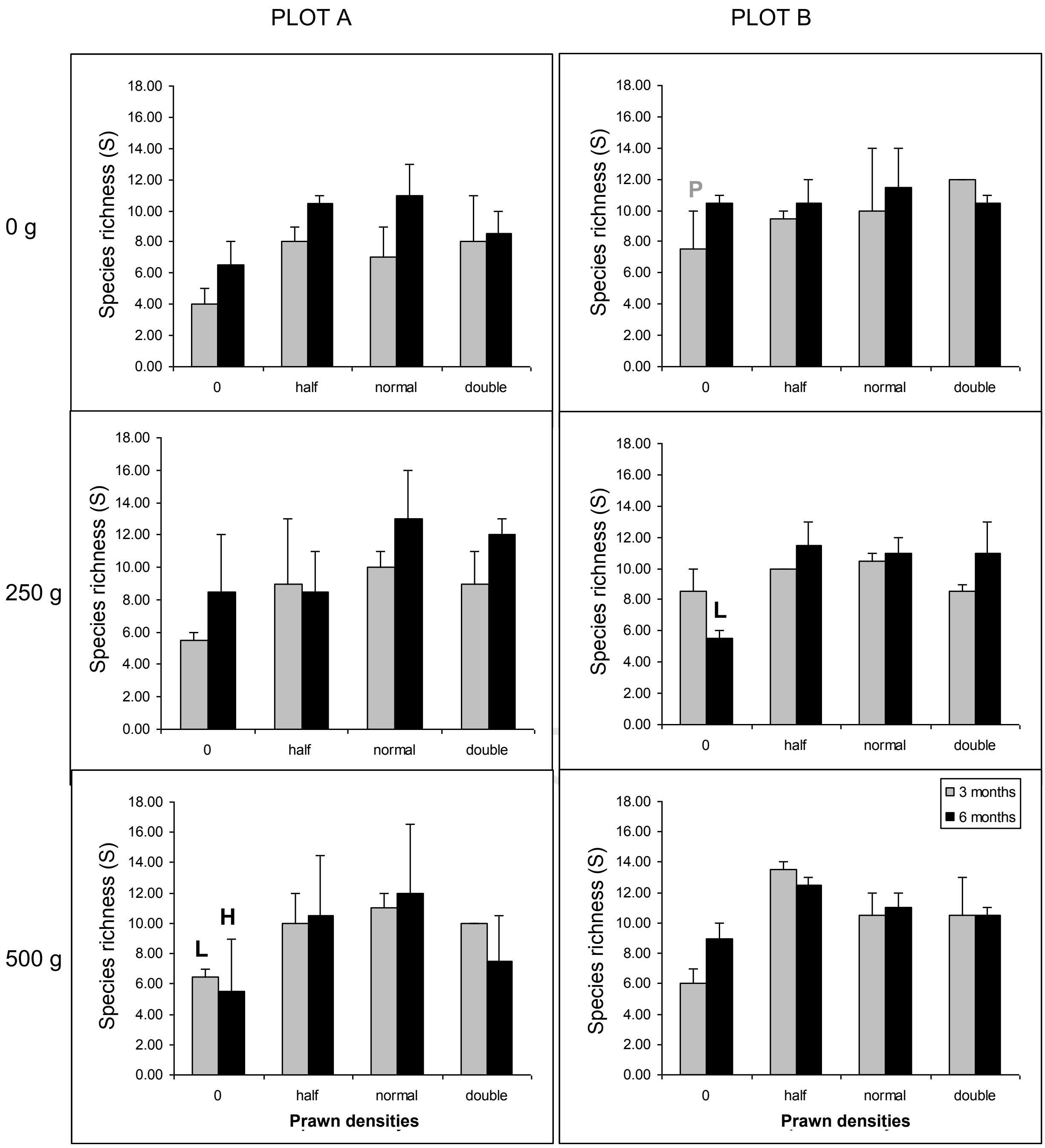


Figure 3.9. Total species richness (S) of macrofauna at all prawn density treatments at the three nutrient levels at three months and six months for plots A and B. Significant relationships ($p < 0.05$) are shown as a black letter and marginally non-significant polynomial relationships ($p < 0.1$) are shown as a grey letter above the time series in which they occur – P for Power, L for logarithmic and H for humped (i.e. second order polynomial).

