A genetic and ecophysiological comparison of co-occurring indigenous (*Perna perna*) and invasive (*Mytilus galloprovincialis*) intertidal mussels

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By

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ABSTRACT

The Mediterranean mussel *Mytilus galloprovincialis* is the most successful marine invasive species in South Africa. Its presence has had significant ecological consequences on the intertidal communities of the west coast. On the south coast, *M. galloprovincialis* co-exists and competes with the indigenous intertidal mussel *Perna perna* in the lower balanoid zone, where they show partial habitat segregation. The upper and the lower mussel zones are dominated by *M. galloprovincialis* and *P. perna* respectively while they co-occur in the mid zone. In this thesis *M. galloprovincialis* and *P. perna* are compared in terms of their population genetics and their ecophysiology.

The success of an invader depends on its ability to react to new environmental factors, especially when compared to indigenous species. The distribution and diversity of intertidal species throughout the world are strongly influenced by periodic sand inundation and hydrodynamic stress. Occupying the lower intertidal zone, *P. perna* is more strongly influenced by sand (burial and sand in suspension) than *M. galloprovincialis*. Despite this, *P. perna* is more vulnerable to the effects of sand, showing higher mortality rates under experimental conditions in both the laboratory and the field. *M. galloprovincialis* has longer labial palps than *P. perna*, indicating a better ability to sort particles. This, and a higher tolerance to anoxia, explains its lower mortality rates when exposed to burial or suspended sand. Habitat segregation is often explained by physiological tolerances, but in this case, such explanations fail.

The ability of a mussel to withstand wave-generated hydrodynamic stress depends mainly on its byssal attachment strength. The higher attachment strength of *P. perna* compared to *M. galloprovincialis* and of solitary mussels compared to mussels living within a bed (bed mussels) can be explained by more and thicker byssal threads. *M. galloprovincialis* also has a wider shell, is subjected to higher hydrodynamic loads than *P. perna* and shows a higher theoretical probability of dislodgement, this is borne out under field conditions. The attachment strength of both species increased from higher to lower shore, in parallel to a gradient of a stronger wave action. Monthly measurements showed that *P. perna* is always more strongly attached than *M. galloprovincialis* and revealed seasonal fluctuations of attachment strength for both species in response to wave height. The gonad index of both species was negatively cross-correlated with attachment strength. The results are discussed in the context of the evolutionary strategy of the alien mussel, which directs most of its energy to fast growth and high reproductive output, apparently at the cost of reduced attachment strength. This raises the prediction that its invasive impact will be more pronounced at sites subjected to low or moderate wave action at heavily exposed sites.

The potential of a species for invasion is also determined by the ability of the invader to disperse. Population genetics provide indirect information about dispersal through a direct measurement of gene flow. The low genetic divergence (measured as mtDNA) of *M. galloprovincialis* confirms its recent arrival in South Africa. In contrast, the population genetics structure of P. perna revealed strong divergence on the south-east coast, resulting in a western lineage (straddling the distributional gap of the Benguela System), and an eastern lineage, with an overlap region of the two on the south coast between Kenton-on-Sea and Haga Haga. This genetic disjunction may be caused by Agulhas Current acting as an oceanographic barrier to larval dispersal, or by different environmental selective forces acting on regional populations. Over the last ten years, *M. galloprovincialis* has shown a decrease or cessation of its spread to the east in exactly the

region of the genetic disjunction in *P. perna*, again suggesting either an oceanographic barrier to larval dispersal, or increasing selection driven by sharp gradients in environmental conditions.

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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

One of the major ecological consequences of globalization is a sharp increase in the number of invasive species being introduced to ecosystems and the frequency with which such introductions are made (Jenkins 1996; McNeely 2001; Levine and D'Antonio 2003). The introduction of new species is related to the development of new markets and trade routes (Cassey et al. 2004; Semmens et al. 2004) and a positive correlation has been shown between the relative abundances of invasive species in different countries and the volume and composition of imports (Dalmazzone 2000; Vilà and Pujadas 2001; Levine and D'Antonio 2003). Introduced species can themselves be an object of trade or can travel incidentally together with traded goods. Most importantly, invasions involve not only species but also their genes and, through hybridization and subsequent introgression, endemic species might be altered by invasive relatives (Hails and Morley 2005).

With the global rise in volume and speed of transoceanic shipping over the last century, coastal regions of the world have been particularly subjected to increasing pressure from alien species (Carlton 1987, 1996, 1999a; Carlton and Geller 1993; Ruiz et al. 1997; Cohen and Carlton 1998). There are ten confirmed alien and 22 cryptogenic marine species along the South African coast. Of these, only the mussel *Mytilus galloprovincialis* has spread extensively, while the other species are restricted to harbours, sheltered lagoons and estuaries (Branch and Steffani 2004; Robinson et al. 2005). *M. galloprovincialis* is thought to be of Mediterranean origin (Barsotti and Meluzzi 1968; Wilkins et al. 1983; Steward and England 1983) and it has become invasive in many parts of the world, including South Africa (Grant and Cherry 1985), Hong Kong (Lee and Morton 1985), Japan (Wilkins et al. 1983), Korea (McDonald et al. 1991), Hawaii (Apte et al. 2000), Mexico (Ramirez and Càceros-Martìnez 1999), California, Washington, and the west coast of Canada (McDonald and Koehn 1988; Heath et al. 1995; Anderson et al. 2002), perhaps the east coast of Canada (Vario et al. 1988), Britain and Ireland (Ahmad and Beardsmore 1976; Skibinski et al. 1978). It now has an antitropical distribution, occurring in the temperate zones of the northern and southern hemispheres while absent from the tropics (Hilbish et al. 2000).

M. galloprovincialis was first recorded in South Africa in 1979 at Saldanha Bay, 150 km north of Cape Town on the west coast, and its identity was later confirmed by morphological and protein electrophoresis analysis (Grant et al. 1984; Grant and Cherry 1985). There are two possible hypotheses that may explain the presence of the Mediterranean mussel in South Africa. The first is that *M. galloprovincialis* may have dispersed along the west coast of Africa, across the tropics, during one of the Pleistocene glacial periods. Both the Mediterranean populations and the southern African populations of *M. galloprovincialis* would then represent relict populations of a wider African distribution. A second possibility is that it was intentionally or inadvertently introduced to South Africa by man. Analysis of mussel shells from shell middens and raised beach deposits, dating from as long ago as 120 000 years ago (last interglacial period), did not show any evidence of *Mytilus* (Grant and Cherry 1985). Moreover, when it was first recorded in Saldanha Bay, it was not present in Cape Town (Grant and Cherry 1985). It is therefore likely that the appearance of *M. galloprovincialis* in southern Africa is a recent event. Extensive surveys of southern African intertidal shores from the 1930s to the 1960s did not reveal the presence of *Mytilus* (Stephenson 1944; Kensley and Penrith 1970; Penrith and Kensley 1970). In addition *Mytilus* is absent from the collections at the Zoology Department of the University of Cape Town (Grant and Cherry 1985). All this suggests that the European species arrived in South Africa in the late 1960s, early 1970s, most probably by shipping, or was intentionally introduced without record (Grant and Cherry 1985). Soon after its first detection, it started to spread to the north at an average rate of 115km year^{-1}, and to the south, at a slower rate of about 25km year^{-1} (Hockey and van Erkom Schurink 1992). It is now the dominant intertidal mussel from the Cape of Good Hope in South Africa to Lüderitz in southern Namibia (Fig. 1.1), constituting 74% of the mussel biomass on wave-exposed shores on this coast (Hockey and van Erkom Schurink 1992, Griffiths et al. 1992; Branch and Steffani 2004).

Prior to 1989, *M. galloprovincialis* was absent from the south coast, but was intentionally introduced to Port Elizabeth (Fig. 1.1) for mariculture purposes. *M. galloprovincialis* spread eastwards from Port Elizabeth at a rate of about 5km year^{-1}, driven by wind generated surface current (McQuaid and Phillips 2000; Branch and Steffani 2004). However, the Port Elizabeth population was removed after approximately 18 months and the daughter populations died out. *M. galloprovincialis* has subsequently invaded much of the south coast, spreading naturally from the Cape of Good Hope eastwards to within 20 km west of East London at Kidd's Beach (Fig. 1.1; Robinson et al. 2005). In the last ten years its rate of expansion on the south coast has drastically decreased, suggesting that it may have reached its biogeographic limit. In South Africa, *M. galloprovincialis* represented only 1% of overall mussel standing stock on the south coast in 1992 (Griffiths et al. 1992). Recent surveys show that it still occurs at generally low densities, but it has become abundant at certain sites such as Plettenberg Bay and Jeffreys Bay where it can form 50% of mussel standing stocks (Fig. 1.1; Rius 2005).

The southern African coastline covers a wide range of climatic and oceanic conditions and hence supports a great biodiversity. The west coast is affected by the Benguela Current and is characterized by intense upwelling and high nutrient levels (Shannon 1985). The east and south coasts are influenced by the warmer, more oligotrophic Agulhas Current (Bustamante et al. 1995). These coasts support different intertidal biotas (Emanuel et al. 1992) and historically different species dominated their mussel populations.

There are three abundant indigenous intertidal mussel species in South Africa: 1. *Aulacomya ater*, which overlaps in intertidal habitat with *M. galloprovincialis* and was the dominant west coast mussel before being displaced by the Mediterranean mussel (van Erkom Schurink and Griffiths 1990); 2. *Choromytilus meridionalis* is primarily a west coast species, but extends onto the south coast. It is most abundant at sand/rock interfaces, thus it is little affected by the European mussel, which lives in habitats with lower levels of siltation. 3. *Perna perna* is the dominant intertidal mussel species along the south and east coasts of South Africa and indeed most of Africa (both east and west coasts).

This thesis focuses on *M. galloprovincialis* and *P. perna*. On the south coast, *P. perna* shows partial habitat segregation with *M. galloprovincialis* in the lower eulittoral zone (referred to here as the mussel zone). The upper and the lower areas of the mussel zone are dominated by *M. galloprovincialis* and *P. perna* respectively, while they co-occur in the mid-mussel zone (Bownes 2005). *M. galloprovincialis* shows similar zonation with other *Perna* species in other parts of the world. *M. galloprovincialis* occupies the high intertidal zone in Hong Kong where the green mussel *Perna viridis* dominates the lower intertidal (Lee and Morton 1985), and the same situation is found with *Perna canaliculus* in New Zealand (Kennedy 1976). The cold waters of the Benguela Upwelling System limit *P. perna* distribution north of the Cape of Good Hope and it is absent from there to Lüderitz Bay (Fig. 1.1) in southern Namibia. From there it extends to the Mediterranean Sea as far as the Gulf of Tunis (van Erkom Schurink and Griffiths 1990). On the west coast, where *P. perna* is absent, *M. galloprovincialis* extends its distribution down the shore to the sublittoral fringe (Bustamante et al. 1998; Branch and Steffani 2004). *P. perna* is indigenous elsewhere in the south Atlantic, including the coasts of Brazil, Uruguay, Venezuela, and the west Indies (Berry 1978) and has become invasive in the Gulf of Mexico (Hicks and Tunnell 1993). *P. perna* is also indigenous in the Indian Ocean, including southern India, Sri Lanka and Madagascar (Berry 1978).

The invasion of the west coast of South Africa by *M. galloprovincialis* affected not only the indigenous mussel *A. ater,* but had also major consequences for overall intertidal community structure, including the elimination of other indigenous species, with strong effects on mussel bed infaunal assemblages and higher trophic levels (van Erkom Schurink and Griffiths 1990; Hockey and van Erkom Schurink 1992; Hammond and Griffiths 2004). Many associated species can live within a mussel bed (Brown and Seed 1977; Suchanek 1985; Suchanek 1992; Seed 1996) and on the west coast *M. galloprovincialis* has extended the cover of mussel beds higher on the shore than the indigenous mussel, *A. ater*, which it replaced. Unlike *A. ater*, *M. galloprovincialis* is able to form multi-layered mussel beds, and consequently it has increased total mussel biomass, the availability of habitat for mussel bed infauna and the abundance of associated infauna (Griffiths et al. 1992; Hockey and van Erkom Schurink 1992;

Hammond and Griffiths 2004). Infaunal assemblages of the Mediterranean mussel are quantitatively larger than those of *A. ater*, but the two are qualitatively similar (Griffiths et al. 1992). The infauna biotas associated with *C. meridionalis*, *P. perna* and *M. galloprovincialis* differ in composition, but not abundance, biomass or diversity (Hammond and Griffiths submitted for publication, see Branch and Steffani 2004). Infaunal communities also do not differ when comparing beds of mussels of different sizes or at different heights on the shore. It has been suggested that this absence of variation is due to uniform environmental conditions inside mussel beds in general (Branch and Steffani 2004).

Two limpets have also been significantly affected by the invasion of the west coast by *M. galloprovincialis*: *Scutellastra granularis* (Branch and Steffani 2004), and *S. argenvillei* (Steffani and Branch 2003a, b). *M. galloprovincialis* excludes *S. granularis* from the primary substratum, but, at the same time, the mussel itself provides a favourable substratum for juveniles of this species, consequently increasing its density but decreasing its mean size (Hockey and van Erkom Schurink 1992). Wave generated hydrodynamic stress regulates the interaction between the Mediterranean mussel and *S. argenvillei*, with the former dominating primary substratum on exposed shores and the latter dominating semi-exposed intertidal rocks. Beside these harmful ecological effects, *M. galloprovincialis* has brought benefits to the African Black Oystercatcher (*Haematopus moquini*), becoming a major component of its diet and increasing its breeding success (Hockey and van Erkom Shurink 1992). It is also economically important, as the mussel culture industry in South Africa is based on the Mediterranean mussel.

Invasion involves two essential stages: transport of an organism to a new location (Williamson 1996; Mack et al. 2000), and establishment and population growth in the invaded locality (Veltman et al. 1996). The responses of an introduced species to variations in time and space of resource availability, natural enemies and the physical environment determine its ability to invade and the dynamics of co-existence with indigenous species (Moyle and Light 1996; Petren and Case 1996; Sher and Hyatt 1999; Davis et al. 2000; Mack et al. 2000). It is the difference in response of indigenous and invasive species to these factors that determines the success of the invader (Chesson 2000; Shea and Chesson 2002). *M. galloprovincialis* shows several characteristics of an aggressive invasive species. When compared to the indigenous South African mussel species *Perna perna, Choromytilus meridionalis* and *Aulacomya ater*, it shows rapid growth rates over a range of water temperatures, high fecundity, high recruitment rate, resistance to desiccation and resistance to parasites (Hockey and van Erkom Schurink 1992; van Erkom Schurink and Griffiths 1991, 1993; Calvo-Ugarteburu and McQuaid 1998). These features should provide competitive advantages to *M. galloprovincialis* and could explain its success as an invader on the South African coast.

Another important factor influencing the dynamics of an invasion is the ability of the invader to disperse, thus spreading from the initial population and extending its domain. The dispersal potential of seeds, spores and larvae has long been recognized for its importance in influencing large-scale patterns of distribution and geographic ranges of sedentary organisms (Perron and Kohn 1985; Richmond 1987; Scheltema 1989; Gaines and Bertness 1992; Emlet 1995). Marine species possessing pelagic larvae have the potential for considerable levels of connectivity among local populations. Mediated by larval dispersal, this connectivity is potentially of considerable importance because it determines the magnitudes of immigration and emigration (Sale 1991; Caley et al. 1996). By moving from one population to another, larvae also transfer their genes, generating gene flow (gene migration) among populations. Gene flow (via larval dispersal) can provide information on the degree of exchange (connectivity) among populations. Migration into or out of a population may be responsible for a marked change in gene pool frequencies (the number of individual members with a particular trait). Immigration may result in the addition of new genetic material to the established gene pool of a particular species or population. Understanding the magnitude and patterns of larval dispersal is critical to understanding questions of population persistence and community dynamics and is also a key element for effective placement of management boundaries and the design of marine protected area networks (Sale 1991; Caley et al. 1996; Sale and Kritzer 2003).

Both *M. galloprovincialis* and *P. perna* reproduce by means of a planktotrophic larval stage that disperses in the water column for a period (weeks to months) of feeding, growth and morphological development, before settling to the substratum and being recruited into an adult population. Given the relatively long period that larvae spend in the water column, both species have a high dispersal potential.

However, several studies have shown discrepancies between the potential and the realized dispersal of larvae (Palumbi 1994; Todd 1998). These discrepancies are mainly due to the influence on larval behaviour of oceanographic conditions, to reproductive timing, and to the availability of suitable habitat in terms of both biotic and abiotic factors (Shanks 1995; Todd 1998; Shanks 2005). Direct measurement of dispersal is difficult because microscopic planktonic larvae are difficult to track and because dispersal can be regulated by multiple complex processes (Cowen et al. 2000; Gilg and Hilbish 2003). Genetic variation among populations of a species can be used as an indirect approach to circumvent the problems of the direct estimation of gene flow via larval dispersal (Palumbi 2003). Knowledge of dispersal capabilities and extent of gene flow in contemporary species may aid in the identification of biogeographic barriers or filters to dispersal that act on evolutionary time scales. The population genetic structure of the indigenous *P. perna* gives indirect information about larval dispersal in time and space, and about dispersal barriers that may affect dispersal. In addition, the genetic structure of *P. perna* populations from the East coast will be useful in predicting patterns in the eastward domain expansion of the invasive *M. galloprovincialis*. In addition, understanding the genetics of *M. galloprovincialis* can help to confirm the origins through either vacariance or dispersal, of South African populations, by contrasting the genetics of *M. galloprovincialis* with a known indigenous species (*P. perna*) with similar reproductive biology.

This thesis is divided into four main chapters, plus the introductory Chapter 1.

Chapter 2 discusses sand stress as a possible cause of habitat segregation between the two mussels species. Chapter 3 deals with wave-induced hydrodynamic stress as a possible factor responsible for habitat segregation and investigates causes and consequences of differences in attachment strength between *M. galloprovincialis* and *P. perna*. Chapter 4 examines the population genetic structure of both species along the Namibian and South African coasts. Finally, Chapter 5 concludes the thesis with a general discussion.

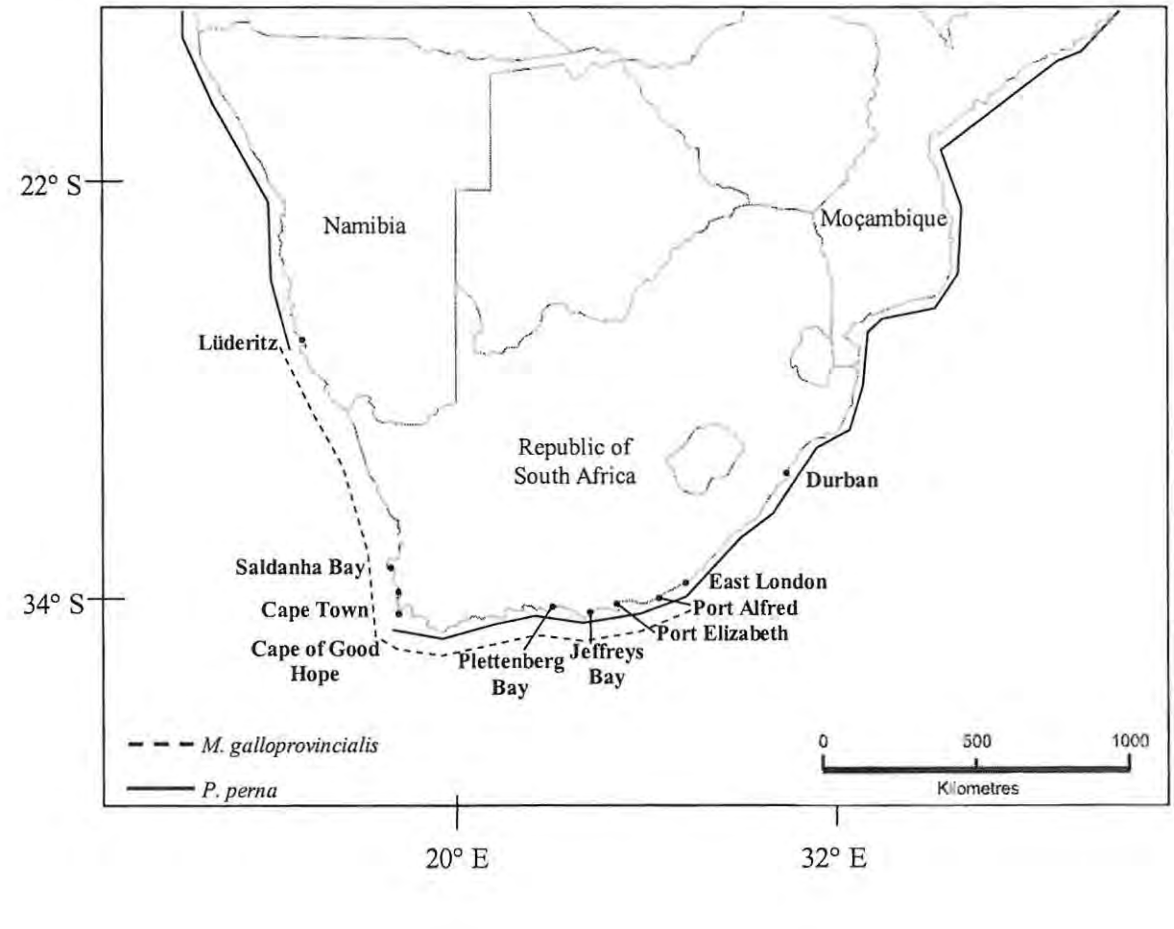

Fig. 1.1 The distribution of *Perna perna* and *Mytilus galloprovincialis* along the southern African coast

CHAPTER 2

SAND STRESS AS A NON-DETERMINANT FACTOR IN HABITAT SEGREGATION BETWEEN *Perna perna* AND *Mytilus galloprovincialis* IN SOUTH AFRICA

Introduction

Rocky shores throughout the world are periodically disturbed by sand inundation or sand scour. In South Africa, rocky shores that are regularly and extensively inundated by sand are more common than non-inundated rocky shores (31% and 27% of the coastline respectively; Bally et al. 1984). Despite this, few studies have investigated such ecosystems. Most recent studies have examined the effects of sand on the survival or lifestyle of single species (D'Antonio 1986; Marshall and McQuaid 1989; Pineda and Escofet 1989), or have analysed the effects of sand inundation on species richness and composition (Bally et al. 1984; McQuaid and Dower 1990). Sand can maintain a balance between sand tolerant and sand intolerant competitors (Taylor and Littler 1982; Littler et al. 1983), it can cause a temporary impoverishment of the biota by selective species elimination (Daly and Mathieson 1977; Devinny and Volse 1978), and it can explain the habitat segregation of intertidal species (Marshall and McQuaid 1989).

Mussels can be subjected to stress generated by sand either through sand burial or because the ambient water carries a heavy load of suspended sand (Fig. 2.1). They sort filtered material using two pairs of labial palps (Ward et al. 1998) and at very high particle concentrations, the filtration rate can be reduced to zero (Widdows et al. 1979; Richardson 1985; Leverone 1995). Thus, the ability to regulate filter-rejecting mechanisms during sudden changes in sediment load concentration can be a prerequisite for survival. In sandy beaches, there is a very rapid decline in oxygen deeper in the sediments (Pearse et al. 1942; Brafield 1964), consequently, when buried under sand, mussels are not only subjected to the negative effects of sand itself, but also to anoxia. In response to periods of depleted oxygen, mussels reduce the valve gape or close the valves

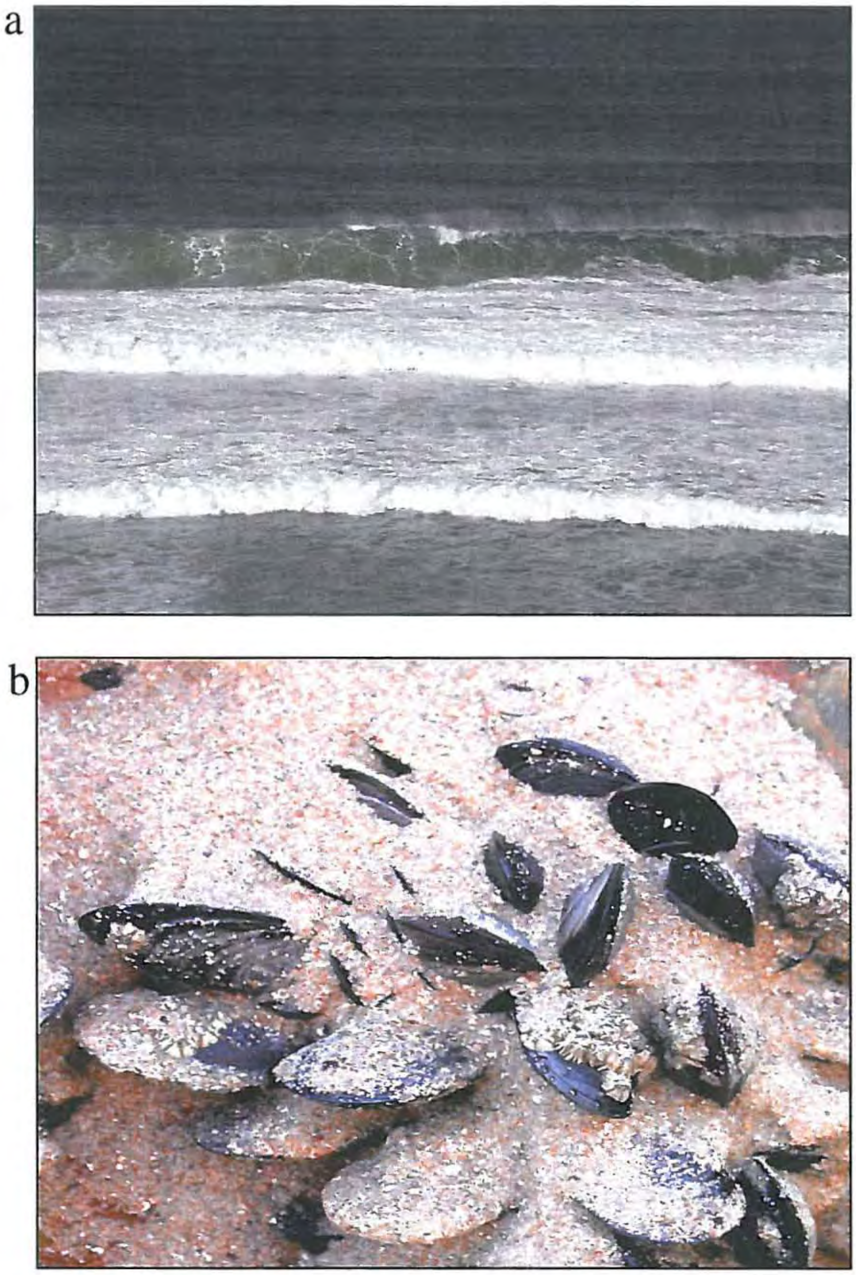

Fig. 2.1 Waves not only subject intertidal organisms to hydrodynamic stress, they can also carry a heavy load of suspended sand (a), note brown colour of the wave, subjecting intertidal communities to sand scour and sand burial (b).

(Jørgensen 1990), and switch from aerobic to anaerobic respiration (Taylor 1976), but anaerobic acidic end products can accumulate to lethal levels (Newell 1970). The south coast of South Africa is characterised by patches of rocky shore interspersed with long stretches of sandy beach. The majority of rocky shores are influenced by sand (Bally et al. 1984) and intertidal organisms living on hard substrata are consequently considerably sand-influenced. Physical disturbances by sand may play an important role in the invasion biology of *Mytilus galloprovincialis* and could influence the interaction between the invasive and the indigenous mussel species. This chapter investigates the effects of sand stress on the survival of *M. galloprovincialis* and *Perna perna*. The hypothesis is that, because sand accumulates from the subtidal and has stronger effects low on the shore, sand scour and burial contribute to habitat segregation (vertical zonation) of the two species. Understanding how the two species behave when subjected to environmental stress helps to predict future interactions and the ecological effects of the invader. Specifically, I tested the hypothesis that *P. perna* has greater tolerance to the effects of sand than *M. galloprovincialis*, so that *M. galloprovincialis* is excluded from the lower shore at least partly through the effects of sand inundation and sand scour. Mortality rates of mussels subjected to sand burial and to sand in suspension were observed in field and laboratory experiments. In order to investigate the possible causes of mortality under sediment load stress, the two species were also subjected to anoxic conditions and the findings were set in the context of field measurements of seasonal fluctuations in sand levels near mussel beds.

Materials and Methods

Laboratory experiments

For all laboratory experiments, adult mussels (3-5cm shell length) of both species were collected from the mid mussel zone of a rocky shore at Plettenberg Bay on the south coast of South Africa (Fig.2.2; 34° 22' S, 23° 22' E). Before each laboratory experiment all individuals were acclimated in re-circulated oxygenated seawater for 24 hours. All experiments were run in a controlled environment chamber at 19°C under a 12:12h light: dark regime (high output fluorescent light). Salinity was maintained at 35‰. Experimental aquaria were aerated and the water was replaced daily. Mussel mortality was assessed as failure to close the valves when disturbed. Mortality rates were calculated as means of three aquaria for each treatment and analysed using repeated measures ANOVA with treatment as a fixed factor.

Sand burial and suspended sand

Mussels (n = 360 for each species) were placed in nylon net bags (6mm mesh) containing 10 mussels each, and subjected to three different sand load conditions in aquaria, using sand collected from the field:

1) Sand in suspension: $12g.l^{-1}$ of fine sand. This concentration was based on the average load of resuspended sand measured in the sea at high tide near the study mussel bed.

2) Buried: covered with sand to a depth of 10-15cm.

3) Control: aerated seawater, no sand.

Each experimental condition was replicated three times (three aquaria). In order to keep sand in suspension in treatment 1, compressed air was pumped through perforated tubes placed on the floor of the aquaria. For treatment 2, air was pumped through the water above the level of the sand. Dead mussels were counted and removed daily.

Anoxia

For each species, 15 individuals were placed in each of three aquaria containing 5l of anoxic sea water. Anoxic conditions ($0.01\text{-}0.03\text{ml.O}_2.\text{l}^{-1}$) were prepared by bubbling Instrumental Grade N_2 gas into the aquaria, and then sealing them. Anoxic seawater was replaced daily and oxygen tension was checked every 12 hours and readjusted if necessary. Dead mussels were counted and removed daily.

Labial palp length

A dissecting microscope was used to measure the lengths of the labial palps of 20 mussels (25-67mm shell length) of each species to the nearest 0.1mm. Regression analysis was used to test the dependence of labial palp length on animal shell length for each species and a Students't test was used to test equality of the two regression coefficients.

Sand content

M. galloprovincialis and *P. perna* (n = 60 for each) were placed in 6 nylon net bags (10 individuals per bag). Twenty mussels of each species were placed in each of three aquaria. The bags were then buried under sand to a depth of 10-15cm. The experiment was run for 72 hours and mortality was checked daily. Every 24 hours, five live individuals were taken from each bag. Sand content of the animals was removed by opening the mussels and carefully washing the tissues. The water used to rinse the internal parts of the animal was drawn through a glass fibre filter, which was then dried at 60°C to constant weight. After the first day, the shell volume of each animal was measured by comparing the weight of NaCl required to fill one valve, to the weight of a known volume of NaCl.

Field experiments

Sand burial

Adult mussels of both species (3-5cm shell length) were collected in November 2004 in the intertidal zone at St. Francis Bay (Fig. 2.2; 34° 13' S, 24° 49' E), about 140km west of Plettenberg Bay. Like Plettenberg Bay, St. Francis Bay is one of a series of "half-heart", or log-spiral shaped bays found on the south coast, facing the south-west Indian Ocean (Fig. 2.2; Branch and Branch 1981, Wooldridge 1988). Three experimental sites, approximately 100m apart, were selected in sand covered areas adjacent to rocks with mussel populations and at approximately the same vertical height as the mussel zone. Mussels ($n = 240$ for each species) were immediately placed in nylon net bags and buried *in situ* under 30-40cm of sand. Each bag contained 20 individuals of the same species; bags were equally distributed among sites. Bags were attached by fishing line to nearby rocks, so that they could be found easily. A table tennis ball was left beside each mussel bag; its presence at the next low tide confirmed that the samples had not been exhumed during high tide. Every day, bags were recovered and the mortality of each species was checked. Dead animals were removed. Cumulative mortality rates were calculated as means for each site and analysed using repeated measurements ANOVA.

Seasonality of sand level fluctuation

Digital photographs of mussel beds populating vertical rocks were taken every month at two intertidal sites (Keurbooms and Robberg, 10km apart) in Plettenberg Bay for a period of 18 months. *M. galloprovincialis* and *P. perna* zones were defined as the areas of the mussel bed that were covered by at least 90% (± 5%) of one of the two mussel species. The mid-mussel zone was the area of co-existence between the *M. galloprovincialis* and *P. perna* zones. The lowest level of sand recorded at each site during the monitoring period was taken to be the reference level from which all other levels were measured. Percentage cover of mussels was determined by analysing digital images of 3m wide transects running c. 15m horizontally in each zone at each site.

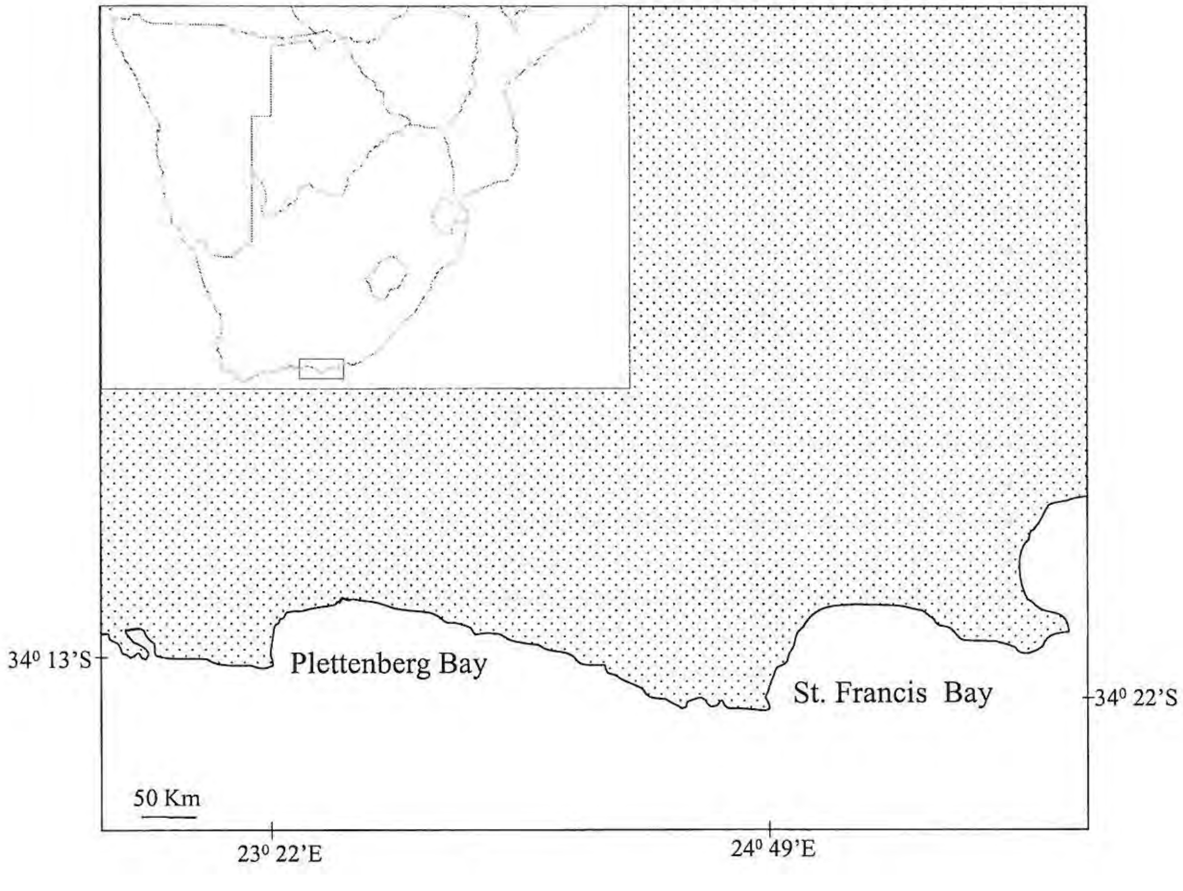

Fig. 2.2 Location of study areas

Results

Laboratory experiments

Mortality rates under conditions of sand burial, sand suspension and anoxia.

Repeated measures ANOVA showed that mortality rates were higher for *Perna perna* than for *Mytilus galloprovincialis* under all sand stress and anoxic treatments ($p < 0.0001$ in all cases; Tables 2.1, 2.2, 2.3). During all experiments, under control conditions of sand free, oxygenated water, *P. perna* suffered 2.5% mortality compared to 4.2% for *M. galloprovincialis*. When subjected to sand burial in the laboratory, mortality for both species began after two days (12.5% and 4.2% for *P. perna* and *M. galloprovincialis* respectively), but there was a significant day x species interaction. Lethal time for 50% mortality (L_T 50) was reached after four days for *P. perna* and six days for *M. galloprovincialis* (Fig. 2.3). When subjected to sand in suspension, mortality was much lower for both species. Again there was a day x species interaction. Mortality for *P. perna* started after five days (0.8%), while the first dead *M. galloprovincialis* were found only after nine days (1.7%). L_T 50 was reached after 10 days and 14 days for *P. perna* and *M. galloprovincialis* respectively (Fig. 2.4). Under anoxic conditions, mortality for both species began later than when buried under sand (after four days, 2.2% for *M. galloprovincialis* and 4.4% for *P. perna*). L_T 50 was reached after 9 days and 12 days for *P. perna* and *M. galloprovincialis* respectively giving a day x species interaction (Fig. 2.5).

	df	MS	F	p
Species	1	206.255	110.720	<0.0001
Aquarium	2	0.161	0.087	0.9173
Species*Aquarium	2	3.193	1.714	0.2082
Error	18	1.863		
Day	7	315.916	661.013	<0.0001
Day*Species	7	8.779	18.369	<0.0001
Day*Aquarium	14	0.501	1.048	0.4114
Day*Species*Aquarium	14	0.449	0.939	0.5197
Error	126	0.478		

Table 2.1 RM-ANOVA for sand burial laboratory experiment. df = degree of freedom; MS = Mean Squares; F = F–Ratio; P = probability value.

	df	MS	F	p
Species	1	599.725	677.631	<0.0001
Aquarium	2	0.572	0.646	0.5358
Species*Aquarium	2	0.211	0.238	0.7906
Error	18	0.885		
Day	17	392.192	1440.369	<0.0001
Day*Species	17	52.656	193.385	<0.0001
Day*Aquarium	34	0.528	1.938	<0.0019
Day*Species*Aquarium	34	0.348	1.278	0.1454
Error	306	0.272		

Table 2.2 RM-ANOVA for suspended sand laboratory experiment. df = degree of freedom; MS = Mean Squares; F = F–Ratio; P = probability value.

	df	MS	F	p
Species	1	2372.84	19.0297	0.0120
Error	4	124.69		
Day	11	3509.54	172.7624	<0.0001
Day*Species	11	131.76	6.4862	<0.0001
Error	44	20.31		

Table 2.3 RM-ANOVA for anoxia laboratory experiment. df = degree of freedom; MS = Mean Squares; F = F–Ratio; P = probability value.

