The epibiotic relationship between mussels and barnacles

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By

Caroline Margaret Bell

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Abstract

Epibiosis is an ecological relationship that has been described as one of the closest possible associations in marine ecosystems. In the space limited rocky intertidal, mussel beds provide important secondary space for barnacles. The epibiotic relationship between mussels and barnacles on the south-east coast of South Africa was considered at different scales, from large-scale, natural patterns of epibiosis on the rocky shore, to fine-scale settlement choices of barnacles and the effects on the condition and growth rates of individual mussels. Mussel and barnacle assemblages were generally stable over a 12-month period. The tracking of individual mussels with and without barnacle epibionts resulted in a significant increase in mortality rate of mussels with epibionts over 12 months (two-way ANOVA, $p = 0.028$). Barnacles on rocks, as well as on mussels, were also tracked with no significant effect of substratum on mortality of barnacles (two-way ANOVA, $p = 0.119$). Prevalence and intensity of barnacle infestations was also examined in relation to coastline topography on two co-occurring mussel species, the indigenous *Perna perna* and invasive *Mytilus galloprovincialis*. The results were complex, but bay status had significant effects on prevalence and intensity for both mussel species, depending on the time and zone. The effect of bay in relation to time was particularly relevant for *M. galloprovincialis* (four-way nested ANOVA, Season X Site(Bay): $p = 0.0002$), where summer prevalence was higher than that of winter in bays, regardless of zone, while in open coast sites, the effect of season was only significant in the mid zone. Patterns of intensity generally showed higher values in summer. Substratum preference by barnacles was investigated by recording settlement, survival and mortality of *Chthamalus dentatus* barnacles on various treatments. There was a strong preference for the rock-like plastic substratum by primary settlers (pair-wise tests of PERMANOVA: Dead < Rock mimic ($p = 0.0001$); Replica < Rock mimic ($p = 0.019$) and Live < Rock mimic ($p = 0.0001$)). This indicates that barnacles settle on mussel shells only as a secondary choice and that micro-topography is an important variable in barnacle settlement. The effect of barnacle epibiosis on condition index and growth of *P. perna* and *M. galloprovincialis* was also examined as a direct indication of the health of mussels subjected to the biological stress of epibiosis. Although not significant (PERMANOVA: *P. perna:* p(perm) = 0.890; *M. galloprovincialis:* p(perm) = 0.395), growth for both mussel species was slower for barnacle-infested individuals in summer, which is the main growing season for mussels in the region. Results from condition index calculations, however, showed no negative impacts of epibiotic barnacles (three-way ANCOVA: *P. perna:* $p = 0.372$; *M. galloprovincialis:* $p = 0.762$).

Barnacle epibionts create a new interface between the mussel and its environment and this interaction can affect other members of the community. The possibility of the barnacle epibiont causing increased drag also needs further investigation. Biological processes operating within a wide range of physical stressors drive the interactions on the rocky shore, such as epibiosis. Overall, the results of this study suggest that the epibiotic relationship between mussels and barnacles on the south-east coast of South Africa does not significantly affect the mussel species present and that barnacles only use mussel shells as a secondary choice of substratum.

Table of Contents

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Chapter One

General Introduction

Epibiosis refers to an ecological relationship in which an organism, the ‘epibiont’, lives on, or attaches itself onto, the outer body surface of another, the ‘basibiont’ (Wahl et al. 1997). These terms describe ecological roles and are rarely species-specific, but potentially there are numerous advantages and disadvantages for both parties involved (Wahl 1989). This interaction can be one of the closest symbiotic associations (Laudien & Wahl 2004) and if the relationship was mutually positive one would expect the basibiont to evolve attraction mechanisms and the epibiont to show signs of settlement specificity. Positive interactions can, however, be indirect and this is often the case in stressful environments, where primary space-holders frequently buffer nearby species against harsh environmental conditions (Bertness & Callaway 1994, Brooker & Callaghan 1998, Bertness et al. 1999, Menge 2000). When living space is in short supply, the colonisation of a living surface (epibiosis) commonly occurs and this can be by a sessile animal, plant, or even bacteria (Wahl & Sonnichsen 1992). Occasionally there are epibionts in terrestrial environments, but these are generally limited to humid climates and to the plant kingdom (Wahl & Mark 1999). Terrestrial examples are vines, or lianas, using trees for support (Putz 1984), as well as ferns and orchids growing on tree branches (Taylor & Wilson 2003). Mosses, algae and lichens are further terrestrial examples, but it is more common to find epibiotic associations in aquatic habitats, both marine and freshwater (Wahl & Mark 1999).

Marine systems, in particular, have high occurrences of plants and animals used as substrata due to space being a limiting resource (Paine 1979, 1984, Witman & Suchanek 1984). This is especially the case for rocky shores (Connell 1961b, Paine 1966, Dayton 1971), which are widely accepted as having a high variety of habitats and high physical heterogeneity (Kostylev et al. 2005) in which, mussel beds provide important secondary space (Murdoch 1969, Dayton 1971, Paine 1979, 1984, Lee & Ambrose 1989, Buschbaum 2000, Erlandsson et al. 2005). Lohse (1993a) points out that, since mussel beds are actually more spatially intricate than rock, the amount of available and suitable substratum for settlement by other organisms is most likely greater where mussels are present. Mussel distribution itself is heterogeneous and exhibits nested patchiness, where smaller patches occur within larger ones (Erlandsson et al. 2005). These patches can act as refuges for species that live in and around the mussel beds. Jones et al. (1994) coined the term ‘ecosystem engineers’ for any organism that changes or controls the availability of resources for other organisms. Mussels are prime examples of such a group of habitat makers, as they provide suitable and often less stressful habitats for other organisms that live on rocky shores (Bertness & Callaway 1994). It is a well-recognised attribute of intertidal systems that neighbouring organisms lessen the obvious stresses of heat and desiccation (Dayton 1975, Menge 1978, Bertness 1989, Bertness & Leonard 1997). These and other stresses form severe gradients of physical stress on rocky shores. These gradients serve as a valuable base to predict biological patterns (e.g. Connell 1972) or to experimentally assess the effects of any changes in abiotic factors on organism interactions (Bertness et al. 1999). Hydrodynamically, they include large-scale effects such as oceanographic mechanisms (Shanks 1983), as well as smaller-scale influences such as micro-hydrodynamics (Pineda 2000). Coastline topography is linked to these effects and can be defined as the alongshore shape of the coast, not including relief features (von der Meden et al. 2008). Coastlines that consist of headlands and associated bays have been thoroughly studied with regards to larval retention, settlement rates and adult abundances (Wing et al. 1998, Archambault & Bourget 1999, Roughan et al. 2005, Mace & Morgan 2006). South Africa’s south coast has a number of large bays within a few hundred kilometres of each other and all in the same biogeographic region (Bustamante & Branch 1996), which makes it logistically and biologically ideal for comparing multiple bay and open coast systems (von der Meden et al. 2008). Generally, the south coast is exposed to high levels of wave action, but certain areas, especially within bays, are relatively sheltered (McQuaid et al. 2000, Erlandsson et al. 2005). How exposed to wave action a particular rocky shore is, can influence its biology in different ways, including the distribution, biomass, composition and interactions of species (Kingsbury 1962, Underwood & Chapman 1998).

The patterns and structures of assemblages generated in an intertidal system have been studied extensively for a number of decades and interactions between species on the rocky shore have also been a common target. These interactions play a large role in determining the distribution and abundance of species (Jones et al. 1994). As epibiosis is one of the closest interactions in intertidal ecosystems (Laudien & Wahl 2004), it provides an opportunity to examine symbiotic associations in much finer detail. The effect of the epibiont on the “host” organism may be positive, negative or neutral (Witman & Suchanek 1984) and according to many authors, the epibiotic associations in the marine realm appear to be commensal (e.g. Seed & O’Connor 1981, Keough 1984, Laihonen & Furman 1986), but it has also been suggested the relationship could be mutualistic or, in some cases, competitive (Dittman & Robles 1991). Wahl (1989) defines epibiosis as non-symbiotic and emphasises the association as facultative, although the significance of the various effects of epibiosis is dependent on the biology and life history traits of the epibionts and basibionts. The abiotic surroundings will have an influence on the interaction, too (Wahl 1989), and it is important to realise that, by settling on another organism, the epibiont has created a new interface between the basibiont and its environment (Laudien & Wahl 1999). Epibiosis has been studied over a wide range of species, from the barnacle, *Balanus crenatus*, settling on the outer shells of *Littorina littorea* (Buschbaum & Reise 1999), to another *Balanus* sp. (*B. improvises*) settling on the eggcases of a dogfish species (*Scyliorhinus canicula*) and the brittle star, *Ophiura texturata* (Bers & Wahl 2004), to epibiotic macroalgae growing on mussels (Witman & Suchanek 1984, Dittman & Robles 1991, O'Connor et al. 2006), as well as sponge species growing on scallops of the genus *Chlamys* (Bloom 1975, Forester 1979, Pond 1992).

Worldwide, mussels are commonly overgrown by epizoans (Witman & Suchanek 1984) and a number of studies have examined the relationship between host and epibiont, in this case the relationship between mussels and barnacles, respectively. One of the possible disadvantages for the infested mussel is increased predation, as shown in a study on *Mytilus edulis* and the starfish predator, *Asterias rubens,* that showed preference for mussels with barnacle epibionts (Laudien & Wahl 1999). Other harmful effects that have been demonstrated include reduction in growth of mussels when overgrown by barnacles (Buschbaum & Saier 2001). Laihonen and Furman (1986), however, found no significant effect of *Balanus improvises* on the growth rates of *M. edulis*. Epibiotic barnacles, however, grew faster on living shells as opposed to dead shells, suggesting a commensal relationship (Laihonen & Furman 1986). The various antifouling properties of mussel shells could also be important in determining the nature of the epibiosis (Bottjer 1981, Bers & Wahl 2004, Bers et al. 2010). Antifouling strategies are employed by potential basibionts to limit or prevent colonisation by epibionts (Wahl 1989) and can be mechanical, chemical or physical (Scardino et al. 2003). Many epibionts that attach to mussel shells, including barnacles, increase the height of the mussel and thus cause increased drag and higher risk of dislodgement (Witman & Suchanek 1984, Wahl 1997, Bers & Wahl 2004). The possibility of the basibiont or host organism being torn loose from the substratum due to its epibionts was originally suggested by Dayton (1973) and Paine (1979), but Witman and Suchanek (1984) also showed this to be the case for mussels that were overgrown with, mostly, the brown alga, *Fucus distichus,* and the barnacles *Semibalanus cariosus* and *Balanus nubilus.*

The mussel species that occur intertidally in South Africa comprise four species, *Aulacomya ater* (Molina), *Choromytilus meridionalis* (Krauss), *Perna perna* (Linnaeus), and the invasive Mediterranean mussel, *Mytilus galloprovincialis* (Lamarck) (van Erkom Schurink & Griffiths 1991), along with the alien mussel species, *Semimytilus algosus*, recently reported from the west coast (Mead et al. 2011). The Mediterranean mussel, *M. galloprovincialis*, and the indigenous *P. perna* have co-occurred along the south coast of South Africa since the introduction of *M. galloprovincialis* (Grant & Cherry, 1985). At sites colonised by both species, *P. perna* tends to inhabit the low to mid mussel zone and *M. galloprovincialis* occupies the high mussel zone (Bownes & McQuaid 2006). The barnacle species of primary importance in the present study include *Chthamalus dentatus* (Krauss), *Tetraclita serrata* (Darwin) and *Octomeris angulosa* (Sowerby). Delafontaine and Flemming (1989) have studied these species on the south-east coast of South Africa. They found that the physical parameters of submergence time and wave exposure determine species distribution on the rocky shore. Generally, *C. dentatus* occurs in the upper intertidal, *T. serrata* in the mid zone and *O. angulosa* in the mid to low zones (Branch et al. 1994).

Of the mussel species in the present study, *M. galloprovincialis* has weaker attachment than *P. perna* (Zardi et al. 2006b) and thus one would expect any epibiotic organisms attached to *M. galloprovincialis* shells to have a negative effect due to greater drag. The pedal glands of most adult bivalve molluscs produce an energetically expensive (Griffiths & King 1979) complex of proteinaceous threads, known as the byssal threads, that attach the individual to the substratum (Price 1983). Mussels can adjust their attachment strength by varying the number and the thickness of these byssal threads (Bell & Gosline 1997, Carrington 2002, Zardi et al. 2006b). Issues of attachment strength can be referred back to wave exposure and coastline topography and the large-scale influences at play when considering mussels and their epibionts.

Extensive work has been done on the effects of epibiosis on the basibiont species. The implications of such a close relationship are important in ecological and evolutionary contexts, for the understanding of assemblage dynamics and strength of biological interactions.

1.1 Structure of the thesis

The present study was intended to give an unbiased view from the perspectives of the epibiont and basibiont (in this case the barnacle and the mussel). The four data chapters give an overview of epibiosis at different scales through a gradient approach, starting from the long-term effects of natural occurrence of epibiosis on the shore to more detailed effects at specific times and at varying spatial scales, including measurements of mortality, settlement choice by epibionts and fitness. Chapter Two describes the changes in overall abundance of mussels and barnacles on the rock surface. It then looks at mortality rates of mussels with or without barnacles attached to them and mortality rates of *Chthamalus dentatus* barnacles attached to rock or mussel shells. Chapter Three involves an extensive study across six sites (three bay sites and three open coast sites). It examines the prevalence (i.e. incidence rate) and intensity (i.e. the percentage of an individual mussel that is covered by barnacles) of barnacle infestations on the different mussel species, *P. perna* and *M. galloprovincialis*. Chapter Four takes up the epibiotic relationship from the barnacle's side and looks at substratum preference shown by *C. dentatus* at settlement. Mortality and survival of adult barnacles and mussels are recorded to evaluate the potential effects at the population level. Chapter Five examines the physiological effects of epibiosis on the two mussel species, *P. perna* and *M. galloprovincialis.* No distinction was made between barnacle species. The parameters measured were growth and condition index. These parameters give an indication of the health of an individual and of any perceived stress that the species is experiencing.

Chapter Two

Temporal changes in abundances of mussels and barnacles on the rocky shore and the long-term effects of epibiosis

2.1 Introduction

A general understanding of patterns can be generated through simultaneous descriptions from one time in several places, or from one place at several times, but sampling at only one time greatly limits the meaningfulness of the data. Underwood and Chapman (1998) found pronounced variations in intertidal assemblages in Australia between sites, shores and over time. There was no sign of temporal consistency and they concluded that measuring and knowing the natural temporal variation of intertidal assemblages within and between sites is essential for ecological studies of systems particularly influenced by environmental variability, such as rocky shores.

Ecologically, environmental conditions and population properties such as densities determine the abundance and shape the distribution of a species (Brown 1984). When studying intertidal assemblages and patterns of abundances of sessile or sedentary species, the level of resistance of the organisms to extrinsic effects such as desiccation, wave action and sand inundation (Zardi et al. 2006a) or biological forces such as predation (Paine 1984), is essential in driving the success of a species on the shore. These extrinsic factors describe various forms of disturbance and contribute to shaping community structure (Dayton 1971), along with initial patterns of settlement. Encompassing all these factors is the subject of interspecific competition and the idea has been proposed that the number of co-existing species will equal the number of resources when a competition-dominated system is at equilibrium (MacArthur & Levins 1964). In contrast, a dominant predator might reallocate and increase the number of actual resources, or limiting factors and, by doing so, permit more species to coexist (Levin 1970). Paine (1971) points out that two-dimensional space is the primary limiting resource in rocky intertidal systems. In a study done in New Zealand on competition, he found that the mussel, *Perna canaliculus*, can virtually form a monoculture in the mid-tidal zone when the dominant starfish predator is removed from the system. The mussels are, however, limited by a large brown alga species, *Durvillea antarctica.* In the absence of the starfish, *Perna* and *Durvillea* exclude other potential competitors from the available space. Of course there is a variety of other species associated with the two zonal dominants, but these do not directly occupy space on the rock surface (Paine 1971). Predation is just one example of disturbance and, although the south coast of South Africa is not necessarily a predator-driven system (Bustamante & Branch 1996), it is important to consider the possible effects of predators on rocky shore communities. In the absence of predation, or any other form of disturbance, one would expect mussels to use the available space by forming persistent monocultures (Paine 1984). It has been found that, on hard substrata, mussels competitively dominate barnacles due to the ability of smaller mussels to smother underlying barnacles, while barnacles are generally more resistant than mussels to physical stress and can persist in refuges where mussels cannot (Stephens & Bertness 1991). Paine (p.1342, 1984) describes the "brute force competitive capacities of *Mytilus*" that he observed where small barnacles are smothered and larger barnacle species are overgrown and then abraded to death.

Topographically-driven resource division can influence the co-existence of barnacles and mussels on rocky shores (Chiba & Noda 2000) and positive intraspecific interactions, rather than interspecific competition, can also maintain the topography-related mosaic of barnacles and mussels (Menge 1976). Positive interactions can include facultative and obligatory facilitations, as well as mutualisms, and only recently have these been getting the attention they deserve in ecological theory (Bertness & Callaway 1994). Facilitative interactions are density-dependent interactions among populations where no party involved is negatively affected (Stephens & Bertness 1991). Particularly in physically harsh environments, such as rocky shores, density-dependent facilitation processes may be far more common than is generally believed (Bertness 1989). The contribution these facilitation processes make to patterns of distribution and abundance can be substantial. Even indirect interactions play an important role in shaping community and ecosystem organisation (Berlow 1999).

Barnacles are known to dominate a distinct zone at intermediate to high tidal heights (Stephenson & Stephenson 1949) and the associated high recruitment rates lead to intraspecific competition for certain species (Connell 1961b). Such competition can cause there to be a uniform distribution in space (Holme 1950, Clark & Evans 1954). At higher tidal heights, high recruitment can result in barnacles protecting each other from thermal stresses and ultimately facilitate survivorship (Bertness 1989). Situations like this stem from the settlement behaviour of barnacle cyprids (see Crisp & Barnes 1954, Crisp 1961) and it is possible that barnacles have an advantage over mussels because of this. Where barnacles recruit quickly and in high densities, successful invasion by mussel recruits will be reduced.

This form of interference competition has been described, more specifically, as inhibition of recruitment, where, generally, interference competition is an interaction in which A and B have direct, mutually negative effects on each other (Menge 1995). Another example of interference competition from the rocky shore is how limpets can inhibit the recruitment of barnacles by forcing recent settlers off the rock (Dayton 1971). Although it is quite likely that barnacles inhibit mussel recruitment, the converse is also possible, where barnacles indirectly enhance the recruitment of mussels by means of their physiology and settlement patterns. The gaps between individual barnacles provide preferential settlement sites, where it is likely that mussels experience reduced mortality (Menge 1976) and thus colonisation by mussels can be enhanced by the presence of barnacles. Menge and Sutherland (1976) have shown that barnacles are outcompeted for space by mussels when predators are absent or excluded. However, this is seldom the case and Connell (1983) reviews the complexities of interspecific competition and how it affects the distribution, abundance and resource use of species in natural communities. When considering competition, there is always an aspect of resource use and it is always in short supply (Birch 1957). This is the case in intertidal ecosystems and primary living space is the major limiting resource (Paine 1966).

Because of this, many colonial, intertidal species have diverged in their characteristics and niche parameters (MacArthur & Levins 1964, 1967) and have evolved life history strategies so that they can still make use of the limited resource (Jackson 1977, Suchanek 1981). Epibiosis is one such example, where barnacles commonly make use of mussel shells as additional hard substratum for settlement (Paine 1974, Dittman & Robles 1991). This increased opportunity for barnacle settlement is most certainly an advantage for the species, but is the epibiotic mode of life optimal for barnacles? By monitoring epibiosis of barnacles over mussels or mussel beds, the present study examines the natural occurrence of epibiosis and its progression.

2.2 Methods

2.2.1 Study site

Data collection for this study was conducted at two bay sites (selected to be comparable in terms of wave exposure, slope, aspect etc.) along the south-east coast of South Africa: Shark Rock Pier in Algoa Bay, Port Elizabeth (33°58'47" S; 25°39'30" E) and St. Francis Bay (34°10'16" S; 24°50'6" E). Seven fixed plots were selected where both barnacles and mussels occurred in varying abundances. This was generally in the mid to high mussel zone of the rocky shores. Both *Mytilus galloprovincialis* and *Perna perna* occurred at these sites (Bownes & McQuaid 2006). The predominant barnacle species encountered in the areas selected was *Chthamalus dentatus* (pers. obs.). Both bay sites that were selected are south facing, gently curved and are open to the sea rather than semi-enclosed (von der Meden et al. 2008). Despite the south coast of South Africa generally experiencing high levels of wave action certain areas, such as, within bays, are relatively sheltered (McQuaid et al. 2000, Erlandsson et al. 2005). All sites also had similar slopes and were dominated by sandstone except for St Francis Bay that was granite.

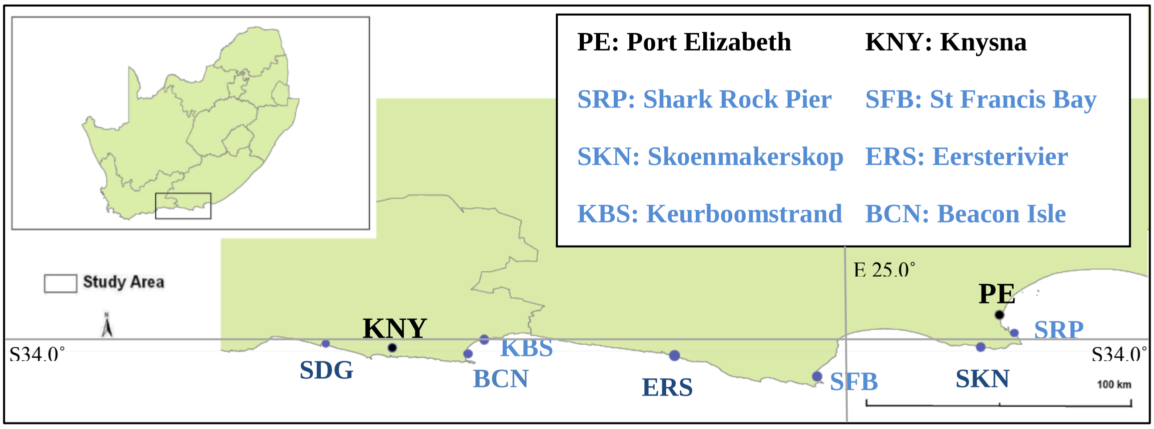

Figure 2.1 Map of South Africa’s south-east coast, with study sites for this and other chapters indicated in blue. Reference points are represented in black. Abbreviations in dark blue indicate open coast sites and those in light blue are bay sites.

2.2.2 Data collection and analysis

The overall relative cover of mussels and barnacles was calculated by digital analysis of photographs (using ImageJ 1.45 software), at seven fixed plots at each site. Barnacles that were living on mussel shells were not considered part of the overall barnacle cover and only those attached to rock were counted. A further two months were sampled at Shark Rock Pier, in 2012, to account for November and December, 2011, when the site was submerged with sand.

Barnacle mortality was calculated by tracking a fixed number of identifiable barnacle individuals over a 12-month period. Any new individuals settling over this time were ignored. *Chthamalus dentatus* individuals on mussels and on rock (six *C. dentatus* individuals of each "treatment" in each plot) were monitored monthly for 12 months (from June 2011 to May 2012 for Shark Rock Pier and September 2011 to August 2012 for St Francis Bay) by means of photographs taken of seven fixed plots (approximately 90cm X 60cm) at each site. Therefore a total of 42 barnacles were tracked on mussels and 42 on rock, at each site. From the same fixed plots and photographs, individual mussels were tracked over the same 12-month period. Mussels were selected for their distinctive position in the mussel bed, i.e. where they could be easily identified in the photographs from month to month. Mussels that were either infested with *C. dentatus* or non-infested (six mussels of each "treatment" at each plot) were followed monthly for the same months and at the same sites as described above. There was no differentiation between mussel species, but the majority of the plots had higher abundances of *P. perna*.

2.2.3 Statistical analysis

Percentage cover of mussels and barnacles

The overall relative cover of mussels and barnacles was analysed by performing a repeated-measures ANOVA on arcsine-transformed ratios of barnacles/mussels (i.e. the proportion of barnacle cover was divided by the proportion of mussel cover) with site (random, two levels) and time (fixed, 12 levels). For each site a t-test was also performed on the ratios for the first and last months of the sampling period (i.e. time 1 and time 12), to test for significant differences over a one-year period. Furthermore, the full time series are presented to describe the month to month change in cover of mussels and barnacles.

Barnacle mortality on rock and mussels

At the end of the study, Month 12, a two-way ANOVA was conducted on arcsine transformed data to test the mortality of barnacles in relation to place of settlement (i.e. mussel or rock, fixed, 2 levels) and site (random, 2 levels). The numbers of dead barnacles were represented as a proportion of the total numbers that were monitored over the 12-month period.

Mussel mortality with or without epibiotic barnacles

At the end of the study, Month 12, a two-way ANOVA was conducted on arcsine transformed data to test the survival of mussels with (infested) or without (non-infested) barnacles (fixed, two levels) and site (random, two levels). The numbers of dead mussels were represented as a proportion of the total numbers that were monitored over the 12-month period. For analyses of mortality, homogeneity and normality were tested and confirmed using the Cochran's and the Kolmogorov-Smirnov tests and all transformed data passed these tests. All statistical analyses were performed using the software Statistica 10.0.

2.3 Results

2.3.1 Overall cover

The repeated-measures ANOVA showed no significant effect of site on the ratios of barnacle percentage cover and mussel percentage cover (p = 0.283; F = 1.159; df = 1) and no significant effect of time (p = 0.768; F = 0.087; df = 11). Despite the lack of a significant effect of site, the patterns over time differed somewhat between Shark Rock Pier (Figure 2.2) and St Francis Bay (Figure 2.3). The t-tests performed on Month 1 and Month 12, showed no significant differences at Shark Rock Pier or St Francis Bay (Table 2.1), meaning that the cover of barnacles and mussels at each site did not differ significantly between the start and end of the 12-month period.

Table 2.1 T-test results between Month 1 and Month 12 at Shark Rock Pier and St Francis Bay.