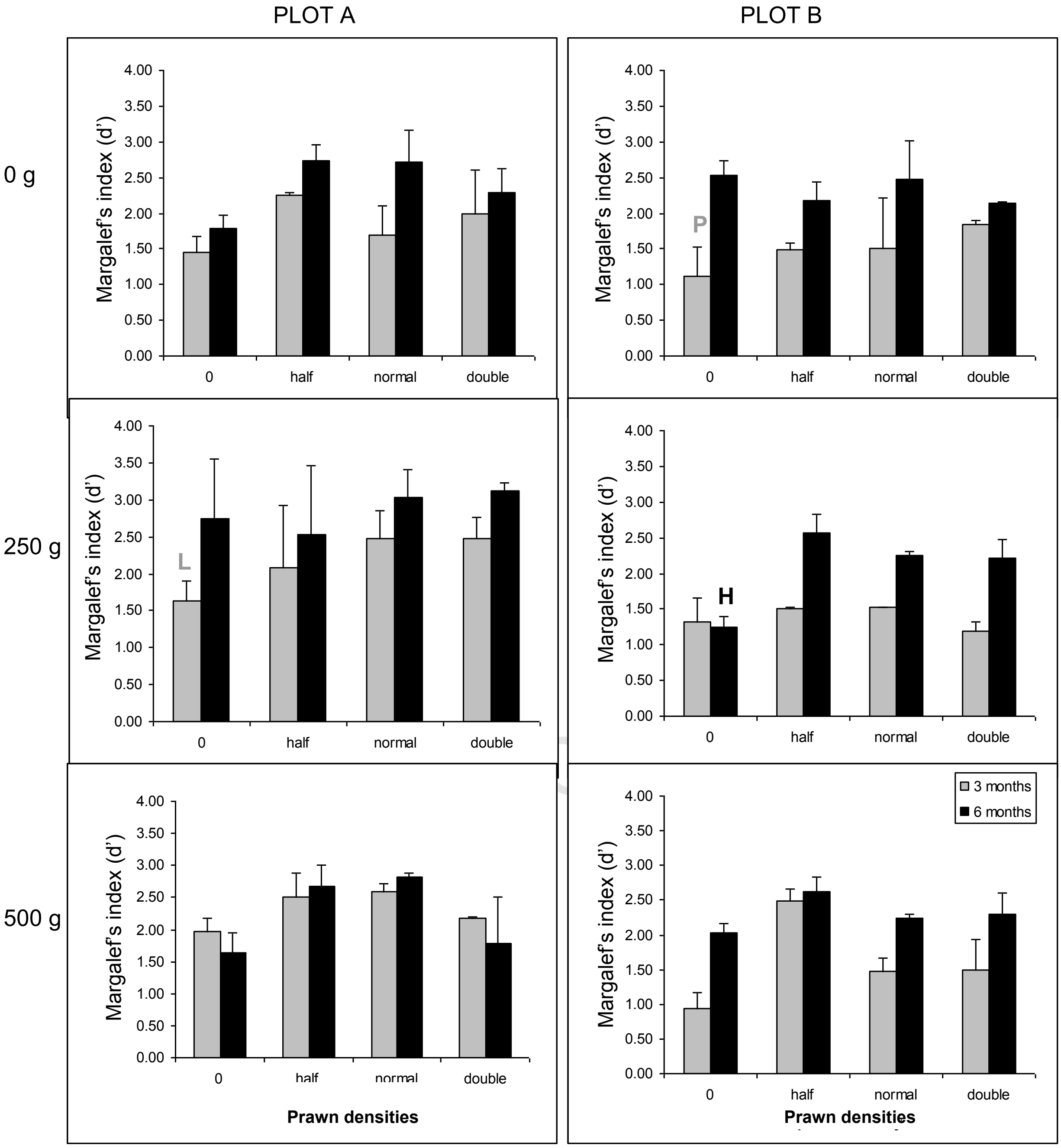


Figure 3.10. Margalef's index (d´) for macrofauna at all prawn density treatments at the three nutrient levels at three months and six months for plots A and B. Significant relationships ($p < 0.05$) are shown as a black letter and marginally non-significant polynomial relationships ($p < 0.1$) are shown as a grey letter above the time series in which they occur – P for Power, L for logarithmic and H for humped (i.e. second order polynomial).

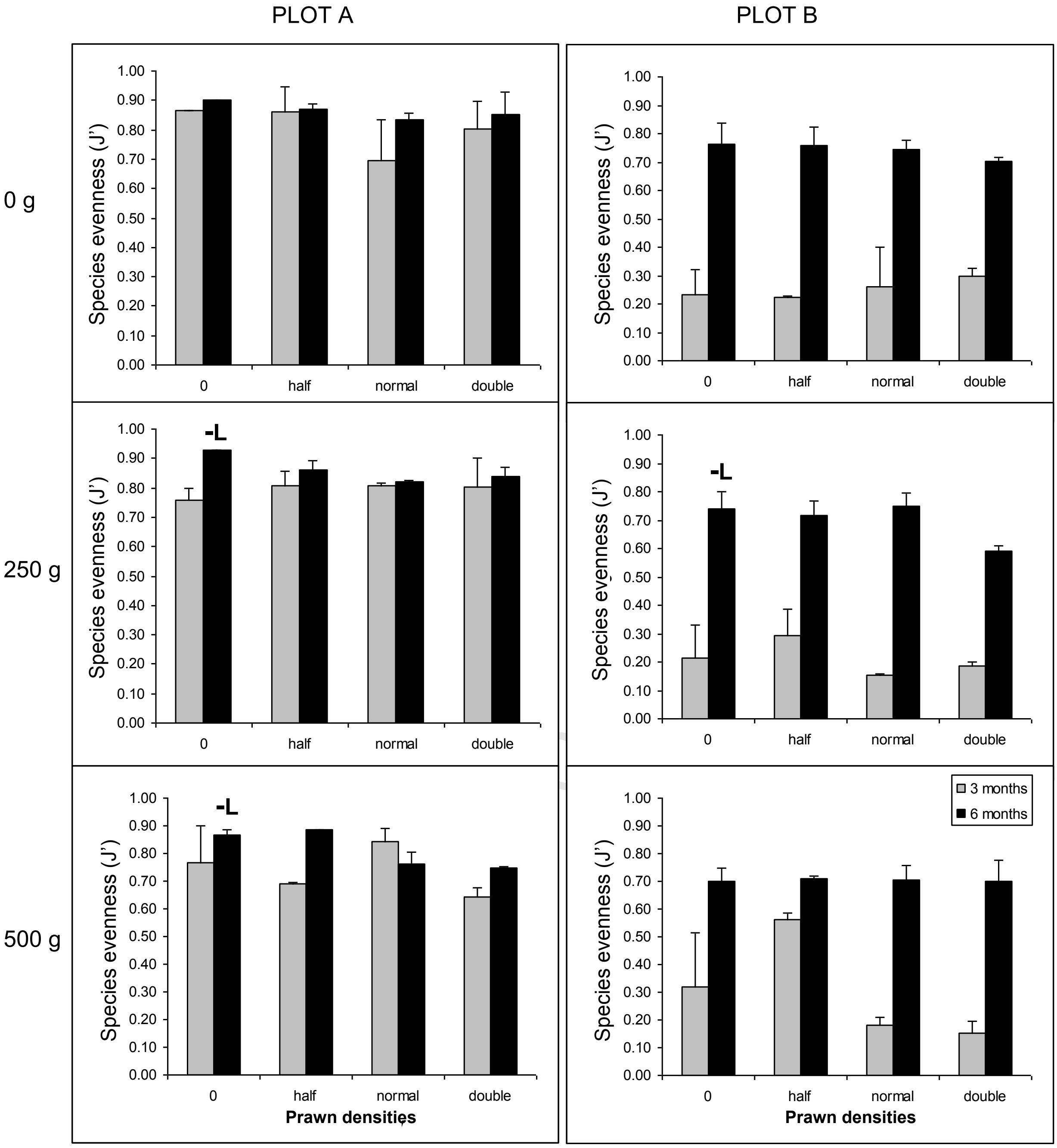


Figure 3.11. Pielou's index of species evenness (J´) for macrofauna at all prawn density treatments at the three nutrient levels at three months and six months for plot A and plot B. Significant relationships ($p < 0.05$) are shown as a black letter (-L for a negative logarithmic relationship) above the time series in which they occur.