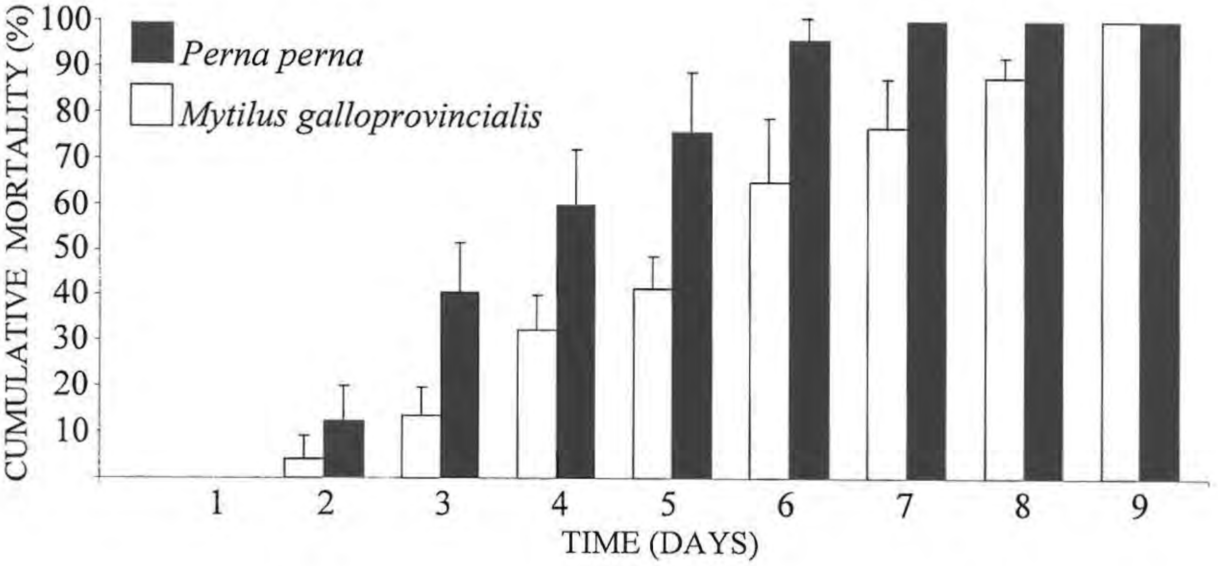

Fig. 2.3 Mean cumulative percentage mortality for three aquaria (+ SD) of *P. perna* and *M. galloprovincialis* when exposed to sand burial in the laboratory.

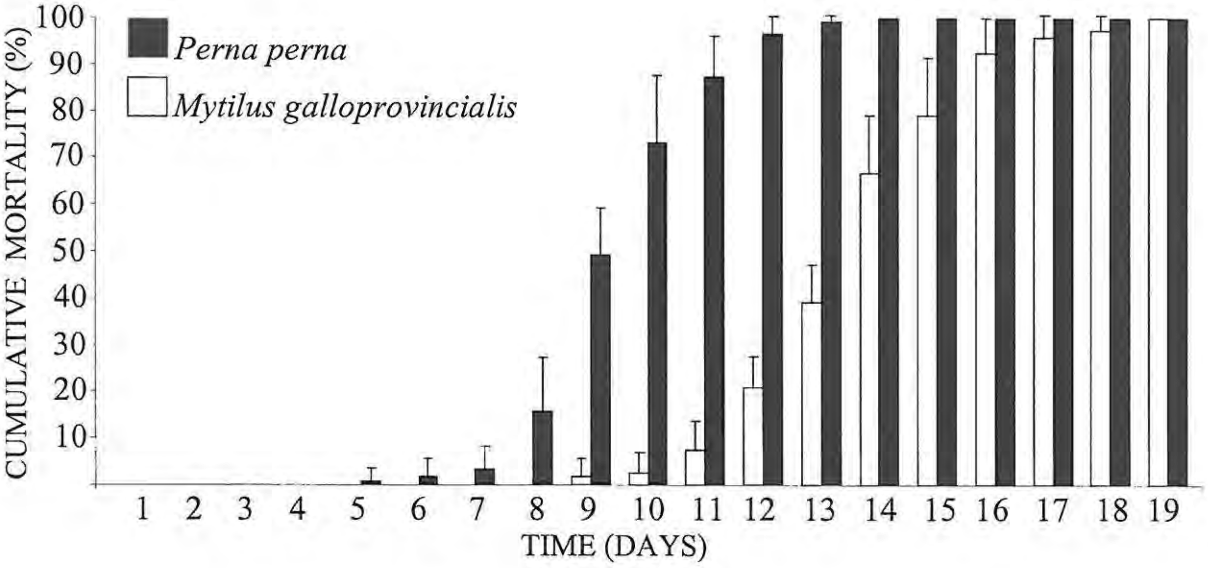

Fig. 2.4 Mean cumulative percentage mortality for three aquaria (+ SD) of *P. perna* and *M. galloprovincialis* when exposed to sand in suspension (12 g.l^{-1}) in the laboratory.

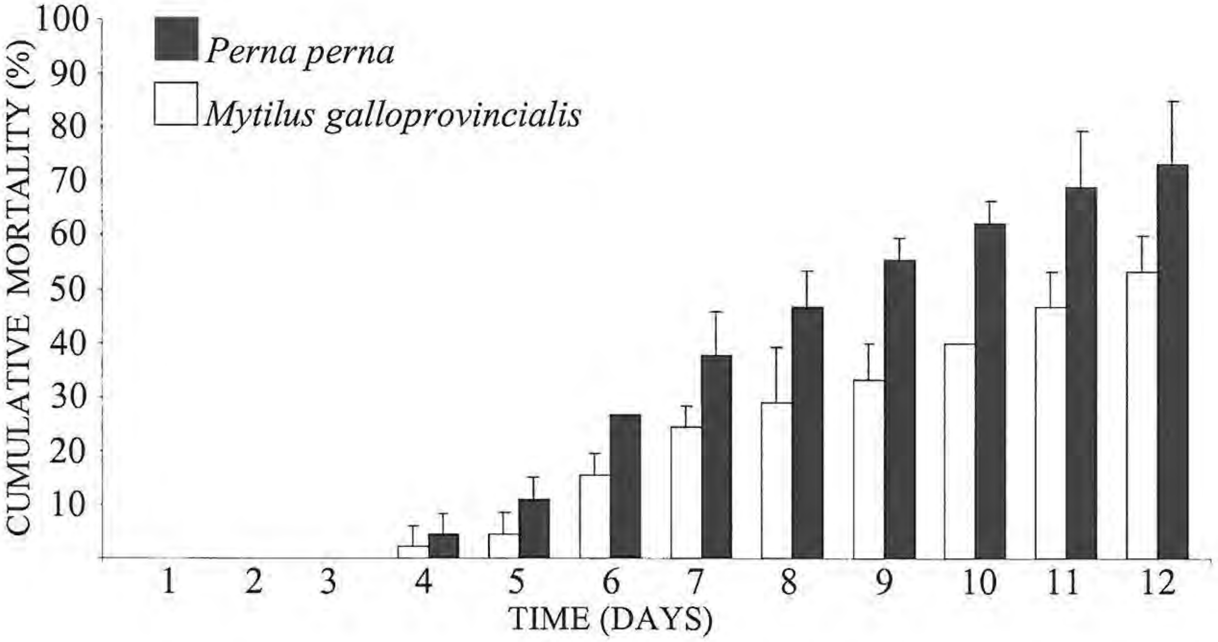

Fig. 2.5 Mean cumulative percentage mortality for three aquaria (+ SD) of *P. perna* and *M. galloprovincialis* when exposed to anoxic conditions in the laboratory.

Labial palp length

Labial palp length increased with animal size for both species (regression analysis, $p < 0.001$ in both cases). *M. galloprovincialis* had significantly (Students't test, $p < 0.0001$) longer labial palps than *P. perna* over the whole size range, averaging 28.3% longer overall (Fig. 2.6).

Sand content

Correlation analysis showed no relation between volume of an animal and sand content. Subsequently a two-way model 1 ANOVA was used to analyse the data, with species and day as factors. Two-way ANOVA showed that the amount of sand present in live mussels buried under sand was significantly ($p < 0.0001$; Table 2.4) higher for *P. perna* than for *M. galloprovincialis*, with no significant time x species interaction. Within the first 24 hours, 47.8mg and 8.4mg of sand were found in *P. perna* and *M. galloprovincialis* respectively. The amount of sand increased slightly during the following days, up to 98.8mg for *P. perna* and 16.9mg for *M. galloprovincialis* (Fig. 2.7), though the effect of time was just non-significant ($p = 0.055$).

	df	MS	F	p
Day	2	6936.9	2.99993	0.0551
Species	1	73159.5	31.63843	<0.0001
Day*Species	2	3684.4	1.59337	0.2093
Error	84	2312.4		

Table 2.4 Two-way ANOVA for sand content laboratory experiment. df = degree of freedom; MS = Mean Squares; F = F–Ratio; P = probability value.

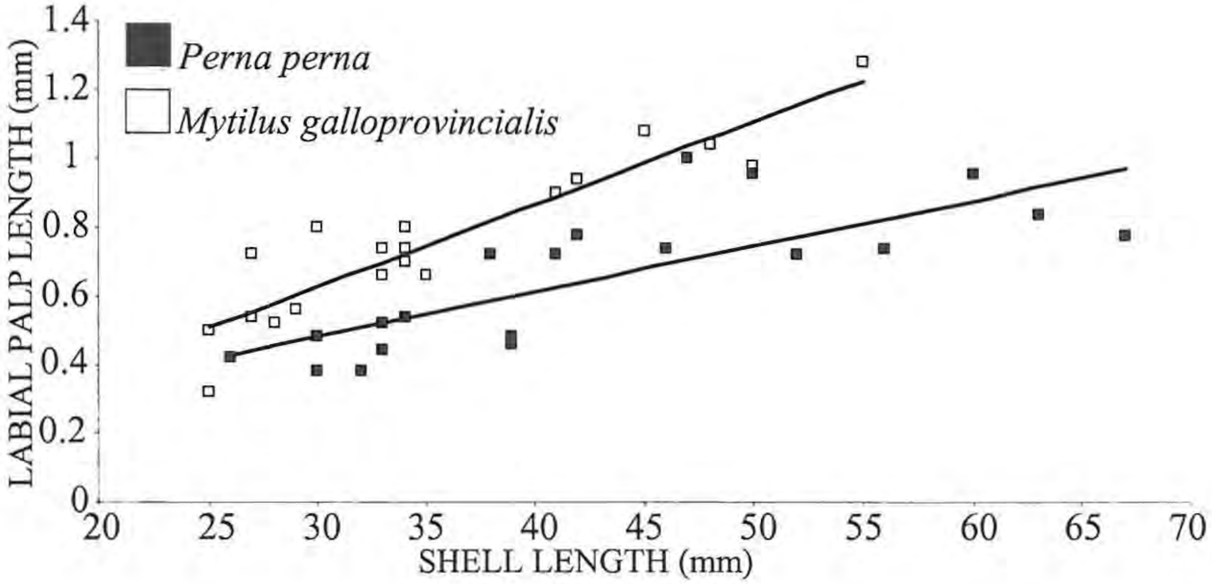

Fig. 2.6 Relationship between shell length and labial palp length for *P. perna* ($y = 0.0133x + 0.0824$; $r^2 = 0.6071$) and *M. galloprovincialis* ($y = 0.0241x - 0.0948$; $r^2 = 0.8607$).

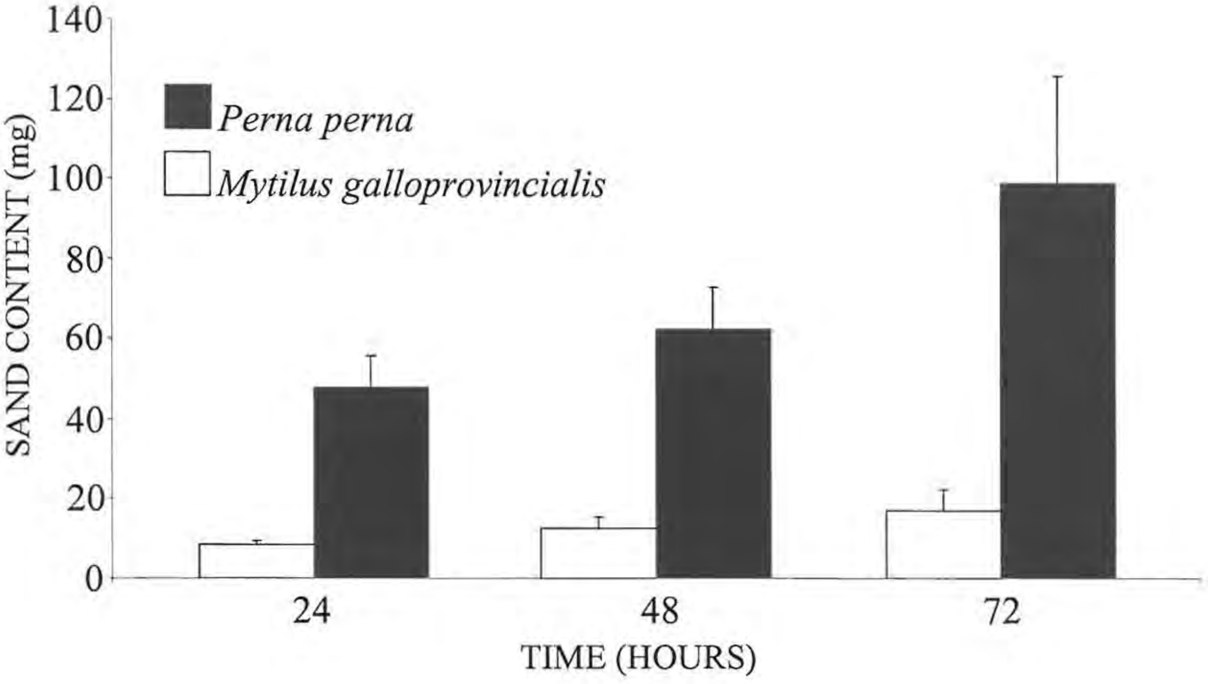

Fig. 2.7 Mean amount of sand (+SD) accumulated within the valves in live mussels when exposed to sand burial in the laboratory.

Field Experiments

Sand burial

Repeated measures ANOVA showed that mortality rates for *P. perna* were significantly higher than for *M. galloprovincialis* ($p < 0.0001$; Table 2.5) with sand burial. Mortality rates followed the same pattern as for the laboratory sand burial experiment. After 48 hours, mortality was 14.6% and 3.3% for *P. perna* and *M. galloprovincialis* respectively; L_T 50 was reached after five days for *P. perna* and six days for *M. galloprovincialis* (Fig. 2.8).

	df	MS	F	p
Species	1	25867.4	68.7040	<0.0001
Site	2	717.4	1.9053	0.1775
Species*Site	2	1409.0	3.7424	0.0437
Error	18	376.5		
Day	5	22251.1	508.8661	<0.0001
Day*Species	5	483.2	11.0503	<0.0001
Day*Site	10	108.2	2.4743	0.0115
Day*Species*Site	10	81.1	1.8549	0.0621
Error	90	43.7		

Table 2.5 RM-ANOVA for sand burial field experiment. df = degree of freedom; MS = Mean Squares; F = F–Ratio; P = probability value.

Seasonality of sand level fluctuation

Monthly photographs of mussel beds taken at Plettenberg Bay showed strong seasonal fluctuation of sand coverage with vertical changes of up to 175cm. At both sites and in both years, sand accumulation increased towards the end of summer and the beginning of autumn (particularly April and March), followed by massive removal at the end of autumn. 100% mortality for both species was observed once sand level decreased to uncover the mussel beds one month later in May. Sand levels then remained low until the following summer. The lowest sand level (y = 0 in Fig. 2.9 a and b) occurred during winter between August and September. At its greatest depth (April-May 2004 in Robberg, April-May 2003 in Keurbooms), sand completely covered the *Perna* and the mid zones, while the *Mytilus* zone was 35% and 21.4% covered at Robberg and Keurbooms respectively (Fig. 2.9).

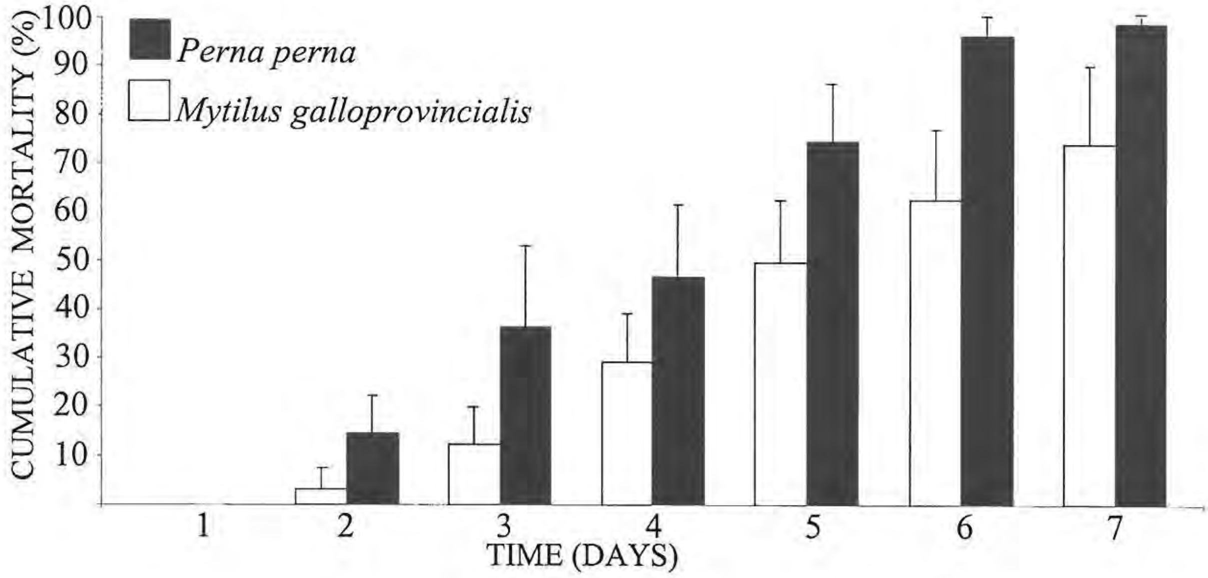

Fig. 2.8 Mean cumulative percentage mortality for three sites (+ SD) of *P. perna* and *M. galloprovincialis* when exposed to sand burial in the field.

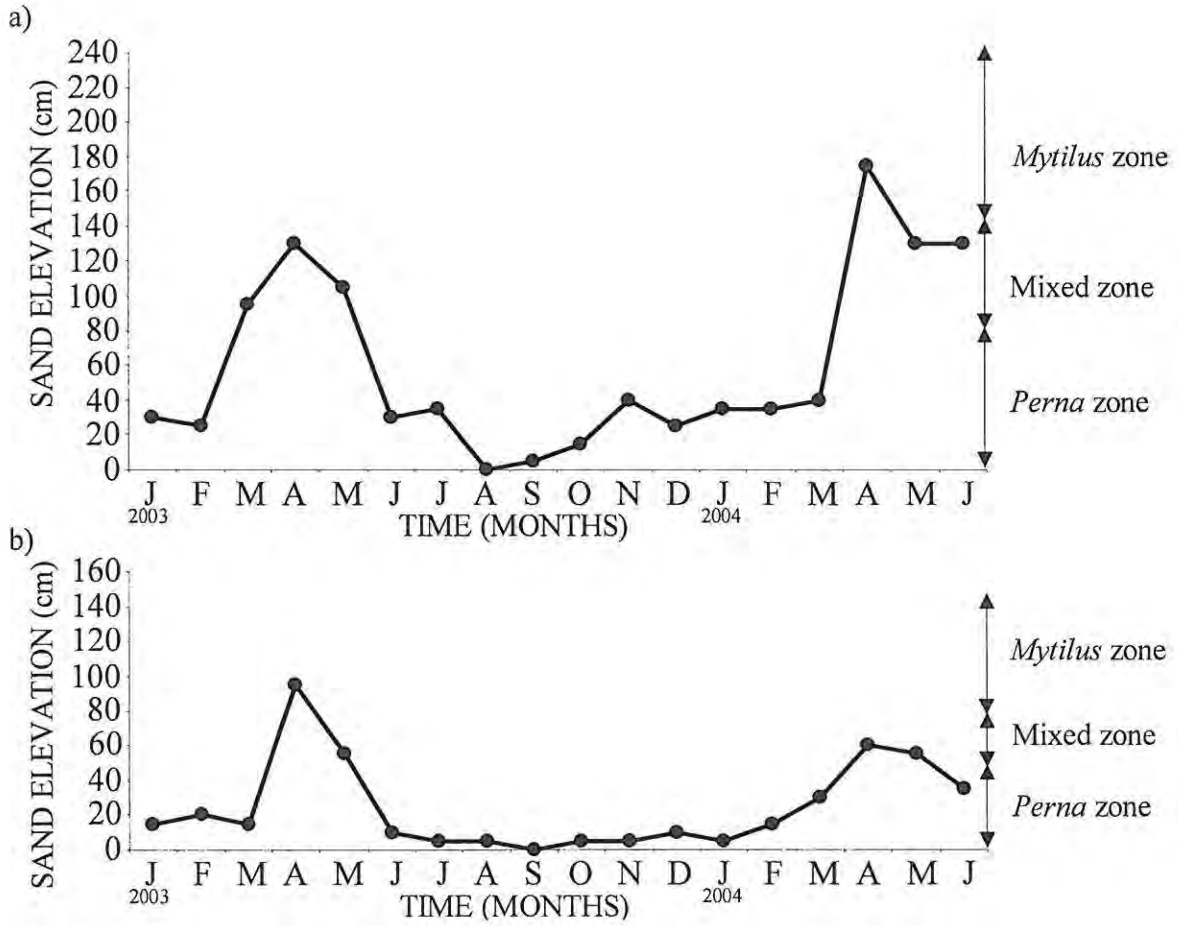

Fig. 2.9 Seasonal sand elevation at Robberg (a) and at Keurbooms (b). The lowest level of sand recorded at each site was taken to be the reference level ($y = 0$). The vertical limits of the three mussel zones at each site are indicated.

Discussion

Digital photographs showed considerable movement of sand, which seasonally covers mussel beds, with enormous effects on mussel survival and consequently on the community. During the experiment, the maximum accumulation of sand to a depth of 1.75m should be seen in the context of a maximum tidal range on this coast of 2-2.5m (Field and Griffiths 1991). Sand inundation does not occur every year, but the dramatic effects of sand burial are obvious from the total mortality of mussels in natural beds covered for one month, while mass mortality of mussels not buried, but exposed to high sediment loads, is observed periodically (personal observation).

In this study I tested the hypothesis that habitat segregation of *Perna perna* and *Mytilus galloprovincialis* could be explained by a greater physiological tolerance of sediment load of the indigenous species. The results were very clear cut and consistent, but rejected this hypothesis.

Although *M. galloprovincialis* was more resistant to the effects of sand than *P. perna*, after a few days, sand burial was lethal to both species; once buried by sand, both species reached L_T50 within four to six days. In nature, burial is likely to last much longer than this (Fig.2.9) so that interspecies differences may have little influence on habitat segregation. The effects of suspended sand are of much briefer duration under natural conditions and it is likely that this is more important in mediating competition between the two species. This is especially so if there are strong sublethal effects that would have been missed by measuring mortality alone. The most obvious could be less feeding efficiency. When exposed to suspended sand in the laboratory, *M. galloprovincialis* showed much greater tolerance than *P. perna*. The greater ability of the invasive species to withstand this stress could be a daily competitive advantage when sediment is brought in suspension during high tide. Mussels are suspension feeding bivalves and consequently they are very vulnerable to high levels of sediment loads in the water (Bricelj and Malouf 1984; MacDonald et al. 1998; Cheung and Shin 2005). The two pairs of labial palps are responsible for rejecting non-food material that has been filtered out (Morton 1987; Seed and Richardson 1999). The material rejected by the palps, together with some large particles rejected directly by the gills, forms mucoid aggregates or pseudofaeces that are expelled by both inhalant and exhalant siphons (Foster-Smith 1975). At high levels of sediment load the sorting mechanism may become overloaded and the equilibrium between filtration and rejection of material is altered. Consequently increasing proportions of filtered material are rejected as pseudofeces until the filtration rate reaches a maximum and then declines to zero (Widdows et al. 1979; Newell et al. 1989).

Ward et al. (1998) showed that, in heterorhabdic bivalve species (ctenidia possessing two types of cilia beating in opposing directions), most of the particle sorting takes place on the ctenidia. In contrast, homorhabdic species, including marine mussels, have ctenidia possessing only one type of cilia and rely predominantly on the labial palps for particle selection. The significantly longer labial palps measured in *M. galloprovincialis* could be a morphological explanation for higher mortality rates of *P. perna* under conditions of sand suspension (Kiørboe and Møhlenberg 1981). In late summer and autumn, massive quantities of sand are moved by storms, covering large portions of the mussel zone on rocky shores. Both laboratory and field experiments showed that *P. perna* is less resistant than *M. galloprovincialis* to sand burial conditions. On sandy beaches, there is a very rapid decline in oxygen deeper in the sediment. At a depth of 2cm the oxygen content is generally about $1.4 ml.l^{-1}$, while at a depth of 5cm there is frequently only about $0.3 ml.l^{-1}$ (Pearse et al. 1942; Brafield 1964). When buried under sand, mussels are exposed to both the abrasive action of the sand itself and to anoxic conditions. Some bivalves live under conditions not only of low oxygen, but also of high sulphide conditions (e.g. Kraus and Doeller 2004) and it can be difficult, or perhaps impossible to separate the effects of hypoxia from increases in hydrogen sulphide under natural conditions (Wu 2002). However, there is no obvious evidence of hydrogen sulphide leading to mortality in the system studied here.

To understand how much anoxia influences mortality during sand burial, mussels were kept in anoxic seawater without sand. *P. perna* was more sensitive to this condition, and this is in accord with published values for oxygen tolerances. *P. perna* and *M. galloprovincialis* can regulate oxygen uptake down to concentrations of approximately 3.4 (Marshall and McQuaid 1993) and 2-2.5ppm (de Zwaan et al. 1991) respectively. However, both species began to die later under anoxic conditions than when buried under sand, indicating that the physical action of sand is a crucial factor influencing mortality. Recent studies show that sediment load stress is strongly correlated to irreversible damage to gill structure in *Perna viridis* (Cheung and Shin 2005). Seriously damaged gill filaments could reduce the effective gill surface area and affect the pumping rate of the organism, leading to hypoxic stress (Jones et al. 1992; Au et al. 2004). When buried, both species accumulated sand within the shell valves while still alive, but the quantities were much higher for *P. perna*. This suggests that *P. perna* gills are more severely damaged and could explain its higher mortality rates, though histological analysis of sand damaged gill tissue of the two species is needed to confirm this. Under control conditions of oxygenated water, mussels in aquaria use the foot to rearrange their position and aggregate in clumps. Valve closure in anoxic water prevents this (personal observation). Moreover, in anoxic water, mussels were not able to replace decayed byssal threads to maintain constant attachment strength (personal observation). These physical limitations could be an important disadvantage when uncovered by sand after temporary burial and re-exposed to hydrodynamic stress.

Although all the results indicate that *P. perna* is less resistant to sand stress, it occupies the lower mussel zone and so, at least at some sites, it is more exposed to the periodic effects of suspended sand and sand burial. The survey showed that the *Perna* zone at the study sites was covered by sand for long periods of the year and digital photographs showed that buried mussel beds were completely removed when sand levels dropped again in summer. In addition, the absence of *M. galloprovincialis* juveniles on the lower shore and of *P. perna* juveniles on the higher mussel zone (personal observation) suggests that interspecific differences in reaction to abiotic stress are not restricted to adults.

In conclusion, despite the fact that *M. galloprovincialis* should have the competitive advantage of being less vulnerable to sand on the low shore, sand is not involved in habitat segregation of *P. perna* and *M. galloprovincialis* and other biotic or abiotic factors are responsible for preventing the invasive species from colonising the lower mussel zone. In particular there is experimental evidence that combinations of wave action (Chapter 3) and competitive displacement by *P. perna* (Rius and McQuaid in press) effectively exclude *M. galloprovincialis* from the lower parts of the mussel zone. Moreover, this study shows that a species that is less well adapted than a competitor to an important abiotic factor both morphologically (in terms of labial palp length) and physiologically (in terms of sand tolerance) can nevertheless maintain dominance.

CHAPTER 3

HYDRODYNAMIC STRESS AS A DETERMINANT FACTOR IN HABITAT SEGREGATION BETWEEN *Perna perna* AND *Mytilus galloprovincialis* IN SOUTH AFRICA

Introduction

Intertidal organisms are often exposed to large hydrodynamic forces associated with wave action (Denny 1985, 1995; Denny and Gaines 1990; Gaylord et al. 1994). Because they can regulate the supply of food or propagules and break or dislodge organisms, wave forces play an important role in determining the structure and dynamics of many intertidal communities (Menge 1976; Lubchenco and Menge 1978; Paine and Levin 1981; Sousa 1985). The ability of an organism to resist the water motion produced by waves is a pre-requisite for life in wave-swept environments and if an organism experiences a force that exceeds its strength of attachment, it will be dislodged.

In South Africa, wave action affects entire intertidal communities, influencing both the vertical distribution of biomass and the trophic composition of that biomass (McQuaid and Branch 1985b) and can have strong species-specific effects (McQuaid and Branch 1984). Mussels are often the major occupiers of primary space on rocky intertidal shores (Seed and Suchanek 1992) and in South Africa, growth rates of mussels are higher at wave exposed sites than sheltered ones, while mussels experiencing high hydrodynamic stress tend to increase shell thickness (McQuaid and Lindsay 2000; Steffani and Branch 2003c).

Waves can be the major cause of mussel mortality in the intertidal zone (Paine and Levin 1981) and the ability to resist dislodgement will be an important attribute for a potentially invasive species. Several studies have reported differences in the strength of byssal attachment for co-existing mussel species that compete for space (Witman and Suchanek 1984; Willis and Skibinski 1992; Bell and Gosline 1997; Hunt and Scheibling 2001; Schneider et al. 2005). Greater attachment strength could contribute to competitive dominance both seasonally, during winter storms, and geographically, along gradients of wave exposure among shores, and vertically within shores, leading to habitat segregation. The success of mussels in withstanding strong wave forces is largely due to the byssus, an extracellular bunch of collagenous threads that is secreted by the byssal gland, which lies in the ventral groove of the foot, and allows mussels to attach firmly to the substratum and to each other (Waite 1992). Each byssal thread is proximally attached to a common stem that connects via the root to the byssus retractor muscle (Brown 1952), and is composed of three distinct parts that differ in their ultrastructure, protein composition and, consequently, in their physical properties (Bairati and Vitellaro-Zuccarello 1976; Benedict and Waite 1986). The proximal region of the thread is more elastic but less strong than the distal region, and the two make up about one and two thirds respectively of total thread length (Qin and Waite 1995). Each thread terminates in an adhesive plaque containing water insoluble adhesive proteins that enable mussels to anchor to solid surfaces (Waite 1992).

Mussels adjust their attachment strength in response to the flow environment. In the laboratory, attachment strength increases with water agitation (Young 1985; Lee et al. 1990) and in the field it increases with increasing wave exposure both between sites (Witman and Suchanek 1984), and between habitats within a site; mussels living in tide pools are less strongly attached than those on emergent rocks (Hunt and Scheibling 2001). Moreover, attachment strength is lower in mussels sheltered within a bed than in those living outside the bed or at its fringe (Witman and Suchanek 1984; Bell and Gosline 1997). Mussel attachment strength responds also to temporal variations in flow; it increases in winter and is correlated with seasonal fluctuations in wind and wave action

(Price 1982; Carrington 2002; Hunt and Scheibling 2001; Witman and Suchanek 1984). Attachment strength also increases with increasing mussel size (Harger 1970; Witman and Suchanek 1984; Hunt and Scheibling 2001). Mussels adjust their attachment strength by varying both the number and the thickness of byssal threads, but differences between species can mainly be explained by differences in thread thickness, while thread number is the primary mechanism affecting attachment strength within a species (Bell and Gosline 1997; Carrington 2002).

The process of byssal thread production can be energetically expensive, forming 8 to 15% of a mussel's monthly energy expenditure (Griffiths and King 1979; Hawkins and Bayne 1985). Reproductive condition varies seasonally, and usually mirrors temporal fluctuations of sea surface temperature (Seed and Suchanek 1992; Carrington 2002). In South African populations of *Perna perna* and *Mytilus galloprovincialis*, gamete production usually takes place twice a year, in winter between June and September and again in summer between January and April (Griffiths 1977; van Erkom Schurink and Griffiths 1991). During gonad development, mussels are subjected to a very high energetic effort, investing up to 90% of their energy budget in gamete production (Seed and Suchanek 1992). Consequently, thread production can be energetically constrained during the reproductive season, making mussels more vulnerable to wave action and thus increasing the risk of dislodgment (Carrington 2002).

The ability of a mussel to survive in a wave-swept environment depends not only on its attachment strength, but also on the magnitude of the hydrodynamic forces to which it is exposed. These hydrodynamic forces are mainly determined by the size and shape of the mussel itself and the flow regime to which it is subjected (Denny et al. 1985; Denny

1988, 1995). Breaking waves can expose mussels to forces due to both the water's velocity, drag and lift, and to its acceleration (Denny 1995), though, because of the small length scales involved, acceleration forces are insignificant relative to lift and drag (Gaylord 2000). Lift is the main hydrodynamic force exerted on mussels living tightly packed in a mussel bed, (referred to here as bed mussels; Denny 1987; Fig. 3.1). Lift is generated by water flowing over the bed and creating a difference in pressure between the top and the bottom of each mussel. This force can be strong enough to pull a mussel up and dislodge it. Bed mussels are usually arranged so that their anterior-posterior axis is perpendicular to the substratum, reducing the area exposed to lift force (flow). Mussels living outside the bed (solitary mussels; Fig. 3.1) are mainly subjected to drag hydrodynamic forces acting in the direction of flow. Solitary mussels are usually arranged with their anterior-posterior axis parallel to the substratum. In South Africa, waves can subject intertidal organisms to extremely intense and powerful hydrodynamic stress (Steffani and Branch 2003c). Moreover, wave exposure has being shown to influence the growth (McQuaid and Lindsay 2000), population structure (McQuaid et al. 2000) and recruitment (McQuaid and Lindsay 2005) of *P. perna*. Consequently, wave action is likely to be a key factor influencing the dynamics of co-existence and competition between *M. galloprovincialis* and *P. perna*.

The influence of wave action on habitat segregation between *M. galloprovincialis* and *P. perna* was investigated by performing two studies:

1) <u>Short-term study</u>:

This study reports differences in byssal attachment strength of the two co-existing and competing mussel species, *M. galloprovincialis* and *P. perna*, and also investigates the mechanical causes contributing to these differences. In addition strength of attachment measurements were coupled with modelled hydrodynamic forces to predict the probability of dislodgement. Thus, the following hypothesis was tested: because hydrodynamic stress is higher on the low shore, it contributes to the vertical zonation of the two species.

2) Seasonal study:

This study examined seasonal fluctuation in the attachment strength of *M. galloprovincialis* and *P. perna*, and relates them to variations in the hydrodynamic environment and reproductive condition. The competitive advantage of being able to resist hydrodynamic stress better could be more or less pronounced in different months of the year due to fluctuations in the reproductive status of the two species and to seasonal changes in sea temperature and wave intensity. Thus the following hypotheses were tested: a) Reproductive condition (calculated by multiplying the mantle gamete fraction by the total mantle dry weight and then dividing by the dry body weight) will be correlated with sea surface temperature, b) Strength of attachment will be positively correlated with wave action, c) Strength of attachment will also be negatively correlated with reproductive effort.

Fig. 3.1 Solitary and bed mussels from the mid-mussel zone.

Materials and methods

Short-term study

All mussels used in this study were collected from a granite shore in Plettenberg Bay (34°05'S, 23°19'E; Fig. 1.1), on the south coast of South Africa, during summer low tides in January 2003. The sampling area was small (approximately 20m^2) and topographically uniform, so all mussels were assumed to be exposed to similar abiotic factors. Mussels living within a monolayered mussel bed (bed mussels) and living outside the bed (solitary mussels) were considered separately. Multilayered mussel beds were not investigated.

