ECOLOGY AND BEHAVIOUR OF BURROWING PRAWNS AND THEIR BURROW SYMBIONTS

ROPAFADZO KELEBUHILE MOYO

Supervisor:

Dr Deena Pillay

Department of Biological Sciences

University of Cape Town

Submitted to the University of Cape Town
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Department of Biological Sciences

University of Cape Town

DECLARATION

I know the meaning of plagiarism and declare that this thesis is to the best of my knowledge my original work except where sources have been acknowledged and that it has not been submitted for any degree or examination in any other university. All the sources I have used or quoted have been indicated and acknowledged by complete references. I am now presenting the thesis for examination for a Degree in Master of Science (Zoology) to the University of Cape Town.

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Ropafadzo K. Moyo

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Date

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ABSTRACT

Ecosystem engineers play important roles as determinants of community dynamics by modulating resource availability for other species. Marine soft-sediment ecosystems are dominated by burrowing engineers which indirectly create biogenic structures that often attract other species, often leading to the evolution of symbiotic relationships. Engineered structures provide non-trophic (e.g. refuge) and trophic functions (e.g. food) for burrow symbionts, however, the relative importance of these functions for symbionts is poorly understood. The behavioural interactions between burrowing engineers and their burrow symbionts are also poorly understood, mainly due to the difficulty in conducting behavioural observations *in situ*. This study aimed to enhance our understanding of the ecological processes and behavioural interactions underlying symbiotic relationships between a dominant South African ecosystem engineer (*Callianassa kraussi*) and its burrow symbiont (*Betaeus jucundus*) in soft sediment systems in Langebaan Lagoon. The study specifically quantified the relative importance of host abundance (a proxy for non-trophic functions) and food availability (trophic functions) provided by burrows of *C. kraussi* in influencing the abundance and distribution of *B. jucundus*. The second aim was to quantify behavioural changes of *C. kraussi* in the presence and absence of the symbiont *B. jucundus* and determine if the sex of *C. kraussi* influences its behavioural response to *B. jucundus*. Results indicate that at a patch scale, trophic functions (food availability) provided by burrows was more important than non-trophic functions in determining symbiont abundance and distribution. However, at an ecosystem scale, non-trophic functions could be a very important determinant of symbiont abundance and distribution. In terms of behavioural interactions, the presence of *B. jucundus* elicited three distinct behavioural responses from *C. kraussi*: intolerance, semi-tolerance and tolerance. In the absence of *B. jucundus*, there was no difference between male and female *C. kraussi* behaviour but this pattern changed in the presence of *B. jucundus*.

Overall, the study has contributed to increasing our understanding of ecological processes that determine the abundance and distribution of burrow symbionts in marine soft-sediments as well as behavioural interactions between burrowers and their symbionts.

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Chapter 1: General Introduction

1.1 Ecosystem Engineering

Community dynamics are governed by the interplay between the abiotic environment and biotic interactions acting from outside and within an ecosystem (Kent *et al*. 2007). Historically, biotic interactions were dominated by research on trophic interactions (predation, grazing etc). However, lately, there is a growing appreciation of the importance of non-trophic and facilitative interactions in driving community dynamics (Ohgushi, 2008). Ecosystem engineering has emerged to be one of the major forms of non-trophic interactions responsible for shaping communities and ecosystems.

Ecosystem engineers are organisms that modify their environments causing state changes in abiotic and biotic material through construction, maintenance or destruction of structures (Jones *et al.* 1994). The effects of ecosystem engineers on communities have been of great interest to ecologists with numerous studies being conducted around the idea (Coleman & Williams, 2002; Berkenbusch & Rowden, 2003; Jouquet *et al.* 2006; Kochmann *et al*. 2008). Ecosystem engineering is ubiquitous with multiple ramificationsfor both abiotic and biotic ecosystem components (Jones *et al*. 2010). Ecosystem engineers are known to strongly influence ecosystem processes and functioning by altering the environment through their activities (Jones *et al*. 1997).

Ecosystem engineers influence other species by modulating availability of resources such as energy, materials, space and food (Jones *et al.* 1997; Jones *et al.* 2010). The beaver for example, which is a well-researched ecosystem engineer, constructs dams that lead to the creation of wetlands where sediments and organic matter are retained. This in turn modifies nutrient cycles and ultimately influences plant and animal communities in the area (Jones *et al*. 1994). The creation of structured habitats is therefore an important function provided by engineers. Engineered habitats generally have facilitative effects on other species by adding structure/habitats to unstructured environments (Gribben *et al.* 2009). Studies have shown that these structure forming engineers can increase and maintain species richness (Dittman 1996; Wright *et al.* 2002),asthe structures formedby ecosystem engineers are often used as habitats by other species (Jones *et al*. 2010).

1.2 Ecosystem Engineering and symbiotic interactions

An important feature of ecosystem engineering is that engineer activities can result in feedbacks to the engineer and co-occurring species at both ecological and evolutionary scales (Jones *et al*. 2010). On an evolutionary scale, feedbacks generated by ecosystem engineering can lead to the evolution of intricate symbiotic relationships with co-occurring organisms (Pillay, 2010). The ability of engineers to create favourable abiotic conditions within environments results in the attraction of certain species (Reise, 1981), leading to varying dependence on the engineered ecosystem and the evolution of various symbiotic relations. Symbiosis refers to a class of biological interaction in which two or more organisms are closely associated with each other, with at least one organism receiving some sort of benefit from the relationship (Mariscal, 1970). The interaction can either be a lifelong interaction (constant) or part of the lifecycle of the symbiont (temporary) (Daida *et al*. 1996; Roossinck, 2005; Kolwzan *et al*. 2006).

Symbiotic associations include interactions such as commensalism, mutualism and parasitism (Stachowicz, 2001; Hooper *et al.* 2005).Commensalism has been defined as an interaction where one organism benefits from forming a relationship with a second organism, but the second one is unaffected by the relationship (Fath& Patten, 1998). The relationship between the whale shark *Rhincodontypus* and several remora fish from the genus *Remora* is an example of a commensal relationship. Remora fish associate themselves with whale sharks and usually attach themselves to the shark in order to gain protection, free rides and food scraps from shark feeding.The shark does not seem to benefit or suffer from this association (Clark & Nelson, 1997). Commensalism is however, difficult to demonstrate in nature. In depth studies of what were considered to be commensal relationships usually reveal some sort of effect on the second species resulting in most of the relationships actually beingeither mutualistic or parasitic (Hogan, 2012).

Parasitism is a negative interaction where one species (parasite) benefits while the other (host) is negatively affected (Johnson et al. 1997), resulting in a reduction in host fitness (Christe *et al*. 1995). However, it is important to note that there are some studies that have identified potential benefits to being parasitized (Bush, 2001). For example, parasites can act as internal sinks for heavy metals in polluted environments. Sures *et al.* (1999) found that intestinal parasites in fish had higher concentrations of heavy metals in their tissue compared to the host fish. The cost of being parasitized was thus offset by the benefits of using the parasites as accumulators of harmful toxins.

Mutualism has been defined as a positive interaction where both species benefit from the interaction (Stachowicz, 2001), with mutualists[1] exhibiting some level of co-evolution (Hay *et al*. 2004). Mutualism has also been defined as an interaction where the benefits of cooperation outweigh the costs for all species

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[1] Mutualists defined here as partners involved in a relationship in which both benefit.

involved without species being co-evolved (Bronstein, 1994b). The latter argues that there are mutualistic interactions that exhibit coevolved traits but have no evolutionary history and that not all mutualisms develop through co-evolutionary processes (Hay *et al.* 2004). There has also been a lot of disagreement on the definitionof benefit within mutualism. Boucher *et al.* (1982) defined benefit as the increase in fitness of species involved in mutualisms.

The relationship between corals and zooxanthallae is a well-studied example of mutualism. Corals are foundation species (Hay *et al.* 2004) that have a mutualistic relationship with algae called zooxanthallae (Goreau *et al.* 1979). The algae increase rates of coral calcification (Knowlton, 2001) and growth of corals by leaking amino acids, sugars, complex carbohydrates and peptides produced during photosynthesis across the cell membrane into the corals (Goreau *et al.* 1979; Hoegh-Guldberg, 1999). In return, the algae receivea stable substrate at depths suitable for photosynthesis and nutrients excreted by the coral, including ammonia and phosphates, that can be used during photosynthesis (Goreau *et al.* 1979; Hoegh-Guldberg, 1999).

There are two main types of mutualism. The first type is coevolved, obligate mutualism, and is a by-product of co-evolution of species, and leads to the evolution of high species interdependence (Dean, 1983). Most mutualists that are co-evolved become specialised mutualists and thus obligate. Obligate mutualists cannot survive in the absence of their mutualist partners (Dean, 1983). Facultative mutualism is the second type of mutualism, in which mutualists are beneficial to each other but can survive independently (Dean, 1983). This type of mutualism is conditional and can change from mutual to neutral to antagonistic depending on local environmental conditions (Hay *et al.* 2004).

1.3 Symbiosis in ecology and evolution

Symbiosis is an important driving force of evolution (Margulis & Bermudas, 1985). As species interact, they evolve in response to each other and this is known as co-evolution (Janzen, 1980). The yucca plant-moth mutualism (Hay *et al*. 2004) is a good example of the latter. The female yucca moth, which is a specialised yucca plant pollinator, has evolved specific mouthparts to collect pollen from yucca flowers (Pellmyr & Huth, 1994). The yucca moth cuts into the ovary of the plant to deposit an egg and also actively transfers the pollen onto the stigma to ensure fertilisation and seed development. The moth larva consumes some of the seeds but leaves most of them intact (Pellmyr & Huth, 1994). Neither species can reproduce without the other because the moth is required to pollinate the yucca flower, which in turn provides the substrate for moth oviposition, thus making this an obligate mutualism (Pellmyr *et al*. 1996).

Species involved in negative interactions(as in parasitism) will have an antagonist co-evolution, which results in an evolutionary arms race. Parasitism has a strong negative effect on the host; therefore, there is a great selection pressure acting on the host to develop mechanisms against the parasites. As the host evolves, the parasite also evolves adaptive strategies to overcome host defences. This leads to an evolutionary arms race where both species try to stay ahead of the other, which is thought to lead to a rapid co-evolution (Soler *et al*. 1998).

1.4 Ecosystem engineering and symbiosis in marine soft sediment ecosystems

Scientists have acknowledged that symbiosis plays an important role in ecological and evolutionary processes, although our understanding of the conditions favouring the evolution and maintenance of these relationships is basic (Bronstein, 2001). The situation is much worse in marine ecosystems, particularly benthic soft-sediments (Charbonneau *et al*. 1997), where several intricate symbiotic relationships have evolved in response to ecosystem engineering. One of the main reasons for the lack of in-depth ecological information on symbiotic interactions in marine soft-sediments relates to the difficulty in conducting *in situ* behavioural observations, because hosts and symbionts are concealed by the matrix in which they live in (Charbonneau *et al*. 1997).

Burrowing invertebrates dominate marine soft-sediments (Peterson, 1991).The burrowing and reworking of sediments result in the formation of biogenic structures (Reise, 1981) that have been viewed as ‘elite structures’ (Bromley, 1996).Burrowing by invertebrates change resource availability for other organisms within the system (Branch & Pringle, 1987), thus making them ecosystem engineers. These burrows offer several advantages to other organisms such as providing refuge from dangers in overlying waters, food resources and oxic microhabitats (Pillay, 2010). In providing such stable microenvironments, these elite structures often stimulate co-inhabitation (Reise, 1981) usually leading to the evolution of elaborate symbiotic assemblages (Pillay, 2010).

Echiuran worms, also known as “Inkeeper Worms”, are one of the most well-known burrowing groups in marine soft-sediments that house elaborate symbiotic assemblages. Anker *et al* (2005) found roughly seventeen different species that are symbionts of the innkeeper worm. However, only are few are obligate symbionts. Echiurans inhabit intertidal and subtidal mudflats in U-shaped burrows (Arp *et al*. 1992) containing water rich in food and oxygen pumped through the burrow by the host (Anker *et al*. 2005). The “fat innkeeper worm” (*Urechis caupo*),which is one of the most well studied echiurans, has been found to form symbiotic relationships with both obligate symbionts such as the scale worm *Hesperonoe adventor* and facultative symbionts such as the crab *Scleroplax granulata* (Anker *et al.* 2005). Most echiuran worms host no more than five symbiont species, but there are other worm species such as *Lissomyemaexilli* that have been found to host as many as eleven different symbiotic species (Anker *et al.* 2005).

Polychaetes are also known to live commensally with other burrowing species.Current estimates indicate that there are roughly 292 species of polychaetes involved in symbiotic relationships (Martin & Britayev, 1998). However, many polychaete species themselves act as hosts to other symbionts. For example, *Amphitrite ornate* and *Chaetopterus variopedatus* burrows are known to house symbionts such as pinnotherid crabs (McDermott, 2005; 2009).

Echinoderms have been known to harbour symbionts within their burrows (Anker *et al.* 2005).For example, the burrowing starfish *Astropecten irregularis* has a symbiotic relationship with the polychaete *Acholoesquamosa,* which obtains an unchallenged access to the stomach contents of the starfishas well as protection provided by the starfish (Freeman *et al.* 1998).

Various burrowing crustaceans form symbiotic relationships with a number of species (Anker *et al.* 2005). The black-claw crab *Panopeus herbstii* found in Mid-Atlantic salt marshes, builds U-shaped burrows that remain filled with water during low tide (Silliman *et al.* 2003). The shrimp *Alpheus heterochaelis* lives commensally within the burrows of the crab where is acquires a habitat, protection from predation and access to food (Silliman *et al.* 2003).Burrows of crustacean stomatopods are known to house various bivalves (Anker *et al.* 2005; Morton,1972).

Thalassinidea, an infraorder of crustaceans, are amongst the most dominant and influential burrowing species globally (Dworschak, 2000). It is important to note that molecular studies have separated this infraorder into two separate groups, the Axiidea and Gebiidea, using nuclear and mitochondrial genes (Robles et al. 2009).

For convenience, the colloquial term “thalassinidean” will be used in this dissertation when referring to the two orders collectively.

Considerable research effort has been dedicated to understanding the effects of thalassinidean burrowing activities on co-occurring non-symbiotic assemblages (Berkenbusch & Rowden, 2003; Siebert & Branch, 2006; Pillay *et al.* 2007a; Henninger & Froneman, 2013). However, thalassinidean are also known to host a variety of symbiont species within soft sediment systems (Branch *et al.* 2010).A brief summary of thalassinidean symbionts and their hosts is presented in table 1.1.

Table 1.1: Summary of symbiotic organisms found with burrowing thalassinideans.

Host	**Symbionts**	**Relationship**	**Study location**
Neotrypaea californiensis	*Hesperonoe complanata*	Commensal (McGinite, 1935)	California, USA
	Cryptomya californica	Commensal (Nara *et al*. 2008)	California, USA
	Ione cornuta	Parasitic – attaches to and feeds on host (Williams &An, 2009)	West coast of Northern America
Upogebia major	*Hesperonoe hwanghaienisis*	Commensal (Sato *et al*. 2001)	Chinese coast in the yellow sea, Japan
	Peregrinamor ohshimai	Commensal (Kato &Itani, 1995)	Japan
	Acmaeopleura sp. (unidentified species)	Ecoparasitic (Itani, 2001)	Japan
	Acmaeopleura toriumii	Commensal (Itani, 2001)	
Upogebia yokoyai	*Cryptomya truncata*	Commensal with a mild form of parasitism (removal of suspended particles from host burrows) (Nara *et al*. 2008)	Kyoto, Japan
	Galeommatoidean bivalves	Commensal (Kato &Itani, 1995)	Japan
	Eutaeniichthys gilli	Commensal with *U. yokoyai* sometimes displaying antagonism towards the symbiont (Henmi & Itani 2014)	Pacific, Japan
Axiposisserratifrons	*Ascidia subterranea*	Mutualistic. (Kneer*et al*. 2013)	Tropics
Sergio trilobata	*Clausidium dissimile*	Commensal (Corsetti & Strasser, 2003)	Western Atlantic tropical environments
Callichirus major	Pea crabs	Symbiotic (true nature of symbiosis is unknown) (Ambrosio & Brooks, 2011, Peiró & Mantelatto, 2011; Peiró *et al*. 2011).	North Carolina USA through Gulf of Mexico to southern Brazil
Sergio mirim	*Pinnixa patagoniensis*	Commensal (Alves & Pezzuto, 1998)	Western Atlantic

Neotrypaea californiensis and *Upogebia yokoyai* are mud dwelling burrowing thalassinidean shrimps (hereafter referred to as shrimps for convenience) that are known to known to form symbiotic relationships with pea crabs, scale worms and bivalves (Peterson, 1991; Nara *et al*. 2008). *Cryptomya californica* and *Cryptomya truncata* are bivalves that live in the burrows of *N. californiensis* and *U. yokoyai* and utilise the water currents generated by the host for feeding, respiration and excretion (Nara *et al*. 2008).

In Southern Brazil, the ghost shrimp *Sergio mirim* builds burrows in intertidal and subtidal zones, providing a suitable habitat for several groups of invertebrates such as pinnotherid crabs (Alves & Pezzuto, 1997). Alves & Pezzuto (1997) observed high incidences of *Pinnixa patagoniensis* in the burrows of *S. mirim*, which were attributed to the space available in the burrows of *S. mirim* along with high concentrations of food and oxygen. Another pinnotherid crab, *Austinixa aidae,* has been described as a symbiont of the burrowing shrimp *Callichirus major* that also inhabits intertidal zones of many beaches in Brazil (Peiró & Mantelatto, 2011).

Goby-shrimp associations are very common (MacGinitie, 1939; Itani *et al*. 1996; Wirtz, 2008). The thalassinidean shrimp *Neotrypaea biffari* lives in pairs and build their burrows in sand. The burrows are also home to the blind gobies (*Typhlogobius californiensis)* which also live in pairs commensally with *N. biffari* (MacGinitie, 1939).Hung Liu *et al.* (2008)recorded the goby *Austrolethopis wardi* in the burrows of the burrowing shrimp *Neaxius acanthus* and noted that the goby is never observed outside the burrows.

In Southern African estuaries and shallow marine systems, *Callichirus kraussi* and *Upogebia africana* (Fig. 1.2 A & B) are the most dominant benthic organisms in terms of their abundance and ecological effects on residents (Pillay *et al*. 2007; Teske *et al*. 2009). *Callichirus kraussi* modifies sedimentary habitats at very large spatial scales (km), primarily through sediment reworking and turnover during burrow construction and maintenance. This in turn modifies several biogeochemical processes such as oxygen and nutrient fluxes across the sediment-water interface. Ecosystem modification by *C. kraussi* affects several other co-occurring organisms across multiple trophic positions (Pillay & Branch 2011). *Upogebia africana,* like *C. kraussi*, is also considered an ecosystem engineer due to its capacity to transform sediments. However,its sediment turnover rate is much lower than that of *C. kraussi* (Wynberg & Branch, 1994).

The distribution ranges of *C. kraussi* and *U. africana* are shown in Figure 1.1. The range of *U. africana* extends from Lamberts Bay north of Cape Town to the Delagoa Bay in Mozambique and it is more abundant in muddy sediments (Forbes, 1973; Branch *et al*. 2010). *Callichirus kraussi* has a much wider range extending from Luderitz Bay in Namibia to the Inhambane region in Mozambique and is most abundant in sandy sediments (Branch *et al*. 2010).

Callichirus kraussi and *U. africana* are known to form symbiotic relationships with an alpheid shrimp (*Betaeus jucundus*), the six-legged crab (*Spiroplax spiralis)* and scale-worm (*Antinoe lactea*) (Fig 1.2 C, D & E) (Branch *et al*. 2010). The goby *Psammogobius knysnaensis* (Fig 1.2F) is also occasionally found in burrows of *C. kraussi* and *U. africana. Betaeus jucundus* may be an obligate symbiont in the burrows of mud and sand prawns. However, the socio-biology of this species is not well known (Anker & Baeza, 2012), and is therefore referred to in literature as either a symbiont or a mutualist.