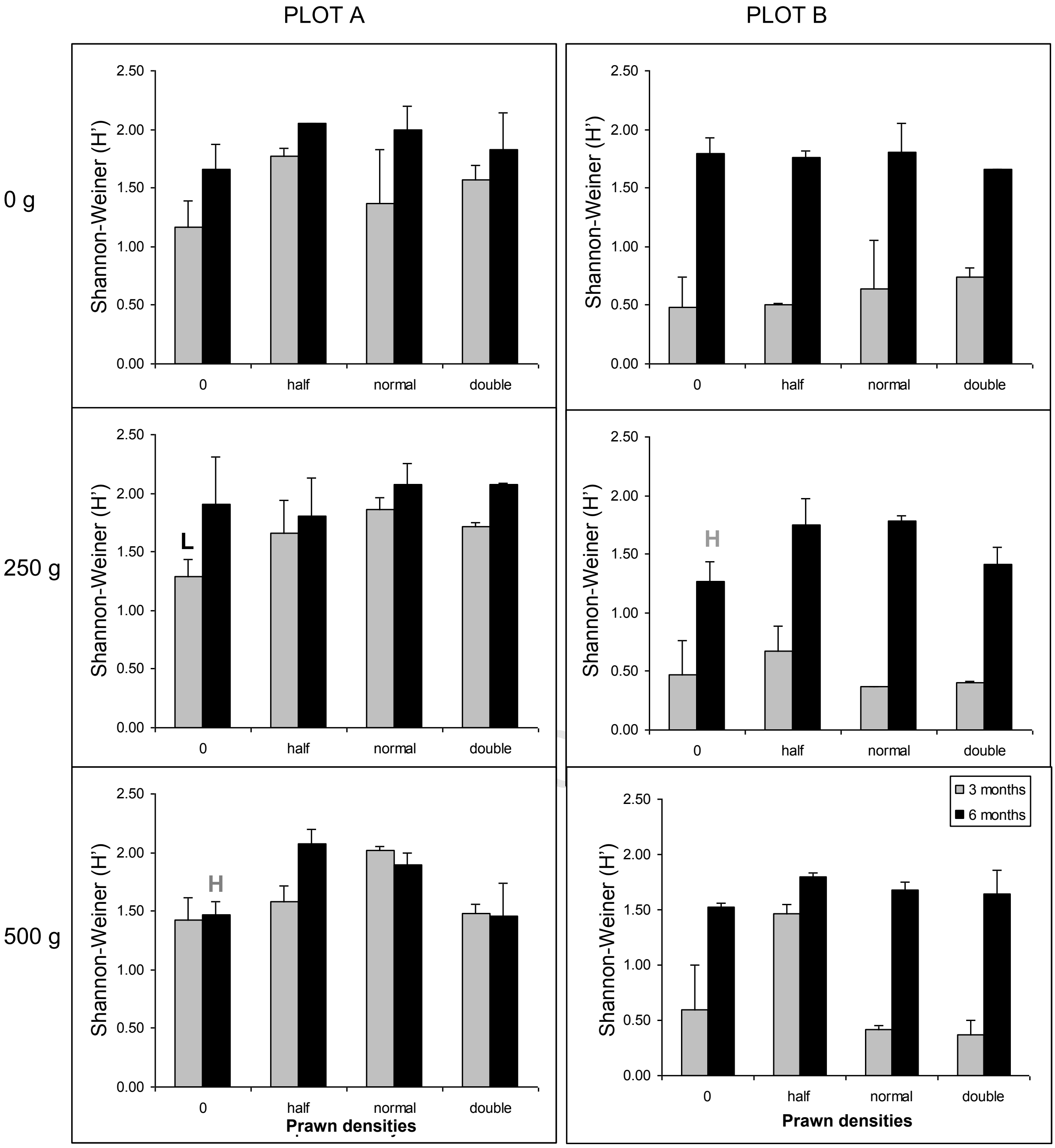


Figure 3.12. Shannon-Weiner index (H´) of macrofaunal species diversity at all prawn density treatments at the three nutrient levels at three months and six months for plots A and B. Significant relationships ($p < 0.05$) are shown as a black letter and marginally non-significant polynomial relationships ($p < 0.1$) are shown as a grey letter above the time series in which they occur– P for Power, L for logarithmic and H for humped (i.e. second order polynomial).

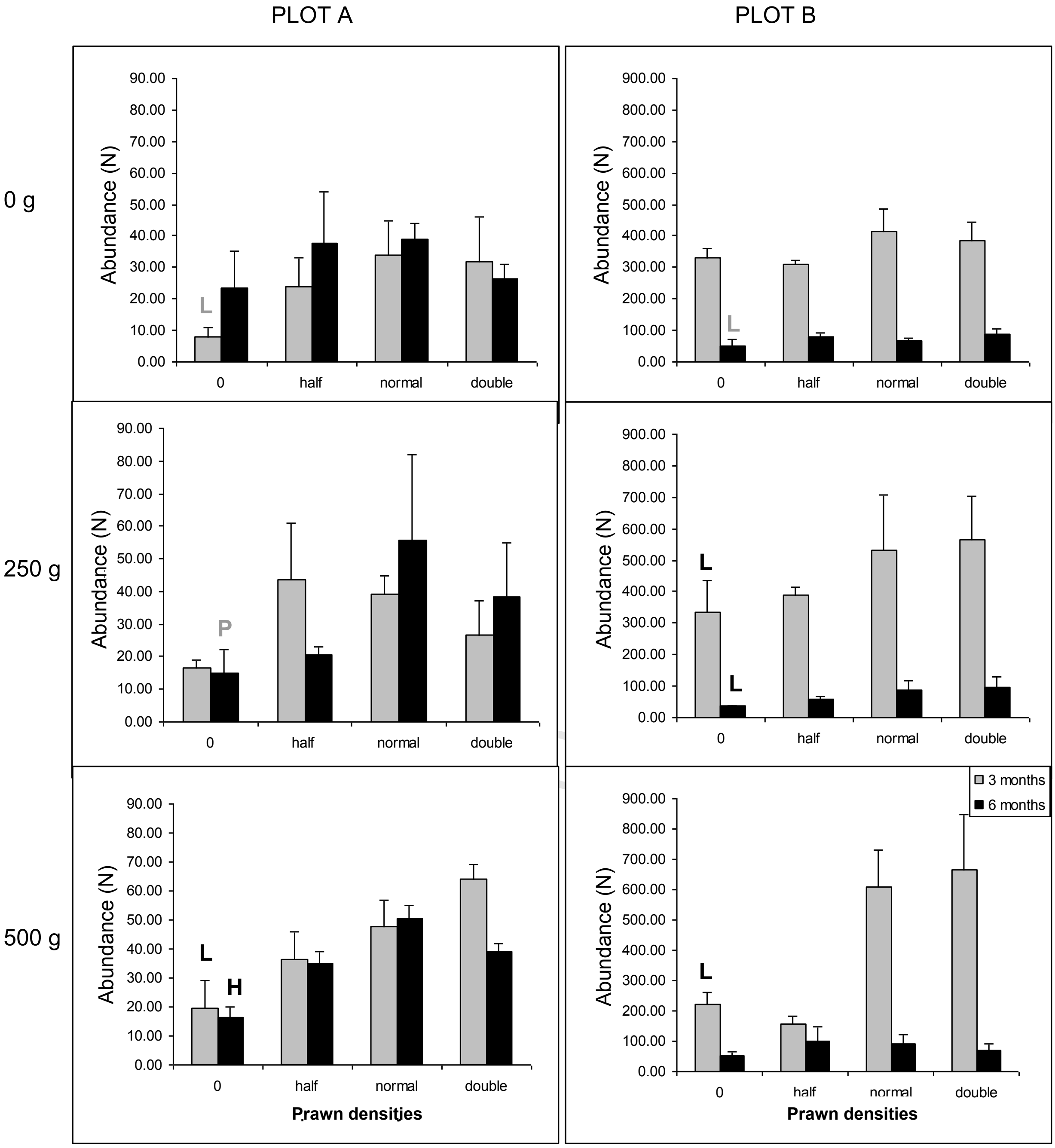


Figure 3.13. Total abundance (N per 157.1 cm^2) of macrofauna at all prawn density treatments at the three nutrient levels at three months and six months for plots A and B. Note that plot B has a scale ten times that of plot A. Significant relationships ($p < 0.05$) are shown as a black letter and marginally non-significant polynomial relationships ($p < 0.1$) are shown as a grey letter above the time series in which they occur – P for Power, L for logarithmic and H for humped (i.e. second order polynomial).

Table 3.4. Differences between macrofaunal communities analysed in terms of species composition in plots A and B as determined by a two-way ANOVA for nutrient and prawn effects for the three-month and six-month sampling periods. Significant differences of $p < 0.05$ are indicated by *.

	Nutrients			**Prawns**			**Prawns*Nutrients**		
	Pseudo-F	df	p	Pseudo-F	df	p	Pseudo-F	df	p
3 months									
A	0.611	2	0.823	1.010	3	0.457	0.984	6	0.510
B	1.187	2	0.308	1.748	3	*0.047	0.888	6	0.652
6 months									
A	0.646	2	0.843	1.991	3	*0.018	0.968	6	0.553
B	0.761	2	0.721	1.648	3	0.058	0.725	6	0.885

Table 3.5. The effects of nutrient and prawn density treatments on various diversity indices for both sampling periods and plots as calculated from factorial ANOVAs or a combination of Kruskal-Wallis (H) and one-way ANOVA (F) as indicated in the table. Polynomial regression p-values are also given. Significant effects are indicated by * (p < 0.05) and ** (p < 0.01).