Attachment strength and failure location

Mytilus galloprovincialis and *Perna perna* individuals (2.3-6.9cm shell length; n = 75 for each species and for each position, i.e. bed or solitary mussels), were tested for attachment strength in the mid mussel zone. To measure mussel attachment strength, a 2mm diameter hole was drilled through the shell valves close to the posterior margin using a hand held battery operated drill (Denny 1987). A fish hook, connected to a recording spring scale, was inserted through the hole. The scale was steadily and uniformly lifted normal to the rock surface until dislodgment occurred (after 1 to 3 sec) and the force required to detach each mussel was recorded in Newtons. The minimum separation of bed mussels was 20cm, so that measurements did not influence one another by disturbing the mussel bed. Two-way ANCOVA, with shell length as a co-variate, was used to evaluate the effects of species and position (fixed factors) on attachment strength. The location of byssus failure for each dislodged mussel from the mid zone was recorded in one of five categories: root, stem, byssal thread, plaque and substratum. In addition, attachment strengths of *M. galloprovincialis* in beds in the high and mid zones, and of *P. perna* in beds from the mid and low zones were measured using similarly sized individuals (3.5-4.5cm shell length; n = 50 for species in each zone). *P. perna* and *M. galloprovincialis* are absent or rare in the high and low mussel zone respectively. A Kruskal-Wallis test was performed to evaluate the effect of zone on attachment strength for each species separately.

Morphometrics

For each dislodged mussel, the shell length (anterior-posterior axis), height (dorso-ventral axis) and width (lateral axis) were measured to the nearest mm with Vernier callipers. The minor shell platform area ($A_{p,min}$, minimum exposed shell area) was approximated as an ellipse and calculated using shell height and width as major and minor axes respectively. The major shell platform area ($A_{p,max}$, maximum exposed shell area) was approximated as an ellipse using shell length and height. Data were log transformed and ANCOVA was used to evaluate the effects of species and shell length on $A_{p,min}$ and $A_{p,max}$, and on shell width and height.

The number of byssal threads was counted from samples (n = 50) of solitary and bed mussels of both species collected in the mid mussel zone. To avoid breaking the byssal threads, each individual was carefully removed from the substratum using a scalpel. For each mussel, diameters of the root and of the proximal region of one newly formed, randomly selected byssal thread were measured. Data on thread numbers were square root transformed and a two-way ANCOVA was performed to evaluate the effects of species, position (fixed factors) and shell length (co-variate) on the number of byssal threads. Regression analysis was used to test the dependence of thread number and root diameter on shell length for each species and for each position. Students' t-test was used to test equality of the coefficients for the shell length-species and shell length-position regressions of the two species.

Wave exposure

Degree of wave exposure was quantified in October 2005 by measuring maximum wave force using dynamometers modelled on those used by Palumbi (1984). Fifteen dynamometers where placed at Port Alfred (33°30'S, 27°10'E; Fig. 1.1), five in each of the three mussel zones, and removed the following day during very rough seas with over five meter swells (South African Weather Service). Each dynamometer gave a single measurement of maximum wave force during the two tidal cycles which was expressed in $N.m^{-2}$. One-way ANOVA was performed to determine whether maximum wave force differed among the three mussels zone.

Hydrodynamic forces

Bed mussels are oriented with their long axis perpendicular to the plane of the substratum. Lift was assumed to be the only hydrodynamic force imposed on such mussels (Denny 1987) and was calculated as:

$$F_{lift} = 1/2\rho U^2 A_{p,min} C_l \qquad (1)$$

where ρ is sea water density ($1024 Kg.m^{-3}$), U is water velocity ($m.s^{-1}$), C_l is the dimensionless coefficient of lift (0.88 for bed mussels, Denny 1987), and $A_{p,min}$ is the minor shell platform area, calculated as described. The posterior-anterior axis of solitary mussels is parallel to the plane of the substratum. For solitary animals, drag was assumed to be the only hydrodynamic force imposed on them (Denny 1987) and was calculated as:

$$F_{drag}=1/2\rho U^2 A_{p,max} C_d \qquad (2)$$

where C_d is the coefficient of drag. For *Mytilus californianus* this coefficient is 0.8 when drag acts perpendicular (worst case scenario) and 0.2 when it acts parallel (best case scenario) to the posterior-anterior axis respectively (Denny et al. 1985). $A_{p,max}$ is the major shell platform area, calculated as described. Acceleration force was not considered because of the small spatial scales of surf zone acceleration (Denny et al. 1985; Gaylord et al. 1994; Gaylord 2000). Formulae (1) and (2) were used to calculate hydrodynamic forces acting on both bed and solitary mussels. Two scenarios were investigated for solitary mussels: the direction of water flow perpendicular (worst case scenario) and parallel (best case scenario) to the posterior-anterior axis. Because differences in shell morphology between the two species were relatively subtle, we assumed that coefficients of drag and lift (C_d and C_l) were the same for the two species.

Probability of dislodgement

Tenacity measurements of bed and solitary (worst case scenario position) mussels of both species were ranked in increasing order. The probability, p, of having a tenacity less than a mussel of rank j was calculated using the equation $p=j(n+1)$, where n is the number of mussels sampled. The probability of dislodgment was calculated as the maximum value of p amongst mussels with tenacities less than or equal to the hydrodynamic force (lift or drag) generated by water flow of given U.

Statistical analysis

Shapiro's and Levene's tests were used to assess normality and homogeneity of variances respectively. When data failed the requirements for parametric analysis, they were transformed as indicated; when transformation was successful, parametric analysis was performed, otherwise non-parametric analysis was used.

Seasonal study

Mussels used in this study were collected monthly between May 2003 and October 2004 from granite shore in Plettenberg Bay (34°05'S, 23°19'E; Fig. 1.1), on the south coast of South Africa. Again, the sampling area was small (approximately 20 m^2) and topographically uniform, so all mussels were assumed to be exposed to similar abiotic factors. Samples included only mussels living within a monolayered mussel bed from the mid-mussel zone.

Attachment strength and reproductive condition

Mytilus galloprovincialis and *Perna perna* individuals (3.5-4.5cm shell length; n = 20 each month for each species) were tested *in situ* for attachment strength as described for the short-term study experiment (Denny 1987; Bell and Gosline 1997). The number of byssal threads of each dislodged mussel was counted. The diameter of the distal region of three randomly chosen byssal threads from each individual was measured using a dissecting microscope (± 1μm). In addition, the reproductive status of 20 female mussels of each species (3.5-4.5cm shell length) collected at the same site was determined each month. Reproductive condition factors of the two sexes, which indicate fluctuations in flesh weight due to gamete maturation, cannot be distinguished (Griffiths 1977; Griffiths and King 1979). The gonad mantle lobes were dissected from each animal. A small portion (~ 0.1g) of gonad tissue was cut from the middle region of the mantle lobe and fixed in Bouin's solution for histological examination; the remaining gonadal tissue and the rest of the body were dried separately to constant weight (2d) at 60°C. The fixed gonadal tissues were then dehydrated in ascending concentrations of alcohol, washed in xylene and then embedded in paraffin. Samples were then cut in 6μm sections and stained with hematoxylin and eosin. A representative section through the gonad tissue was used to determine the reproductive status of each individual using a video microscope system. A grid of 64 points was drawn on the video monitor and the fraction of mantle tissue that was reproductive (mantle gamete fraction) was calculated by dividing the number of points that fell on developing or ripe eggs by the total number of points. The gonadosomatic index (GSI), a measure of reproductive condition, was calculated by multiplying the mantle gamete fraction by the total mantle dry weight and then dividing it by the flesh dry body weight (Roff 1992).

Kruskal-Wallis tests were used to evaluate the effects of species and month on attachment strength, number of byssal treads, diameter of the distal region and gonad index. Significant results for Kruskal-Wallis tests were further examined using post hoc LSD analysis (except for GSI).

Oceanographic data

Wave heights were obtained from data for a virtual buoy located at 34° 51' S : 23° 53' E as recorded by the USA National Data Buoy Centre (www.ndbc.noaa.gov). A virtual buoy gives a wave model prediction of a buoy report. Wave height, wave direction, wind speed, wind direction and period are extracted from the NOAA WAVEWATCH III model (Tolman 1999). Mean daily wave heights were calculated from hourly values, and then used to calculate mean monthly values. Daily sea surface temperature (SST) data were recorded at Tsitsikamma (32°21'S, 26°58'E) by the South African Weather Service, and were used to determine mean monthly values.

Cross-correlation

Mean attachment strengths of *M. galloprovincialis* and *P. perna* were cross-correlated with wave height, GSI and SST. GSI of each species was cross-correlated with SST using the computer program Statistica 7.

Results

Short-term study

Attachment strength and failure location

Mussel size, species and position (bed or solitary animals) were highly significant factors influencing attachment strength (two-way ANCOVA, $p < 0.0001$; with no significant interaction among factors; Table 3.1; Fig. 3.2). On average, attachment strength of *Perna perna* was 24-26% higher than for *Mytilus galloprovincialis*, for solitary and bed mussels respectively. Solitary mussels were 19 and 21% more strongly attached to the substratum than those sheltered within a mussel bed for *M. galloprovincialis* and *P. perna* respectively. The effects of species and position increased at greater shell lengths. Comparisons of attachment strengths for mussels dislodged from the high, mid and low mussel zones showed a clear vertical gradient, with mussels living lower on the shore having stronger attachment in both species (Kruskal-Wallis test, $p < 0.0001$ in both cases). On average, *M. galloprovincialis* in the mid zone were 46% more strongly attached than those in the high zone, while *P. perna* attachment strength was 35% higher in the low zone than in the mid zone (Fig. 3.3).

	df	MS	F	p
Shell length (co-variate)	1	98383.43	1956.047	<0.0001
Species	1	15621.07	310.576	<0.0001
Position (bed/solitary)	1	7596.09	151.025	<0.0001
Species*Position (bed/solitary)	1	81.24	1.615	0.2047
Error	295	50.30		

Table 3.1 Two-way ANCOVA for attachment strength measurements of mussels from the mid-mussel zone. df = degree of freedom; MS = Mean Squares; F = F–Ratio; p = probability value.

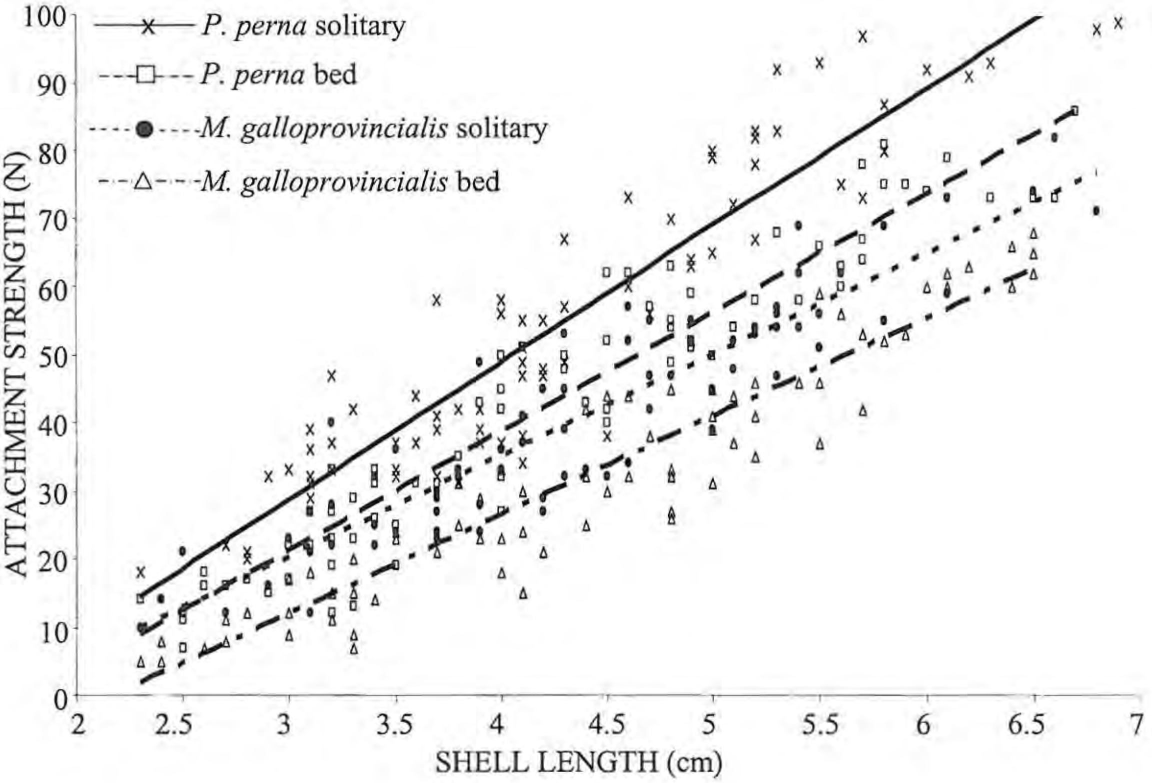

Fig. 3.2 Relationship between shell length and attachment strength in *P. perna* solitary mussels ($y = 20.188x-31.807$, $r^2 = 0.873$), *P. perna* bed mussels ($y = 17.492x-31.32$, $r^2 = 0.9173$), *M. galloprovincialis* solitary mussels ($y = 14.868x-24.337$, $r^2 = 0.8421$), and *M. galloprovincialis* bed mussels ($y = 14.49x-31.52$, $r^2 = 0.8922$).

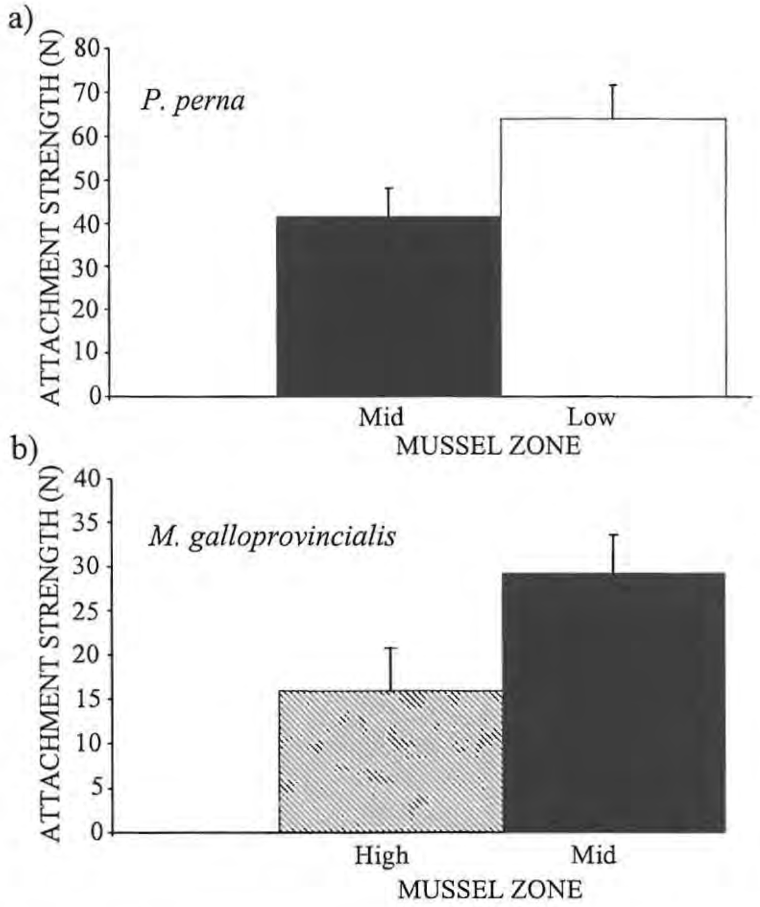

Fig. 3.3 Relationship between mean attachment strength (+SD), and mussel zone. (a) *M. galloprovincialis*, mid/high zones. (b) *P. perna*, mid/low zones.

Stem failure was never observed (Table 3.2). For both species, the most frequent failure location was substratum for bed and the plaque for solitary mussels. Root failure was rare but more frequent in solitary mussels (8-11%) than in bed mussels (1.5-2%). The frequency of substratum failure was higher in bed mussels (about 50%) than in solitary mussels (12-16%). Plaque was the weakest attachment location for the solitary mussels, accounting for 42 and 49% for *M. galloprovincialis* and *P. perna* respectively, while in bed mussels the values were 31 and 36%. Byssal threads failed more often than the root, and again, this was more frequent in solitary mussels (27-35%) than in bed mussels (11-19%).

Failure location	*Mytilus galloprovincialis*		*Perna perna*	
	solitary	bed	solitary	bed
Root	11.1	2.1	8	1.5
Thread	34.9	18.9	27	11.2
Plaque	42	30.9	48.9	36.3
Substratum	12	48.1	16.2	51

Table 3.2 Location of attachment failure (%) for *M. galloprovincialis* and *P. perna* individuals experimentally dislodged in the field. Sample size is 75 mussels for each column.

Morphometrics

The co-variate, shell length, significantly influences all morphometrics, while species affected some morphometrics but not others. Shell height did not differ significantly (ANCOVA, $p = 0.4$; Table 3.3) between the two species, while *M. galloprovincialis* shells were 22% wider than *P. perna* shells for a given shell length (ANCOVA, $p < 0.0001$; Table 3.4; Fig. 3.4). Consequently, *M. galloprovincialis* had a significantly bigger (ANCOVA, $p < 0.0001$; Table 3.5) $A_{p,min}$ than *P. perna*, but the morphometric relationship between shell length and $A_{p,max}$ did not differ significantly between species (ANCOVA, $p = 0.37$; Table 3.6; data not shown). Byssal thread number increased with shell length and both species and position significantly affected byssal thread number (two-way ANCOVA, $p < 0.0001$ with no significant interaction; Table 3.7; Fig. 3.5). On average, the number of byssal threads for *P. perna* was 23.6 and 18.5% higher than for *M. galloprovincialis*, for solitary and bed mussels respectively. Solitary mussels had 22.6 and 27.5% more byssal threads than bed mussels for *M. galloprovincialis* and *P. perna* respectively. Thread diameter increased with animal size for both species and for both positions (regression, $p < 0.001$). *P. perna* had significantly (Students't test, $p < 0.0001$) thicker threads than *M. galloprovincialis* (approximately 14% for both solitary and bed) and solitary mussels had significantly (Students't test, $p < 0.0001$) thicker threads than bed mussels (approximately 12% for both species; Fig. 3.6). Root diameter increased with animal size for both species and for both positions (regression, $p < 0.001$).

	df	MS	F	p
Shell length (co-variate)	1	2.609668	1707.182	<0.0001
Species	1	0.001183	0.774	0.3798
Error	297	0.001529		

Table 3.3 ANCOVA for shell height measurements. df = degree of freedom; MS = Mean Squares; F = F–Ratio; p = probability value.

	df	MS	F	p
Shell length (co-variate)	1	3.606458	1259.748	<0.0001
Species	1	0.861910	301.068	<0.0001
Error	297	0.002863		

Table 3.4 ANCOVA for shell width measurements. df = degree of freedom; MS = Mean Squares; F = F–Ratio; p = probability value.

	df	MS	F	p
Shell length (co-variate)	1	12.3960	1741.86	<0.0001
Species	1	0.9353	131.42	<0.0001
Error	297	0.0071		

Table 3.5 ANCOVA for $A_{p,min}$ measurements. df = degree of freedom; MS = Mean Squares; F = F–Ratio; p = probability value.

	df	MS	F	p
Shell length (co-variate)	1	12.5023	8280.9	<0.0001
Species	1	0.0012	0.8	0.368
Error	297	0.0015		

Table 3.6 ANCOVA for $A_{p,max}$ measurements. df = degree of freedom; MS = Mean Squares; F = F–Ratio; p = probability value.

	df	MS	F	p
Shell length (co-variate)	1	744.607	605.276	<0.0001
Species	1	94.269	76.629	<0.0001
Position (bed/solitary)	1	134.977	109.720	<0.0001
Species*Position (bed/solitary)	1	1.999	1.625	0.2039
Error	195	1.230		

Table 3.7 Two-way ANCOVA for thread number. df = degree of freedom; MS = Mean Squares; F = F–Ratio; p = probability value.

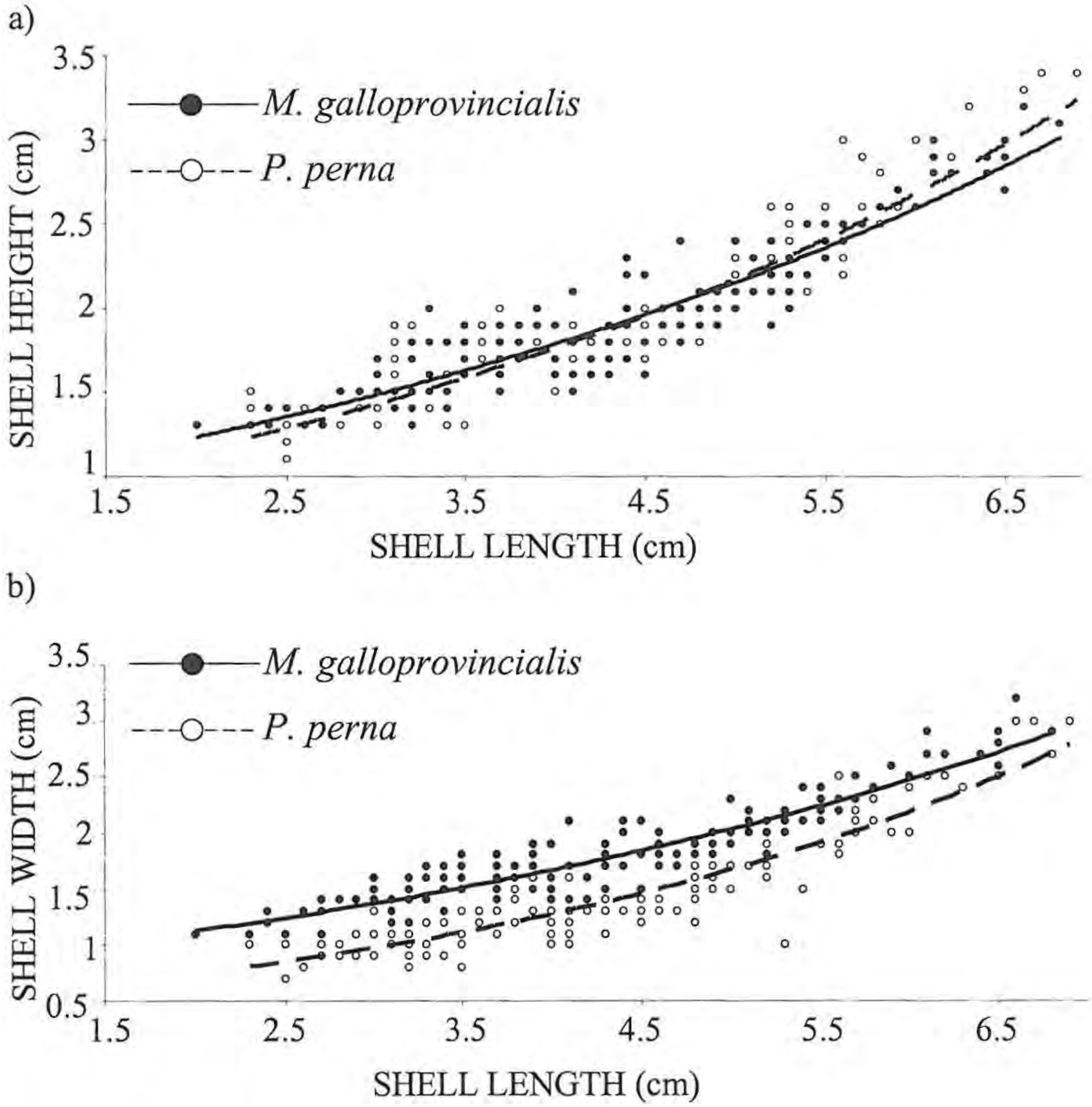

Fig. 3.4 Relationships between shell length and (a) shell height (*P. perna* $y = 0.7587e^{0.2104x}$, $r^2 = 0.8618$; *M. galloprovincialis* $y = 0.8519e^{0.1858x}$, $r^2 = 0.8466$), and (b) shell width (*P. perna* $y = 0.4321e^{0.2701x}$, $r^2 = 0.8236$; *M. galloprovincialis* $y = 0.7645e^{0.1956x}$, $r^2 = 0.8422$).

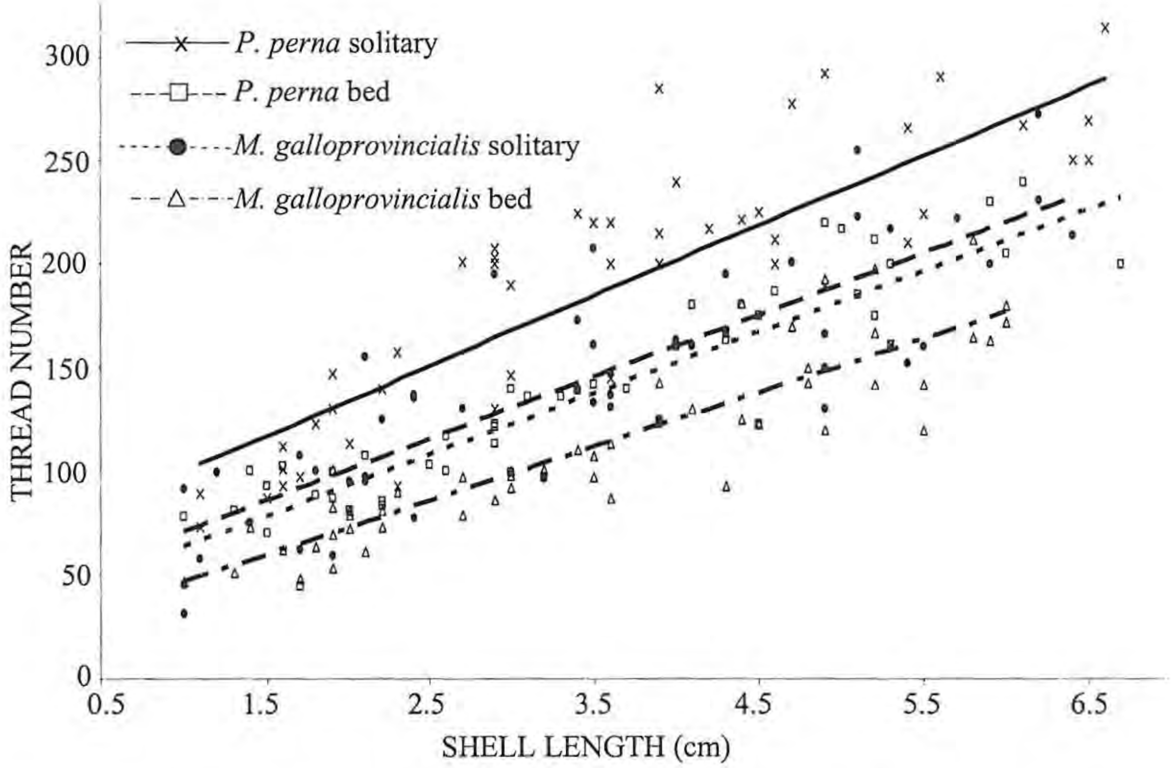

Fig. 3.5 Relationship between shell length and thread number in *P. perna* solitary mussels ($y = 33.977x+65.964$, $r^2 = 0.7107$), *P. perna* bed mussels ($y = 29.917x+40.629$, $r^2 = 0.7133$), *M. galloprovincialis* solitary mussels ($y = 29.672x+33.543$, $r^2 = 0.825$), and *M. galloprovincialis* bed mussels ($y = 26.144x+20.125$, $r^2 = 0.806$).

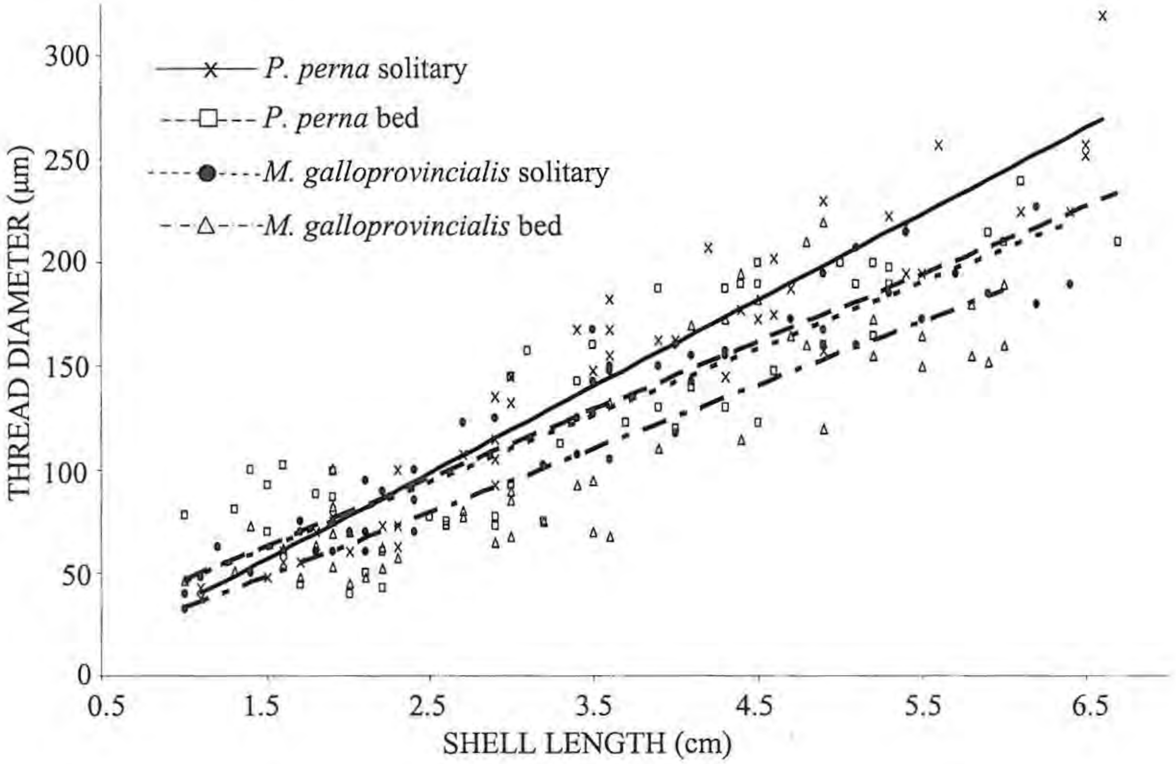

Fig. 3.6 Relationship between shell length and thread diameter in *P. perna* solitary mussels (y = 41.759x-5.7193, r^2 = 0.9156), *P. perna* bed mussels (y = 32.858x+13.73, r^2 = 0.7588), *M. galloprovincialis* solitary mussels (y = 32.191x+13.426, r^2 = 0.9018), and *M. galloprovincialis* bed mussels (y = 30.837x+1.9683, r^2 = 0.7566).

Wave exposure

One-way ANOVA showed that there was a significant ($p < 0.0001$) difference in the maximum wave forces recorded at different levels of the mussel zone (Fig. 3.7). Mean maximum wave forces increased from the high (9 682$N.m^{-2}$), through the mid (19 206$N.m^{-2}$) to the low (30 476$N.m^{-2}$) zone.

Hydrodynamic forces

Estimated drag forces acting on $A_{p,max}$ of solitary mussels did not differ between species, because there was no significant difference in morphometric scaling of $A_{p,max}$ on shell length. On average, the predicted scaled hydrodynamic drag force for a solitary mussel exposing $A_{p,max}$ to water flow (worst case scenario) was 60.5% higher than for mussels exposing $A_{p,min}$ (solitary in best case scenario and bed mussels). Under both bed and best case solitary conditions, forces acting on $A_{p,min}$ for *M. galloprovincialis* were approximately 22% higher than for *P. perna* for a shell length of 4.5cm (Fig. 3.8). Scaled hydrodynamic force was size dependent: it increased by approximately 66% between shell lengths of 4.5cm and 7cm (data not shown). At velocities lower than 6$m.s^{-1}$ the predicted hydrodynamic force was approximately $2.13\times10^4N.m^{-2}$, while at higher water velocities (14 to 20$m.s^{-1}$) it was $31.86\times10^4N.m^{-2}$ and $80.57\times10^4N.m^{-2}$ for mussels exposing $A_{p,min}$ and $A_{p,max}$ respectively.

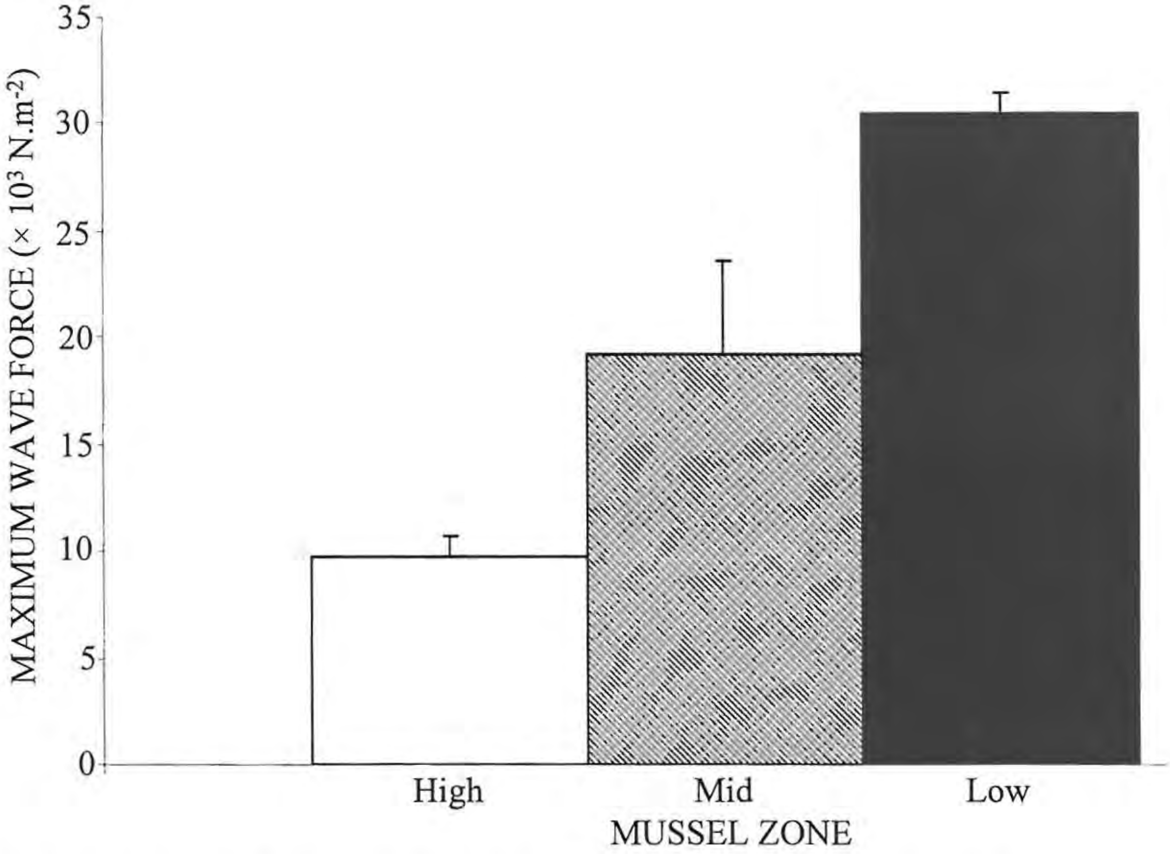

Fig. 3.7 Mean maximum wave forces in the high, mid and low mussel zones (+SD).

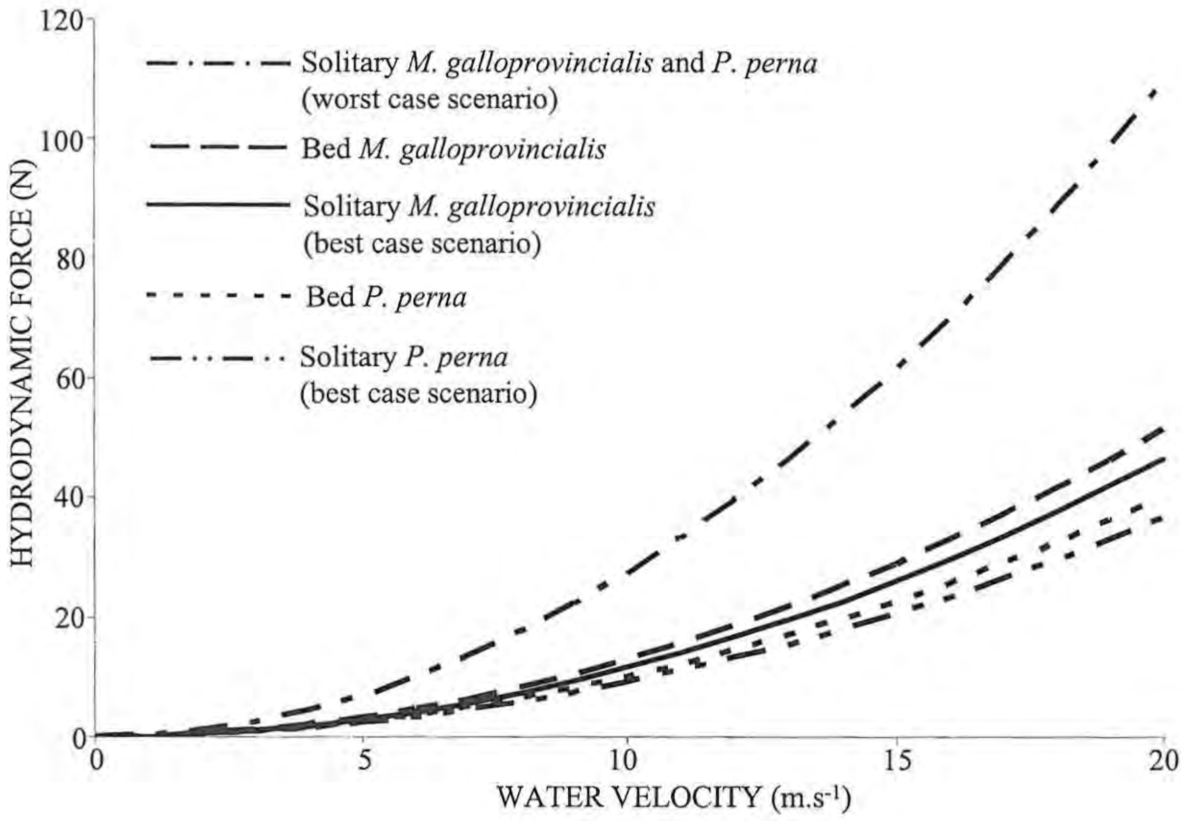

Fig. 3.8 Predicted scaled hydrodynamic forces as a function of water velocity for solitary (worst and best case scenario) and bed mussels (4.5cm shell length). *P. perna* solitary worst case ($y = 0.274x^2+6.10^{-14}x$) and best case scenario ($y = 0.091x^2-7.10^{-15}x+3.10^{-14}$), *P. perna* mussel bed ($y = 0.1x^2+10^{-14}x-3.10^{-14}$), *M. galloprovincialis* solitary worst case($y = 0.274x^2+6.10^{-14}x$) and best case scenario ($y = 0.116x^2+10^{-14}x$), *M. galloprovincialis* mussel bed ($y = 0.128x^2+3.10^{-14}x-6.10^{-14}x$).