Shark Rock Pier	*Df*	t-value	p
Ratio (%barnacles / %mussels)	1	-0.119	0.907
St Francis Bay			
Ratio (%barnacles / %mussels)	1	-1.3360	0.206

Df = degrees of freedom; p = significance level

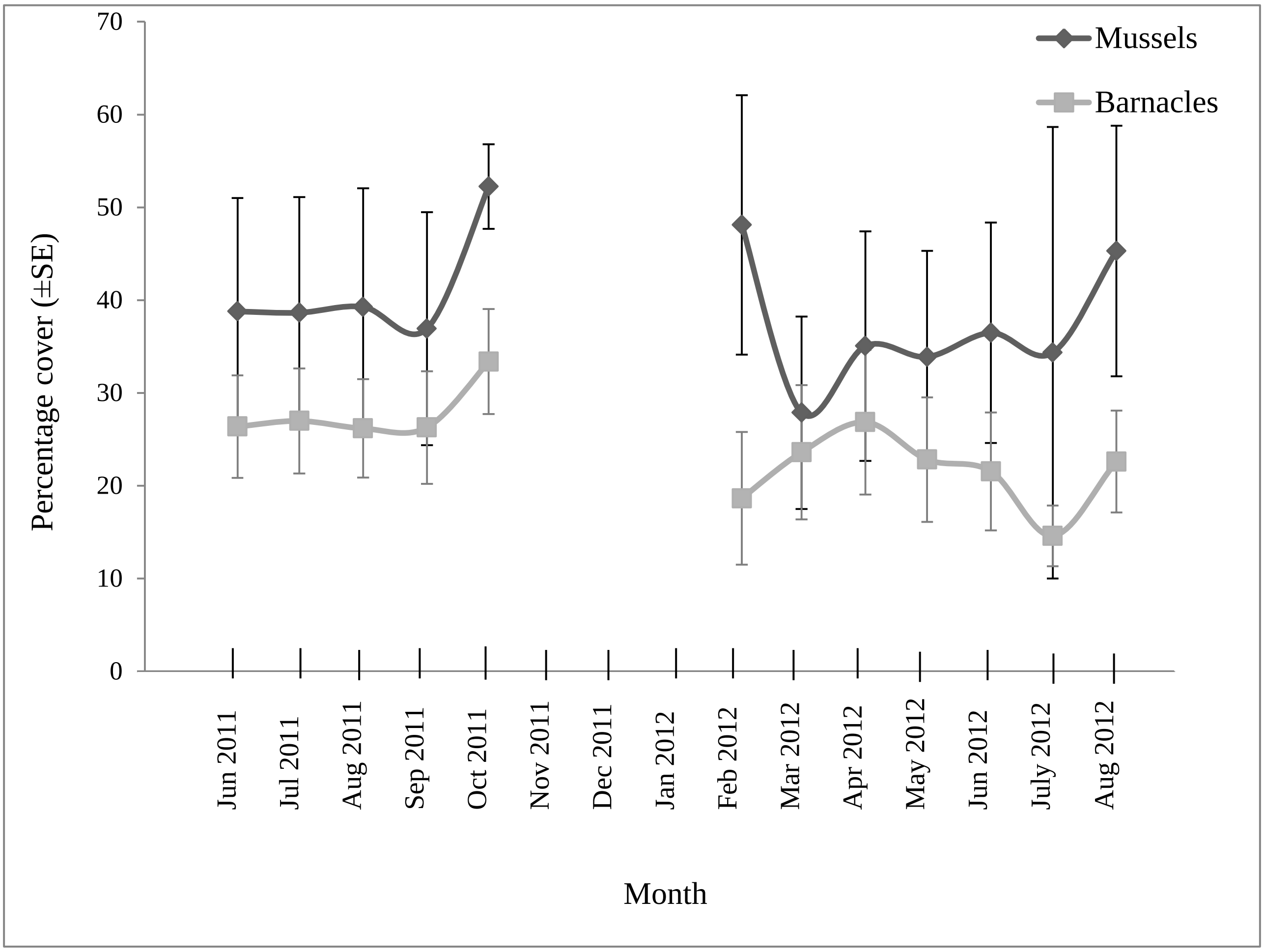

Figure 2.2 Average percentage cover of mussels and barnacles from June 2011 to August 2012 at Shark Rock Pier, Algoa Bay. November 2011, December 2011 and January 2012 could not be sampled due to sand inundation. Error bars indicate standard errors.

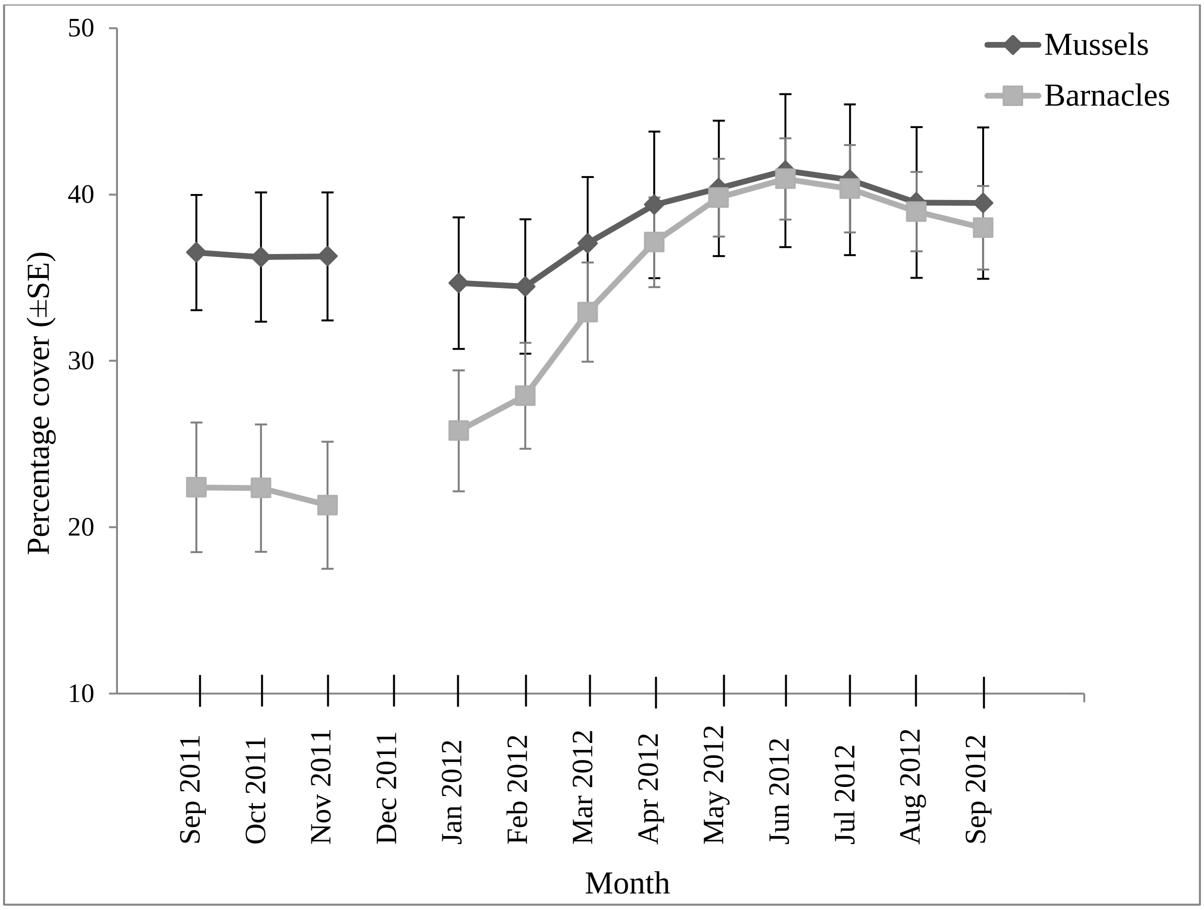

Figure 2.3 Average percentage cover of mussels and barnacles from September 2011 to September 2012 at St Francis Bay. December 2011 not sampled. Error bars indicate standard errors.

2.3.2 Mussel mortalities

There was a significant effect of "mussel state" on the number of mussel deaths (Table 2.2), but no significant effect of site or interaction. Mussels infested with barnacles had a significantly higher mortality rate than those that were not infested (Figure 2.4). Of the total number of mussels monitored over the 12 months, half of the infested mussels had been washed off by Month 12 and only approximately a third of non-infested mussels had died.

Table 2.2 Results of the two-way ANOVA showing the effects of site and mussels state (infested or non-infested) on mortality of mussels.

	Df	SS	MS	F	p
Site	1	0.037	0.037	0.617	0.442
Mussel state	1	0.331	0.331	5.503	**0.028***
Site X Mussel state	1	0.045	0.045	0.737	0.399
Error	24				

Df = degrees of freedom; SS = sum of squares; MS = mean squares; F = f-ratio; p = significance level

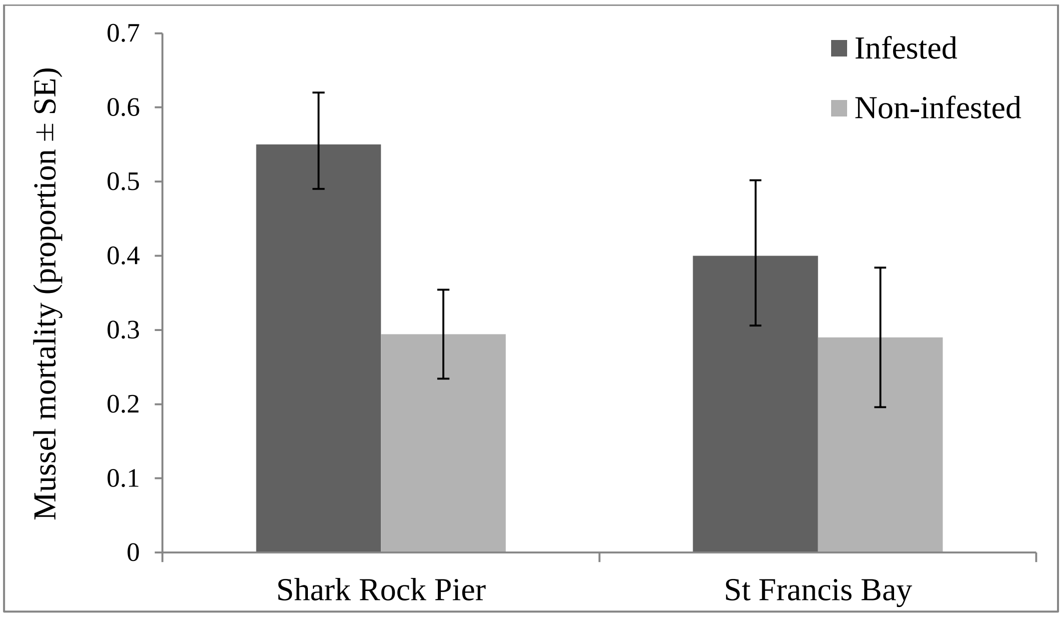

Figure 2.4 Average proportions of mussel deaths, comparing mussels infested with barnacles (n = 6) and non-infested (n = 6), from the seven fixed plots at the two sites. Error bars indicate standard errors.

2.3.3 Barnacle mortalities

There was no significant effect of place of settlement for barnacles, nor was the effect of site significant (Table 2.3). The average mortality for barnacles on either rock or mussel shell showed a slight trend of higher mortality rates for barnacles on mussels at Shark Rock Pier (Figure 2.5), but the difference was not significant.

Table 2.3 Results of the two-way ANOVA showing the effects of site and place of settlement (on mussels or rock) on mortality of barnacles.

	Df	SS	MS	F	p
Site	1	0.084	0.084	1.728	0.200
Place of settlement	1	0.125	0.125	2.606	0.119
Site X Place of settlement	1	0.053	0.053	1.104	0.304
Error	24				

Df = degrees of freedom; SS = sum of squares; MS = mean squares; F = f-ratio; p = significance level

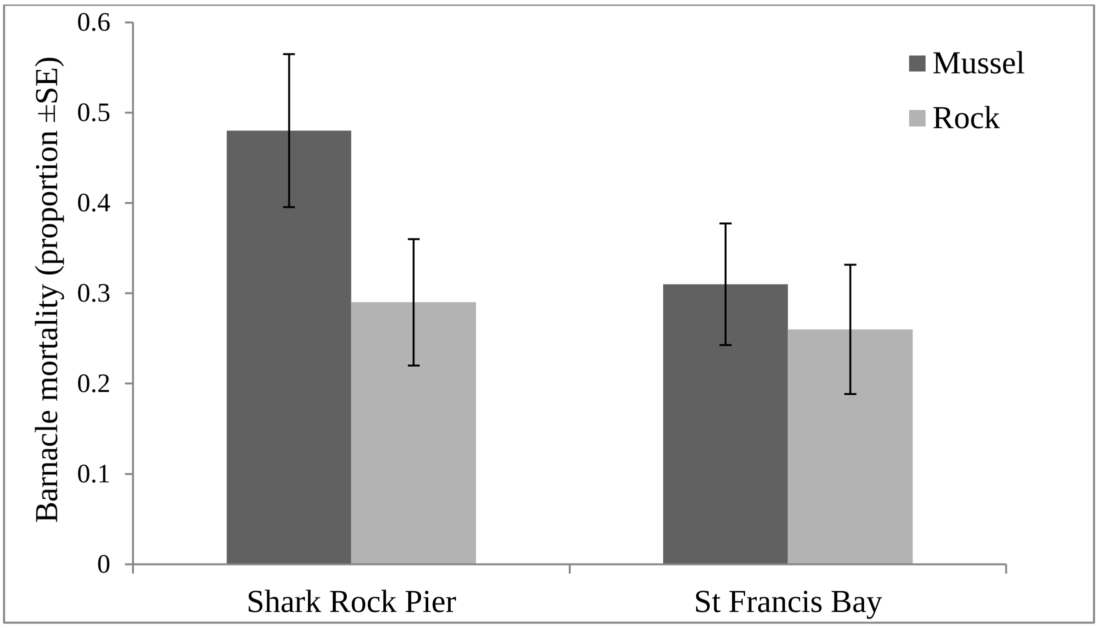

Figure 2.5 Average proportions of barnacle deaths, comparing the place of settlement, either on rock (n =6) or on mussel shells (n = 6), from the seven fixed plots at each site. Error bars indicate standard errors.

2.4 Discussion

It is well known that mussels dominate rocky shores worldwide and their success as principal space-occupiers is most distinct at exposed or semi-exposed sites in temperate habitats (Suchanek 1985). Mussel dominance, although not significant, was evident in the present study as, on average and at both sites, there was higher cover of mussels than of barnacles. Barnacles, however, occupied relatively large areas, too, and this is an indication of their gregarious nature (Knight-Jones 1953, Crisp & Meadows 1962) and possibly their preference for cracks and pits when settling (Crisp & Barnes 1954). When such factors interact, the result can be clumped distributions, but Wethey (1984) found that surface contour was the predominant factor causing patterns of higher settlement intensity in certain areas. Although studies on long-term series of barnacle settlement are not available for this coast, the intensity of barnacle settlement seems to vary locally, regionally and temporally (Wethey 1985) and so, despite 12 months being a substantial observation time, the results presented may be due to localised effects or variations in factors such as predation (Dayton 1971) and larval supply to these sites (Buschbaum 2000).

Although there were no significant patterns from the repeated measures ANOVA the different trends for abundances at Shark Rock Pier compared to St Francis Bay show varying dynamics of the mussel-barnacle co-existence. The most striking difference was most likely driven by a major disturbance event at Shark Rock Pier, through heavy sand inundation of the site over the main summer months. Episodic sand inundations influence intertidal species (McQuaid & Dower 1990) and mussel beds are known to experience high mortality rates due to sand burial (Littler et al. 1983). In February 2012, at Shark Rock Pier, barnacle abundances were reduced far more than the mussels, but, by March 2012, mussel cover dropped off to a percentage cover close to that of barnacles. This pattern could be explained by the sand receding and being washed away from the site and the plots being, once again, entirely visible for analysis. There is also the possibility of one species being more resistant to sand inundation than the other, given the different levels of sand tolerance of different species (Taylor & Littler 1982, Littler et al. 1983). Bownes and McQuaid (2006) found that the dynamics of the mussel populations at a site in Plettenberg Bay, South Africa, are clearly driven by sand inundation in the low shore. *Mytilus galloprovincialis* and *Perna perna*, the mussel species in the present study, cannot tolerate sand stress for long periods of time (Marshall & McQuaid 1993, Zardi et al. 2006a). This was apparent at Shark Rock Pier, where sand inundation is undoubtedly a prominent factor in the austral summer months.

The slopes of the fixed plots in the present study were vertical or inclined and this could affect the distribution of mussels and barnacles, as it has been shown that mussels compete less successfully with barnacles on vertical surfaces (Menge 1976). When testing the effects of horizontal or inclined slopes it was found that, while barnacles rapidly settled and covered most available space on all treatments, mussels outcompeted and replaced barnacles in the following five to seven months (Menge 1976). Along with slope, that height on the shore affects rates of recovery (Suchanek 1985). Plots in the present study were chosen in the mid- to high- zones of the intertidal, but if some plots were slightly higher and closer to the balanoid zone, it follows that barnacles could have an advantage over mussels.

Despite both sites being situated within bays, another factor that may well influence the dynamics of the intertidal communities being considered is wave force and how it can break or dislodge organisms (Menge 1976, Paine & Levin 1981, Bustamante & Branch 1996). At two plots in St Francis Bay there were sudden decreases in the percentage cover of mussels, but barnacle cover was relatively stable. Reviewing the figure of percentage cover at St Francis Bay, there was a levelling out of mussels around January and February 2012 and this could be an effect of dislodgement by wave action. Menge (1976) found that varying wave exposure had little effect on the interspecific competition for space between mussels and barnacles (*Mytilus* and *Balanus*), so either this is not the case for the study species at St Francis Bay or there was some other influence on the results for those plots.

All arguments presented thus far revolve around the concept of these species competing for space and do not allow for the use of mussel shells as a secondary substratum (Bustamante et al. 1997) by barnacles and other epibiotic species. As in the study by Chiba and Noda (2000), the co-existence of mussels and barnacles in an area might be a function of resource division rather than competition and, taking into account the empty space on mussel shells that is often used by barnacles as a substratum, the limiting resource is not necessarily space. The mussel individual and attached barnacle, however, form a two-species unit (Laihonen & Furman 1986) and if the mussel gets washed away, harvested or preyed on, the barnacle(s) most likely die/s along with the mussel. This occurred in a few instances in the present study, where barnacle mortality resulted when the entire mussel was absent from the plot. Additional replication would increase the accuracy of any future studies with regards to epibiont mortalities. In future, an environmental variable could be tested to make the study a confirmatory one, rather than simply using an exploratory, observation-based approach (Bustamante et al. 1997) as was done in the present study.

The significant effect of mussel condition on mortality is of particular interest. The fact that mussels infested with barnacle epibionts are washed off or die more often supports work done by Witman and Suchanek (1984) on the increased drag caused by epibionts on bivalves. It makes sense that increased hydrodynamic forces exerted on a mussel will increase the chance of dislodgement and determine whether the mussel survives or not (Witman & Suchanek 1984). The increased drag exerted on an infested mussel will also mean more energy needs to be allocated to attachment to the substratum and this would leave less energy available for reproduction (Zardi et al. 2007). Although further data collection is required to determine the effect of increased drag, it appears that mussels have a lower chance of survival when infested with barnacles, but for barnacles there is little difference in mortality rates when settled on mussels rather than on rock. This would lead one to think that the epibiotic relationship might be parasitic due to the barnacles benefitting from additional settlement space and the mussels being harmed through increased drag. However, considering that the epibiotic barnacles get washed away with the dislodged mussels, it seems the epibiotic lifestyle is not ideal for barnacle survival rates and there may be trade-offs for the epibiotic barnacles.

The dynamics of the mussel-barnacle co-existence is, overall, relatively stable over a 12-month period, despite disturbance events. Time scales are important when trying to establish the “stability” of a system. Connell and Sousa (1983) explore the possibility of multiple stable states and what an acceptable time-scale might be to confirm the existence of alternative stable states. They also discuss the finer details of ecological stability and consider a stable system one where there are one or more equilibrium points at which the system remains when it experiences a disturbance, or that it returns to when perturbed or disturbed. Much work has been done on the modelling of these systems, but as Dayton et al. (1984) point out, ecologists are becoming more aware that simplified, general theoretical models of stability are not applicable to natural systems. Natural communities are made up of patches and guilds that involve strong biological interactions (Paine 1980) and these can determine the level of local stability. There is also the issue of the term “stability” assuming that equilibrium exists (Barkai & Branch 1988). This is not necessarily true for mussel beds and related communities, so the term “alternative states” might be more accurate (Paine & Levin 1981). This notion has stemmed from work by Sutherland (1974), who considered “multiple stable points” as an undeniable reality in space and time for natural communities. Therefore the patterns described from the present study might be just one state or form at which the community is stable, but it is a fair assumption that the data represent the natural changes in abundances of mussels and barnacles on the rocky shores at these sites.

Understanding the relationship between mussels and barnacles at the species level is fundamental to the research conducted into the individual relationships between a mussel and its epibiotic barnacles. From the results in the present study, it seems that there is a trade-off for barnacles between the importance, for the species, of secondary space on mussel shells and, for the individual, the possibility of mortality if the mussel dies or becomes dislodged.

Chapter Three

Prevalence and intensity of infestations of mussels by barnacle epibionts

3.1 Introduction

Spatial variability is typical of intertidal systems, where steep environmental gradients occur and shape the abundance of species and the patterns of assemblages (Brown 1984). What regulates intertidal ecosystems and the biological communities that comprise them has been widely studied, with an initial focus on the responses of individual species to predation, disturbance, competition or recruitment (e.g. Connell 1961b, Paine 1966). As the understanding of the intertidal ecosystems evolved, ecologists began to concentrate on the effects of environmental conditions on the whole community and species assemblages (Menge & Sutherland 1987, Menge & Farrell 1989, Menge & Olson 1990, Bustamante et al. 1997, Underwood & Chapman 1998). These and other studies have recognised that, of the multitude of environmental factors, the two most important abiotic forces that influence the make-up of the rocky intertidal at local scales are gradients of desiccation, which often result in vertical zonation, and the effects of wave exposure, which lead to horizontal zonation (Dayton 1971, Connell 1972, Dayton et al. 1984, Menge & Sutherland 1987, Menge & Farrell 1989, Menge & Olson 1990).

Vertical zonation is common around the world and, although tide was generally thought of as the primary cause of this feature (Colman 1933, Hewatt 1937, Doty 1946), Stephenson and Stephenson (1949) maintained that this idea is mistaken and it is, in fact, the existence of an interface between air and water. Stephenson and Stephenson (1949) reasoned that there is a gradient of light-penetration below the surface of the water which, along with other factors such as changes in sedimentation, drives the formation of zones. Above water, zonation also occurs due to the interaction of the rock surface with the associated water body, in the form of spray or moisture from evaporation (Stephenson & Stephenson 1949). Zonation is, however, not caused simply by the physical rise and fall of the tide, but is due to biological interactions interplaying with physical environmental factors (Underwood 1978). More stress-tolerant organisms are found higher up the shore, where they experience greater exposure to sunlight and air. For example, strong sunlight may kill juveniles close to the high tide mark and orientation (north-facing/south-facing) may also play an important role in survival for some species (Connell 1972). Plants or sessile animals have frequently been used to study the causes of limits in vertical zonation (Connell 1972) and barnacles have often been considered when investigating patterns of distribution. For example, Grosberg (1982) documented the zonation of two *Balanus* species in California, USA, and found that the vertical zonation of cyprids in the plankton mirrored the vertical distribution of adults on the substratum and highlighted the strong influences pre-settlement behaviour can have on the vertical distribution of sessile organisms.

Horizontal zonation occurs at larger scales and is determined by changes in water movement (Connell 1972). Water movements, including coastal oceanography, are strongly influenced by the topography of the coastline and globally it is common to find bays with accompanying headlands (Geyer & Signell 1992, Gan & Allen 2002, von der Meden et al. 2008). Bays often experience reduced wave action due to their enclosed or semi-enclosed nature (von der Meden et al. 2008). How sheltered or exposed a site is will influence the distribution, biomass and composition of communities on rocky shores (Kingsbury 1962, Bustamante et al. 1997, Underwood & Chapman 1998). Bays differ from the open coast, not only in wave exposure (Burrows et al. 2008, von der Meden et al. 2008), but also in temperature structure (Graham et al. 1992, Schumann et al. 1995) and food availability (Archambault et al. 1999). Worldwide, it has also been shown that, relative to open coasts, bays have higher larval densities and greater settlement, recruitment and adult abundances (Helson & Gardner 2004, Roughan et al. 2005, Mace & Morgan 2006). In South Africa, the south-east coast has a number of bays that allow for multiple bay/open coast comparisons (von der Meden et al. 2008). Sites in this study were chosen to create replication at the level of ‘bay’.

In addition to large-scale variations, such as vertical and horizontal zonation, rocky shores display variation on a smaller scale due to the physical environment, as well as direct or indirect activities of other organisms (Connell 1972). The ‘patchiness’ that results is common to hard substratum communities (Taylor & Wilson 2003) and many rocky shores are highly irregular and not continuous, so considerable variation in slope and surface orientation occurs (Connell 1972). In the intertidal, hard substratum is broken, irregular and in short supply (Connell 1961b, Jackson 1977, Paine 1984). Successful primary space occupiers must be effective at competing for existing space, or the organisms must locate new space in the system (Bertness & Grosholz 1985). Mussels, as efficient ecosystem engineers, can create this new or secondary space for the settlement of epibionts (Lohse 1993a). They also provide habitats for infaunal organisms, retain receding sea water and organic material and protect certain organisms from predators (Commito & Boncavage 1989, Lohse 1993a). Within mussel beds, patches are created and maintained by physical disturbances and biological interactions (Paine & Levin 1981). Other than predation, bed age (Commito & Rusignuolo 2000), mussel species and size (Iwasaki 1995) and sediment accumulation (Crooks & Khim 1999) influence the spatial mosaic of mussels. Therefore, although mussels are generally accepted as competitively dominant on rocky shores, patches resulting from disturbance can provide primary space for other species (Paine 1966, Dayton 1971, Menge 1976).

On the south-east coast of South Africa the invasive species, *Mytilus galloprovincialis*, coexists with the indigenous *Perna perna* and, while the latter dominates the lower shore, *M. galloprovincialis* reaches its highest abundance in the high mussel zone of the intertidal (Bownes & McQuaid 2006). The competitive interactions between these two species have been examined in the different zones within the mussel zone and found to be relatively stable through co-existence due to partial habitat segregation (Rius & McQuaid 2006). Hanekom (2008) studied how the invasive mussel species affected associated fauna along the south coast and found a significant difference in the densities of associated species when comparing pre-invasion and post-invasion conditions. In particular, the toothed barnacle, *Chthamalus dentatus*, experienced a significant, but temporary decline in density and biomass values on the south coast when *M. galloprovincialis* first invaded this part of the coast (Hanekom 2008). Mussels often inhabit grooves, while barnacles can live on flat vertical or horizontal surfaces and so do not necessarily compete for the same type of space (Erlandsson et al. 2005). Competition for space is also reduced by barnacles using mussel shells as a secondary substratum (Laihonen & Furmen 1986). The distribution of barnacle epibionts has not been studied in conjunction with larger-scale effects such as the level of bay topography, shore level and through time. The impact of barnacle infestations on co-occurring mussel species, such as the ones found on the south-east coast of South Africa, *P. perna* and *M. galloprovincialis*, provides an ideal example for a comparison of infestation in indigenous and invasive species.

The proportion of mussel individuals infested with barnacles in a sampled area gives the incidence rate of this relationship on the rocky shore and is known as the *prevalence* of barnacle infestation. If infested, an individual mussel can be evaluated to determine how much of its outer surface is covered with barnacles and, in the present study, this is considered to be the *intensity* of infestation. Intensity can either be quantified as the mean number of barnacles per mussel or as the percentage of the mussel shell covered by barnacles. In this study, percentage cover has been used.

Prevalence and intensity of epibiosis by barnacles on *P. perna* and *M. galloprovincialis* along the shore at replicated exposed and bay sites were measured to test the hypothesis that these two mussels experience different degrees of infestation.

3.2 Methods

3.2.1 General site description

The collection of specimens for the study on intensity and prevalence of epibiosis on mussel beds was conducted in three bays along the south coast of South Africa and three adjacent open-coast sites; these being Shark Rock Pier in Algoa Bay (33˚58' 48" S; 25˚39' 31" E) and Skoenmakerskop (34˚2' 28" S; 25˚32' 1" E), St. Francis Bay (34˚10' 16" S; 24˚50' 6" E) and Eersterivier (34˚4' 23" S; 24˚13'27" E), Keurboomstrand in Plettenberg Bay (34˚0' 18" S; 23˚ 27' 30" E) and Sedgefield (34˚1' 45" S; 22˚46' 6" E).

These sites were selected due to the co-presence of *P. perna* and *M. galloprovincialis*, as well as the occurrence of epibiotic barnacles on mussels. The area of the shore occupied by mussels can be divided into the high, mid and low zones, with *M. galloprovincialis* colonising the high zone and *P. perna* dominating the low zone. The mid-zone is the area between the high and low zone, where the two species overlap and coexist, forming a mixed zone (Bownes & McQuaid 2006).