		Nutrients			Prawns			Prawns*Nutrients		
		F/H	df	p	F/H	df	p	F	df	p
S	3 months									
	A	2.030	2	0.174	3.117	3	0.066	0.086	6	0.997
	B	0.193	2	0.827	2.771	3	0.087	1.021	6	0.457
	6 months[1]	0.318	2	0.853	12.306	3	**0.006			-
N	3 months									
	A	3.164	2	0.079	4.659	3	*0.022	0.977	6	0.481
	B[2]	1.085	2	0.581	5.288	3	**0.008			-
	6 months									
	A	0.114	2	0.893	3.568	3	*0.047	0.551		0.760
	B	0.132	2	0.877	1.610	3	0.239	0.416		0.855
d'	3 months									
	A	1.470	2	0.269	1.576	3	0.247	0.431	6	0.845
	B	0.473	2	0.635	2.635	3	0.098	1.517	6	0.254
	6 months[1]	0.566	2	0.754	8.462	3	*0.037			-
J'	3 months									
	A	0.955	2	0.412	0.225	3	0.877	1.128	6	0.403
	B[2]	0.695	2	0.707	1.765	3	0.186		6	-
	6 months									
	A	3.689	2	0.056	6.946	3	**0.006	1.105	6	0.414
	B	0.852	2	0.451	1.243	3	0.337	0.510	6	0.790
H'	3 months									
	A	0.873	2	0.443	3.092	3	0.068	1.072	6	0.431
	B[2]	1.095	2	0.578	1.813	3	0.177			-
	6 months									
	A	1.207	2	0.333	1.348	3	0.305	0.743	6	0.626
	B	2.011	2	0.177	2.376	3	0.121	0.904	6	0.523

[1] Kruskal-Wallis tests for both nutrient and prawn density treatments on diversity index for plot A and B together.

[2] Kruskal-Wallis test for nutrient treatment effects on index in plot B only.

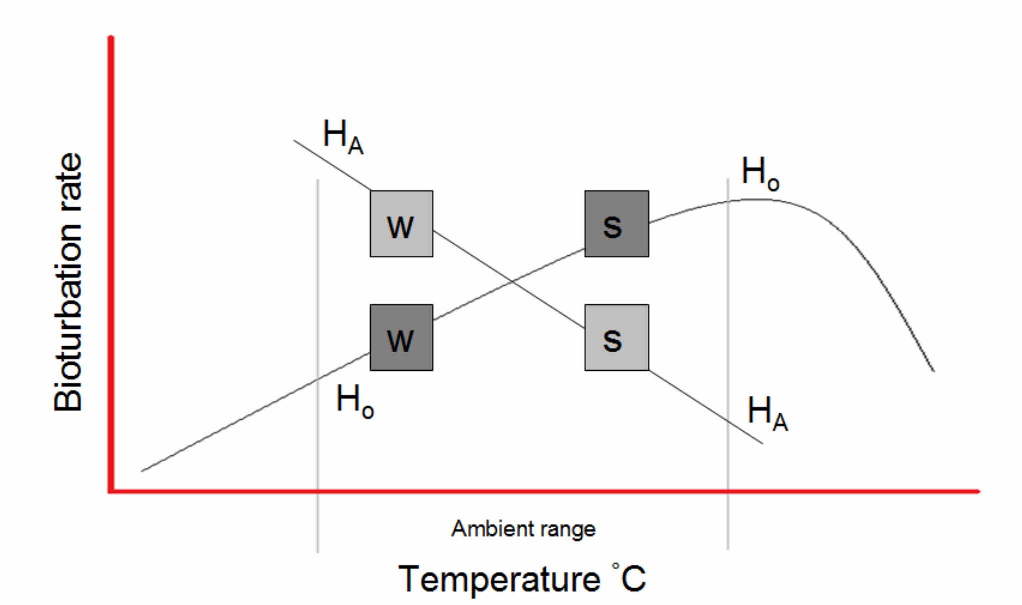


Figure 4.1. Alternative hypotheses about the effects of increasing ambient temperature on bioturbation. Either bioturbation rate is higher at summer (S) temperatures, due to an increase in metabolism and activity with increasing temperature (H_o) or, alternatively, activity is higher in winter (W) than summer-like temperatures, because feeding rate and corresponding bioturbation decrease with increased food availability at higher temperatures (H_A).

Figure 4.2. Photograph of the experimental set-up of 220-L buckets containing four cores, prior to prawn installation, with an inset showing how luminophores were applied to the sediment surface, following prawn installation and acclimation, and how the four tubes (three with prawns and one control without prawns) were individually covered with meshing.