Probability of dislodgement

The probability of mussel dislodgement for a given water velocity was influenced by both species and position. For water velocities higher than $13 m.s^{-1}$, dislodgement probability was always higher for *M. galloprovincialis* than for *P. perna*, and within species, it was higher for solitary than bed mussels. For water velocities between10 and $13 m.s^{-1}$, the likelihood of dislodgment decreases in the order *M. galloprovincialis* solitary, *M. galloprovincialis* bed, *P. perna* solitary, *P. perna* bed. At water velocities lower than $10 m.s^{-1}$ there is an extremely low probability of any mussels being dislodged.

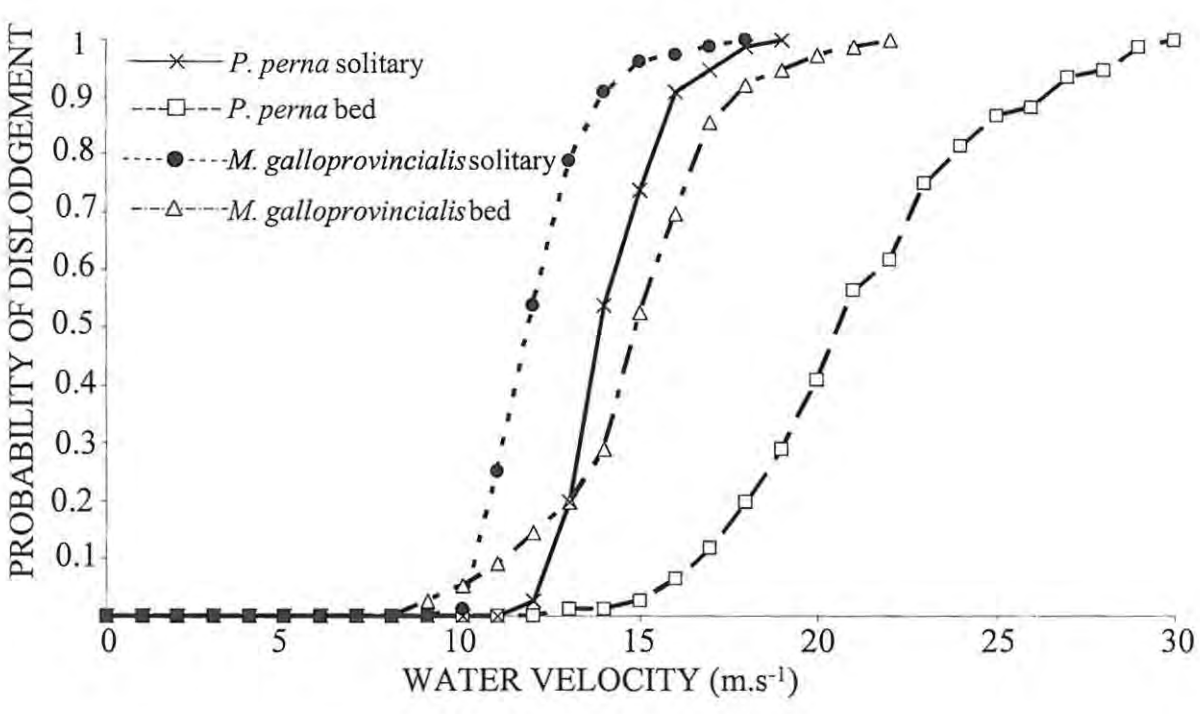

Fig. 3.9 Predicted probability of dislodgement as a function of water velocity for solitary (in a worst case scenario position) and bed mussels for both species.

Seasonal study

Attachment strength and reproductive condition

Mean attachment strength varied seasonally (Fig. 3.9), showing almost exactly the same pattern for both species. There was a rapid decrease in late winter/early spring (between August and October) and a more gradual increase in summer (between December and January). Maximum attachment strength values were recorded in August 2003 (73.4N) and in September 2004 (96.6N) for *M. galloprovincialis* and *P. perna* respectively. The two species showed the weakest attachment in November 2003 (32.2N) and in December 2004 (43.6N) for *M. galloprovincialis* and *P. perna* respectively (Fig. 3.9). A Kruskal-Wallis test showed that *P. perna* was significantly ($p < 0.0001$) more strongly attached than *M. galloprovincialis*. Mean thread diameter and number followed the same seasonal pattern decreasing between August and October and increasing between December and February (Figs 3.10 and 3.11). Kruskal-Wallis tests showed that *P. perna* had significantly more (42.5%) and thicker (14.4%) threads than *M. galloprovincialis* ($p < 0.0001$ in both cases). A post hoc LSD analysis showed that mean monthly attachment strength and thread number were significantly different between the two species during all months. In contrast, mean monthly thread diameter was not significantly different in December, February, June, July and October 2004 (post hoc LSD analysis; $p > 0.05$ in all cases). For each species, mean attachment strength was significantly correlated with thread number ($p < 0.001$; Fig. 3.12 a, b) and diameter ($p < 0.05$ for *M. galloprovincialis* and $p < 0.001$ for *P. perna*; Fig. 3.13 a, b).

Fluctuations in gamete maturation and release showed that both *M. galloprovincialis* and *P. perna* had two major spawning each year, the former from October to December 2003 and from April to June 2004, the latter from June to July 2003 and 2004 and from January to March 2004 (Fig. 3.14).

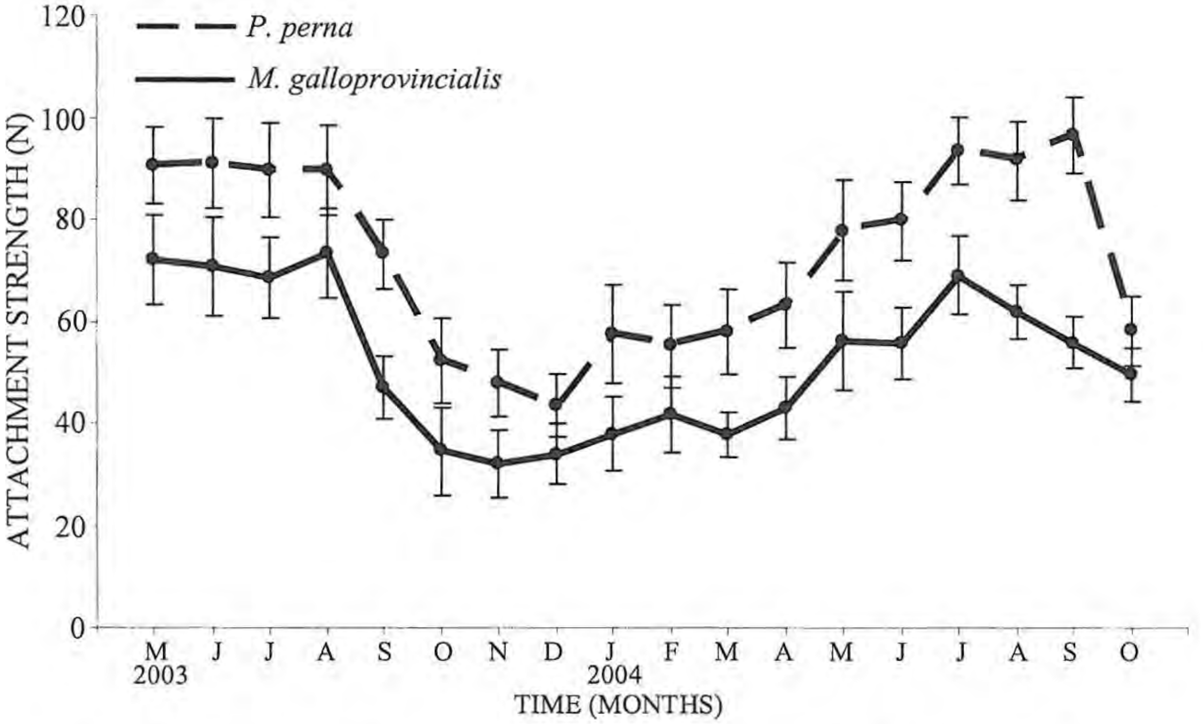

Fig. 3.9 Mean (± SD) monthly attachment strength of *P. perna* and *M. galloprovincialis* in Plettenberg Bay from May 2003 to October 2004.

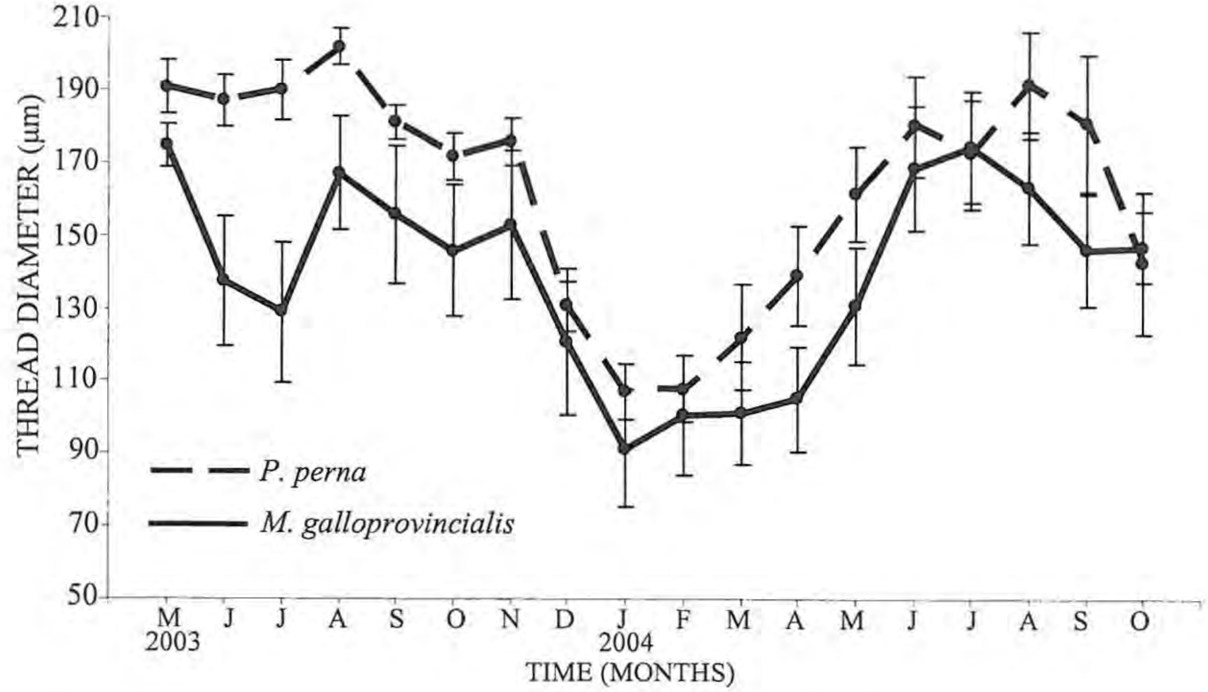

Fig. 3.10 Mean (± SD) monthly thread diameter of *P. perna* and *M. galloprovincialis* in Plettenberg Bay from May 2003 to October 2004.

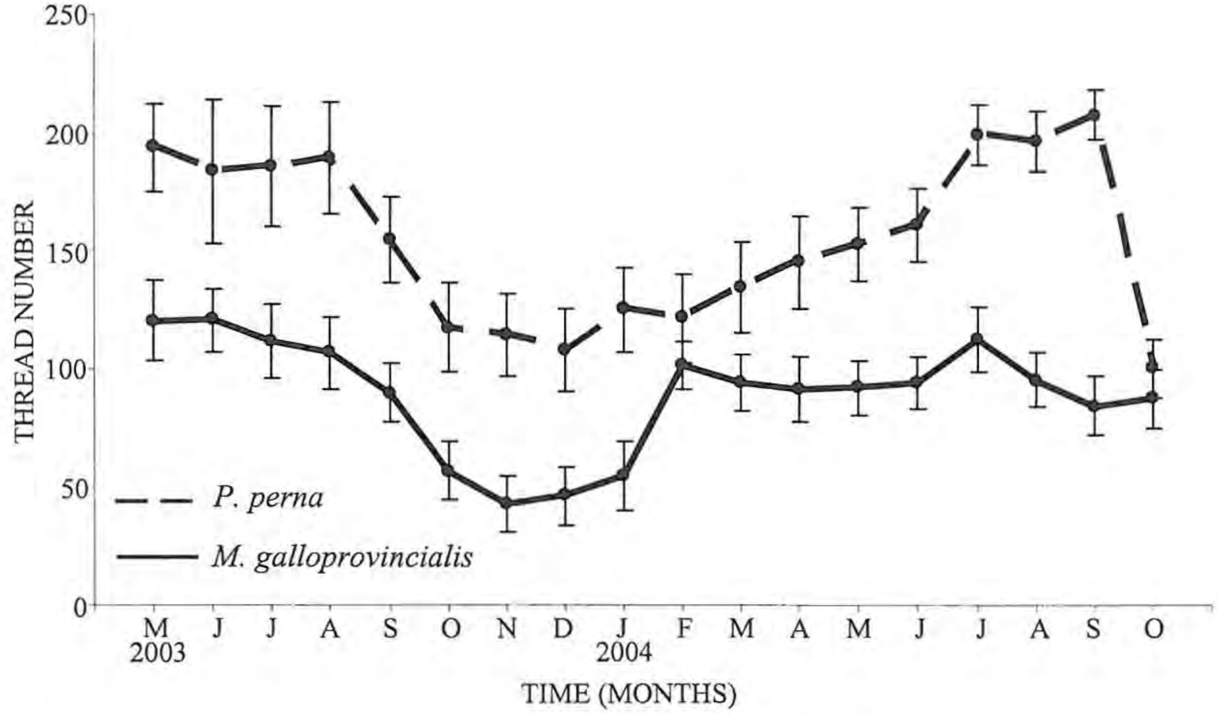

Fig. 3.11 Mean (± SD) monthly number of threads for *P. perna* and *M. galloprovincialis* in Plettenberg Bay from May 2003 to October 2004.

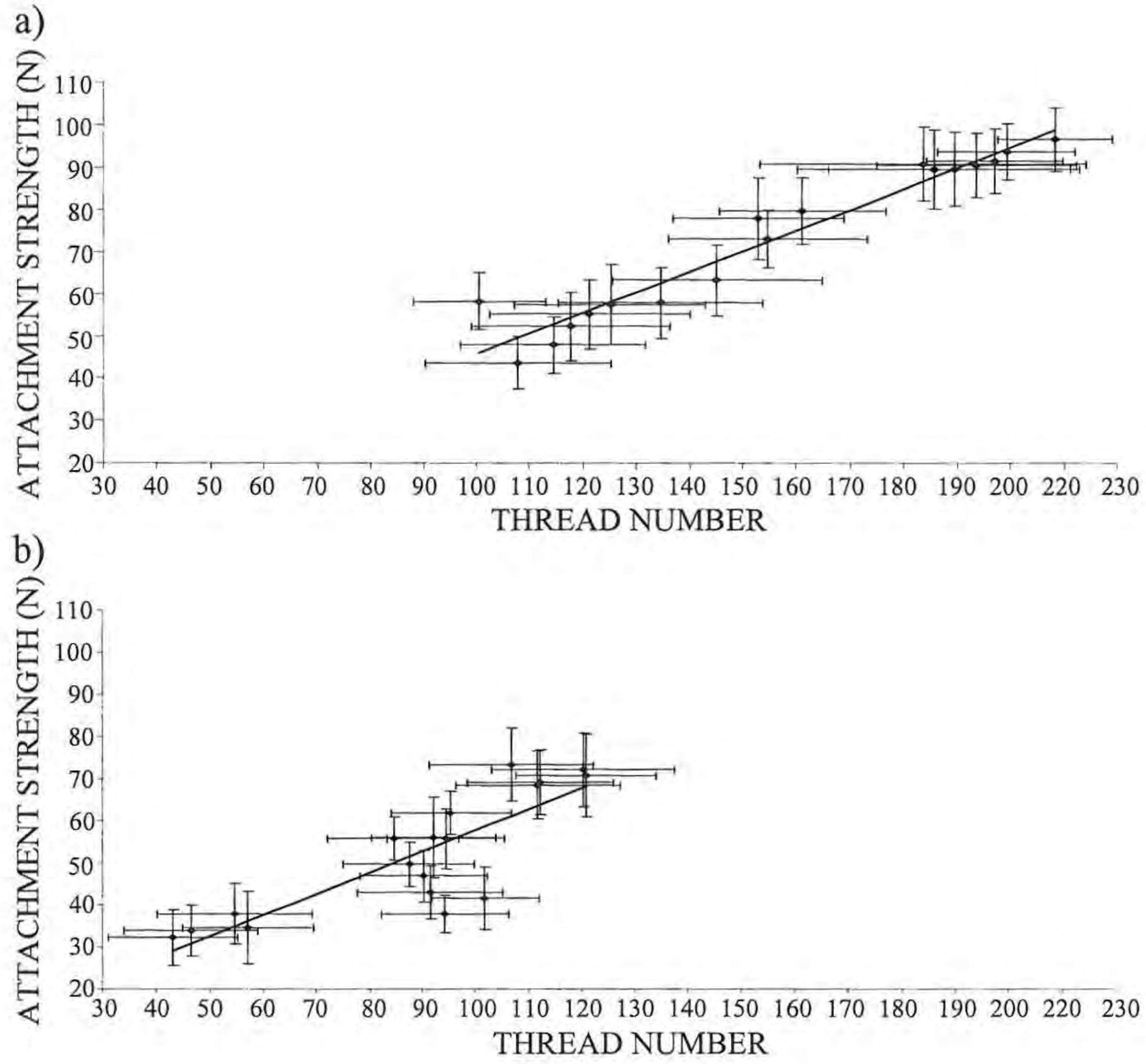

Fig. 3.12 Correlation between mean monthly attachment strength and mean monthly thread number for (a) *P. perna* ($y = 0.4905x - 3.3182$; $r^2 = 0.9381$) and (b) *M. galloprovincialis* ($y = 0.5054x + 7.2668$; $r^2 = 0.7002$).

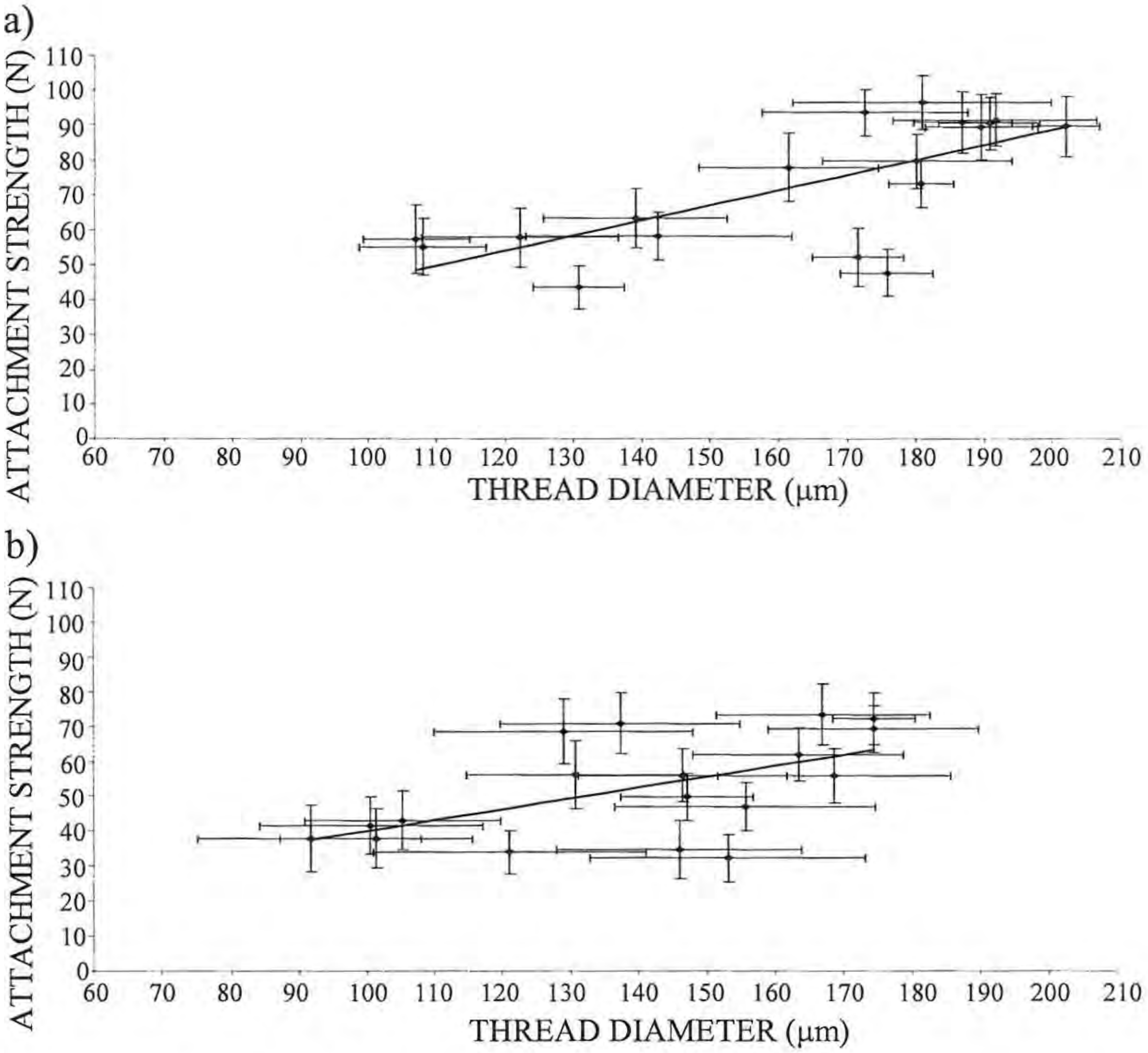

Fig. 3.13 Correlation between mean monthly attachment strength and mean monthly thread diameter for (a) *P. perna* ($y = 0.4334x + 2.118$; $r^2 = 0.5199$) and (b) *M. galloprovincialis* ($y = 0.3115x + 8.8785$; $r^2 = 0.3313$).

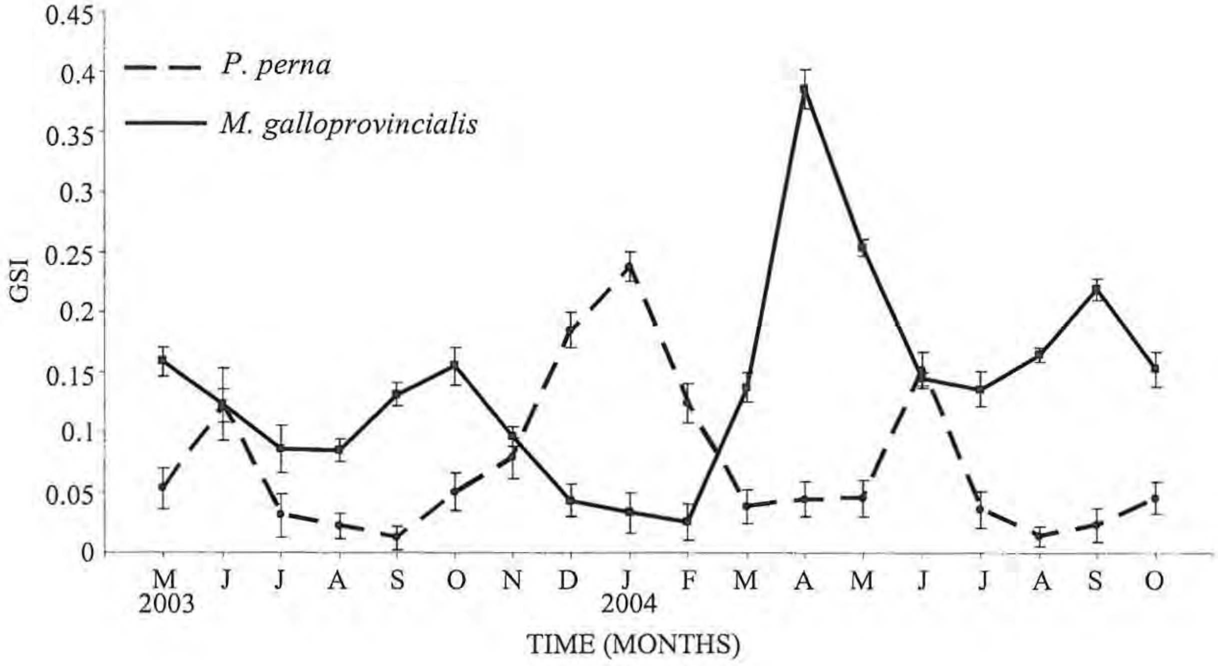

Fig. 3.14 Mean (± SD) monthly gonadosomatic index (GSI) of *P. perna* and *M. galloprovincialis* in Plettenberg Bay from May 2003 to October 2004.

Oceanographic data

Mean monthly wave height increased in late autumn-winter and decreased in late spring with minima in summer (i.e. January, February; Fig. 3.16). Mean monthly sea surface temperature (SST) showed a seasonal cycle with a maximum peak in late summer (21.8°C, February) and two minima in early spring 2003 (15.6°C, September) as winter 2004 (16.2°C, July; Fig. 3.17).

Statistical analyses

The best positive cross correlation between attachment strength and mean wave height was observed with zero lag for both species, and when attachment strength lagged one month behind (*P. perna*) or preceded wave height by one month (both species; Fig. 3.18 a, b). Attachment strength was negatively correlated with GI 1-2 months earlier (i.e. lag = 1-2 months) for *P. perna* and 6 months earlier (lag 6) for *M. galloprovincialis* (Fig. 3.19 a, b). Attachment strength of both species was negatively correlated with SST with zero lags and when it preceded SST by one or two months (Fig. 3.20 a, b). *P. perna* gonad index showed the highest positive correlation with SST at lags of zero and +1; *M. galloprovincialis* was not significantly correlated with SST (Fig. 3.21 a, b).

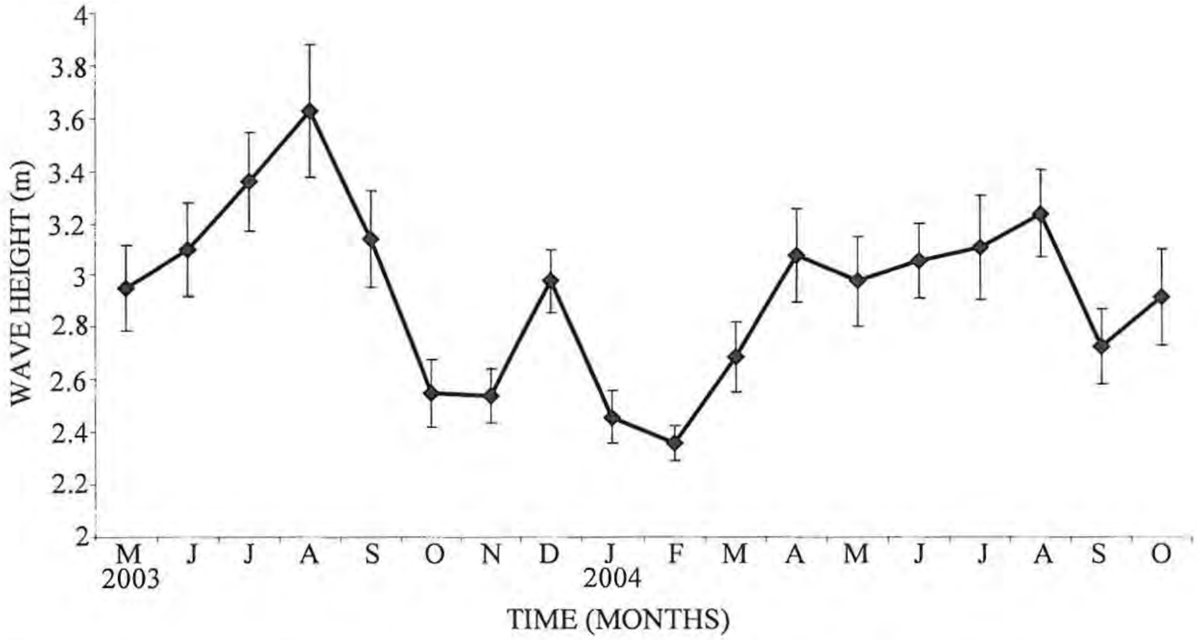

Fig. 3.16 Mean (± SD) monthly wave height in Plettenberg Bay from May 2003 to October 2004.

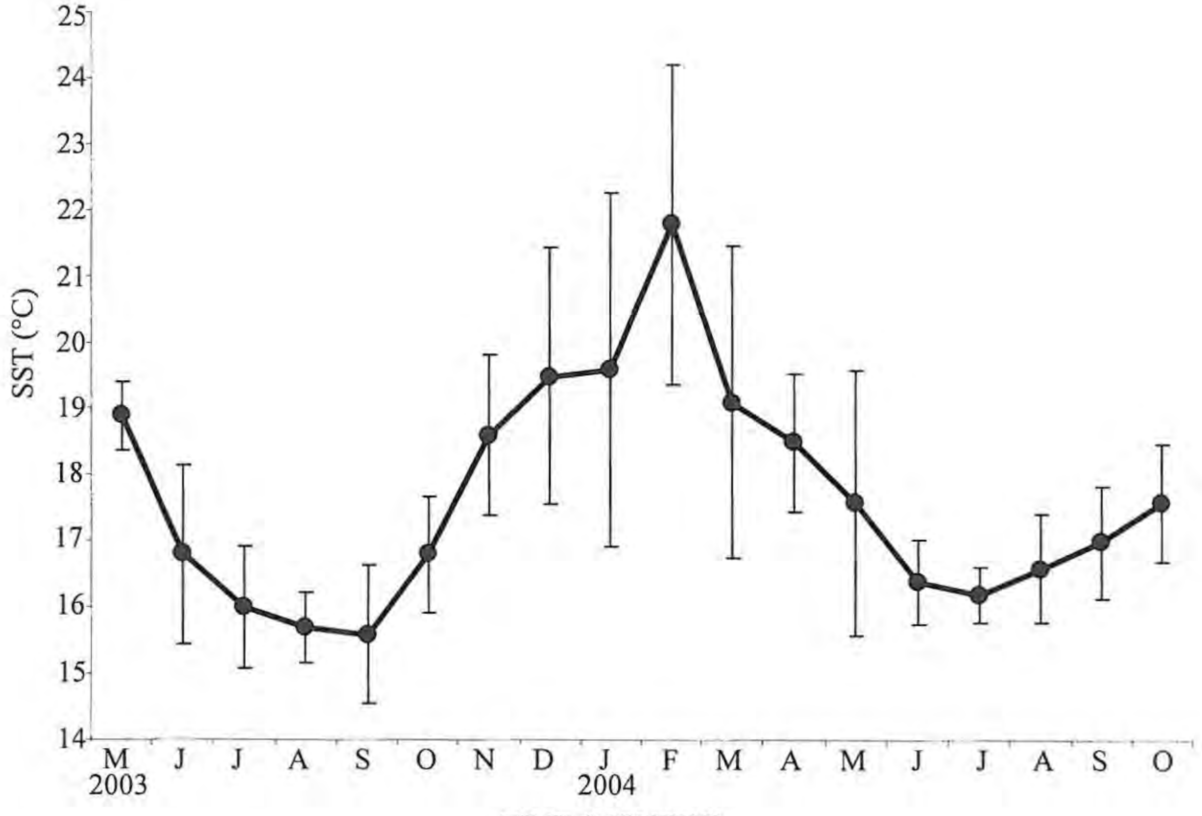

Fig. 3.17 Mean (± SD) monthly sea surface temperature (SST) in Plettenberg Bay from May 2003 to October 2004.

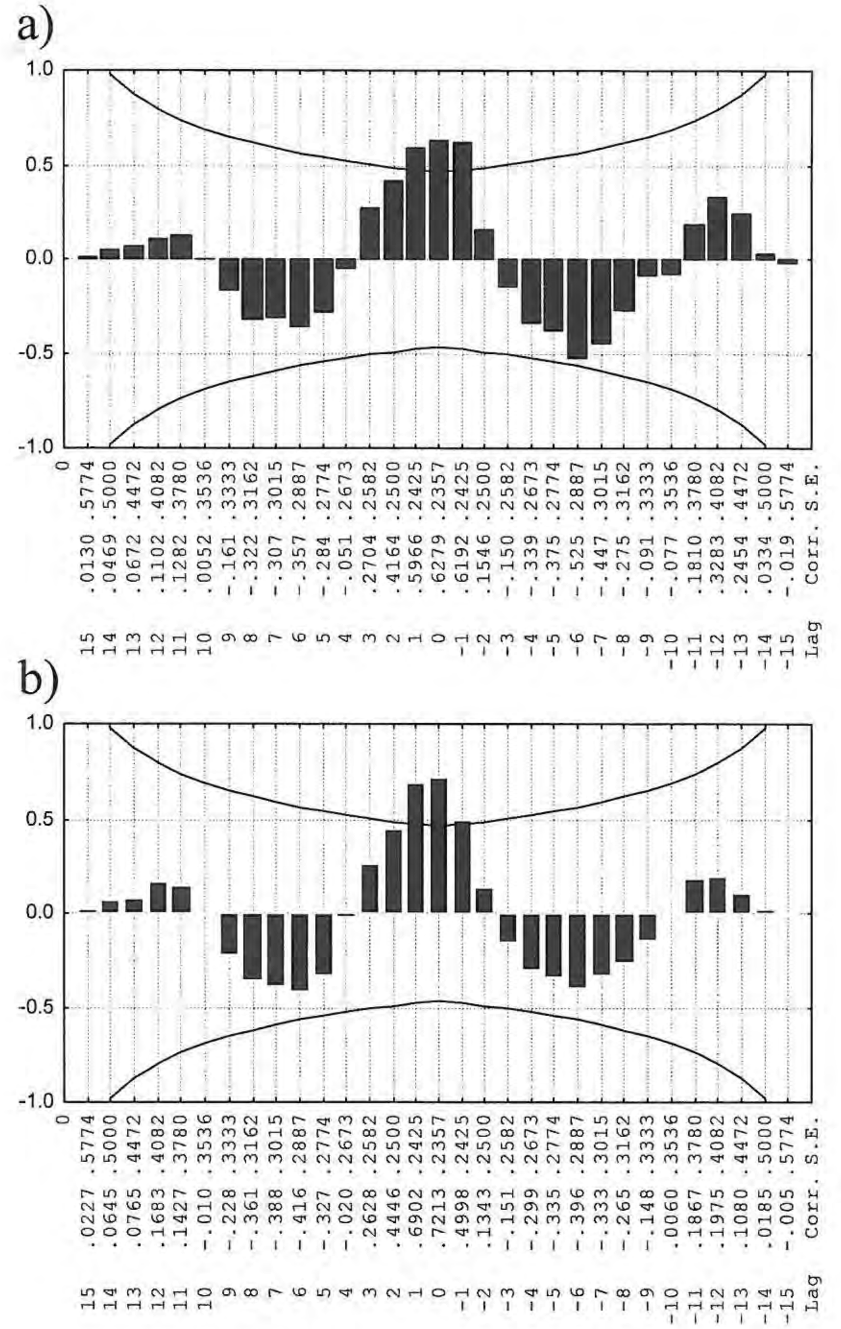

Fig. 3.18 Cross-correlation analyses of mean monthly attachment strength with wave height for (a) *P. perna*, (b) *M. galloprovincialis*. Bars are correlation coefficients; curved lines are approximate 95% confidences levels for the significance of each correlation.