Other species that characterise the low mussel zone include the limpet *Scutellastra cochlear* and encrusting coralline algae (e.g. *Spongites yendoi*), as well as branching red algae (*Plocamium* spp.) and articulated coralline algae as epibionts on the mussel patches. In the mid zone, the barnacle *Octomeris angulosa* is common, especially on wave-exposed shores, and patches of red algae such as *Gelidium pristoides* are dominant in most mid-zones on the south coast. The species typically found in the high zone along with mussels are toothed barnacles (*Chthamalus dentatus),* volcano barnacles (*Tetraclita serrata*), gastropod snails (*Afrolittorina* spp.), dwarf cushion stars (*Parvulastra exigua*), winkles (*Oxystele* spp.) and limpets (particularly *Scutellastra granularis*) (Branch & Branch 1981, Branch et al. 1994).

All three barnacle species mentioned above can be epibiotic on mussels and, unless stated otherwise, no distinction is made here among barnacle species.

3.2.2 Data collection

Percentage cover of *P. perna* and *M. galloprovincialis* in the different zones, prevalence (percentage of mussels infested with barnacles from replicates of 100% cover mussel beds) and intensity (percentage barnacle cover per infested mussel) were considered during the austral summer and winter to compare them among mussel species, zones and sites. Ten replicates from each of the three zones (low, mid and high) were sampled on 100% mussel cover beds, using a 50 x 50 cm quadrat, calculating the percentage cover of *P. perna* and *M. galloprovincialis* in each quadrat. Photographs of each quadrat were taken to compare direct methods of estimates of percentage cover with digital image analysis. In the low, mid and high zones, five 20 x 20 cm quadrats were scraped from each zone and preserved in alcohol for further laboratory analysis.

3.2.3 Laboratory

Samples were handled carefully, to ensure that any epibiotic barnacles were not dislodged from the mussel shells and only mussels equal to, or more than, 10 mm in length were considered for the study. Mussels >10 mm were recognised as adults/sub-adults; they are more likely to reproduce and survive (Erlandsson & McQuaid 2004). Prevalence of mussel infestation by barnacles was calculated by counting how many mussels of each species had at least one barnacle attached to the shell (as presence/absence) and how many did not. A prevalence index was calculated by taking the number of infested mussels as a proportion of the total number collected in each sample.

Intensity was determined by placing each infested mussel into a category that was dependent on the percentage of the total shell (i.e. both shell valves) covered by barnacles. There were eight categories and the percentage cut-off points were: <5; 5-10; 11-20; 21-30; 31-40; 41-50; 51-60; 61-70. Photographs were taken of 30 mussels from each category to validate the accuracy of the classing method used. Photographs were then analysed, using ImageJ 1.45 software. Mussels were measured to the nearest mm using Vernier callipers. The number and species of barnacles were noted.

3.2.4 Statistical analysis

Percentage cover of adult mussels

In order to determine if percentage cover of the two species of mussels reflected the patterns reported by the literature (Bownes & McQuaid 2006), two separate four-way nested ANOVAs were performed, haphazardly selecting five quadrats for *M. galloprovincialis* and five for *P. perna*. Season (fixed, 2 levels), bay status (fixed, 2 levels), site (random, 6 levels, nested in Bay status) and zone (fixed, 3 levels) were the independent parameters against which arcsine transformed cover of mussel, the dependent variable, was tested. Homogeneity and normality were tested using the Cochran's and the Kolmogorov-Smirnov tests, respectively, and none of the data met these assumptions ($p < 0.01$ in all cases), even when arcsine transformed. However, Underwood (1997) considers ANOVA to be robust to non-normal, non-homogeneous data, especially in the case of large overall sample sizes, with multiple treatments, as on this occasion ($n = 180$ per species).

Prevalence

To test for the effect of bay status (fixed, 2 levels), site (random, nested in bay status, 6 levels), season (fixed, 2 levels) and zone (fixed, 2 levels) on the prevalence of barnacle infestation, two four-way nested ANOVAs were computed, one for *M. galloprovincialis* on the high and mid zone and one for *P. perna* on the low and mid zone, after the data had been arcsine transformed. As *M. galloprovincialis* is known to be dominant in the high zone, *P. perna* in the low zone (Bownes & McQuaid 2006, von der Meden et al. 2008 and this study), the relevant zones were compared with the mid zone data, so as not to bias results. Homogeneity and normality were tested using the Cochran's and the Kolmogorov-Smirnov tests, respectively, and although these assumptions were not met, the sample size was large enough ($n=120$ per species) for the analysis to be considered robust (Underwood 1997). Significant results were further analysed by the post hoc test, Student Newman Keuls. All statistical analyses were performed using the software Statistica 10.0.

Intensity

To test for the effect of season (fixed, 2 levels), bay status (fixed, 2 levels), site (random, nested in bay status, 6 levels) and zone (fixed, 2 levels), on the intensity of barnacle infestation, with mussel size as a covariate, two separate PERMANOVAs (permutational ANOVA) with ANCOVA set up, were computed, one for *M. galloprovincialis* on the high and mid-zone and one for *P. perna* on the low and mid-zone, after the data had been arcsine transformed. Again, relevant zones were compared with the mid zone data, as described above. This analysis was done using the PERMANOVA+ adds-in to PRIMER (v. 6.1.10; PRIMER-E Ltd.). Significant results were further analysed by the post hoc test, Student Newman Keuls.

3.3 Results

3.3.1 Percentage cover of mussels

Perna perna

The abundance of *Perna perna* was considered across the high, mid and low zones and it was always highest in the low zone, except at Shark Rock Pier in winter, where the mid-zone abundance was slightly higher, and at Eersterivier, where summer abundance in the mid zone was greater than the low zone. These exceptions explain the significant interaction of season, site and zone (Table 3.1).

Table 3.1 Four-way nested ANOVA to test the abundance of *Perna perna* using arcsine transformed proportions (percentage cover) as the dependent variable. The symbol * indicates a significant p-value, where * is p<0.05, ** is p<0.01 and *** is p< 0.001.

	Df	SS	MS	F	p
Season	1	0.208	0.208	3.427	0.138
Bay	1	0.237	0.237	1.101	0.352
Site (Bay)	4	0.859	0.215	3.669	0.550
Zone	2	10.929	5.464	33.863	**0.0001*** **
Site (Bay) X Zone	8	1.291	0.161	0.986	0.508
Season X Bay	1	0.001	0.001	0.011	0.923
Season X Site (Bay)	4	0.243	0.061	0.371	0.823
Season X Zone	2	0.027	0.013	0.081	0.923
Bay X Zone	2	0.557	0.279	1.727	0.238
Season X Bay X Zone	2	0.184	0.092	0.561	0.592
Season X Site (Bay) X Zone	8	1.309	0.164	3.038	**0.004** **
Error	144	7.756	0.054		

Df = degrees of freedom; SS = sum of squares; MS = mean squares; F = f-ratio; p = significance level

Mytilus galloprovincialis

As with *P. perna*, there was a significant effect of zone for *M. galloprovincialis* and also a significant season, site and zone interaction (Table 3.2). This interaction was significant because there were instances, such as at St Francis Bay, where the abundance was higher in the mid zone in summer. This differs from the other sites that all showed the expected pattern of *M. galloprovincialis* being dominant in the high zone. Occasionally, there was also a significant difference between seasons and, although for *M. galloprovincialis* winter abundances were generally higher, at Shark Rock Pier and Sedgefield, in both the mid and high zones, there was a greater abundance in summer.

Table 3.2 Four-way nested ANOVA to test the abundance of *Mytilus galloprovincialis* using arcsine transformed proportions (percentage cover) as the dependent variable. The symbol * indicates a significant p-value, where * is p<0.05, ** is p<0.01and *** is p< 0.001.

	Df	SS	MS	F	P
Season	1	0.041	0.041	0.621	0.475
Bay	1	0.119	0.119	0.569	0.493
Site (Bay)	4	0.841	0.210	1.857	0.229
Zone	2	1.268	0.634	8.309	**0.011***
Season X Bay	1	0.012	0.012	0.184	0.610
Season X Site (Bay)	4	0.267	0.067	2.238	0.154
Season X Zone	2	0.219	0.109	3.684	0.073
Bay X Zone	2	0.039	0.011	0.256	0.780
Site (Bay) X Zone	8	0.610	0.076	2.557	0.103
Season X Bay X Zone	2	0.007	0.003	0.113	0.894
Season X Site (Bay) X Zone	8	0.239	0.029	3.148	**0.003****
Error	144	1.365	0.009		

Df = degrees of freedom; SS = sum of squares; MS = mean squares; F = f-ratio; p = significance level

3.3.2 Prevalence of barnacle infestations

Perna perna

The prevalence of barnacle infestations (percentage of infested mussels over total sample) on *P. perna* was significantly affected by a combination of season, site and zone, as well as the interaction of bay and zone (Table 3.3). In general, prevalence was higher in summer than winter, with the exceptions of Shark Rock Pier (SRP) and Sedgefield (SDG), where prevalence in the low zones was higher in winter (Figure 3.1). There were significant differences between seasons (denoted by asterisks in Figure 3.1) at most sites and zones. Sedgefield was the only site that showed no significant effect of season in either zone. Most sites showed only small within-season differences between zones, with exceptions at Shark Rock Pier in winter and at Eersterivier (ERS) in summer.

Table 3.3 Four-way nested ANOVA of barnacle prevalence for *Perna perna* using arcsine transformed proportions as the dependent variable. The symbol * indicates a significant p-value, where * is $p<0.05$, ** is $p<0.01$and *** is $p< 0.001$.

	Df	SS	MS	F	p
Season	1	3.733	3.733	3.906	0.119
Bay	1	0.007	0.007	0.014	0.912
Site (Bay)	4	1.966	0.491	0.678	0.668
Zone	1	0.269	0.269	3.424	0.138
Season X Bay	1	0.592	0.592	0.620	0.475
Season X Site (Bay)	4	3.823	0.956	3.085	0.150
Season X Zone	1	0.099	0.099	0.320	0.602
Bay X Zone	1	0.671	0.671	8.529	**0.043***
Site (Bay) X Zone	4	0.315	0.079	0.254	0.894
Season X Bay X Zone	1	0.001	0.001	0.002	0.971
Season X Site (Bay) X Zone	4	1.239	0.310	5.141	**0.0009*****
Error	96	5.785	0.060		

Df = degrees of freedom; SS = sum of squares; MS = mean squares; F = f-ratio; p = significance level

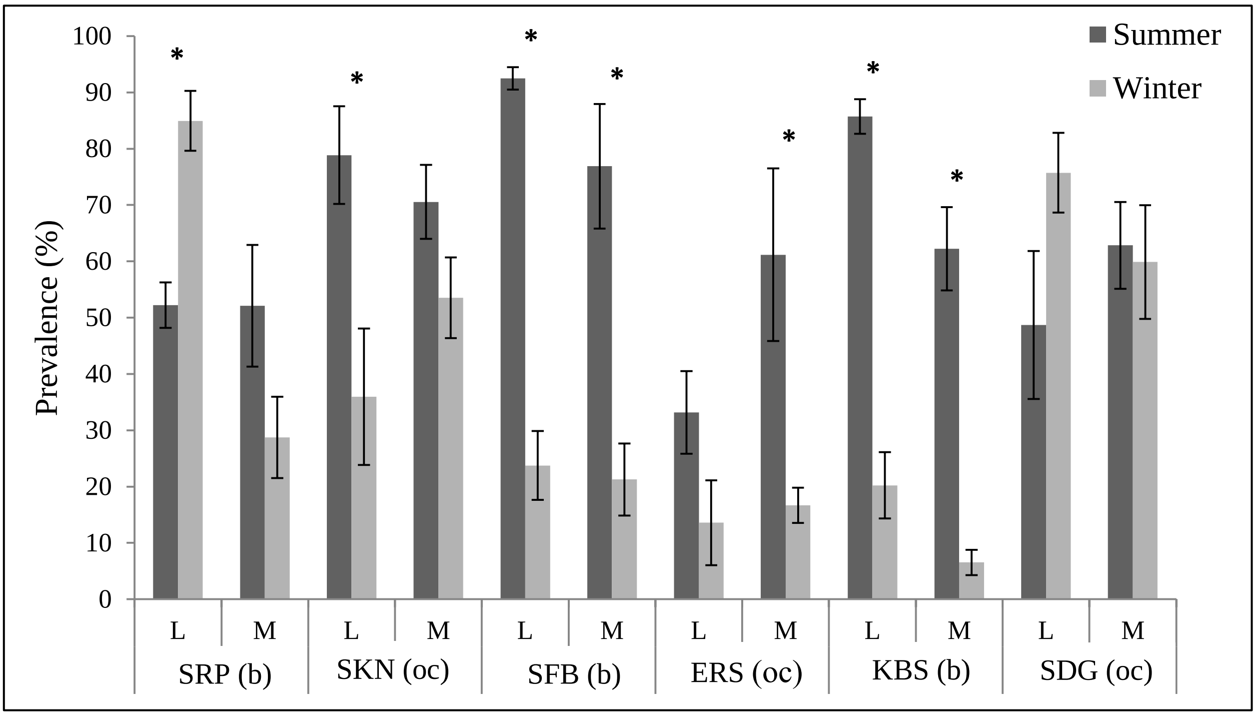

Figure 3.1 Prevalence of barnacle infestations on *Perna perna* across the two seasons sampled, where L = low zone; M = mid zone; oc = open coast and b = bay. Sites are arranged from east to west, the error bars indicate standard errors and * indicates significant differences between seasons.

The significant effect of bay at different zones can be seen in Figure 3.2, with an effect of topography evident only for the low zone.

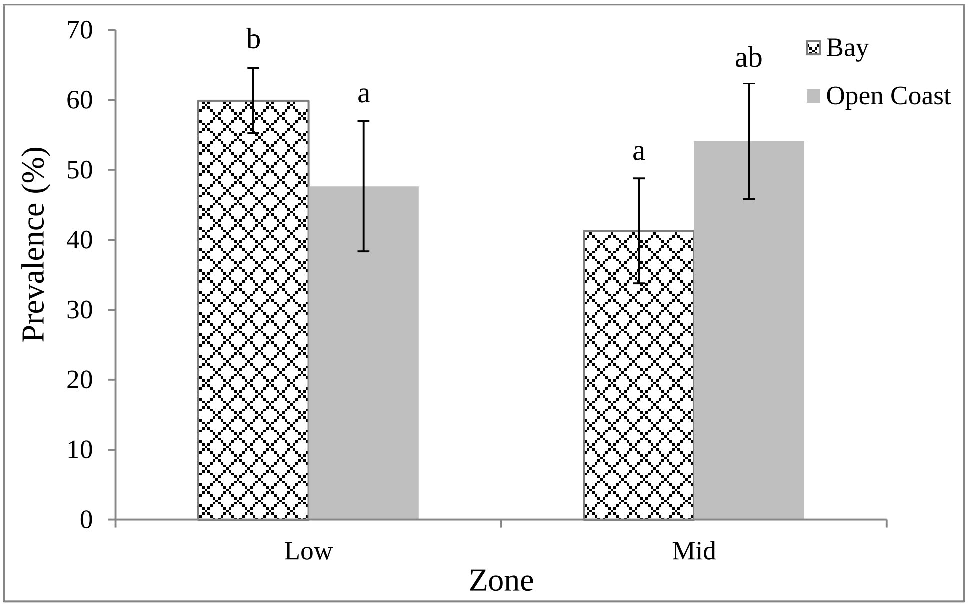

Figure 3.2 Mean prevalence of barnacle infestations on *Perna perna*, in bay and open coast situations and across the two relevant zones. The error bars indicate standard errors and the letters represent homogeneous groups from the results of the Student Newman-Keuls post-hoc tests.

Mytilus galloprovincialis

The prevalence of barnacle infestation on *M. galloprovincialis* mussels was significantly affected by a combination of season, bay and zone (three-way interaction), as well as a number of two-way interactions, which included season x site (bay), season x zone and site (bay) x zone (Table 3.4). The effect of the three-way interaction is illustrated in Figure 3.3. It can be seen that summer prevalence was significantly higher than that of winter in bays, regardless of zone, while in open coast sites the effect of season was only significant in the mid shore.

Table 3.4 Four-way nested ANOVA of barnacle prevalence for *Mytilus galloprovincialis* using arcsine transformed proportions as the dependent variable. The symbol * indicates a significant p-value, where * is p<0.05, ** is p<0.01 and *** is p< 0.001.

	Df	SS	MS	F	P
Season	1	2.594	2.594	8.809	**0.041***
Bay	1	0.291	0.291	1.314	0.316
Site (Bay)	4	0.887	0.222	0.652	0.649
Zone	1	0.832	0.832	17.435	**0.014***
Season X Bay	1	0.737	0.737	2.502	0.189
Season X Site (Bay)	4	1.178	0.295	139.450	**0.0002*****
Season X Zone	1	0.618	0.618	292.728	**0.0001*****
Bay X Zone	1	0.060	0.060	1.264	0.324
Site (Bay) X Zone	4	0.191	0.048	22.597	**0.005****
Season X Bay X Zone	1	0.170	0.170	0.446	**0.0009*****
Season X Site (Bay) X Zone	4	0.008	0.002	0.051	0.995
Error	96	3.972	0.041		

Df = degrees of freedom; SS = sum of squares; MS = mean squares; F = f-ratio; p = significance

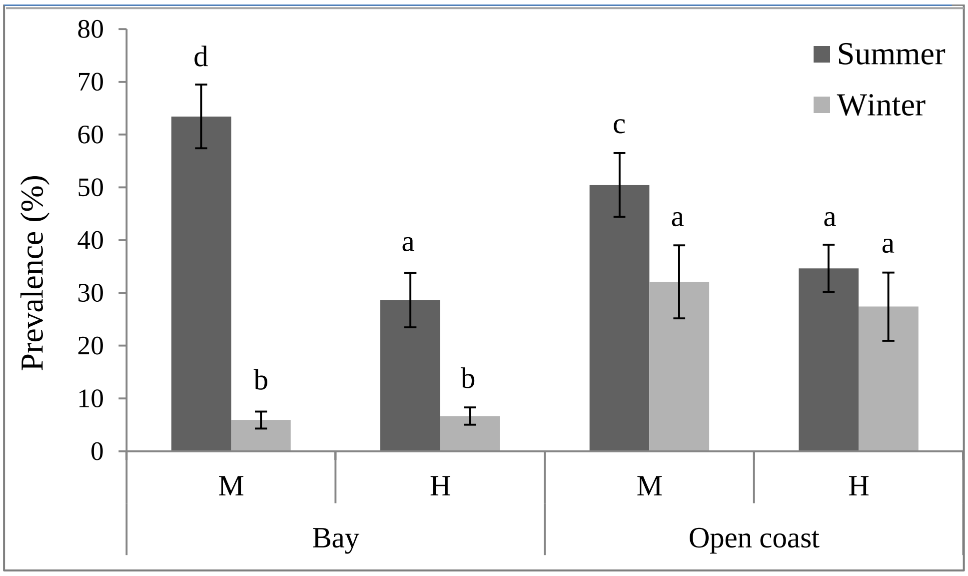

Figure 3.3 Prevalence of barnacle infestations on *Mytilus galloprovincialis* mussels, across the two seasons sampled and the relevant zones, where M = mid zone and H = high zone. Error bars indicate standard errors and the letters represent homogeneous groups from the results of the Student Newman-Keuls post-hoc tests.

The significant interaction between site (bay) and season found for *M. galloprovincialis* can be seen in Figure 3.4. At all sites except Sedgefield (SDG), prevalence was higher in summer than in winter. Most sites show a significant difference between seasons, but at Skoenmakerskop (SKN) and Sedgefield summer and winter values were not significantly different from each other. When considering the significant interaction between zone and season a similar pattern was evident, with summer prevalence being significantly higher in both the mid and high zones (Figure 3.5). In winter there was no effect of zone on prevalence, but in summer prevalence of barnacle infestation was, on average, highest in the mid zone.

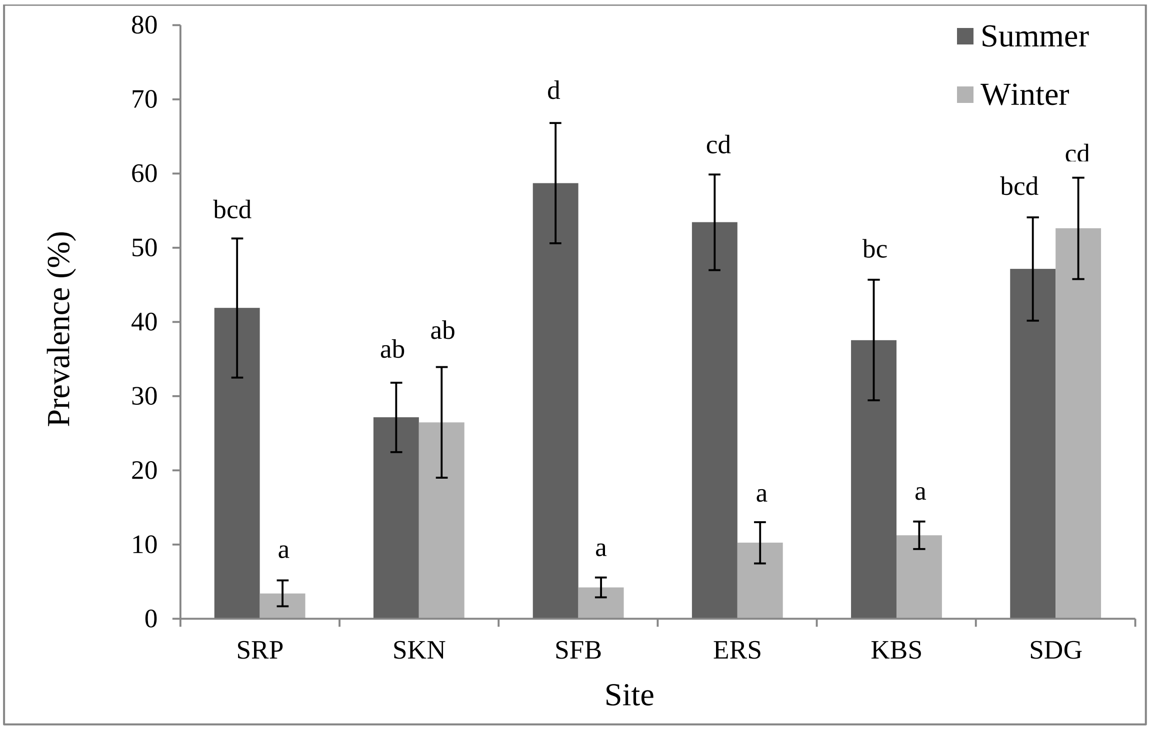

Figure 3.4 Mean prevalence of barnacle infestations on *Mytilus galloprovincialis*, across the two seasons sampled. Sites are arranged from east to west, error bars indicate standard errors and the letters represent homogeneous groups from the results of the Student Newman-Keuls post-hoc tests**.**

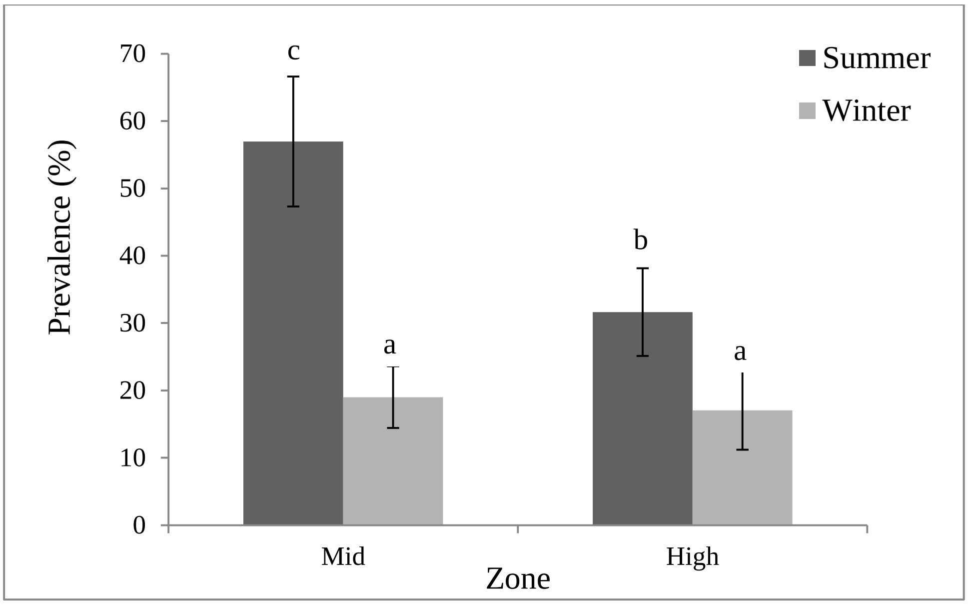

Figure 3.5 Prevalence of barnacle infestations on *Mytilus galloprovincialis* mussels, across the two seasons sampled in the relevant zones. Error bars indicate standard errors and the letters represent homogeneous groups from the results of the Student Newman-Keuls post-hoc tests**.**

Overall, *Perna perna* had a higher average prevalence of infestation (51%) than *Mytilus galloprovincialis* (31%). Combining data for both species and across all sites, prevalence was highest in the low zone overall and lowest in the high zone. In the mid zone, *P. perna* had a higher average prevalence of infestation (54%) than *M. galloprovincialis* (42%). There was variation between sites for both species, but the effects of bay status and season are of real interest and the patterns of prevalence for the separate species in their relative zones are also important.

3.3.3 Intensity

Perna perna

The PERMANOVA performed on the intensity of barnacle infestation on *P. perna* showed that three of the four significant three-way interactions included size, the covariate (Table 3.5). The interaction of site (bay), season and zone was the fourth significant three-way interaction and site also featured in many of the two-way interactions. The significance of the site (bay) x season x zone interaction can be explained by certain zones at particular sites having intensities that stand out as noticeably different in either summer or winter

(Figure 3.6). For example, Sedgefield (SDG) and Shark Rock Pier (SRP) both had unusually high infestation intensities in the low zone during winter, compared to other sites and zones.

Table 3.5 PERMANOVA to test the main effects on infestation intensity of *Perna perna* with size as a covariate. The symbol * indicates a significant p-value, where * is $p < 0.05$, ** is $p < 0.01$ and *** is $p < 0.001$.

	Df	SS	MS	pseudo-F	p (perm)	U perms	p (MC)
Bay	1	724.240	724.240	3.120E-2	0.860	548	0.975
Season	1	2654.7	2654.7	0.683	0.471	998	0.484
Site (Bay)	4	92252	23063	50.323	**0.001**	999	**0.001****
Zone	1	1301.7	1301.7	0.233	0.693	999	0.785
Size	1	4345.8	4345.8	0.755	0.420	999	0.472
Size X Bay	1	8129.7	8129.7	17.739	**0.001**	999	**0.001****
Size X Season	1	128.090	128.090	0.125	0.848	999	0.855
Size X Zone	1	1013.6	1013.6	0.592	0.494	999	0.537
Bay X Season	1	109.28	109.28	4.736E-2	0.871	998	0.952
Bay X Zone	1	1978.9	1978.9	0.416	0.548	999	0.644
Season X Zone	1	12490	12490	1.157	0.298	999	0.332
Size X Site (Bay)	4	21815	5453.8	11.900	**0.001**	998	**0.001****
Site (Bay) X Season	4	9780.9	2445.2	5.335	**0.001**	998	**0.002****
Site (Bay) X Zone	4	18310	4577.5	9.988	**0.001**	999	**0.001****
Size X Bay X Season	1	2280.8	2280.8	4.977	**0.02**	998	**0.017***
Size X Bay X Zone	1	3234.6	3234.6	7.058	**0.004**	997	**0.003****
Size X Season X Zone	1	781.280	781.280	0.259	0.739	999	0.756
Bay X Season X Zone	1	9969.1	9969.1	1.497	0.286	999	0.292
Size X Site (Bay) X Season	4	367.4	917.1	2.001	0.087	997	0.076
Size X Site (Bay) X Zone	4	12956	3239	7.067	**0.001**	998	**0.001****
Site (Bay) X Season X Zone	4	27402	6850.5	14.947	**0.001**	999	**0.001****
Size X Bay X Season X Zone	1	119.6	1194.6	2.607	0.089	998	0.098
Size X Site (Bay) X Season X Zone	4	1870.5	467.640	1.020	0.357	997	0.389
Res	2458	1.127E6	458.300				

Df = degrees of freedom; SS = sum of squares; MS = mean squares; U = unique permutations;

p (MC) = Monte Carlo significant values; p = significance level.