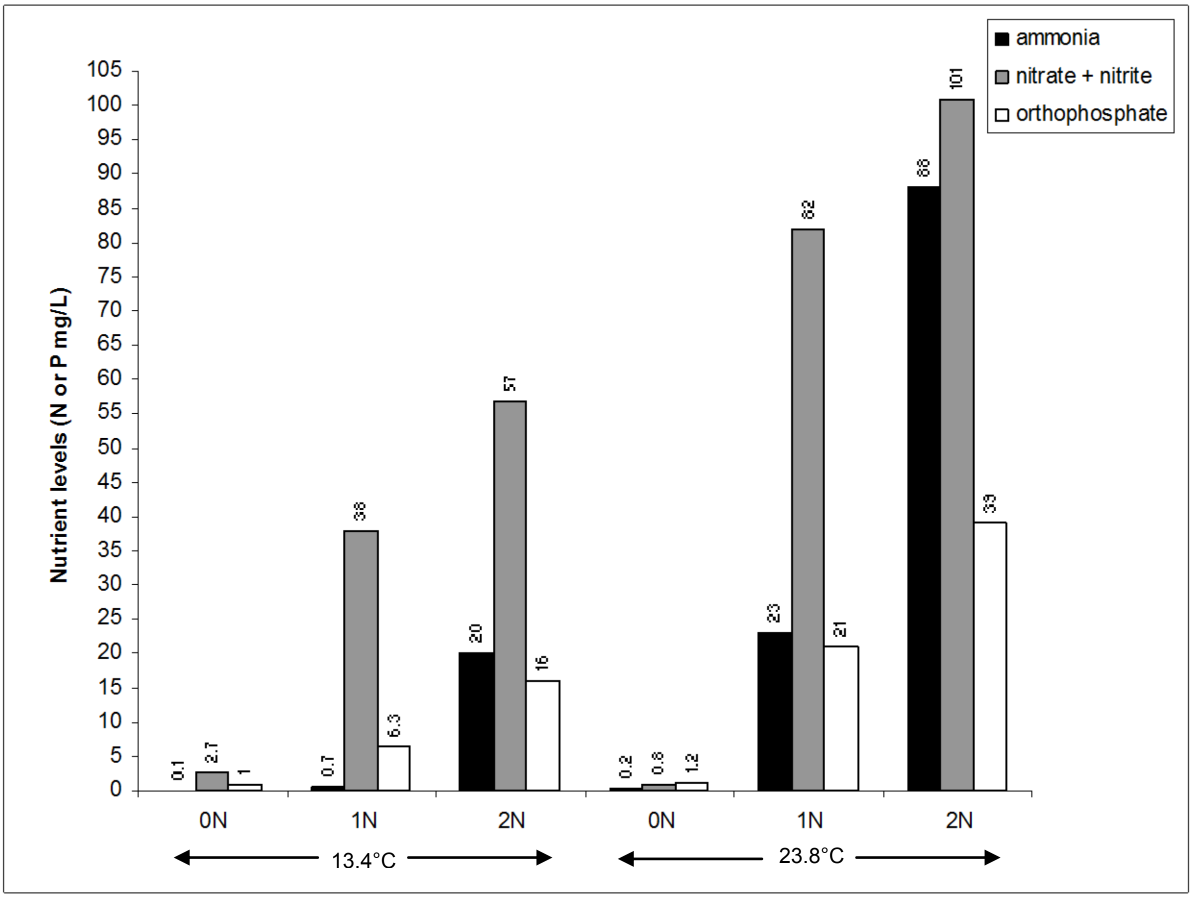


Figure 4.3. Levels of nutrient species, ammonia, nitrates with nitrites and orthophosphate, as measured in untreated 0N (zero Plantacote Plus® 8M fertilizer), 1N treated (250 g fertilizer) or 2N treated (500 g fertilizer) aquaria.

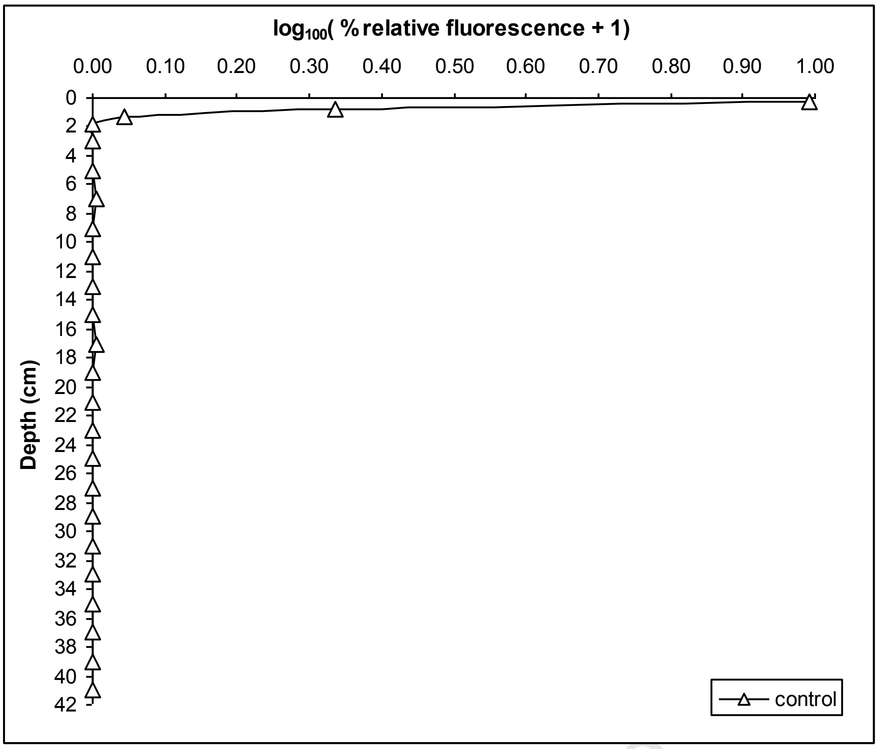


Figure 4.4. Profile of relative luminophore abundance with depth in the control core lacking *C. kraussi* (for 23.8°C).

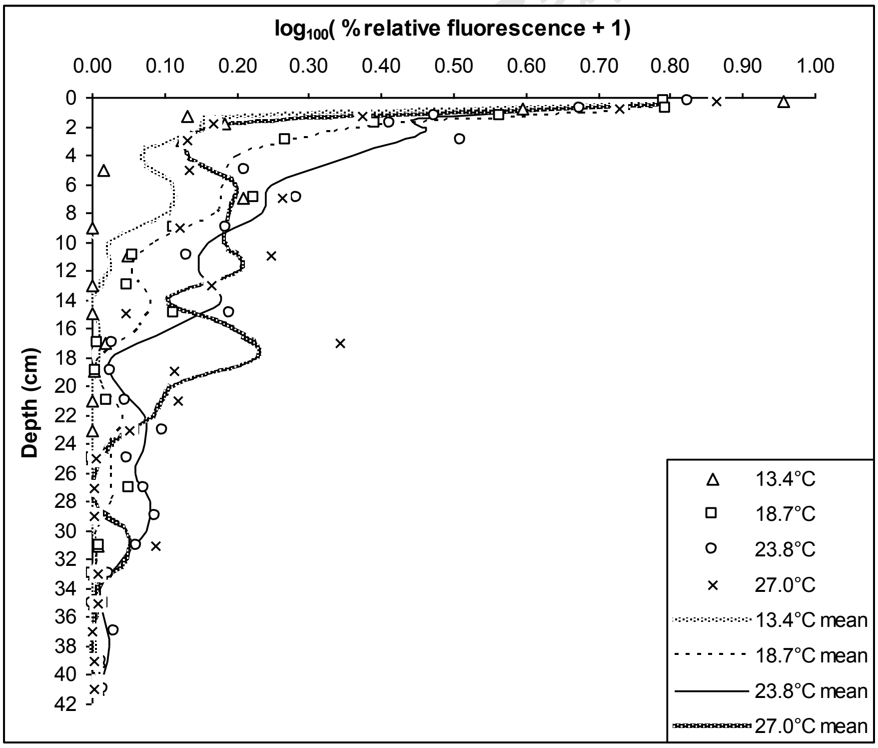
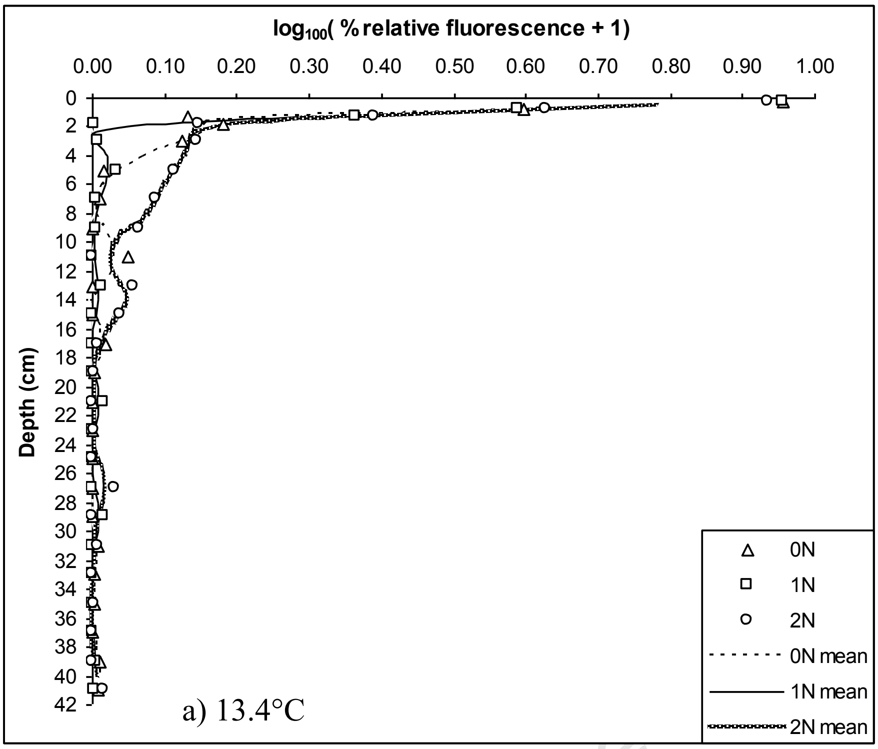


Figure 4.5. Average profiles of luminophore abundance (as relative fluorescence) relative to depth, for cores containing background levels of *C. kraussi* under a range of temperature manipulations. Running means for consecutive values are shown as lines whereas the true means are indicated by symbols.



