Effects of coastal topography on physiology, behaviour and genetics of indigenous (*Perna perna*) and invasive (*Mytilus galloprovincialis*) mussels

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By

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ABSTRACT

Organisms inhabit environments that have many dimensions, each of which can vary temporally and spatially. The spatial-temporal variations of environmental stressors and disturbances may have major but different effects on indigenous and invasive species, favouring either of them at different times and places.

The invasive mussel *Mytilus galloprovincialis* invaded the South African coast 30 years ago and, on the south coast of South Africa, it now competes and co-exists with the indigenous *Perna perna* in the lower eulittoral zone (referred to here as the mussel zone) The invasive and indigenous species dominate the upper and the lower mussel zones respectively, while the two co-exist in the mid-zone.

My results show that intertidal mussels experience, and respond to, spatial and temporal fluctuations of several biotic and abiotic stressors. The invasive and the indigenous species adopt different strategies when reacting to environmental factors and their physiological and behavioural responses vary in time and in different habitats as different pressures become of overriding importance.

Attachment strength of both species decreased in summer and increased in winter, and was higher on the open coast than in bays for both species, showing a strong positive correlation with wave force in time and space. *P. perna* had significantly higher attachment strength than *M. galloprovincialis* but, contrary to previous studies, the difference in gonad index between the two species varied according to the habitat. In bay habitats, *M. galloprovincialis* had a higher maximum reproductive effort than *P. perna*, however, on the open coast, there was no significant difference between the two species, suggesting that for the invasive species wave action is a limiting factor not only in terms of the attachment strength but also of energy availability for reproductive tissue

i

development. Major spawning events occurred during periods of low wave action while minor spawning coincided with periods of intense hydrodynamic stress. On the open coast, gonad index was negatively correlated with attachment strength for both species while, in bays, there was no correlation between these two factors for either. The two species also showed different behaviour. In the field, *M. galloprovincialis* moved significantly more than *P. perna* over a period of six months. The higher mobility of the invasive species was also confirmed in the laboratory where, in general, *M. galloprovincialis* formed clumps more readily than *P. perna*. Taken collectively, these results suggest that channelling more energy into attachment strength limits reproductive tissue development and that, while the indigenous species invests more in byssal production, the invasive species adopts a more dynamic strategy looking for aggregation or a safer arrangement.

Higher endolithic infestation and a greater expression of heat shock proteins (Hsps) in mussel populations on the open coast than in bays indicate that this habitat is a more stressful environment not only in terms of wave action. Endolith damaged mussels had significantly lower attachment strengths and condition indices than clean mussels, probably due to the need to channel energy into shell repair. The constant shell repair and expression of Hsps typical of open coast populations are energetically demanding processes. These observations suggest that on the open coast, mussels are subjected to more severe energetic constraints than in bay habitats.

Wave and sand stress fluctuated seasonally with the former having a greater effect on mussel mortality on the open coast and the latter a higher impact on bay populations. Overall, mussel mortality rates were higher on the open coast than in bays. My results show that populations on the open coast had fewer private haplotypes and less genetic

endemism than those inside bays. Gene flow analysis showed the relatively stable bay habitats act as source populations with greater genetic migration rates out of bays than into them. These differences in genetic structure on scales of 10s of kilometers show that coastal configuration strongly affects selection, larval dispersal and haplotype diversity.

Environmental gradients that are key factors in species distribution over large geographical scales can also be responsible for micro-scale distributions. My results show that *M. galloprovincialis* colonizes the upper mussel zone where temperature is high, but is less tolerant to this stressor and has to maintain a high expression of Hsps. This suggests that temperature is probably a limiting factor in its invasion towards the sub-tropical east coast.

There are inter- and intra-specific differences in responses to the environment which highlight the efforts of *M. galloprovincialis* and *P. perna* to optimize resource utilization for survival and reproduction. Determining these differences is crucial to understanding patterns of co-existence between competing indigenous and invasive species.

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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

Biological invasions are affecting the earth's ecosystems profoundly (Mark et al. 2000), and a major challenge in invasion biology lies in identifying the processes regulating invasion success (Lawton and Brown 1986; Ehrlich 1989; Davis et al. 2000; Ruiz et al. 2000). Invasions can be considered from a variety of view points including the characteristics of invaders (Kolar and Lodge 2001), the characteristics of invaded communities (Lonsdale 1999), resources (Davis et al. 2000) and natural enemies (Keane and Crawley 2002). These factors are not independent and it is important to consider them together (Shea and Chesson 2002).

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). In some cases, a third stage, involving a regional spread from an initial, successful population, is applicable. There are ten alien and 22 cryptogenic invasive 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 probably of Mediterranean origin (Barsotti and Meluzzi 1968; Steward and England 1983; Wilkins et al. 1983) and 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 in Saldanha Bay, 150 km north of Cape Town on the west coast, and its identity was later confirmed by morphological and protein electrophoretic analysis (Grant et al. 1984; Grant and Cherry 1985). Its recent arrival is confirmed by genetic (Zardi et al. 2007a) and archaeological data (Grant and Cherry 1985). The population genetic structure based on mitochondrial DNA of *M. galloprovincialis* shows low genetic divergence, which is typical of a recent invasion (Zardi et al. 2007a). Analysis of mussel shells from shell middens and raised beach deposits, dating from as long ago as 120 000 years (last interglacial period), did not show any evidence of *Mytilus* (Grant and Cherry 1985). The Mediterranean species most probably arrived in South Africa by shipping, or else it was intentionally introduced without a 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 that 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 it 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

3

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 (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.

The invasion of *M. galloprovincialis* in South Africa has had major consequences for overall intertidal community structure, including the elimination of 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 *Aulacomya 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). In contrast, the infaunal biotas associated with *M. galloprovincialis* and two indigenous intertidal mussel species, *Choromytilus meridionalis* and *Perna perna*, differ in composition, but not abundance, biomass or diversity (Branch and Steffani 2004; Hammond and Griffiths 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.*

4

argenvillei (Steffani and Branch 2003a,b). *M. galloprovincialis* excludes adult *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 overall population density but decreasing 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. Besides 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 (Robinson et al. 2005).

Here, I will focus on *M. galloprovincialis* and the indigenous mussel *P. perna* on the south coast of South Africa. On this section of 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 and McQuaid 2006). *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

5

to the Mediterranean Sea as far as the Gulf of Tunis (van Erkom Schurink and Griffiths 1990). On the west coast of South Africa, 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).

Three main factors contribute to an invader's population growth rate: resources (Petren and Case 1996; Sher and Hyatt 1999; Davis et al. 2000), natural enemies (Settle and Wilson 1990; Mack et al. 2000), and the physical environment, all of which vary in time and space (Moyle and Light 1996; Sutherst et al. 1999). However, as both indigenous species and invaders respond to these factors, it is the difference in their responses that determines the success of the invader and the dynamics of their co-existence (Chesson 2000; Shea and Chesson 2002).