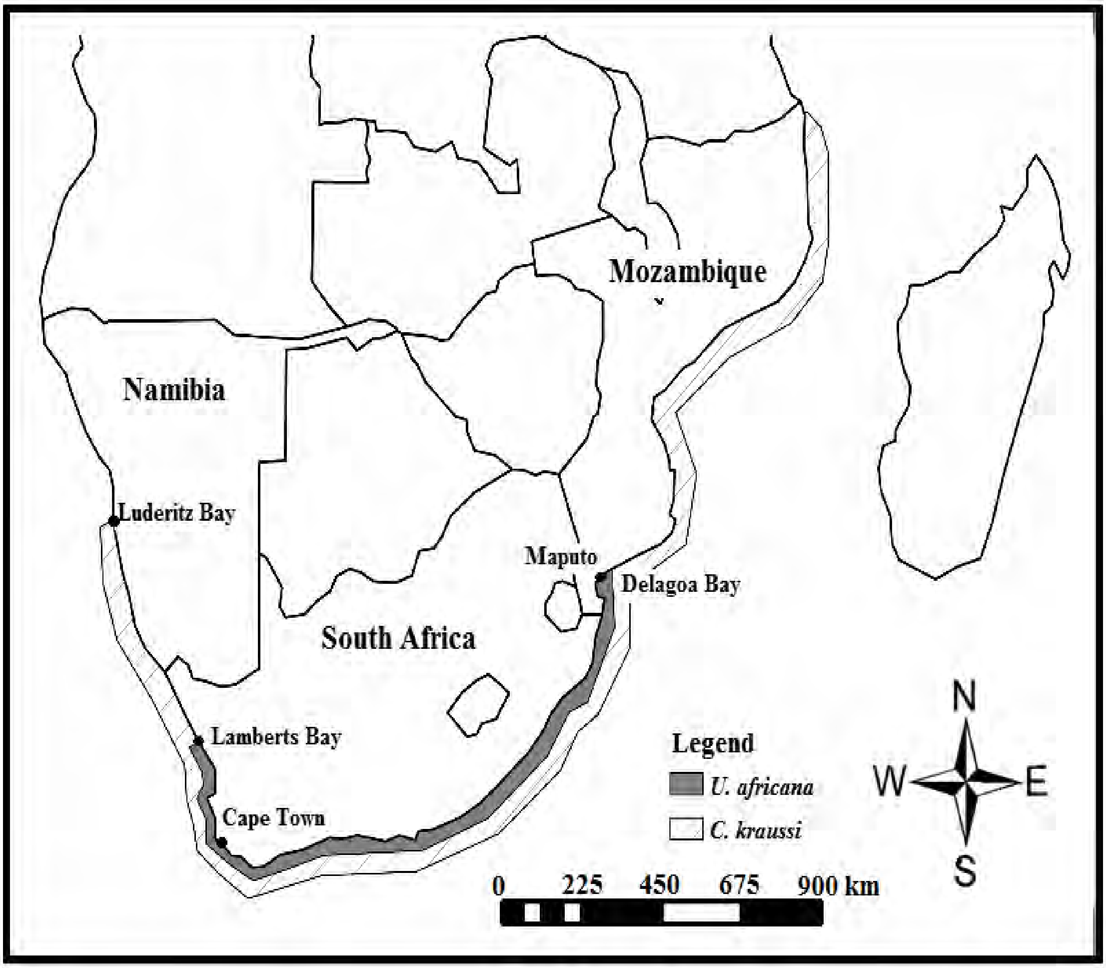

Fig. 1.1: Distribution ranges of *C. kraussi* and *U. africana* along the Southern African coast.

The distribution of the burrow symbionts is embedded within the distribution range of the burrowing prawns. The known distribution of *A. lactea* is limited to the Saldanha Bay and Langebaan area while the distribution ranges of *B. jucundus* and *S. spiralis* extend from Saldanha Bay in the Western Cape to KwaZulu-Natal (Branch *et al*. 2010).

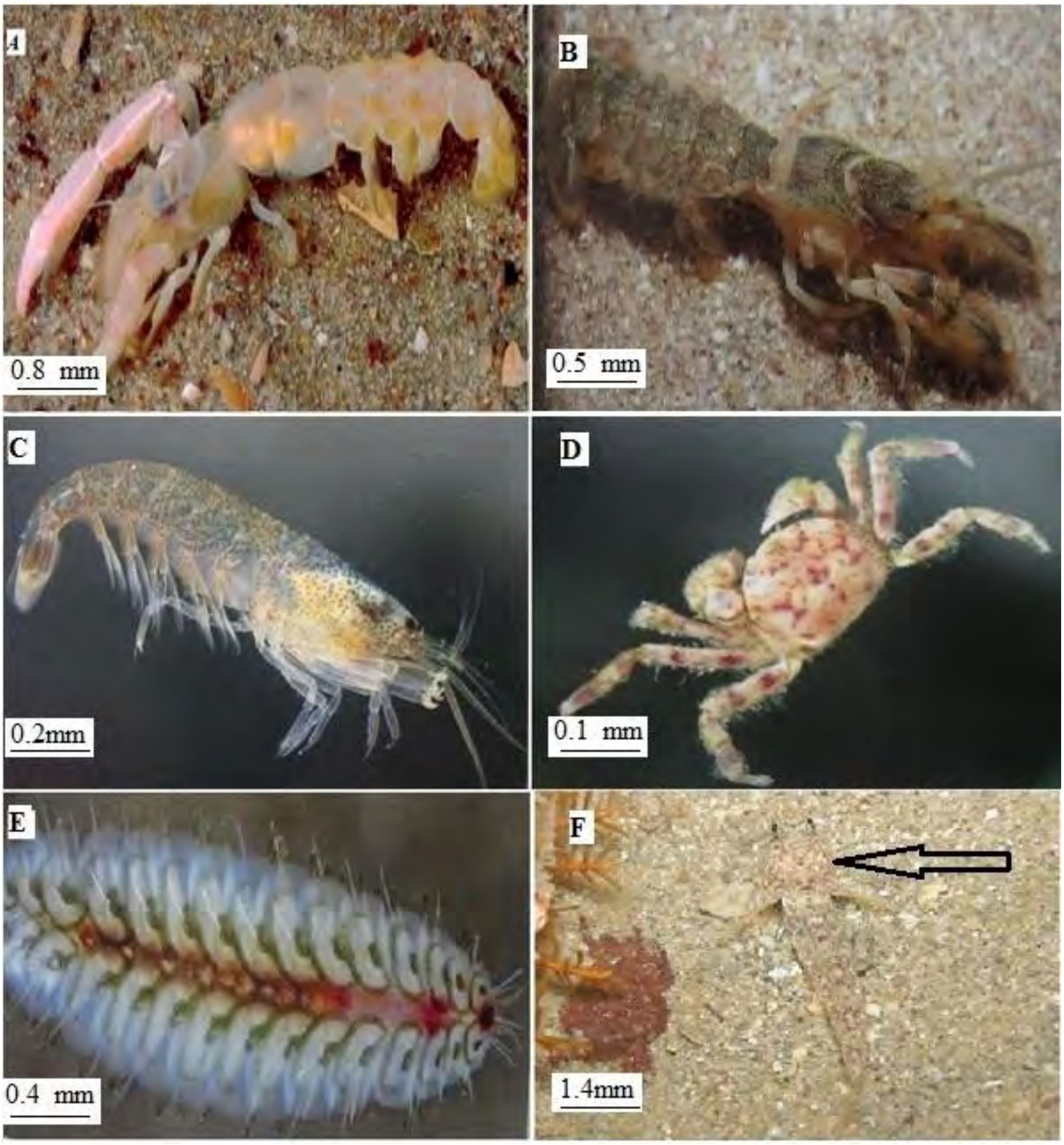

Fig. 1.2: Thalassinidean prawns (A) *Callichirus kraussi* and (B) *Upogebia africana* and burrow symbionts (C) *Betaeus jucundus,* (D) *Spiroplax spiralis* (E)*Antinoe lactea* and (F) *Psammogobius knysnaensis* (Photos: Griffiths CL).

1.5 Dissertation layout

This dissertation broadly aims to contribute to improving our understanding of the ecological processes and behavioural interactions underlying symbiotic relationships between ecosystem engineers (*C. kraussi* and *U. africana*) and symbionts (*A. lactea, B. jucundus* and *S. spiralis*) in soft sediment systems in Langebaan Lagoon.

The arrangement of this dissertation showing the chapter layout and aims is provided in Fig 1.3. Following this introductory chapter, chapter two focuses on the ecological factors determining the distribution and abundance of burrow symbionts in Langebaan Lagoon. Engineered systems provide non-trophic (e.g. refuge) and trophic functions (e.g. food) that attract symbionts into burrows. However, most studies have not distinguished between these two functions in determining symbiont densities. The aim of this chapter is to quantify the relative importance of host abundance (a proxy for non-trophic functions) and food availability (trophic functions) in burrows in influencing the abundance and distribution of burrow symbionts. If non-tropic functions are better predictors of symbiont abundance, then a positive relationship between host abundance and symbiont abundance is expected. However, if trophic functions best predict symbiont abundances, symbiont abundance is expected to increase with food availability in burrows.

Dissertation layout

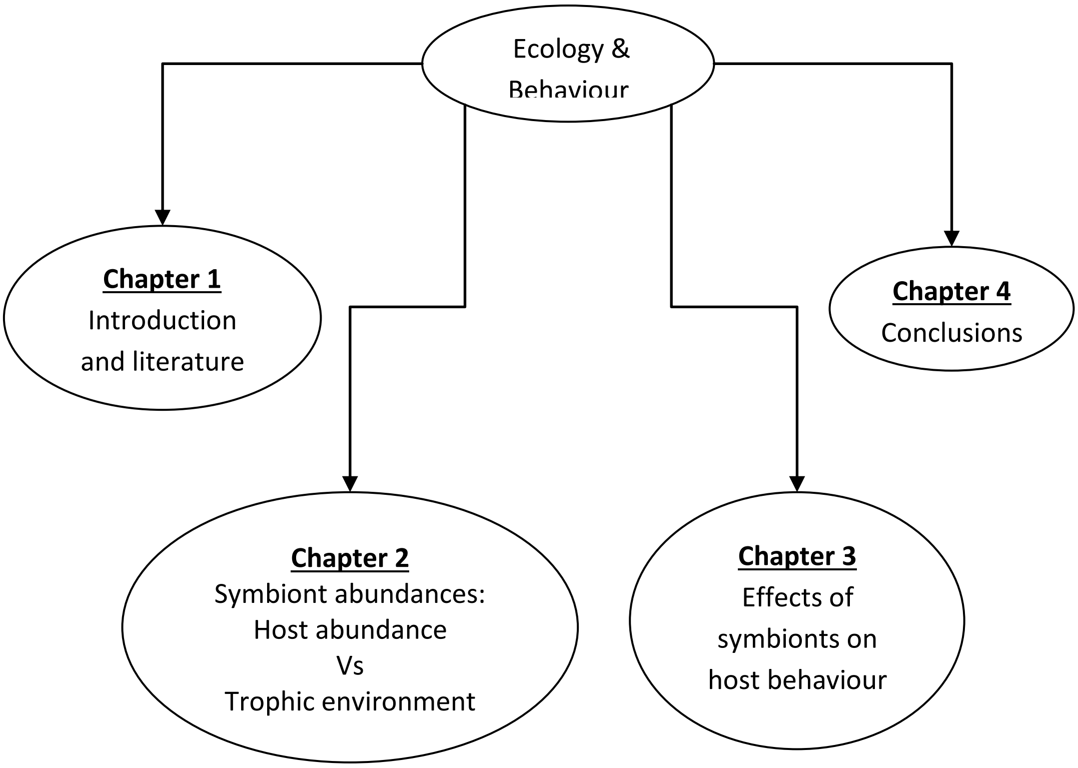

Fig. 1.3: General layout of thesis showing a breakdown of component chapters.

Chapter three focuses on the influence of the burrow symbiont *B. jucundus* on the behaviour of the burrowing prawn *C. kraussi.* In behavioural ecology studies, the alteration of host behaviour by symbionts in marine soft-sediments has rarely been quantified. The aim of this chapter is to contribute to improving knowledge of the latter by determining behavioural changes of *C. kraussi* in the presence and absence of the symbiont *B. jucundus*. A secondary issue addressed is the effect of the presence of *B. jucundus* on the time spent by *C. kraussi* on bioengineering activities. As an ecosystem engineer, *C. kraussi* has a major influence on soft sediment functions such as sediment turnover and nutrient fluxes from sediments to the overlying water column. If *B. jucundus* affects the time spent by *C. kraussi* on engineering activities, then the presence of *B. jucundus* could indirectly influence the ecological functioning in soft-sediments. This chapter also aims to determine if the sex of *C. kraussi* influences their response to the presence *B. jucundus*. Chapter 4 concludes the dissertation by presenting a synthesis of findings and the major conclusions drawn from the study.

Chapter 2: Distribution and abundance of thalassinidean burrow symbionts: host abundance vs burrow trophic environment

2.1. Introduction

Marine soft-sediments are the most common habitat in the world, covering about 70% of the world's seafloor (Wilson, 1991; Lohrer & Hancock, 2004). In terms of coastal habitats, soft-sediment systems offer a different habitat matrix to organisms than hard substrata (Fairweather & Quinn, 1995). Unlike hard, rocky habitats, soft-sediment ecosystems are three dimensional, permitting organisms to burrow several meters deep within the matrix (Little, 2000; Lohrer & Hancock, 2004). Life in soft-sediments is therefore very different from life on rocky habitats (Little, 2000). On rocky substrates, animal assemblages are dominated by organisms that attach directly on to the rock whereas soft sediments are dominated by invertebrates living beneath the sediment surface (Peterson, 1991). Most rocky substrate organisms are sessile and often have relatively little refuge from threats such as predation, competition and desiccation but sediment dwellers can burrow to avoid these threats (Little, 2000). The unique features of soft-sediments and their assemblages in comparison to rocky shores are shown in table 2.1.

Table 2.1: Comparison of habitat features characteristic of soft-sediment and rocky shore systems and their effects on biological processes and resident communities. (Summarised from Peterson, 1991; Fairweather & Quinn, 1995; Little, 2000).

Feature	**Soft-sediments**	**Rocky shores**
Dimensions	Three dimensional	Two dimensional
Substrate	Sandy or muddy flats	Hard substrate
Desiccation and temperature stress	Low. Finer particles can retain moisture during low tideand cool sediments.	High. Periodic exposure to air during low tidecreates desiccation threats.
Competitionfor space	Low. Organisms capable of burrowing in sediment.	High.Limited space available for attachment of organisms.
Larval settlement	Settlement space not an issue except in area with very high adult densities.	Limited space for settlement therefore very high competition.
Animal assemblages	Mobile invertebrates living within the sediments with many soft bodied organisms	Hard shells, sessile or slow moving attaching themselves to the rocks.

Soft-sediment systems harbour a variety of taxonomic groups, including polychaetes, crustaceans, echinoderms and molluscs (Ellingsen, 2002). Within these taxonomic groups are key organisms that engineer and structure the habitat at different scales (Widdows and Brinsley, 2002). In soft-sediment systems there are two functional categories of organisms that engineer and structure the substrate: sediment stabilisers and bioturbators that destabilise sediments (Volkenborn *et al.* 2009). Sediment stabilizers such as tube building worms and sea grass bind sediments, decrease sediment re-suspension and enhance sediment cohesiveness. In contrast, bioturbators re-suspend, erode or move sediments through digging and burrowing (Widdows and Brinsley, 2002).

Of the two functional groups, bioturbators are the focal group in this investigation. The burrowing and reworking of sediments result in the formation of biogenic structures that are attractants of other species (Reise, 1981).Burrows act as microhabitats for other organisms and provide refuge from predation, wave action and desiccation whilst also providing food and a stable environment for attracted organisms (Little, 2000; Kristensen, 2000; Reise 2002). Therefore, bioturbating species are an important component of soft-sediment systems (Wynberg & Branch 1997). Of the known burrowers in marine soft-sediment ecosystems, thalassinideans are considered to rank amongst the most influential (Pillay & Branch, 2011).

Thalassinideans have been found to greatly influence benthic ecosystems and their communities (Berkenbusch & Rowden, 2003; Pillay *et al.* 2007; D'Andrea & DeWitt 2009). Bioturbation by thalassinideans causes redistribution of particles and an increase in fluxes of water at the water-sediment interfaces, thereby altering the physical and chemical properties of the substratum and interstitial water (Blondin & Rosenberg, 2006) which in turn impact a range of organisms (Pillay & Branch, 2011).

Many thalassinidean species build complex burrows with elaborate branches and chambers as shown in Fig. 2.1A, which can be connected communal burrows (Frey & Howard, 1975). Burrowing by thalassinideans increases sediment permeability and porosity, improves oxygenation and material exchange at the sediment-water interface, thus creating microhabitats for other organisms (Fig. 2.1B; Pillay & Branch, 2011). Burrows also provide refuges from predators (Dittmann, 1996; Reise 2002), turbulence, exposure at low tide and high wave action (Little 2000). In a study conducted to determine the effects of burrows on smaller infauna, Dittmann (1966) found that densities of organisms in burrows exceeded densities in the surrounding areas.

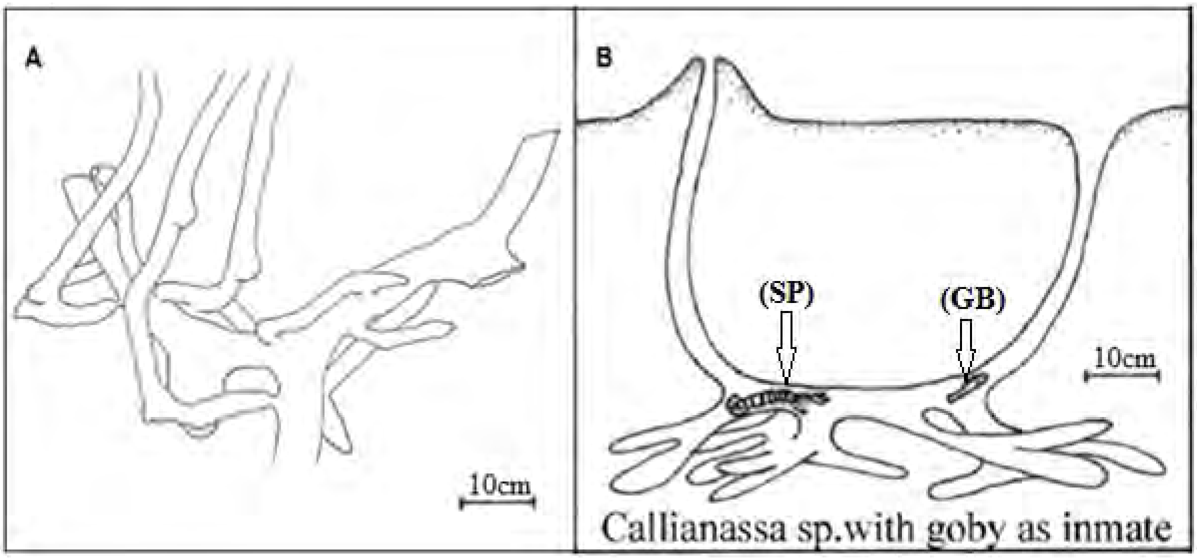

Fig. 2.1: (**A**) Complex burrow architecture of a typical thalassinidean sandprawn with numerous interconnected tunnels branching off the main burrow. (**B**) Burrow compartments provide microhabitats for smaller symbionts. SP: thalassinidean sand prawn, GB: goby (Frey & Howard 1975; Reise, 2002).

Not only do these burrows provide suitable habitat for smaller infauna, they also acts as substrate on which food sources can accumulate, which in turn provide a source of food for burrow dwellers. Burrow walls are often rich in organic matter derived from phytoplankton, microphytobenthos and detritus (Papaspyrou *et al.* 2005). Studies have shown that bacterial biomass is much higher in burrow walls relative to surrounding area (Branch & Pringle, 1987; Pillay & Branch, 2011). *Pestarella tyrrhena* is a deposit feeding thalassinidean shrimp that accumulates poor quality detritus and uses it to 'garden' bacteria, which are more nutritious food sources (Demiri *et al*. 2009).

Therefore, bioengineered structures act as a substrate for complex interactions between species (Reise, 2002).Burrows of thalassinidean prawns attract numerous burrow symbionts with varying dependence on the burrow habitat (Pillay *et al*. 2011).These bioengineered structures provide trophic and non-trophic functions to the burrow symbionts(Branch & Pringle, 1987;Papaspyrou*et al*. 2005;Pillay & Branch, 2011). However, most studies have not separated between these two functions and the relative importance of these in determining symbiont densities is poorly understood. There has been little attempt to quantitatively understand the relative importance of trophic and non-trophic functions in determining symbiont abundances.