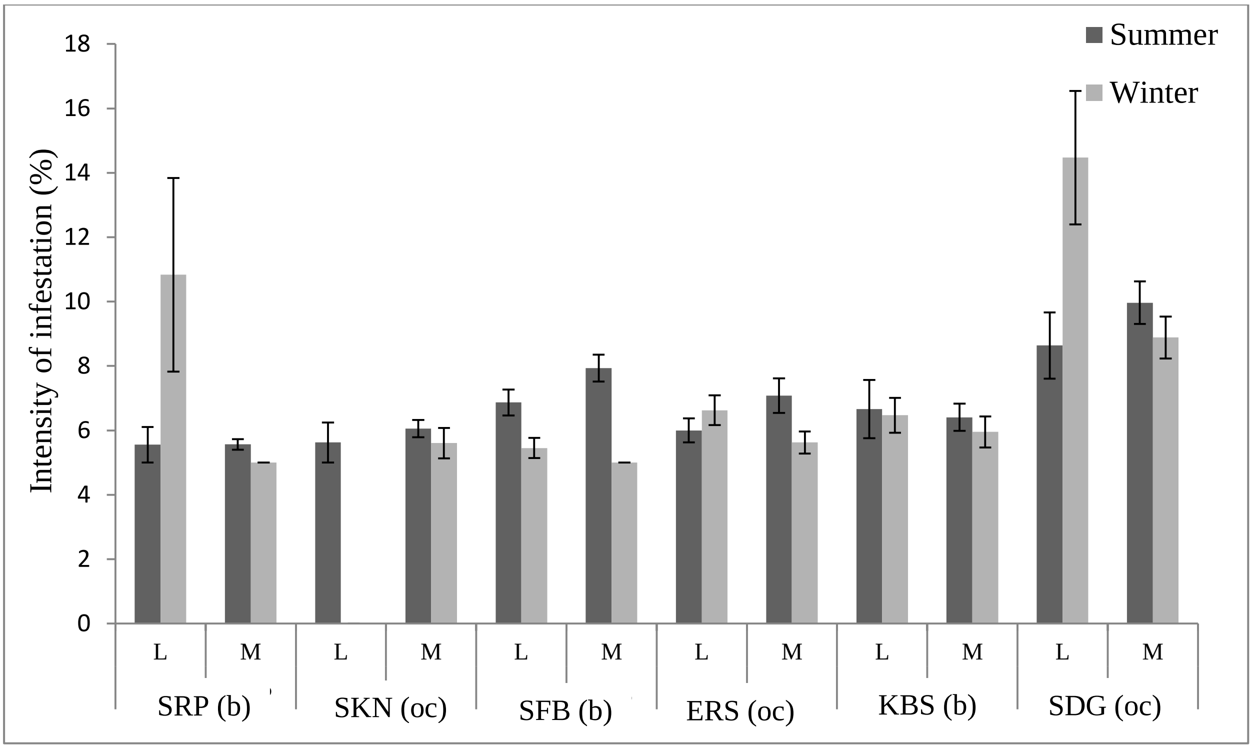

Figure 3.6 Intensity of barnacle infestation on *Perna perna*, across the two seasons sampled, where M = mid zone; H = high zone; oc = open coast and b = bay. Sites are arranged from east to west. The error bars indicate standard errors**.**

The significant interaction of size x site (bay) x zone is represented in the panels in Figure 3.7. Although there was little to no relationship between mussel size and intensity of infestations at most of the sites, occasionally there was a significantly positive relationship, though in all cases the R^2 values were extremely low. For example, at Shark Rock Pier and Skoenmakerskop there were significant positive relationships in the low zones and there was a more pronounced, significant positive trend at Sedgefield, in both zones. Focussing on the differences between zones, there was a weak pattern of higher infestation intensities for smaller mussels in the mid zone and this was most noticeable at St Francis Bay, Keurboomstrand and Sedgefield.

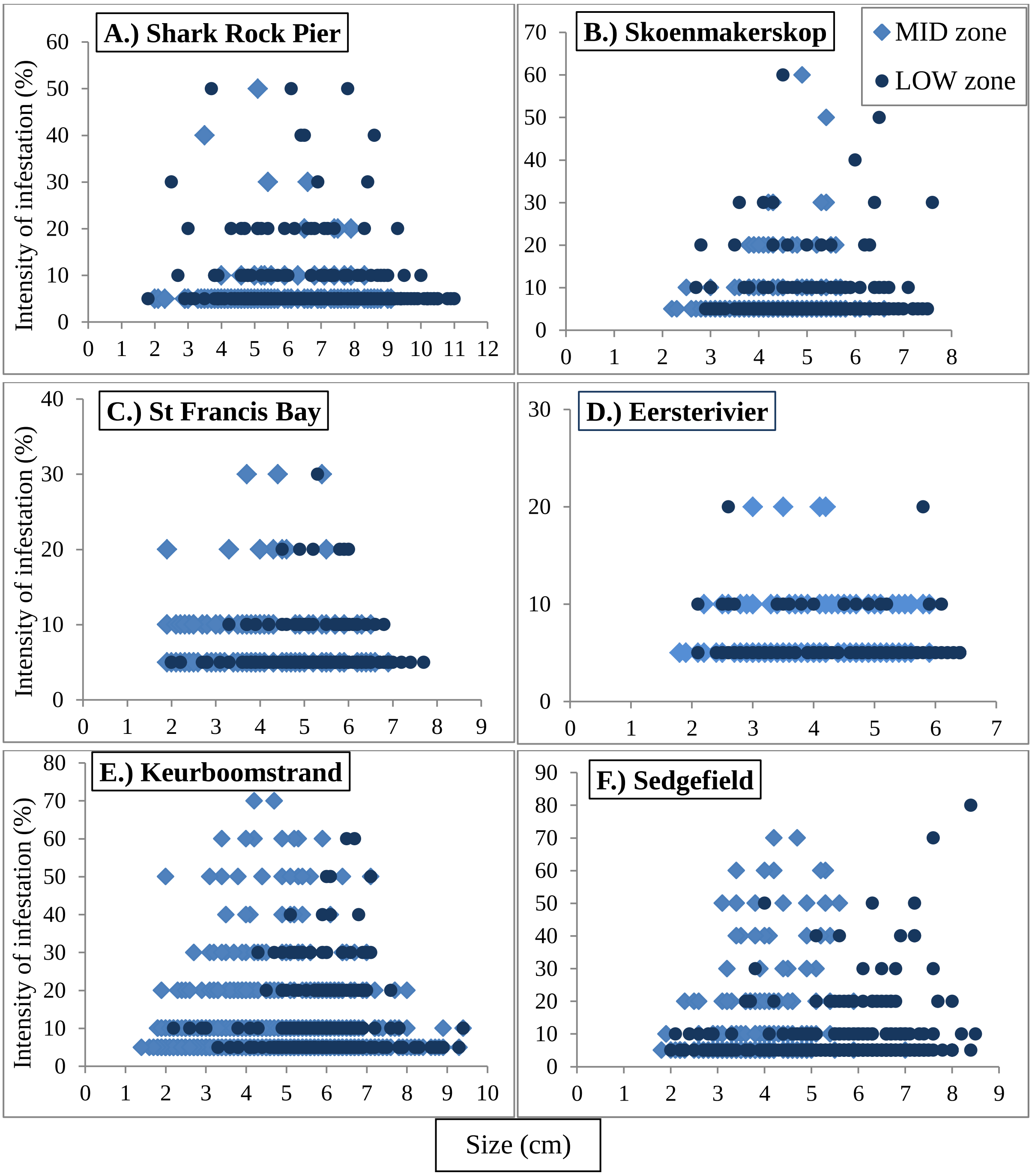

Figure 3.7 Mussel sizes and intensities of infestations as percentage of shell covered by barnacles on *Perna perna* mussels, across the two relevant zones. Sites are arranged from east to west: **A.)** Shark Rock Pier (mid: $p = 0.386$, $R^2 = 0.004$, $n = 214$; low: $p =$ **<0.00001***, $R^2 = 0.111$, $n = 310$); **B.)** Skoenmakerskop (mid: $p = 0.063$, $R^2 = 0.117$, $n = 296$; low: $p =$ **0.00001***, $R^2 = 0.061$, $n = 240$); **C.)** St Francis Bay (mid: $p = 0.857$, $R^2 = 0.0002$, $n = 149$; low: $p = 0.351$, $R^2 = 0.005$, $n = 188$); **D.)** Eersterivier (mid: $p = 0.677$, $R^2 = 0.001$, $n = 157$; low: $p = 0.815$, $R^2 = 0.0004$, $n = 126$); **E.)** Keurboomstrand (mid: $p = 0.095$, $R^2 = 0.020$, $n = 141$; low: $p = 0.613$, $R^2 = 0.001$, $n = 194$); **F.)** Sedgefield (mid: $p =$ **0.0001***, $R^2 = 0.063$, $n = 234$; low: $p =$ **0.00002*,** $R^2 = 0.065$, $n = 269$). Note that the scales of the *x* and *y* axes vary slightly between sites.

Mytilus galloprovincialis

From the PERMANOVA performed on *M. galloprovincialis*, for the intensity of barnacle infestation, it was shown that any three-way interaction involving size or bay was significant and the two-way interaction between these factors was also significant (Table 3.6). Another significant three-way interaction was that among size, season and zone, where there was a significant relationship between size and intensity of infestation for both seasons in the mid zone (Figure 3.8). In the high zone, the significant, positive, relationship between size and intensity was more obvious in summer, winter less so, but it was still significant, again with extremely low coefficients of determination (Figure 3.8). The results also showed that any two-way interaction involving site (nested in bay) was significant (Table 3.6).

Table 3.6 PERMANOVA to test the main effects on infestation intensity of *Mytilus galloprovincialis* with size as a covariate. The symbol * indicates a significant p-value, where * is $p < 0.05$, ** is $p < 0.01$ and *** is $p < 0.001$.

	Df	SS	MS	pseudo-F	p (perm)	U perms	p (MC)
Bay	1	18914	18914	0.994	0.481	535	0.403
Season	1	1662.4	1662.4	0.487	0.544	997	0.664
Site (Bay)	4	58475	14619	37.629	**0.001**	999	**0.001****
Zone	1	2740.3	2740.3	0.649	0.453	998	0.536
Size	1	1759.5	1759.5	0.938	0.361	999	0.384
Size X Bay	1	1763.5	1763.5	4.539	**0.029**	999	**0.023***
Size X Season	1	5206.9	5206.9	11.377	**0.001**	999	**0.002****
Size X Zone	1	283.02	283.02	0.337	0.655	998	0.651
Bay X Season	1	5331.1	5331.1	2.336	0.211	999	0.174
Bay X Zone	1	199.23	199.23	8.132E-2	0.874	999	0.987
Season X Zone	1	266.88	266.88	0.381	0.581	997	0.937
Size X Site (Bay)	4	5959.3	1489.8	3.835	**0.003**	996	**0.004****
Site (Bay) X Season	4	5089.3	1272.3	3.275	**0.010**	999	**0.009****
Site (Bay) X Zone	4	11880	2970	7.645	**0.001**	999	**0.001****
Size X Bay X Season	1	3224.2	3224.2	8.299	**0.006**	999	**0.003****
Size X Bay X Zone	1	1373.5	1373.5	3.536	**0.057**	997	**0.049***
Size X Season X Zone	1	3734.9	3734.9	8.514	**0.004**	997	**0.001****
Bay X Season X Zone	1	1570.7	1570.7	1.259	0.354	999	0.363
Size X Site (Bay) X Season	4	1411.1	352.77	0.908	0.493	999	0.487
Size X Site (Bay) X Zone	4	1418.6	354.64	0.913	0.472	999	0.443
Site (Bay) X Season X Zone	4	3225.4	806.35	2.076	0.073	998	0.075
Size X Bay X Season X Zone	1	97.418	97.418	0.251	0.712	999	0.716
Size X Site (Bay) X Season X Zone	4	2092	523	1.346	0.239	999	0.226
Res	183	7.125E5	388.49				

Df = degrees of freedom; SS = sum of squares; MS = mean squares; U = unique permutations; p (MC) = Monte Carlo significant values; p = significance level.

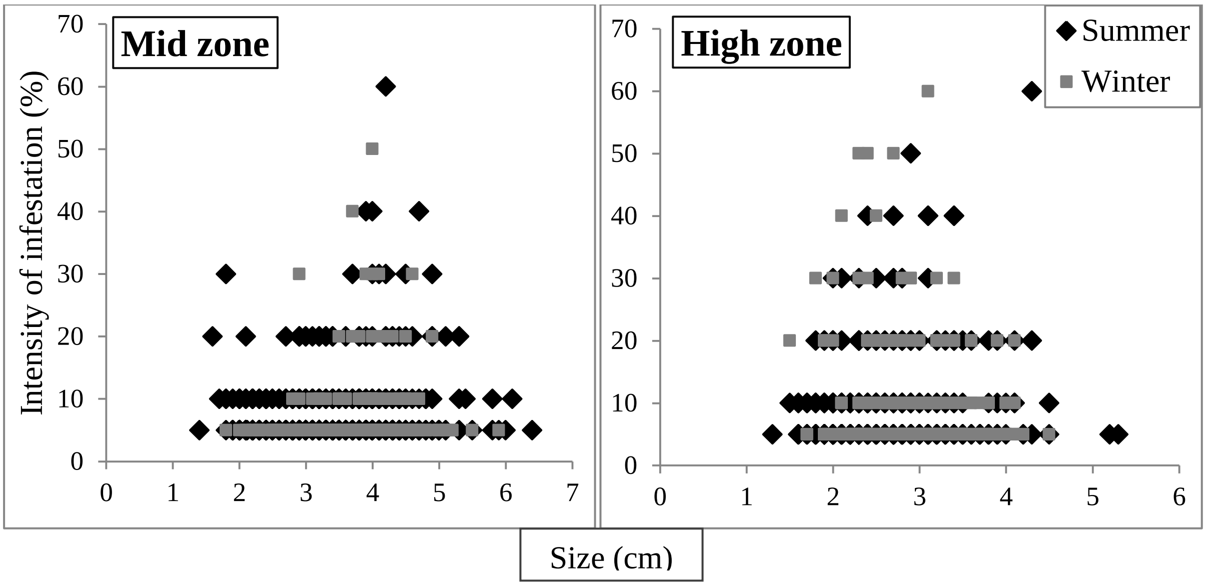

Figure 3.8 Mussel sizes and intensity of barnacle infestations as percentage of shell covered by barnacles on *Mytilus galloprovincialis* mussels, across the two seasons sampled and in the mid zone (summer: p = **0.008***, R^2 = 0.023, n = 644; winter: p = **0.025*,** R^2 = 0.018, n = 271) and high zone (summer: p = **0.0007***, R^2 = 0.017, n = 664; winter: p = **0.014*,** R^2 = 0.020, n = 304).

To clarify the significant interaction among size, bay and season, size can be considered as having a different effect in bay sites compared with open coast sites, but only in certain seasons. There is no clear pattern in the data presented in Figure 3.9. For both seasons in bays there were significant, positive trends between intensities of infestation and mussel size and, for open coast sites, only summer showed a significant correlation.

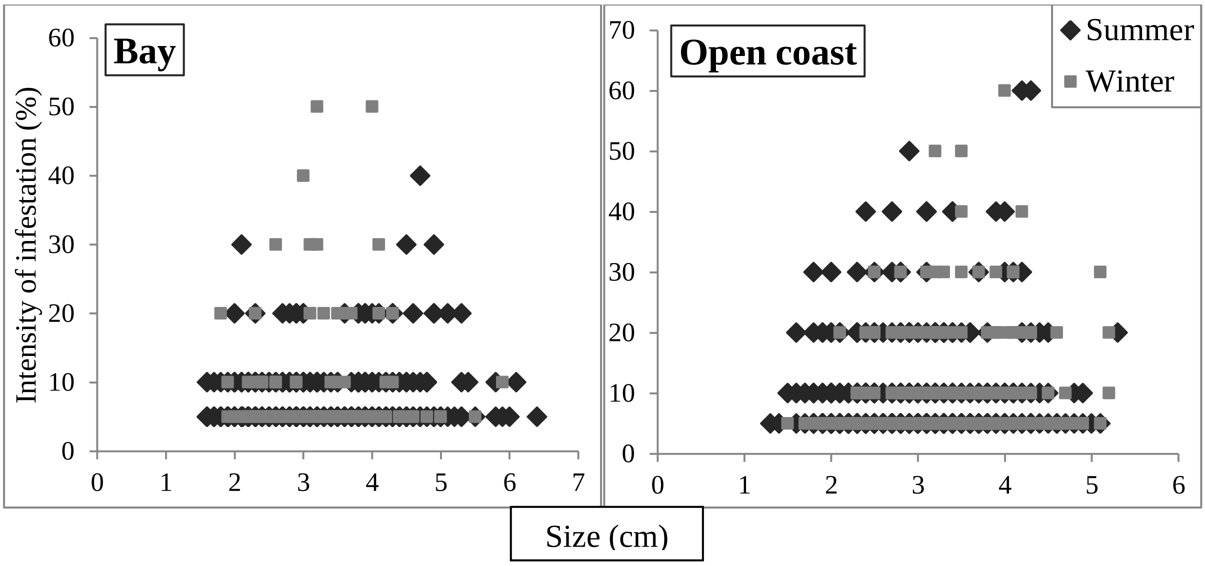

Figure 3.9 Mussel sizes and intensity of barnacle infestations on *Mytilus galloprovincialis* mussels, across the two seasons sampled and comparing bay (summer: p = **0.00008***, R^2 = 0.028, n = 702; winter: p = **0.0009*,** R^2 = 0.061, n = 176) to open coast (summer: p = **0.011***, R^2 = 0.0001, n = 605; winter: p = 0.823, R^2 = 0.0001, n = 399). Note that the scales of the *y* axes are different.

The significant two-way interactions of site (bay) x season and site (bay) x zone are combined in Figure 3.10. Although the differences between summer and winter were minimal at most sites and zones, there were notable differences at certain sites and zones. These included seasonal differences in the mid zone at St Francis Bay (SFB) and the high zones of Keurboomstrand (KBS) and Sedgefield (SDG) (Figure 3.10). There were also only three cases of winter intensities being higher than summer and these occurred in the high zones of St Francis Bay (SFB), Eersterivier (ERS) and Keurboomstrand (KBS).

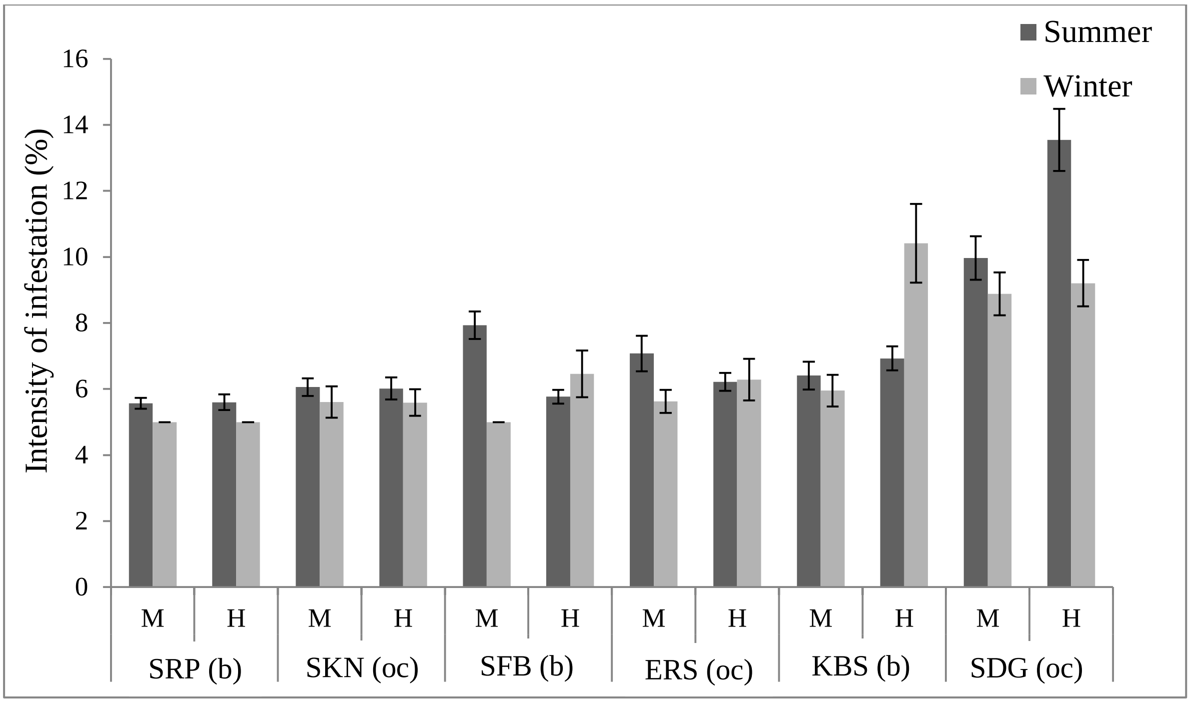

Figure 3.10 Intensity of barnacle infestations on *Mytilus galloprovincialis* mussels, across the two seasons sampled, where M = mid zone; H = high zone; oc = open coast and b = bay. Sites are arranged from east to west. The error bars indicate standard errors**.**

The factors with significant effects on the intensity of infestation were similar for *P. perna* and *M. galloprovincialis* and the only differences lay in the interaction of size and season being significant for *M. galloprovincialis,* but not for *P. perna*. In addition, the three-way interactions of site (bay), season and zone, as well as site (bay), size and zone, were significant for *P. perna*, but not for *M. galloprovincialis.* Significant interactions involving either zone or season were common and occasionally both interacted with either size, for *M. galloprovincialis,* or site, for *P. perna*. Although these results for infestation intensities are complex, it must be noted that bay status, interacting with other factors, comes out as significant for both mussel species. The covariate size had significant effects, but these reflect the large sample sizes and size is a poor predictor of intensity of infestation. The small-scale effect of size interacted with multiple other factors to produce an array of significant results.

3.4 Discussion

When investigating the percentage cover of *Perna perna* and *Mytilus galloprovincialis* across the different vertical zones of the intertidal, it was found that the indigenous *P. perna* was dominant in the low zone, while *M. galloprovincialis* was more common in the mid to high zones, as was first reported by Bownes and McQuaid (2006). Results for both *P. perna* and *M. galloprovincialis* showed a significant effect of not only zone, but also of the interaction among season, site (nested in bay) and zone. Bearing in mind that the study was only conducted over one austral summer and one austral winter (i.e. no replication of season), the effect of the factor 'season' is in reality an effect of 'time' and this interaction could be explained by an unusual temporal event. For instance, *P. perna* abundance at Shark Rock Pier was higher in the mid zone than the low zone in winter and at Eersterivier the same was true in summer. *Mytilus galloprovincialis* also showed variation at certain sites and, although winter abundances were generally higher at Shark Rock Pier and Sedgefield in both the mid and high zones, there was a greater abundance of *M. galloprovincialis* in summer. This supports work done by Reaugh-Flower (2011), who noted unpredictable temporal patterns at certain sites and for different mussel species in South Africa. The fact that site (bay) appears in the interaction is not unprecedented, as von der Meden et al. (2008) similarly found site-specific deviations from a *M. galloprovincialis*-dominated upper zone. Therefore, despite the significant interaction involving season, site and zone, the patterns found here were consistent with other studies (Bownes & McQuaid 2006, von der Meden et al. 2008).

Prevalence was measured as the proportion of infested mussels in a sample and the two mussel species were each considered in the zones where they were abundant. Generally, *P. perna* showed higher prevalence rates and in the mid zone *P. perna* had a higher average prevalence, so one would think this is not merely an effect of zone. Although the upper intertidal has been classified as the Balanoid zone, and is characterised by numerous barnacle genera such as *Balanus, Chthamalus* and *Tetraclita* (Stephenson & Stephenson 1949), the lower zones of the intertidal experience longer submergence times, which could mean more opportunity for the settlement of larvae (Pineda & Caswell 1997, Porri et al. 2007). The effect of bay interacting with either zone for *P. perna* or season and zone for *M. galloprovincialis* is also important for larval settlement, as it has been suggested that bays are demographically more closed systems than open coast sites (McQuaid & Phillips 2000). Other studies have recorded increased zooplankton abundance in oceanographic features such as fronts and eddies that are created by headlands (Murdoch 1989, Wing et al. 1998). The retention of larvae within bays has been demonstrated by a number of authors (Wing et al. 1995a, Archambault & Bourget 1999, Roughan et al. 2005). Water temperature and salinity also differ between bay and open coast sites and this may influence larval survival (Schumann et al. 1982) and ultimately the prevalence of barnacle infestation on mussel shells.

Along the south-east coast of South Africa, mussel abundance is often also high in the low zone of the rocky shore, whereas there is relatively poor cover of mussels in the high zone (von der Meden et al. 2008). This would suggest that, in the high zone, there is still available rock substratum for barnacle settlement. If, indeed, barnacles only settle on mussel shells as a secondary choice (see Chapter Four on barnacle settlement), this would explain the generally lower prevalence of infestation in the high zone. Barnacle larvae have also been shown to use adult conspecifics as an indication of where to settle (Grosberg 1982, Pineda & Caswell 1997, Kent et al. 2003). This gregarious behaviour may influence the prevalence as well as the intensity of barnacle settlement on individual mussels. Other smaller-scale effects would include micro-hydrodynamic processes and surface textures of the substratum (Raimondi 1990). These finer scale influences are discussed in more detail in Chapter Four.

The effect of water temperature is also important when considering seasonal change and ‘season’ in the present study featured as significant in many of the interactions for prevalence and intensity for both *P. perna* and *M. galloprovincialis*. The extent of barnacle infestations on mussels has been found to show strong temporal variation and to differ between years (Buschbaum 2000). Therefore the strong effect of season in the present study may simply be due to the particular time of the study. The unusual results for prevalence on *P. perna* at Shark Rock Pier and Sedgefield in the low zones, as well as Sedgefield for *M. galloprovincialis*, illustrate these temporal variations, where winter prevalence was higher than summer’s, contrary to the results of all other sites. The pronounced effect of bay status for prevalence on *M. galloprovincialis* is quite likely linked to the effect of bay on this species’ patterns of abundance on the south-east coast of South Africa. Von der Meden et al. (2008) compared abundances of *M. galloprovincialis* and *P. perna* along the south coast and found a stronger effect of bay for *M. galloprovincialis.* This is not surprising, considering *M. galloprovincialis* has lower attachment strength and a wider shell (Rius & McQuaid 2006, Zardi et al. 2006b, 2008), so any increase in drag caused by barnacle epibionts would increase the chance of the individual mussel being dislodged, which is a common effect of epibiosis (Dayton 1973, Paine 1979). If infested mussels are washed off the rocks more readily, it would explain why barnacle infestation prevalence, as well as intensities of infestations, were lower in winter when strong storms occur (Zardi et al. 2006b). Such large-scale (>100m) effects on barnacle abundances can explain much of the spatial and temporal variability and these effects can be linked to overall physical transport processes (Shanks 1983, Farrell et al. 1991, Pineda 1991, 1994, Bertness et al. 1996).