Habitat segregation of *P. perna* and *M. galloprovincialis* is not explained by physiological tolerance to sand stress. When buried under sand, *P. perna* mortality rates were significantly higher than those of *M. galloprovincialis* in both laboratory and field experiments (Zardi et al. 2006a), yet it is *P. perna* that dominates the low shore where sand inundation is frequent (Zardi et al. 2006a).

Zardi et al. (2006b) showed that *P. perna* has stronger byssal attachment than *M. galloprovincialis* due to the presence of more and thicker byssal threads, and that hydrodynamic stress is a determinant of the vertical zonation of the two species, relegating the less strongly attached *M. galloprovincialis* to the higher mussel zone,

6

where water action is less strong. The benefit of being more firmly attached to the substratum is especially clear when mussels are subjected to strong winter storms (Zardi et al. 2006b). After an exceptionally strong storm occurred on the south coast of South Africa, mortality rates of *M. galloprovincialis* were significantly higher than those of the indigenous species at all three mussel zones (Zardi et al. 2006b). At the same time, competition seems also to play a role in the zonation of the two species. *P. perna* improves survival of *M. galloprovincialis* on the low shore in the short term, by providing protection against wave action, but excludes it competitively in the longer term (Rius and McQuaid 2007). On the other hand, *M. galloprovincialis* is a better exploitation competitor, colonizing free space more rapidly after disturbances (Erlandsson et al. 2006).

Wave and sand stress vary also in time (Zardi et al. 2007b) and can alter the mortality rates of the two populations on a monthly basis (Zardi et al. in press). During an 18 month survey, *P. perna* attachment strength was always significantly higher than that of *M. galloprovincialis*, while the latter had a greater reproductive output (Zardi et al. 2007b). It can be suggested that, although high gamete production improves the ability of *M. galloprovincialis* to colonise free space (Erlandsson et al. 2006), it may energetically limit its capacity to invade wave exposed shores. Mortality rates of the two species over a period of six months showed different timing. The indigenous species had higher mortality than *M. galloprovincialis* during periods of high sand accumulation in mussel beds, while the pattern was reversed during winter, when wave action was high (Zardi et al. in press).

Determining variation of environmental stress over different spatial scales (from micro scales to meso scales) is crucial if one is to connect an organism's stress responses to

7

patterns of structure in the resident community (Halpin et al. 2002). Moreover, a quantitative and qualitative analysis of environmental gradients at scales relevant to organisms is important to understand species distributions and interactions between indigenous and invasive populations (Shea and Chesson 2002, Helmuth et al. 2007).

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. Based on an analysis of rocky shore invertebrates, Emanuel et al. (1992) divided the South African coast into three zoogeographic regions: from Lüderitz (Namibia) to Cape of Good Hope (cool-temperate Namaqua Province); from Cape of Good Hope eastward to East London (warm-temperate Agulhas Province); from East London north to Mozambique (subtropical Natal Province). However, sites that may be geographically within a defined terrestrial or oceanographic eco-region may experience dramatically different oceanic and climatic conditions (Menge et al. 1997a,b). For example, it has been suggested that regional patterns of tidal regime and local patterns of wave splash can overwhelm the effects of large-scale climate in driving patterns of body temperature, leading to complex thermal mosaics of body temperatures rather than simple latitudinal gradients (Helmuth et al. 2006).

Although the south coast of South Africa is all part of a distinct biogeographic region (the warm-temperate Agulhas Province), meso-scale (tens of kilometers) topographic features can generate environmental gradients within the same region, affecting the responses of intertidal organisms and their distributions. In particular, this stretch of coast is characterised by a series of bays, each with a prominent cape, facing the south-west Indian Ocean. Bays are a common feature of the world's coastlines, and populations in bays are often subjected to strong selective gradients, e. g. high

8

temperature variation, lower salinities, lower flow velocities and less hydrodynamic disturbance (Ricketts and Calvin 1968; Bertness and Gaines 1993). In addition to these selective gradients, bay morphology can influence oceanographic features by retaining water and consequently affecting larval dispersal (Roughan et al. 2005).

Micro scale (tens of centimetres) variations of stress gradients can also determine the distribution of intertidal communities. The rocky shore contains a variety of microhabitats (e. g. tidal pools, emergent rocks, mussel beds and canopy forming algae), and their interactions with environmental factors (e. g. solar radiation, waves, wind) can be extremely complex, resulting in variations of stress intensity over scales of centimeters (Halpin et al. 2002; Denny et al. 2003; Helmuth and Denny 2003). For example, temperature variation can play an important role in setting the upper limits to the vertical distribution range of intertidal organisms and confine them to distinct bands on the shore with species-specific upper and lower vertical limits (Tomanek and Helmuth 2002). This view is also supported by numerous laboratory studies demonstrating that physiological resistance to physical conditions is greater in species that live at higher tidal heights (Newell 1979; Somero 2002).

The aim of this thesis is to understand the effects of environmental factors related to coastal topography (i. e. bays and the open coast) in regulating adult distribution and larval dispersal of the invasive *M. galloprovincialis* and the indigenous *P. perna* at different spatial and temporal scales.
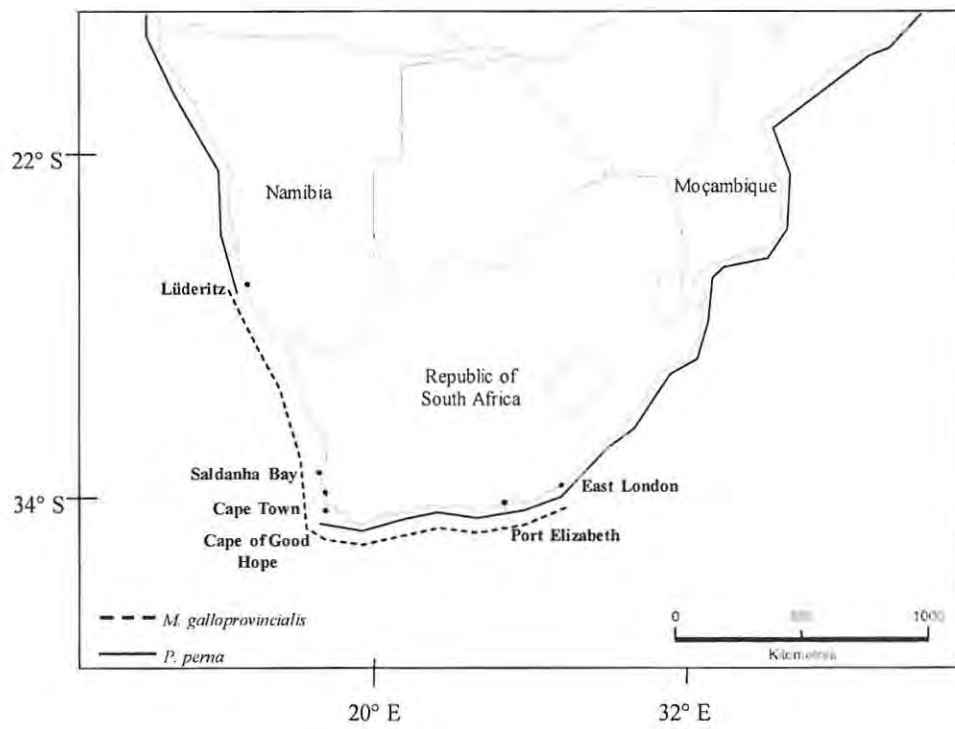
Fig. 1.1 The distribution of *Perna perna* and *Mytilus galloprovincialis* along the southern African coast

Structure of the thesis

The thesis is divided into seven chapters.