The aim of this chapter is to determine spatio-temporal distribution patterns in thalassinidean prawn hosts (*Callichirus kraussi* and *Upogebia africana*) and symbionts (*Betaeus jucundus, Spiroplax spiralis, Antinoe lactea* and *Psammogobius knysnaensis)* in Langebaan Lagoon. It was hypothesised that abundances for both the hosts and the symbionts would be higher at mid shore positions. In intertidal systems, organisms living at the high water mark face the problem of desiccation and temperature stress, while those living at the low water mark often have to withstand high wave action and predation. The mid shore would therefore be a suitable habitat compared to the high and low water marks (Little, 2000). This chapter also aims to understand the relative importance of non-trophic (physical refuge) and trophic (food availability) benefits provided by burrows in influencing the abundance and distribution of burrow symbionts. Specifically, the chapter questions whether host abundance, as a proxy of number of burrows that can provide refuge (non-trophic benefit), is more important than burrow extracellular polymeric substances[1] (EPS) and

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[1] Polymers exuded by microbes

chlorophyll *a* concentrations (trophic benefits) in determining the distribution and abundance of symbionts in Langebaan Lagoon. It was hypothesised that food availability in the burrows would best predict symbiont abundances. Since burrow symbionts do not exist outside of burrows independently, symbionts have to obtain their nutritional requirements from burrows within which they live. Therefore, within sandflats dominated by sandprawns, burrows with high food availability should be the main determinant of symbiont abundance.

This chapter also seeks to determine whether the amount of food available in burrows for symbionts is dependant in sediment granulometry. Studies have shown that finer muds have more organic material and higher microbial biomass than coarse sediments due to the cohesive nature of mud, which allows for a stable environment for microbes (Underwood & Smith, 1998; Little, 2000). It was predicted that burrow EPS and chlorophyll *a* concentrations would increase with greater mud content in burrow walls, which in theory, could be important in determining burrow symbiont abundances.

2.2. Materials and Methods

2.2.1. Study Area

The study was conducted in Langebaan Lagoon (18°03'E; 33°08'S) on the West coast of South Africa (Fig. 2.2). The lagoon is about 17km long and stretches from Saldanha Bay in the north to Geelbek, which is the southernmost point of the lagoon. Unlike most lagoonal systems, Langebaan Lagoon is a purely marine lagoon with no river inputs (Siebert & Branch, 2005). The Lagoon is divided into three management zones; zone A is a multi-functional section used for fishing, bait collecting and most water sports. Zone B is controlled, and prohibits bait collecting, but does allow non-destructive recreational activities. Zone C is a prohibited area that is closed off to public use (no boating or fishing allowed) (Hanekom *et al.* 2009). For the purposes of this study, three sites (Oesterval, Klein Oesterval and Bottelary) within the lagoon were sampled (Figure 2.2).

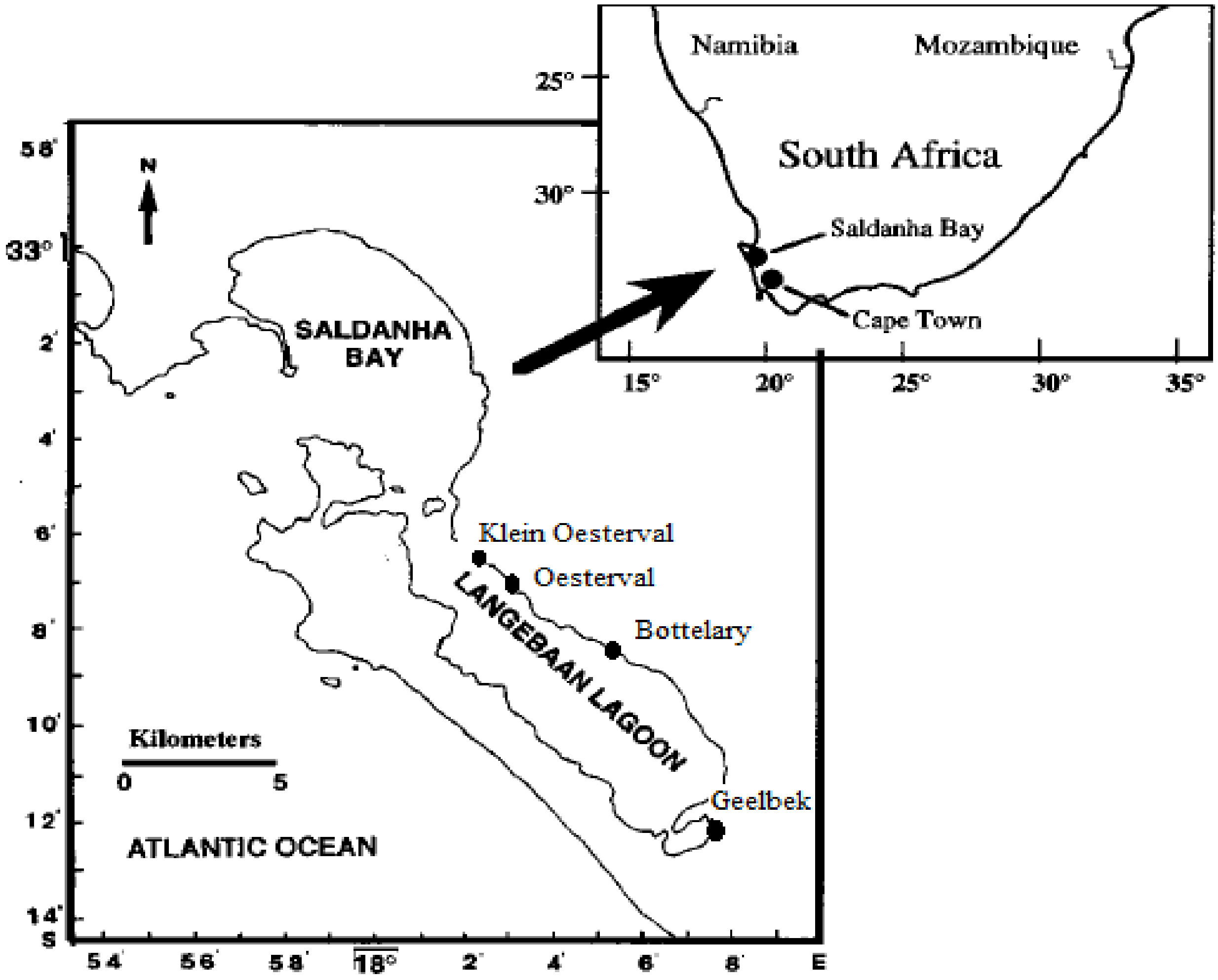

Fig. 2.2: Map of Langebaan Lagoon showing its location in South Africa as well as sampling sites (Klein Oesterval, Oesterval and Bottelary) (adapted from Wynberg & Branch, 1997).

2.2.2. Sampling design and data collection

Field surveys were undertaken during spring (September) and autumn (April) to determine spatio-temporal patterns in distribution and abundance of burrowing prawns and burrow symbionts, and ultimately the relative contribution of trophic and non-trophic burrow resources as determinants of symbiont abundances. Sampling was undertaken during a 4 to 5 day period around spring low tides. A nested sampling design was utilised for this study as summarised in Fig 2.3.

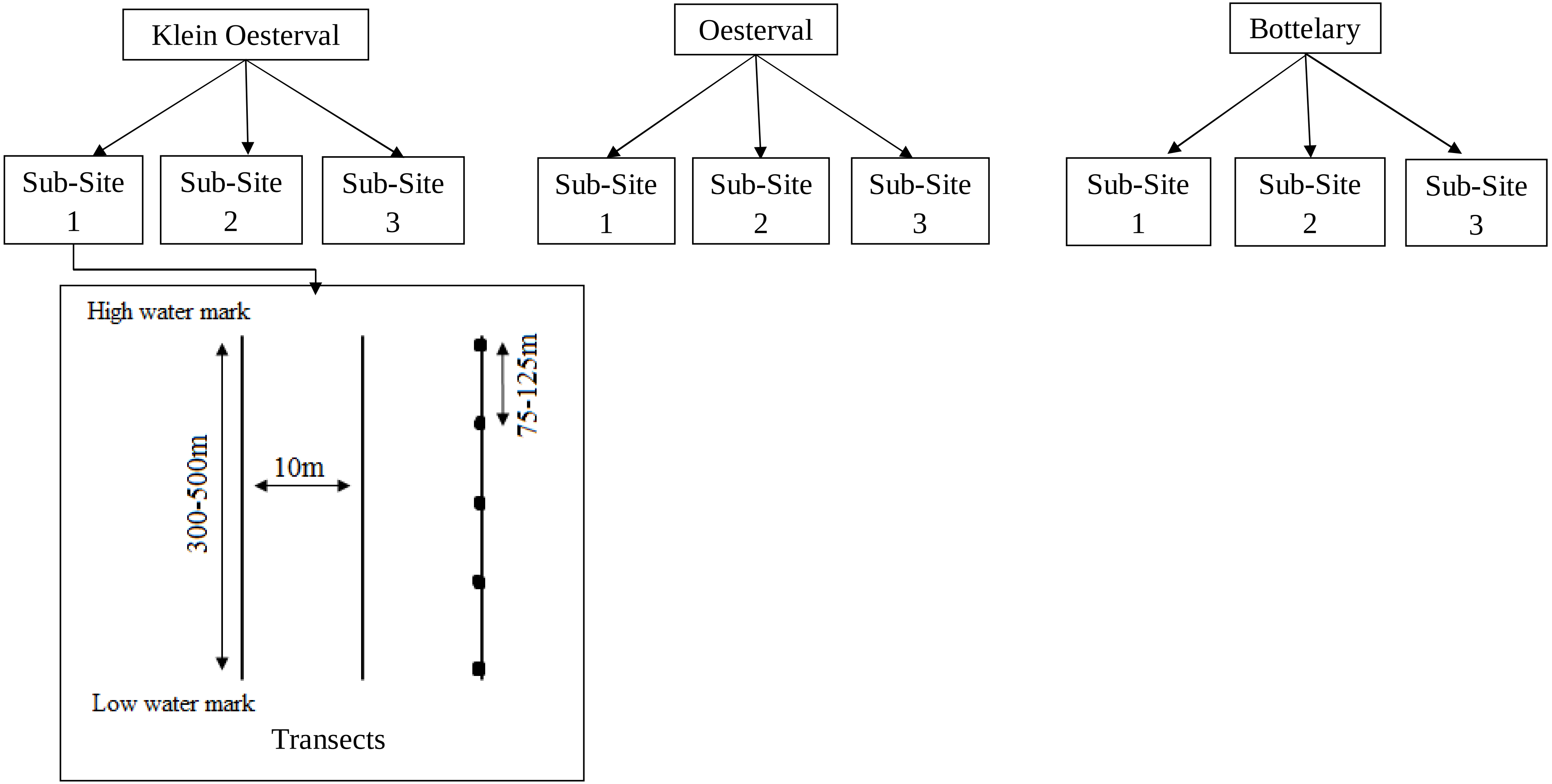

Fig 2.3: Nested sampling design showing the three sites sampled, with three sub-sites per site and three transects per sub-site. Sampling was done from high to low water marks with five shore positions sampled per transect. Black squares in on transect line denote sampling stations.

At each site, 3 sub-sites were sampled roughly 50m apart. At each sub-site, line transect sampling was conducted with 3transects 10m apart running from the high to low watermark. The distance from the high to low watermark varied between 300-500m at the different sites. On each transect, sampling was done at 5 shore positions equidistant from each other. Six sediment cores were collected at each shore position per transect using a stainless steel prawn pump (Length = 75cm; Diameter = 5cm) to sample thalassinidean hosts and symbionts. The six sediment cores were sieved on a 2000µm mesh and the retained burrowing prawns and symbionts were counted and recorded.

2.2.3. Sediment sampling and analysis

Sediment particle size

Sediment samples for particle size analysis were collected from the sediments obtained during coring for prawns and symbionts. Samples were taken from the cored sediments at a depth of about 35cm at the high, mid and low water mark and placed in 20ml vials for further analysis in the laboratory, which involved sieving sediments on a series of sieves with geometrically decreasing mesh sizes (2000µm to 63µm)(Folk, 1980; Gee & Or, 2002). Sediment fractions retained by each sieve were placed in aluminium foil dishes, dried at 50°C for 24hours and then weighed (Gee &Or, 2002). Plots of cumulative particle size against sediment phi values were produced per sample, and the median phi value determined as the phi value corresponding to 50% of the cumulative mass of sediment. Mud content was determined as the fraction of sediment retained by a 63µm mesh.

Extracellular polymeric substances

Extra-cellular polymeric substances (EPS) are exuded by bacteria and diatoms (Little, 2000). Sediment samples for EPS were collected from the burrow walls at depths of 10cm at the mid-shore position, where abundances of symbionts peaked (See results: Fig. 2.7).Ten samples at mid-shore position per sub-site were collected. The burrows were split open and about 5 grams of sediment was scraped from the burrow wall and placed into 20ml vials. The samples were kept in a freezer for 24hrs before analysis. EPS concentrations were determined using the phenol-sulfuric acid assay according to Underwood *et al*(1995), in which a sub-sample (1gram) of the homogenised sediment sample was placed a test-tube to which 2ml of distilled water was added followed by 1ml of 5% phenol and 5ml of concentrated sulphuric acid (90%). The samples with the phenol-sulfuric acid mixture were diluted 10 fold and the absorbance measured using a Merck Spectroquant Pharo 100 spectrophotometer against a reagent blank at 485nm.

Chlorophyll a

Sediment samples for chlorophyll *a* concentration were also collected from the burrow walls at the mid-shore position as specified for EPS above. Burrow sediment samples of roughly 10g for each sample were placed in vials containing 30ml of 90% acetone for extraction of chlorophyll *a* and kept in a freezer for 48hrs. A sub-sample (2ml) for each homogenised chlorophyll *a* sample was then measured using a Turner Designs Trilogy fluorometer (Dawson & Pillay 2011).

2.2.4. Data Analysis

Two statistical software packages were used to analyse the data. Statistical Package for the Social Sciences (SPSS) was used for univariate testing and PRIMER6.1.11 with PERMANOVA+ 1.0.1was used for the multivariate analyses.

In SPSS, a nested analysis of variance (ANOVA) was used to test the effect of spatial and temporal variables on the abundance of burrowing prawns and burrow symbionts. Nested ANOVA was also used to determine spatial and temporal differences in mud content, EPS and chlorophyll *a*. Post hoc tukey tests were employed to assess differences within treatments. To test the assumptions required for parametric analyses, a one sample K-S was performed to determine whether the data were normally distributed and a Levene's test was performed to test for equality of variances. A significance level of 0.05 was used in all statistical tests. A stepwise multiple regression analysis was performed to determine the relative importance of *C. kraussi* abundances, burrow EPS and chlorophyll a concentrations and mud content in determining the abundance of *B. jucundus*, which emerged to be the most abundant of the symbionts sampled. Collinearity tests were performed as part of the regression analysis by running a Durbin-Watson and a collinearity diagnostics test. *Upogebia africana* abundance was not included in the multiple regression analysis due to its extremely low abundance.

In primer, a nested PERMANOVA was performed to determine differences in symbiont community structure (all symbiont species; *Betaeus jucundus, Spiroplax spiralis, Antinoe lactea* and *Psammogobius knysnaensis*) between the two seasons and amongst the sites and sub-sites.

2.3. Results

Spatial differences in abundances of burrowing prawns and symbionts

Host abundances

Table 2.2 gives the summary statistics of effects of time and space on the abundances of the burrowing prawns. *Upogebia africana* abundance was unaffected by any of the spatial and temporal variables. *C. kraussi* abundance differed statistically between sites, transects and shore positions. Post hoc analysis showed that abundances of *C. kraussi* were higher in Bottelary than in Klein Oesterval and Oesterval ($p = 0.001$). This pattern was more prominent in autumn than in spring (Fig. 2.4). The mid-shore had the highest abundances of *C. kraussi* for all sites except at Oesterval (Fig. 2.5).

Table 2.2: Summary statistics of ANOVA showing effects of seasons, site, sub-site, transects and shore position on the abundance of *C. kraussi* and *U. africana*. *F* = test statistic, df = degrees of freedom, *p* = significance level. * denotes statistically significant differences.

Organism	**Level**	***F***	**Df**	***p***
C. kraussi	**Season**	1.635	1	0.202
	Site	10.341	4	0.001*
	Sub-site	3.226	12	0.001*
	Transect	0.766	36	0.829
	Shore position	2.258	72	0.001*
U. africana	**Season**	2.648	1	0.105
	Site	0.907	4	0.461
	Sub-site	1.051	12	0.404
	Transect	0.708	36	0.892
	Shore position	1.340	72	0.070

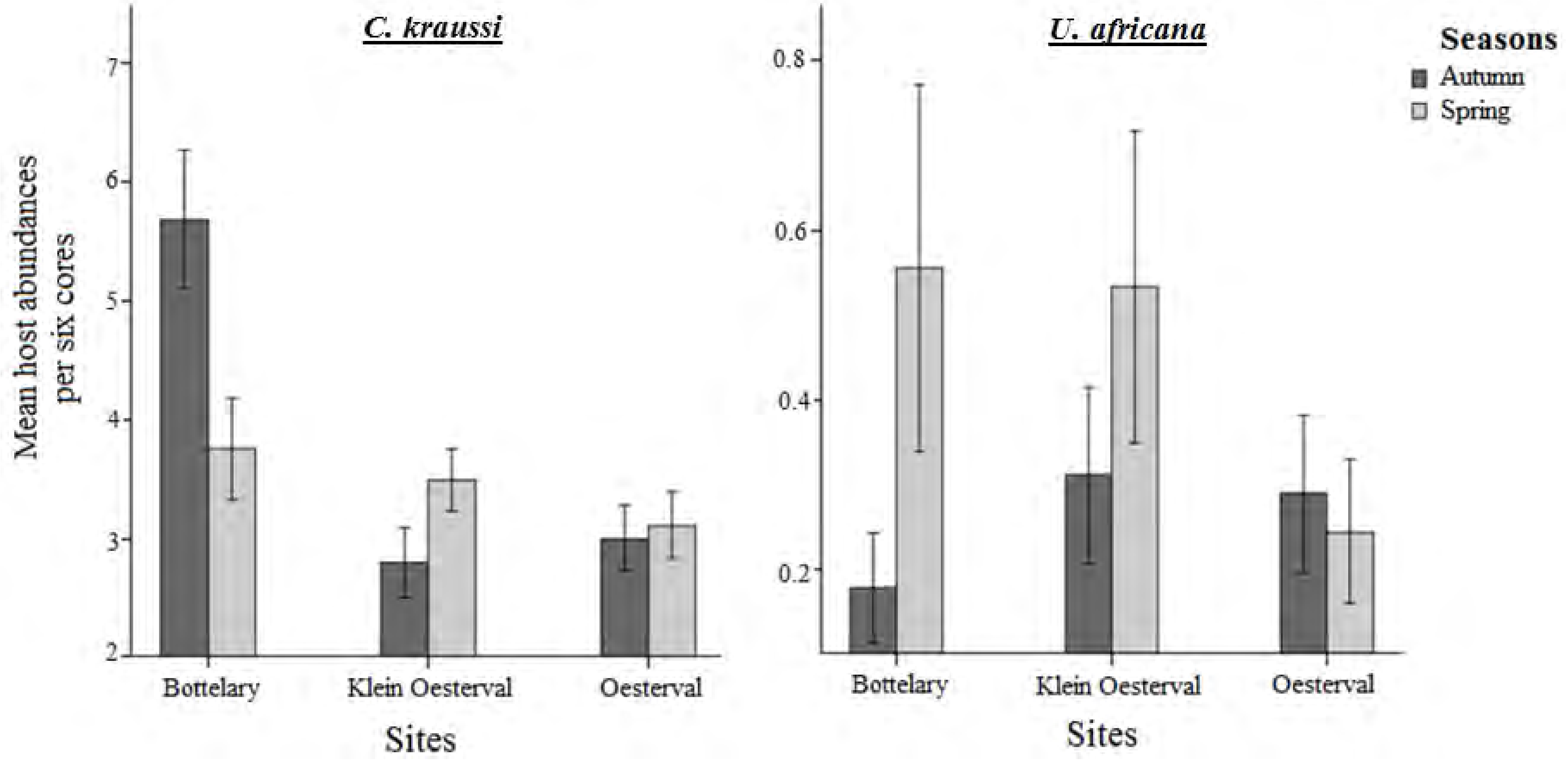

Fig 2.4: Abundances of burrowing prawns, *Callichirus kraussi* and *Upogebia africana* (Means ± 1SE) in autumn and spring within each site.