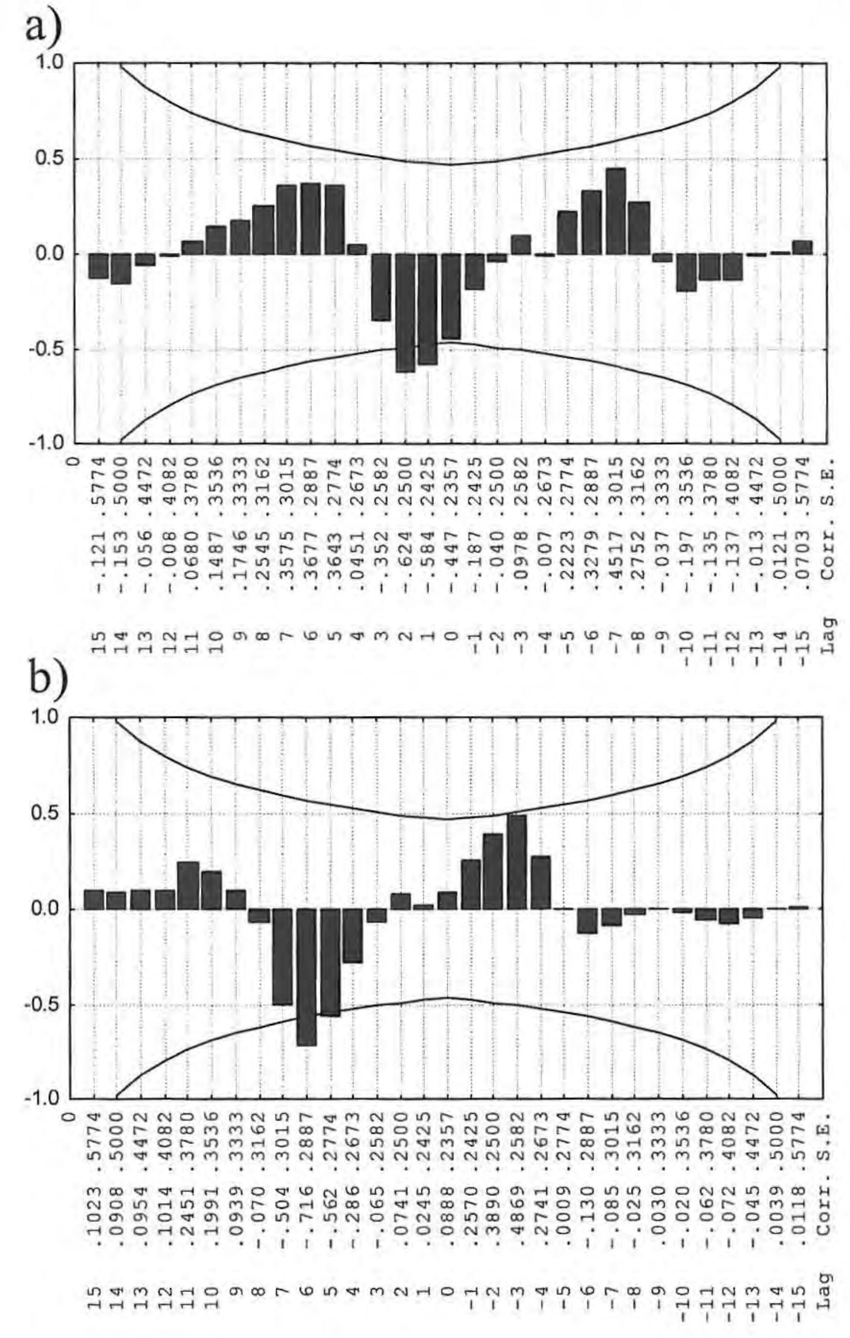

Fig. 3.19 Cross-correlation analyses of mean monthly attachment strength with GSI for (a) *P. perna*, (b) *M. galloprovincialis*. Bars are correlation coefficients; curved lines are approximate 95% confidences levels for the significance of each correlation.

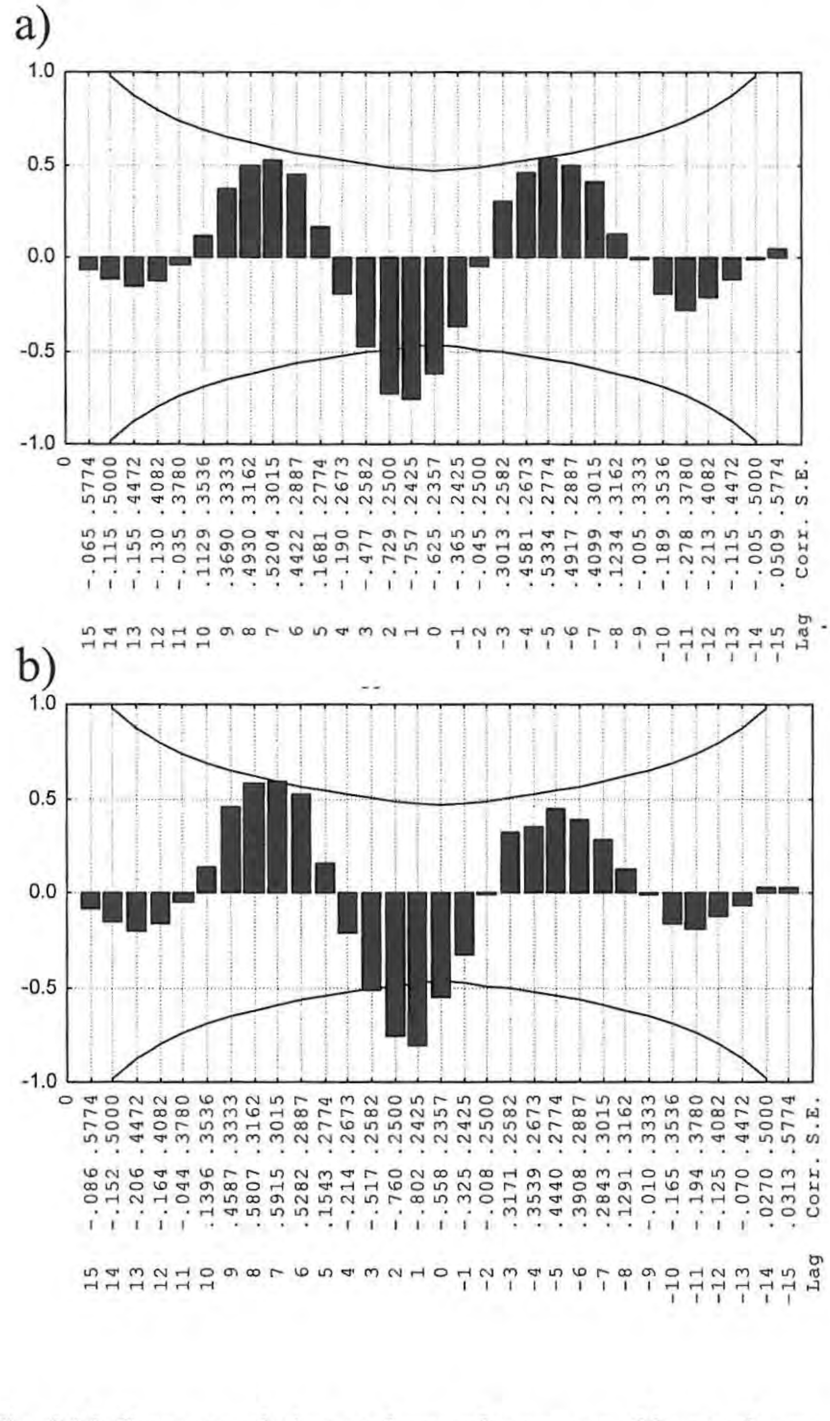

Fig. 3.20 Cross-correlation analyses of mean monthly attachment strength with SST for (a) *P. perna*, (b) *M. galloprovincialis*. Bars are correlation coefficients; curved lines are approximate 95% confidences levels for the significance of each correlation.

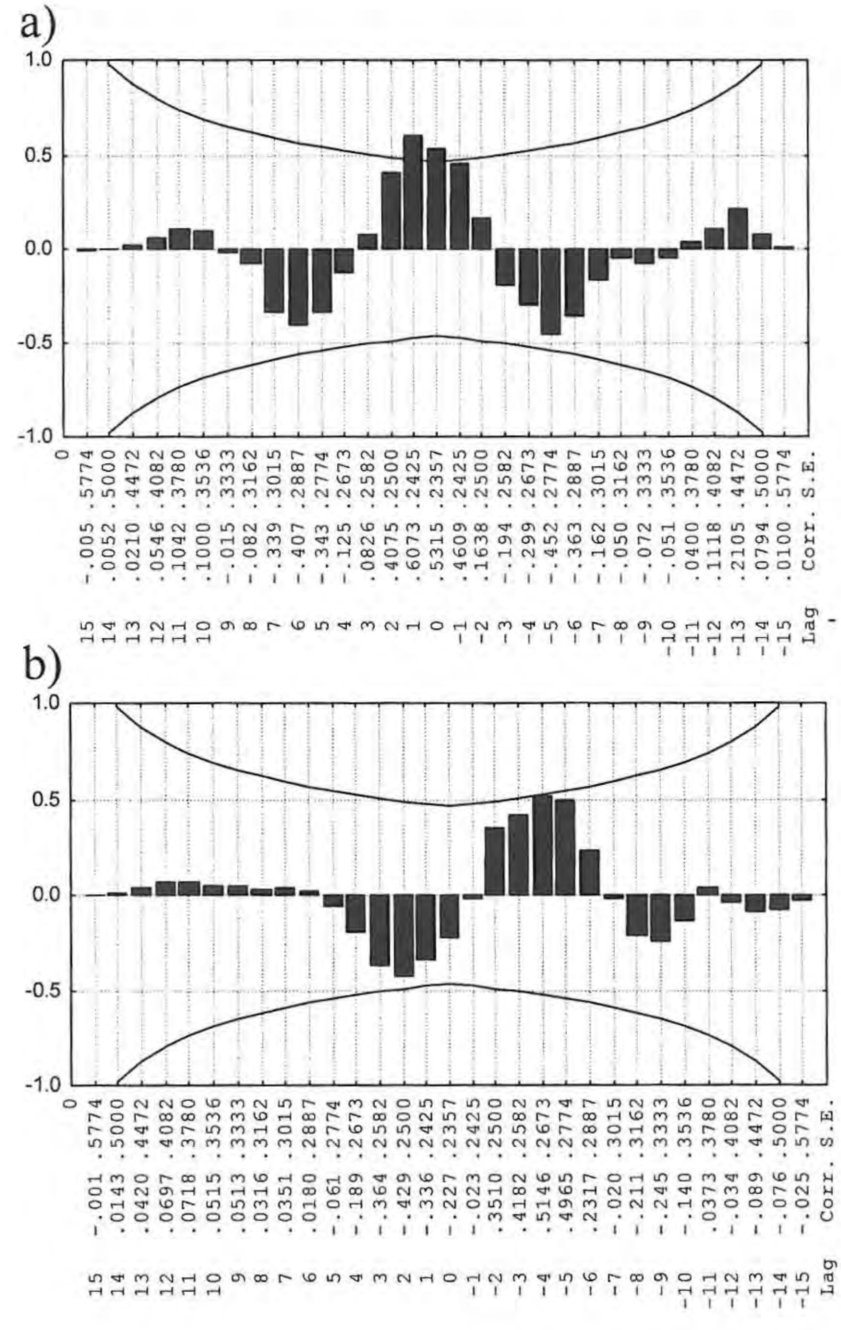

Fig. 3.21 Cross-correlation analyses of mean monthly GSI with SST for (a) *P. perna*, (b) *M. galloprovincialis*. Bars are correlation coefficients; curved lines are approximate 95% confidences levels for the significance of each correlation.

Discussion

The risk of dislodgement for a mussel is proportional to the square of the maximum water velocity it experiences. Dynamometers showed a vertical gradient of maximum wave forces in the mussel zone, with more intense hydrodynamic forces lower on the shore. This mirrors a vertical gradient in mussel attachment strength for both species. Moreover, *Perna perna* living lower on the shore will experience not only higher maximum water velocity but, during a single tide cycle, it will be subjected to hydrodynamic stress for a longer period than *Mytilus galloprovincialis* living in higher intertidal areas.

Hydrodynamic stress depends not only on water velocity but also on the shape of the mussel and the area over which the force acts. Scaled to mussel shell length, *M. galloprovincialis* has a wider shell than *P. perna*, increasing its hydrodynamic load. *M. galloprovincialis* has an $A_{p,min}$ 21.7% larger than *P. perna*, consequently, when exposing $A_{p,min}$ to drag or lift forces, it will experience about 22% greater hydrodynamic stress than *P. perna*. Possible morphometric differences between bed and solitary mussels of the same species were not readily obvious and were not investigated. The morphometric relationship for shell length vs. $A_{p,max}$ did not differ significantly between the two species; thus they will experience similar hydrodynamic drag forces when exposing $A_{p,max}$ to water flow.

The risk of dislodgement, of course, depends on a mussel's attachment strength. Hydrodynamic forces can affect the integrity of the byssus by damaging or breaking some of the byssal threads, or by dislodging the whole animal when the forces exceed total byssal strength. This study showed that, on average, *P. perna* is more strongly attached than *M. galloprovincialis*, and that the attachment strength of solitary mussels was higher than that of bed mussels. On average, the difference in attachment strength between the two species was maintained during the 18 months survey, but attachment strength for both was 40% higher in winter compared to summer. On average *P. perna* had 28% higher attachment strength than *M. galloprovincialis*, with a greater difference during winter. The wider shell of *M. galloprovincialis* and its weaker byssal attachment indicate a disadvantage during hydrodynamic stress that could explain the vertical zonation of the two species, and the restriction of *M. galloprovincialis* to the higher mussel zones, where water velocity is less intense and exposure to wave action is briefer. Due to their orientation and position on the rocks, bed and solitary mussels are primarily subjected to lift and drag force respectively. Laboratory studies showed that solitary mussels tend to arrange the posterior-anterior axis perpendicular to water flow and parallel to the substratum thus exposing a smaller area and reducing hydrodynamic stress (Dolmer and Svane 1994). In the field, this ideal orientation is difficult to maintain due to the variable and unpredictable flow of breaking waves. On average, a 4.5cm solitary mussel in a worst case scenario position (broadside to the flow) exposes a 60% greater surface area than a solitary mussel of the same size in a best case scenario position (end on), and will experience proportionally larger hydrodynamic stress. In contrast, bed mussels are arranged with the posterior-anterior axis perpendicular to the substratum, always exposing the smallest shell area to lift, which is independent of the direction of water flow. Consequently bed mussels are subject to a more predictable hydrodynamic environment (Denny 1987). On average, a solitary mussel is about 20% more strongly attached than a bed mussel; however its increased attachment strength is not enough to compensate for the higher hydrodynamic load, leading to a higher probability of dislodgement.

In this study, attachment strength was quantified by pulling the mussel normal to the substratum until dislodgment occurred; this method simulates lift forces, but not drag and thus it is an adequate method for bed but not for solitary mussels. Bell and Gosline (1996) predicted that the tenacity measured parallel to the substratum for a solitary mussel with uniformly distributed byssal threads is 53-57% of that measured perpendicular to the substratum. According to this prediction, solitary mussels would be even more prone to dislodgement. A mussel living in a densely packed bed exploits the physical protection from hydrodynamic stress and predation offered by nearby mussels and can decrease the metabolic cost of byssus production, potentially investing more energy into growth and reproduction. However, in a mussel bed, reduced food availability leads to slower growth and lower reproductive output (Okamura 1986, Frechette et al. 1989, O'Riordan et al. 1993, Butman et al. 1994). Moreover, mussel beds can form a very dense and intricate matrix of byssal threads attaching to the substratum and linking mussels to each other. When mussel density is very high, the byssal matrix can lose contact with the substratum, resulting in a slight elevation of the mussel bed - a hummock. These hummocks are relatively easily dislodged by wave action, forming gaps (e.g. Seed and Suchanek 1992, personal observation). Removed individuals deprive neighbouring mussels of shelter and of area to which byssal threads can attach, so that their attachment strength is lowered. In addition, being connected to each other, the hydrodynamic stress suffered by one mussel can be partially transferred to nearby individuals.

The primary mechanisms of increasing attachment strength are: increased thread number, increased thread thickness and increased thread strength. The third mechanism was not investigated. Our results show that thread number and diameter both contribute to species and position differences in attachment strength, and that the former is the major factor. The short-term study showed that on average *P. perna* was 25% more strongly attached than *M. galloprovincialis*; this difference is reflected by an increased byssal thread number and diameter, on average 22 and 14% respectively. Mussels living outside the bed are 20% more strongly attached than mussels in beds; this is again due to an increased thread number (on average 25%) and thread diameter (on average 12%). In *Mytilus edulis* seasonal changes of attachment strength are mirrored by thread number fluctuations (Carrington 2002). Our results showed that, despite the lower contribution than thread number (37%) to strength of attachment, thread thickness is also correlated to seasonal strength of attachment.

Generally, the plaque was the most common failure location for both species. Plaque fallibility could have been overestimated due to difficulties in distinguishing between plaque failure and failure of a thin layer of substratum to which the plaque adheres. This miscalculation would have been more pronounced in bed mussels than solitary mussels because of the higher amount of sediment (sand, shell fragments etc.) which tends to accumulate within a bed. The large amount of sediment could also explain the higher substratum fallibility of bed mussels compared to those not living in patches. Stem failure never occurred and our results show that the root was the least common failure location. Threads failed more in solitary mussels than in bed mussels; this is probably because the former are attached to the primary substratum, while at least some of the threads of the latter are attached to less secure secondary substratum. Root failure occurs only when the root is weaker than the total byssal thread strength and may be more common in solitary mussels because they have more and thicker threads attached to primary substratum.

The time required for mussels to react to changes in hydrodynamic stress can vary even within the same species; *M. edulis* attachment strength can both precede or react to increasing wave height (Price 1982; Carrington 2002). In the present study, the highest correlation of attachment strength with wave height was observed at zero lag for both species, showing that changes in attachment strength in both *M. galloprovincialis* and *P. perna* react to, rather than precede increased hydrodynamic stress. Despite the ability of mussels to produce a byssal thread in less than five minutes (personal observation) and thus to adjust their attachment strength in a relatively short time, there are periods when high hydrodynamic stress does not coincide with high strength of attachment (e.g. December 2003; Figs 3.9, 3.16). Seasonal variation in attachment strength could also negatively respond to temperature, with higher SST affecting the production of byssal threads (Van Winkle 1970). Mussel dislodgements are sporadic events, that can occur unpredictably throughout the year, but strong storms during winter render mussels particularly prone to hydrodynamic disturbances (Brundrit and Shannon 1989). Rius and McQuaid (in press) showed that, under experimental conditions in the field, mortality of *M. galloprovincialis* during a storm was higher than that of *P. perna* in the same zone, highlighting the ability of the indigenous *P. perna* mussel to withstand hydrodynamic stress better. In addition, mortality showed a marked decreased from the low zone, through the mid zone to the high zone, indicating that mussels on the low shore were more susceptible to storms.

The success of *M. galloprovincialis* as an invader is largely due to its fast growth and very high reproductive output, which is between 20 and 200% greater than that of the indigenous South African mussel species (van Erkom Schurink and Griffiths 1991). My results confirm this pattern; on average *M galloprovincialis* GSI was 38% higher than that of *P perna* while the maximum value was nearly double. The high reproductive output of *M. galloprovincialis* leads to densities up to 20 000 recruits per 100cm^2 on the west coast (Harris et al. 1998) and also on the south coast *M. galloprovincialis* recolonizes free space significantly faster (Erlandson et al. in revision). Gonad development can place high energetic demands on mussels (forming up to 90% of a mussel's energy budget; Seed and Suchanek 1992), while the replacement of decayed byssal threads can take up 8-15% of total energy expenditure (Griffiths and King 1979; Hawkins and Bayne 1985). Thus the high reproductive effort of *M. galloprovincialis* may come at the cost of poorer attachment strength.

Water flow at wave-exposed shores not only puts mussels at risk of dislodgement and of damage to their shells, it also delivers more food compared to sheltered sites (Sanford et al. 1994; Bustamante and Branch 1996; Dhalhoff and Menge 1996). Food provides energy for metabolically costly processes such as byssal thread replacement, increases in shell thickness and gamete synthesis (Griffiths and King 1979; Hawkins and Bayne 1985; Seed and Suchanek 1992). Consequently, attachment strength could react not only to wave forces, but could also be influenced by energetic constraints due to limited food delivery or to energetic investment in other physiological needs. Both species showed a major and a minor spawning event each year, and in almost all cases the major event was preceded by gamete production during periods of relatively low hydrodynamic stress.

The only exception was December 2003. In contrast, minor spawning events (i.e. those following minor peaks in June for *P. perna* and October 2003 and September 2004 for *M. galloprovincialis*) coincided with periods of relatively high wave height. This suggests that more energy was used to increase attachment strength, consequently limiting gonad tissue development. The major period of gamete production was more protracted for *P. perna* (four months) than for *M. galloprovincialis* (two months) and the GSI reached higher values for the invasive species. Differences in attachment strength between the two species can be seen as a consequence of the evolutionary strategy of the invasive mussel, which directs most of its energy into fast growth and high reproductive output.

Many attempts have been made to determine the factors controlling mussel gametogenesis and spawning. Of these, temperature and food supply seem to be particularly important (Hilbish and Zimmerman 1988). Rising, falling and fluctuating temperatures have all been reported to stimulate spawning in *Mytilus* (Wilson and Seed 1974; Hines 1979; Wilson 1987; Seed and Suchanek 1992). A few studies have suggested that spawning occurs only within a critical temperature range (Zhanget al. 1980; Sprung 1983), while others did not find any relation between spawning and temperature (Sunila 1981). Newell et al. (1982) showed that populations of *Mytilus edulis* living at the same latitude and experiencing the same temperature regime, exhibited large temporal differences in gametogenesis and suggested that these differences were due to variations in food availability. Bayne and Worral (1980) showed that gamete production in *M. edulis* is stimulated by increasing temperature only if enough food is available. In addition, reproduction can be influenced by several other environmental factors, such as rough weather, phase of the moon and tidal fluctuations, salinity and by endogenous factors (Battle 1932; Suchanek 1978; Parulekar et al. 1982; Wilson 1987; Seed and Suchanek 1992).

In South Africa, different spawning periods are reported in different regions for *P. perna* and *M. galloprovincialis* (Berry 1978; van Erkom Schurink and Griffiths 1991). *P. perna* and *M. galloprovincialis* individuals analyzed in this study were sampled from the same restricted area at the same time, and they are consequently presumed to be exposed to the same environmental factors. Despite this, the two species showed different reproductive timing, suggesting that species-specific endogenous factors or responses to environmental conditions play a crucial role in regulating gametogenesis and spawning. *P. perna* gonad index values were positively cross-correlated, with lags of 0 and +1, with sea surface temperature. Gonad index increased in September, reaching its highest values in January and sea surface temperature mirrors this fluctuation. In contrast, the minor gonad index peak took place during a temperature decrease. The gonad index of *M. galloprovincialis* was not significantly cross-correlated with sea surface temperature with any lag period. If we consider mussel spawning periods as sudden decreases of gonad index from relatively high values, *M. galloprovincialis* spawning events always took place at temperatures ranging between 16.4 and 19.5°C, while *P. perna* spawned at the highest and the lowest temperatures recorded in the 18 months of survey.

In conclusion, attachment strength of both species constantly tracked fluctuations in wave height, while GSI was negatively correlated with attachment strength. This suggests that gamete production is energetically constrained by the energy needed to increase attachment strength during periods of high wave action. This study shows that two coexisting species can maintain different energetic strategies and that, although the reproductive strategy of *M. galloprovincialis* seems to have played a crucial role in its ability to invade rocky shores worldwide, it can also set limits to its invasive ability. Our results and the decreased growth rates and condition values shown for *M. galloprovincialis* on extremely exposed shores on the west coast of South Africa (Steffani and Branch 2003c), support the prediction that the ability of *M. galloprovincialis* to displace *P. perna* will be more pronounced at sheltered or moderately exposed sites. The results of this study, and those of Bownes and McQuaid (submitted) and Rius and McQuaid (in press), contrast with the situation on the cold water, upwelling dominated west coast where *M. galloprovincialis* extends to the low shore (personal observation). A possible explanation for this difference is that the intense upwelling on the west coast and the consequent higher concentrations of phytoplankton compared to the south coast (Shannon 1985; Brown et al. 1992; Bustamante et al. 1995) can provide the invasive species with enough energy to increase attachment strength and to withstand the high hydrodynamic stress that characterises the lower shore. Another explanation comes from field experiments indicating that the exclusion of *M. galloprovincialis* from the low zone on the south coast may be due not only to the effects of wave action, but also to competitive interaction with *P. perna* (Rius and McQuaid, in press). Moreover, the low biodiversity which characterises the west coast is a broad community feature generally seen as promoting the success of invasive species (Bustamante and Branch 1996; Mack et al. 2000).

CHAPTER 4

POPULATION GENETIC STRUCTURE OF *Perna perna* AND *Mytilus galloprovincialis*

Introduction

The determination of larval dispersal distances and larval origins is a central challenge in contemporary marine ecology. In particular, when adult organisms are sessile or exhibit limited dispersal, the dispersal of early life stages is critical to their population dynamics (Sale 1991; Caley et al. 1996). Information about larval dispersal is available for very few species (Shanks et al. 2003) and most of these are species that disperse over very short distances and can be tracked visually (Olson 1985; Stoner 1992). Methods of estimating dispersal involve an analysis of either the dispersal process or of the resultant population structure (Largier 2003). Population genetics can provide information on the degree of exchange between populations through a direct estimation of gene flow via larval dispersal (Edmands et al. 1996; Burton 1998). Gene flow and population subdivision leave signatures in the spatial distribution of neutral molecular variation that can be utilized to estimate the amount of gene exchange (Slatkin 1985) and to reconstruct biogeographical history (Avise et al. 1987).

Population biologists adopted protein electrophoresis to measure genetic variation in the 1960s when Harris (1966), Lewontin and Hubby (1966) and Johnson et al. (1966) demonstrated that electrophoretic surveys of protein variation could be used to estimate levels of genetic variation within a species and to describe population structure. More recently, the development of the polymerase chain reaction (PCR) has allowed the analysis of DNA sequences (Innis et al. 1990). PCR is used to amplify greatly the number of copies of specific fragments of DNA from complex DNA samples, thereby facilitating the subsequent analysis of the amplified fragments (Innis et al. 1990). The primary requirement for using PCR resides in the development of short (about 20 bases long)

DNA primers complementary to the ends of the DNA sequence of interest. High levels of DNA sequence conservation across broad taxonomic boundaries are common in numerous places in the genome. This allows the development of "universal primers", which are gene-specific primers that allow PCR amplification of DNA from a broad array of taxa. A number of such primer sequences are known in the literature (e.g., Kocher et al. 1989; Palumbi et al. 1991; Folmer et al. 1994). Allozymes and mitochondrial DNA have become widely used tools in molecular phylogeny and phylogeography (Avise 2000). Allozyme studies of marine invertebrates have often revealed little indication of population subdivision, whereas mtDNA data have more frequently identified population genetic differentiation. For example, discrepancies between results obtained from allozymes and mtDNA analyses were reported for blue mussel species, *Mytilus galloprovincialis* (Karakousis and Skibinski 1992), *M. edulis*, *M. trossulus*, *M. galloprovincialis* (Quesada et al. 1995), and oysters, *Crassostrea virginica* (Reeb and Avise 1990; Karl and Avise 1992). Explanations for such discrepancies have focused on the higher mutation rate of mtDNA (Brown et al. 1979; Powell et al. 1986), undetected genetic variation in allozymes (Nei 1987), balancing selection acting on allozymes (Karl and Avise 1992; McDonald et al. 1996), neutrality of allozymes and directional selection acting on mtDNA (Hare and Avise 1998), as well as historical factors resulting from the different evolutionary forces acting on mitochondrial and nuclear genomes (Rigaa et al. 1997).

The animal mitochondrial genome exhibits several characteristics that make it suitable for population genetic and phylogenetic analysis. First, substitution rates are generally high, with much polymorphism occurring as nucleotide substitutions at third codon positions

(Brown et al. 1979). In mammals, substitutions accumulate up to 10 times faster in mitochondrial DNA (mtDNA) than in single-copy nuclear DNA (scnDNA; Brown et al. 1979, 1982). Rates of mtDNA evolution, however, have been shown to be higher in mammals than in fish, amphibians and a range of invertebrates (sea urchins, insects and nematodes; Lynch & Jarrell 1993), which sometimes exhibit substitution rates that are roughly similar between invertebrate mitochondrial and nuclear genomes (e.g. Vawter & Brown 1986; Sharp & Li 1989; Lynch & Jarrell 1993). Second, animal mitochondrial genomes are generally maternally inherited and non-recombining. Hence, the entire mitochondrial genome has the same historical pattern of common descent (Wilson et al. 1985). Although many mitochondrial genes are highly conserved at the amino acid level, third codon position substitutions are often silent and thus selectively neutral (Brown et al. 1979), providing many potentially informative phylogenetic characters. The mtDNA transmission system of mussels of the families Mytilidae (sea mussels) and Unionidae (freshwater mussels) is exceptional because it possesses two mtDNA lineages, one transmitted through the egg, the F lineage, and one transmitted through the sperm, the M lineage (Skibinski et al. 1994a, 1994b; Zouros et al. 1994a, 1994b; Liu et al. 1996). Sperm mtDNA is delivered into the oocyte and eliminated within the first 24 h after fertilization in females (Sutherland et al. 1998) but is retained in males; consequently females are homoplasmic for the F genome, whereas males are usually heteroplasmic for both genomes, with their gonads dominated by the M molecule and their somatic tissues dominated by the F molecule (Zouros et al. 1994*b*; Garido-Ramos et al. 1998; Steward et al. 1995; Passamonti and Scali 2001). This phenomenon has become known as doubly uniparental inheritance (DUI; Zouros et al. 1994*a*). M and F DNA sequences normally differ by as much as 20% (Hoeh et al. 1997).

In this study, I explore the population genetic structure of the two dominant intertidal mussel species in South Africa, the invasive *M. galloprovincialis* and the indigenous *Perna perna*, over the entire South African and southern Namibia coastlines. The potential for larval dispersal among populations can be assessed with knowledge of pelagic larval duration and behaviour and movement of water masses. Both species reproduce by means of a planktotrophic larval stage that disperses in the water column for a period (weeks to months) of feeding, growth and morphological development, before settling to the substratum and being recruited in an adult population. Thus, they have high dispersal potential. The swimming abilities of mussel larvae are very limited and they are considered to have only a very limited influence on their actual dispersal. Consequently these larvae are often considered passive particles, mainly transported by currents (Scheltema 1986, Shanks 1995), though Shanks and Brink (2005) have recently shown that larval behaviour of certain bivalves can be critical in determining dispersal during upwelling events.

The primary oceanographic influence on the east coast of South Africa is the Agulhas Current. This warm current flows down the east coast of South Africa (from 27°S to 40°S) at rates of 10 to 20 km d^{-1}, following the 200 m bathymetry line of the continental shelf from Maputo to the tip of the Agulhas Bank (Lutjeharms 2004; Fig. 4.1a). At Cape Padrone the shelf becomes wider, pushing the current farther from the coast (Fig. 4.1a). Further west the current undergoes a dramatic retroflection, moving hundreds of miles away from the mainland. About once every two months the retroflection loop closes on itself forming a retroflection eddy (or Agulhas ring) about 320Km in diameter, which is transported north-westward across the Atlantic Ocean by the Benguela Current (Lutjeharms 1998). Agulhas rings carry water with thermal and salinity characteristics of the Indian Ocean. The Agulhas Current has a volume flux of about 65 million cubic meters per second; its surface speed is about $2ms^{-1}$ and it becomes slower with depth (a few cms^{-1}at 2500m). The Agulhas Current is about 60 to 100Km wide (Lutjeharms 1998). The inshore edge is well defined by a temperature increase (up to 10°C), while the seaward edge is less sharp with a gradual decline of temperature and speed. The surface temperature is on average 28°C in summer and drops to about 20°C in winter (Lutjeharms 1998). The inshore thermal front of this current varies geographically and in time, and may alter patterns of along-shelf larval dispersal. It usually lies 14 to 38km offshore, but it can flow onto the coast at 0 to 1km offshore (Goschen and Schumann 1990). Downstream of Durban the continental shelf is narrow (Fig. 4.1a). Over this shelf region currents are parallel to the Agulhas, but in both alongshore directions, suggesting that they are driven largely by wind (Anderson et al. 1988). On the east coast, where the shelf width is minimal (about 10Km), the Agulhas Current can overpower these counter currents (Lutjeharms 2004) consequently influencing the along shore transport of larvae. On the south coast, where the shelf is wider, the Agulhas Current still influences an extensive area of the shelf (Schumann 1987; Goschen and Schumann 1988) though wind becomes the main forcing function (Schumann 1981; McQuaid and Phillips 2000).

The coastal environment of western South Africa, Namibia and southern Angola is strongly influenced by the Benguela Current. From Cape Point it flows northwards until offshore of Lüderitz, where the main flow deflects to the northwest, moving away from the coast (Peterson and Stramma 1991; Wedepohl et al. 2000; Fig. 4.1a). It is bounded by two warm currents, the Angola current in the north and the Agulhas Retroflection in the south. The Benguela Current is characterized by Ekman-driven coastal upwelling. The intense and consistent upwelling off Lüderitz (27-28°S) separates the Northern Benguela from the Southern Benguela and creates a semi-permanent environmental barrier (Boyd and Cruickshank 1983; Agenbag and Shannon 1988).

Gene flow may be limited by selection as well as by dispersal (Hilbish and Koehn 1985; Koehn et al. 1980; Powers 1987). The South Africa coastline covers a wide range of climatic and oceanic conditions and can be divided into biogeographic regions that support a great diversity of algae and animals. The boundaries of these regions depend on the organisms investigated (Stephenson and Stephenson 1972; Day 1981; Potter et al 1990; Prochazka 1994; Turpie et al. 2000), but in general they can be defined as a Cool Temperate West Coast Province, a Warm-Temperate South Coast Province and a Subtropical East Coast Province (Harrison 2002). Stephenson and Stephenson (1972) examined the distribution of rocky-shore biota and defined three biogeographic provinces: a West Coast Province down the west coast to Cape Point, characterized by cold-waters forms; a warm-temperate South Coast Province, with mainly cooler-water species, from approximately Cape Point to Port St Johns; and a subtropical East Coast Province, characterized by warm-water species, from approximately Port St Johns to Mozambique(Fig. 4.1a). Based on an analysis of rocky shore invertebrates, Emanuel et al. (1992) divided the South African coast into three zoogeographic regions (Fig. 4.1b): from Lüderitz (Namibia) to Cape Point (cool-temperate Namaqua Province); from Cape Point eastward to East London (warm-temperate Agulhas Province); from East London north to Mozambique (subtropical Natal Province).

In this study, I sequenced mitochondrial DNA (mtDNA) from *M. galloprovincialis* and *P. perna* populations along the South African and Namibian coastline to reconstruct haplotype phylogenies. The possible influence of biogeographic regions on phylogeographic patterns through selection, and the influence of local currents as oceanographic barriers regulating dispersal is investigated.

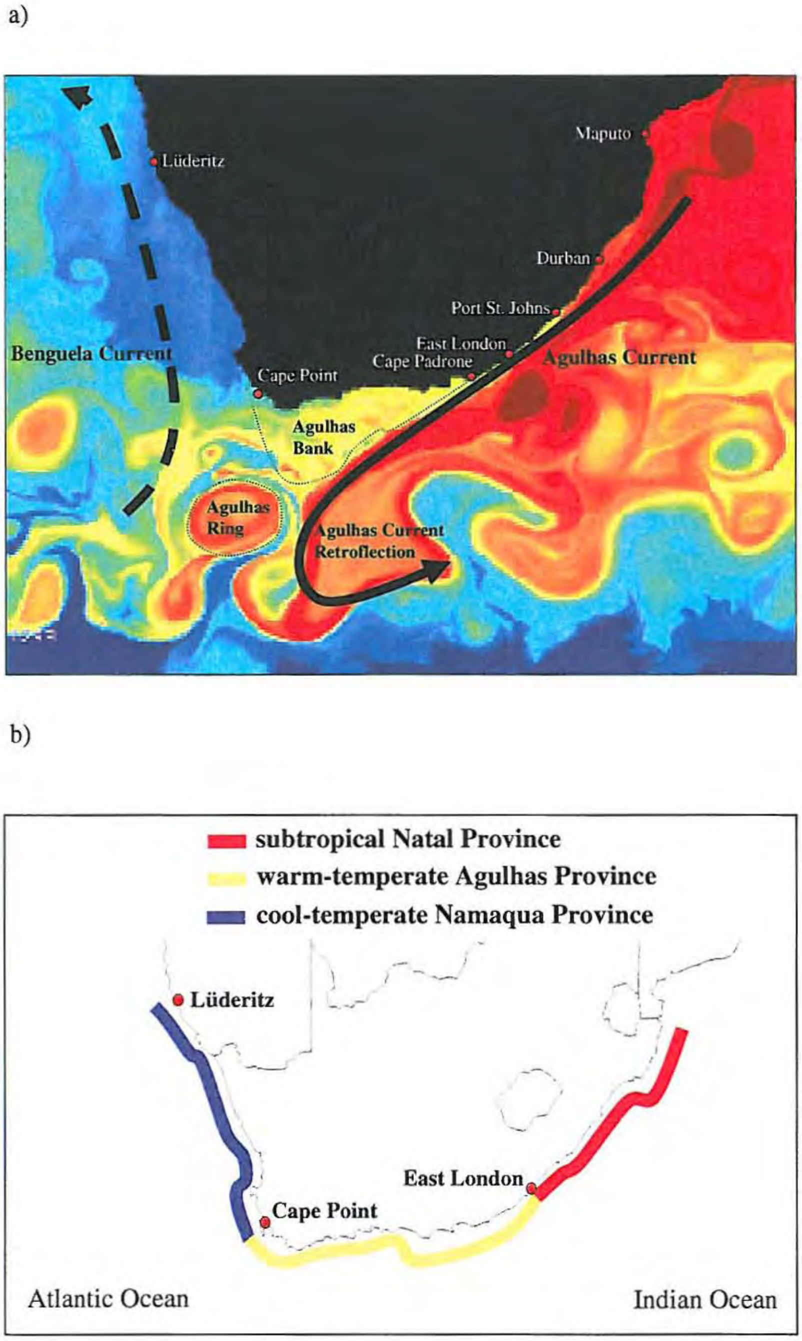

Fig. 4.1 a) Sea Surface Temperature (SST) image showing Agulhas Current and Benguela Current dynamics; b) South African coastline divided in biogeographical regions according to Emanuel et al. (1992).