Chapter 1 forms a general introduction on the invasion of *Mytilus galloprovincialis* in South Africa, highlighting features of its co-existence with the indigenous *Perna perna* and the importance of variations in time and space of environmental factors in regulating the distribution of the two species at both meso and micro scales. Chapter 2 compares the physiological responses of the two species to several environmental factors in bays and on the open coast over a period of 12 months. Chapter 3 examines the expression of heat shock proteins in mussels at bay and open coast sites and at micro scales in response to heat stress during low tide in the three mussel zone. Chapter 4 investigates the population genetic structure of bay and open coast populations of *Perna perna*. Chapter 5 investigates the effects of predation and hydrodynamic stress on the behaviour of the two species. Chapter 6 deals with the degree of endolith infestation at bay and open coast sites. Finally, Chapter 7 concludes the thesis with a general discussion.

CHAPTER 2

ENERGY CONSTRAINTS OF INVASIVE (*MYTILUS GALLOPROVINCIALIS*) AND INDIGENOUS (*PERNA PERNA*) MUSSELS AND ENVIROMENTAL DISTURBANCES AT BAYS AND ON THE OPEN COAST

INTRODUCTION

When studying the interactions between invasive and indigenous species it is important to take into account the particular habitat into which the invader is introduced (Gerlach and Rice 2003; Burns 2006; Leicht-Young et al. 2007).

An invasive species can have varying degrees of success in establishing and proliferating depending on environmental conditions (Steffani and Branch 2003b; Burns 2006; Richards et al. 2006; Pranovi et al. 2006). Some native environments, especially if they are "extreme", can resist invaders (Daehler 2003), and thus an invader can often fail to establish (Mack 1996). If environmental conditions to which a native species is well adapted change, the native species may lose its prior advantage over non-indigenous species (Byers 2002; Pranovi et al. 2006). However, changes of environmental stress can also challenge well established invasive species, such as *Mytilus galloprovincialis* in South Africa, and possibly influence their distribution (see Chapter 3).

Intertidal populations are shaped by a wide range of biotic and abiotic factors. Among the most important physical factors are height on the shore, degree of wave exposure and sand scouring and sand burial (e.g. McQuaid and Branch 1985; Marshall and McQuaid 1989; Gaylord et al. 1994; Denny 1995; Zardi et al. 2006a,b).

Tidal action leads to steep gradients in temperature, desiccation and oxygen availability (Newell 1979; Zandee et al. 1986) that can drive the distribution and vertical zonation of intertidal species (Wethey 1983; Menconi et al. 1999). Intraspecific variability in sensitivity to thermal stress may contribute to patterns in the latitudinal distribution of mussels within the genus *Mytilus* (Hofmann and Somero 1996), and can also set both the upper intertidal and geographic limits of many other intertidal invertebrates (e.g.

13

Connell 1961; Wethey 1984; Seed and Suchanek 1992; see Chapter 3). Wave action affects entire intertidal communities because it can regulate the supply of food (Bustamante and Branch 1996), dislodge organisms (Paine and Levin 1991; Rius and McQuaid 2006), and influence both the vertical distribution of biomass and the trophic composition of that biomass (McQuaid and Branch 1985). Breaking waves generate hydrodynamic forces on organisms in the intertidal zone (Bell and Gosline 1997; Denny 1987, 1988; Witman and Suchanek 1984). Storms represent one of the largest survival risks for intertidal organisms because these events increase wave action over a period of one to two days and can quickly dislodge individuals with weak attachment (macroalgae: Carrington 1990; Gaylord et al. 1994; Blanchette 1997; crustaceans: Lau and Martinez 2003; various molluscs: Denny 1987, 1995; Denny and Blanchette 2000; Carrington 2002a,b).

A mussel will be dislodged from the substratum when hydrodynamic stress exceeds its attachment strength (Denny et al. 1985; Carrington 2002a,b). The ability of a mussel to withstand strong wave forces is largely due to the byssus, an extracellular bunch of collagenous threads that is secreted in the ventral groove of the foot, which allows mussels to attach firmly to the substratum and to each other (Waite 1992). The strength of byssal attachment has been shown to vary spatially and temporally. For example, attachment strength increases with wave exposure between sites (Witman and Suchanek, 1984) and between habitats within a site (tidepools *versus* emergent rock, Hunt and Scheibling, 2001). Attachment strength also follows an annual cycle, with twofold variation among seasons (Price 1980, 1982; Carrington 2002a,b; Zardi et al. 2007b) It has often been suggested that the dynamics of attachment strength reflect adaptive responses of mussels to increased flow; producing a greater number of byssal

threads so that they can withstand higher wave action (Van Winkle 1970; Witman and Suchanek 1984; Young 1985; Lee et al. 1990; Dolmer and Svane 1994; Hunt and Scheibling 2001; Zardi et al. 2007b). However recent studies have suggested that variation in attachment strength does not only reflect increased thread production in response to wave action, and that other factors, such as seasonal variability in both the material properties of byssal threads and thread decay may be important (Moeser and Carrington 2006).

Periodic sand inundation affects rocky shores throughout the world, and can maintain a balance between sand tolerant and sand intolerant competitors (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), explain the habitat segregation of some intertidal species (Marshall and McQuaid 1989) and can be responsible for massive mortality of intertidal organisms (Zardi et al. 2006a). Mussels can be affected by sand stress either through sand burial or because the ambient water carries a heavy load of suspended sand (Cheung and Shin 2005; Zardi et al. 2006a). 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 can 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). In addition, during sand burial, 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 (Jørgensen 1990),

and switch from aerobic to anaerobic respiration (Taylor 1976), but acidic anaerobic end products can accumulate to lethal levels (Newell 1970).

Mytilus galloprovincialis and *Perna perna* show different tolerances to wave and sand stress (Zardi et al. in press). *P. perna* has higher attachment strength than the invasive species and consequently it is able to withstand wave action better (Zardi et al. 2006b). In contrast, *M. galloprovincialis* is less vulnerable than the indigenous species to the scouring and anoxic stresses induced by sand inundation and burial (Zardi et al. 2006a). Moreover, a recent survey over a period of six months showed that the indigenous species has higher mortality rates than *M. galloprovincialis* during periods of high sand accumulation in mussel beds, while the pattern reversed during winter when wave action is intense (Zardi et al. in press).