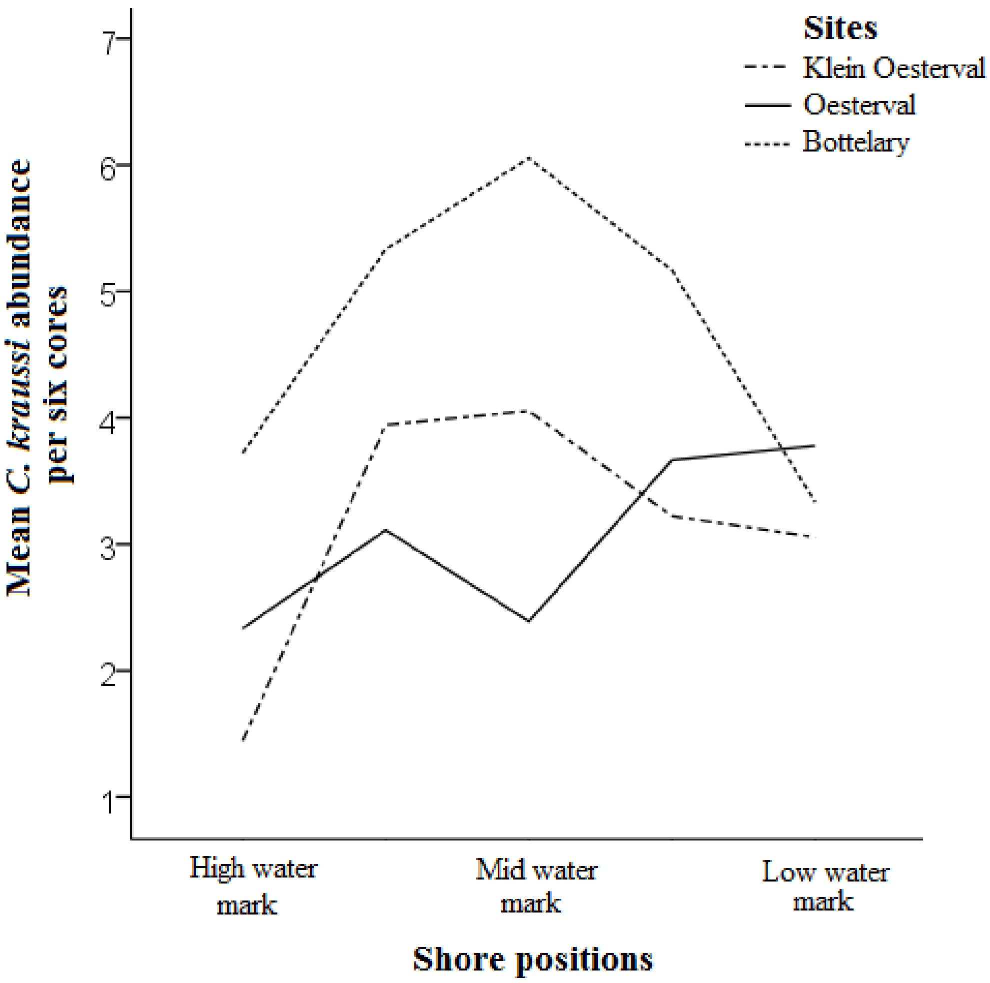

Fig. 2.5: Abundances of *C. kraussi* from high to low water mark at the three sampling sites.

Symbiont abundances

With the exception of the gobies, none of the other symbionts were ever observed outside of the thalassinidean burrows in the field. *Betaeus jucundus* abundances differed significantly between seasons, sites and shore positions (Table 2.3). Abundances were higher in autumn than in spring with the exception of Oesterval, where the trend was reversed (Fig. 2.6). At site level, differences observed were between Oesterval and Bottelary (Post hoc: *p* = 0.001) and Oesterval and Klein Oesterval (Post hoc: *p* = 0.001).The highest abundances occurred at mid-shore in Bottelary and Klein Oesterval and between mid and low-shore in Oesterval (Fig 2.7). Within seasons, *B. jucundus* abundances differed between sites in spring, with post hoc analyses showing differences between Oesterval and Klein Oesterval (*p* = 0.001) and between Oesterval and Bottelary (*p* = 0.001).

Spiroplax spiralis abundances were significantly different between seasons, with greater abundances recorded in autumn than in spring (Fig. 2.6). *Antinoe lactea* abundances differed statistically between shore positions, with abundances being highest at mid-shore positions for all sites (Fig. 2.7). *Psammogobius knysnaensis* abundances differed between seasons with abundances higher in spring than autumn (Fig. 2.6).

Table 2.3: Summary statistics of ANOVA showing effects of seasons, site, sub-site, transects and shore position on the abundance of burrow symbionts. *F* = test statistic, df = degrees of freedom, *p* = significance level. * denotes statistically significant differences.

Organism	**Level**	***F***	**df**	***p***
B. jucundus	**Season**	19.991	1	0.001*
	Site	35.786	4	0.001*
	Sub-site	0.672	12	0.777
	Transect	0.352	36	1
	Shore position	11.764	72	0.001*
S. spiralis	**Season**	4.878	1	0.028*
	Site	0.558	4	0.693
	Sub-site	0.706	12	0.745
	Transect	0.904	36	0.630
	Shore position	1.344	72	0.068
A. lactea	**Season**	3.448	1	0.065
	Site	2.034	4	0.091
	Sub-site	0.897	12	0.551
	Transect	0.793	36	0.795
	Shore position	1.412	72	0.041*
P. knysnaensis	**Season**	4.500	1	0.035*
	Site	2.611	4	0.036*
	Sub-site	0.889	12	0.559
	Transect	0.944	36	0.564
	Shore position	0.769	72	0.892

Symbiont community structure was significantly different at season (PERMANOVA, Pseudo-*F*=32.413, *p*=0.024) and site levels (PERMANOVA, Pseudo-*F*=31.062, *p*=0.008). However, pair-wise tests indicated non-significant differences between all site and sub-site comparisons.

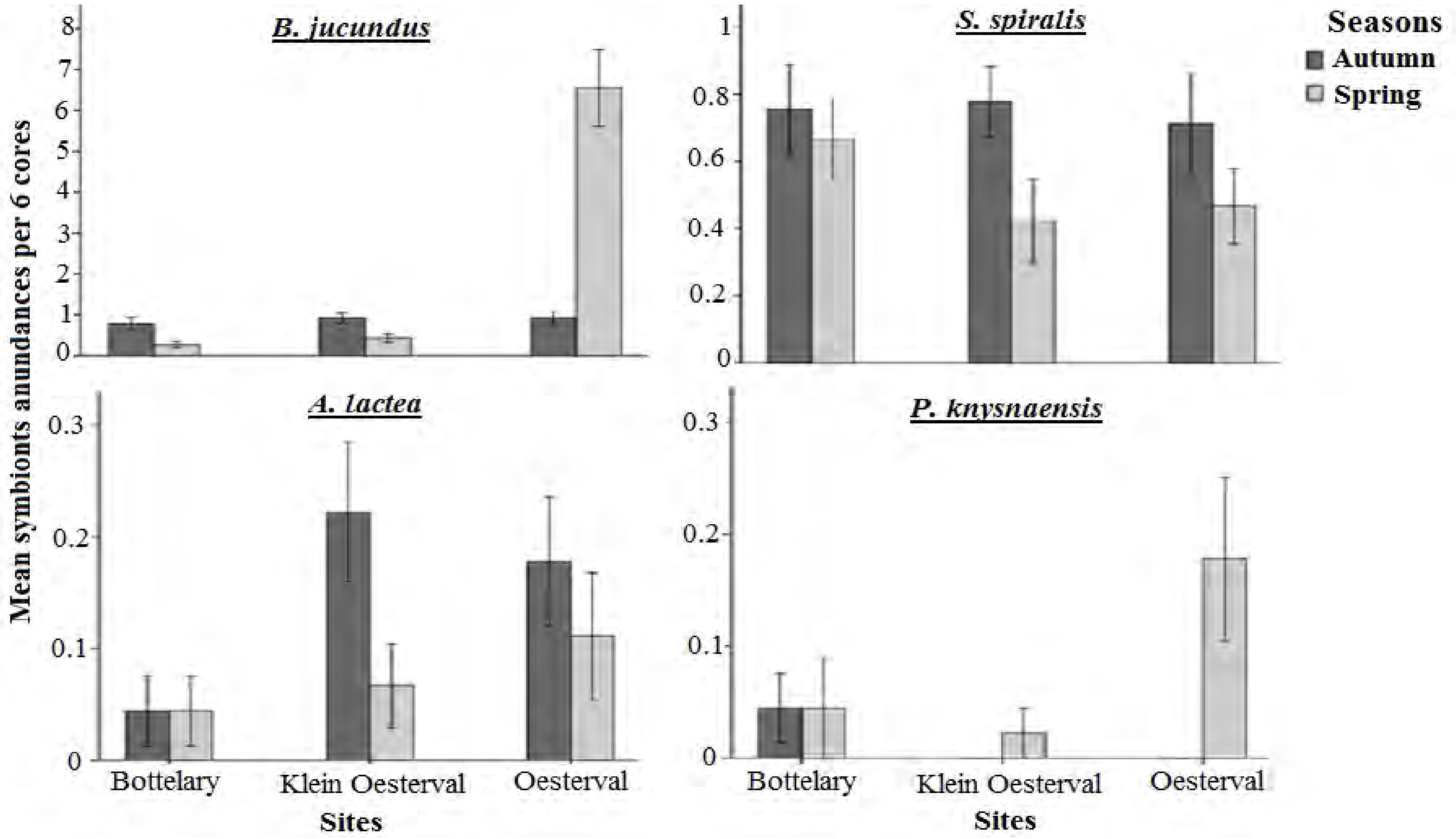

Fig. 2.6: Abundances of burrowing symbionts, *Betaeus jucundus*, *Spiroplax spiralis*, *Antinoe lactea* and *Psammogobius knysnaensis* (Means ± 1SE) in autumn and spring within each site.

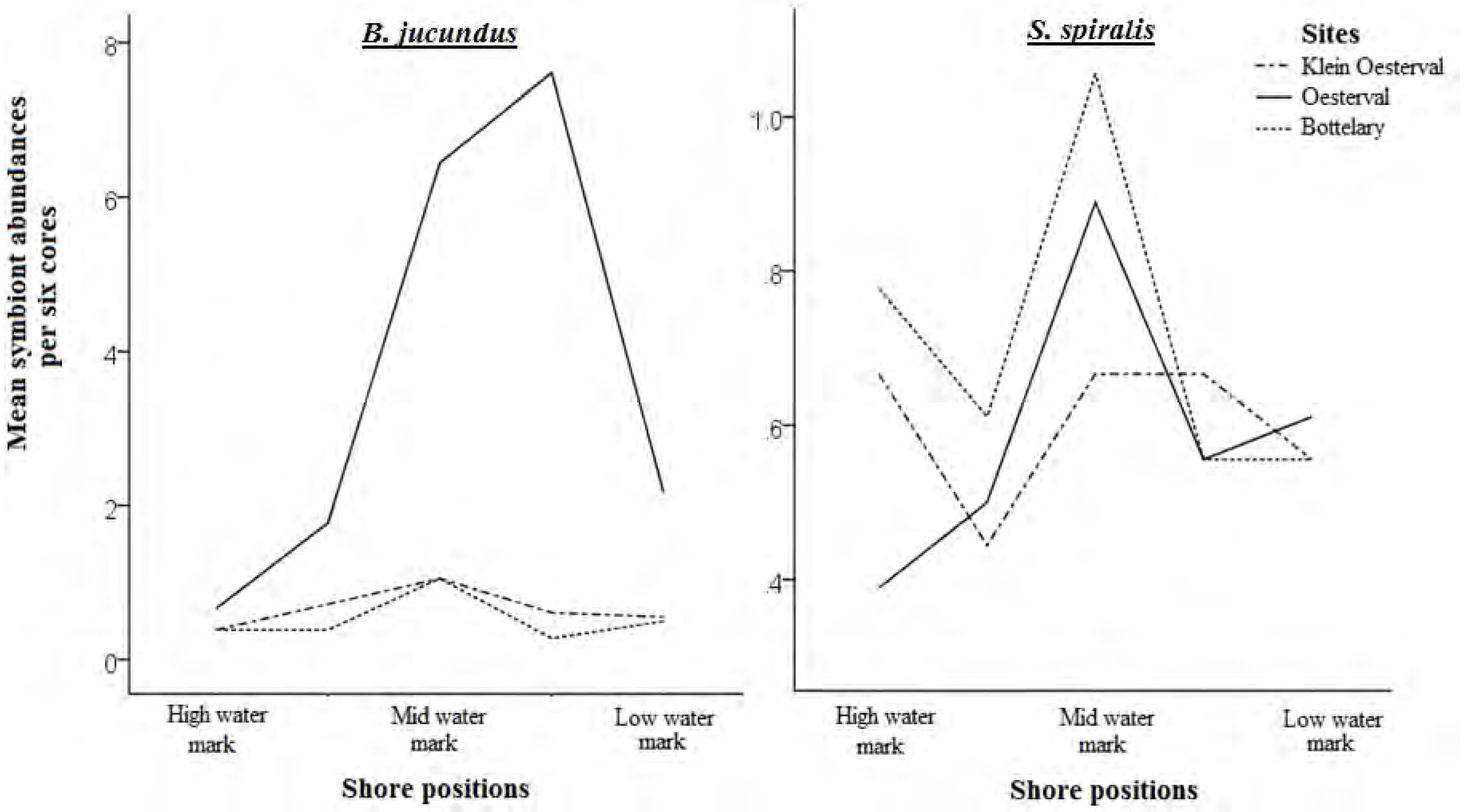

Fig. 2.7: Distribution and abundances of *B. jucundus* and *S. spiralis* along the intertidal from the high to low water mark.

Spatial and temporal differences of abiotic factors

Phi and mud content

Sediment particle size (phi) was unaffected by any of the spatial and temporal variables except for shore position. Post hoc tests showed significant differences between the high- and mid-water marks (p = 0.014). Mud content was marginally significant at site level but not for season, sub-site and shore position (Table 2.4). Post hoc analysis was unable to detect any site differences.

Table 2.4: Summary statistics of ANOVA showing effects of season, site, sub-site, transects and shore position on phi and mud content. F = test statistic, df = degrees of freedom, p = significance level. * denotes statistically significant differences.

		F	**df**	***p***
Phi	**Season**	0.139	1	0.711
	Site	1.525	4	0.216
	Sub-site	1.059	12	0.429
	Shore position	4.365	72	0.018*
Mud	**Season**	0.107	1	0.745
	Site	2.613	4	0.051*
	Sub-site	1.586	12	0.140
	Shore position	0.453	72	0.638

In spring, Oesterval had a mean mud content of 1.63%, Bottelary of 0.93% and Klein Oesterval of 0.695% with the lowest value recorded at Klein Oesterval. However, in autumn Oesterval had the lowest mean mud content (1.01%) of all the three sites and Bottelary had the highest (1.52%) (Fig. 2.8).

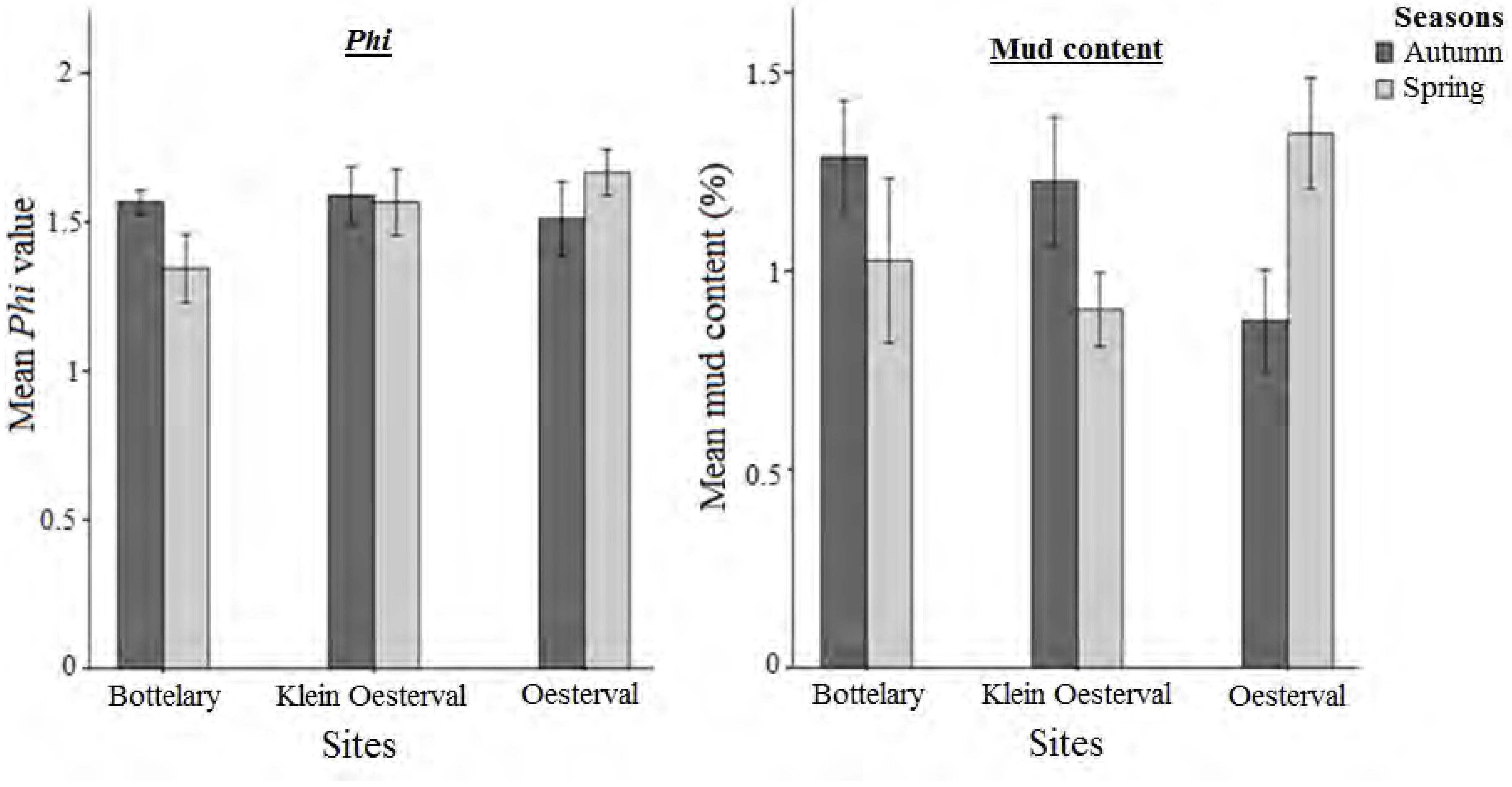

Fig. 2.8: Mean phi values and mud content (percentage of total sediment) at the different sites in autumn and spring.

Burrow EPS and chlorophyll a concentrations

Overall burrow chlorophyll and EPS concentrations varied at large temporal (between seasons)and spatial (site) scales (Table 2.5). Concentrations were significantly higher in spring than autumn (Fig. 2.9) with the latter trend being prominent in Oesterval for EPS concentrations and in Oesterval and Bottelary for chlorophyll *a* concentrations. Post hoc tests showed differences between Klein Oesterval and Oesterval (p = 0.001) and between Klein Oesterval and Bottelary (p = 0.001) for EPS concentrations. For chlorophyll *a* concentrations, differences were observed between Oesterval and the other two sites (p = 0.001). At a smaller temporal scale i.e. within seasons, in autumn, chlorophyll *a* concentrations were significantly different between Oesterval and Klein Oesterval and between Oesterval and Bottelary (Post hoc tests: p = 0.001) whereas EPS concentrations were significantly different between Bottelary and Klein Oesterval (Post hoc test: p = 0.024). In spring, both chlorophyll *a* and EPS concentrations differed amongst all 3 sites (Post hoc tests: p = 0.001).Oesterval had the highest chlorophyll *a* and EPS concentrations relative to Bottelary and Klein Oesterval (Fig. 2.9).

Table 2.5: Summary statistics of ANOVA showing effects of seasons, sites and sub-sites on EPS and chlorophyll *a*. *F* = test statistic, df = degrees of freedom, *p* = significance level. * denotes statistically significant differences.