Materials and methods

Sampling, DNA extraction, amplification and sequencing

Populations of *Mytilus galloprovincialis* and *Perna perna* were sampled at 11 and 14 sites respectively along the Namibian and South African coasts (Fig. 4.2). Mussels were opened in the laboratory and a piece of gonad tissue was examined under the microscope to determine the sex of the animal by the presence of eggs or sperm. Because of doubly uniparental inheritance (DUI), only female individuals were used in this study. Whole genomic DNA was extracted from approximately 1mm^3 of gonad tissue (attached to the mantle) using a standard phenol-chloroform extraction method and re-dissolved in 50μl water. The primers LCOI 1490 (5'-GGTCAACAAATCATAAAGATATTG-3') and HCO 2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994) were used in a polymerase chain reaction (PCR; Mullis et al. 1986; Saiki et al. 1988) to amplify a portion of the mitochondrial cytochrome oxidase subunit I gene (mtDNA COI). Amplifications were performed in a 100μl solution containing 10-100μg DNA, 0.4μM each primer, 5μl Qiagen PCR buffer, 200μM each dNTP, and 2.5U Taq DNA polymerase (Qiagen). The PCR cycling profile had an initial denaturation step at 94°C for two min., 35 cycles of denaturation at 94°C for 60sec., annealing at 54°C for 60sec, extension at 72°C for 90sec, and a final extension at 72°C for 5min. PCR products from each individual were purified with a Qiaqick gel extraction Kit (Qiagen) and cycle sequenced in the forward and reverse direction with the same primers used in the amplification, using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystem) and sequenced on a ABI 3100 genetic analyser.

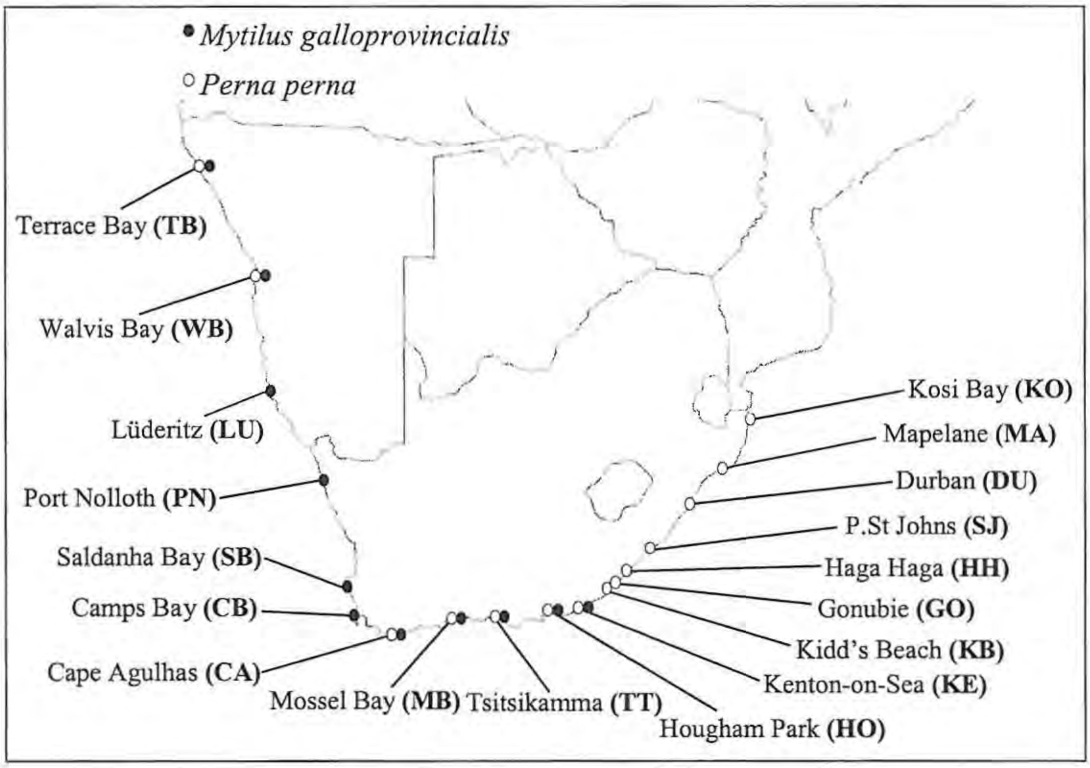

Fig. 4.2 Sample locations for *M. galloprovincialis* and *P. perna*. Location codes in brackets

Data analysis

Alignment of 400-bp sequence data was carried out using ClustalW (Thompson et al. 1994). DNA sequences were translated into amino acid data using the invertebrate mitochondrial genetic code to verify their mitochondrial origin. One has confidence that this is the case because: 1) there was at least one reading frame with no stop codon, 2) all translated sequences matched with published records of COI sequences in GenBank, and (3) no variable nucleotide positions consistently showing double peaks were encountered in chromatograms, as might be expected in the case of nuclear pseudogenes.

PAUP* version 4.0b10 (Swofford 2002) was used to generate neighbour-joining trees using the most suitable model of sequence evolution for each species as suggested by the Akaike Information Criterion (AIC) as implemented in the program MODELTEST 3.06 (Posada and Crandal 1998). Bootstrap values were generated from a re-sampling of 1000 neighbour-joining trees.

All analyses that involved estimates of sequence divergence of *M. galloprovincialis* used the Hasegawa-Kishino-Yano (HKY+G) model of nucleotide substitutions while for *P. perna* the Tamura-Nei (TrN) model of nucleotide substitutions was used (Hasegawa et al. 1985; Tamura and Nei 1993). Gamma distribution parameters (α) estimated in MODELTEST were 0.335 and 0.22 for *M. galloprovincialis* and *P. perna* respectively.

A triangular matrix of p distances among *P. perna* individuals was generated using PAUP* version 4.0b10 (Swofford 2002) and imported into Exel to calculate mean intra and inter lineages average sequence divergence.

Isolation by distance

mtDNA lineages identified using genealogical reconstructions were examined for evidence of isolation by distance (Slatkin 1993). ARLEQUIN version 2.001 (Schneider et al. 2000) was used to calculate F_{st} values (sequence divergence among haplotypes; Excoffier et al. 1992) among populations associated with specific lineages. Pairwise F_{st} values were related to corresponding pairwise geographic distances; the strength and significance of the relationship between genetic differentiation and geographical distance was assessed by performing Mantel tests using the program MANTEL for Windows version 1.16 (Cavalcanti 2005). The geographic distance between populations was measured as the shortest continuous water-surface distance. The significance of the Mantel statistic Z is tested by a permutation procedure in which values in one data matrix are randomly reshuffled (20 000 permuted data-sets were created).

Results

Sequence characteristics

Sequence data (400-bp) from 110 and 140 individuals for *Mytilus galloprovincialis* and *Perna perna* respectively were aligned and analysed. For M. *galloprovincialis,* 29 nucleotides sites were polymorphic, 18 of which were parsimony informative; a total of 21 haplotypes were identified. *P. perna* exhibited 50 unique haplotypes and 58 polymorphic sites, 27 of which were parsimony informative.

Phylogeographical patterns

When the COI haplotypes of *P. perna* were used to produce a phylogenetic tree (Neighbour-joining tree; Fig. 4.3), they formed two distinct lineages. One lineage included samples from Terrace Bay to Haga Haga (i. e. from the Namibian coast to the south-east South African coast). Samples of the other lineage extended from Kosi Bay to Kenton-on-Sea (South African east coast). The distributions of the two *P. perna* lineages thus overlap for about 200Km between Haga Haga and Kenton-on-Sea (south-east coast; Fig. 4.4). Average within lineage sequence divergences were 1.1% and 1% for the east and western lineage respectively. Average sequence divergence between the two lineages was 2.9%.

The COI Neighbour-joining tree of *M. galloprovincialis* comprised only one lineage from Terrace Bay to Kenton-on-Sea (Fig. 4.5).

P. perna showed greater nucleotide diversity than *M. galloprovincialis*. An unrooted haplotype network of *P. perna* sequences shows two main haplotypes. Haplotypes no. 1 and no. 2 are the most common and ancestral haplotypes in populations from the eastern lineage (east coast) and the western lineage (south and west coasts) respectively (Fig.

4.6). Haplotype 1 was never sampled in the Namibian populations, and south of Gonubie it was found in only two individuals (from Kenton-on-Sea and from Kidd's Beach; Fig. 4.7). Haplotype 2 was the most common haplotype on the south coast and was also sampled once in Walvis Bay. It was never found farther north than Kidd's Beach (Fig. 4.7). The western lineage shows a higher nucleotide diversity than the east lineage; many haplotypes are several (up to 6) mutational steps away from the central haplotype, and are presumably ancient in origin (Fig. 4.6). In the eastern lineage, most haplotypes differ from haplotype 1 by one change, with the exception of haplotype 12 which differs by 5 steps (Fig. 4.6). Haplotype 3 was most common on the south coast but was also sampled at Terrace Bay and Haga Haga. Haplotypes restricted to one site (private haplotypes) were found at all locations, and were more frequent in the western lineage than to the eastern lineage. The highest frequencies (80-90%) were found in the two Namibian populations (Fig. 4.7). Haplotype 4 was private to the east coast. As with haplotype 1, haplotype 5 was sampled on both the south and the east coasts. Haplotype 6 was private to Mapelane and Durban. Haplotype 10 was private to Cape Agulhas and Mossel Bay. Haplotype 11 was only found twice, in Walvis Bay and Kidd's Beach which are thousands of kilometers apart.

Low haplotype diversity characterised populations of *M. galloprovincialis*. Haplotype 1, which occupies a central (ancestral) position in the unrooted haplotype network, was the most common at all sites (Fig. 4.8, Fig. 4.9). All other haplotypes differed from haplotype 1 by one to three changes, except for haplotype 2 which differed by 6 changes (Fig. 4.8). Private haplotypes were found at all locations except three sites from the west coast (Terrace Bay, Luderitz, Saldanha Bay). Haplotype 3 was private to Mossel Bay and Cape

Agulhas. Haplotypes 4, 5 and 6 were all private to the Namibian and South African west coasts.

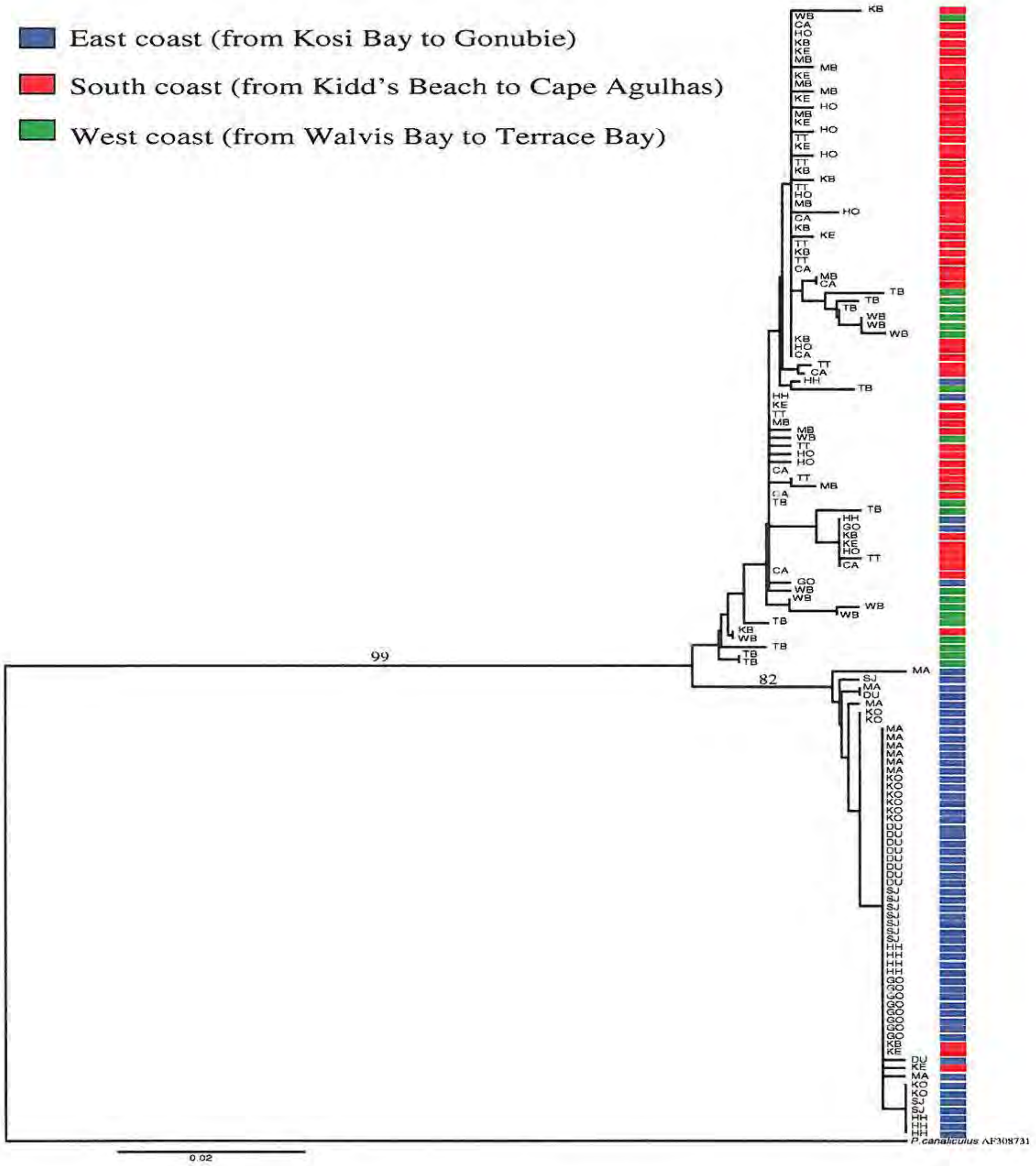

Fig.4.3 Neighbour-joining phylogram based on sequences from cytochrome oxidase I (COI) for *P. perna*. The tree was rooted using *Perna canaliculus* as an outgroup. Clade support from 1000 bootstrap replications (>80%) was added to some branches. See Fig. 4.2 for name codes. The geographical region where each haplotype was found is shown above.

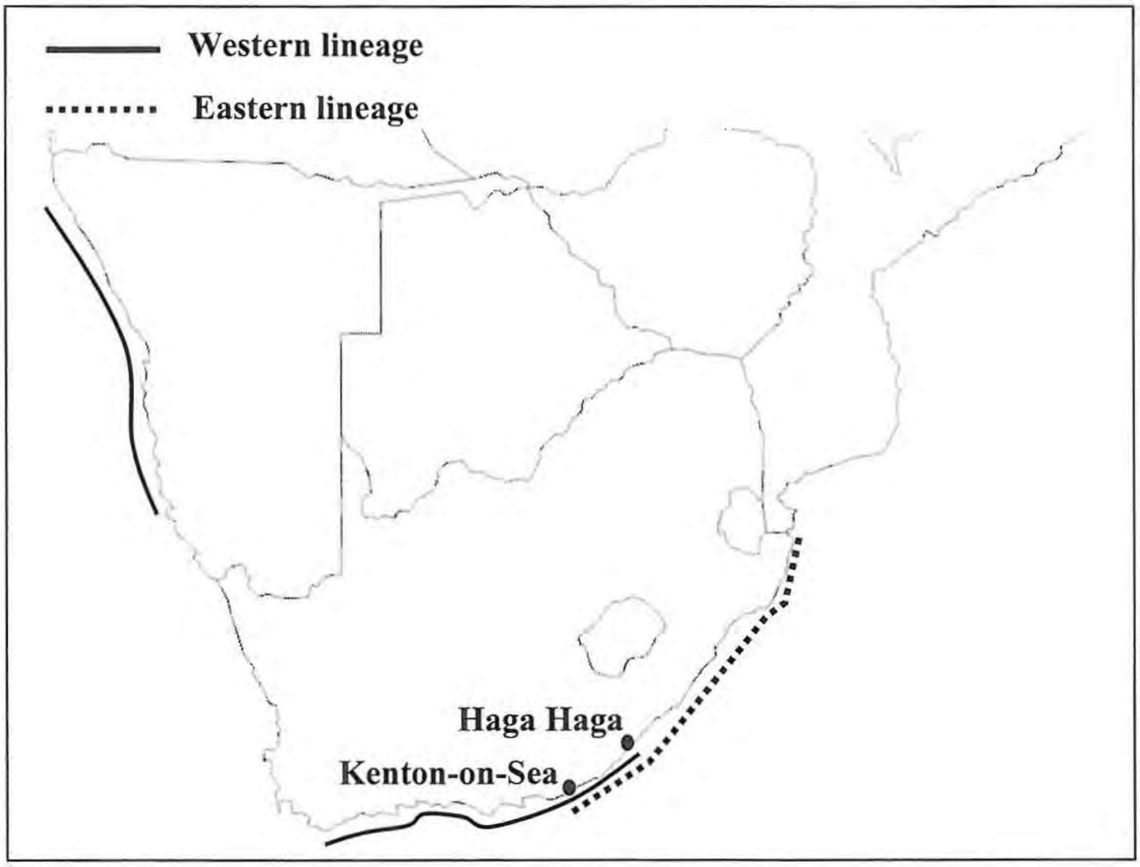

Fig. 4.4 Geographic distribution pattern of the mtDNA lineages of *P. perna*

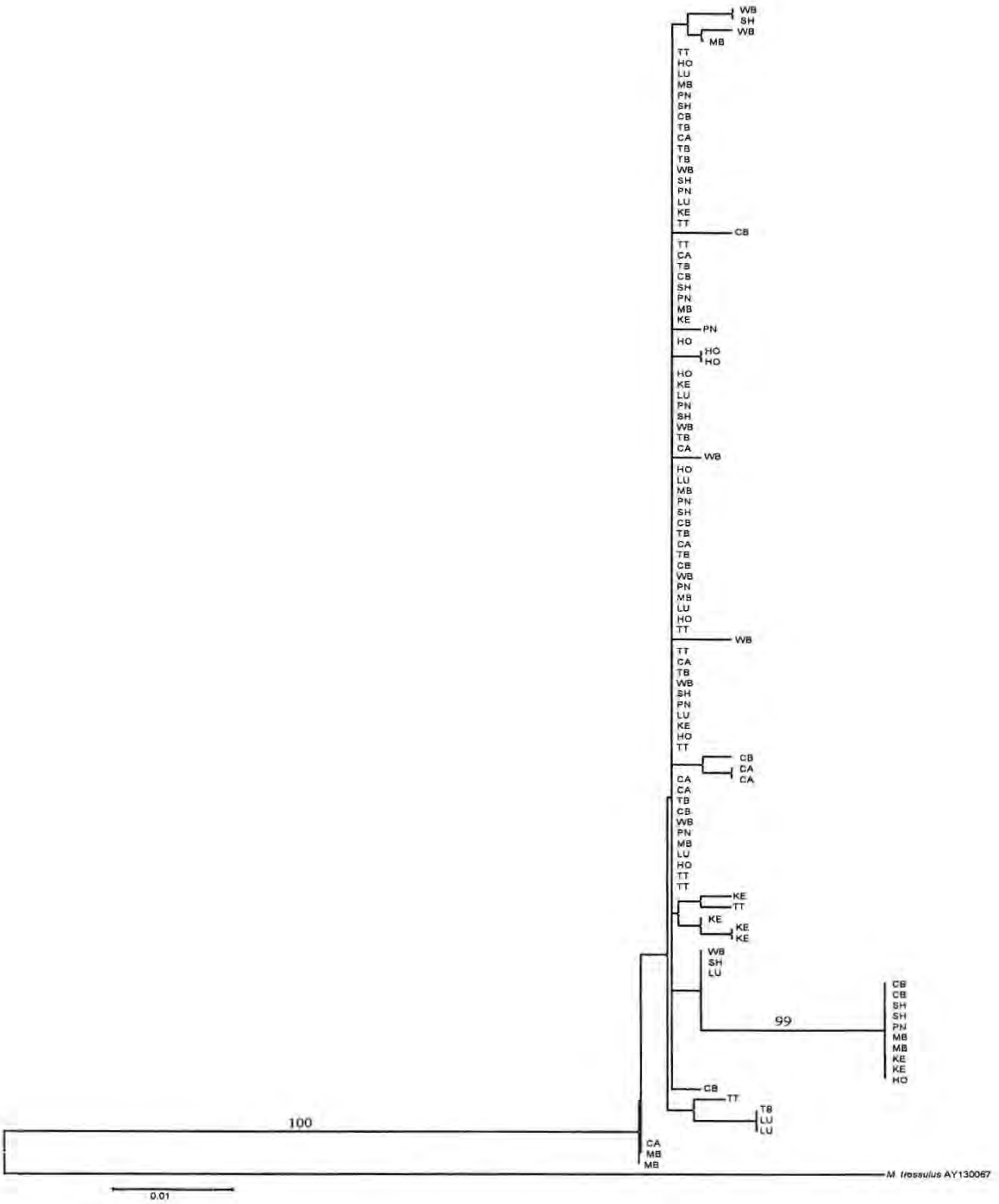

Fig.4.5 Neighbour-joining phylogram based on sequences from cytochrome oxidase I (COI) for *M. galloprovincialis*. The tree was rooted using *Mytilus trossulus* as an outgroup. Clade support from 1000 bootstrap replications (>80%) was added to some branches. See Fig. 4.2 for name codes.

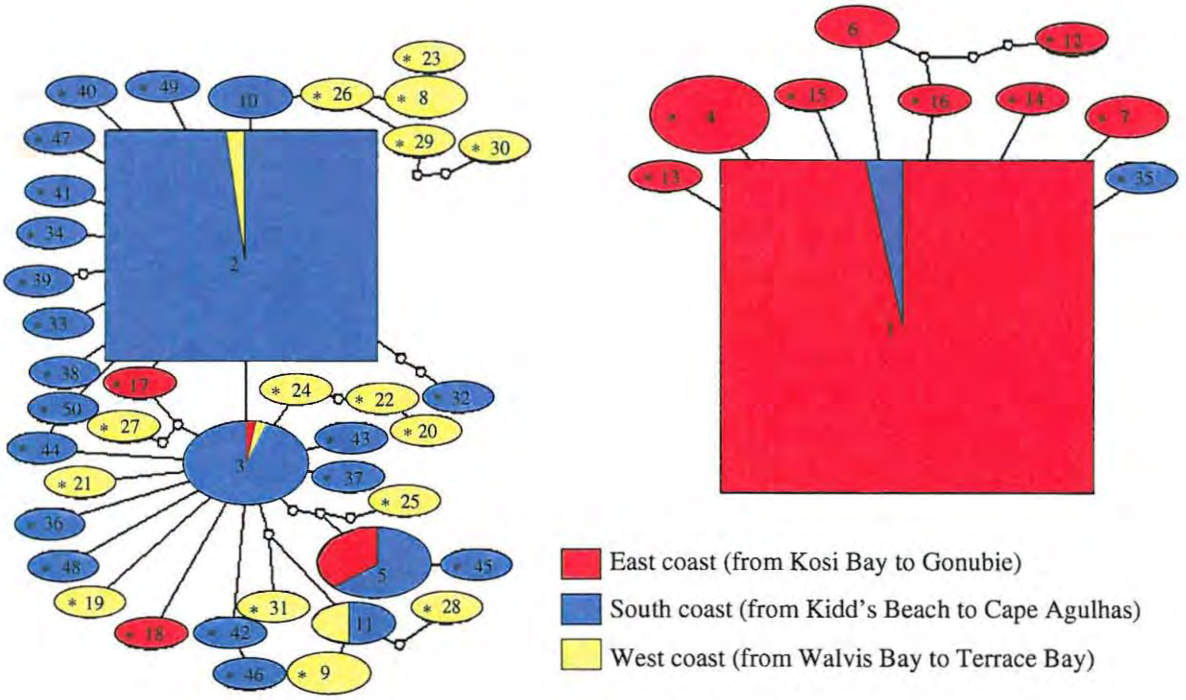

Fig. 4.6 *P. perna*: statistical parsimony cladogram of cytochrome oxidase I haplotypes (1-50). The size of each area reflects the relative abundance of the haplotype in the dataset, open circles indicate nucleotide substitutions between haplotypes and the ancestral haplotypes are indicated by a rectangle. The geographical region where each haplotype was found is shown in the legend and private haplotypes are denoted with an asterisk.

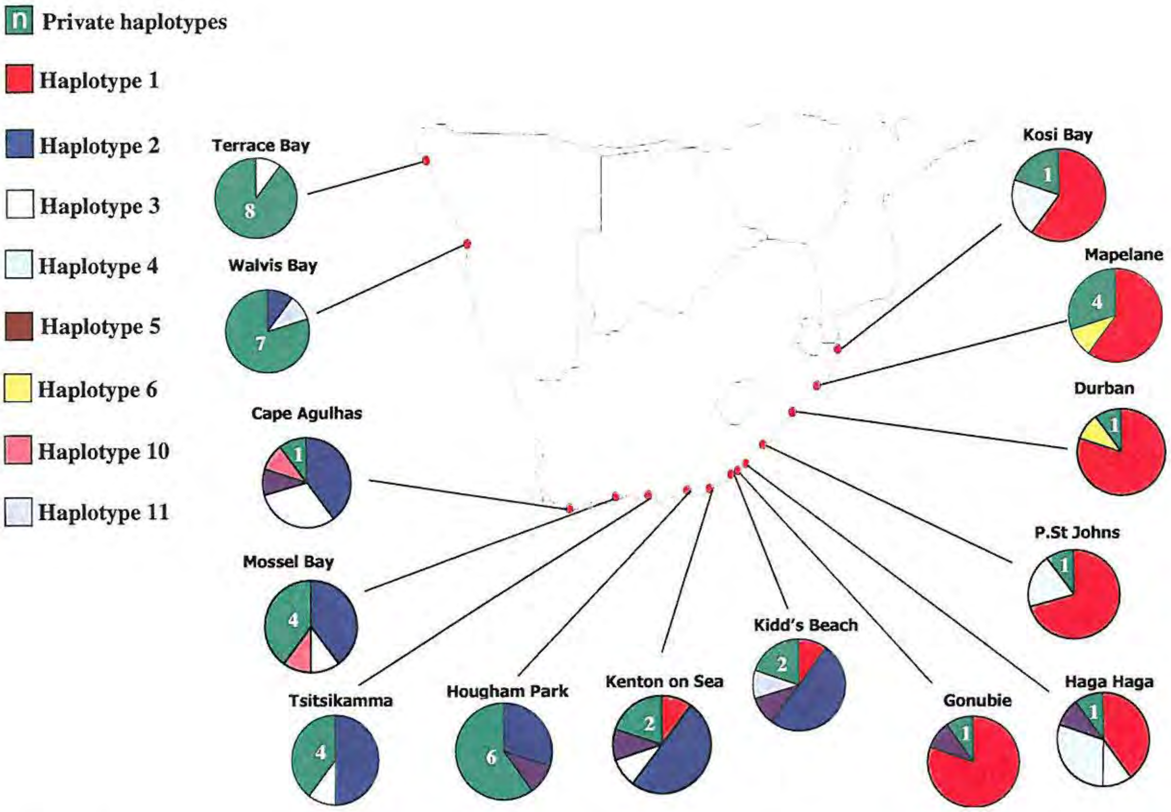

Fig. 4.7 Haplotypes frequencies at each sample location for *P. perna*

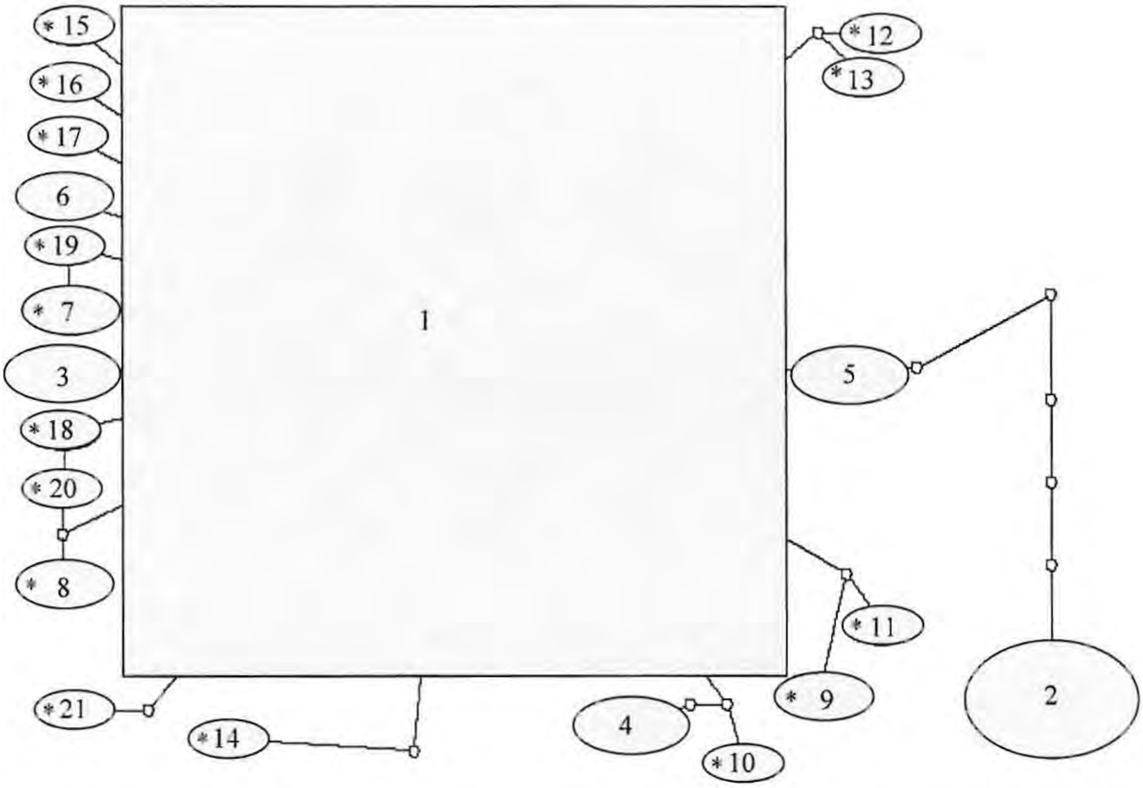

Fig. 4.8 *M. galloprovincialis*: statistical parsimony cladogram of cytochrome oxidase I haplotypes (1-21). The size of each area reflects the relative abundance of the haplotype in the dataset, open circles indicate nucleotide substitutions between haplotypes and the ancestral haplotype indicated by a rectangle. Private haplotypes are denoted with an asterisk.

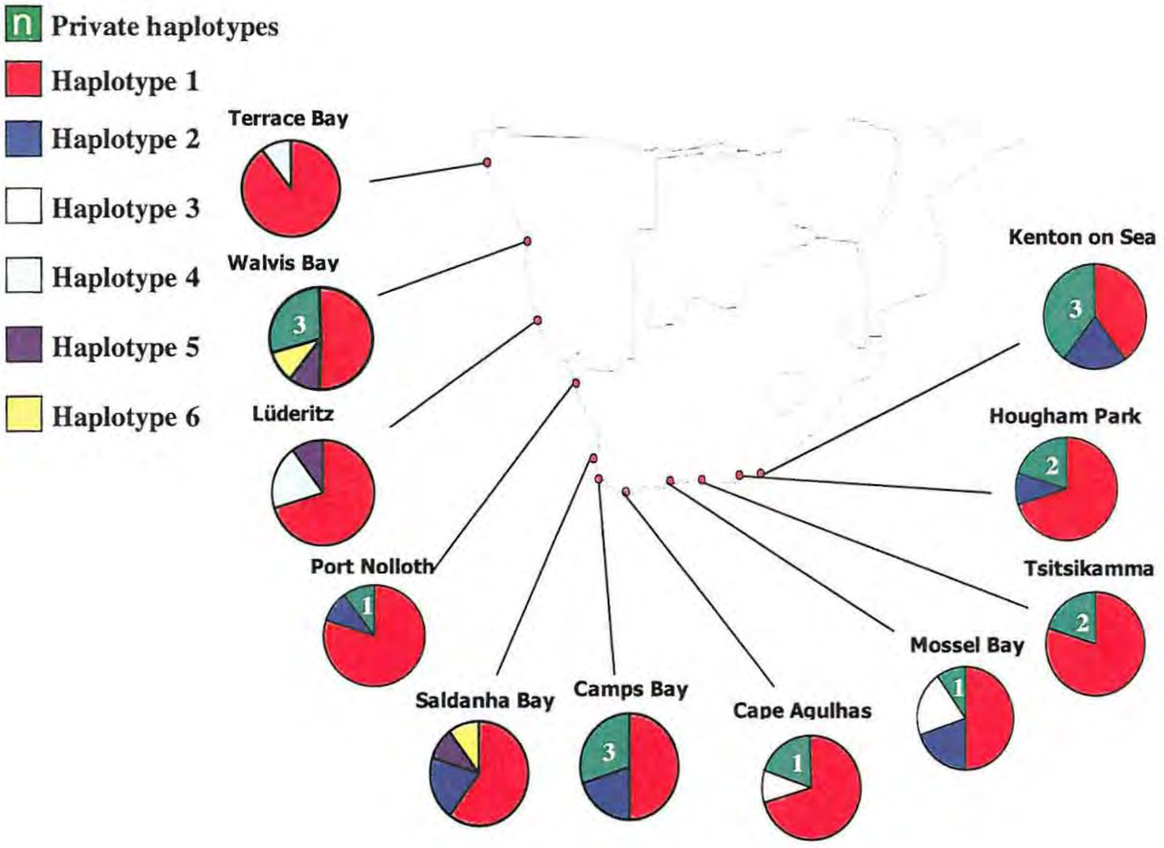

Fig. 4.9 Haplotypes frequencies at each sample location for *M. galloprovincialis*

Isolation by distance

Variance of pairwise F_{st}-values was not explained by geographical distance in the *P. perna* eastern lineage (p=0.8; Table 4.1). A Mantel test indicated a significant relationship (p=0.0122) between F_{st} and geographical distance among populations of the western lineage, indicating isolation by distance. However, when the two Namibian locations (Terrace Bay and Walvis Bay) were excluded, the relationship between F_{st}-values and geographic distance was not significant (p=0.833). The Mantel test was non-significant for the *M. galloprovincialis* lineage (p=0.35).

Species	Grouping	*r*	*t*	*p*
Perna perna	Western	-0.358	-2.252	**0.0122**
	Western excluding Terrace Bay and Walvis Bay	0.202	-0.966	0.833
	Eastern	0.192	0.850	0.80
	Total (Western + Eastern)	-0.141	-1.354	0.09
Mytilus galloprovincialis	Total	-0.052	-0.374	0.35

Table 4.1 Results of Mantel tests on matrices of genetic differentiation among populations (pairwise F_{ST} using p-distances) and geographic distance of the *M. galloprovincialis* mtDNA lineage, and of *P. perna* Western lineage, Eastern Lineage and Western lineage excluding the two Namibian populations. Numbers shown are: *r* = Pearson product-moment correlation (shown instead of the Mantel *Z* statistic for simplicity of interpretation), *t* = approximate Mantel *t*-test statistic and *p* = probability that random $Z <$ observed Z.

Discussion

The population genetics of *Perna perna* in southern Africa have already been investigated using geographical variations in allozymes frequencies of four highly polymorphic enzymes: glucose-6-phosphate isomerase (*Gpi*), peptidase (Pep-1), 6-phosphogluconate dehydrogenase (*Pgd*), and mannose-6-phosphate isomerase (*Mpi*) (Grant et al. 1992). The results showed that there was essentially no allozyme-frequency differentiation along a 1700Km transect on the south coast from False Bay (Cape Town) to Cape Vidal (approximately 170Km south of the Mozambique border) on the east coast. However, there were strong differences in allozyme frequencies for *Gpi* and *Mpi* at the western edge of the geographic distribution of *P. perna* on the south-west coast. In my study this region was not investigated because *P. perna* could not be found on the western shore of the Cape Peninsula. This seems to confirm the suggestion of Grant et al. (1992) that these populations are not self-sustaining, but originate by chance recruitment from more eastern subpopulations and thus can be subjected to dramatic changes in population structure. Grant et al. (1992) collected an additional sample at Swakopmund (Namibia), about 2000Km north of the western boundary for *P. perna* on the south coast. Only a single significant allele-frequency difference (*Pgd*) between this sample and the South African samples was found and, together with the absence of alleles unique to the Namibian samples, indicated close genetic affinity to southern populations. In contrast, the Mantel test results reported here indicate that the gap of about 1000Km in distribution of *P. perna* between Cape Agulhas and Walvis Bay on the west coast results in significant isolation by distance. Nevertheless, some haplotypes occurring in the South African populations are also present in the Namibian samples, suggesting a certain level of gene flow between the two regions. It is unlikely that *P. perna* larvae are now able to connect Namibian and South African populations by dispersing through the cold waters of the Benguela Upwelling System. However, the Pleistocene or Quaternary oceanic warming (Shannon 1985; Thackeray and Herbert 1991) could have allowed colonization of this stretch of coast and dispersal through the present distribution gap.

Mitochondrial DNA sequences for *P. perna* indicated a strong phylogeographic break on the east coast between East London and Gonubie that was not identified by Grant et al. (1992) using allozymes. Similar phylogeographic patterns have been found in two other species with planktonic larvae, the estuarine prawn *Upogebia africana* (Teske et al. in press) and the limpet *Patella granularis* (Ridgway et al. 1998). Upwelling events occurring on the south-east coast are believed to control the pelagic environment, limiting larval dispersal (Beckley and Hewiston 1994). However, it is important to note that stronger upwelling events like the one off Lüderitz do not seem to limit larval dispersal of *M. galloprovincialis*. A more plausible explanation, also suggested by Teske et al. (in press), is that the influence of the Agulhas Current acts as an oceanographic barrier to the dispersal of mussel larval. On the south-east coast, the current flows near to the shore due to the minimal width of the continental shelf, and its powerful flux could force back northward migrating larvae and, via the Agulhas Retroflection, carry larvae dispersing from northern regions into the open sea.

As an alternative to explaning the observed phylogenetic switch between Kenton-on-Sea and Haga Haga by low gene flow, we can invoke differential selection. Many studies have investigated the limits of the marine biogeographic regions if South Africa using different organisms; the general agreement is that the boundary between the warm temperate region and the subtropical region lies on the south-east coast (Harrison et al. 2002). Environmental factors characterizing these areas may subject *P. perna* populations to different selective forces leading to genetic divergence.