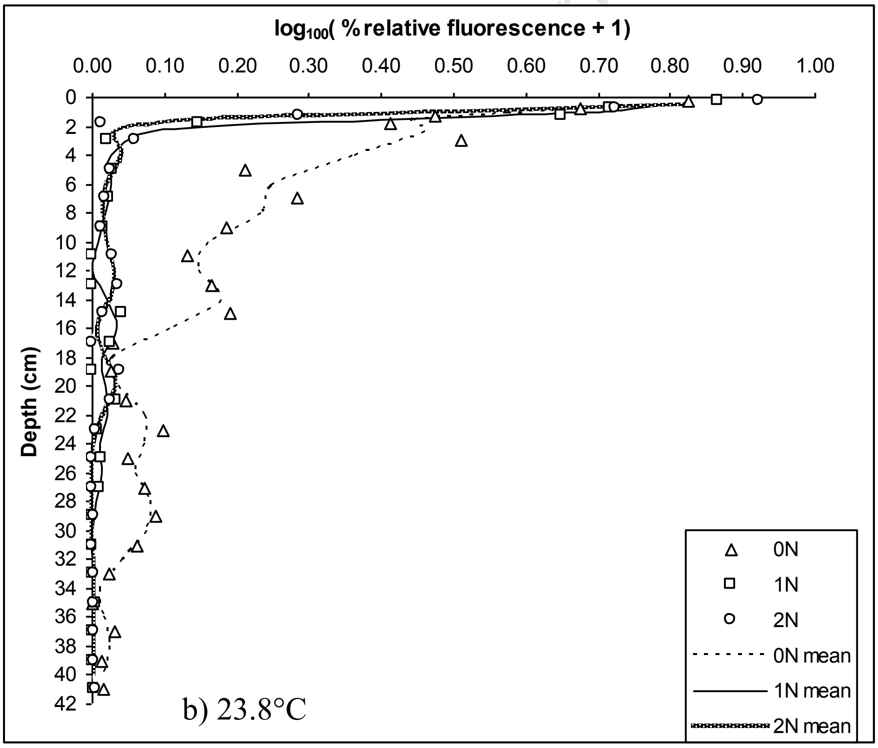


Figure 4.6. Average profiles of luminophore abundance (as relative fluorescence) relative to depth, for cores containing background levels of *C. kraussi* under a range of nutrient levels at (a) winter-type (13.4°C) and (b) summer-type temperature (23.8°C). The running mean is shown (empty symbols) along with the true means (solid symbols).

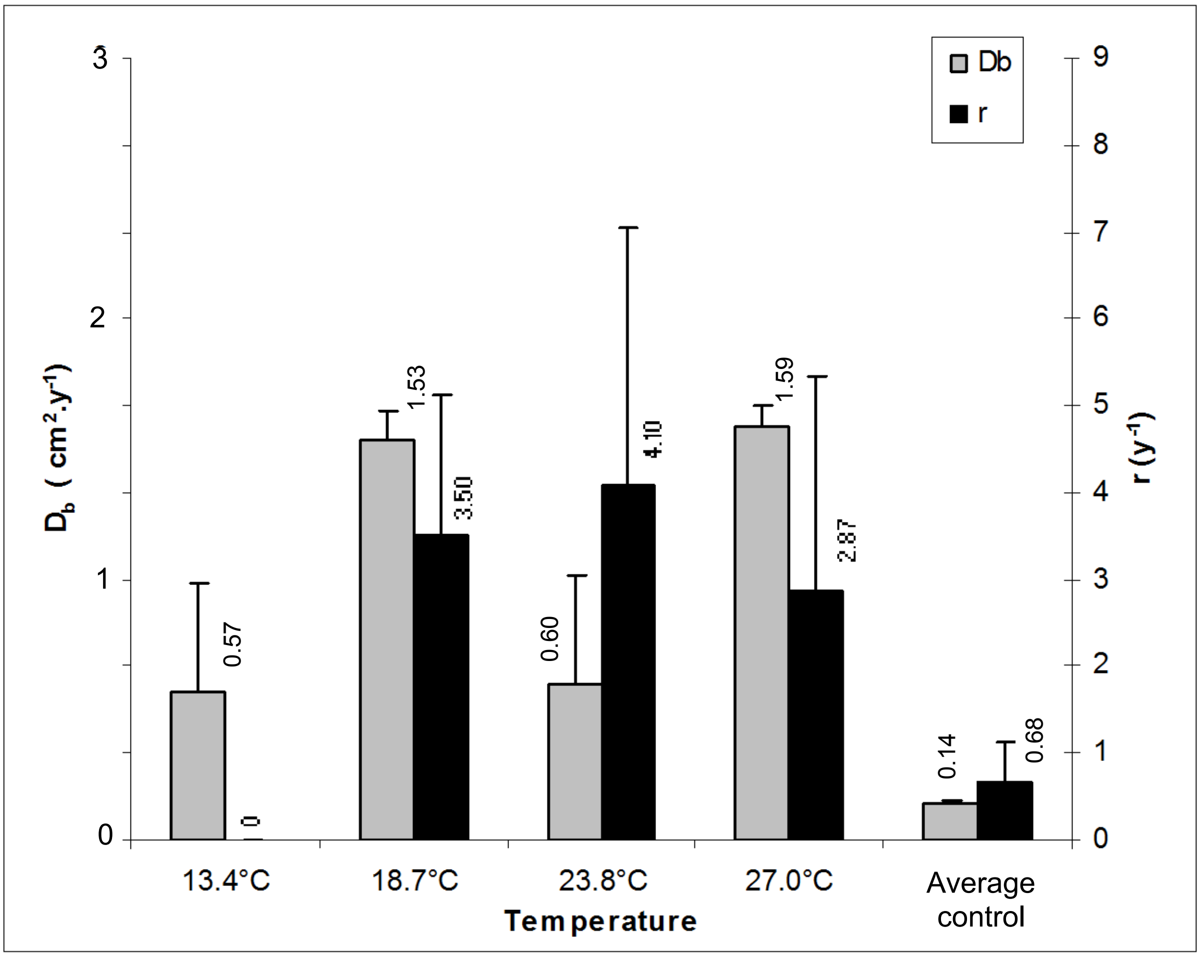


Figure 4.7. The biodiffusion (D_b) and bioadvection (r) coefficients with standard error are shown for the range of manipulated temperatures. No significant relationships are found. The average value for the control, for both the temperature and nutrient experiments, is also given.