In South Africa, invasion of the open coast appears to be restricted by the strong wave action typical of this region; out of 21 invasive species only two are present on wave-exposed shores (Robinson et al. 2005; Mead et al. in press). A similar pattern is reported for marine alien species in Germany (Gollasch and Nehring 2006).

Food provides energy for metabolically costly processes such as byssal thread replacement, somatic growth, increase of shell thickness and gamete synthesis (Griffiths and King 1979; Seed and Suchaneck 1992). During gonad development, mussels are subjected to very high energetic demands and invest up to 90% of their energy in gamete production (Seed and Suchaneck 1992), while the replacement of decayed byssal threads can take up to 8 - 15% of total energy expenditure (Hawkins and Bayne 1985; Seed and Suchaneck 1992). Attachment strength increases with wave exposure (Witman and Suchanek 1984) but exposed sites are also characterised by a greater water flow that delivers more food to the shore (Bustamante and Branch 1996; Dahlhoff and

16

Menge 1996; Sanford and Menge 2001). More sheltered sites have lower food supply but a lower risk of dislodgment. These trade-offs can result in differences in the availability of energy, in the partitioning of this energy and in the physiological performance of intertidal organisms.

Here I investigate the effects of wave action and sand stress on *M. galloprovincialis* and *P. perna* living in two different habitats, bays and the open coast.

MATERIALS AND METHODS

Physiological parameters

Along the south coast of South Africa, rocky headlands bound large "half heart" bays. The experiments were run monthly between November 2006 and October 2007 in two of these bays (Plettenberg Bay, Algoa Bay) and two open coast locations at headlands (Robberg, Cape Recife; Fig. 2.1).

Each location had two sites 200m apart, approximately $20m^2$ in area and topographically uniform, so all mussels in the same site were assumed to be exposed to similar abiotic factors. Samples included only mussels living within a monolayered bed from the mid-mussel zone where the two species co-exist.

Attachment strength

Mytilus galloprovincialis and *Perna perna* individuals (3.5 - 4.5cm; n = 12 each month for each species at each site) were tested *in situ* for attachment strength. A 2mm diameter hole was carefully drilled through the shell valves close to the posterior margin without damaging any byssal threads. A fishhook was inserted through the hole and connected to a recording spring scale via a fishing line. Mussels living within a mussel bed are mainly subjected to lift forces, which act perpendicularly to the substratum (Denny 1987), thus the scale was lifted normal to the rock surface until dislodgment occurred (1 - 3s) and the force required to detach each mussel was recorded in Newtons (N). All dislodged mussels were at least 20cm from each other so that attachment strength measurements were not influenced by previous ones. Data were analysed using a 3-way ANOVA with species, month and habitat (open coast or bays) as fixed factors.

Reproductive condition

The wet mantle was dissected from each mussel (3.5 - 4.5cm; n = 25 each month for each species at each site) and both body and mantle were dried at 60°C to a constant weight. Samples were weighed to the nearest 0.001g and gonad index (GI) was then calculated as the dry mantle weight divided by the dry body weight (Carrington 2002a). Data were analysed using a 3-way ANOVA with species, habitat (open coast or bays) and month as fixed factors.

Mortality measurements

At each site, digital pictures of 12 quadrats (20 x 20cm) were taken monthly, and new quadrats were selected each month (i. e. each quadrats photographed twice only). In each quadrat, 20 mussels were chosen and their mortality was estimated every month as mortality between consecutive photographs. Data were analysed using a 3-way ANOVA with species, month and habitat (open coast or bays) as fixed factors.

Environmental factors

Seasonal sand depth

Digital photographs of mussel beds populating vertical rocks were taken every month at four sites 200m apart at each location (Plettenberg Bay, Robberg, Algoa Bay, Cape Recife) between November 2006 and October 2007. Seasonal sand depth was determined by analysing digital images of 3m wide transects running c. 15m horizontally across a vertical rock face at each site. Sand depth tended to be uniform across the vertical width of the transects and was measured relative to the lowest level from which all other levels were measured. Data failed the requirements of Shapiro's and Levene's test and were analysed using Kruskal-Wallis tests to investigate the effects of habitat (bays or open coast) on sand elevation (months pooled).

Wave force measurements

Degree of wave exposure was quantified on four different occasions (4 October, 9 October, 3 November, 22 November 2006) by measuring maximum wave force using dynamometers modelled on those used by Palumbi (1984). Five dynamometers were placed in three bays (Plettenberg Bay, Jeffreys Bay, Algoa Bay) and three at the open coast locations (Cape St Francis, Cape Recife, Kenton-on-Sea; Fig. 2.1) in the mid-mussel zone and removed the following day. Each dynamometer gave a single measurement of maximum wave force during two tidal cycles, which was expressed in $N.m^{-2}$. 1-way ANOVA was used to evaluate the effect of habitat (bays or open coast) on wave force measurements.

Off-shore wave heights were obtained from 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 real buoy report. Wave heights, wave direction, wave period, wind speed and wind direction were extracted from the NOAA WAVEWATCH III model (Tolman 1999). Estimated mean daily wave heights were calculated from hourly values and then used to calculate mean monthly values. Offshore wave height values for 4 October, 9 October, 3 November, 22 November 2006 were correlated with bay and open coast wave forces measured during the same occasion (n = 15 for bays and open coast).

Cross-correlation analyses

Mean attachment strengths of *M. galloprovincialis* and *P. perna* were cross-correlated with GI, using GI as the lagged variable. Wave forces were cross-correlated with mean attachment strengths of each species, using attachment strength as the lagged variable. Mortality rates of each species were cross-correlated with seasonal sand elevation and

wave force, using mortality as the lagged variable. Cross-correlations had a lag interval of one month and were performed with the computer program Statistica 7. Seasonal bay and open coast wave force data were obtained by converting offshore wave height values according to the regression equation derived above (see Fig. 2.7).

Fig. 2.1

Study locations

RESULTS

Physiological parameters

Attachment strength

There was a significant species x time x habitat interaction because attachment strengths between *Mytilus galloprovincialis* on the open coast and *Perna perna* in bays were not significantly different during all months (Tukey test, 0. 85 < p > 1; Table 2.1). There was no significant difference between habitats within species in three months for *P. perna* (January, February, October) and eight months for *M. galloprovincialis* (December, January, February, March, April, July, September, October).

Overall, *P. perna* had significantly higher attachment strength than *M. galloprovincialis*, with higher attachment strength measured on the open coast for both species (Fig. 2.2).