		F	**df**	***p***
EPS	**Season**	4.131	1	0.044*
	Site	10.213	4	0.001*
	Sub-site	1.230	12	0.267
Chl *a*	**Season**	15.347	1	0.001*
	Site	23.055	4	0.001*
	Sub-site	5.262	12	0.001

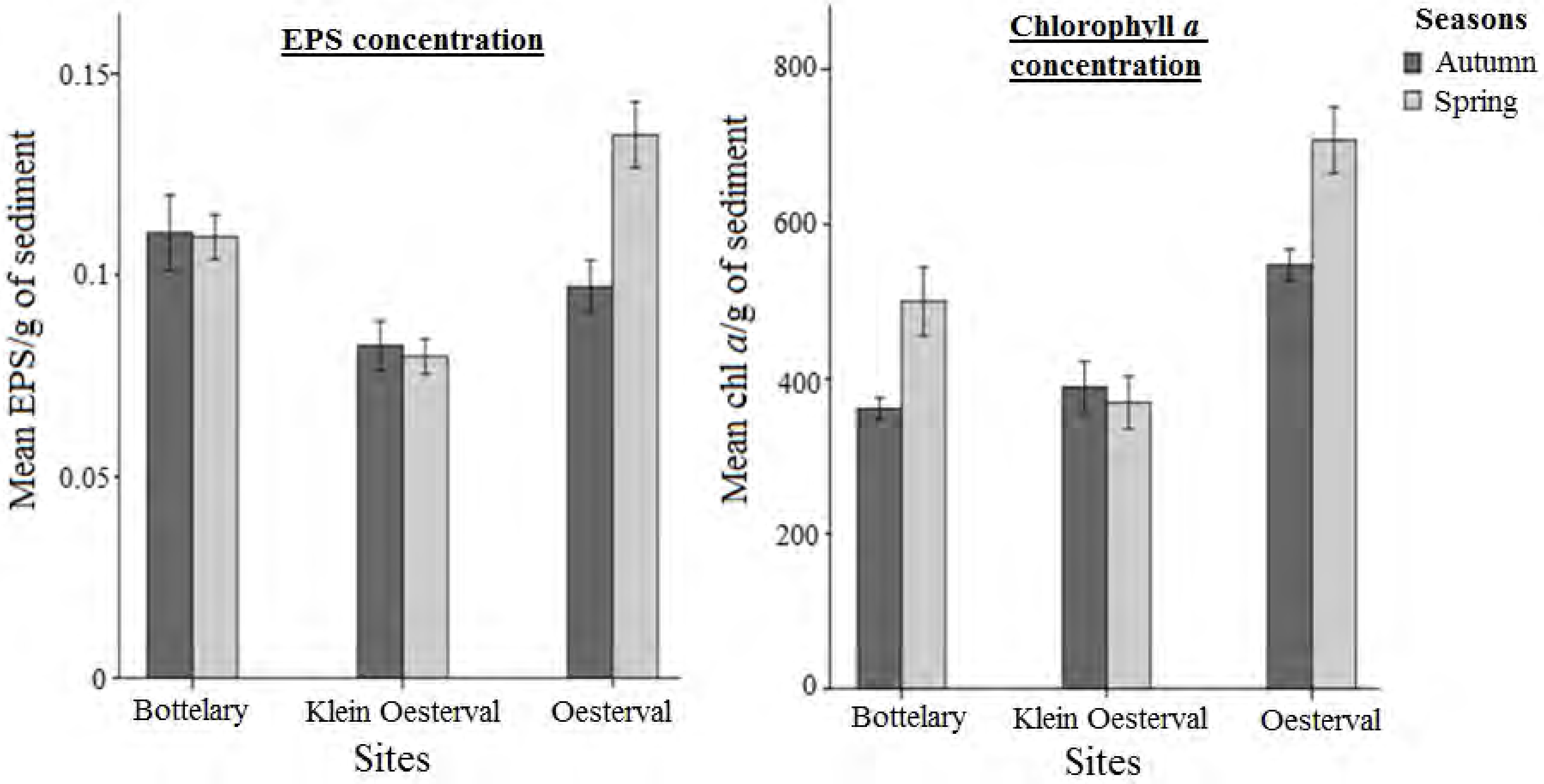

Fig. 2.9: Mean EPS and chlorophyll *a* concentrations at the different sites in autumn and spring.

Determinants of B. jucundus distribution and abundance

Betaeus jucundus was considered for this analysis because it was the most dominant symbiont with abundances high enough to correlate with trophic and non-trophic data.

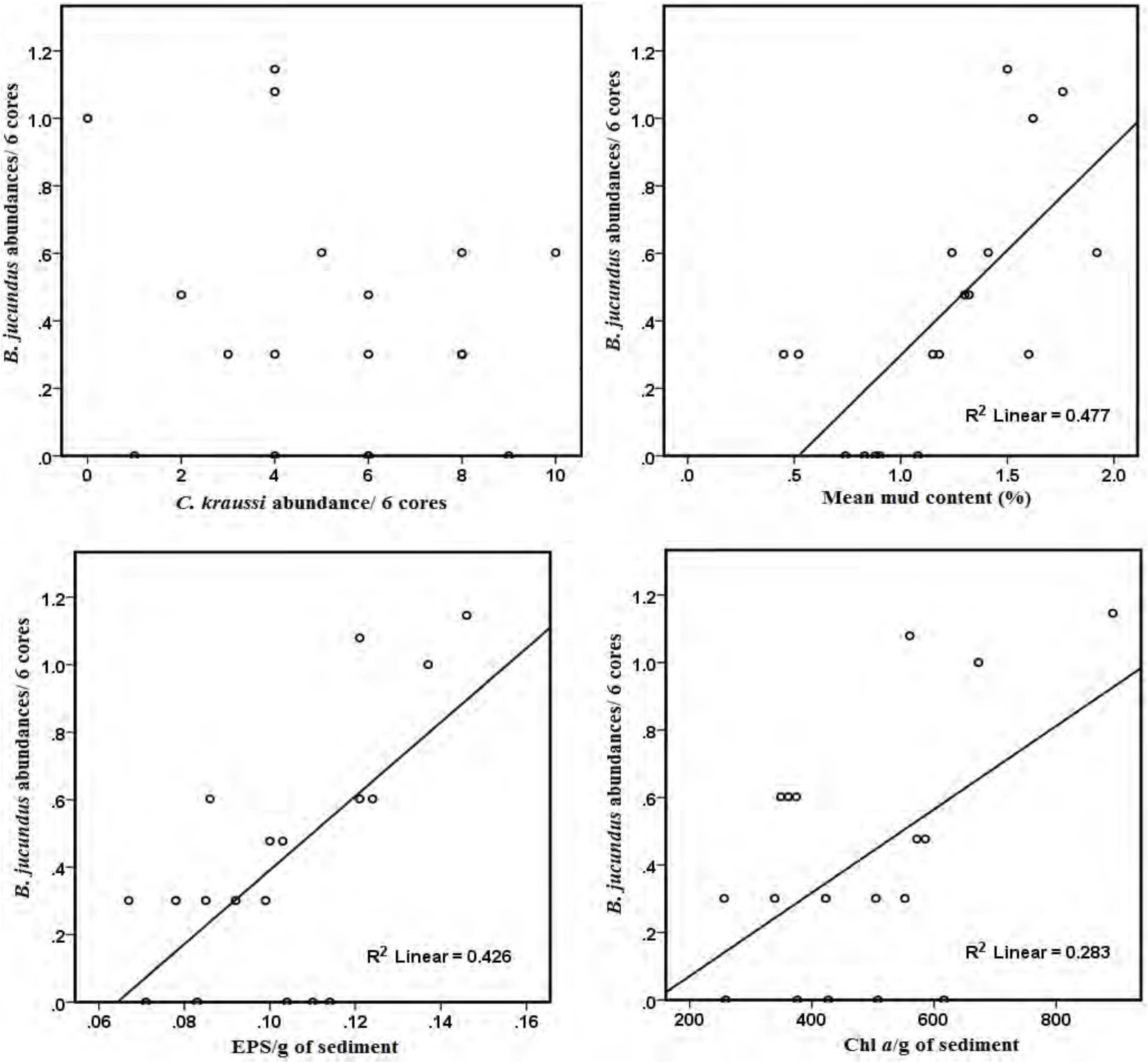

Fig. 2.10: Relationships between *B. jucundus* abundances and (a) *C. kraussi* abundances, (b) mud content, (c) EPS and (d) chlorophyll *a*. R²: coefficient of determination.

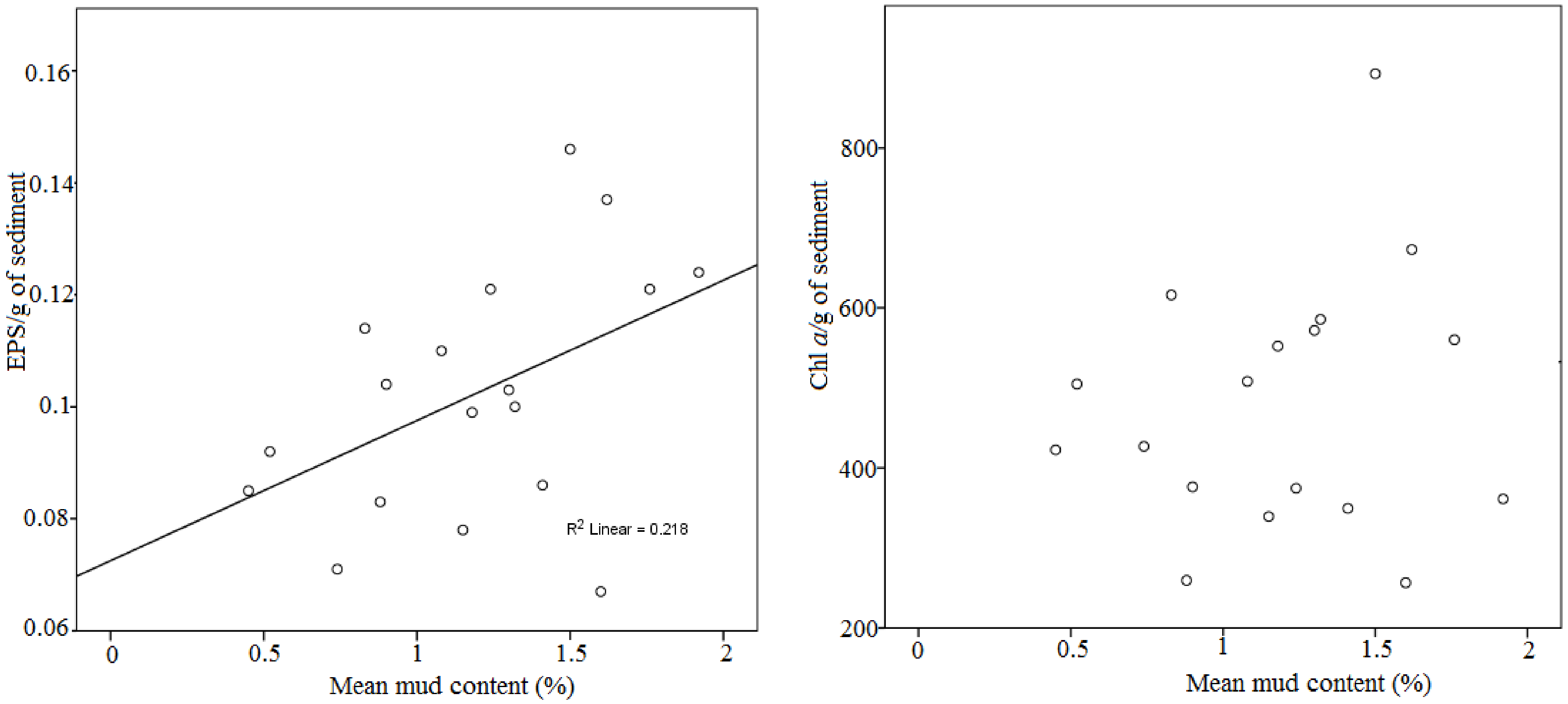

Fig. 2.11: Relationships between mud content and (a) EPS and (b) chlorophyll *a*. R^2: coefficient of determination.

Of the four determinants mentioned above, *C. kraussi* abundance was the only determinant that had no discernable relationship with *B. jucundus* abundance (Fig. 2.10). Of the remaining three determinants, mud content had the strongest relationship ($R^2 = 0.447$) with *B. jucundus* abundance followed by EPS concentration ($R^2 = 0.426$) and chlorophyll a concentration ($R^2 = 0.283$). All three determinants were positively correlated to *B. jucundus* abundances. Mud content was positively correlated to EPS concentrations ($F = 4.465$; $p = 0.051$) but not chlorophyll a concentrations ($F = 0.365$; $p = 0.552$) (Fig. 2.11).

Multiple regression analysis identified a model with burrow EPS and mud content as the best explanatory variables for *B. jucundus* distribution and abundance ($F = 14.038$, $df = 2$, $p < 0.001$).Both EPS concentration and mud content were significant individual contributors to the model ($t = 2.937$, $p = 0.010$ and $t = 2.607$, $p = 0.020$ respectively). EPS was the most significant contributor to the model ($\beta = 0.502$), followed by burrow mud content ($\beta = 0.445$). Burrow chl *a* levels and sandprawn abundances were excluded from the model by regression analysis as they were both insignificant predictors of *B. jucundus* abundance ($t = 0.928$, $p = 0.369$ and $t = 0.135$, $p = 0.895$ respectively).

A Durbin-Watson value of 1.251 suggested that there might be autocorrelation between predictor variables. Collinearity diagnostics tests indicated a tolerance value of greater than 0.2 (0.794) and a VIF value of less than 4 (1.259). These values supported the results of the bivariate plots which showed no signs of collinearity, indicating that autocorrelation was not a problem for the model.

2.4. Discussion

Animal communities are complex and are determined by a number of factors from organismal interactions to physical features of habitats (Rabeni & Minshall, 1977; Hall *et al.*

1992; Little, 2000). However, in marine soft-sediments, there has been very little attempt to separate the factors that determine the abundance of burrow symbionts. Thalassinidean prawns construct burrows that provide trophic and non-trophic functions to the co-inhibiting burrow symbionts (Branch & Pringle, 1987; Papaspyrou *et al*. 2005; Pillay & Branch, 2011) but the relative importance of trophic and non-trophic functions in shaping symbiont communities is relatively unknown. Therefore, this study attempted to determine the importance of non-trophic functions (using host abundances as a proxy) versus trophic functions (EPS and chlorophyll *a* concentrations) provided by burrows in influencing the abundance and distribution of burrow symbionts.

Little (2000) notes that one of the most significant factors governing the distribution of benthic organisms is sediment type. Studies have shown that soft sediment organisms have a preference for mud rather than sand (Zajac & Whitlatch, 1982). Oesterval had higher mud content in spring than in autumn (1.63% and 1.01% respectively). The densities of *C. kraussi*, *B. jucundus* and *P. knysnaensis* were also higher in spring than in autumn (Fig. 2.5 and 2.6). Oesterval also had the highest mud content in spring compared to the other sites and the highest abundances of symbionts. In a study by Zajac & Whitlatch (1982), they found higher densities of organisms in mud dominated areas of the estuary compared to sand dominated areas.

Burrow chlorophyll *a* levels were higher in spring than in autumn for Bottelary and Oesterval but relatively similar in Klein Oesterval. This is probably due to phytoplankton blooms that occur in spring on the West coast of South Africa (Monteiro & Largier, 1999). Different studies have recorded short episodes of phytoplankton blooms in spring on the West coast of South America, Africa and North America which result in high chlorophyll *a* concentrations relative to other seasons (Cloern, 1991; Monteiro & Largier, 1999; Iriarte *et al*. 2007). Langebaan Lagoon is located in the southern Benguela system which is a region of intense upwelling cycles and winds drive inflow of upwelled nutrient rich water into the lagoon thus stimulating primary production (Monteiro & Largier, 1999). This inflow of nutrient rich water from the Benguela might be the reason for the high levels of burrow chlorophyll *a* in spring.

Bacteria and algae exude extracellular polymeric substances (EPS) which are important in food webs, as they provide a nutritious food source for benthic consumers (Underwood & Smith 1998; Hirst *et al*. 2003). Burrow EPS concentrations were relatively similar in Klein Oesterval and Bottelary for both seasons but higher in Oesterval in spring than autumn. Similar patterns in EPS and chlorophyll a concentrations were observed (high EPS concentrations in areas of high chlorophyll *a* concentrations). Underwood & Smith (1998) found a significant correlation between EPS and chlorophyll *a* concentrations in the mudflats of Colne Estuary on the east coast of the United Kingdom. Oesterval had the highest mud content in spring compared to all the other sites in either season. This high mud content corresponds with the highest levels of EPS and chlorophyll *a* recorded within the sampling period with a significant correlation between mud content and EPS concentration. Little (2000) notes that deposited mud and silt in soft shores contain higher levels of organic material than any other sediment type and often leads to increases in microphytobenthic biomass than coarse sediments (Underwood & Smith, 1998). The increased microbial biomass in finer sediments might possibly explain why the highest levels of EPS and chlorophyll a were recorded at Oesterval in spring.

Betaeus jucundus, which was the most abundant symbiont recorded in this study, showed greater abundances in spring than in autumn especially at Oesterval, where EPS and chlorophyll *a* levels were highest in spring. These patterns suggest that the trophic environment plays a strong role in governing the distribution and abundance of *B. jucundus*. The latter is supported by the work of Branch & Pringle (1987) and Wynberg & Branch

(1994) who suggested that the high concentration of food resources in the burrow attracts smaller infauna to the burrow habitat.

It is also possible that the high abundance of *B. jucundus* observed in spring may have been due to a recruitment pulse of *B. jucundus*. During sampling, it was noted that most of the shrimps in spring were smaller than those recorded in autumn, possibly indicating a dominance of juveniles in spring samples. It is conceivable that the timing of recruitment of *B. jucundus* may have evolved to be synchronised with seasonal nutrient pulses associated with upwelling events. Numerous studies have shown that marine larvae recruit during upwelling seasons, taking advantage of the food availability associated with upwelling. For example, recruitment of sea urchins in the Araruama Lagoon in Brazil peaks from August to October, which corresponds to the upwelling season in the region (Junqueira *et al.* 1997). A study by Morgan *et al.* (2009) determined that recruitment of crabs in Northern California corresponded with the upwelling season and stronger upwelling was linked with greater recruitment. In the Benguela upwelling system, copepod egg production and recruitment are correlated with high nutrients associated with upwelling (Richardson *et al.* 2003).

The high numbers of *B. jucundus* in burrows in Oesterval rather than Klein Oesterval or Bottelary might indicate preferential site selection based on burrow sediment type and food availability during recruitment. Studies have shown that marine larvae make use of upwelling and downwelling for dispersal (Otero *et al.* 2009). The larvae of several infaunal species then actively choose settling sites, by selecting sediments most similar to those the adult stages live in (Butman *et al.* 1988). In a study by Moksnes & Heck (2006) to assess processes affecting distribution of blue crabs, it was found that juvenile blue crabs actively selected and colonised structurally complex habitat at settlement that could provide protection from predation. In a separate study, Butman *et al.* (1988) observed that when looking for settlement sites, *Capitella* (Polychaeta) larvae move horizontally within a centimetre of the substrate and frequently swim down to the bottom to test the substrate. Larvae reject the substrate in the absence of appropriate cues. The larvae preferred muddy substrate, consistent with the food requirements of adult *Capitella* sp (Butman *et al*. 1988).

Studies have shown that some species of *Betaeus* have a planktonic larval stage with the larvae spending considerable time in the pelagic habitat (Thatje, 2001; Thatje *et al*. 2003). Very little is known about the life cycle of *B. jucundus* (Anker & Baeza, 2012), but assuming that *B. jucundus* also has a planktonic larval stage like other *Betaeus* species, it is very likely that the larvae are capable of actively choosing settling sites, resulting in non-random abundance patterns.

Studies have suggested that bacteria produce biofilms on sediment surfaces, and together with chemicals produced by the adults, serve as attractants for larvae of some species (Gray, 1967; Gerlach & Atema, 2012). Larval recognition of settlement habitats can be based on detection of conspecifics and characteristics of the habitat using chemical, visual, acoustics or olfactory cues (Gerlach & Atema, 2012). Studies have shown that chemo-sensitive receptors occur in the antennular filaments of species of *Betaeus*, which make them sensitive to chemicals released by other organisms (Ache & Davenport, 1972; Ache & Case, 1969). This chemo-sensory ability may therefore direct the larvae of *B. jucundus* to suitable settling sites.