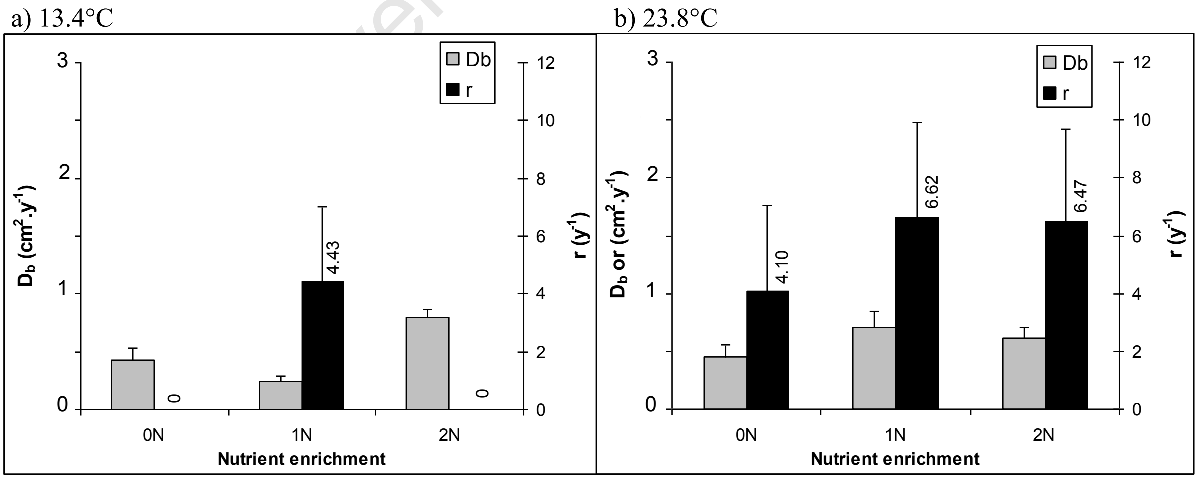


Figure 4.8. The biodiffusion (D_b) and bioadvection (r) coefficients with standard error are shown for three nutrient enrichment levels at (a) winter-type (13.4°C) and (b) summer-type (23.8°C) temperatures.

Appendix 1: Species list for Langebaan Lagoon and Durban Bay in winter 2009 at sites Harbour Roundbush (HR), Yacht Club (YC) and Wilson's Wharf (WW) in Durban and Oesterwal (O), Klein Oesterwal (KO) and Bottelary (B) in Langebaan.

	Durban									Langebaan								
Species	**HR**	**HR**	**HR**	**YC**	**YC**	**YC**	**WW**	**WW**	**WW**	**O**	**O**	**O**	**KO**	**KO**	**KO**	**B**	**B**	**B**
Cirratulus chrysoderma	1														15		1	
Prionospio sexoculata	16	26	26	**1**		2	5	4	1	12	1		1	17	4			
Scoloplos johstonei	1			**3**	6	3	5	6	8		6	20	8	2	5	5		7
Prionospio ehlersi	2		1															
Glycera tridactyla	1	6			2		4	1	4		2			1		1	2	1
Callianassa kraussi	4		24	**17**	43	2			8			3	1		3			1
Nassarius kraussianus	1		7															
Haminoea sp.	1																	
Melita orgasmos		2	5															
Orbinia angrapequensis		2		3	2	1	5	9	1				1			10	10	3
Ceratonereis sp.		1																
Nereid		3																
Anthuriid isopod		1	6															
Syllidae		1										8	9					
Nereid 2		2	9						1		3							
Dosinia hepatica		1						4			3							
Pitar abbreviatus		1	1				1		1									
Cirolanid		1					5	2	6									
Spiroplax spiralis		1	1	1	1	1	2	1	2									
Solen capensis			1															
Melita spp.			1															
Cumacean			1															
Leptanthura laevigata			1															
Eurydice longicornis			3															
amphipod morph 2			1															
Siphunculid			1															
Hydrobia				13	2	9			3									
Cerithium sp.				1					1									
Spionidae				1		1					5		24					
leech (Hirudinea)					1	6		4	1									
Solenocera sp.							1											
cumacean 2							1		1									

Appendix 1 continued

	Durban									Langebaan								
Species	**HR**	**HR**	**HR**	**YC**	**YC**	**YC**	**WW**	**WW**	**WW**	**O**	**O**	**O**	**KO**	**KO**	**KO**	**B**	**B**	**B**
Urothoe grimaldii										2	11	6	4	1	1	13	1	13
Ampelisca palmata										5	4	57	18	6	12	1	2	
Paridotea fucicola										4				1	7	1	1	
Nassarius plicatellus										3				3	4			
Volvarina capensis										4	2			5		2		
Euclymene sp.											7	9	2					
Notomastus sp.											2	1	7			1		
Lysidice sp.											11	5	7			4	6	6
Marphysa depressa											2	6				7	5	1
Nebalia capensis											3	10	19	1	1	1	2	
Ceratonereis erythraeensis											1	14						
Ostracod											3	7	1		1	1		2
Upogebia africana											2							1
Excirolana natalensis											1						1	
Lysianassidae											1	6	5	1		7	27	6
Betaeus jucundus												1						
Nucula nucleus													1					
Notomastus latericeus													2					
Thelepus sp.													1			4		1
Natica tecta													1					
Venerupis corrugatus													1					
Tellimya trigona														2	1	1		
Nephtys sp.														1	1			
Hymenosoma orbiculare															1		1	
Lumbrineris tetraura																1	1	
Tanais philetaerus																1		
Carditella capensis																12	2	4
Diogenes brevirostris																		1

Appendix 2: Species list for Langebaan Lagoon and Durban Bay in summer 2010 at sites Harbour Roundbush (HR), Yacht Club (YC) and Wilson's Wharf (WW) in Durban and Oesterwal (O) Klein Oesterwal (KO), and Bottelary (B) in Langebaan.

	Durban									Langebaan								
Species	**HR**	**HR**	**HR**	**YC**	**YC**	**YC**	**WW**	**WW**	**WW**	**O**	**O**	**O**	**KO**	**KO**	**KO**	**B**	**B**	**B**
Cirratulus chrysoderma										4				3	2	1	1	1
Prionospio sexoculata								2	1					1				
Scoloplos johstonei								2		1	3	5						
Glycera tridactyla	1		3		1	1		5						1		1		1
Callianassa kraussi	1	3						2		7	6	2	1		4	5	2	2
Melita orgasmos	1	1																
Orbinia angrapequensis	7	2	1	1	2	3	1	6	1	16		1		1	1	3	24	6
Ceratonereis sp.										1								
Nereid												2						
Anthuriid isopod								1										
Syllidae										10								
Nereid 2											1							
Pitar abbreviatus								1										
Spiroplax spiralis								2										1
Melita spp.																1	8	1
Siphunculid										1								
Spionidae																3		
leech (Hirudinea)				1				1										
Solenocera sp.							2						1					
Cumacean 2	1																	
Urothoe grimaldii										9	4	3			1	4	14	1
Ampelisca palmate										14		9			1		1	2
Volvarina capensis													1		1		1	1
Euclymene sp.										2	2							
Notomastus sp.										2	2	2						
Lysidice sp.										1	2	2	1			4	14	6
Marphysa depressa										2								4
Nebalia capensis										5	10	5						
Ceratonereis erythraeensis												6			1			
Ostracod												1					4	3
Upogebia africana								1										
Excirolana natalensis		1				1												
Sphaerosyllis semiverrucosa			1							21	4	1				4	51	3
Notomastus latericeus	3									7	3	1					4	5
Thelepus sp.										4		1						1
Tellimya trigona															1	2		1
Nephtys sp.													1					
Hymenosoma orbiculare												1			1	1		
Tanais philetaerus																	2	
Carditella capensis																2	6	5
Diogenes brevirostris												1						
Lasaea adansoni turtoni										2	3	1					5	1
Paratylodiplax edwardsii																	1	
Ostracod *2*										1								
Ostracod *3*											1							
Gastrosaccus psammodytes																	1	
Glycera tridactyla										1	1	2						1
Desdemona sp.										1								3
Polychaete unidentified													1	10	2			
Dotilla fenestrata		2																
Echiuran										7	2	3						