Reproductive condition

Analysis of Gonad Index (GI) showed a significant species x time x habitat interaction. While *M. galloprovincialis* had a higher GI than *P. perna* within each habitat, the differences were not significant in all months. This was particularly true for the open coast (including January, when the maximum GI occurred for both species), where GI was lower than bays in all months.

M. galloprovincialis had two spawning events. The highest GI values were reached in January followed by a sudden decrease indicating strong spawning. A weaker more protracted spawning event was observed between August and October.

In the bay habitat, *P. perna* maximum gonad index (GI) was 64% lower than that of *M. galloprovincialis*. *P. perna* reached maximum gonad index values between December

23

(Plettenberg Bay) and January (other locations; Fig. 2.3). A second spawning event was not detectable in *P. perna*.

Mortality measurements

Monthly photographs of mussel beds showed strong seasonality of mortality rates (Fig. 2.4). Overall, open coast had significantly higher mussel mortality rates than bays (ANOVA, $p < 0.0001$; Table 2.3) but there were significant habitat x time and species x habitat interactions. In bays, two peaks in mortality occurred at the end of summer (February) and in winter (June), with much lower rates for the rest of the year. On the open coast there was a dramatic peak in mortality again in late summer (February), but no such peak in June. Instead there was a protracted period of relatively high mortality over winter/spring (June- October).

There was a species x habitat interaction because, although the effect of habitat was similar for both species, it was stronger for *M. galloprovincialis* than for *P. perna*. The absence of a species x time interaction indicates similar timing between species.

Table 2.1 Results of a 3-way ANOVA applied to attachment strength with species, month and habitat as fixed factors

Source of variation	df	MS	F	p
Species	1	2815.03	713.13	<0.0001
Habitat	1	1821.28	461.38	<0.0001
Time	11	270.03	68.41	<0.0001
Species x Habitat	1	91.31	23.13	<0.0001
Species x Time	11	5.17	1.31	0.21
Habitat x Time	11	12.00	3.04	<0.001
Species x Habitat x Time	11	8.61	2.18	<0.05
Error	2256	3.95		

Table 2.2 Results of a 3-way ANOVA applied to GI with species, habitat and month as fixed factors

Source of variation	df	MS	F	p
Species	1	9.86	1553.9	<0.001
Habitat	1	1.077	169.71	<0.001
Time	11	1.17	184.66	<0.001
Species x Habitat	1	1.87	295.04	<0.001
Species x Time	11	0.25	39.65	<0.001
Habitat x Time	11	0.2	31.19	<0.001
Species x Habitat x Time	11	0.16	24.97	<0.001
Error	4752	0.006		
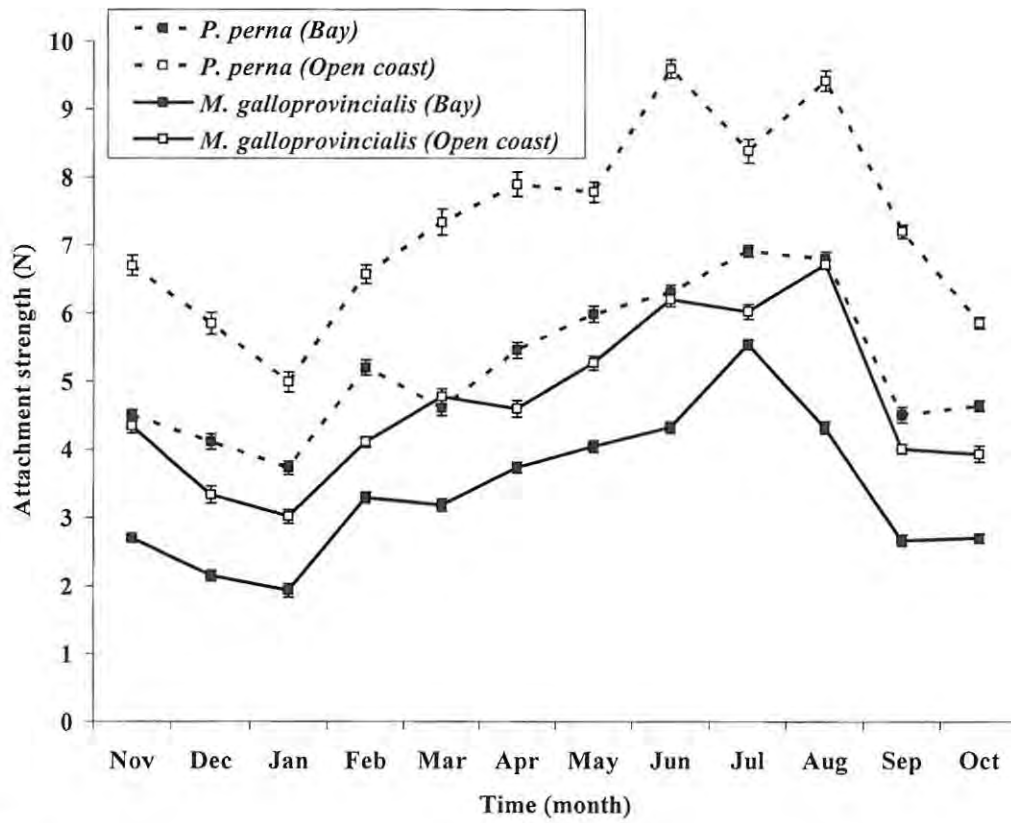
Fig. 2.2

Mean (±SE) monthly attachment strength of *P. perna* and *M. galloprovincialis* at bay and open coast habitats from November 2006 to October 2007.
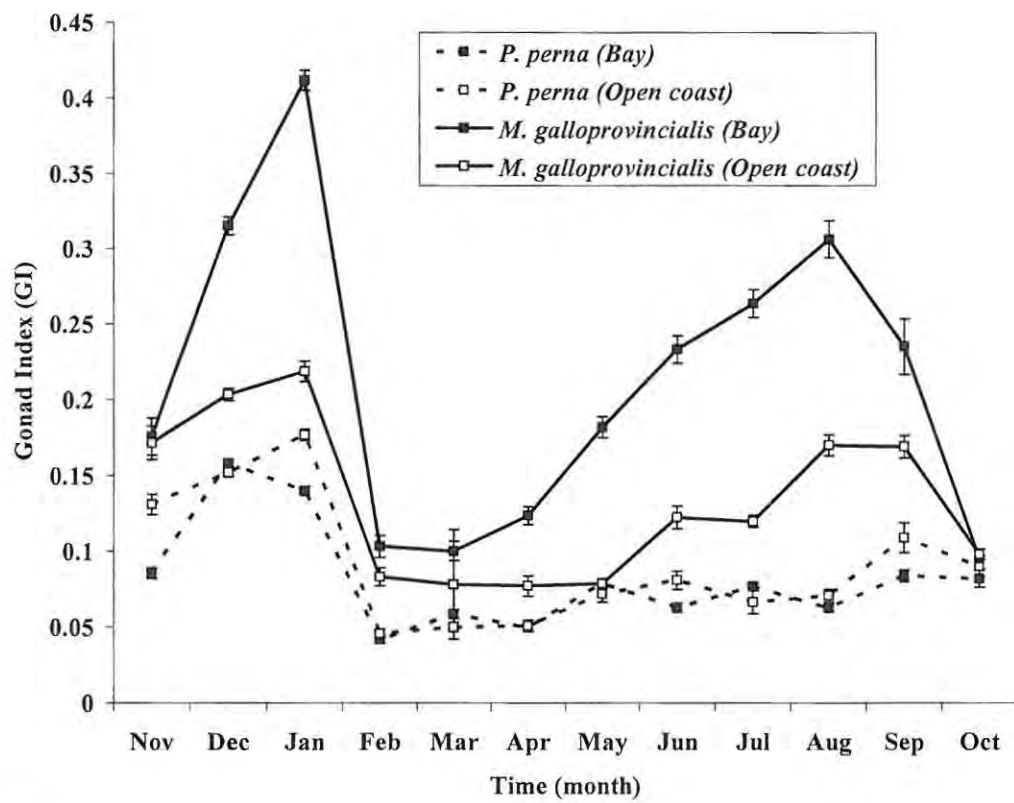
Fig. 2.3