One possible benefit that epibionts provide for their host organism is protection from predators. Many studies have been done on the relationship between basibiont (host), epibiont and predator (Bloom 1975, Vance 1978, Pitcher & Butler 1987, Laudien & Wahl 1999, Buschbaum 2002, Enderlein et al. 2003). Although the majority of these studies focus on the protection given to the basibiont, there is also the situation where the basibiont suffers increased predation pressure because of the epibiont. This has been termed the 'shared doom' effect (Enderlein et al. 2003). Predation was beyond the factors examined in the present study, but may well account for some of the patterns found, particularly when considering the intensity of barnacle infestations. The results showed that mussel size was a key variable and, when interacting with other factors, it often had significant effects. Size seems to vary in importance, depending on the type of predator. Laudien and Wahl (1999) found no effect of mussel size when studying seastar predation on *Mytilus edulis* and the effects of epibiosis, but Enderlein et al. (2003) established an understanding of a hierarchy involving shore crabs, *Carcinus maenas*, where initially mussel size was selected for and epibiosis only mattered within a given prey size class. Predators on the rocky shores of the present study may have influenced numbers of infested mussels (prevalence) or selected for mussels with fewer epibiotic barnacles. At certain sites, such as at Sedgefield and Shark Rock Pier for *P. perna*, it is possible that sub-tidal predators affected results in the low zone more than the mid and high zones. Predators may also select for certain size classes and this would be an interesting consideration in future studies of epibiosis of mussels.

Despite the absence of a strong correlation between mussel size and infestation intensity, there were weak positive relationships that indicate larger mussels have a slightly higher cover of barnacles. This is logical, since larger mussels would be exposed to possible colonisation by barnacles for longer. Buschbaum (2000) found that available shell surface did not decrease when barnacle epibionts declined over the period of a year. The decrease was related to barnacle recruitment and mortality, rather than to their mussel hosts. It seems this may be the case in the present study, where there was no consistent trend of larger mussels having significantly more barnacle epibionts. Annual variations in recruitment of the barnacle *Semibalanus balanoides* have been reported (Carroll 1996, Buschbaum 2000). It is quite possible that the seasonal changes observed for prevalence and intensity at the six sites sampled were reflections of comparable fluctuations.

The fact that the results showed that the majority of mussel individuals had low infestation intensities (< 20%) might be an indication of limited shell surface available for settlement or limited selection for mussel shell during barnacle settlement. Mussel beds are often densely packed, with individuals side by side and only the posterior part of the shell exposed, diminishing space for epibionts to settle (Laihonen & Furman 1986, pers. obs.). Even if barnacles were to settle further down the shell initially, they might be eliminated by unfavourable conditions before long (Laihonen & Furman 1986). Specific areas of the mussel shell might also offer benefits for the barnacles and it is known that strong currents encourage barnacle settlement (Crisp 1955). Thus, when settling on a mussel, a barnacle could seek out the area near the mussel's siphonal aperture. Studies that support this theory include those of Brande (1982), Laihonen and Furman (1986) and Buschbaum (2001). These fine scale effects are of interest, but should not be considered without taking into account other environmental and biological impacts such as predation and coastal oceanography, like those already discussed. The significant interactions for infestation intensity were numerous and often complex, but considering the similar results for both *M. galloprovincialis* and *P. perna,* it can be proposed that, despite variation among sites, there is an important effect of bay, temporal changes and mussel size (under certain circumstances). Exposed shores have been shown to have higher abundances of sessile filter-feeders (McQuaid & Branch 1984, 1985), so it is not surprising that bay status had a significant effect and comparisons within these systems, such as zones, are important because they can be made concurrently, under comparable conditions (Bustamante et al. 1997).

Patterns of prevalence and intensity of infestations generally showed higher values in summer, but for future studies these trends should be monitored over a number of years. The relationship between available rock substratum and intensity of infestation on nearby mussel beds could also be examined more closely. Knowing what the patterns of barnacle epibiosis are along the South African south-east coast can lead to a better understanding of the effects of epibiosis on the indigenous mussel species, *P. perna,* as well as on the invasive mussel, *M. galloprovincialis.*

Chapter Four

Barnacle preferences of habitat: settlement and survival

4.1 Introduction

Marine benthic organisms have planktonic propagules that show initial settlement patterns highly variable in space and time (e.g. Connell 1961a, Hawkins & Hartnoll 1982, Kendall et al. 1982). Settlement on a hard substratum involves the sedentary post-larval stages interacting with each other, as well as facing the challenges of predation and inter-specific competition for space and disturbance by both biotic and abiotic events (Pineda et al. 2009). Patterns of distribution are seldom uniform for organisms in space-limited systems (de Wolf 1973) and interactions in the intertidal create a typical spatial mosaic in which individuals grow and reproduce (Paine & Levin 1981). Post-settlement mortality needs to be examined closely if attempting to understand what determines the continuation of a species (Connell 1985, Minchinton & Scheibling 1993). The distinction between settlement and recruitment is also important (Porri et al. 2006). Recruitment can be defined as the rate at which juveniles join the population (Pineda 2000), or as the number of juveniles that survive for a defined period of time after settlement and are then counted by an observer (Bayne 1964, Keough & Downes 1982, Connell 1985). For benthic invertebrates, this period of time may be a few days or weeks (Connell 1985, Nakaoka 1993, Pineda et al. 2009). There are also multiple spatial scales to consider in the different environments (Connell 1985, Nakaoka 1993, Eckman 1996, Sponaugle et al. 2006). Spatial patterns of settlement for barnacle populations develop from several, at times opposing, factors (Wethey 1984), such as a preference for cracks and pits (Crisp & Barnes 1954), clustered distributions due to gregarious behaviour by larvae (Knight-Jones 1953, Crisp & Meadows 1962, 1963) and the minimum distance needed for internal fertilisation (Crisp 1990). It is long-standing knowledge that barnacles show some degree of preference for settlement sites (Crisp 1961) and once the barnacle cyprids reach the near shore environment they actively seek out and evaluate the microenvironment, rejecting a position if it is not suitable (e.g. Rittschof et al. 1984).

What constitutes a suitable position involves the micro features of the surface, its texture and contours (Crisp & Barnes 1954, Crisp 1961) and the presence of conspecifics

(Knight-Jones & Stevenson 1950, Knight-Jones 1953, Crisp 1961, Crisp & Meadows 1962). Surface topography has been classified according to scale, as texture (the irregularities of a surface, smaller than the size of the larvae) and contour (irregularities larger than that of the larvae) (Crisp & Barnes 1954, Le Tourneux & Bourget 1988). One measure of texture is roughness and most studies have shown that surface roughness generally promotes barnacle settlement (see review in Berntsson et al. 2000a). Marine invertebrate larvae need to locate a refuge to maximise adhesion and avoid high shear stress. This could explain the pattern of preference for rough surfaces (Walters & Wethey 1996), but avoiding desiccation may be another explanation for this preference (Raimondi 1990). These are mostly physical cues, but there are also chemical cues from conspecifics (Knight-Jones 1953, Crisp & Meadows 1962 & 1963, Chabot & Bourget 1988, Raimondi 1988). It is likely that there would be combinations of chemical and physical cues that influence the final larval choice at settlement (Wethey 1984). For example, selection of microhabitat, at scales of heterogeneity larger than 10 cm, in combination with the presence of conspecifics, at scales smaller than 1.5 cm, seems to drive settlement of *Semibalanus balanoides* (Chabot & Bourget 1988).

A more cryptic factor influencing barnacle settlement may be the bacterial film that develops on surfaces that have been immersed in the sea and many marine larvae settle readily on these surfaces (Zobell & Allen 1935, Tighe-Ford et al. 1970). A study by Maki et al. (1988), however, found that bacterial films generally inhibited the attachment of barnacle larvae. When larval age was considered, older larvae attached to clean surfaces in higher percentages than biofilmed areas. This has been supported by Nasrolahi et al. (2012) in a similar study. Certain marine larvae are induced to attach and metamorphose by a specific bacterium (Neumann 1979, Schmahl 1985, Weiner et al. 1985) and the influence of these biofilms can be species specific for barnacle cyprids (Keough & Raimondi 1995). It is also possible that the bacterium only produces the stimulatory substance when at a certain growth stage (e.g. Schmahl 1985, Weiner et al. 1985) or that further cues (like algal epigrowth on surfaces: Jernakoff 1985, Jenkins et al. 1999) or a combination of them, affect recruitment. The shells of bivalves in the intertidal increase the accessible surface for larval settlement and, in spite of the shells offering substantial space, mytilids often appear less fouled than adjacent non-biological substrata (Wahl et al. 1998, Bers & Wahl 2004). One possible explanation is that many marine species have evolved behavioural (Becker & Wahl 1996), chemical, physical or mechanical defence mechanisms to prevent or minimise epibiotic settlement (Wahl 1989). Given the possibility of harmful effects of epibiosis, one might expect surfaces of endemic species to evolve preventative measures to inhibit the settlement of local epibionts, while cosmopolitan species should show a generalised antifouling strategy (Bers et al. 2006). The texture of the periostracum influences the antifouling capacity of mussel shells for *Mytilus edulis* (Wahl et al. 1998, Bers & Wahl 2004) and *M. galloprovincialis* (Scardino et al. 2003) and this might be an indication of adaptations to local, abundant epibionts (Bers et al. 2006). The periostracum is a thin, proteinaceous outer shell layer secreted near the shell margins (Bottjer & Carter 1980) and it is known that the periostracum prevents boring organisms that damage mussel shells (Kaehler 1999). General fitness is also improved by intact periostracum textures, which reduce the incidence of epibiosis on *M. galloprovincialis* (Scardino & de Nys 2004).

Considering the factors that affect settlement, it is clear that they cross a wide range of scales; from kilometres to sub-millimetres (Hills et al. 1998). For barnacles, settlement may be limited by multiple elements, from planktonic larval supply (Buschbaum 2000), linked to oceanic and tidal features (Raimondi 1990, Pineda 1994, Hills & Thomason 1996), to micro-heterogeneity of the available surface (Le Tourneux & Bourget 1988). Over and above the many influencing factors, one would expect that a sessile organism, such as a barnacle, would settle at the most favourable site available due to the fact that after settlement the ability to move to a better location is lost (Laihonen & Furman 1986). Therefore, when barnacles settle on mussel shells, is this an indication of site preference? An experiment was set up to address this question and to investigate the habitat choices made by *Chthamalus dentatus* when settling on different surfaces. Survival of adult *C. dentatus* was also considered, so that conclusions about long-term positive or negative effects of substratum choice could be made. The use of settlement tiles allows for replication in experiments from which generalisations can be inferred after appropriate analysis (Thomason et al. 2002) and this concept was employed in the present study.

4.2 Methods

4.2.1 General site description

To investigate substratum preference by settling barnacles, artificial plates were deployed during the months of March and April 2012, at two rocky shore sites on the south coast of South Africa, within Plettenberg Bay: Beacon Isle (34˚3' 35" S; 23˚22' 49" E) and Keurboomstrand (34˚0' 18" S; 23˚ 27' 30" E). Both sites are situated within the bay and epibiosis by barnacles is naturally present in both areas (per. obs.).

4.2.2 Experimental set up

Artificial settlement plates were deployed haphazardly within the mid mussel zone. Four treatments were considered: live mussels, dead mussels (shell only), resin shells (replicas of shells to mimic architectural micro-surface but not chemical characteristics of natural shells) and a rock mimic (perspex plates). All treatments were prepared in the weeks leading up to the spring low-tides of March and April 2012. Live individuals were collected off the rocks two days prior to field deployment and kept alive in aerated sea water that was changed daily. The mussels for the dead shell treatment were also collected two days prior to setting up the experiment (to ensure the periostracum was as intact as possible and chemical "cues" still present) and were prepared by scraping out the soft tissue, drying the shells off and filling the two halves with two-component epoxy adhesive (Abe epidermix 372). The shells were glued back together and left for 24 hours to set. Resin replicas were made from natural mussel shells from which the soft tissues had been removed. The shells were then gently washed and dried. Moulds were made of the outer surfaces of the two valves separately, using silicone (Loctite, RTV Silicone 587, Blue 80ml Tube). These moulds were left to set for 20 to 30 minutes at room temperature. Resin was mixed at the ratio of 5 ml of catalyst to 300 ml of resin, kept bubble-free by slowly stirring with a teaspoon for five minutes. The resin was then carefully poured into the silicone moulds and left overnight to cure at room temperature. Once solid, the resin casts were removed from the moulds and the two halves of the replica mussel were glued together using clear, two-component epoxy (Alcolin rapid-epoxy).

Live, dead and replica mussels were glued onto clear, perspex plates that had been roughened for better attachment of the glue. The rock mimic treatments were settlement plates that had a film of hard plastic (3M^{TM} Safety-Walk^{TM}, Medium duty, Gray) that resembled natural rock surface as closely as possible. All settlement plates were 8.0 x 5.5 cm in size and the mussels used for all treatments were *P. perna*, between 3.0 and 5.0 cm shell length in size.

Settlement plates were attached to the rocks using battery powered drills and self-tapping screws. The immediate area around each plate was scraped clean of any barnacles or mussels to avoid attraction by conspecifics. In March 2012, at Beacon Isle, 44 plates were arranged covering a total along-shore stretch of 10 m, while, at Keurboomstrand, 40 plates were arranged, all within 15 m of each other, in April 2012. All plates were removed from the rocky shore in August 2012.

4.2.3 Data collection

Once a month, photographs of each individual plate were taken and new barnacles on all treatment were counted, using ImageJ 1.45 software. Barnacles were also allocated to a size class, so that survival over the months could be monitored. Considering basal diameter, barnacle sizes were approximated and divided as follows:

Size class I: visible to the naked eye – 1.00 mm
Size class II: 1.01 mm – 2.50 mm
Size class III: 2.60 mm – 3.50 mm
Size class IV: 3.60 mm – 5.50 mm

Size class IV was conservatively considered to be adult, although other studies have found other *Chthamalus* species (*C. fissus*) to mature at 2 mm basal diameter (Hines 1978). Prior to analysis, the counts for the rock mimic plates were adjusted by taking the area of the plastic film attached to the plate and scaling it to correspond to the average size of the mussels used in the other treatments. At the end of the study, month six for Beacon Isle and month five for Keurboomstrand, final recruitment on different treatments was estimated. In order to normalise for surface area, the numbers of barnacles were calculated per square centimetre of the different treatments. Because the shells were small and shell curvature minimal, they were treated as flat surfaces. Due to unforeseen circumstances, certain settlement plates needed to be replaced, so a total of five sampling events were available for analysis but only events 1 – 4 were used. This was because too few plates remained for statistical analysis after sampling event 5.

4.2.4 Statistical analysis

To determine any possible substratum preference, three separate 3-way PERMANOVAs, for initial (primary) settlement, adulthood and barnacle deaths, were performed. A repeated measure design was used with treatment (fixed, 4 levels) and site (random, 2 levels), considered orthogonal to look at possible interactions between the two factors, while time was considered random, but was excluded from interactions due to a lack of true replication. Pair-wise tests were performed for significant results relevant to the study on each of the PERMANOVAs run. All statistical analyses were done using PERMANOVA+ adds-in to PRIMER (v. 6.1.10; PRIMER-E Ltd.).

4.3 Results

4.3.1 Primary settlers

Settlement of *Chthamalus dentatus* size class I individuals (termed primary settlers hereafter) showed significant differences between sites, treatments and over time (Table 4.1). There was, however, no significant interaction of site and treatment, and the pattern for both sites was clearly similar (Figure 4.1), though the numbers of primary settlers at these sites were significantly different and primary settlement was higher at Beacon Isle across all treatments.

Pairwise tests for the term treatment showed clearly, that rock mimic plates had significantly higher settlement rates than the other three treatments (Figure 4.1): dead < rock mimic (p = 0.0001); replica < rock mimic (p = 0.019) and live < rock mimic (p = 0.0001). Replicas also had higher settlement than dead mussels (p = 0.001), as well as the live treatment (p = 0.0004). There was no significant difference between the live and dead treatments for primary settlers (p = 0.193).

Table 4.1 PERMANOVA to test the main effects on substratum preference shown by primary settlers. The symbol * indicates a significant p-value, where * is p < 0.05, ** is p < 0.01 and *** is p < 0.001.

	Df	SS	MS	pseudo-F	p (perm)	U perms	p (MC)
Site	1	19477	19477	8.571	**0.0001**	9936	**0.004****
Treatment	3	59382	19794	8.711	**0.0001**	9925	**0.0001*****
Time	3	95883	31961	14.065	**0.0001**	9932	**0.0001*****
Site X Treatment	3	6426.8	2142.3	1.943	0.462	9931	0.464
Res	251	5.703E5	2272.3				

Df = degrees of freedom; SS = sum of squares; MS = mean squares; U = unique permutations; p (MC) = Monte Carlo significant values; p = significance level.

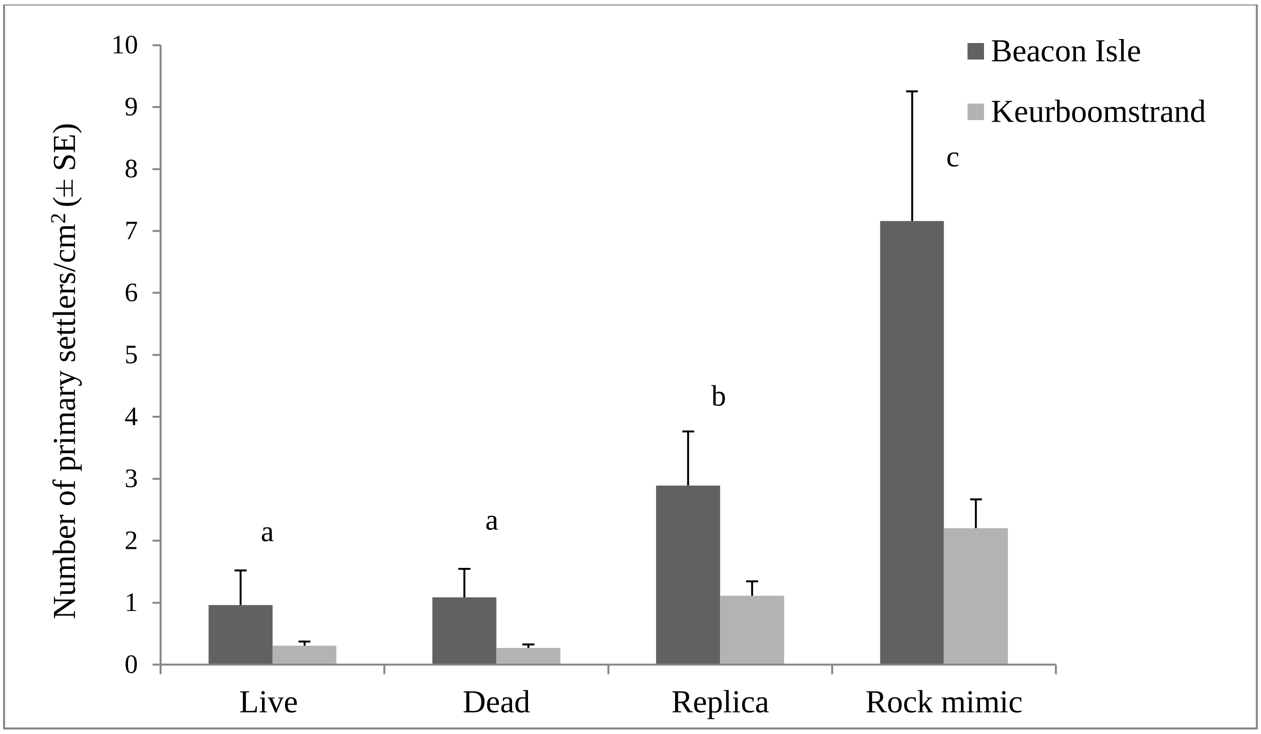

Figure 4.1. Primary settlers across the different treatments at the two sites, Beacon Isle and Keurboomstrand. Error bars indicate standard errors and the letters represent differences between treatments from the results of the pair-wise tests.

Considering the significant effect of time at Beacon Isle, on average, the numbers of primary settlers at time 1 were so high that they could not be represented on the same scale as the primary settlers at times 2, 3 and 4 (Figure 4.2). These extremes were mostly due to the high settlement on the rock mimic plates at time 1. At Keurboomstrand, the initial settlement on the rock mimic was not as high, so data are shown on one scale (Figure 4.3). At both sites it can be seen that there is a general decline in numbers of primary settlers on all treatments from time 1 to time 4.

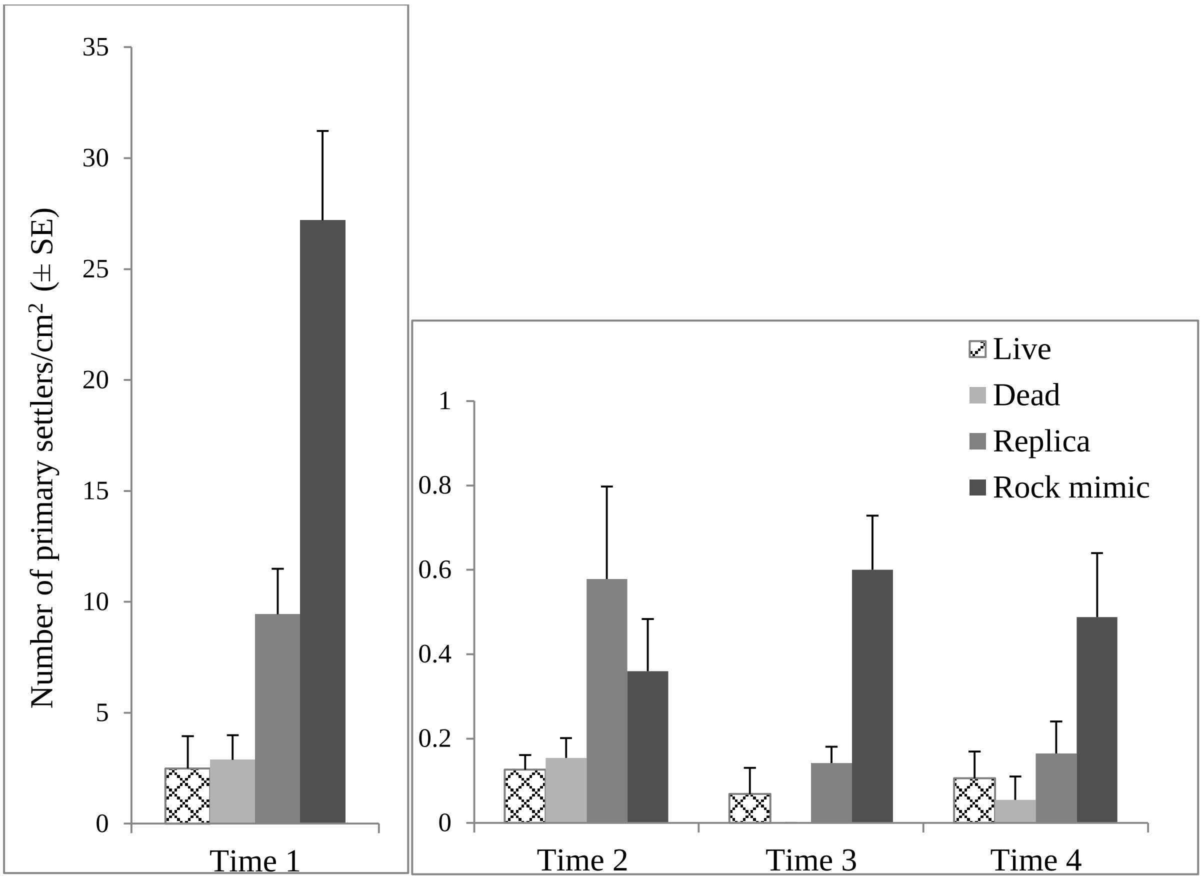

Figure 4.2. Primary settlers on the different treatments at Beacon Isle, showing sampling events (time 1 – time 4). Note that the scales of the *y* axes are different. Values are means and error bars indicate standard errors.

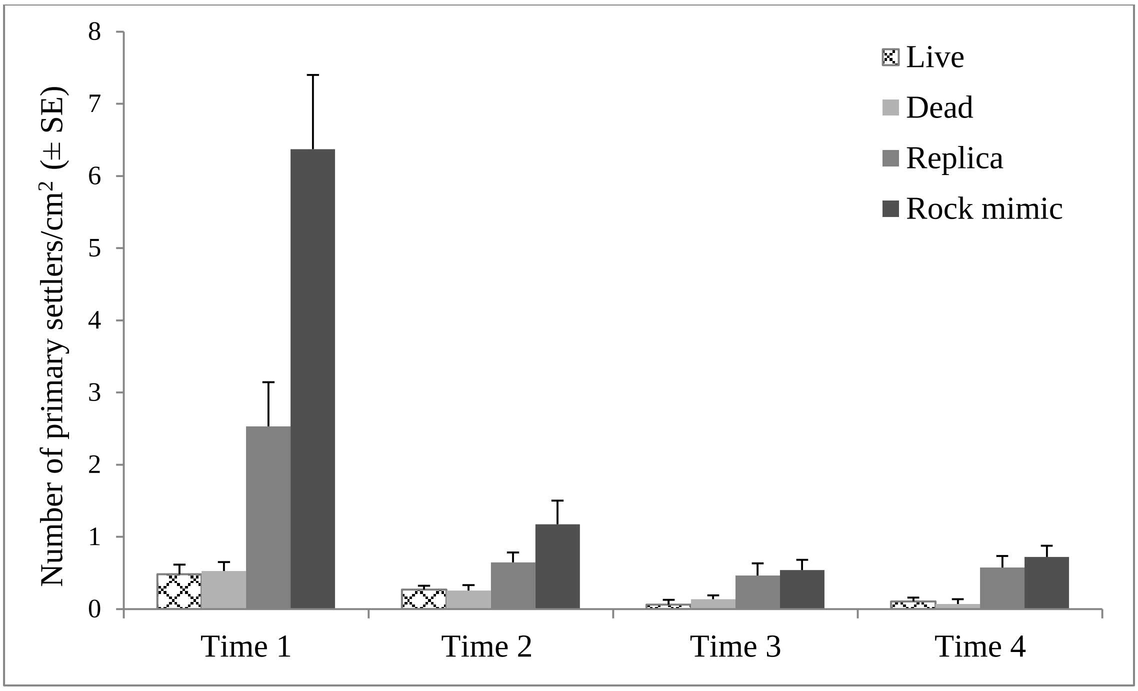

Figure 4.3. Primary settlers on the different treatments at Keurboomstrand, showing sampling events (time 1 – time 4). Values are means and error bars indicate standard errors.

4.3.2 Adult survivors

Barnacles that survived to size class IV were significantly affected by site and time, but not by the factor of interest, treatment (Table 4.2). On average, time 4 showed the highest adult survivor numbers at each site (Figure 4.4). The fact that Beacon Isle had far higher adult numbers than Keurboomstrand is in accordance with the higher numbers of settlers there (Fig. 4.1). Interestingly, there was no correlation between total numbers of settlers and adult survivors, not even when primary settlers from time 1 were used against adults from time 4 (plotting the abundances relative to treatment).

Table 4.2 PERMANOVA to test the main effects of treatment on adult survivorship. The symbol * indicates a significant p-value, where * is $p < 0.05$, ** is $p < 0.01$ and *** is $p < 0.001$.

	Df	SS	MS	pseudo-F	p (perm)	U perms	p (MC)
Site	1	20451	20451	17.417	**0.0001**	9953	**0.0001*** **
Treatment	3	4908.6	1636.2	1.3935	0.2163	9938	0.2111
Time	3	1.179E5	39315	33.482	**0.0001**	9939	**0.0001*** **
Site X Treatment	3	1205.2	401.73	0.3421	0.919	9954	0.913
Res	251	2.948E5	1174.2				

Df = degrees of freedom; SS = sum of squares; MS = mean squares; U = unique permutations; p (MC) = Monte Carlo significant values; p = significance level.