Appendix 3: List of macrofaunal species together with the functional group to which they were assigned for the purposes of this thesis.

Species	**Notes**	**Assigned Functional Group**
Cirratulus chrysoderma	Cirratulidae	Deposit feeders -surface
Prionospio sexoculata	Spionidae	Deposit feeders -surface
Scoloplos johstonei	Orbiniidae	Deposit feeders - burrowers
Prionospio ehlersi	Spionidae	Deposit feeders -surface
Glycera tridactyla	Glyceridae	Predators and Scavengers - burrowers
Callichirus kraussi	Callianassidae	Deposit feeders - burrowers
Nassarius kraussianus	Gastropoda, Prosobranchia	Predators and Scavengers - surface
Haminoea sp.	Gastropoda, Opisthobranchia	Grazers - surface
Melita orgasmos	Amphipoda	Deposit feeders -surface
Orbinia angrapequensis	Orbiniidae	Deposit feeders - burrowers
Ceratonereis sp.	Nereidae	Deposit feeders - burrowers
Nereid	Nereidae	Deposit feeders - burrowers
Anthuriid isopod	Isopoda	Deposit feeders -surface
Syllidae	Polychaeta, Syllidae	Predators and Scavengers - surface
Nereid 2	Polychaeta, Nereidae	Deposit feeders - burrowers
Dasinia hepatica	Bivalvia, clam	Suspensions feeders - surface
Pitar abbreviatus	Bivalvia, clam	Suspensions feeders - surface
Cirolanid	Isopoda, Cirolanidae	Predators and Scavengers - surface
Spiroplax spiralis	Decapoda, crab	Deposit feeders - burrowers
Solen capensis	Bivalvia, clam	Suspensions feeders - surface
Melita spp.	Amphipoda	Deposit feeders -surface
Cumacean	Cumacea	Deposit feeders -surface
Leptanthura laevigata	Isopoda	Predators and Scavengers - surface
Eurydice longicornis	Isopoda	Predators and Scavengers - surface
Amphipod morph 2	Amphipoda	Deposit feeders -surface
Siphunculid	Siphunculida	Deposit feeders -surface
Hydrobia	Gastropoda	Grazers - surface
Cerithium sp.	Turret shell	Grazers - surface
Spionidae	Spionidae	Deposit feeders -surface
leech (hirudinea)	Hirudinea	Predators and Scavengers - surface
Solenocera sp.	Decapoda, Penaeoidea	Deposit feeders -surface
cumacean 2	Cumacea	Deposit feeders -surface
Urothoe grimaldii	Amphipoda	Deposit feeders - burrowers
Ampelisca palmate	Amphipoda	Suspensions feeders - surface
Paridotea fucicola	Isopoda	Grazers - surface
Nassarius plicatellus	Gastropoda	Predators and Scavengers - surface
Volvarina capensis	Gastropoda	Predators and Scavengers - surface
Euclymene sp.	Polychaeta	Deposit feeders -surface
Notomastus sp.	Polychaeta	Deposit feeders - burrowers
Lysidice sp.	Polychaeta	Predators and Scavengers - burrowers

Appendix 3 continued

Species	Notes	Assigned Functional Group
Marphysa depressa	Polychaeta, Eunicidae	Predators and Scavengers - burrowers
Nebalia capensis	Phyllocarida	Suspensions feeders - surface
Ceratonereis erythraeensis	Nereidae	Deposit feeders - burrowers
Ostracod	Ostracoda	Deposit feeders -surface
Upogebia Africana	Decapoda	Deposit feeders - burrowers
Excirolana natalensis	Isopoda, Cirolanidae	Predators and Scavengers - surface
Lysianassidae	Amphipoda	excluded from functional group analysis
Betaeus jucundus	Decapoda, shrimp	Deposit feeders - burrowers
Nucula nucleus	Bivalvia, clam	Suspensions feeders - surface
Sphaerosyllis semiverrucosa	Polychaeta, Syllidae	Predators and Scavengers - surface
Notomastus latericeus	Polychaeta	Deposit feeders - burrowers
Thelepus sp.	Poychaeta, Terrebellidae	Suspensions feeders - surface
Natica tecta	Gastropoda, Prosobranchia	Predators and Scavengers - surface
Venerupis corrugatus	Bivalvia, clam	Suspensions feeders - surface
Tellimya trigona	Bivalvia. Clam	Suspensions feeders - surface
Nephtys sp.	Polychaeta, Nephtidae	Predators and Scavengers - burrowers
Themisto gaudichaudi	Amphipoda	Pelagic - excluded from analyses
Hymenosoma orbiculare	Decapoda, crab	Predators and Scavengers - surface
Lumbrineris tetraura	Polychaeta, Eunicidae	Predators and Scavengers - burrowers
Tanais philetaerus	Tanaiidacea	Predators and Scavengers - surface
Carditella capensis	Bivalvia, Clam	Suspensions feeders - surface
Diogenes brevirostris	Decapoda, hermit crab	Predators and Scavengers - surface
Lasaea adansoni turtoni	Bivalvia, Clam	Suspensions feeders - surface
Paratylodiplax edwardsii	Decapoda, crab	Deposit feeders - burrowers
Ostracod *2*	Ostracoda	Deposit feeders -surface
Ostracod *3*	Ostracoda	Deposit feeders -surface
Gastrosaccus psammodytes	Mysidacea, Shrimp	Deposit feeders -surface
Glycera tridactyla	Polychaeta, Glyceridae	Predators and Scavengers - burrowers
Desdemona sp.	Polychaeta, Sabellidae	Suspensions feeders - surface
Polychaete unidentified	Polychaeta	excluded from functional group analysis
Dotilla fenestrate	crab-decapod	Deposit feeders -surface
Echiuran	Echiura	Deposit feeders -surface
Exosphaeroma truncatitelson	Isopoda	Grazers - surface
Marphysa elettueni	Polychaeta, Eunicidae	Predators and Scavengers - burrowers
Scallibregmidae	Polychaeta, Scallibregmidae	Deposit feeders - burrowers
Assiminea globulus	Gastropoda	Grazers - surface
Perinereis nuntia vallata	Nereidae	Deposit feeders - burrowers
Spionidae	Polychaeta, Spionidae	Deposit feeders -surface
Eunicidae	Polychaeta, Eunicidae	Predators and Scavengers - burrowers