Mean (±SE) monthly gonad index (GI) of *P. perna* and *M. galloprovincialis* at bay and open coast habitats from November 2006 to October 2007.

Table 2.3 Results of a 3-way ANOVA applied to mussel mortality with species, month and habitat as fixed factors

Source of variation	df	MS	F	p
Species	1	0.11	53.35	<0.0001
Habitat	1	0.31	155.76	<0.0001
Time	10	0.27	133.68	<0.0001
Species x Habitat	10	0.008	4.14	<0.05
Species x Time	10	0.001	0.66	0.72
Habitat x Time	10	0.026	13.17	<0.0001
Species x Habitat x Time	10	0.0009	0.47	0.91
Error	2068	0.002		
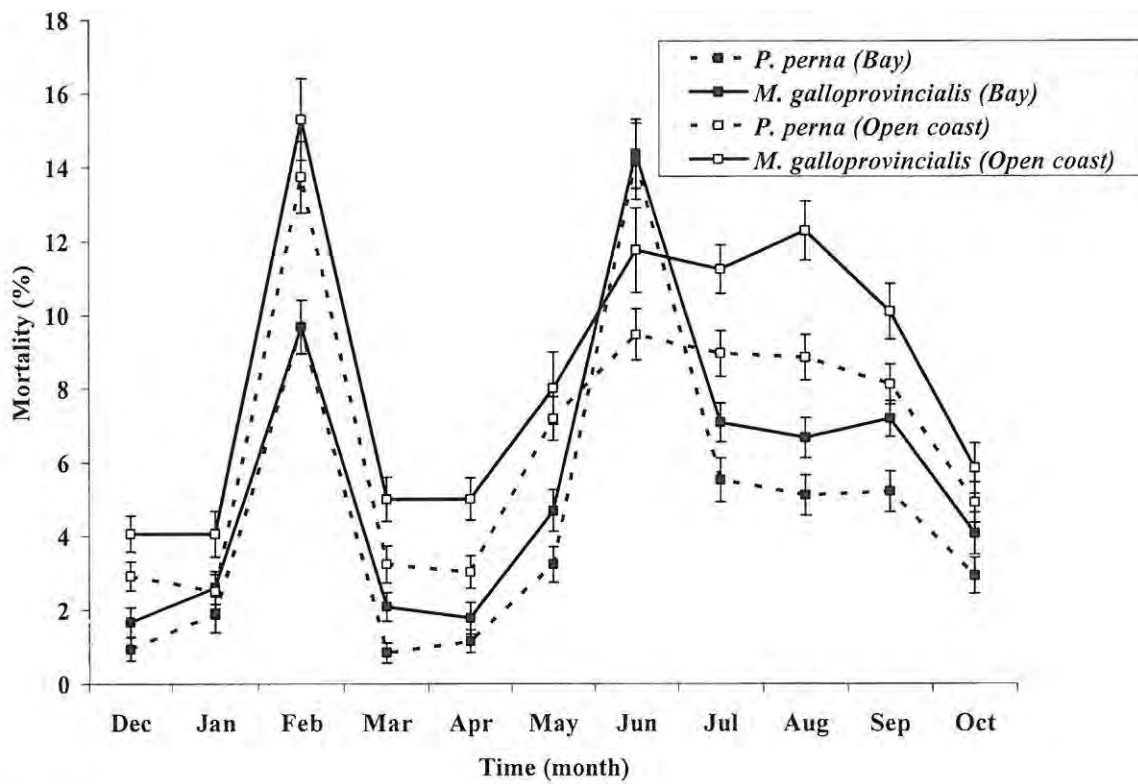
Fig. 2.4

Mean (±SE) monthly percentage mortality rates at open coast and bay habitats from November 2006 to October 2007.

Environmental parameters

Seasonal sand depth

Monthly photographs of mussel beds showed strong seasonal fluctuation of sand coverage with vertical changes of up to 160cm (Fig. 2.5). Overall, bays had a significantly higher sand depth than did the open coast (Kruskal-Wallis, $p < 0.05$). At both habitats, sand accumulation increased towards the end of summer (February) and the beginning of winter (June), followed by massive removal in the subsequent months. Sand levels then remained low through spring and autumn. The lowest sand level ($y = 0$) occurred in March for the open coast and in April in bays.

Wave force measurements

Pooling the data for four different occasions, with an offshore wave height ranging from 1.85 to 5.11m, maximum wave force ($N.m^{-2}$) was significantly higher (ANOVA, $p < 0.0001$; Fig. 2.6) in open coast than in bay habitats. Wave forces both in bays and on the open coast were significantly correlated with offshore wave height ($p < 0.01$ in both cases; Fig. 2.7). Mean monthly wave height increased in late autumn-winter with a peak in July, and decreased in spring and summer with a minimum in January (Fig. 2.8).