Grant and Cherry (1985) examined shells taken from middens and the shell collection at the Zoology Department of the University of Cape Town and concluded that the introduction of *Mytilus galloprovincialis* to southern Africa took place within the previous twenty years. The low haplotype diversity over the total geographic range of *M. galloprovincialis* in South Africa confirms its recent arrival. After spreading along the south coast, it seems that *M. galloprovincialis* has reached its biogeographic limit in the East London area (McQuaid and Philips 2000; Robinson et al. 2005). This suggests that, as with *P. perna,* the influence of the Agulhas Current or of the biogeographic boundaries may be responsible for limiting the expansion of the invasive species. *M. galloprovincialis* is a strong invader world wide and it is well adapted to a wide range of environmental factors, including different temperatures (Branch and Steffani 2004). Invasions frequently constitute rapid evolutionary events (Reznick and Ghalambor 2001), resulting in populations that are genetically dynamic over both space and time. Invasive species are often assumed to penetrate habitat boundaries through their broad tolerance (eurytolerance) or phenotypic plasticity (Baker and Stebbins, 1965; Ricciardi and MacIsaac, 2000; Wolff, 2000; Reid and Orlova, 2002). However, certain populations cannot tolerate or acclimatize to the full range of environmental factors occupied by the species complex, but instead experience strong selection when invading new habitats (Lee 1999; Lee and Petersen 2003). Consequently, the environmental conditions prevailing in the different biogeographic regions of South Africa could exert strong selection on the physiological tolerance and performance of *M. galloprovincialis* during habitat invasion. Another possible explanation for the apparent drop in rate of spread near East London is that *M. galloprovincialis* does not reach its physiological limits but that under east coast environmental conditions it is a weaker competitor in the interaction with *P. perna*. It is known that *P. perna* excludes *M. galloprovincialis* from the low shore in the Kenton-on-Sea area (Rius and McQuaid in press), and it is possible that the lower performance of *M. galloprovincialis,* coupled with the higher performance of *P. perna* farther east excludes the invasive species not only from the lower shore but from the entire intertidal habitat, leaving *P. perna* as the sole mussel.

The west coast of South Africa is influenced by the upwelling of cool, nutrient-rich water. *P. perna* is excluded from this region because of the cold water. Strong perennial upwelling off Lüderitz effectively separates the Northern Benguela from the Southern Benguela. A northwesterly moving tongue of upwelled, turbulent water acts as a semi-permanent environmental barrier to the longshore transport of pelagic fish eggs and larvae (O'Toole 1977; Agenbag 1980; Boyd and Cruickshank 1983; Agenbag and Shannon 1988). However, this oceanographic barrier did not prevent the expansion from Saldanha of *M. galloprovincialis* that was rapidly able to colonise this stretch of coast as far as central Namibia (Branch and Steffani 2004).

High levels of genetic divergence either within and/or among populations of marine taxa are not uncommon (e.g. Foltz et al. 1996; Etter et al. 1999; Quattro et al. 2001; Tarjuelo et al. 2001; Baker et al. 2003; Papakostas et al. 2005; Remerie et al. in press) and they have often been related to the presence of cryptic species. *P. perna* mtDNA sequence data indicated continuous coastal distribution with cryptic phylogeny, with a 2.9%

sequence divergence between the east and the western lineage. Should the two *P. perna* mtDNA lineages identified in this study be formally described as two species? Past work has provided conflicting perspectives on the likely efficacy of mtDNA markers in delineating species boundaries. Some studies, including extensive analyses of GenBank data, have indicated that even closely related species ordinarily show marked mitochondrial divergence (Avise and Walker 1999; Hebert et al. 2003). However, others suggest that mtDNA markers will often encounter problems in species resolution (Funk and Omland 2003; Lipscomb et al. 2003; Mallet and Willmott 2003). For example, a review of case studies (Will and Rubinoff 2004) concluded that nearly one-fourth of all animal species fail the test of mitochondrial monophyly. Future studies could be important in determining whether there are previously unnoticed phenotypic differences between the two *P. perna* mtDNA lineages. Breeding experiments would show if the two lineages are reproductively isolated. Transplant experiments, moving different haplotypes between regions would help us to understand whether adaptation to different environmental conditions is evident. In addition, mtDNA sequences of *P. perna* larvae sampled in the east warm-temperate and south subtropical biogeographic regions will be crucial to understanding if the Agulhas Current operates as an oceanographic barrier, physically limiting dispersal or if the genetic divergence results from the selective action of different environments.

The invasion of *M. galloprovincialis* in South Africa has had major ecological consequences on the structure of intertidal communities, including the replacement of indigenous species and effects at higher trophic levels (see chapter one). At the same time, this invasive species is testing our ability to predict future dynamics and consequences of invasion. Knowledge of the population structure of an indigenous species with a similar larval dispersal and of environmental factors determining it can be helpful in forecasting future development of this invasion.

CHAPTER 5

GENERAL DISCUSSION

Global change and degradation of biodiversity are currently two main concerns in both political and scientific debates. Human environmental exploitation has lead to multiple species extinctions and to dangerous alterations to biodiversity equilibria. Bioinvasions, that is the colonization by non-indigenous species of other areas of the planet, have been considered a manifestation of global change (Vitousek et al. 1996; Bright 1999), altering biodiversity (Robinson and Dickerson 1987; Soulè 1990; Levine 2000), and being related to global environmental changes (Bianchi and Morri 1993; Duarte et al. 1999). In recent decades, the frequency of biological invasions has increased, stimulating much research on population biology and community ecology. Several studies relate individual species introductions to the development of markets and trade routes. For example, the probability of successful invasion by parrot species is positively correlated with the development of the international trade in exotic birds (Cassey et al. 2004). Similarly Semmens et al. (2004) showed that there is a relationship between the occurrence of non-native marine fishes and the volume of imports for the aquarium trade. There is also a relationship between the volume of all trade and general invasion risks, including a positive correlation between the relative abundances of invasive species in different countries and the volume and composition of imports (Dalmazzone 2000; Vilà and Pujadas 2001). In recent years, many reviews have been published highlighting ongoing modifications of the marine environment in many regions of the world (Carlton 1989; Eno 1996; Carlton 1999b; Occhipinti-Ambrogi 2001). Research conducted in several parts of the world (Carlton 1985; Williams et al. 1988; Macdonald and Davidson 1997; Gollasch et al. 2000a,b; Olenin et al. 2000) has shown that unwanted organisms are present in ballast water and related sediments, as well as attached on ship's hulls

(Gollasch 2002; Coutts et al. 2003). On average 12 billion tons of ballast water are transported every year throughout the world (IMO/MEPC 1998), carrying more than 7000 species every day (Carlton 2001). Despite these extremely high numbers, relatively few cases of "successful" invasion have been reported (Hayes and Sliwa 2003). This is because invasion must include successful transition through a series of consecutive, obligatory steps, including uptake, transport, release, introduction and establishment. Even of those invaders that establish, only a small subset will proliferate and spread in the new environment (Williamson 1996; Kolar and Lodge 2001).

In the southern hemisphere, several marine invasive species have been reported to be expanding rapidly, affecting rocky intertidal and shallow inshore water communities. For example the northern hemisphere barnacles *Balanus amphitrite* and *B. glandula* invaded the coast of Argentina in the 1960s-1970s, and since then have expanded by more than 10° of latitude (Orensanz et al. 2002; Bastida et al. 1980). The kelp *Undaria pinnatifida*, native to Japan, Korea, and regions of China, is an aggressive invader not only in the boreal waters of the Mediterranean, Spain, United Kingdom, Belgium, Netherlands, but also in New Zealand, Tasmania and Argentina (Piriz and Casas 1994*)*. The chlorophyte *Codium fragile*, originally from Japan, has invaded Europe, Australia, New Zealand, Canada, the United States and Chile where it is considered a pest (Castilla et al. 2005). The Mediterranean *Mytilus galloprovincialis*, one of "the 100 world's worst invasive alien species" (Lowe et al. 2000), and an aggressive invader in many parts of the world, is the most successful marine invader in South Africa (Robinson et al. 2005).

Biological invasion generally involves two main steps: (1) the introduction and establishment of the species, generally over a restricted area and (2) the demographic expansion of the population in the new environment (Vermeij 1996; Williamson 1996). *M. galloprovincialis* was first recorded in South Africa in the late 1970s, 150 Km north of Cape Town, and it most probably arrived attached on ship's hulls (Grant et al. 1984; Grant and Cherry 1985). That South African *M. galloprovincialis* is a new introduction rather than a relict population is clearly confirmed by the results of Chapter 4. For establishment and growth, a species must be able to increase in abundance at the invaded locality. This depends on the opportunities that the invaded community provides for the invader. Soon after its arrival in South Africa, *M. galloprovincialis* expanded its domain, becoming the dominant intertidal mussel on the West coast and becoming well established on the South coast where it competes with the indigenous *Perna perna* (Griffiths et al. 1992). The interaction between *P. perna* and *M. galloprovincialis* on the south coast offers interesting opportunities to investigate the dynamics of invasion in intertidal habitats. *M. galloprovincialis* is a very aggressive invasive species that was able to generate drastic changes in the intertidal community on the South African west coast, occupying the entire fundamental niche of *Aulacomya ater* (Griffiths et al.1992). Ecological niches are defined by the relationships between organisms and their physical and biological environment, taking into account both time and space. How a species responds to these factors, including their spatial and temporal variation, determines its ability to invade (Shea and Chesson 2002). On the south coast, *P. perna* and *M. galloprovincialis* show partial habitat segregation; the upper and the lower areas of the mussel zone are dominated by *M. galloprovincialis* and *P. perna* respectively, while they co-occur in the mid-mussel zone (Robinson et al. 2005). Whether *M. galloprovincialis* will eliminate *P. perna* from the south coast as it did *A. ater* on the west coast depends on whether the invasive species can occupy the entire fundamental niche of *P. perna*. Otherwise, the present co-existence, through partial habitat segregation will persist. Branch and Steffani (2004) suggest that the interactions between *M. galloprovincialis* and *P. perna* should favour the invasive species, not only because of its higher growth rate and reproductive output, but also because *P. perna* is not a competitive dominant. Prior to the arrival of *M. galloprovincialis* on the south coast, experimental removal or exploitation of *P. perna* was followed by domination by coralline algae or barnacles (Hockey and Bosman 1986; Lambert and Steinke 1986). Now there is evidence that *M. galloprovincialis* is a better exploitation competitor, occupying freed space more effectively than *P. perna* (Erlandsson et al. in revision). There is, however, the possibility of interference competition, which, for mussels, is likely to be mediated through differences in mortality due to abiotic factors and growth rates.

Three main factors that contribute to the spread of an invader are: resource availability (Sher and Hyatt 1999, Davis et al 2000, Jefferies 2000), natural enemies (Settle and Wilson 1990; Torchin et al 1996, Mack et al 2000) and the physical environment (Moyle and Light 1996), all of which vary in time and space. Some studies have highlighted the importance of the physical environment as a constraint on invasion (Moyle and Light 1996, Sutherst et al. 1999). As both residents and invaders respond to environmental harshness, it is the difference in their responses that determines whether invasion is promoted or inhibited by harshness (Chesson and Huntly 1997). In chapters 2 and 3, I analyzed two of the major environmental stresses acting on rocky intertidal communities worldwide: siltation and waves. Sand has been shown to have strong effects on community structure, through both direct physiological effects and by influencing interactions among competitors for space (Taylor and Littler 1982). While some hard substratum species are eliminated by the effects of sand, others, such as the pulmonate limpet *Siphonaria*, have physiological adaptations that allow them to tolerate both suspended sand and burial (Marshall and McQuaid 1989), while still others appear to depend on the presence of sand and are rarely or never found in areas totally unaffected by sand. In South Africa for example this is true for *Choromytilus meridionalis* and the large chiton *Dinoplax gigas* (Dower 1989). It is difficult to imagine a directly beneficial effect of sand, and the advantages are presumably largely through the exclusion of competitors, as for the anemone *Anthopleura elegantissima* in California (Taylor and Littler 1982). Thus sand has a counter-intuitive effect on species richness by increasing habitat diversity. While psammophobic species are eliminated from parts of the shore, sand tolerant and psammophilic species are provided with habitats resulting in an increase in species diversity when the entire shore is considered (McQuaid and Dower 1990). While it is clear from field observations that sand powerfully affects the low shore in South Africa through stochastic mass mortalities of mussels, we cannot invoke sand as a mechanism for habitat segregation between *P. perna* and *M. galloprovincialis*. Although sand stress has dramatic effects on both species, it does not explain the habitat portioning between the invasive and the indigenous species. *P. perna* is morphologically and physically less well adapted to sand-induced stress than *M. galloprovincialis* and can nevertheless dominate the lower mussel zone which is more exposed to the periodic effects of suspended sand and sand burial.

Wave stress is one of the main factors responsible for preventing the invasive species from colonising part of the niche of *P. perna*. Steffani and Branch (2003c) showed that, on the south coast, growth rates and condition values of *M. galloprovincialis* decrease on extremely exposed shores. These observations together with my results predict that the invasive potential of *M. galloprovincialis* will be higher at sheltered or moderately exposed sites. Surprisingly, on the west coast, *M. galloprovincialis* extends its domain to the low mussel zone. This implies greater tenacity of *M. galloprovincialis* in the cold waters of the west coast. It is important to note that the interaction of environmental factors with resources offered by the environment and natural enemies can play a potentially important role (Tilman 1982; Chesson and Huntly 1997). Concentrations of phytoplankton on the upwelling dominated west coast are generally much higher than on the south coast (Shannon 1985; Brown 1992; Bustamante et al. 1995) and could be an important resource, providing the invasive species with enough energy to increase attachment strength and to withstand the high hydrodynamic stress that characterises the lower shore. On the south coast, field manipulative experiments have shown that *M. galloprovincialis* is excluded from the lower shore not only by wave action, but also by being outcompeted by *P. perna* (Rius and McQuaid in press). There is no such evidence for the interaction between *M. galloprovincialis* and *A. ater* on the west coast, but clearly *M. galloprovincialis* was competively dominant. This contrast could be related to the abilities of *P. perna* as a competitor, or to decreased performance of *M. galloprovincialis* in the warmer, more oligotrophic waters of the south coast. In addition, the low biodiversity which characterises the west coast is generally regarded as a feature allowing more ready success for invasive species (Bustamante and Branch 1996; Mack et al. 2000).

Once established, the demographic expansion of an invasive population in the new environment is a key factor in determining the magnitude of the invasion. Both *M. galloprovincialis* and *P. perna* possess pelagic larvae that are presumably highly dispersive and have the potential to connect adult populations and to increase the geographic range of the species. There are both advantages and disadvantages to dispersing from one site to another. Advantages include inbreeding avoidance, the possibility of colonizing a new site with more resources, and a potential escape from unfavorable conditions such as limited resources, predators, pathogens and parasites. Disadvantages include an inability to locate a suitable new site, predation during dispersal, failure to locate a mate and outbreeding depression (Stenseth and Lidicker 1992). Because of the weak swimming abilities of planktonic larvae, larval dispersal depends mainly on local hydrodynamic features (Scheltema 1986, Shanks 1995). However, a recent study has shown that larval behavior and vertical distribution can play an important role in the dispersal of certain bivalve species. Unexpectedly, some species of bivalve larvae did not behave as passive particles and were not swept offshore by upwelling or onshore by downwelling (Shanks and Brink 2005). This effect is attributed to differences in vertical migration between species.

Two major current systems sweep the South African coast: the warm Agulhas flowing down the east coast, and the cold Benguela up the west coast (Branch and Branch 1981). On the east coast, cooler pockets of water close inshore flow parallel to the coast but in a direction opposite to the Agulhas Current, i.e. to the north-east. On the south-east coast the shelf width is minimal, so that these counter currents are squeezed close to the shore and are often overpowered by the Agulhas (Lutjeharms 2004). On the south coast, where the continental shelf is wider, the Agulhas Current still influences an extensive area of the shelf (Schumann 1987; Goschen and Schumann 1988; Lutjeharms 2004), though wind becomes the main forcing function (Schumann 1981; McQuaid and Phillips 2000). All of these currents strongly influence the life of animals and plants living along the South African coastline.

Three main biogeographic regions are recognized around the southern African coast: a Cool Temperate West Coast Province, a Warm-Temperate South Coast Province and a Subtropical East Coast Province (Stephenson and Stephenson 1972; Day 1981; Potter et al 1990; Prochazka 1994; Turpie et al. 2000). Based on an analysis of rocky shore invertebrates, Emanuel et al. (1992) divided the South African coast in to three zoogeographic regions: a cool-temperate Namaqua Province from Lüderitz (Namibia) to Cape Point; a warm-temperate Agulhas Province from Cape Point eastward to East London; and a subtropical Natal Province from East London north to Mozambique (Fig. 4.1b).

The major findings of Chapter 4 were that *M. galloprovincialis* shows genetic structure typical of a recently introduced species, with far less genetic variability than *P. perna* and that there is a strong shift in population genetics in the population structure of *P. perna* between Kenton-on-sea and Haga Haga. Both currents and environmental factors associated with biogeographic regions can act as barriers to larval dispersal, limiting gene flow and leading to genetic divergence. Previous studies attributed changes in species composition in this region to temperature changes (Stephenson and Stephenson 1972, Wallace and Van der Elst 1975; Whitfield 1994, Maree et al. 2000). Changes in the selective environmental regime are often related to species genetic divergence and speciation (Koehn et al. 1980; Powers 1987; Palumbi 1994). Differences in temperature between the warm-temperate Agulhas Province and the subtropical Natal Province could have selected differently adapted genotypes. It also possible that the southward flowing Agulhas Current physically limits the northward dispersal of larvae. Towards the southwest, the Agulhas Current is deflected away from the African continent by the Agulhas Bank and undergoes a massive retroflection, moving hundreds of miles away from the mainland. Consequently, off southern Natal, where the influence of the Agulhas Current on the coast is most pronounced, dispersing larvae might be caught in the flux of the Agulhas Current and transported far away from the coast. The Agulhas Current can reach a speed of $2m.s^{-1}$ and "There is no hope for the larvae of intertidal species which are caught up in this system"(McQuaid and Phillips 2000).

Data on the population genetic structure of *P. perna*, together with information about the history of the invasion of *M. galloprovincialis* and oceanographic patterns of the region help us to predict how the invasion will develop. After the introduction of *M. galloprovincialis* to Port Elizabeth, it soon expanded its geographic domain. The yearly average increase in distributional range was 42Km to the east (McQuaid and Phillips). At the present its expansion seems to have stopped or at least drastically decreased (Robinson et al. 2005). This seems to suggest that the biogeographic boundaries responsible for the phylogeographic break of *P. perna* also influence the dispersal of *M. galloprovincialis* larvae, although it may be that *M. galloprovincialis* simply reaches its physiological limits in terms of sea temperature near East London. The definition of the population genetic structure of an indigenous species can be important in determining the presence of environmental barriers that could prevent or limit the demographic expansion of an invasive species with similar potential dispersal.

Once a species has become established and has spread from the point of introduction it is virtually impossible to stop the invasion (Mack et al. 2000). Invasions should not be treated uniquely on a case-by-case basis, but rather integrated into large scale ecology and evolutionary biology. My results, integrated with previous studies, show the potential importance of knowing the abilities of an invader when coping with local communities and environment. They also show that knowledge about co-existing indigenous species with similar behaviour may be exploited to predict invader performance in the new habitat. To turn invasion biology into a predictive science might be illusory. It is very difficult to predict unequivocally the effects of a new species and to separate benign introductions from those that cause vast changes to the system they invade. However, predictions (and consequent applications) about possible future invasions and about developments of ongoing invasions can be attempted only through a detailed investigation of the potential of the invader and of the target communities.

REFERENCES

Agenbag JJ (1980) General distribution of pelagic fish off South West Africa as deduced from aerial fish spotting (1971-1975 and 1977) and as influenced by hydrology. Fish Bull S Afr 13: 55-67

Agenbag JJ, Shannon LV (1988) A suggested physical explanation for the existence of a biological boundary at 24°30'S in the Benguela system. S Afr J Mar Sci 6: 119-132

Ahmad M, Beardsmore JA (1976) Genetic evidence that the "Padstow mussel" is *M. galloprovincialis*. Mar Biol 35: 139-147

Anderson AS, Bilodeau AL, Gilg MR, Hilbish TJ (2002) Routes of introduction of the Mediterranean mussel (*Mytilus galloprovincialis*) to Puget Sound and Hood. J Shellfish Res 21: 75-79

Anderson PM, Barnosky CW, Bartlein PJ, Behling PJ and 29 others (1988) Climatic changes of the last 18 000 years: observations and model simulations. Science 241: 1043-1052

Apte S, Holland BS, Godwin LS, Gardner JPA (2000) Jumping ships: a stepping stone event mediating transfer of a non-indigenous species via a potentially unsuitable environment. Biol Invasions 2: 75-79

Arndt A, Smith MJ (1998) Genetic diversity and population structure in two species of sea cucumber: differing patterns according to mode of development. Mol Ecol 7: 1053-1064

Au DWT, Pollino CA, Wu RSS, Shin PKS, Lau STF, Tang JYM (2004) Chronic effects of suspended solids on gills structure, osmoregulation, growth, and triiodothyronine in juvenile green grouper *Epinphelus coioides*. Mar Ecol Prog Ser 266: 255-264

Avise JC (2000) Phylogeography. Harvard University Press, Cambridge, MA

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Ann Rev Ecol Syst 18: 489-522

Avise JS, Wolker D (1999) Species realities and numbers in sexual vertebrates: Perspectives from an asexually transmitted genome. Proc Natl Acad Sci USA 96: 992-995

Ayre DJ, Hughes TP (2000) Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. Evolution 54: 1590-1605

Bairati A, Vitellaro-Zuccarello L (1976) The ultrastructure of the byssal apparatus of *Mytilus galloprovincialis*. IV. Observation by transmission electron microscopy. Cell Tissue Res 166: 219-234

Baker AM, Bartlett C, Bunn SE, Goudkamp K, Sheldon F, Hughes JM (2003) Cryptic species and morphological plasticity in long-lived bivalves (Unionoida: Hyriidae) from inland Australia. Mol Ecol 12: 2707-2717

Baker HG, Stebbins GL (1965) The genetics of colonizing species. Academic Press, New York.

Bally R, McQuaid CD, Brown AC (1984) Shores of mixed sand and rock: an unexplored marine ecosystem. S Afr J Sci 80: 500-503

Barsotti G, Meluzzi C (1968) Osservazioni su *Mytilus edulis* L. e *M. galloprovincialis* Lmk. Conchiglie 4: 50-58

Bastida R, Trivi de Mandri M, Lichtschein de Bastida V, Stupak M (1980) in V Congreso Internacional de Corrosion Marina e Incrustaciones, Madrid (Garsi, Madrid), pp 229-320

Battle H (1932) Rhythmical sexual maturity and spawning of certain bivalve molluscs. Contrib Can Biol Fish. 7: 257-276

Bayne BL, Worral CM (1980) Growth and production of mussels (*Mytilus edulis*) from two populations. Mar Ecol Prog Ser 3: 317-328

Beckley LE, Hewiston JD (1994) Distribution and abundance of clupeoid larvae along the east coast of South Africa in 1990/1991 S Afr J Mar Sci 14: 205-212

Bell CM, Gosline JM (1996) Mechanical design of mussel byssus: material yield enhances attachment strength. J Exp Biol 199: 1005-1017

Bell CM, Gosline JM (1997) Strategies for life in flow: tenacity, morphometry, and probability of dislodgement of two *Mytilus* species. Mar Ecol Prog Ser 159: 197-208

Benedict CV, Waite JH (1986) Composition and ultrastructure of the byssus of *Mytilus edulis*. J Morph 189: 261-270

Berry PF (1978) Reproduction, growth and production in the mussel, *Perna perna* (Linnaeus) on the east coast of South Africa. Investl Rep Oceanogr Res Inst, Durban 48: 1-28

Bert TM (1986) Speciation in western Atlantic stone crabs (genus *Menippe*): the role of geological processes and climatic events in the formation and distribution of species. Mar Biol 93: 157-170

Bert TM, Harrison RG (1988) Hybridization in western Atlantic stone crabs (genus *Menippe*): evolutionary history and ecological context influence species interactions. Evolution 42: 528-544

Bianchi CN, Morri C (1993) Range extensions of warm-water species in the Northern Mediterranean: evidence for climatic fluctuations? Porcupine Newslett 5: 156-159

Botsford LW, Micheli F, Hastings A (2003) Principles for the design of marine reserves. Ecol Appl 13: 25-31

Bownes SJ (2005) Habitat segregation in competing species of intertidal mussels in South Africa. PhD thesis, Rhodes University, South Africa

Boyd AJ, RA Cruickshank (1983) An environmental basin model for west coast pelagic fish distribution. S Afr J Sci 79: 150-151

Brafield AE (1964) The oxygen content of interstitial water in sandy shores. J Anim Ecol 33: 97-116

Branch GM, Steffani CN (2004) Can we predict the effects of alien species? A case-history of the invasion of South Africa by *Mytilus galloprovincialis* (Lamarck). J Exp Mar Biol Ecol 300: 189-144

Branch M, Branch GM (1981) The Living Shores of Southern Africa. C Struik, Cape Town

Bricelj VM, Malouf RE (1984) Influence of algal and suspended sediment concentrations on the feeding physiology of the hard clam *Mercenaria mercenaria*. Mar Biol 84: 155-165

Bright C (1999) Invasive species: pathogens of globalisation. Foreign Policy 116: 50-64

Brown CH (1952) Some structural proteins of *Mytilus edulis* L. Q J Microsc Sci 93: 487-502

Brown PC (1992) Spatial and seasonal variation in chlorophyll distribution in the upper 30m of the photic zone in the southern Benguela/Agulhas ecosystem. In: Benguela Trophic Functioning, Payne AIL, Brink KH, Mann KH, Hilborn R (eds), S Afr J Mar Sci 12: 515-525

Brown RA, Seed R (1977) *Modiolus demissus* (L) - an autecological study. In: Keegan BF, Oceidingh P, Boaden PJS (eds), Biology of Benthic Organisms, Pergamon Press, Oxford, pp 93-100

Brown WM, George M Jr, Wilson AC (1979) Rapid evolution of animal mitochondrial DNA. Proc Natl Acad Sci USA 76: 1967-1971

Brown WM, Prager EM, Wang A, Wilson AC (1982) Mitochondrial DNA sequences of primates: Tempo and mode of evolution. J Mol Evol 18: 225-239

Brundrit GB and Shannon LV (1989) Cape storms and the Agulhas current: a glimpse of the future? S Afr J Mar Sci 85: 619-620

Burton RS (1998) Intraspecific phylogeography across the Point Conception biogeographic boundary. Evolution 52:734–745

Bustamante RH, Branch GM (1996) Large scale patterns and trophic structure of southern African rocky shores: the roles of geographic variation and wave action. J Biogeogr 23: 339-351

Bustamante RH, Branch GM, Eeekhout S (1998) The influences of physical factors in the distribution and zonation patterns of South African rocky-shore communities. S Afr J Mar Sci 18: 93-105

Bustamante RH, Branch GM, Eekhout S, Robertson B, Zoutendyk P, Schleyer M, Dye A, Hanekom N, Keast D, Jurd M, McQuaid CD (1995) Gradients of intertidal primary productivity aroud the coast of South Africa and their relationships with consumer biomass. Oecologia 102: 189-201

Butman CA, Frechette M, Geyer WR (1994) Flume experiments on food supply to the blue mussel *Mytilus edulis* L. as a function of boundary layer flow. Limnol Oceanogr 39: 1755-1768

Caley MJ, Carr MH, Hixon MA, Huges TP, Jones GP, Menge BA (1996) Recruitment and the local dynamics of open marine populations. Annu Rev Ecol Syst 27: 477-500

Calvo-Ugarteburu G, McQuaid CD (1998) Parasitism and invasive species: effects of digenetic trematode on mussels. Mar Ecol Prog Ser 169: 149-163

Carlton JT (1985) Transoceanic and interoceanic dispersal of coastal marine organisms: The Biology of Ballast Water. Oceanogr Mar Biol Annu Rev 23: 313-317

Carlton JT (1987) Patterns of transoceanic marine biological invasions in the Pacific Ocean. Bull Mar Sci 41: 452-465

Carlton JT (1989) Man's role in changing the face of the ocean: biological invasions and implications for the conservation of near-shore environments: Biol Conserv 3: 265: 273

Carlton JT (1996) Pattern, process, and prediction in marine invasion ecology. Biol Conserv 78: 97-106

Carlton JT (1999a) Molluscan invasion in marine and estuarine communities. Malacologia 41: 439-454

Carlton JT (1999b) The scale and ecological consequences of biological invasions in the World's oceans. In: Sandlund OT, Schei PJ, Viken A (eds), Invasive species and Biodiversity Management. Kluwer Academic Publishers, Dordrecht pp 195-212

Carlton JT (2001) Introduced Species into US Waters. Carlton JT for Pew Oceans Commission

Carlton JT, Geller JB (1993) Ecological roulette: the global transport of nonindigenous marine organisms. Science 261: 78-82

Carrington E (2002) Seasonal variation in the attachment strength of the blue mussel: Causes and consequences. Limnol Oceanogr 47(6): 1723-1733

Cassey P, Blackburn TM, Russell GJ Jones KE (2004) Influences on the transport and establishment of exotic bird species: an analysis of the parrots (Psittaciformes) of the world. Glob Change Biol 10: 471-426

Castilla JC, Uribe M, Bahamonde N, Clarke M, Desqueyroux-Faundez R, Kong I, Moyano H, Rozbaczylo N, Santelices B, Valdovinos C, Zavala P (2005) Down under the southeastern Pacific: a survey of marine non-indigenous species in Chile. Biol Invasions 7: 213-232

Chesson P (2000) Mechanisms of maintenance of species diversity. Annu Rev Ecol Syst 31: 343-366

Chesson P, Huntly N (1997) The roles of harsh and fluctuating conditions in the dynamics of ecological communities. Am Nat 150: 519-553

Cheung SG, Shin PKS (2005) Size effects of suspended particles on gill damage in green-lipped mussel *Perna viridis*. Mar Poll Bull 51: 801-810

Cohen AN, Carlton JT (1998) Accelerated invasion rate in a highly invaded estuary. Science 279: 555-558

Coutts AD, Moore KM, Hewitt CL (2003) Ships' sea-chests: an overlooked transfer mechanism for non-indigenous marine species? Mar Poll Bull 46: 1510-1513

Cowen RK, Lwiza KMM, Sponaugle S, Paris CB, Olson DB (2000) Connectivity of marine populations: Open or closed? Science 287: 857-859

D'Antonio CM (1986) Role of sand in domination of hard substrata by the intertidal alga *Rhodomela larics*. Mar Ecol Prog Ser 27: 263-275

Dahlhoff EP, Menge BA (1996) Influence of phytoplankton concentration and wave exposure on the ecophysiology of *Mytilus californianus*. Mar Ecol Prog Ser 14: 97-107

Dalmazzone S (2000) Economic factors affecting vulnerability to biological invasions. In: The Economics of Biological Invasions. Perrings C, Williamson M, Barbier EB, Delfino D, Dalmazzone S, Shogren J, Simmons P, Watkinson A (eds), Edward Elgar, pp 17-30

Daly MA, Mathieson AC (1977) The effects of sand movement on intertidal seaweeds and selected invertebrates at Bound Rock, New Hampshire, USA. Mar Biol 43: 45-56

Davis MA, Grime JP, Thompson K (2000) Fluctuating resources in plant communities: a general theory of invasibility. J Ecol 88: 528-534

Day JH (1981) Summaries of current knowledge of 43 estuaries in the southern Africa. In: Estuarine Ecology with Particular Reference to Southern Africa. JH Day (ed), AA Balkema, Cape Town, pp 251-329

De Moor IJ, Bruton MN (1988) Atlas of alien and translocated indigenous aquatic animals in southern Africa. South African National Scientific Programmes Report, Pretoria, South Africa, vol 144

De Zwaan A, Cortesi P, van den Thillart G, Roos J, Storey KB (1991) Differential sensitivities to hypoxia by two anoxia-tolerant marine molluscs: a biochemical analysis. Mar Biol 111: 343-351

Denny MW (1985) Wave forces on intertidal organisms: a case study. Limnol Oceanogr 30: 1171-1187

Denny MW (1987) Lift as a mechanism of patch initiation in mussel beds. J Exp Mar Biol Ecol 113: 231-245

Denny MW (1988) Biology and mechanism of the wave swept environment. Princeton University Press, Princeton, NJ

Denny MW (1995) Predicting physical disturbance: mechanistic approaches to the study of survivorship on wave-swept shores. Ecol Monogr 65: 371-418

Denny MW, Daniel TL, Koehl MAR (1985) Mechanical limits to size in wave-swept organisms. Ecol Monogr 55: 69-102

Denny MW, Gaines SD (1990) On the prediction of maximal intertidal wave forces. Limnol Oceanogr 35: 1-15

Devinny JS, Volse LA (1978) Effect of sediments on the development of *Macrocystis pyrifera* gametophytes. Mar Biol 48: 343-348

Dolmer P, Svane I (1994) Attachment and orientation of *Mytilus edulis* L. in flowing water. Ophelia 40: 63-74

Dower KM (1989) Sand inundation on rocky shores: its effects on species richness and the structure of species assemblages. MSc thesis, Rhodes University

Duarte CM, Agustí S, Kennedy H, Vaqué D (1999) The Mediterranean climate as a template for Mediterranean marine ecosystems: the example of the northeast Spanish littoral. Prog Oceanogr 44: 245-270

Edmands S, Moberg PE, Burton RS (1996) Allozyme and mitochondrial DNA evidence of population subdivision in the purple sea urchin *Strongylocentrotus purpuratus*. Mar Biol 126: 443-450

Emanuel BP, Bustamante RH, Branch GM, Eekhout S, Odendaal FJ (1992) A zoogeographic and functional approach to the selection of marine reserves on the west coast of South Africa. S Afr J Mar Sci 12: 341-54

Emlet RB (1995) Developmental mode and species geographic range in regular sea urchins (Echinodermata: Echinoidea). Evolution 42: 476-489

Eno NC (1996) Non-native species in British waters: effects and controls. Aquat Conserv: Mar Freshwat Ecosyst 6: 215-228

Erlandsson J, Pal P, McQuaid CD Re-colonization rate differs between co-existing indigenous and invasive intertidal mussels following major disturbance Mar Ecol Prog Ser (in revision)

Etter RJ, Rex MA, Chase MR, Quattro JM (1999) A genetic dimension to deep-sea biodiversity. Deep-Sea Res 46: 1095-1099

Field JG, Griffiths CL (eds) (1991) Littoral and sublittoral ecosystems of southern Africa. In Mathieson AC, Nienhus PH. Ecosystems of the world, Elsevier, Amsterdam, pp 323-346

Folmer O, Black M, Hoeh R, Lutz R, Vrijenhoek (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotech 3: 294-299

Foltz DW, Breaux JP, Campagnaro EL, Herke SW, Himel AE, Hrincevich AW, Tamplin JW, StickleWB (1996) Limited morphological differences between genetically identified cryptic species within the *Leptasterias* species complex (Echinodermata: Asteroidea). Can J Zool 74: 1275-1283

Foster-Smith RL (1975) The effect of concentration of suspension on filtration rates of pseudofaecal production for *Mytilus edulis* (L), *Cerastoderma edule* (L) and *Venerupis pullastra* (Montagu). J Exp Mar Biol Ecol 17: 1-22

Frechette M, Butman CA, Geyer WR (1989) The importance of boundary-layer flow in supplying phytoplankton to the benthic suspension feeder, *Mytilus edulis* L. Limnol Oceanogr 34: 19-36

Funk DJ, Omland KE (2003) Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annu Rev Ecol Evol Syst 34: 397-423

Gaines SD, Bertness MD (1992) Dispersal of juveniles and variable recruitment in sessile marine species. Nature 360: 579-580

Gardner JPA, Skibinski DOF (1991) Biological and physical factors influencing genotype-dependent mortality in hybrid mussel populations. Mar Ecol Prog Ser 71: 235-243

Garido-Ramos M, Stewart TD, Sutherland WB, Zouros E (1998) The distribution of male-transmitted and female-transmitted mitochondrial DNA types in somatic tissues of blue mussels: implications for the operation of doubly uniparental inheritance of mitochondrial DNA. Genome 41: 818-824

Gaylord B (2000) Biological implications of surf-zone complexity. Limnol Oceanogr 45: 174-188

Gaylord B, Blanchette CA, Denny MW (1994) Mechanical consequences of size in wave-swept algae. Ecol Monogr 64: 287-813

Gilg MR, Hilbish TJ (2003) Patterns of larval dispersal and their effect on the maintenance of a blue mussel hybrid zone in southwestern England. Evolution 52: 1061-1077

Gollasch S (2002) The importance of the ship hull fouling as a vector of species introductions into the North Sea. Biofouling 18: 105-121

Gollasch S, Lenz J, Dammer M, Andres HG (2000a) Survival of tropical ballast water organisms during a cruise from the Indian Ocean to the North Sea. J Plankton Res 22: 923-937

Gollasch S, Rosenthal H, Botnen H, Hamer J, Laing I, Leppakoski E, Macdonald E, Minchin D, Nauke M, Olenin S, Utting S, Voigt M, Wallentinus I (2000b) Fluctations of zooplankton taxa in ballast water during short-term and long-term ocean-going voyages. International Review of Hydrobiology 85: 597-608

Goschen WS, Shumann EH (1988) Ocean current and temperature structures in Algoa Bay and beyond in November 1986. S Afr J Mar Sci 7: 101-116

Goschen WS, Schumann EH (1990) Agulhas current variability and inshore structures off the Cape Province, South Africa. J Geophys Res 95: 667-678

Grant WS, Cherry MI, Lombard AT (1984) A cryptic species of *Mytilus* (Mollusca: Bivalvia) on the west coast of South Africa. S Afr J Mar Sci 2: 149-162

Grant WS, Cherry MI (1985) *Mytilus galloprovincialis* Lmk. in South Africa. J Exp Mar Biol Ecol 90: 179-191

Grant WS, Schneider AC, Leslie RW, Cherry MI (1992) Population genetics of the brown mussel *Perna perna* in southern Africa. J Exp Mar Biol Ecol 165: 45-58