In terms of host abundances, prawn burrows are known to influence densities of smaller organisms such as the symbionts (Griffis &Suchanek, 1991; Pillay & Branch, 2011). Dittmann (1996) found that the burrows of the thalassinidean *C. australiensis* (now *Trypaea australiensis*) had a great influence on the densities of infauna, including symbionts. Densities of co-occurring species declined significantly in low burrow density areas. However, this raises the question of whether the densities of these symbionts are a result of burrow availability or other factors such as the mud content or food availability within the burrow. If burrow abundance is the primary determinant of symbiont abundance, as suggested by Dittmann (1996), then the results of this study should have shown a strong positive correlation between host abundance (which is a measure of burrow availability) and symbiont abundance. However, there was no discernable evidence in this study that host abundance has any influence on symbiont abundance. In fact, multiple regression analysis excluded sandprawn as a factor in all models, thus eliminating sandprawn abundance from the factors affecting the distribution and abundance of *B. jucundus*. The relationship between host and symbiont abundances in this study is therefore not as fundamental as thought to be in determine the densities of the symbionts.

Multiple regression analysis showed evidence to support the notion that the number of hosts is not as important as the burrow trophic environment (food availability) and mud content when it comes to the distribution and abundances of burrow symbionts in soft sediment systems. Although chlorophyll *a* was excluded from the model, EPS concentrations and mud content were shown to be better and more significant predictors of *B. jucundus* abundance and distribution. However, it is important to acknowledge that even though host abundance are not as important as the burrow trophic environment in determining *B. jucundus* abundance, they still play an integral part in the distribution of symbionts as symbionts like *B. jucundus* do not exist independently (Reise 2002). It is also important to acknowledge that the trophic environment, which is seemingly more important in the distribution and abundance of the symbionts, is created through the burrowing action of hosts. Therefore, on a patch scale, the trophic environment is more significant in determining distribution and abundance of burrow symbionts but on an ecosystem scale (burrowed and non-burrowed patches), burrow availability would be a very important determinant of symbiont abundance.

Chapter 3: The influence of *Betaeus jucundus* on the behaviour of the burrowing prawn *Callichirus kraussi*

3.1. Introduction

Ecologists are constantly trying to determine the importance of interactions between individuals in populations and communities in order to understand patterns in ecosystems (Bronstein, 1994b; Hay *et al*. 2004). Of these interactions, negative ones such as competition and predation have received more attention than positive interactions (Hay *et al*. 2004). However, ecologists have recently acknowledged that positive interactions are possibly as important as negative interactions in community and ecosystem dynamics (Stachowicz, 2001). One such positive interaction is mutualism.

Mutualism develops when resources become scarce and co-operation between organisms has greater advantages than solitary existences (Odum, 1985). When benefits of co-operation are greater than costs, organisms tend to form mutualistic relationships (Ferriere *et al*. 2002). Stressful environments have been found to promote mutualism as a way of increasing organism survival (Odum, 1985), and it also has been found that potential enemies can become mutualists in stressful environments to enhance survival (Hay *et al*. 2004).

Mutualism develops because one of the mutualists[1] can provide a limiting resource (e.g. refuge) to the partner. Some plants like acacia trees form mutualisms with ants (Boucher *et al*. 1982),which defend the plants from herbivores and in turn obtain shelter and food from the trees (Collinge, 2009). Other organisms form mutualistic relationships to gain refuge from competitors. Corals, for example, have evolved mutualisms with herbivores such as fish that graze on seaweeds that smother corals. Corals in turn provide the herbivores with refuge and

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[1] Mutualists defined here as partners involved in a relationship in which both benefit.

food (Stachowicz, 2001). Subtidal mussels (*Modiolus modiolus*) form mutualistic relationships with sea urchins which graze on kelps, which in turn dislodge mussels rock, causing mortality. Grazing of kelp by urchins increases survival of mussels by decreasing mussel dislodgement. A greater force is required to dislodge urchins off mussel beds than on bare rock, making urchins less susceptible to predation and dislodgement while in mussel beds (Witman, 1987).

Even though both partners benefit from mutualisms, these interactions are still vulnerable to exploitation (Bronstein, 2001). Exploitation occurs when one mutualist obtains a resource from its partner without returning a benefit (Bronstein, 2001). The greatest conflict for all mutualisms is that each of the mutualists is under selection to further exploit the other (Sagers *et al*. 2000). Bronstein (2001) argues that the greatest benefit is gained by each mutualist when each maximises benefit relative to investment. However, models predict that mutualisms can only be stable if all participants can prevent excessive exploitation from each other. Therefore mutualism is not necessarily about maximising benefits but also reducing exploitation (Pellmyr &Huth, 1994).

Population models have shown that increasing numbers of mutualists that depend on a particular partner can lead to a breakdown in the mutualism (Bronstein *et al*. 2003). In this case, the costs become greater than the benefits (Ferriere*et al*. 2002). This tipping of the balance causes destabilisation of the mutualistic interaction. For example, the mutualistic interaction between plants and their pollinators becomes destabilised when pollinator numbers increase, because predation on seeds by pollinator progeny becomes more costly than beneficial for the plants (Bronstein *et al*. 2003).

Bronstein (1994b) notes that mutualisms are ubiquitous throughout nature and many studies on mutualisms have been very descriptive (e.g. identifying the mutualists of a particular species). She also argues that very little is known about broader biological processes underlying mutualistic relationships. The change in behaviour of hosts in response to mutualists is one such gap that is generally poorly quantified, but the situation is worse in marine soft-sediment systems. These ecosystems are three dimensional, permitting organisms to burrow several meters deep within the matrix (Little, 2000; Lohrer & Hancock, 2004) making it difficult to study mutualist behaviour *in situ*.

This chapter broadly aims to contribute to improving knowledge of behavioural interactions between mutualists in marine soft-sediments. The study focuses on clarifying the relationship between *Callichirus kraussi* and *Betaeus jucundus*, with the specific aim being to understand how the behaviour of *C. kraussi* changes in the presence and absence of the *B. jucundus*. A secondary question posed is the effect of the presence of *B. jucundus* on the time spent by *C. kraussi* on ecosystem engineering activities. The reworking and flushing of burrows by *C. kraussi* affects sedimentary and biochemical properties and processes, which in turn can affect benthic communities in soft sediments (Pillay and Branch, 2011). It is hypothesized that the presence of *B. jucundus* should alter the activity budget of *C. kraussi*, which would involve an increase in time spent interacting with *B. jucundus*. However, this would lead to decrease in time spent by *C. kraussi* on activities such as feeding and burrowing irrigation and maintenance, which in turn should alter the ecosystem engineering functions provided by *C. kraussi*. Lastly, the question of whether the sex of *C. kraussi* influences behavioural responses of *C. kraussi* to *B. jucundus*, and whether this influenced the time spent by *C. kraussi* on ecosystem engineering activities was also addressed. The latter question is relevant given that previous studies have shown that behavioural interactions are influenced by organism traits such as size and sex (Magurran, 1986). If host sex does influence ecosystem engineering activities by *C. kraussi*, then this factor could also be an important in indirectly influencing ecosystem functioning in marine soft-sediments.

3.2. Materials and methods

3.2.1. Experimental design

To understand the relationship between the burrowing sandprawn *C. kraussi* and *B. jucundus*, mesocosm experiments were undertaken in which behavioural responses of *C. kraussi* to *B. jucundus* were assessed. Purpose-built tanks were used for the experiments. The dimensions of the tanks were 50cm (length) X 30cm (height) X 0.7cm (width).The narrow width of the tank limited 3 dimensional movements of the burrowing prawns into 2 dimensions and therefore allowed for direct observations of interactions in burrows. A closed circulation system with 4 tanks within a shallow (15cm) tub was used (Figure 3.1). The tub, which acted as a water reservoir, was filled with 75L of water and the 4 tanks were placed upright inside the tub. Tanks were placed on stilts to raise them above the water level in the tub. Water was pumped into the tanks from the reservoir using an HQB water pump with an output of 3000L/hr (Fig. 3.1) and back into the reservoir as overflow from the tanks. The water was kept oxygenated and clean using3 Elite 802 air pumps placed evenly across the reservoir and an Elite Jet-Flo filter.

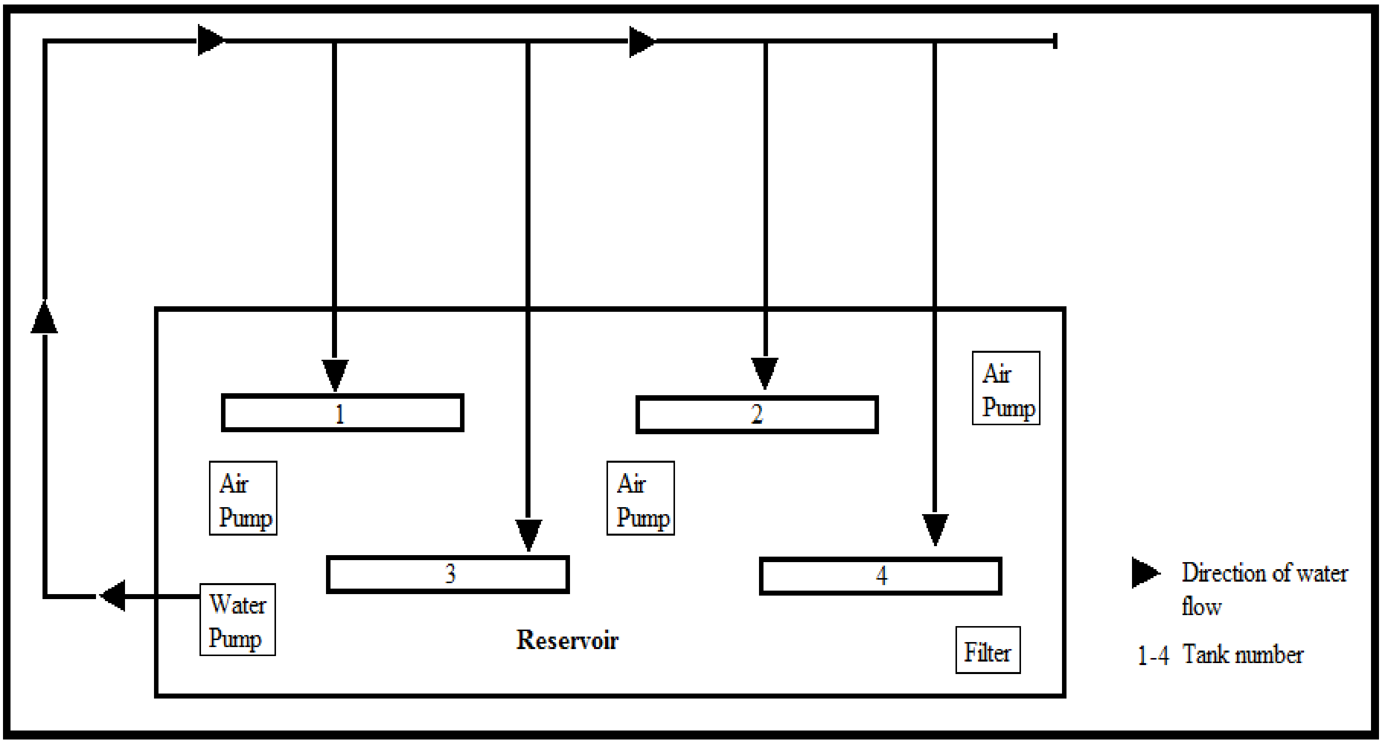

Fig. 3.1: Design of aquarium experiment (top view) used for observations of interactions between *C. kraussi* and *B. jucundus*. Arrows indicate direction of water flow and the numbers (1-4) indicate number of tanks used. Air pumps and a filter were used for aeration and filtration of water in the reservoir.

Tanks were filled to a depth of 20cm with sand collected from Langebaan Lagoon and left to settle for 10 minutes before the *C. kraussi* were introduced into the tank. The prawns were allowed 3 days to construct burrows and acclimatise to their new environment before observations commenced. The sandprawns used had a carapace width of approximately 5.5mm.

Prior to commencing the experiment, observations were made to establish the behaviours of *C. kraussi* exhibited that would form the basis of observation in the final experiment. The behaviours of five *C. kraussi* individuals were observed in the absence and presence of *B. jucundus* for 45 minutes.

Two separate experiments were undertaken using the aquaria setup in Fig. 3.1. This involved observations of the behaviour of the sandprawns for 45mins before *B. jucundus* was introduced and repeated in the presence of *B. jucundus* for another 45mins. One *B. jucundus* was introduced to each tank per observation. A stopwatch with a time lap function was used to record time (in seconds) spent by *C. kraussi* on each behaviour.

The first experiment aimed to determine behavioural changes by *C. kraussi* in the presence and absence of *B. jucundus*. Twelve observations using twelve different *C. kraussi* and *B. jucundus* were made. The second experiment manipulated *C. kraussi* sex with the aim of determining responses of males and females to the presence of *B. jucundus*. Ten observations using five males and five females were made.

3.2.2. Data Analysis

Multidimensional scaling (MDS) ordinations were constructed in PRIMER6.1.11 based on resemblance matrices produced from Bray-Curtis similarities to illustrate variability in *C. kraussi* behavioural responses to *B. jucundus* and variability in male and female *C. kraussi* behaviour in the presence of *B. jucundus.* A square root transformation was applied to the data. PERMANOVA and pair-wise tests were performed to statistically quantify differences in behaviour of *C. kraussi*.

Statistical package for the social sciences (SPSS) was used for univariate data analyses. Analysis of variance (ANOVA) was used to test the effect of presence/absence of *B. jucundus* and host sex on *C. kraussi* behaviour. One sample K-

S and Levene's tests were performed to test for normality and equality of variances. A significance level of 0.05 was used in all statistical tests.

3.3. Results

A summary of behaviours exhibited by *C. kraussi* in the absence of *B. jucundus* is presented in table 3.1.

Table 3.1: Description of behaviours of *C. kraussi* recorded in preliminary observations.

Behaviour	Description
Irrigation	Irrigation of the burrow through back and forth movement of pleiopods.
Feeding	Taking sediment to the mouthpart and sifting out organic matter.
Locomotion	Movement of prawn through tunnels.
Tunnelling	Extension of burrow tunnels through removal of sediment.
Excavation	Sediment ejection from the burrow to form mounds outside the burrow.
Cleaning	Movement of pereiopods from side to side to remove sediment on the body.
Aggression	Physical attack and pursuit of *B. jucundus*.
Burrow sealing	Collecting sediment from within the burrow to close off the burrow opening.
Positive interaction	Contact between host and mutualist followed by non-aggressive behaviour.
Negative interaction	Contact between host and mutualist followed by pursuit of *B. jucundus* out of the burrow.

3.3.1. Experiment 1: effect of B. jucundus presence on C. kraussi behaviour

The responses exhibited by the sandprawn *C. kraussi* to *B. jucundus* were divided into three behavioural categories. The categories consisted of intolerant sandprawns, which pursued *B. jucundus* out of the burrow and sealed off the burrow. The second category, the semi-tolerant individuals, occasionally pursued *B. jucundus* out of the burrow but allowed it to return. The third category, the tolerant individuals, chased *B. jucundus* out of the burrow initially but then formed a relationship with *B. jucundus*, in which *B. jucundus* was allowed to move freely within the burrow system.

MDS ordinations (Fig. 3.2) and PERMANOVA indicated discrete behavioural responses of *C. kraussi* to the presence of *B. jucundus* (PERMANOVA, Pseudo-F= 8.2625, p= 0.001) and different behavioural patterns in the presence and absence of *B. jucundus* (PERMANOVA, Pseudo-F= 28.489, p= 0.001). Behavioural differences were observed between the intolerant and semi-tolerant individuals and between intolerant and tolerant individuals (pair-wise, $p < 0.005$).

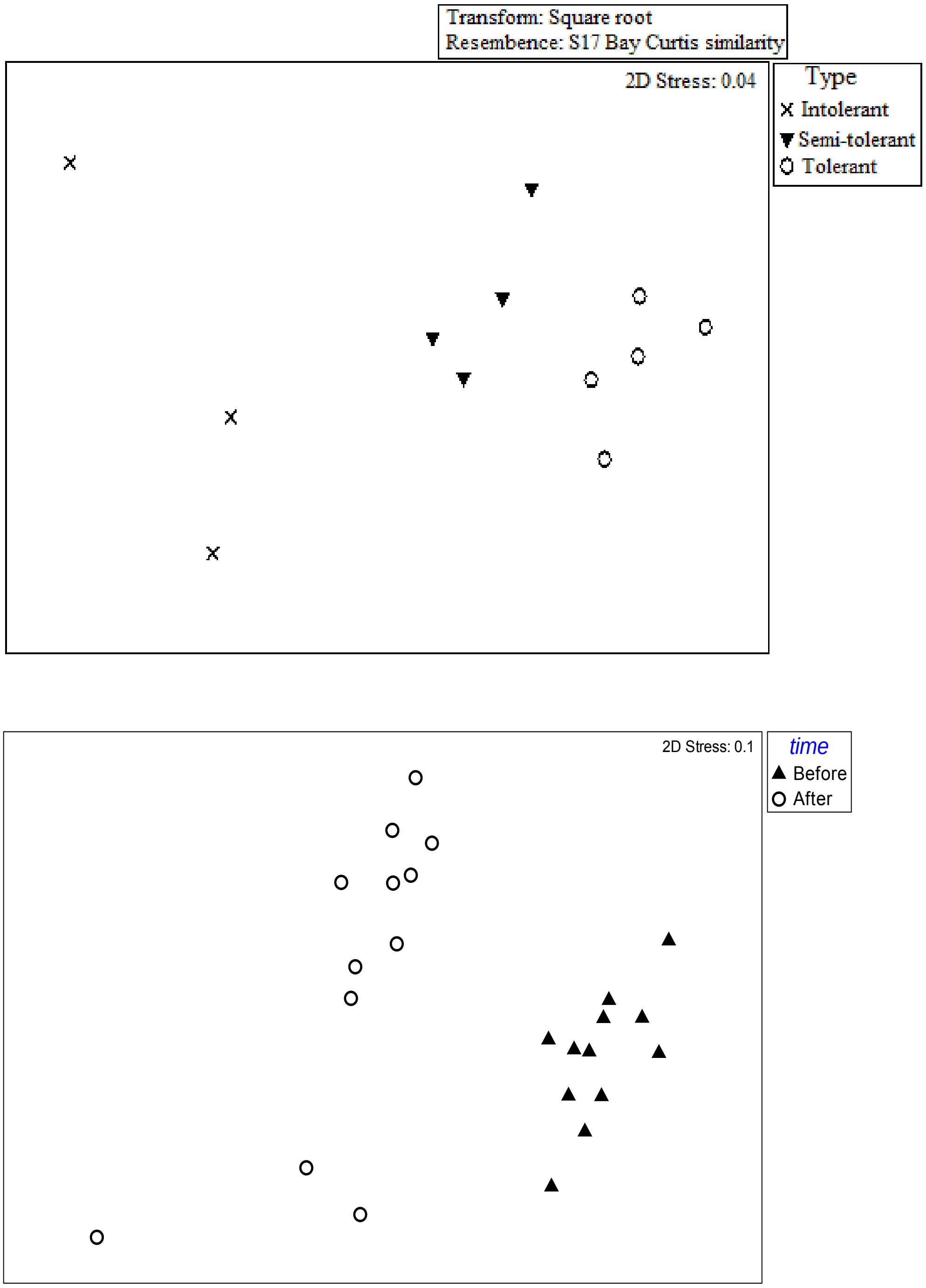

Fig. 3.2: Multidimensional scaling (MDS) ordination illustrating variability in *C. kraussi* behavioural responses to the presence of *B. jucundus* (top) and differences in *C. kraussi* behaviour in the presence and absence of *B. jucundus* (bottom).

Table 3.2: Summary statistics of ANOVA showing individual and interactive effects of behavioural categories and presence/absence of *B. jucundus* on behaviour of *C. kraussi*. *F* = test statistic, df = degrees of freedom, *p* = significance level. * denotes statistically significant differences.