Table 2.5 Results of a 1-way ANOVA applied to wave force with habitat as a fixed factor

Source of variation	df	MS	F	p
Habitat	1	292.74	25.51	$p < 0.0001$
Error	22	11.48		
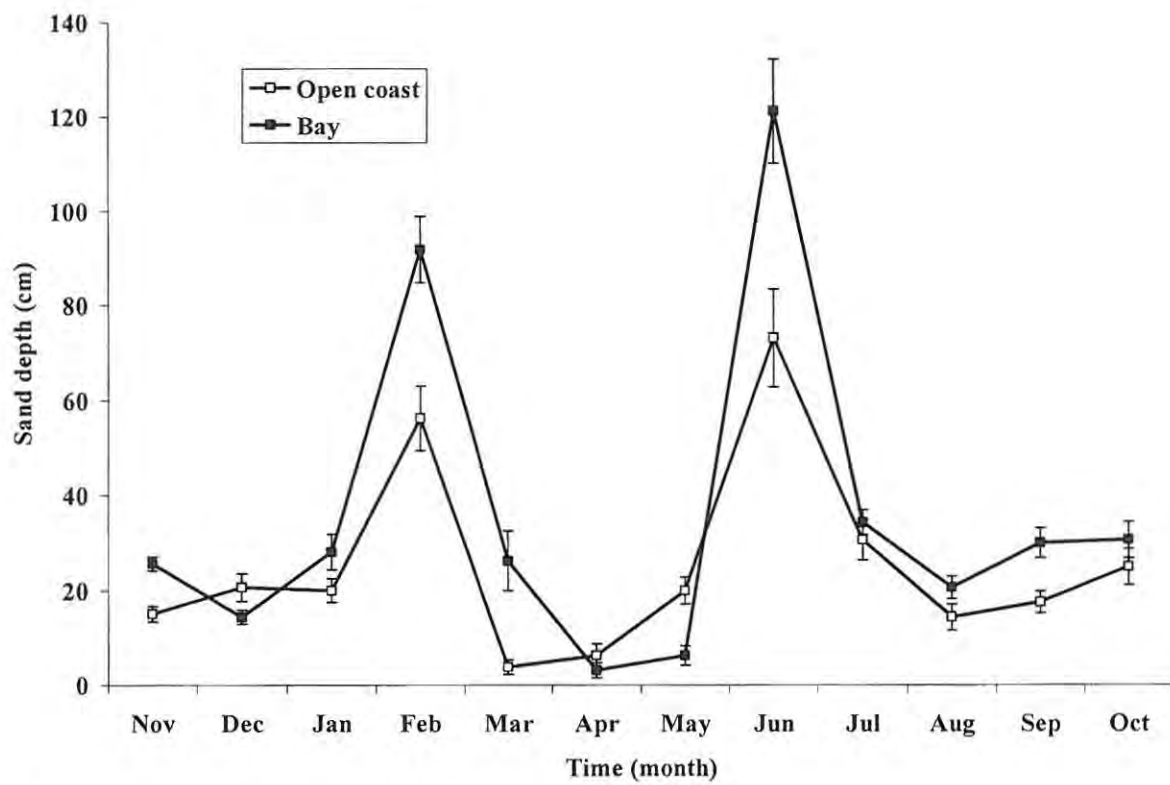
Fig. 2.5

Mean (±SE) monthly sand depth at bay and open coast habitats from November 2006 to October 2007.
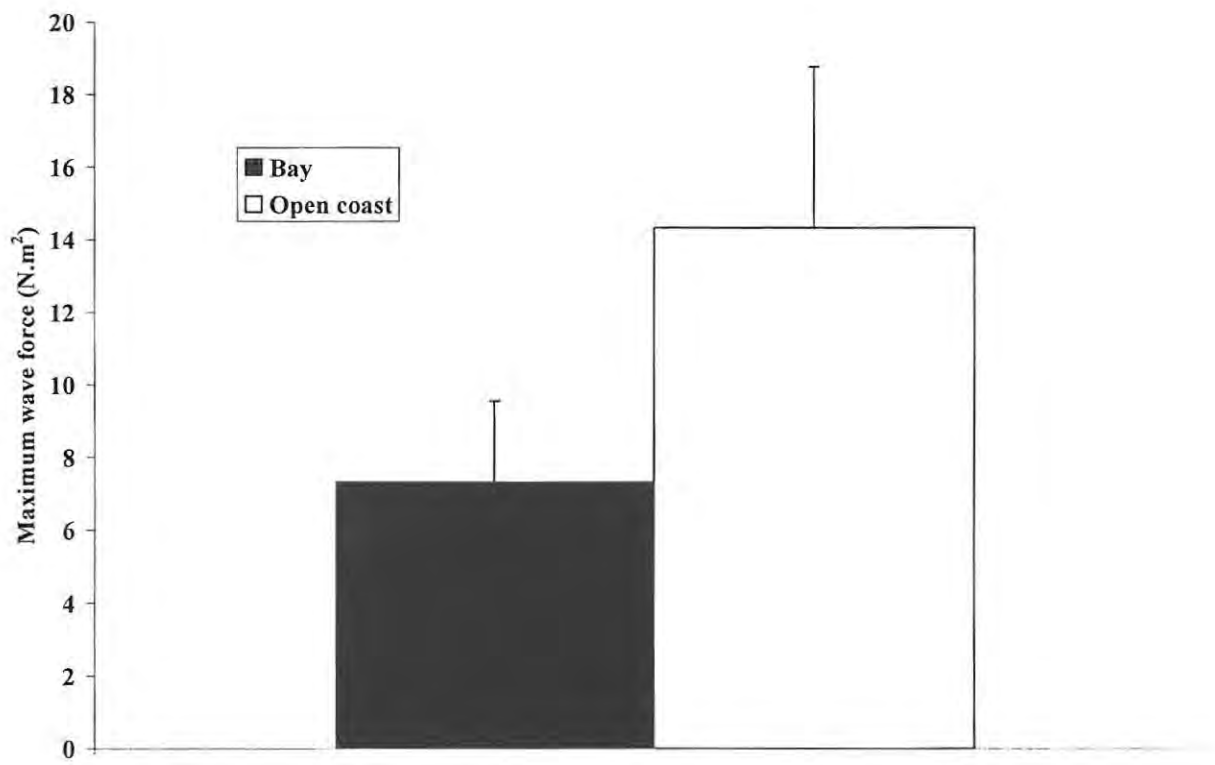
Fig. 2.6