Griffiths CL (1977) Reproductive cycles in littoral populations of *Choromytilus meridionalis* (Kr) and *Aulacomya ater* (Molina) with a quantitative assessment of gamete production in the former. J Exp Mar Biol Ecol 30: 53-71

Griffiths CL, King JA (1979) Energy expended on growth and gonad output in the ribbed mussel *Aulacomya ater*. Mar Biol 53: 217-222

Griffiths CL, Hockey PAR, Van Erkom Shurink C, Le Roux PJ (1992) Marine invasive aliens on South African shores: Implications for community structure and trophic functioning. S Afr J Mar Sci 12: 713-722

Hails RS, Morley K (2005) Genes invading new populations: a risk assessment perspective. Trends Ecol Evol 20: 245-252

Hammond W, Griffiths CL (2004) Influence of wave exposure on South African mussel beds and their associated infaunal communities. Mar Biol 144: 547-552

Hare MP, Avise JC (1998) Population structure in the American oyster as inferred by nuclear gene genealogies. Mol Biol Evol 15: 119-128

Harger JRE (1970) The effect of wave impact on some aspects of the biology of sea mussels. Veliger 12: 401-414

Harris JH (1996) Enzyme polymorphisms in man. Proc R Soc Lond, B 164: 298-310

Harris JM, Branch GM, Elliott BL, Currie B, Dye AH, McQuaid CD, Tomalin BJ, Velasquez C (1998) Spatial an temporal variability in recruitment of intertidal mussels around the coast of southern Africa. S Afr J Zool 33: 1-11

Harrison TD (2000) Preliminary assessment of the biogeography of the fishes in South African estuaries. Mar Freshwater Res 2002 53: 479-490

Hasegawa, Kishino H, Yano T (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol 22: 160-174

Hawkins AJS, Bayne BL (1985) Seasonal variation in the relative utilization of carbon and nitrogen by the mussel *Mytilus edulis*: budgets, conversion efficiencies and maintenance requirements. Mar Ecol Prog Ser 25: 181-188

Hayes KR, Sliwa C (2003) Identifying potential marine pests-a deductive approach applied to Australia. Mar Poll Bull 46: 91-98

Heath DD, Rawson PD, Hilbish TJ (1995) PCR-based nuclear markers identify alien blue mussel (*Mytilus* spp.) genotypes on the west coast of Canada. Can J Fish Aquat Sci 52: 2621-2627

Hebert PDN, Cywinska A, Ball SL, de Waard JR (2003) Biological identifications through DNA barcodes Proc R Soc London Ser B 270: 313-321

Hicks DW, Tunnell JW Jr (1993) Invasion of the south Texas coast by the edible brown mussel *Perna perna* (Linnaeus, 1758). Veliger 36: 92-94

Hilbish TJ, Koehn RK (1985) The physiological basis of natural selection at the Lap Locus. Evolution 39: 1302-1317

Hilbish TJ, Zimmerman KM (1988) Genetic and nutritional control of th gametogenic cycle in *Mytilus edulis*. Mar Biol 98: 223-228

Hilbish TJ, Mullinax A, Dolven SI, Meyer A, Koehn RH, Rawson PD (2000) Origin of the antitropical distribution pattern in marine mussels (Mytilus spp.): Routes and timing of transequatorial migration. Mar Biol 136: 69-77

Hines AH (1979) Effects of a thermal discharge on reproductive cycles in *Mytilus edulis* and *Mytilus californianus* Fish Bull 77: 498-503

Hockey PAR, Bosman AL (1986) Man as an intertidal predator in Transkei: disturbance, community convergence and management of a natural food supply. Oikos 46: 3-14

Hockey PAR, van Erkom Schurink C (1992) The invasive biology of the mussel *Mytilus galloprovincialis* on the southern African coast. Trans Roy Soc S Afr 48: 123-139

Hoeh WR, Stewart DT, Saavedra C, Sutherland BW, Zouros E (1997) Phylogenetic evidence for role-reversals of gender-associated mitochondrial DNA in *Mytilus* (Bivalvia: Mytilidae). Mol Biol Evol 14: 959-967

Hunt HL, Scheibling RE (2001) Predicting wave dislodgement of mussels: variation in attachment strength with body size, habitat, and season. Mar Ecol Prog Ser 213: 157-164

IMO/MEPC (1998) Harmful aquatic organisms in ballast water, Report of the Working Group on the Ballast Water convened during MEPC 41. MEPC 42/8, IMO

Innis MA, Gelfand DH, Sninsky JJ, White TJ (1990) PCR Protocols. A Guide To Methods and Applications. Academic Press, San Diego, CA

Jefferies RL (2000) Allochthonous inputs: integrating population changes and food web dynamics. Trends Ecol Evol 15: 19-22

Jenkins PT (1996) Free trade and exotic species introductions. Biol Conserv 10: 300-302

Johnson FM, Kanapi CG, Richardson RH, Wheeler MR, Stone WS (1966) An analysis of polymorphisms among isozyme loci in dark and light *Drosophila ananassae* strains from America and Western Samoa. Proc Natl Acad Sci USA 56: 119-125

Jones HD, Richards OG, Southern TA (1992) Gill dimension, water pumping rate and body size in the mussel *Mytilus edulis* L. J Exp Mar Biol Ecol 155: 213-237

Jørgensen CB (1990) Bivalve filter feeding: hydrodynamics, bioenergetics, physiology and ecology. Olsen and Olsen Press, Fredensborg, Denmark

Karakousis Y, Skibinski DOF (1992) An analysis of allozyme, mitochondrial DNA and morphological variation in mussel (*Mytilus galloprovincialis*) populations from Greece. Experientia 48: 878-881

Karl SA, Avise JC (1992) Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. Science 256: 100-102

Keane RM, Crawley MG (2002) Exotic plant invasion and the enemy release hypothesis. Trends Ecol Evol 17: 164-170

Kennedy VS (1976) Crawling and clustering behaviour of three species of intertidal mussels in New Zealand. N Z J Mar Freshw Res 18: 417-423

Kensley B, Penrith ML (1970) New records of Mytilidae from the northern south west African coast. Ann S Afr Mus 57: 15-24

Kiørboe T, Møhlenberg F (1981) Particle selection in suspension-feeding bivalves. Mar Ecol Prog Ser 5: 291-296

Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci USA 86: 6196-6200

Koehn RK, Newell RI, Immerman F (1980) Maintenance of an aminopeptidase allele frequency cline by natural selection. Proc Natl Acad Sci USA 77: 5385-5389

Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. Trends Ecol Evol 16: 199-204

Kraus DW, Doeller JE (2004) Sulfide consumption by mussel gill mitochondria is not strictly tied to oxygen reduction: measurements using a novel polarographic sulphide sensor. J Exp Biol 207: 3667-3679

Kyle CJ, Boulding EG (2000) Comparative population genetic structure of marine gastropods (*Littorina* spp.) with and without pelagic larval dispersal. Mar Biol 137: 835-845

Lambert D, Steinke TD (1986) Effects of destroying juxtaposed mussel-dominated and coralline algal communities at Umdoni Park, South Africa. S Afr J Mar Sci 4: 203-217

Largier JL (2003) Considerations in estimating larval dispersal distances from oceanographic data. Ecol App 13: S71-S89

Lee CE (1999) Rapid and repeated invasions of fresh water by the saltwater copepod *Eurytemora affinis*. Evolution 53: 1423-1434

Lee CE, Petersen CH (2003) Genotype by environment interaction for salinity tolerance in the freshwater invading copepod *Eurytemora affinis*. Phys Biochem Zool 76: 296-301

Lee SY, Morton BS (1985) The introduction of the Mediterranean mussel *Mytilus galloprovincialis* into Hong Kong. Malacol Rev 18: 107-109

Lee SY, Lim SS, Owen MD (1990) The rate and strength of the byssal reattachment by the blue mussels (*Mytilus edulis* L.). Can J Zool 68: 2005-2009

Leverone GR (1995) Growth and survival of caged adult bay scallops (*Argopecten irradians concentricus*) in Tampa Bay with respect to levels of turbidity, suspended solids and chlorophyll *a*. Florida Science 58: 216-227

Levine JM (2000) Species diversity and biological invasions: relating local process to community pattern. Science 288: 852-853

Levine JM, D'Antonio CM (2003) Forecasting biological invasions with increasing international trade. Biol Conserv 17: 322-326

Lewontin RC, Hubby JL (1966) A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of Drosophila pseudoobscura. Genetics 54: 595-609

Lipscomb D, Platnick N, Wheeler Q (2003) The intellectual content of taxonomy: a comment on DNA taxonomy. Trends Ecol Evol 18: 65-66

Littler MM, Martz DR, Littler DS (1983) Effects of recurrent sand deposition on rocky intertidal organisms: importance of substrate heterogeneity in a fluctuating environment. Mar Ecol Prog Ser 11: 129-139

Liu HP, Mitton JB, Wu SK (1996) Paternal mitochondrial DNA differentiation far exceeds maternal DNA and allozyme differentiation in the freshwater mussel *Anodonda grandis grandis*. Evolution 50 952-957

Lonsdale WM (1999) Global patterns of plant invasions and the concept of plant invisibility. Ecology 80: 1522-1536

Lovette IJ, Seutin G, Ricklefs RE, Bermingham E (1999) The assembly of an island fauna by natural invasion: sources and temporal patterns in the avian colonization of Barbados. Biol Investig 1: 33-41

Lowe S, Browne M, Boudjelas S, De Poorter M (2000) 100 of the World's Worst Invasive Alien Species A selection from the Global Invasive Species Database. The Invasive Species Group (ISSG)

Lubchenco J, Menge BA (1978) Community development and persistence in a low rocky intertidal zone. Ecol Monogr 48: 67-94

Lutjerharms JRE (1998) Coastal hydrography. In Eastern & Southern Cape Coasts, R Lubke and I de Moor (eds), University of Cape Town Press and the Grahamstown Branch of The Wildlife and Environment Society of Southern Africa, pp 50-61

Lutjerharms JRE (2004) The coastal oceans of south-eastern Africa. The Sea 14: 781-832

Lynch M, Jarrell PE (1993) A method for calibrating molecular clocks and its application to animal mitochondrial DNA. Genetics 135: 1197-208.

MacDonald BA, Bacon GS, Ward JE (1998) Physiological response of infaunal (*Mya arenaria*) and epifaunal (*Placopecten magellanicus*) bivalves to variations in the concentration and quality of suspended particles II. Absorption efficiency and scope of growth. J Exp Mar Biol Ecol 219: 127-141

Macdonald E, Davidson R (1997) Ballast Water Project, Final Report, FRS Marine Laboratory Aberdeen, pp 83

Mack MC, Simberloff D, Lonsdale W, Evans H, Clout M, Bazzaz F (2000) Biotic invasions: causes, epidemiology, global consequences, and control. Ecol Appl 10: 689-710

Mallet J, Willmott K (2003) Taxonomy: renaissance or Tower of Babel? Trends Ecol Evol 18: 57-59

Maree RC, Whitfield AK, Booth AJ (2000) Effect of water temperature on the biogeography of South African estuarine fishes associated with the subtropical/warm temperate subtraction zone. S Afr J Mar Sci 96: 184-188

Marko PB (2004) 'What's larvae got to with it?' Disparate patterns of post-glacial population structure in two benthic marine gastropods with identical dispersal potential. Mol Ecol 13: 597-611

Marshall DJ, McQuaid CD (1989) The influence of the respiratory response on the tolerance to sand inundation of the limpets *Patella granularis* (L) (Prosobranchia) and *Siphonaria capensis* (Q et G) (Pulmonata). J Exp Mar Biol Ecol 129: 191-201

Marshall DJ, McQuaid CD (1993) Differential physiological and behavioural responses of the intertidal mussels, *Choromytilus meridionalis* (Kr.) and *Perna perna* L., to exposure to hypoxia and air: a basis for spatial separation. J Exp Mar Biol Ecol 171: 225-237

McDonald JH, Koehn RK (1988) The mussel *Mytilus galloprovincialis* and *M. trossulus* on the Pacific coast of North America. Mar Biol 99: 111-118

McDonald JH, Seed R, Koehn RK (1991) Allozymes and morphometric characters of three specie of *Mytilus* in the Northern and Southern Hemispheres. Mar Biol 111: 323-333

McDonald JH, Verrelli BC, Geyer LB (1996) Lack of geographic variation in anonymous nuclear polymorphisms in the American oyster, *Crassostrea Virginica*. Mol Biol 13: 1114-1118

McNeely JA (2001) An introduction to human dimensions of invasive alien species. In The Great Reshuffling. Human Dimensions of Invasive Alien Species (McNeely JA, ed) IUCN pp 5-20

McQuaid CD, Branch GM (1984) Influence of sea temperature, substratum and wave exposure on rocky intertidal communities: an analysis of faunal and floral biomass. Mar Ecol Prog Ser 19: 145-151

McQuaid CD, Branch GM (1985) Trophic structure of rocky intertidal communities: response to wave action and implications for energy flow. Mar Ecol Prog Ser 22: 153-161

McQuaid CD, Dower KM (1990) Enhancement of habitat heterogeneity and species richness on rocky shores inundated by sand. Oecologia 84: 142-144

McQuaid CD, Lindsay JR (2005) Interacting effects of wave exposure, tidal height and subustratum on spatial variation in densities of mussel *Perna perna* plantigrades. Mar Ecol Prog Ser 301-173-184

McQuaid CD, Lindsay JR, Lindsay TL (2000) Interactive effects of wave exposure and tidal height on population structure of the mussel *Perna perna*. Mar Biol 137: 925-932

McQuaid CD, Lindsay TL (2000) Effect of wave exposure on growth and mortality rates of the mussel *Perna perna*: bottom-up regulation of intertidal populations. Mar Ecol Prog Ser 206: 147-154

McQuaid CD, Phillips TE (2000) Limited wind-driven dispersal of intertidal mussel larvae: *in situ* evidence from the plankton and the spread of the invasive species *Mytilus galloprovincialis* in South Africa. Mar Ecol Prog Ser 201: 211-220

Menge BA (1976) Organization of the New England rocky intertidal community: Role of predation, competition, and environmental heterogeneity. Ecol Monogr 46: 355-393

Morton B (1987) The functional morphology of the organs of the mantle of *Perna viridis* (L 1758) (Bivalvia: Mytilacea). Am Malacol Bull 5: 159-169

Moyle PB (1986) Fish introduction in North America: patterns and ecological impact. In Mooney HA (ed) Ecology of Biological invasions of North America and Hawaii. Springer, New York Berlin Heidelberg, pp 27-43

Moyle PB, Light T (1996) Fish invasions in California: do abiotic factors determine success. Ecology 77: 1666-1670

Nei M (1987) Molecular evolutionary genetics/Masatoshi Nei. New York, Columbia

Newell CR, Shumway SE, Cucci TL, Selvin R (1989) The effects of natural seston particle size and type on feeding rates, feeding selectivity and food resources availability for the mussel *Mytilus edulis* L., 1758 at bottom culture sites in Maine. J Shellfish Res 8: 187-196

Newell RC (eds) (1970) Biology of intertidal animals. Logos Press London

O'Riordan CA, Monismith SG, Koseff JR (1993) A study of concentration boundary-layer formation over a bed of model bivalves. Limnol Oceanogr 38: 1712-1729

O'Toole MJ (1977) Investigations into the early life history of the South West African anchovy *Engraulis capensis* Gilcrest. In Investigations into some important fish larvae in the South East Atlantic in relation to hydrological environment, PhD Thesis, University of Cape Town

Occhipinti-Ambrogi A (2001) Transfer of marine organisms: a challenge to the conservation of coastal biocoenoses. Aquat Conserv: Mar Freshwat Ecosyst 11: 243-251

Okamura B (1986) Group living and the effects of spatial position in aggregations of *Mytilus edulis*. Oecologia 69: 341-347

Olenin S, Gollasch S, Jonusas S, Rimkute I (2000) En-route investigation of plankton in ballast water on ship's voyage from the Baltic Sea to the open Atlantic coast of Europe. Int Rev Hydrobiol, Spec Issue 85: 577-596

Olson RR (1985) The consequences of short-distance larval dispersal in a sessile marine invertebrate. Ecology 66: 30-39

Orensanz JM, Schwindt E, Pastorino G, Bortolus A, Casas G, Darrigran G, Elias R, Lopez GJJ, Obenat S, Pascual M, Penchaszadeh P, Luz Piriz M, Scarabino F, Spivak ED, Vallarino EA (2002) No longer the pristine confines of the world ocean: a survey of exotic marine species in the southwestern atlantic. Biol Invasions 4: 115-143

Paine RT, Levin SA (1981) Intertidal landscapes: disturbance and the dynamics of pattern. Ecol Monogr 51: 145-178

Palumbi SR (1984) Measuring intertidal wave force. J Exp Mar Biol Ecol 81: 171-179

Palumbi SR (1994) Genetic divergence, reproductive isolation, and marine speciation. Ann Rev Ecol Syst 25: 547-572

Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. Ecol Appl 13: 146-158

Palumbi SR, Martin AP, Romano S, McMillan WO, Stice I, Grabowski G (1991) The simple fool's guide to PCR. Vers 2 Dept Zoology, University of Hawaii, Honolulu, HI

Papakostas S, Triantafyllidis A, Kappas I, Abatzopoulos TJ (2005) The utility of the 16S gene in investigatingcryptic speciation within *Brachionus plicatilis* species complex. Mar Biol 147: 1129-1139

Parulekar AH, Dalal SF, Ansari ZA, Harkantra SN (1982) Environmental physiology of raft grown mussels *Perna viridis* in Goa, India. Aquaculture 29: 83-94

Passamonti M, Scali V (2001) Gender-associated mtDNA heteroplasmy in the venerid clam *Tapes philippinarum* (Mollusca Bivalvia). Curr Genet 39: 117-124

Paulay G (1990) Effects of the late Cenozoic sea-level fluctuations on the bivalve faunas of tropical oceanic islands. Paleobiology 16: 415-434

Pearse AS, Humm HJ, and Wharton GW (1942) Ecology of beaches at Beauford, N.C. Ecol Monogr 12: 135-90

Penrith ML, Kensley BF (1970) The constitution of the fauna of rocky intertidal shores of South West Africa. Part I. Luderitz. Cimbebasia (A) 189-239

Perron FE, Kohn AJ (1985) Larval dispersal and geographic distribution in coral reef gastropods of the genus *Conus*. Proceedings of the Fifth International Coral Reef Congress, Tahiti 5(4): 95-100

Peterson RG, Stramma L (1991) Upper-level circulation in the South Atlantic Ocean. Prog Oceanogr 26: 1-73

Petren K, Case TJ (1996) An experimental demonstration of exploitation competition in an ongoing invasion. Ecology 77: 118-132

Pineda J, Escofet A (1989) Selective effects of disturbance on populations of sea anemones from northern Baja California, Mexico. Mar Ecol Prog Ser 55: 55-62

Piriz ML, Casas GN (1994) Occurrence of *Undaria pinnatifida* in Golfo Nuevo, Argentina. Appl Phycol Forum 10: 4

Potter IC, Beckley LE, Whitfield AK, Lenanton RCJ (1990) Comparison between the roles played by estuaries in the life cycles of fishes in temperate Western Australia and southern Africa. Envir Biol Fish 28: 143-78

Powell JR, Caccone A, D Amato G, Yoon C (1996) Rates of nucleotide substitution in *Drosophila* mitochondrial DNA and nuclear DNA are similar. Proc Natl Acad Sci USA 83: 9090-9093

Powers DA (1987) A multidisciplinary approach to the study of the genetic variation within species. In New Directions in Physiological Ecology, ME Feder (ed), A Bennet, W Burggren, RB Huey, pp 38-70. Cambridge UK: Univ Cambridge Press, 364 pp

Price HA (1982) An analysis of factors determining seasonal variation in the byssal attachment strength of *Mytilus edulis* L. J Mar Biol Assoc UK 62: 147-155

Prochazka K (1994) Habitat partitioning in shallow-water cryptic ichthyofaunal communities in the western and south-western Cape, South Africa. PhD Thesis, University of Cape Town, South Africa

Qin X, Waite JH (1995) Exotic collagen gradients in the byssus of the mussel *Mytilus edulis*. J Exp Biol 198: 633-644

Quattro JM, Chase MR, Rex MA. Greig TW, Etter RJ (2001) Extreme mitochondrial DNA divergence within populations of the deep-sea gastropod *Frigidoalvania brychia*. Mar Biol 139: 1107-1113

Quesada H, Wenne R, Skibinski DOF (1995) Differential introgression of mitochondrial DNA acr species boundaries within the marine mussel genus *Mytilus*. Proc Roy Soc B 262: 51-56

Ramirez SC, Càceros-Martìnez J (1999) Settlement of the blue mussel *Mytilus galloprovincialis* Lamarck on artificial substrates in Bahìa de Todos Santos, BC, México. J Shellfish Res 18: 33-39

Reeb CA, Avise JC (1990) A genetic discontinuity in a continuously distributed species: mitochondrial DNA in the American oyster, *Crassostrea virginica*. Genetics 124: 397-406

Reid D, Orlova MI (2002) Geological and evolutionary underpinnings for the success of Ponto-Caspian species invasions in the Baltic Sea and North American Great Lakes. Can J Fish Aquat Sci 59: 1144-1158

Rejmanek M (1996) What attributes make some plant species more invasive? Ecology 77: 1655-1661

Remerie T, Bourgois T, Peelaers D, Vierstraete A, Vanfleteren J, Vanreusel A Phylogeographic patterns in the mysid *Mesopodopsis slabberi* (Crustacea, Mysida) in Western Europe: evidence for high molecular diversity and cryptic speciation. Mar Biol (in press)

Reznick DN, Ghalambor CK (2001) The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. Genetica 112: 183-198

Ricciardi A, MacIsaac HG (2000) Recent mass invasion of the North American Great Lakes by Ponto-Caspian species. Trends Ecol Evol 15: 62-65

Richardson BA (1985) The impact of forest road construction on the benthic invertebrate fauna of a coastal stream in southern New South Wales. Bull Aust Soc Limn 10: 65-88

Richmond RH (1987) Energetics, competency, and long-distance dispersal of planula larvae of the coral *Pocillopora damicornis*. Mar Biol 93: 527-533

Ridgway TM, Stewart BS, Branch GM, Hodgson AN (1998) Morphological and genetic differentiation of *Patella granularis* (Gasteropoda: Patellidae): recognition of two sibling species along the coast of southern Africa. J Zool Lond 245: 317-333

Rigaa A, Cellos D, Monnerot M (1997) Mitochondrial DNA from the scallop *Pecten maximus*: an unusual polymorphism detected by restriction fragment length polymorphism analysis. Heredity 79: 380-387

Rius M (2005) The effects of the invasive mussel *Mytilus galloprovincialis* and human exploitation on the indigenous mussel *Perna perna* on the south coast of South Africa. MSc thesis, Rhodes University, South Africa

Rius M, McQuaid CD Wave action and competitive interaction between the invasive mussel *Mytilus galloprovincialis* and the indigenous *Perna perna* in South Africa. Mar Biol (in press)

Robinson JV, Dickerson JE (1987) Does invasion sequence affects community structure? Ecology 68: 587-595

Robinson TB, Griffiths CL, McQuaid CD, Rius M (2005) Marine alien species of South Africa – status and impacts. Afr J Mar Sci 27: 297-306

Roff DA (1992) The evolution of life histories: theory and analysis. Chapman & Hall, New York, New York, USA

Ruiz GM, Carlton JT, Grosholz ED, Hines AH (1997) Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent and consequences. Am Zool 37: 621-632

Sale PF (1991) Reef fish communities: open nonequilibrial system. In : Sale PF (ed.) The Ecology of Fishes on Coral Reefs. Academic Press, San Diego, CA, pp 564-598

Sale PF, Kritzer JP (2003) Determining the extent and spatial scale of population connectivity: decapods and coral reef fishes compared. Fish Res 65: 153-172

Sanford RL JR, Paaby P, Luvall JC, Phillips E (1994) Climate, geomorphology, and aquatic systems. In La Selva: Ecology and Natural History of a Neotropical Rain Forest (LA McDade, KS Bawa, HA Hespenheide, and GS Hartshorn, eds.), University of Chicago Press, Chicago, pp 19-33

Scheltema RS (1986) On dispersal and planktonic larvae of benthic invertebrates: an eclectic overview and summary of problems. Bull Mar Sci 39: 290-322

Scheltema RS (1989) Planktonic and non-planktonic development among prosobranch gasteropods and its relationship to the geographic range of species. In JS Ryland and PA Tyler, editors. Reproduction, genetics, and distributions of marine organisms. Olsen and Olsen, Fredensborg, Denmark, pp 183-188

Schiffman PM (1997) Animal-mediated dispersal and disturbance: driving forces behind alien plant naturalization. In Luken JO, Thieret JM (eds) Assessment and management of plant invasions. Springer, New York Heidelberg Berlin, pp 87-94

Schneider KR, Wethey DS, Helmuth BST, Hilbish TJ (2005) Implications of movement behavior on mussel dislodgement: exogenous selection in a *Mytilus* spp. hybrid zone. Mar Biol 146: 333-343

Schumann EH (1981) Low frequency fluctuations off the Natal coast. J Geophys Res 86: 6499-6508

Schumann EH (1987) The coastal ocean off the east coast of South Africa. Trans Roy Soc S Afr 46: 215-29

Seed R (1996) Patterns of biodiversity in the macro-invertebrate fauna associated with mussel patches on rocky shores. J Mar Biol Assoc UK 76: 203-210

Seed R, Suchanek TH (1992) Population and community ecology of *Mytilus*. In: Gosling EG (ed) The mussel *Mytilus*: ecology, physiology, genetics and culture. Elsevier, New York, p 87-169

Seed R, Richardson CA (1999) Evolutionary traits in *Perna viridis* (L) and *Septifer virigatus* (Weigmann) (Bivalvia: Mytilidae). J Exp Mar Biol Ecol 239: 273-287

Semmens BX, Buhle ER, Salomon AN, Pattengill-Semmens CV (2004) A hotspot of non-native marine fishes: evidence for the aquarium trade as an invasion pathway. Mar Ecol Prog Ser 266: 239-244

SettleWH, Wilson LT (1990) Invasion by the variegated leafhopper and biotic interactions: parasitism, competition and apparent competition. Ecology 71: 1461-1470

Shanks AL (1995) Mechanisms of cross-shelf dispersal of larval invertebrates and fish. In McEdward L (ed), Ecology of Marine Invertebrate Larvae. CRC Press, New York, pp 323-368

Shanks AL, Grantham BA, Carr MH (2003) Propagule dispersal distance and the size and spacing of marine reserves. Ecol App 13: 159-169

Shanks AL, Brink L (2005) Upwelling, downwelling, and cross-shelf transport of bivalve: test of a hypothesis. Mar Ecol Progr Ser 302: 1-12

Shannon LV (1985) The Benguela ecosystem. Part I. Evolution of the Benguela, physical features and processes. Oceanogr Mar Biol Annu Rev 23: 105-182

Sharp PM, Li WH (1989) On the rate of DNA sequence evolution in *Drosophila*. J Mol Evol 28: 398-402

Shea K, Chesson P (2002) Community ecology theory as a framework for biological invasions. Trends Ecol Evol 17: 170-176

Sher AA, Hyatt LA (1999) The disturbed resource-flux invasion matrix: a new framework for patterns of plant invasion. Biol Invasions 1: 107-114

Skibinski DOF, Ahmad M, Beardsmore JA (1978) Genetic evidence for naturally occurring hybrids between *Mytilus edulis* and *Mytilus galloprovincialis* Evolution 32: 354-364

Skibinski DOF, Gallagher C, Beynon CM (1994a) Mitochondrial DNA inheritance. Nature 368: 817-818

Skibinski DOF, Gallagher C, Beynon CM (1994b) Sex-limited mitochondrial DNA transmission in the marine mussel *Mytilus edulis*. Genetics 138: 801-809

Slatkin M (1985) Rare alleles as indicators of gene flow. Evolution 39: 53-65

Soulé ME (1990) The onslaught of alien species, and other challenges in the coming decades. Biol Conserv 4: 233-239

Sousa WP (1985) The role of disturbance in natural communities. Annu Rev Ecol Syts 15: 353-391

Sprung M (1983) Reproduction and fecundity of the mussel *Mytilus edulis* at Helgoland (North Sea). Mar Ecol Progr Ser 17: 283-293

Steffani CN, Branch GM (2003a) Spatial comparisons of populations of an indigenous limpet *Scutellastra argenvillei* and an alien mussel *Mytilus galloprovincialis* along a gradient of wave energy. Afr J Mar Sci 25: 195-212

Steffani CN, Branch GM (2003b) Temporal changes in an interaction between an indigenous limpet *Scutellastra argenvillei* and an alien mussel *Mytilus galloprovincialis*: effects of wave exposure. Afr J Mar Sci 25: 213-229

Steffani CN, Branch GM (2003c) Growth rate, condition, and shell shape of *Mytilus galloprovincialis*: responses to wave exposure. Mar Ecol Prog Ser 246: 197-209

Stenseth NC, Lidicker WC Jr (1992) The study of dispersal: a conceptual guide. In Animal Dispersal: Small Mammals as a Model. NC Stenseth, WC Lidicker Jr (ed), New York/London: Chapman & Hall, pp 5-20

Stephenson TA (1944) The constitution of the intertidal fauna and flora of South Africa. Part 2. Ann Natal Mus 10: 261-358

Stephenson TA, Stephenson A (1972) Life Between Tidemarks on Rocky Shores. WH Freeman: San Francisco

Steward TG, England J (1983) Holocene sea-ice, variations and paleoenvironmental change, northernmost Ellesmere Island, NWT, Canada. Arct Alp Res 15: 1-17

Stewart DT, Saavedra C, Stanwood RR, Ball RR, Zouros E (1995) Male and female mitochondrial DNA lineages in the blue mussel (*Mytilus edulis*) species group. Mol Biol Evol 12: 735-747

Stoner DS (1992) Vertical distribution of a colonial ascidian on a coral reef: the roles of larval dispersion and the life history variation. Am Nat 139: 802-824

Suchanek TH (1978) The ecology of *Mytilus edulis* L. in exposed rocky intertidal communities. J Exp Mar Biol Ecol 31: 105-120

Suchanek TH (1985) Mussels and their role in structuring rocky shores communities. In: Moore PG, Seed R (eds) The ecology of rocky coasts. London, Hodder and Stoughton, pp 70-96

Suchanek TH (1992) Extreme biodiversity in the marine environment: mussel bed communities of *Mytilus californianus*. Northw Environ J 7: 150-152

Sunila I (1981) Reproduction of *Mytilus edulis* L. (Bivalvia) in a brackish water area, the Gulf of Finland. Ann Zool Fenn 48: 121-128

Sutherland BW, Stewart DT, Kenchington ER, Zouros E (1998) The fate of paternal mitochondrial DNA in developing female mussels, *Mytilus edulis*: implications for the mechanism of doubly uniparental inheritance of mitochondrial DNA. Genetics 148: 341-347

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10: 512-526

Tarjuelo I, Posada D, Crandall KA, Pascual M, Turon X (2001) Cryptic species of *Clavelina* (Ascidiacea) in two different habitats: harbours and rocky littoral zones in the northwest Mediterranean. Mar Biol 139: 455-462

Taylor AC (1976) Burrowing behaviour and anaerobiosis in the bivalve *Arctica islandica* (L). J Mar Biol Ass U K 56: 95-109

Taylor PR, Littler MM (1982) The role of compensatory mortality, physical disturbance, and substrate retention in the development and organization of a sand-influenced, rocky-intertidal community. Ecology 63: 135-146

Teske PR, McQuaid CD, Froneman WP, Barker NP Impacts of marine biogeographic boundaries on phylogeographic patterns of three South Africa estuarine crustaceans. Mar Ecol Progr Ser (in press)

Thackeray JF, Herbert RS (1991) Environmental indices from planktonic foraminifera in Late Holocene sediments off Walvis Bay. S Afr J Sci 87: 611-618

Tilman D (1982) Resource Competition and Community Structure. Princeton University Press

Todd CD (1998) Larval supply and recruitment of benthic invertebrates: do larvae always disperse as much as we believe? Hydrobiologia 375/376: 1-21

Tolman HL (1999) User manual and system documentation of WAVEWATCH-III version 1.18. NOAA/NWS/NCEO/OMB tecnical note 166, 110 pp

Torchin ME, Lafferty KD, Kuris AM (1996) Infestation of an introduced host, the European green crab, *Carcinus maenas*, by a symbiotic nemertean egg predator, *Carcinonemertes epalti*. J Parasitol 82: 449-453

Turpie JK, Beckley LE, Katua SM (2000) Biogeography and the selection of priority areas for conservation of South African coastal fishes. Biol Conserv 92: 59-72

Van Erkom Schurink C, Griffiths CL (1990) Marine mussels of southern Africa- their distribution, standing stocks, exploitation and culture. J Shellfish Res 9: 75-85

Van Erkom Schurink C, Griffiths CL (1991) A comparison of reproductive cycles and reproductive output in four southern African mussel species. Mar Ecol Prog Ser 76: 123-134

Van Erkom Schurink C, Griffiths CL (1993) Factors affecting relative rates of growth in four South African mussel species. Aquaculture 109: 253-273

Van Winkle W (1970) Effect of environmental factors on byssal thread formation. Mar Biol 7: 143-148

Vario SL, Koehn RK, Väinöla R (1988) Evolutionary genetics of the *Mytilus edulis* complex in the North Atlantic region. Mar Biol 98: 51-60

Vawter L, Brown WM (1986) Nuclear and mitochondrial DNA comparisons reveal extreme rate variation in the molecular clock. Science 234: 194-196

Veltman CJ, Nee S, Crawley MJ (1996) Correlates of introduction success in exotic New Zealand birds. Am Nat 147: 542-557

Vermeij GT (1996) An agenda for invasion biology. Biol Conserv 78: 3-9

Vilà M, Pujadas J (2001) Land-use and socio-economic correlates of plant invasions in European and North African countries. Biol Conserv 100: 397-401

Vitousek PM, D'Antonio CM, Loope LL, Wsetbrooks R (1996) Biological invasions as global environmental changes. Am Sci 84: 218-228

Waite JH (1992) The formation of mussel byssus: anatomy of a natural manufacturing process. In Results and Problems in Cell Differentiation, Vol 19, Biopolymers (ed ST Case), Berlin: Springer-Verlag, pp 27-54

Wallace JH, van der Elst RP (1975) The estuarine fishes of the east coast of South Africa. IV Occurrence of juveniles in estuaries. V Ecology, estuarine dependence and status. Oceanographic Research Institute, Investigational Report No. 42 Durban , South Africa

Ward JE, Levinton JS, Shumway SE, Cucci TL (1998) Particle sorting in bivalves: in vivo determination of the pallial organs of selection. Mar Biol 131: 283-292

Wedepohl PM, Lurjeharms, Meeuwis JM (2000) Surface drift in the south-east Atlantic Ocean. S Afr J Mar Sci 22: 71-79

Whitfield AK (1994) An estuary-association classification for the fishes of southern Africa. S Afr J Sci 90: 411-417

Widdows J, Fieth P, Worrall CM (1979) Relation between seston, available food and feeding activity, in the common mussel *Mytilus edulis*. Mar Biol 50: 195-207

Wilkins NP, Fujino K, Gosling EM (1983) The Mediterranean mussel *Mytilus galloprovincialis* Lmk. in Japan. Biol J Linn Soc 20: 365-374

Will K, Rubinoff D (2004) Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. Cladistics 20: 47-55

Williams RJ, Griffiths FB, Van der Wal EJ, Kelly J (1988) Cargo vessel ballast water as a vector for the transport of non-indigenous marine species. Estuar Coast Shelf Sci 26: 409-420

Williamson M (1996) Biological Invasion. Chapman and Hall, London

Willis GL, Skibinshi DO (1992) Variation in strength of attachment to the substrate explains differential mortality in hybrid mussel (*Mytilus galloprovincialis* and *Mytilus edulis*) populations. Mar Biol 112: 403-408

Wilson AC, Cann RL, Carr SM, George M, Gyllensten UB, Helm-Bychowski KM, Higuchi RG, Palumbi SR, Prager EM, Sage RD, Stoneking M (1985) Mitochondrial DNA and two perspectives on evolutionary genetics. Biol J Linn Soc 26: 375-400

Wilson JH (1987) Mussels: The problem of early spawning. Aquacult Ir 30: 20-21

Wilson JH, Seed R (1974) Reproduction in Mytilus edulis L. (Mollusca: Bivalvia) in Carlingford Lough, Northern Ireland. Ir Fish Invest Ser B (Mar) 15: 1-30

Witman JD, Suchanek TH (1984) Mussel in flow: drag and dislodgement by epizoans. Mar Ecol Prog Ser 16: 259-268

Wolff WJ (2000) Recent human-induced invasions of fresh waters by saltwater animals? Aquat Ecol 34: 319-321

Wu RSS (2002) Hypoxia: from molecular responses to ecosystem responses. Mar Poll Bull 45: 35-45

Young GA (1985) Byssus-thread formation by the mussel *Mytilus edulis*: effects of environmental factors. Mar Ecol Prog Ser 24: 261-271

Zardi GI, Nicastro KR, Porri F, McQuaid CD Sand stress as a non-determinant of habitat segregation of indigenous (*Perna perna*) and invasive (*Mytilus galloprovincialis*) mussels in South Africa. Mar Biol (in press)

Zhang F, He Y, Liu X, Li S, Ma J, Lou Z (1980) The breeding season of *Mytilus edulis* in Jiaszhou Bay, Shandong Province, China (Chinese with English Abstract). Oceanogr Limnol Sin 11: 341-350

Zouros E, Ball AO, Saavedra C, Freeman KR (1994a) Mitochondrial DNA inheritance. Nature 368: 817-818.

Zouros E, Ball AO, Saavedra C, Freeman KR (1994b) A new type of mitochondrial DNA inheritancein the blue mussel Mytilus. Proc Natl Acad Sci USA 91: 7463-7467