Behaviour	**Behavioural category**			**Presence/absence of mutualist**			**Interaction**		
	F	**df**	***p***	***F***	**df**	***p***	***F***	**df**	***p***
Irrigation	1.217	2	0.319	7.395	1	0.014*	1.509	2	0.248
Feeding	6.912	2	0.006*	1.304	1	0.268	1.232	2	0.315
Locomotion	1.311	2	0.294	0.059	1	0.810	1.607	2	0.228
Tunnelling	3.785	2	0.042*	2.097	1	0.165	1.506	2	0.249
Excavation	5.112	2	0.017*	1.791	1	0.197	3.131	2	0.148
Cleaning	0.588	2	0.566	0.241	1	0.629	0.471	2	0.632
Aggression	3.304	2	0.057						
Burrow sealing	7.475	2	0.004*						
Positive interaction	3.867	2	0.037*						
Negative interaction	1.222	2	0.315						

Time allocated to feeding by *C. kraussi* differed between individuals that were intolerant, semi-tolerant or tolerant of *B. jucundus* (Table 3.2) with tolerant individuals spending more time feeding compared to the other individuals (Fig. 3.3). The intolerant and tolerant individuals spent significantly more time irrigating burrows in the absence of *B. jucundus* (Fig. 3.3). The intolerant individuals spent more time digging and excavating than semi-tolerant and the tolerant individuals especially in the presence of *B. jucundus* (Fig. 3.3).

C. kraussi that were intolerant of *B. jucundus* sealed off their burrows and displayed more aggression towards *B. jucundus* relative to other groups. Unlike the tolerant and semi-tolerant individuals, there was no positive interaction between the intolerant individuals and *B. jucundus* (Fig 3.4). Tolerant *C. kraussi* spent significantly more time interacting positively with *B. jucundus* compared to the intolerant and the semi-tolerant individuals with less time spent on negative interaction (Fig. 3.4).

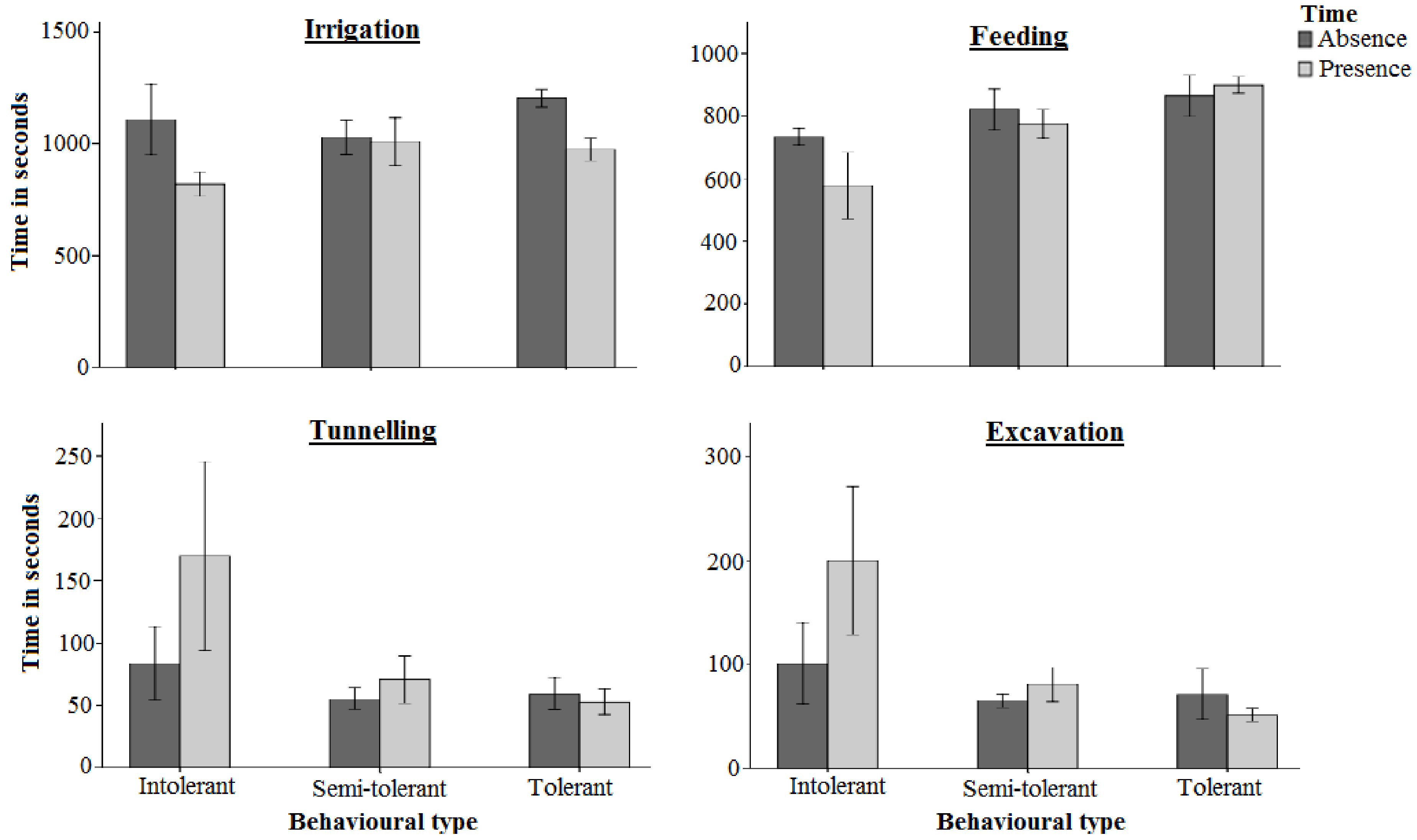

Fig. 3.3: Time (Means ± 1SE) spent by *C. kraussi*on each behaviour in the absence and presence of *B. jucundus*.

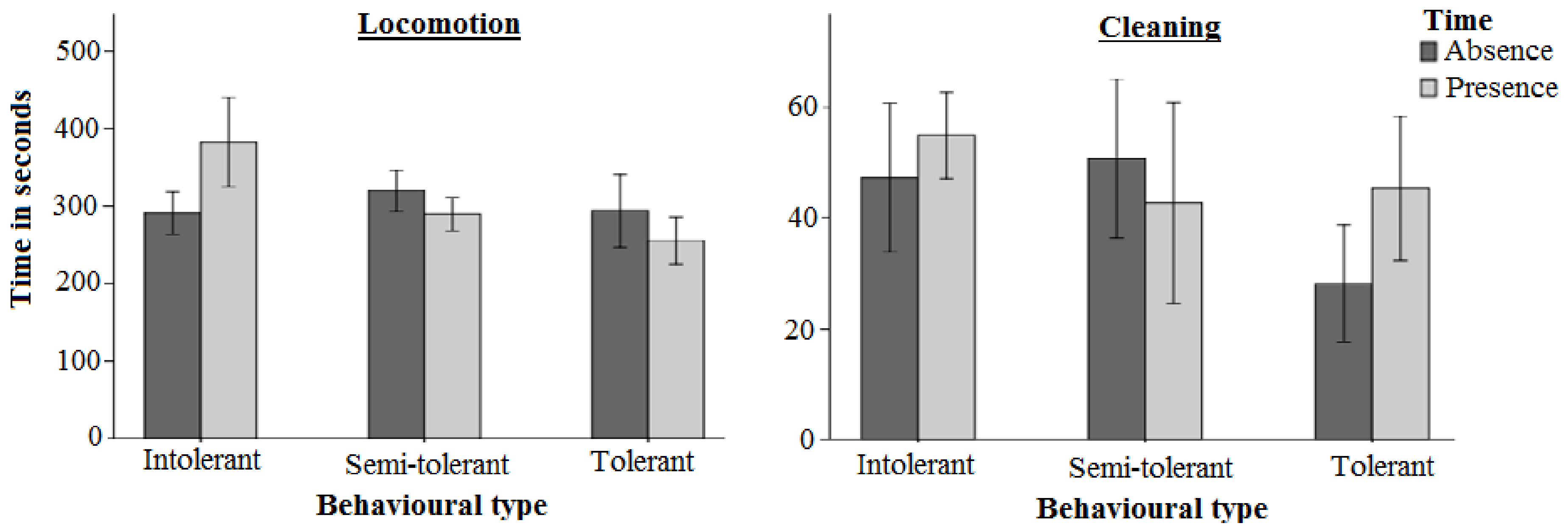

Fig. 3.3 Continued

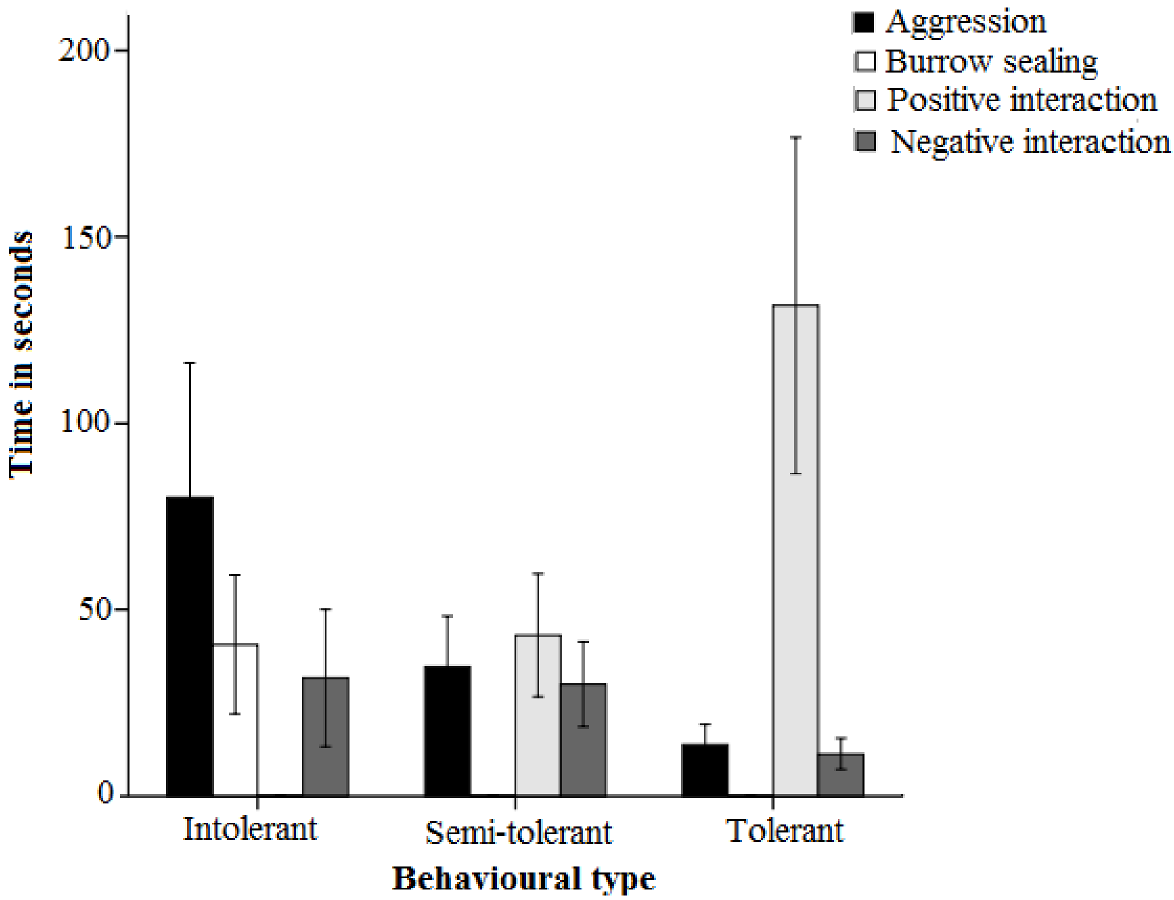

Fig. 3.4: Time (Means ± 1SE) spent on activities performed by *C. kraussi* only in the presence of *B. jucundus*.

3.3.2. Experiment 2: effect of <u>C. kraussi</u> sex on their responses to <u>B. jucundus</u>

MDS ordination indicated no differences in male and female *C. kraussi* behaviour in the absence of *B. jucundus* and distinct behavioural responses between male and female individuals to the presence of the *B. jucundus* (Fig. 3.5).

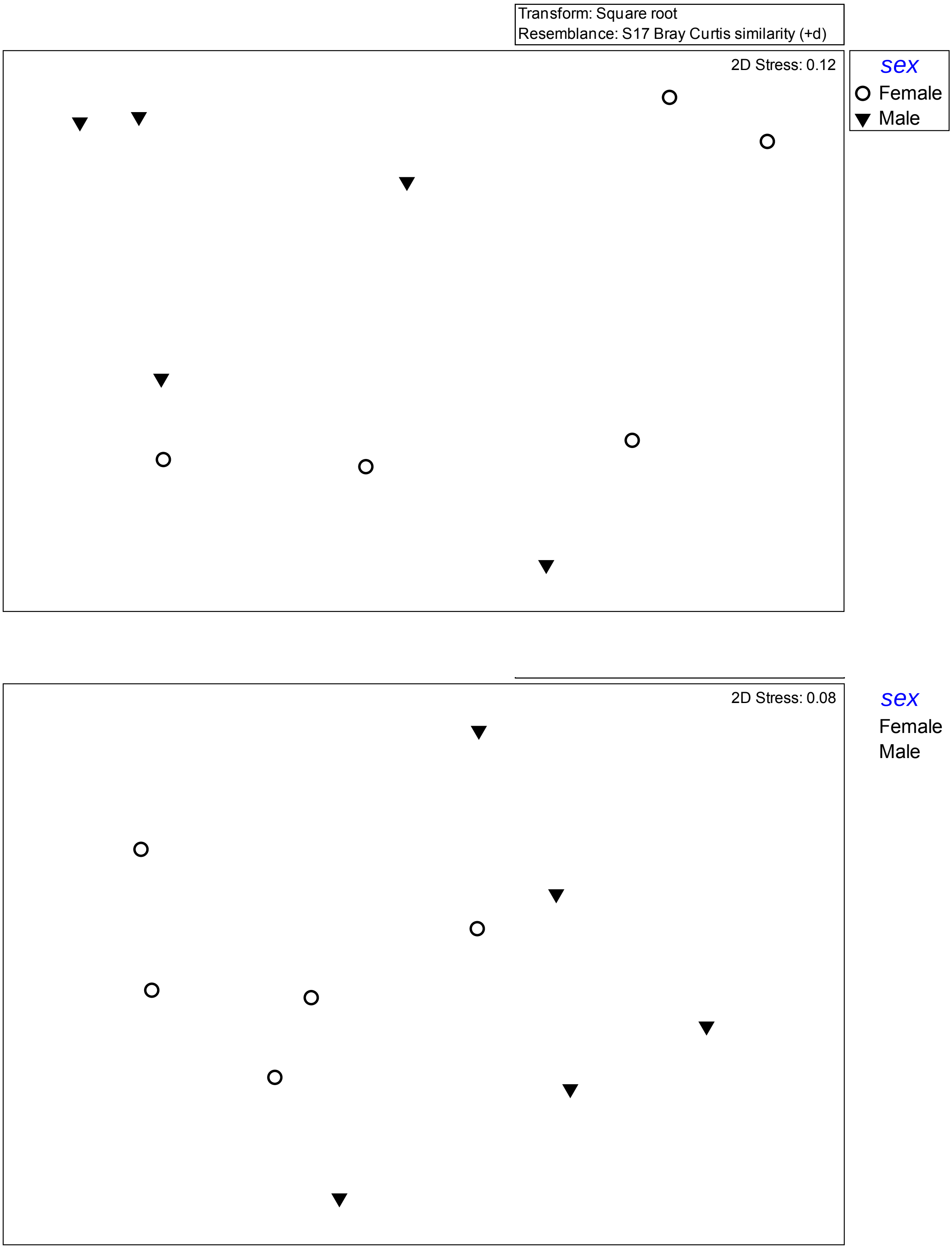

Fig. 3.5: Multidimensional scaling (MDS) ordination illustrating variability in male and female *C. kraussi* behaviour in the absence (top) and presence (bottom) of *B. jucundus*.

MDS ordination patterns were further supported by PERMANOVA (Pseudo-F= 0.99411, p= 0.431) showing no difference in *C. kraussi* behaviour in the absence of *B. jucundus* and the opposite trend (Pseudo-F= 2.9815, p= 0.027) in the presence of *B. jucundus*.

Table 3.3: Summary statistics of ANOVA showing effects of host sex and presence/absence of mutualist on behaviour of host and an interaction of the two variables. F = test statistic, df = degrees of freedom, p = significance level. * denotes statistically significant differences.

Behaviour	**Host sex**			**Presence/absence of mutualist**			**Interaction**		
	F	**df**	***p***	***F***	**df**	***p***	***F***	**df**	***p***
Irrigation	2.837	1	0.112	15.673	1	0.001*	0.059	1	0.811
Feeding	4.856	1	0.043*	0.113	1	0.741	0.461	1	0.507
Locomotion	0.531	1	0.477	4.034	1	0.062	0.150	1	0.704
Tunnelling	0.312	1	0.584	1.321	1	0.267	1.755	1	0.204
Excavation	1.288	1	0.273	3.411	1	0.083	0.832	1	0.375
Cleaning	0.050	1	0.989	15.213	1	0.001*	2.794	1	0.114
Aggression	0.544	1	0.470						
Positive interaction	0.170	1	0.685						
Negative interaction	0.959	1	0.340						

Female *C. kraussi* allocated marginally more time to feeding compared to males (Fig. 3.6), but this was not influenced by the presence or absence of *B. jucundus* (Table 3.3). In the presence of *B. jucundus*, both sexes spent less time irrigating, cleaning themselves and on locomotion (although not statistically significant) (Fig. 3.6). Both sexes spent significantly more time interacting positively with *B. jucundus*. Males allocated more time to aggression towards *B. jucundus* compared to the females, however, this was not significantly different (Fig. 3.7).

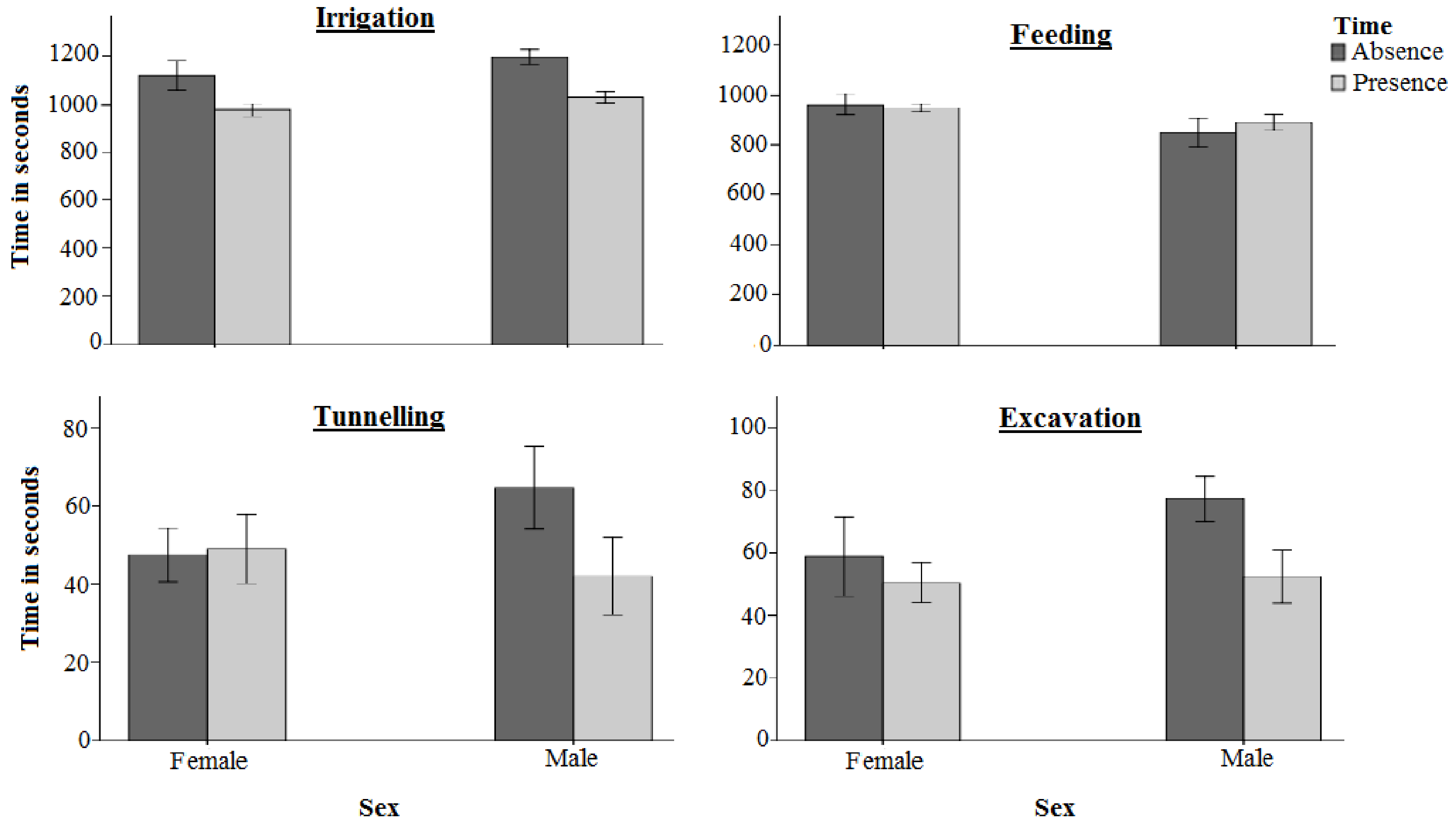

Fig. 3.6: Time (Means ± 1SE) spent by male and female *C. krauss*ion each behaviour in the absence and presence of *B. jucundus*.