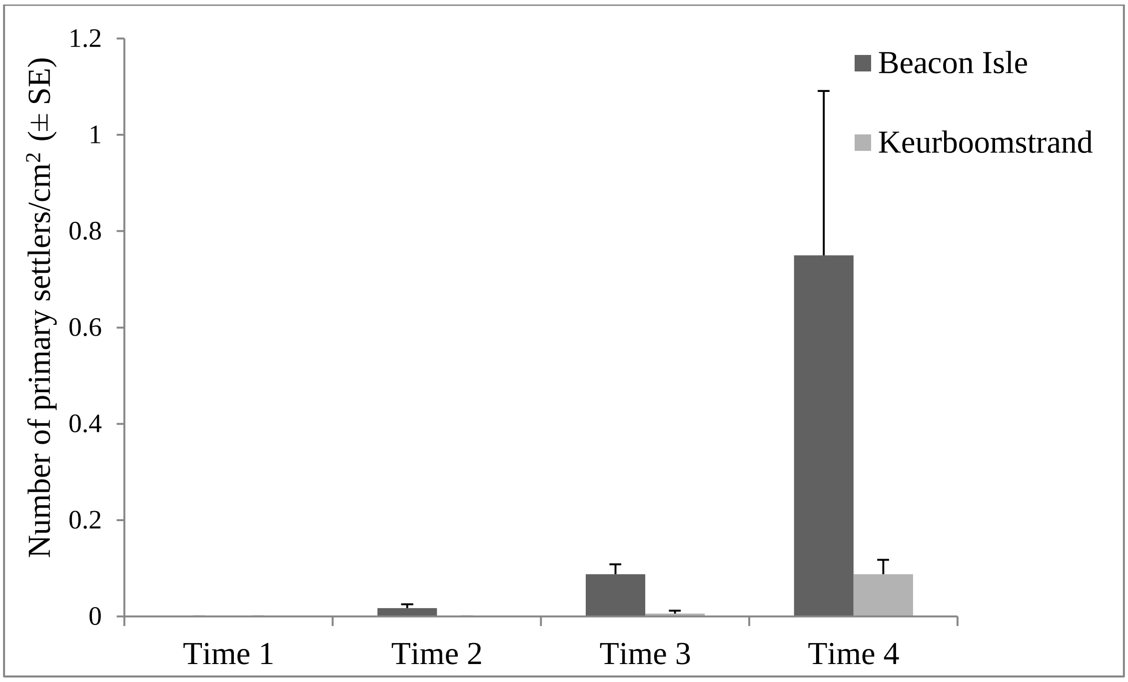

Figure 4.4. The general pattern of adult survivors at the two sites, Beacon Isle and Keurboomstrand, showing sampling events (time 1 – time 4). Error bars indicate the standard errors.

Despite neither treatment nor the treatment x site interaction being significant, it is still of interest that at Beacon Isle the rock mimic treatment had the most adults by time 4 (Figure 4.5). At Keurboomstrand, numbers of adult barnacles were extremely low and standard errors were large, but the replica treatment was the only treatment with adults at time 3 and had the most adults at time 4, although not significantly more than the other treatments (Figure 4.5). These patterns, however, might be a reflection of the total number of initial settlers throughout the study so, to represent the data fairly, proportions of adult barnacles are shown in Figure 4.6. These proportions are calculated from the total number of primary settlers. Again, there was no obvious pattern by treatment, though at time 4, the replica treatment had the highest values at both sites.

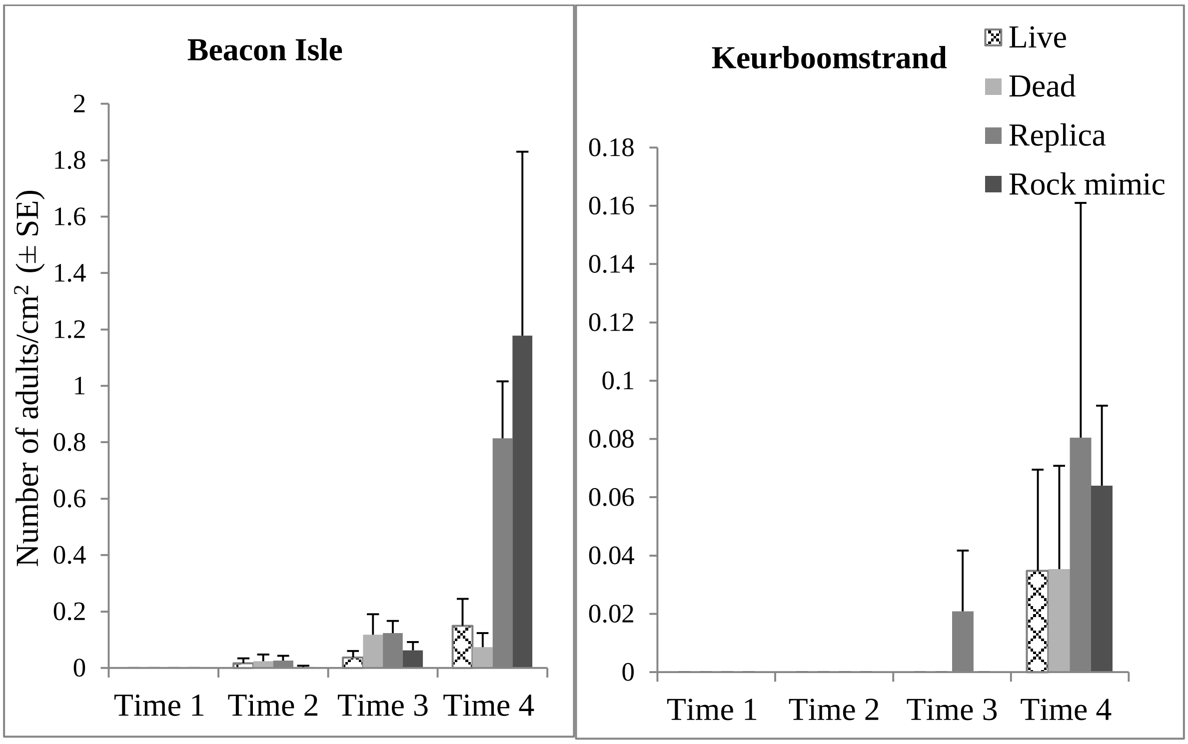

Figure 4.5. Adult survivors on the different treatments at Beacon Isle and Keurboomstrand, showing sampling events (time 1 – time 4). Error bars indicate standard errors. Note that the scales of the *y* axes are different.

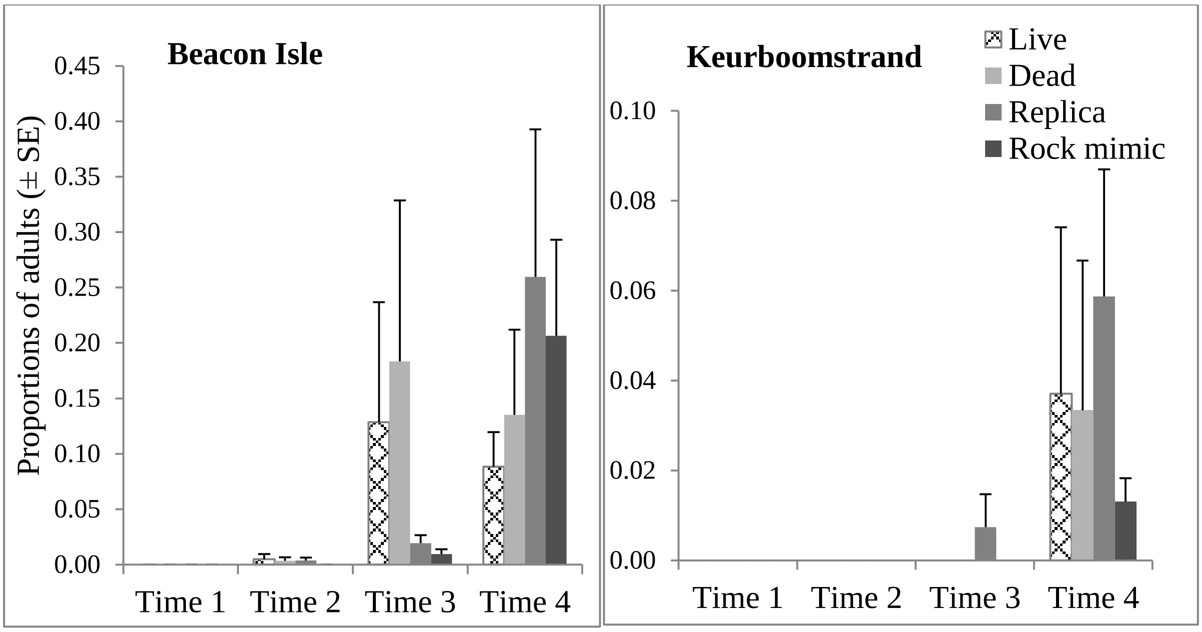

Figure 4.6. Adult survivors as proportions of total numbers of settlers on the different treatments at Beacon Isle and Keurboomstrand, showing sampling events (time 1 – time 4). Error bars indicate standard errors. Note that the scales of the *y* axes are different.

4.3.3 Barnacle deaths

The number of observed dead barnacles was significantly affected by type of substratum (treatment) as well as time (Table 4.3). The overall pattern shows significantly higher deaths on the rock mimic plates (Figure 4.7) and the pair-wise tests confirmed that deaths in the rock mimic were significantly higher than in any other treatment, which, on the other hand, did not differ among each other (Figure 4.7). The rock mimic treatment had many more mortalities than any other treatment, but again this may be an effect of high initial settlement. Thus barnacle deaths are also shown as proportions of the total number of settlers in Figure 4.9.

Table 4.3 PERMANOVA to test the main effects of substratum on barnacle deaths. The symbol * indicates a significant p-value, where * is $p < 0.05$, ** is $p < 0.01$ and *** is $p < 0.001$.

	Df	SS	MS	pseudo-F	p (perm)	U perms	p (MC)
Site	1	4665.9	4665.9	4.009	0.033	9921	0.032
Treatment	3	76114	25371	21.801	**0.0001**	9937	**0.0001*** **
Time	3	16463	5487.6	4.715	**0.002**	9938	**0.0012** **
Site X Treatment	3	1596.2	532.08	0.457	0.787	9959	0.792
Res	251	2.921E5	1163.8				

Df = degrees of freedom; SS = sum of squares; MS = mean squares; U = unique permutations; p (MC) = Monte Carlo significant values; p = significance level.

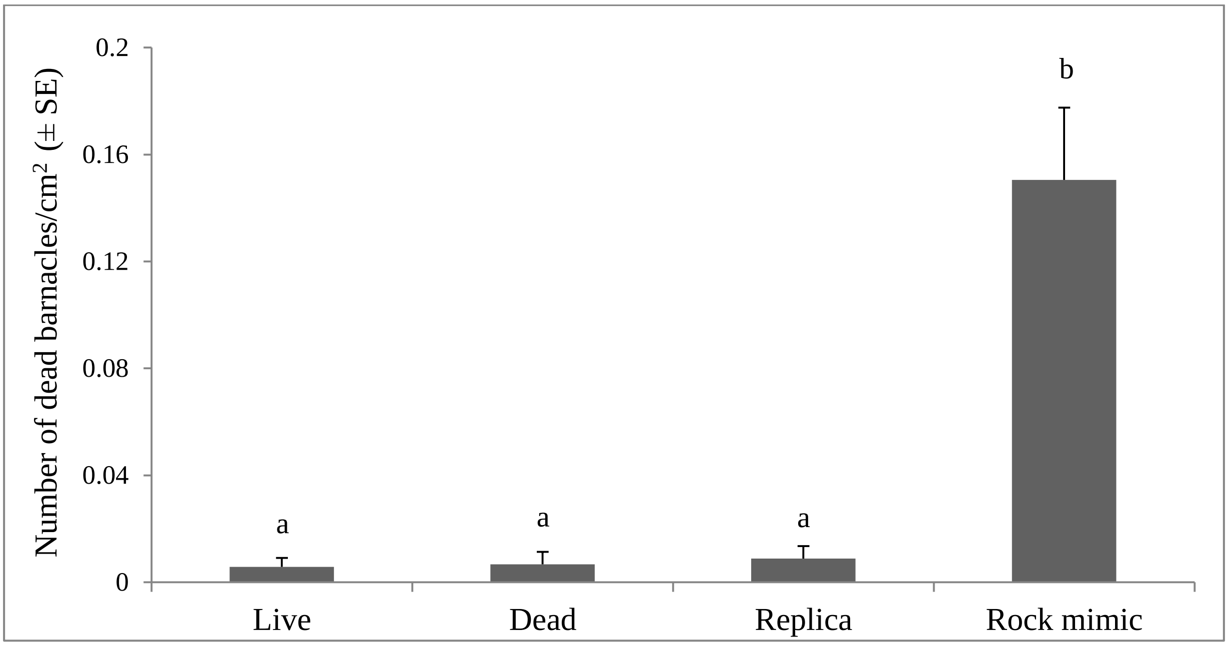

Figure 4.7. Barnacle mortality on different treatments. Error bars indicate standard errors and the letters represent differences between treatments from the results of the pair-wise tests.

The significant effect of time can be seen in Figure 4.8. The number of barnacle deaths on the rock mimic were much higher than any of the the other treatments, so are presented on a different y-axis, but, in general, averages of dead barnacles were low. The low numbers of recorded barnacle deaths does not account, however, for barnacles that died and broke off or were washed off the settlement plates. In Figure 4.8 it can be seen that numbers were relatively high at times 1 and 3 for the live treatment. The dead treatment had its highest number of dead barnacles at time 2 (albeit with a large variability) and the replica treatment had the highest numbers of dead barnacles at times 2 and 4. The rock mimic treatment had the most dead barnacles at time 4 and the lowest number were at time 1. When considering the barnacle deaths as proportions of total number of settlers the rock mimic treatment still had the most deaths proportionaly at times 2, 3 and 4 (Figure 4.9). The dead treatment had its highest proportions of deaths at time 2, whereas live mussels showed the highest proportions of deaths at time 3.

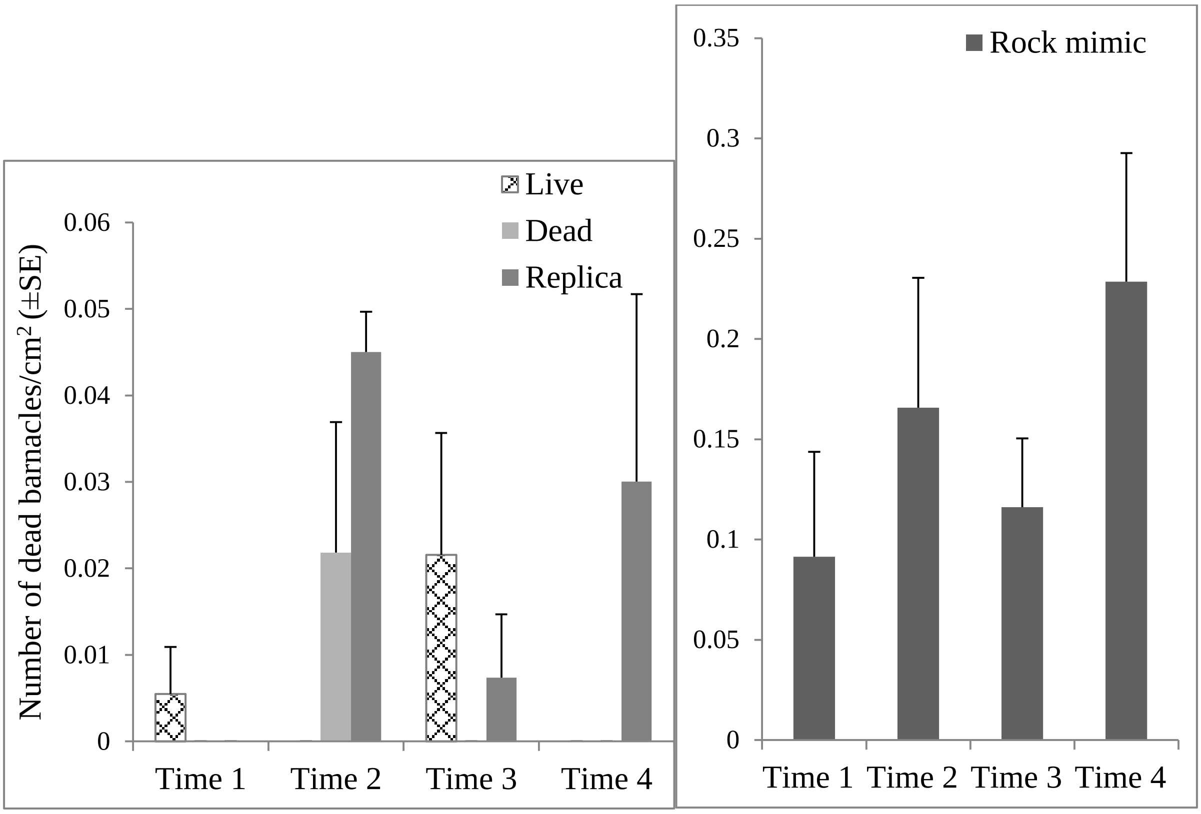

Figure 4.8. Dead barnacles on the different treatments at sampling events, time 1 – time 4. Error bars indicate standard errors. Rock mimic treatment is shown separately due to large differences in numbers (note that the scales of the *y* axes are different).

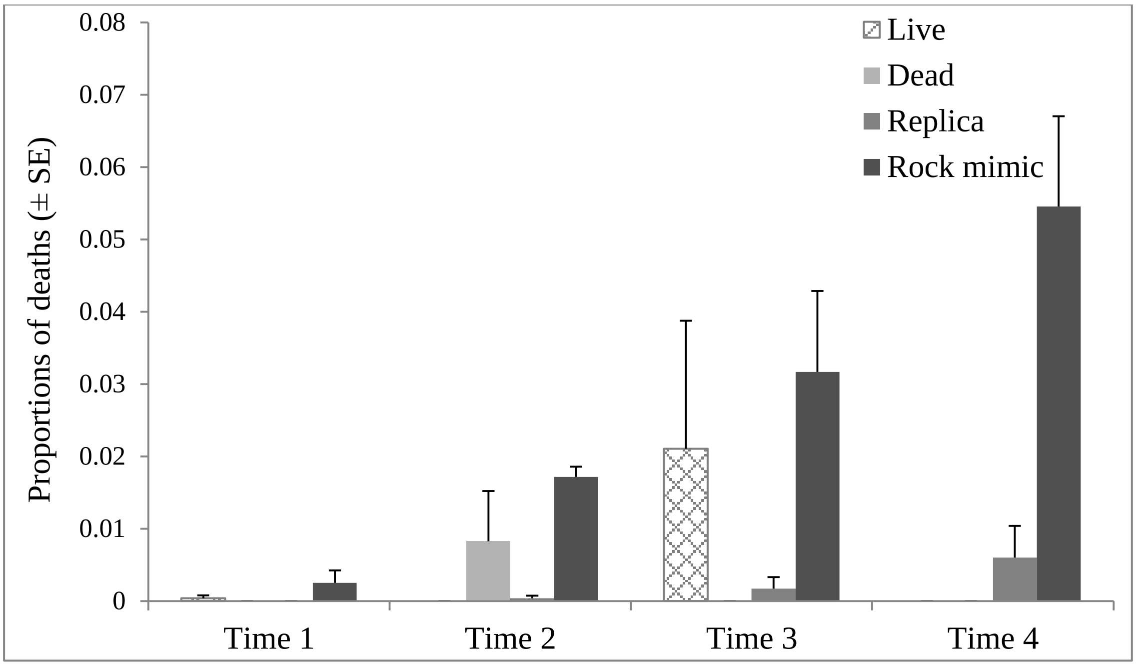

Figure 4.9. Barnacle deaths as proportions of total numbers of settlers on the different treatments, showing sampling events (time 1 – time 4). Error bars indicate standard errors.

It was recognised that, for all the results of this chapter seasonality cannot be distinguished from age of substratum but for this reason 'time' was not considered in any interation. The main objective of the chapter was to examine barnacle preference of micro-topography and chemical cues.

4.4 Discussion

Barnacles showed a clear preference for settlement on rock-like substratum (the rock mimic treatment). On average, numbers of primary settlers on the rock mimic plates were double those of the replica mussels at both sites. The numbers of settlers on replica treatments were significantly higher than live and dead treatments, so this would imply that there are features of a mussel's shell that deter barnacle larvae from settling. This inhibition of fouling has been widely studied and the defence mechanisms of an organism have been categorised as mechanical, chemical or physical (Scardino et al. 2003) and certain organisms may combine these different modes of action to deter or prevent epibionts (Wahl et al. 1998). The surface roughness of a mussel's shell, measured as surface irregularities, has been closely investigated as a possible physical defence (Crisp & Barnes 1954, Chabot & Bourget 1988, Le Tourneux & Bourget 1988, Raimondi 1990, Hills & Thomason 1996, Walters & Wethey 1996, Hills & Thomason 1998a, Wahl et al. 1998).

In the present study, *Perna perna* individuals were used on the settlement plates as the 'live' and 'dead' treatments and it has been shown that the properties of the periostracum of *P. perna, Mytilus edulis* and *M. galloprovincialis* are all similar (Bers et al. 2006). Reduced fouling has been shown on *M. galloprovincialis* (Scardino et al. 2003), as well as on different populations of *M. edulis* (Bers et al. 2006). These anti-settlement properties of the mussel surfaces are due to the micro-topography rather than the thickness of the periostracum which has been found to vary between species (Harper 1997). Micro-texture is another aspect of the periostracum and this differs from surface roughness in that it is the regular, defined surface structure (Andersson et al. 1999, Köhler et al. 1999, Berntsson et al. 2000a, Callow et al. 2002). Investigations into micro-texture have often used artificial micro-topographies and these include the scales of 2-4 mm (Hills & Thomason 1998b), 50-100 mm (Andersson et al. 1999) and 30-45 mm (Berntsson et al. 2000b). At these scales, settlement of barnacle larvae was reduced. Wethey (1986) proposed that surface contour is the most important factor affecting recruitment of *Semibalanus balanoides*. Considering the results of primary settlement in the present study, it is clear that *Chthamalus dentatus* cyprids avoid live mussels as well as dead mussels that still have an intact periostracum, leading to the supposition that there must be particular antifouling cues given off by the protein of the periostracum. This idea has previously been presented by Scardino et al. (2003), who suggested a possible species-specific surface bound chemical that repels larvae, but they acknowledged that this anti-fouling mechanism requires further investigation. A hierarchy can be established from the present study, where primary settlers preferentially settle on rock-like substratum, then replica treatments, followed by live and dead treatments. This would suggest that chemical cues are avoided over micro-topography, with primary settlers showing a strong preference for the rock-like substratum.

There is such a broad range of settlement cues for marine invertebrate larvae (Berntsson et al. 2004) that inferring acceptance or rejection of different cues from final settlement on plates or panels in the field was the most effective means of studying substratum preference. There are multiple aspects to consider, such as differences in settlement and/or recruitment between sites (Raimondi 1990), which were evident in the significant effect of site, for primary settlers as well as adults. Additionally, factors that have been shown to influence settlement of cyprids include any biofilms that have formed on the substratum (Maki et al. 1988, Wiezorek & Todd 1998), physio-chemical properties (Crisp & Barnes 1954, Becker 1993, Hills & Thomason 1996, Holm et al. 1997, Holmes et al. 1997), macro- and micro-scale hydrodynamics (Crisp 1955, Shanks 1986, Bertness et al. 1996), predation and competition (Connell 1961a, Dayton 1971, Wethey 1984, Berlow & Navarrete 1997), the age of the cyprid (Satuito et al. 1996, Jarret 1997, Jarret & Pechenik 1997) and the presence of other barnacles (Knight-Jones 1953). There is, however, a clear effect of treatment in the present study and there was no interaction of site and treatment, so there is an undeniable effect of substratum on the preference shown by barnacles when choosing a settlement site. Gregarious behaviour is widespread among barnacles and chemical cues from conspecific adults have been found in laboratory experiments, to encourage settlement (Knight-Jones 1953, Crisp & Meadows 1962, Rittschof et al. 1984). These chemical cues have also been found in the field (Raimondi 1988, Jarrett 1997). Biochemical cues from other invertebrate species (Raimondi 1988), biofilms (Maki et al. 1998) and macroalgae (Le Tourneux & Bourget 1988**)** are also known to affect barnacle settlement and many of the settlement plates were overgrown with algae in the present study (pers. obs.).

A further consideration is the availability of space and recruitment rates. Numbers of primary settlers at times two, three and four were much lower than at time one. This could simply be a result of settlement events and variations in larval supply, i.e. timing, but Raimondi (1990) has found similar patterns for *C. anisopoma*, where recently settled barnacles seemed to inhibit further settlement after 14 days in the field. *Semibalanus balanoides* also displayed a positive relationship between settlement rate and recruit density until approximately 30% of the free space was covered, after which settlement rate decreased (Chabot & Bourget 1988). There appears to be a threshold level of available free space, therefore, below which cyprids will not readily settle (Minchinton & Scheibling 1993). From the high initial settlement on some treatments, this possibility may have been the case in the present study. Gaines and Roughgarden (1985), however, regarded settlement of barnacles as a simple function of the availability of free space, but adjusting for planktonic larval supply, time of site immersion and average bulk flow over the substratum. They found that settlement plays as important a role as post-settlement processes such as predation and competition through physical dislodgement. Logically, if there is higher settlement in an area there will be higher recruitment rates, unless there is a major disturbance. With a larger, denser population, the community will be more stable over time, but if settlement is low the community will be recruitment-limited and sensitive to fluctuations in settlement (Underwood et al. 1983), due to the unoccupied space or gaps between the patches of barnacles (Gaines & Roughgarden 1985). The matter of larval supply is particularly important when unoccupied space becomes rare. The strength of the effect that local features have on settlement and recruitment depends heavily on this supply (Bertness et al. 1992). Despite the local variability and effects of larval supply, the fact remains that, in the present study, there were clear preferences shown for rock mimic plates over the other treatments.

Adult abundances showed no significant effect of treatment and, although initial settlement is an indication of preference, ultimately how many individuals survive to reproduce is fundamental, both ecologically and evolutionarily (Pineda et al. 2009). It has been shown that high settler densities reliably predict adult barnacle densities (Menge 2000), but in this study primary settlers did not consistently determine the numbers of adults. Barnacle deaths were the number of dead barnacle carapaces counted on the settlement plates and do not account for barnacles that died and were washed off entirely from the settlement plates, leaving no trace and therefore not being counted. The numbers of adult survivors may be a more accurate representation of substratum effect over time. It is well known that marine invertebrates have extremely high fecundities, but very few individuals survive to reproduce (Pineda et al. 2009), due to extremely high post-settlement mortality (Gosselin & Qian 1996, Hunt & Scheibling 1997). Raimondi (1991) concludes that adult distribution of sessile species, such as barnacles, is determined either by their initial settlement patterns, or by post-settlement mortality that occurs before maturity. Although there was no significant difference between treatments for adult survivors it must be remembered that various other processes shape the adult population and survival rates are continuously influenced by biotic factors such as predation (Connell 1961a, Dayton 1971, Wethey 1984) and abiotic effects such as extreme weather events (Connell 1961b, de Wolf 1973). These processes are variable in space and time, so it was not surprising that the effect of treatment on adult survival could have been obscured. Also the fact that there was no correlation between primary settlers and adults survivors showed that factors other than initial settlement are determining adult populations. In a similar study examining the influence of different surface characteristics on fouling communities, Thomason et al. (2002) concluded that field tests reveal the complexities of the interactions between substratum and time. In the present study, the strong preference shown for the rock-like substratum by primary settlers demonstrates just how important micro-topography is during the primary intertidal establishment phase and, although primary settlers did not consistently determine adult numbers, there is a clear effect of treatment in the present study.

Chapter Five

The effects of epibiotic barnacles on the growth and condition index of mussels

5.1 Introduction

The growth rates of marine mussels have been extensively studied, and despite the plasticity that growth often displays and the many influencing factors that can determine the rates of growth, growth is still commonly used to measure the health of individuals or the success of a particular species. Factors influencing rates of growth can be intrinsic, such as different genes in different species or populations (Gentili & Beaumont 1988, Peterson & Beal 1989), or extrinsic, like the environmental conditions which could influence the quantity of food available and its quality, water temperature, population density, rate of water exchange and the amount of time the mussels are exposed between tides (Seed 1976, Griffiths & Griffiths 1987). As noted by Seed and Richardson (1999), food supply is perhaps the factor most directly affecting the rate of growth. Sustained growth is not possible without food (Seed & Richardson 1999), but the importance of food supply may also depend on which parameter is being measured. Growth is defined as the increase in body size (Seed 1976). This includes weight and volume, but for a mussel the shell is an essential feature, so growth is usually measured as changes in shell length (Seed 1976). It has been recognised that growth in shell length is not necessarily paralleled with growth in soft tissue and, in *Mytilus edulis*, shell growth precedes soft tissue growth (Hilbish 1986). The deposition of soft tissue also fluctuates, depending on the stage of the reproductive cycle, whereas shell growth continues at a more-or-less regular rate, with only slight variations throughout the year (van Erkom Schurink & Griffiths 1993). One possible explanation for this is that mussel shells have lower organic content than soft tissues (Jørgensen 1976), so seasonal changes in food availability will not have the same effect on shell growth as they do on soft tissue growth (Borrero & Hilbish 1988). Soft tissue (or flesh weight) is therefore a well-integrated indicator of the health status of mussels and a common method for assessing this status or ‘fitness’ is the condition index (Davenport & Chen 1987).

The condition index (CI, hereafter) provides an almost instantaneous indicator of the state of the individual and, in bivalve molluscs, condition indices have been used for over half a century (Baird 1958). By measuring the amount of shell, or the volume enclosed by the shells, and relating this to the quantity of living tissue, the fitness of the mussel can be assessed (Baird 1958). Using the dry flesh weight is the preferred method, due to slight variations in techniques of weighing wet flesh (Lawrence & Scott 1982). In a comparison of methods for the calculation of condition in *Mytilus edulis*, Davenport and Chen (1987) concluded that, although dry weight measurements are time-consuming, this method needs less refinement to acquire accurate results. Factors that may affect results include season and reproductive condition (Baird 1958) and the wave exposure of the site where the mussels were collected (Steffani & Branch 2003). Condition index is influenced by reproductive condition, which varies with season and is linked to seasonal changes in sea surface temperature (Barber & Blake 1981, Carrington 2002). The energy required for gonad development reduces the flesh weight, unless food uptake is sufficient (Hancock & Franklin 1972). In the months before spawning an increase in body energy is necessary for the successful release of gametes (Griffiths & King 1979). In a study done on *Mytilus galloprovincialis* in South Africa by Steffani and Branch (2003), there was only a slight tendency towards lower condition indices in October and April. These trends are not surprising, considering these months coincide with the beginning of the two spawning periods that *M. galloprovincialis* experiences yearly (Zardi et al. 2007). The decrease in condition index of *M. edulis* in relation to spawning has been shown often in Europe (Seed 1969, De Zwaan & Zandee 1972, Dare & Edwards 1975, Dethlefsen 1975) and similar decreases in body weight have been found for other bivalves (Ansell & Trevallion 1967, Ansell 1972). In South Africa, *Perna perna* also has two main spawning events in a year, but not over the same period as *M. galloprovincialis* (van Erkom Schurink & Griffiths 1991). Different mussel species seem to have specific, endogenous factors, or possibly different responses to environmental conditions, that play a key role in the timing of gamete production and spawning (Zardi et al. 2007).

For both condition index and growth there are other factors to consider, such as intraspecific competition for food when mussel densities are high, which can also reduce growth (Bertness & Grosholz 1985, Wildish & Kristmanson 1985, Okamura 1986). The effects of epibionts on mussels have been studied widely and Dittman and Robles (1991) found that *M. californianus* had reduced shell lengths when overgrown by algae and that experimental removal of epiphytes led to an increase in growth rates. Flow velocities, drag and dislodgement of mussels also intensify when epizoans such as kelp and/or barnacles are present on the mussel shell (Witman & Suchanek 1984). Such amplified drag may increase the demand for byssal production to improve attachment strength. This could reduce the energy available for growth and flesh production of the infested mussel (Steffani & Branch 2003). Griffiths and King (1979) pointed out just how energetically expensive the process of byssal thread production can be. For *Aulacomya ater* (Molina) they found it formed 8% to 15% of a mussel's monthly energy expenditure.

The mussels being examined in the current study (*Perna perna* and *Mytilus galloprovincialis*) are sedentary filter feeders which depend on highly variable planktonic food in the surrounding intertidal waters (Lesser et al. 2010). The aim of this chapter is to determine the potential added stress of having barnacles as epibionts on the two species by assessing the physiological characteristics of growth and condition index of infested and non-infested mussels.

5.2 Methods

5.2.1 Site description

The study of the effect of barnacle epibiosis on mussel growth was conducted at Shark Rock Pier (33˚58′48″ S 25˚39′31″ E) in Port Elizabeth, on the south-east coast of South Africa. Both mussel species used for this study, *Perna perna* and *Mytilus galloprovincialis*, occur at this site, where there was also a sufficient infestation by barnacles to carry out the tagging procedure to follow growth. Mussels used for the measurement of condition index were collected from four of the six sites used for the prevalence and intensity part of the study (for GPS co-ordinates and site descriptions see Chapter 3). These were Sedgefield, Keurboomstrand, Eersterivier and St Francis Bay.

5.2.2 Growth

Growth of *P. perna* and *M. galloprovincialis* was measured by tagging 30 infested (approximately 10-50% of mussel shell covered with barnacles) and 30 non-infested individuals of each species at the beginning of the austral winter (May, 2011) and another 30 infested and 30 non-infested of each species at the beginning of the austral summer (December, 2011). Mussels were tagged by gluing blue paper triangles onto the shell, with the point of the triangle on the growing edge margin, using superglue (Loctite Superglue

Easy Brush). Only mussels infested with the barnacle, *Chthamalus dentatus*, were tagged. Due to practical constraints, all mussels were of medium size and fell within a range of shell lengths from 25-45 mm. After 15 weeks, in each season, all tagged mussels were collected, and returned to the laboratory. The sampling periods covered the core months of austral summer and austral winter.

In the laboratory, growth of the mussels was measured under a dissecting microscope fitted with an ocular micrometer from the tag point to the new limit of the shell margin.

5.2.3 Condition index

To understand the possible effect of epibiosis on the condition index (CI) of mussels, adult *P. perna* and *M. galloprovincialis* (>10 mm) were chosen with a range of percentages (0-70%) of the shell covered by epibiotic barnacles (30 mussels of each species). These mussels were selected during the two sampling events that occurred from May to July, 2011 and January to February, 2012. These months were representative of the austral seasons, winter and summer, respectively. It must be noted that, strictly speaking, the season should be replicated in other years, but due to time limitations season was not duplicated.

The CI was calculated as a percentage of the dry weight of the soft tissue over the dry shell weight. The soft tissue of each animal was dissected out and dried on a piece of previously weighed, aluminium foil, at 60°C, for a minimum of 48 hours. The shell was dried separately. Following Davenport and Chen (1987), CI was calculated as:

$$CI = (\text{dry soft tissue weight}/\text{dry shell weight}) * 100$$

Percentage of the total area of each mussel shell that was covered by barnacles was calculated by analysing digital photographs, using the ImageJ 1.45 software.

5.2.4 Statistical analysis

To analyse the growth data, separate PERMANOVAs (permutational ANOVA), with ANCOVA set up, were performed on *P. perna* and *M. galloprovincialis*, respectively. Season (fixed factor, two levels), infestation (fixed, two levels) and size (continuous variable) were considered as predictor variables, with growth as the dependent variable. This analysis was done using the PERMANOVA+ adds-in to PRIMER (v. 6.1.10; PRIMER-E Ltd.). When necessary, separate ANCOVAs for the two seasons were performed and regressions were done using Statistica 10.0. Homogeneity and normality were tested using the Cochran's and the Kolmogorov-Smirnov tests.

In order to assess the relationships surrounding the CI of mussels, separate two-way ANCOVAs were done for *P. perna* and *M. galloprovincialis*. The effects on the dependent factor CI, of season (fixed factor, two levels), bay status (fixed, two levels), site (random, four levels, nested in bay status) and percentage infestation of barnacles on the mussel shells (continuous variable), were tested. Significant effects were examined further, using Student-Newman-Keuls post-hoc tests. Correlation analyses were run to examine possible links between percentage infestation and CI. All statistical analyses were performed using the software Statistica 10.0.

5.3 Results

5.3.1 Growth

Perna perna

The results of PERMANOVA showed that the presence of epibiotic barnacles (percentage of infestation) had no significant effect on the growth of *Perna perna.* There was, however, a significant effect of the interaction between size and time and a significant effect of both of these variables individually (Table 1.1).

Table 5.1 PERMANOVA to test the effect of barnacle infestation on the growth of *Perna perna*. The symbol * indicates a significant p-value, where * is p<0.05, ** is p<0.01 and *** is p< 0.001.

	Df	SS	MS	pseudo-F	p (perm)	U perms	p (MC)
Infestation (%)	1	9.86E-03	9.86E-03	1.73E-02	0.890	9825	0.898
Season	1	10.732	10.73	18.831	**0.0002**	9843	**0.0001*** **
Size	1	4.479	4.479	7.8579	**0.008**	9823	**0.006** **
Size X Season	1	2.589	2.589	4.542	**0.040**	9812	**0.040***
Size X Infestation (%)	1	0.261	0.261	0.458	0.489	9810	0.503
Season X Infestation (%)	1	0.991	0.991	1.739	0.191	9842	0.199
Size X Season X Infestation (%)	1	1.87E-02	1.87E-02	3.27E-02	0.860	9814	0.852
Residual	72	41.035	0.570				

Df = degrees of freedom; SS = sum of squares; MS = mean squares; U = unique permutations;

p (MC) = Monte Carlo significant values; p = significance level.

An ANCOVA, performed on the summer and winter data separately, showed that growth was affected by the size of mussels in summer only. The regression (Figure 5.1) showed that, although the R^2 values were very low and the correlations marginally non-significant, the patterns of growth were similar for both seasons, where larger mussels grew faster over the 15 weeks that they were on the rocky shore. When seasons are pooled, the effect of mussel size on growth was enhanced, but due to season having a significant effect, this was considered irrelevant.

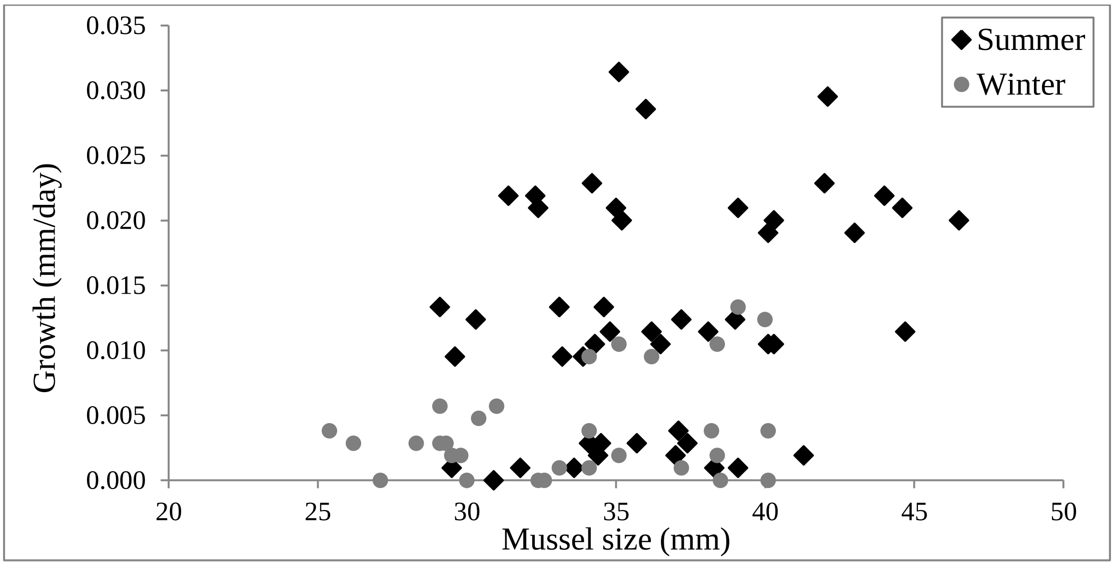

Figure 5.1 Correlation of growth (mm/day) and the covariate, mussel size (mm), for *Perna perna* mussels in different seasons (summer: $p = 0.0532$, $R^2 = 0.0757$; $n = 50$; winter: $p = 0.0795$, $R^2 = 0.1058$, $n = 30$).

Growth for *P. perna* was significantly higher in summer for both infested and non-infested mussels (Figure 5.2). It must be noted that, although not statistically significant, growth was slightly higher in summer for the non-infested mussels measured. The opposite is true for winter growth, but again the results are not significantly different.

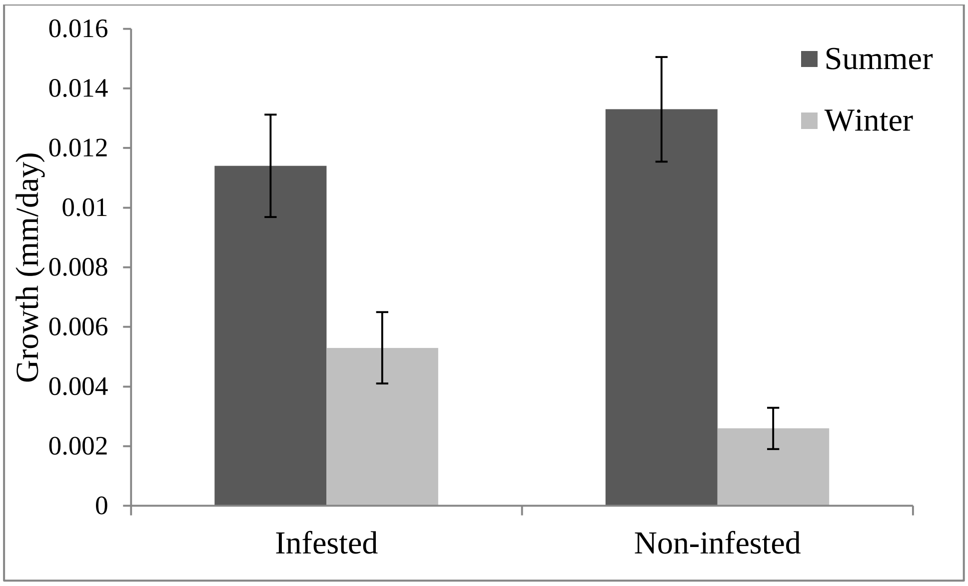

Figure 5.2 Growth of infested and non-infested *Perna perna* mussels, across the two seasons sampled. Values are means and error bars indicate standard errors.

Mytilus galloprovincialis

The presence of epibiotic barnacles had no significant effect on the growth of mussels and there was also no significant effect of size of mussel on the growth. The only significant effect was that of season (Table 5.2), with *M. galloprovincialis* growing more in summer than in winter, regardless of their state of infestation. No interactions between factors were significant. In contrast to *P. perna*, the covariate, size, had no significant effect for *M. galloprovincialis.*

Table 5.2 PERMANOVA to test the effect of barnacle infestation on the growth of *Mytilus galloprovincialis.* The symbol * indicates a significant p-value, where * is p < 0.05, ** is p < 0.01and *** is p < 0.001.

	Df	SS	MS	pseudo-F	p (perm)	U perms	p (MC)
Infestation (%)	1	0.648	0.648	0.781	0.395	9815	0.374
Season	1	4.050	4.050	4.879	**0.025**	9858	**0.030***
Size	1	0.303	0.303	0.365	0.538	9837	0.551
Size X Season	1	5.24E-02	5.24E-02	6.31E-02	0.804	9839	0.799
Size X Infestation (%)	1	8.07E-02	8.07E-02	9.72E-02	0.755	9825	0.753
Season X Infestation (%)	1	2.116	2.116	2.549	0.113	9855	0.116
Size X Season X Infestation (%)	1	0.149	0.149	0.179	0.667	9820	0.669
Res	72	59.760	0.830				

Df = degrees of freedom; SS = sum of squares; MS = mean squares; U = unique permutations; p (MC) = Monte Carlo significant values; p = significance level.

Growth for infested *M. galloprovincialis* was similar in both summer and winter, but for non-infested mussels there was a significant difference between seasons (Figure 5.3). The growth in summer was always higher, but not necessarily significantly so.

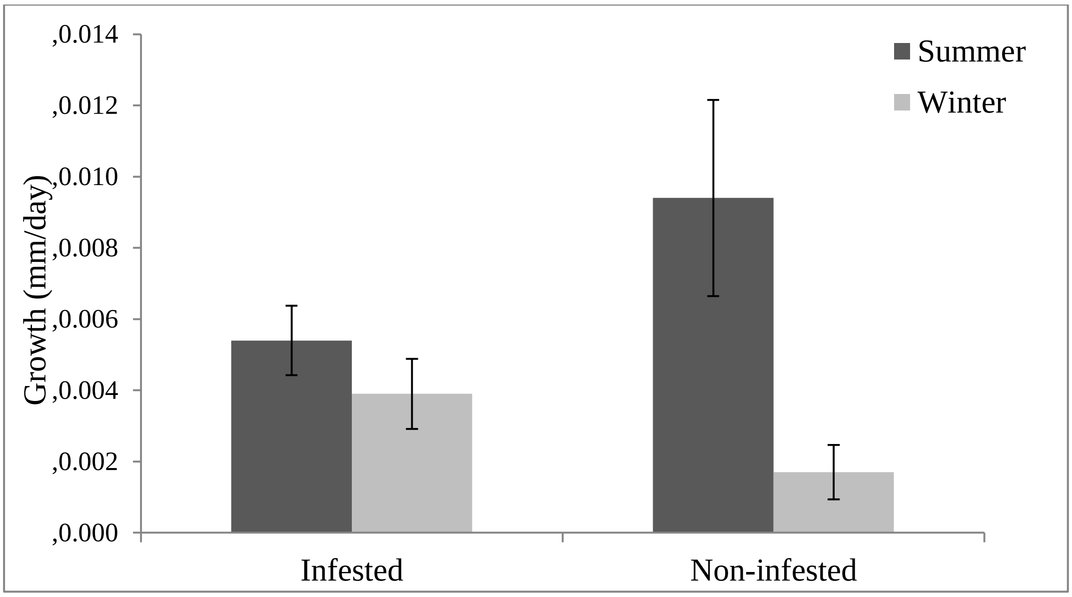

Figure 5.3 Growth of infested and non-infested *Mytilus galloprovincialis* mussels, across the two seasons sampled. Values are means and error bars indicate standard errors.

5.3.2 Condition Index

Perna perna

The intensity of barnacle infestation, measured as percentage of shell covered by barnacles, had no significant effect on the condition of *P. perna* (Table 5.3). There was, however, a significant effect of the interaction between time and site (Table 5.3).

Table 5.3 Three-way ANCOVA to test the effect of barnacle infestation on the condition index (CI) of *Perna perna.* The symbol * indicates a significant p-value, where * is $p < 0.05$, ** is $p < 0.01$and *** is $p < 0.001$.

	Df	SS	MS	F	p
Bay	1	38.587	38.587	0.494	0.554
Season	1	6.230	6.230	0.082	0.801
Site (Bay)	2	156.277	78.139	1.030	0.492
Infestation (%)	1	14.432	14.432	1.276	0.372
Site (Bay) X Infestation (%)	2	23.251	11.626	1.463	0.406
Bay X Infestation (%)	1	25.133	25.133	2.221	0.270
Season X Bay	1	7.193	7.193	0.095	0.787
Season X (Site) Bay	2	151.726	75.863	16.746	**0.00001*** **
Season X Infestation (%)	1	19.258	19.258	2.470	0.250
Season X Site (Bay) X Infestation (%)	2	15.897	7.949	1.755	0.178
Error	104	471.152	4.530		

Df = degrees of freedom; SS = sum of squares; MS = mean squares; F = f-ratio; p = significance level.

The Student-Newman-Keuls post-hoc tests produced groupings (Figure 5.4) that showed similarities between the summer data of St Francis Bay (SFB) and Sedgefield (SDG), which both had higher condition scores than any other sites. The data from Keurboomstrand (KBS) in winter stood out as the only case of CI being higher in winter than summer. The effect of season was significant for both bay sites (KBS and SFB), but only one of the open coast sites (ERS). At the other open coast site, SDG, there was no significant difference between seasons, with summer CI being only slightly higher than winter.

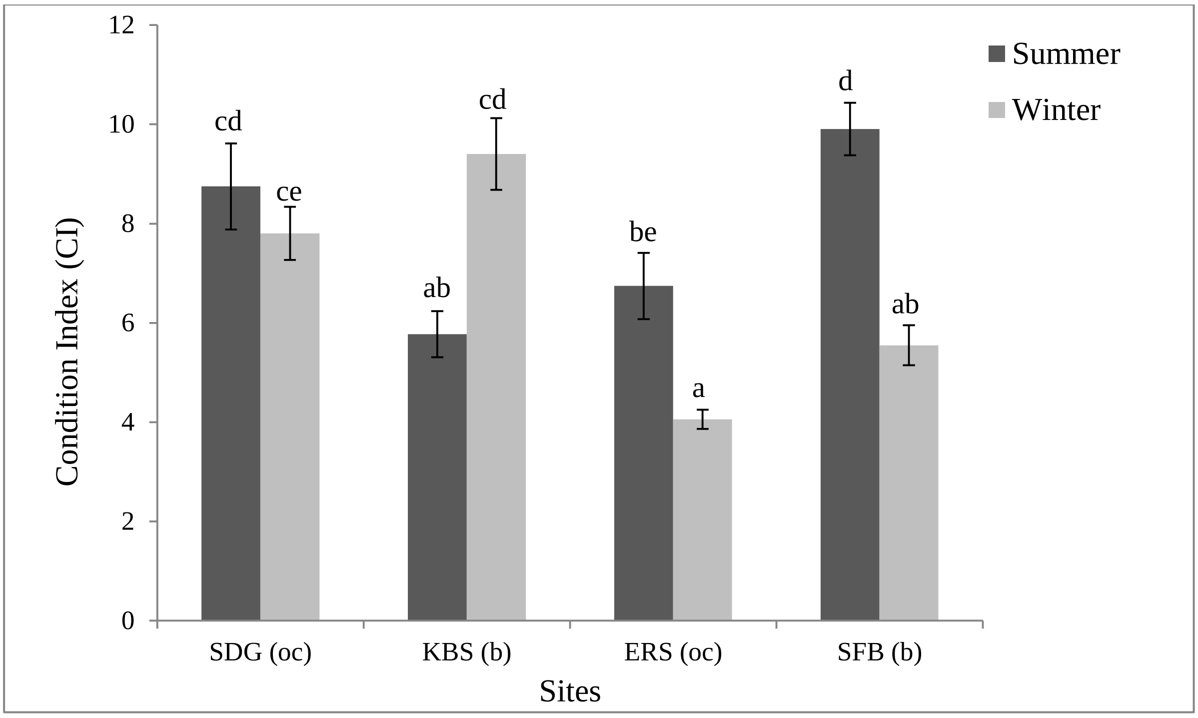

Figure 5.4 Condition Index of *Perna perna* at the different sites (where oc = open coast and b = bay) and across the two seasons sampled. Sites are arranged from west to east and the error bars indicate standard errors and the letters represent homogeneous groups from the results of the Student Newman-Keuls post-hoc tests**.**

The effect of season on CI, at each site, can be seen in Figure 5.5. Although there are no strong trends, weakly negative, but non-significant, correlations between percentage of shell covered by barnacles and CI were found for all sites and seasons except at Eersterivier (Figure 5.5C), where the correlation was significantly positive for summer ($p = 0.0151$; $R^2 = 0.3760$; $n = 15$). The winter data for Eersterivier showed low infestation levels (maximum = 12 % of mussel shell infested) and there was no correlation between barnacle infestation and CI ($p = 0.9125$; $R^2 = 0.0311$; $n = 15$).

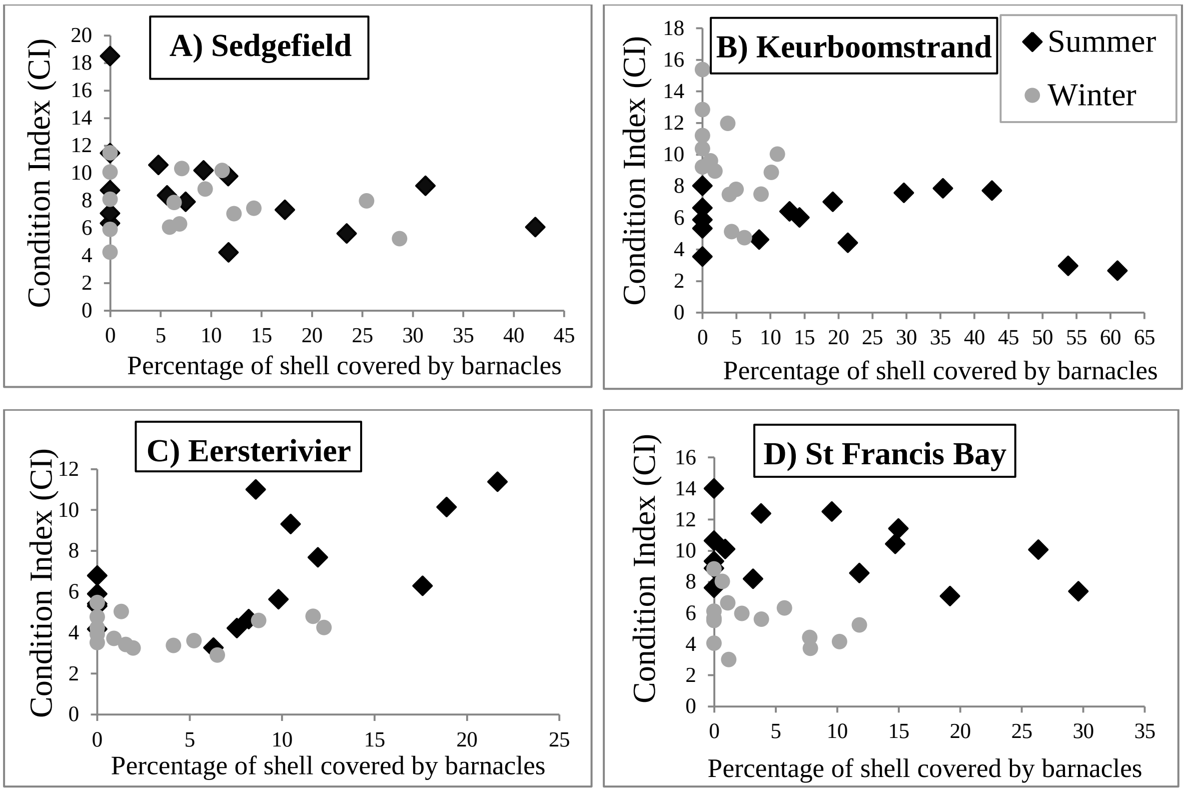

Figure 5.5 Correlation of percentage barnacle cover (infestation) and condition index of *Perna perna* at the different sites: **A)** Sedgefield (summer: p = 0.1603, R^2 = 0.1457, n = 15; winter: p = 0.4936, R^2 = 0.0368, n = 15), **B)** Keurboomstrand (summer: p = 0.3431, R^2 = 0.0693, n = 15; winter: p = 0.0845, R^2 = 0.2115, n = 15), **C)** Eersterivier (summer: p = 0.0151*; R^2 = 0.3760, n = 15; winter: p = 0.9125; R^2 = 0.0311, n = 15) and **D)** St Francis Bay (summer: p = 0.3160, R^2 = 0.0772, n = 15; winter: p = 0.1453, R^2 = 0.1559, n = 15). Note that different scales were used on the y axes.