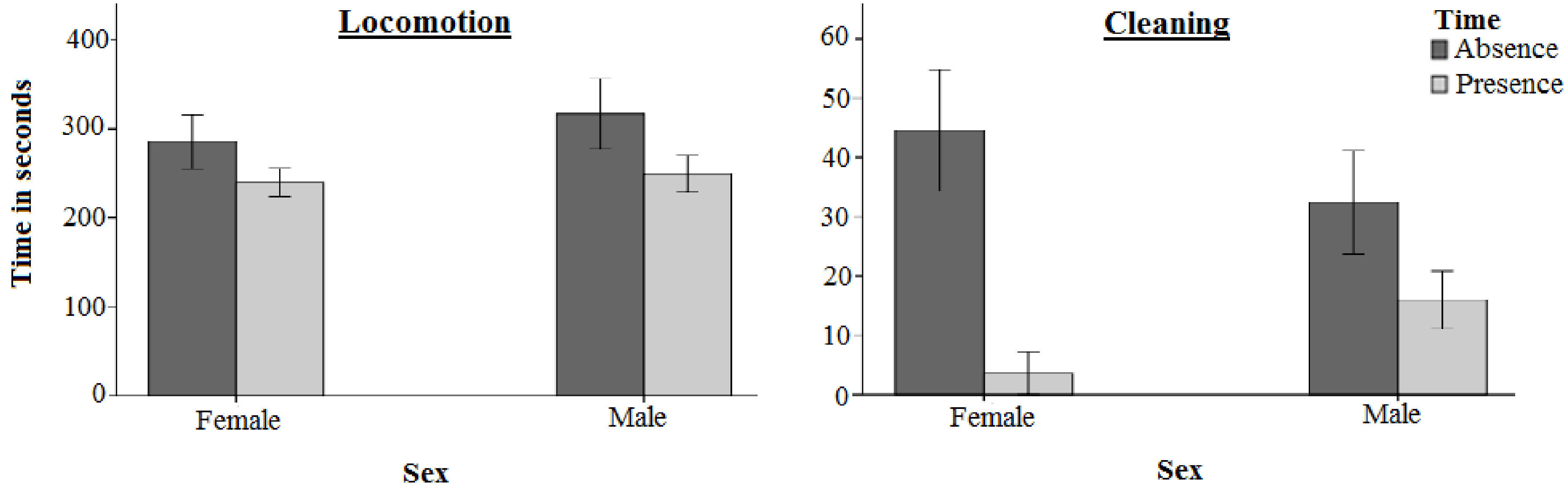

Fig: 3.6: Continued

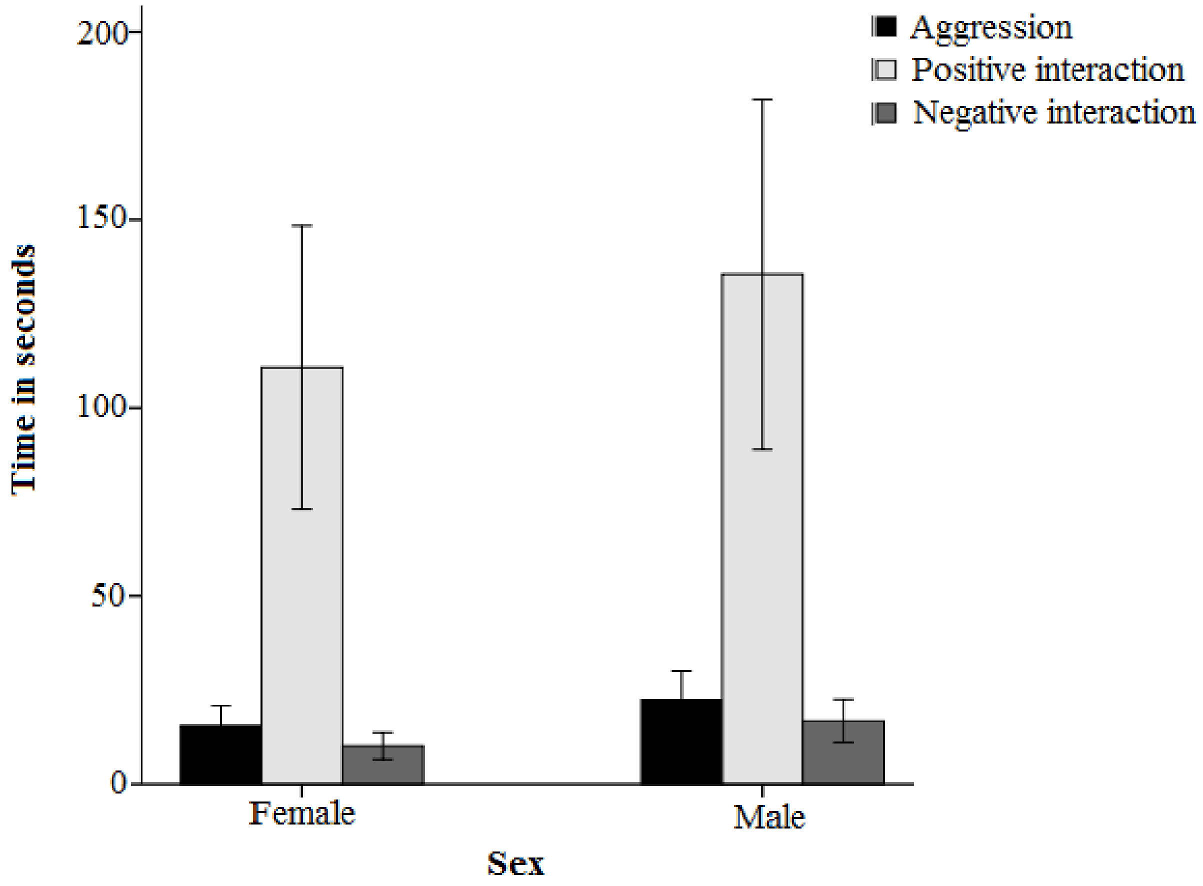

Fig. 3.7: Time (Means ± 1SE) spent on activities performed by male and female *C. kraussi* only in the presence of *B. jucundus*.

3.4. Discussion

Bronstein (1994b) points out that very little is known about ecological and evolutionary processes underlying symbiotic relationships, which hampers understanding of such relationships. Although the relationship between *B. jucundus* and *C. kraussi* is well documented in the literature (Branch *et al*. 2010; Anker & Baeza 2012), there has been little attempt to move beyond the descriptive nature of this relationship. This chapter therefore attempted to understand the behavioural responses of *C. kraussi* in the presence and absence of *B. jucundus*, as well as the role of host sex within this symbiosis.

3.4.1. Response of C. kraussi to B. jucundus

The presence of *B. jucundus* elicited three distinct behavioural responses from *C. kraussi*. The first response was intolerance, where individuals displayed aggression toward *B. jucundus*. Intolerant responses involved *C. kraussi* chasing *B. jucundus* out of the burrow followed by sealing of burrows to prevent re-entry of *B. jucundus*. The second response was semi-tolerance, where *C. kraussi* occasionally pursued *B. jucundus* out of the burrow but permitted its re-entry into the burrow. The third response was tolerance, where *C. kraussi* were initially chased *B. jucundus* out of the burrow and subsequently permitted *B. jucundus* to reside in the burrow, followed by the formation of a relationship. At this point, it is not quite clear why *C. kraussi* displayed such variable responses to the presence of *B. jucundus*.

Pillay (2010) described the relationship between *C. kraussi* and *B. jucundus* as a mutualism. However, the wide spectrum of responses observed in the study challenges the conclusions made by Pillay (2010). The contrasting results between this study and that of Pillay (2010) could be due to the differences in sample sizes and observations made. Pillay (2010) had only one observation, which did not show the non-mutualistic behaviours observed in this study with a greater sample size.

The fact that *C. kraussi* demonstrates such a wide spectrum of responses to the presence of *B. jucundus* suggests that the relationship between *C. kraussi* and *B. jucundus* might not be a strong mutualism, as previously described (Pillay 2010), but more of a facultative mutualism than an obligate one. Obligate mutualists cannot survive in the absence of their mutualist partners (Dean, 1983).If the mutualism between *C. kraussi* and *B. jucundus* was truly obligate, then intolerant responses by *C. kraussi* would not have been observed. Facultative burrow mutualists are associated with several burrowing taxa, and it is likely that the burrow habitat is the main attractant for the mutualists rather than the host itself (Anker *et al*. 2005). *B. jucundus* belongs to the family Alpheidae, which has at least 20 species known to be facultative mutualists of echiurans, stomatopods, other thalassinideans, and fishes (Anker *et al*. 2005). However, like most Alpheidae, *B. jucundus* has a very cryptic lifestyle (Anker *et al*. 2005; Anker & Baeza, 2012) which is a major hindrance to developing our understanding of the species (Anker & Baeza, 2012).

The different responses of *C. kraussi* to *B. jucundus* may be linked with some aspect of the biology of specific *B. jucundus* used in experiments. The appeal of a mutualist to a host might depend on size, age and sex of the other partner (Bronstein, 1994a). Heil (2013) suggests that hosts can possibly identify a future partner in advance and associate only with “high quality” (those that provide maximum benefits) mutualists. *C. kraussi* that displayed intolerance to *B. jucundus* could have rejected the mutualist because it was not of a high enough “high quality”. The size, age and sex of *B. jucundus* used in the experiment could not be accurately determined due to their small size, and these features could have contributed to the different *C. kraussi* behavioural responses observed.

C. kraussi used in the experiment were of similar size, with an average caparace lengths of 5.5mm. It is therefore unlikely that *C. kraussi* size could have contributed to its variable behavioural responses observed. The sex of the host may have been responsible for observed differences in their behavioural responses, therefore necessitating a second experiment that manipulated host sex to determine behavioural responses to the presence of *B. jucundus*. In the absence of *B. jucundus*, there was no difference between male and female *C. kraussi* behaviour. However, this pattern changed in the presence of *B. jucundus*. Some of the responses shown by *C. kraussi* to the presence of *B. jucundus* could therefore be a result of the host sex.

The strength of a mutualism is determined by the services required by the two partners at any given time. A mutualism is dependent on each partner providing a required service and receiving benefits for the services provided without compromising its fitness (Boucher *et al*. 1982; Bronstein, 1994; Stachowicz, 2001). If the service being provided by the mutualist is not required, then the strength of the mutualism becomes weak and the partner might choose to terminate the relationship. In other words, the decision by a mutualist to participate in a mutualistic relationship is dependent on its expected payoff (Axen *et al*. 1996). Considering intraspecific variation in behaviour due to sex, (Magurran, 1986; Krebs & Davies, 2009), *C. kraussi* males and females may require different services from *B. jucundus* at any given time. However, based on the data obtained, there was no clear indication of what these services might be.

Differences in male and female *C. kraussi* behaviour were observed in time allocated to feeding. Female *C. kraussi* spent significantly more time feeding compared to the males. Since the sizes of the males and females were approximately the same, it is unlikely that body size is the cause of the differences observed. Boinski (1988) studied the foraging behaviour of squirrel monkeys and found that female monkeys spent more time foraging compared to the males. Boinski (1988) argued that nutritional requirement might differ due to different activities performed by males and females, with females possibly having a greater nutrient and energetic demand due to their investment in reproduction.

Ginnett & Demment (1997) also demonstrate that male giraffes spent less time foraging compared to females but had higher consumption rates. Therefore, males consumed more food than females by increasing their bite size and chewing rates and by reducing chewing effort. Male *C. kraussi* could therefore spend less time on feeding than females because they have also developed strategies to maximise feeding over a shorter time.

Both sexes of *C. kraussi* tolerated the presence of *B. jucundus* to some degree as none of the individuals sealed off their burrows to keep the *B. jucundus* out. This was not the case in experiment 1, in which some *C. kraussi* pursued *B. jucundus* out of burrows and sealed the opening. In a study by Bshary & Noe (2003) on cleaner fish and their clients, it was established that fleeing and aggression between cleaner and client rarely ever occur once a relationship has been established. Apparently, cleaners and clients need to build a relationship to eventually gain benefits from the interaction. The aggressive pursuit of *B. jucundus* by intolerant *C. kraussi* might be due them not being able to form a relationship. In tolerant and semi- tolerant *C. kraussi*, initial interactions with *B. jucundus* were also aggressive, but this was followed by contact and tactile communication, which was then followed by tolerance. It is possible that during contact, some aspect of *B. jucundus* is assessed regarding the quality of service it could provide to *C. kraussi*, which would then determine if a relationship between host and mutualist could develop.

Male *C. kraussi* displayed marginally more aggression than females to the presence of *B. jucundus*, though this was not statistically significant. In a study of territorial behaviour of anemone fish, Moyer & Sawyers (1973) they found that when defending nests against invaders, both males and females displayed aggressive defence against invaders, but males showed slightly more aggression than females. Paull *et al* (2010) found that in dominance hierarchies of zebrafish, dominant females were less aggressive to their subordinates than dominant males. The finding that male

C. kraussi are marginally more aggressive than females are consistent with several other studies showing the same trend (Moyer & Sawyers, 1973; Paull *et al*. 2010).

The findings of the experiments conducted in this study generally indicate a decline in time allocated to certain behaviours by *C. kraussi* in the presence of *B. jucundus*. The time budget of *C. kraussi* is expected to shift given that the presence of *B. jucundus* in burrows would necessitate more time spent by *C. kraussi* on interacting with *B. jucundus*. Morales *et al.* (2008) argues that within a mutualism, interspecific communication is important in regulating and coordinating investments between mutualistic partners. Therefore, some of the shifts in time budgets of *C. kraussi* could be related to time spent of communication with *B. jucundus*.

3.4.2. Indirect effects of B. jucundus on ecosystem functions

Mutualism between foundation species and their residents are very important for the functioning of entire ecosystems (Hay *et al*. 2004). Foundation species are species that define much of the local community structure by creating stable conditions for other species and by modulating ecosystem processes (Rohr *et al*. 2009). *C. kraussi* is one such species (Pillay & Branch, 2011). Residents often provide services that benefit foundation species and these benefits could have a cascading effect to the system (Hay *et al*. 2004).

In experiment 1 of the present study, time spent by *C. kraussi* excavating sediment and building mounds increased in the presence of *B. jucundus*, specifically for intolerant *C. kraussi.* Studies have shown that the reworking of sediment by *C. kraussi* influences sediment processes and properties (Pillay & Branch, 2011). The mounds formed during excavation result in sediment unevenness which enhances oxygen transfer into burrow, resistance to wave action and water swirling and eddying over the burrows. In addition, these sediment reworking activities can alter the structure of surface dwelling filter- and deposit-feeders (Pillay & Branch, 2011).Therefore, subtle indirect changes in sediment reworking by *C. kraussi* due to the presence of *B. jucundus* may translate to subtle changes in benthic community structure at very fine scales.

On a similar note, burrow irrigation rate by *C. kraussi* was reduced in the presence of *B. jucundus* in experiment 1 and 2 of this study. Irrigation by thalassinidean is known to affects biochemical properties and processes in porewater in soft-sediment systems (Pillay & Branch, 2001). Callianassids primarily irrigate their burrows for gaseous exchange and to pump burrow waters out when concentrations of excretory wastes become too great (Stamhuis *et al* 1996). Therefore, irrigation by thalassinideans influences the depth to which nutrients and gases (primarily oxygen) penetrate into sediments, which in turn influence various biochemical processes and ultimately the distribution and abundance of benthic organisms (Pillay & Branch, 2011).Therefore, declines in irrigation rates by *C. kraussi* in the presence of *B. jucundus* could potentially have important ramifications for the functioning of soft-sediment ecosystems.

Chapter 4: Final summation and conclusions

Marine soft-sediments are the most common habitat in the world, covering about 70% of the world's seafloor (Wilson, 1991) and harbouring a variety of taxonomic groups (Ellingsen, 2002). One of the most important groups is the thalassinideans, which greatly influence the structure and composition of benthic communities through burrowing (Pillay *et al.* 2007). These burrowers create biogenic structures that lead to co-habitation by other species (Reise, 1981) and to the evolution of elaborate symbiotic assemblages (Pillay, 2010).

We know that these elaborate symbiotic assemblages exist (Branch *et al.* 2010) but the factors determining the abundance and distribution of the diverse range of co-inhabiting symbiotic fauna is poorly understood. The 2^{nd} chapter of this dissertation sought to determine if the distribution and abundance of the burrow symbiont *Bateaus jucundus* was primarily determined by trophic or non-trophic functions provided by burrows of *Callichirus kraussi*. It was hypothesised that food availability would best predict symbiont abundance in the burrows.

This study showed no evidence of a positive relationship between *C. kraussi* and *B. jucundus* abundances (Fig. 2.10) as would have been the case if the distribution and abundance of the symbiont was dependent on number of burrows available. However, mud content together with the burrow trophic environment (EPS and chlorophyll *a* concentrations) had a positive effect on *B. jucundus* abundance.

Multiple regression analysis selected a model with EPS and mud content as the best variables to explain *B. jucundus* abundance and distribution. Chlorophyll *a* and sandprawn abundance were excluded from the model.It would thus appear that the primary determinants of the distribution and abundance of *B. jucundus* in Langebaan Lagoon system were sediment mud content and food availability (specifically EPS) in burrows. Mud content is unlikely to provide a direct effect on the abundance of *B. jucundus*, but could indirectly affect *B. jucundus* abundance by influencing the burrow trophic environment. Studies have shown that muddy sediments are more cohesive and have higher levels of organic matter than sandy sediments (Little, 2000). The cohesive nature of mud allows for a stable environment and microorganisms (diatoms, bacteria) can flourish leading to high biomass and thus food availability to burrow symbionts. Chapter 2 concluded that although burrowing prawns are important ecosystem engineers in marine soft sediment systems, it is the burrow trophic environment rather than the abundance of the engineer itself or the burrows they create, that determined the abundance of symbionts occurring in burrow systems.

Chapter 3 aimed to quantify the behavioural interactions that occur between *C. kraussi* and *B. jucundus* by experimentally determining responses of the host to the presence of the *B. jucundus* and the role of host sex in determining their responses to the *B. jucundus*.

Callichirus kraussi exhibited a variety of responses to the presence of *B. jucundus*. The first response was intolerance, where *C. kraussi* pursued *B. jucundus* out of the burrow and sealed off the burrow. The second response, semi-tolerance, involved *C. kraussi* occasionally pursuing *B. jucundus* out of the burrow but allowed it to return. The third response, tolerance, involved *C. kraussi* chasing *B. jucundus* out of the burrow initially but then formed a relationship with *B. jucundus*, in which *B. jucundus* was allowed to move freely within the burrow system. In view of the range of behaviours exhibited by *C. kraussi*, it seems likely that this particular mutualism was more facultative than obligate, as obligate mutualists do not survive in the absence of their mutualist partners (Dean, 1983).

The different responses of *C. kraussi* to *B. jucundus* may have been linked to size, age and sex of either mutualist or the host. The age and sex of *B. jucundus* used in the experiment could not be determined due to their small size and the *C. kraussi* used in the experiment were of similar size. The possibility that host sex may have been responsible for observed differences in host behavioural responses called for a second experiment that involved exposure of *B. jucundus* to *C. kraussi* of different sexes.

In the absence of *B. jucundus*, there was no difference in behaviour between male and female *C. kraussi*. However, in the presence of *B. jucundus*, male and female *C. kraussi* interacted differently in response to the burrow symbiont. These differences could be due to male and female *C. kraussi* requiring different services from *B. jucundus* at any given time. However, based on the data obtained, there was no clear indication of what these services might be.

Taken collectively, the study has contributed to increasing our understanding of ecological processes that determine the abundance and distribution of burrow symbionts in marine soft-sediments as well as the behavioural interactions that occur between burrowers and their symbionts.

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