Mytilus galloprovincialis

The level of infestation by barnacles had no significant effect on the conditions of *M. galloprovincialis* (Table 5.4), although the effect of the interaction between season and percentage infestation was only marginally non-significant. As with the results for *P. perna*, there was a strong effect of the interaction between season and site interaction (Table 5.4).

Table 5.4 Three-way ANCOVA to test the effect of barnacle infestation on the condition index of *Mytilus galloprovincialis.* The symbol: * indicates a significant p-value, where * is p<0.05, ** is p<0.01and *** is p< 0.001.

	Df	SS	MS	F	p
Bay	1	7.762	7.762	0.382	0.599
Season	1	1.197	1.197	0.044	0.853
Site (Bay)	2	40.657	20.329	0.753	0.570
Infestation (%)	1	1.431	1.431	0.119	0.762
Site (Bay) X Infestation (%)	2	24.931	12.466	15.705	0.070
Bay X Infestation (%)	1	8.006	8.006	0.667	0.497
Season X Bay	1	10.476	10.476	0.388	0.597
Season X Site (Bay)	2	53.967	26.983	7.409	**0.00098*** **
Season X Infestation (%)	1	8.825	8.825	9.348	0.052
Season X Site (Bay) X Infestation (%)	2	1.587	0.794	0.218	0.805
Season X Bay X Infestation (%)	1	2.485	2.485	2.632	0.199
Error	104	2378.773	23.642		

Df = degrees of freedom; SS = sum of squares; MS = mean squares; F = f-ratio; p = significance level.

For *M. galloprovincialis,* the interaction between season and site was most pronounced in the results from Keurboomstrand, where winter data were significantly higher than those for summer (Figure 5.6). At all other sites the winter data were lower than summer. The differences between sites for *M. galloprovincialis* were not as varied as they were for *P. perna,* but it should be noted that, for both *P. perna* and *M. galloprovincialis,* Keurboomstrand was unique, in that at all other sites the winter data were lower than the summer data, though the difference was not always significant.

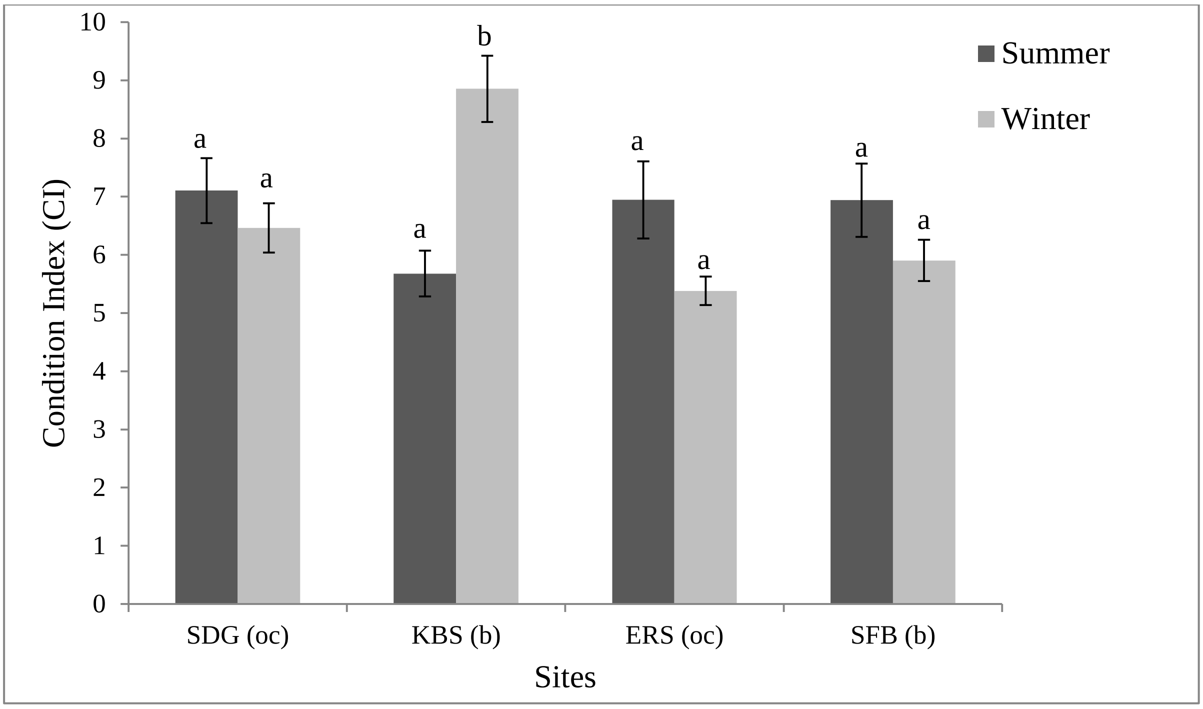

Figure 5.6 Condition index of *Mytilus galloprovincialis* at the different sites (where oc = open coast and b = bay) and across the two seasons sampled. Sites are arranged from west to east and the error bars indicate the standard errors and the letters represent homogeneous groups from the results of the Student Newman-Keuls post-hoc tests**.**

At all sites the correlations for summer were non-significant, while for winter there were weak correlations. These were significant for Eersterivier and St Francis Bay (Figure 5.7B).

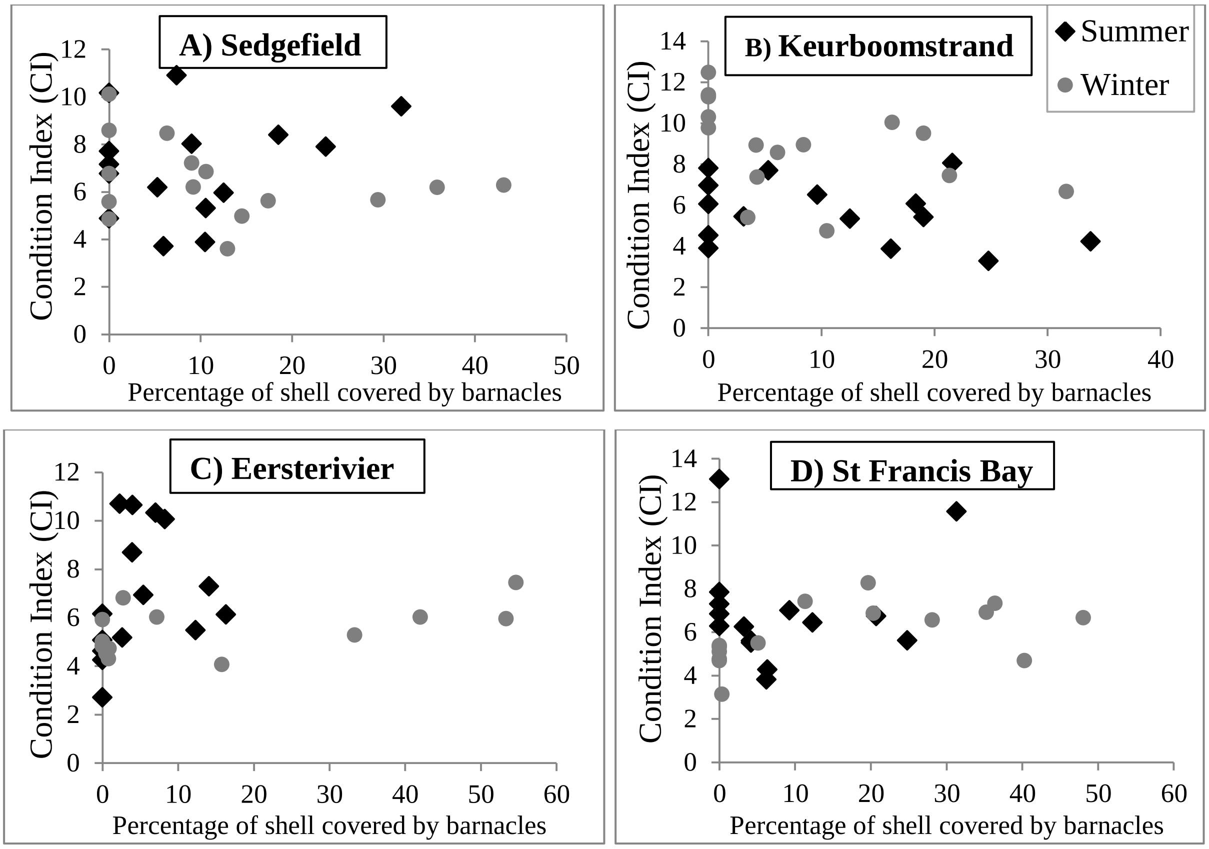

Figure 5.7 Correlation of percentage barnacle cover (infestation) and condition index of *Mytilus galloprovincialis* at the different sites, **A)** Sedgefield (summer: $p = 0.4101$, $R^2 = 0.0528$, $n = 15$; winter: $p = 0.2862$, $R^2 = 0.0869$, $n = 15$), **B)** Keurboomstrand (summer: $p = 0.2747$, $R^2 = 0.0909$, $n = 15$; winter: $p = 0.1044$, $R^2 = 0.1899$, $n = 15$), **C)** Eersterivier (summer: $p = 0.3826$, $R^2 = 0.0591$, $n = 15$; winter: $p = 0.0336$*, $R^2 = 0.3026$, $n = 15$) and **D)** St Francis Bay (summer: $p = 0.5863$, $R^2 = 0.0234$, $n = 15$; winter: $p = 0.0474$*, $R^2 = 0.2695$, $n = 15$). Note that different scales were used on the y axes for some sites.

Sites within bays or on the open coast did not stand out as having significantly different CIs. The two mussel species, however, showed significant results for the interactions site and season. Seasons were significantly different for *P. perna* at three sites, but only at one site for *M. galloprovincialis*. Considering the correlation of barnacle infestation and CI, the summer data for *P. perna* at Eersterivier indicated a significant, positive relationship. The winter data for *M. galloprovincialis* at both Eersterivier and St Francis Bay were significant and also positive.

5.4 Discussion

Seed (1976) summarises the patterns of growth in mussel shell length, pointing out that growth is generally rapid during the spring and summer, but slow or absent during the colder winter months. *Mytilus galloprovincialis* and *P. perna* show the same pattern and season stood out as a significant factor, although, for *P. perna*, its effect depended on individual size. Van Erkom Schurink and Griffiths (1991), however, argued that in South Africa shell growth does not cease during winter as it does in Europe and that South African mussels should grow fairly uniformly throughout the year. Slower growth rates in winter may be caused by the reduced availability of food (Seed 1976), which is commonly used to explain winter loss of flesh weight (Ansell & Trevallion 1967, Ansell 1972, Hancock & Franklin 1972, Dare & Edwards 1975). Flesh weight is a component of the CI used to determine the state of *M. galloprovincialis* and *P. perna* health. The results revealed better condition (higher CI values) for both species in summer, with the exception of CI in Keurboomstrand, Plettenberg Bay. This may be due to the particular characteristics of this site, such as the degree of wave exposure which, being within a bay, would be reduced. The mussel beds at Keurboomstrand might be more sheltered than the mussel beds at other sites and therefore less energy is put into byssal thread production for attachment. This has already been shown to be energetically expensive (Griffiths & King 1979) and, if less energy was required for attachment, more energy would be available for tissue growth. However, St Francis Bay is also a sheltered site and did not show this trend. So either there are finer scale site characteristics, or the better CI values at Keurboomstrand are a reflection of just the "right amount" of wave action. Steffani and Branch (2003) found that *M. galloprovincialis* had higher condition values at moderately exposed sites than at sheltered sites and suggested that more food is delivered with increased water flow. Several other authors have found that mussels or other inter-tidal filter-feeders with faster growth rates exhibit better physiological condition in exposed than in sheltered locations (Robles & Robb 1993, Leichter & Witman 1997, Sanford & Menge 2001). Steffani and Branch (2003), however, found that at sites with extreme wave exposure, both growth and condition decreased for *M. galloprovincialis.* Their results confirm that, above certain hydrodynamic thresholds, further increases in water flow and related food supply are not likely to have additional positive, but rather harmful, effects (Prins & Smaal 1989). Excessive current speeds may also result in a physiological or behavioural response of shell closure (Wildish & Kristmanson 1985), which would then reduce food intake and thus growth. The effect of bay was tested in the CI analyses and was not significant for either species, so there was no apparent effect of coastal topography, but this still does not exclude the role of wave exposure on mussel fitness status, especially because direct measurements of water flow and wave action were not made during this study.

In the present study, CI values varied significantly between seasons at three sites for *P. perna*, yet for *M. galloprovincialis* there was a significant difference between summer and winter at only one site and, once again, this was at Keurboomstrand. One would not expect *M. galloprovincialis* to be significantly influenced by season in terms of temperature alone as many species in the genus *Mytilus* occur under extreme arctic conditions, where the temperature can easily be below 0^{o}C (Seed 1976). Therefore any seasonal changes in *M. galloprovincialis'* flesh weight probably result from complex interactions of food availability and temperature with growth and reproductive processes (Dare & Edwards 1975). Along a similar line, gonad index of *M. galloprovincialis* seems not to be affected by sea-surface temperature, whereas the values for *P. perna* reflect changes in sea-surface temperature more accurately (Zardi et al. 2007). The timing of spawning events may be important when examining the condition of mussels, because if sampling was done in the months leading up to, or just after, a spawning event, the results would reflect this. The precise status of reproductive condition was not examined histologically so, if the specimens were collected during a time of gonad development, the CI may have been higher than expected. For bivalves in the boreal region, prior to summer spawning, renewal of reserves is needed, which results in growth and gametogenesis (Ansell & Trevallion 1967). In South Africa, *P. perna* and *M. galloprovincialis* have two main spawning events within a year: a major, summer event and a minor, winter event (van Erkom Schurink & Griffiths 1991). In Plettenberg Bay, on the south-east coast, the months at which these events occur do not seem to coincide for the two species (Zardi et al. 2007). Depending on the region, *P. perna* and *M. galloprovincialis* spawn at different times (van Erkom Schurink & Griffiths 1991), but the months of spawning reported by Zardi et al. (2007) would be relevant to the sites used for CI in the present study, as these sites are all within 150 km of Plettenberg Bay. The CI results indicate that, for *M. galloprovincialis,* at Keurboomstrand only, there was a difference between seasons and, although there was no replication of seasons in this study, the difference might be due to samples being collected in the build-up period before a spawning event. If this "build-up" was not visible to the naked eye, the mussel would still have been included in the analysis. The fact that *M. galloprovincialis* at Keurboomstrand showed such different responses to all other sites might also be a reflection of the general success of the species in the Plettenberg Bay region, where it has been shown to thrive in terms of abundance (von der Meden et al. 2008). It is possible that this increased abundance is linked to the differing spawning times for *P. perna* and *M. galloprovincialis* (Zardi et al. 2007) and this gives *M. galloprovincialis* an opportunity to dominate in this particular bay.

With regards to how barnacles attached to mussel shells influence the basibiont’s growth and health status, not a great amount of work has been done. Laihonen and Furman (1986) found that epibiotic barnacles had no effect on the growth rates of blue mussels (*Mytilus edulis*) in Finland. Other studies, not specifically focusing on epibiotic barnacles, found reduced growth in bivalves and snails that were infested with algal or artificial epibionts (Dittmann & Robles 1991, Wahl 1997). Various other factors may influence the condition indices of mussels and many authors have looked at the effects of parasites such as *Mytilicola* (Copepoda, Cyclopoidea) on the flesh weight of mussels (see examples in Dethlefsen 1975). Conflicting results may be due to the localities where the studies were carried out; where environmental conditions are highly favourable for mussels, the impact of parasitism may be compensated for and not substantial (Dethlefsen 1975). Another possible influence on mussel growth and condition is the level of the shore where the mussels live. Decreases in mussel reproductive and growth rates have been recorded for individuals living higher up the shore, most likely due to shorter feeding times, as feeding is only possible during submersion (Seed 1976, Suchanek 1981). Intraspecific competition may be another factor affecting growth and CI results. Intraspecific competition during the season of highest growth rates reduces growth in the ribbed mussel, *Geukensia*, where it occurs at high densities (Bertness & Grosholz 1985). Where mussel densities are high, smaller, juvenile mussels do not grow as much as larger ones (Kautsky 1982, Bertness & Grosholz 1985) and by including size as a covariate in the growth analyses, this effect was taken into account. Mussel size had a significant effect on *P. perna*, alone and when interacting with season, which may reflect the effects of density and intraspecific competition.

Surprisingly, the few significant correlations between CI and barnacle infestation indicated a positive relationship. So it would seem barnacles that settle on mussel shells do not increase drag and shift the energy balance of the mussel towards byssus production, as has been suggested in other studies (Steffani & Branch 2003). These positive correlations were limited to winter, occurring at Eersterivier, for both *P. perna* and *M. galloprovincialis*, and at St Francis Bay for *M. galloprovincialis* alone. This pattern in the winter season is surprising, considering that wave action generally increases during winter; however, other factors may have been important. For example, upwelling events, that bring nutrients up from colder, deeper waters offshore, result in increased productivity (Schumann et al. 1982) and, in turn, increased condition of mussel populations.

In some instances at the other sites, the data showed non-significant negative trends, leading one to believe that the effects of epibiosis are site-specific. In terms of growth, mussels were not significantly affected by barnacle infestation, although there was a trend of lower growth rates for infested mussels in summer. Growth rates in winter were, in fact, slightly higher for infested mussels, but this is also possibly influenced by site and other factors such as competition. In an effort to reduce variables, the growth experiment was run at one site (Shark Rock Pier in Algoa Bay) and tagged mussels were all at the same level on the rocky shore. This might explain the generally low growth rates and perhaps the differences between infested/non-infested and seasons. Sampling across various zones, and at multiple sites, might give further insight into the effects of barnacle epibiosis on growth. An additional consideration for CI might be to sample heavily infested mussels to explore possible thresholds at which the drag from the barnacles has an effect. Histological analyses of reproductive state could also supplement a study such as this on epibiosis. One further point is that a wide range of barnacle cover used in the CI analyses and in future narrower ranges could be used.

The physiological parameters, growth and condition give an indication of health. An organism that is under stress will show reduced fitness through these factors. Although not significant, growth for both *P. perna* and *M. galloprovincialis* was less for barnacle-infested individuals in summer, which is the main growing season for mussels. This may well be evidence of barnacles negatively effecting individual mussels, but results from CI calculations generally showed no negative impacts of epibiotic barnacles. It is possible, therefore, that there is no overall effect on the health of the infested mussels.

Chapter Six

General discussion

The possibility of fouling (epibiosis) in the sea is omnipresent and the list of fouled species is extensive (Wahl 1989). This associative state is particularly fundamental when a habitat has limited primary space for settlement (Dayton 1971). On the rocky shore, mussel beds can provide essential secondary space (Murdoch 1969, Dayton 1971, Paine 1979, Paine 1984, Lee & Ambrose 1989, Buschbaum 2000, Erlandsson et al. 2005). If a species is sessile and uses secondary space, it becomes entirely dependent on the well-being of the “host” (Dayton 1971). This close, interspecific relationship includes both direct and indirect interactions between the two parties (Wahl et al. 1997). Rocky shore communities are structured by the interactions between the physical and biological disturbances, including the competitive nature of the dominant species (Seed & O’Connor 1981). These interactions were assessed at various scales and the present study aimed to examine the interactions between mussels and barnacles, along with certain abiotic factors. From a wider perspective, temporal changes in the abundances of mussels and barnacles on the rocky shore were considered. This aspect of the study dealt with physical disturbance events and interactions between the two species. The abundances and direction of the interactions were fairly consistent over a 12-month period. This supports the assumptions made about interactions being constant in certain communities (Menge & Sutherland 1976). A “stable” environment does not necessarily mean an unchanging state, but rather one in which the scales and degree of environmental variation make the relationships in the system persist (Holling 1973). Useful generalisations can be generated from these persistent relationships, but we must consider that biological interactions, such as the degree of competition will vary temporally and/or spatially. These variations depend on a number of factors, including the levels of recruitment of the relevant species (Keough 1984), the external disturbances (Connell & Sousa 1983) and the mere nature of the species present (Dayton 1971).

It has been suggested that the relationship between mussels and barnacles is, in certain cases, competitive (Dittman & Robles 1991). Competitive effects are, however, lessened by the fact that barnacles frequently use mussel shells as additional substratum (Laihonen & Furman 1986), which means barnacles do not necessarily compete with mussels for primary space. The present study, however, did not address competition directly. When there is no other available space for settlement and barnacles make use of mussels as a secondary substratum, they face the risk of being reliant on the well-being of the basibiont mussel. The benefit of increased space for settlement must outweigh the risk. Epibiosis has been associated with trade-offs before, such as when increased drag is counterbalanced by the protection from predators for periwinkles (Wahl 1997). Barnacles, specifically, have been shown to make trade-offs with regards to the growth patterns of their larval stages (Lucas et al. 1979), adult shell shapes (Lively 1986) and the use of space in the habitat (Jackson 1977). The concept of compensation and possible trade-offs has been modelled using barnacles to explain community diversity and patterns of disturbance (Petraitis et al. 1989). In the present study, if barnacles are making a trade-off, it is not by choice, as the settlement study shows barnacle cyprids prefer rock-like substratum over mussels. It is possible, however, that the epibiotic relationship between mussels and barnacles plays an important role in shaping species assemblages on the rocky shore and may have wide-reaching implications for the intertidal community.

To gain a better understanding of the association between mussels and epibiotic barnacles, the prevalence and intensity of barnacle infestations were investigated. This resulted in a finer scale study of species distributions and the effects of various factors relating to the physiological adaptations of the relevant species. The factors taken into account included the vertical and horizontal zones of the intertidal (Colman 1933, Hewatt 1937, Doty 1946, Dayton 1971, Connell 1972), mussel species and season (Reaugh-Flower et al. 2011). The larger scale coastline topography also contributed to the variable nature of epibiosis. The effect of bay was significant when considering the intensity of barnacle infestations, in numerous interactions and results, and for both mussel species. In addition, *Perna perna* showed higher infestation intensities for smaller mussels in the mid zone at certain sites. *Mytilus galloprovincialis* showed positive trends between intensities of infestation and mussel size, in bays, for both seasons. At open coast sites there was a significant correlation of mussel size and infestation intensities in summer only for *M. galloprovincialis.* Other important effects on infestation intensities were seasonal changes and mussel size (under certain circumstances). There was an absence of strong trends when analysing the patterns of prevalence, but the incidence of barnacle infestations was generally higher in summer. The results show the complexity of factors that drive patterns of prevalence on the shore and the need for additional large to micro-scales studies in an ecological, as well as physiological, context.

Due to available substratum being limited on the rocky shore, it is important to consider any form of substratum preference shown by barnacles. It has been suggested that, in certain situations, at the millimetre scale, passive supply cannot explain the resulting recruitment patterns alone (e.g. Bourget et al. 1994, Pineda 1994, Gregoire et al. 1996, Hills & Thomason 1996), especially when considering that barnacle larvae can be highly selective when choosing settlement sites (Wethey 1986, Lemire & Bourget 1996). Results from the settlement experiment support this, with strong preference being shown by primary settlers for rock-like substratum. The topography of mussel periostracum seems to have anti-settlement properties (Bers et al. 2006). This was demonstrated in the experiment on patterns of initial settlement, where primary settlers appear to be avoiding "shell-like" micro-topography (replica treatment), first, and the possible chemical cues produced by the periostracum (comparison of live and dead mussel treatments). The low numbers of adult barnacle survivors on mussel shells, and the lack of their correlation with numbers of primary settlers, strengthen the argument that barnacles settle on mussel shells as a secondary choice and will primarily choose rock or alternative substratum, if available.

Despite barnacle cyprids avoiding mussel shells, the prevalence of epibiosis by barnacles was still relatively high and it is essential to develop an understanding of the physiological effects that the epibiont has on the individual mussel. Growth is a popular physical parameter to measure when assessing the health status of an organism and, in mussels, it is commonly measured as change in shell length (Seed 1976). Sustained growth is not possible without food (Seed & Richardson 1999) and food supply can be reduced by epiphytes (Paine & Suchanek 1983) and possibly other epibionts, too. Alternatively, epibionts can increase drag on mussels (Witman & Suchanek 1984) due to changes in the micro-hydrodynamics around the mussel (Buschbaum & Saier 2001). Mussel growth can also be enhanced by the presence of epibiotic barnacles (see Laihonen & Furman 1986), but this was not the case in the present study. The slower (but not significant) growth of mussels with epibiotic barnacles recorded in the present study may be an indication of barnacles disadvantaging their hosts.

Condition was also measured as an indication of the fitness of individual mussels, using a shell to living flesh ratio (Baird 1958). Although the reproductive state may have been a confounding factor in the results, the large sample size, and sampling in two seasons, allowed for the conclusion that condition index was not affected by barnacle epibionts. Seasonal influences at specific sites reflect the role that wave exposure may play in influencing the condition of mussels. Higher condition values have been recorded at exposed sites and the reason is likely to be increased food supply from the increase in water flow (Steffani & Branch 2003). Such variability in the condition of mussels could be addressed specifically in a laboratory experiment. Studying an interaction in nature, however, is far more realistic and can be used as a powerful tool by ecologists (Menge 1991). A further consideration is that organisms are usually physiologically adapted to the environmental stresses they have to face (Menge & Sutherland 1976). In the intertidal, severe physiological stress is caused by the merging of the marine environment into the terrestrial environment and intertidal organisms are subject to these challenging conditions (Dayton 1971). From the results of the present study it seems plausible that the added stress of having barnacle epibionts is not significant and mussels are not affected in terms of their growth or condition.

Interactions on the rocky shore are, undoubtedly driven by biological processes operating within a wide range of physical stressors and evidence for these driving forces has been provided by many authors (e.g. Connell 1961a, 1961b, Paine 1966, 1974, Dayton 1971, Menge 1978). The potential for biological interactions on the rocky shore is also high, due to many species coexisting in a structurally complex environment (Menge & Sutherland 1976). Competition, in various forms, increases diversity through increased habitat specialisation and mussel beds, in particular, support a diverse assemblage of associated invertebrates (Lintas & Seed 1994). When barnacles attach to mussel shells they create a new interface between the mussel and its environment (Laudien & Wahl 1999) and this interaction can, in turn, affect other members of the community, especially potential predators (Wahl et al. 1997, Laudien & Wahl 1999).

Epibiosis can result in a direct interaction between basibiont and epibiont. The consequences of epibiosis, however, can also be indirect and/or have slow, but cumulative effects for the entire population. One example of an indirect effect of the epibiont is the additional drag caused and the potential for increased mortality of the host organism (Dittman & Robles 1991, Buschbaum & Saier 2001). Any increase in mortality would be a concern for the species as a whole, since survival and reproduction are fundamental processes, both ecologically and evolutionarily (Pineda et al. 2009). Any increases in fitness for a species involved in an epibiotic relationship would also have important consequences for the entire population. This has been suggested for barnacles living on mussel shells, where the currents produced by the mussel’s siphons enhance the feeding abilities of the barnacles (Laihonen & Furman 1986). Although physical parameters of the barnacles were not measured in the present study, one can consider the additional space provided by mussel shells as a benefit. Barnacles opportunistically settle on mussel shells only as a secondary choice and mussels generally experience low infestation intensities. Patterns of prevalence and intensity of barnacle infestations, however, showed higher values in summer for both *M.*

galloprovincialis and *P. perna.* Seasonal changes interacting with the effect of bay were significant factors for prevalence and intensity of infestations. Ultimately, I found no significant, negative growth or health effects on mussels that had epibiotic barnacles, but the possibility that mussels experience increased drag and higher rates of dislodgement needs further investigation, particularly for the invasive species, *M. galloprovincialis*, as there may be sub-lethal effects such as decreased reproductive output.

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