Mean (+SD) maximum wave force (N.m^{-2}) at open coast and bay habitats.
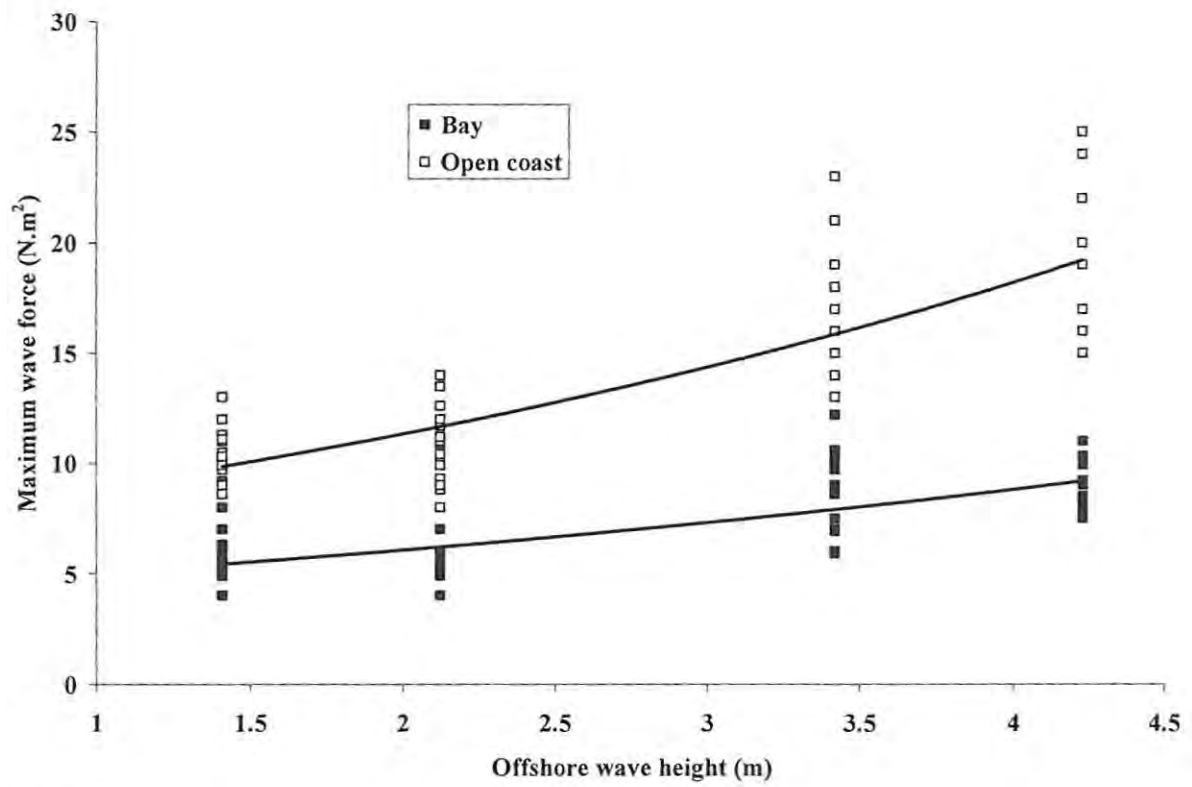
Fig.2.7

Regression between offshore wave height and maximum wave forces at bays and on the open coast (n = 60, y = $4.1809e^{0.1859x}$, R^2 = 0.5094, p < 0.01 for bays; n = 60, y = $7.066e^{0.2365x}$, R^2 = 0.7308, p < 0.01 for open coast).
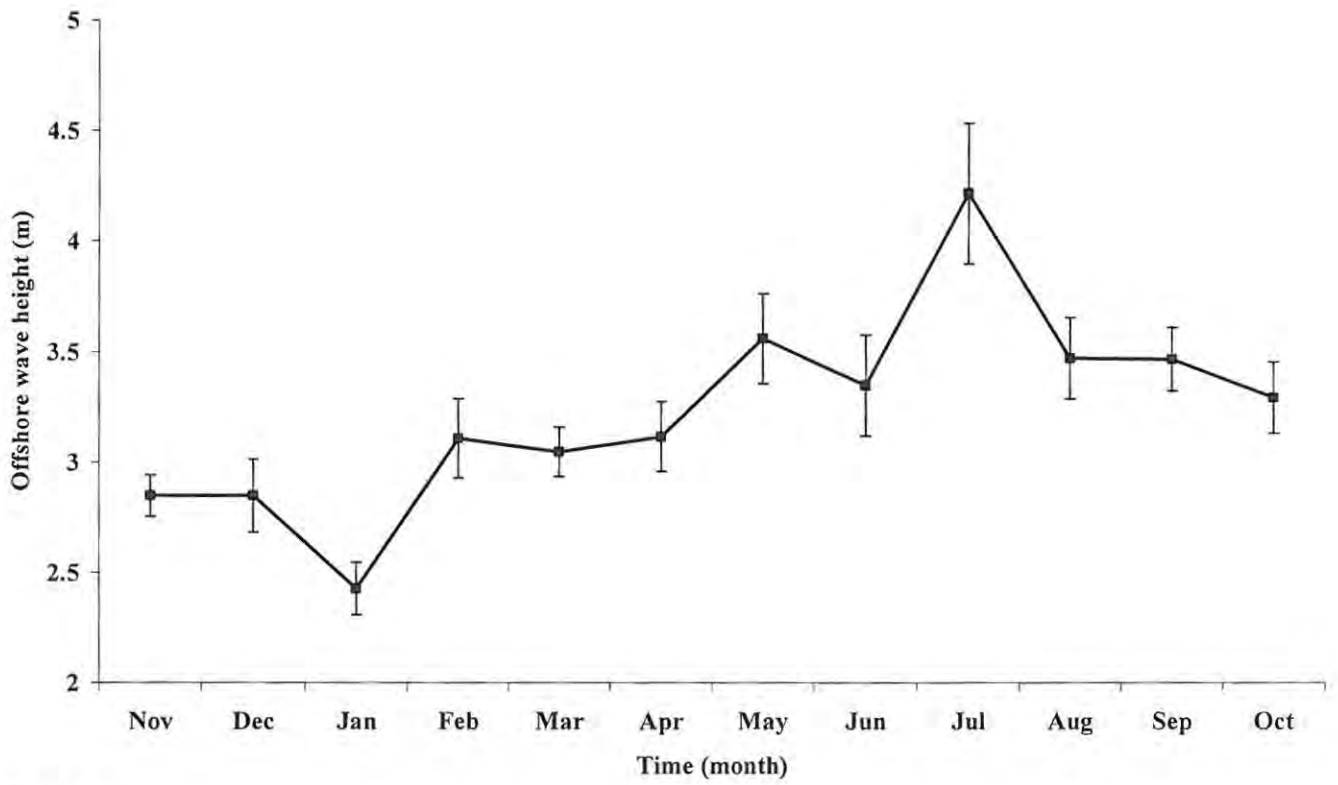
Fig. 2.8

Mean (±SE) monthly offshore wave height from November 2006 to October 2007. Data from a virtual buoy located at 34° 51' S, 23° 53' E, as predicted by the USA National Data Buoy Centre (www.ndbc.noaa.gov).

Cross-correlation analyses

No significant correlation between attachment strength and GI was observed for bays, but there was a significant negative correlation on the open coast for both species (Table 2.6; lag = +1 for *M .galloprovincialis* and lag = +1, 0 for *P. perna*; Fig. 2.9a,b,c,d).

For both species, significant positive correlations between attachment strength and wave force were observed at a lag of zero and +1 in the bays and at lags of zero and ±1 on the open coast (Fig. 2.10 a,b,c,d).

Significant positive correlations between mortality rates and sand elevation were observed at zero lag for both species in both habitats, but with a higher correlation at bay sites (Fig. 2.11a,b,c,d).

No significant correlation between mortality rates and wave force was observed on the open coast, but there was a significant positive correlation in bays for both species (lag = +1; Fig. 2.12 a,b,c,d).
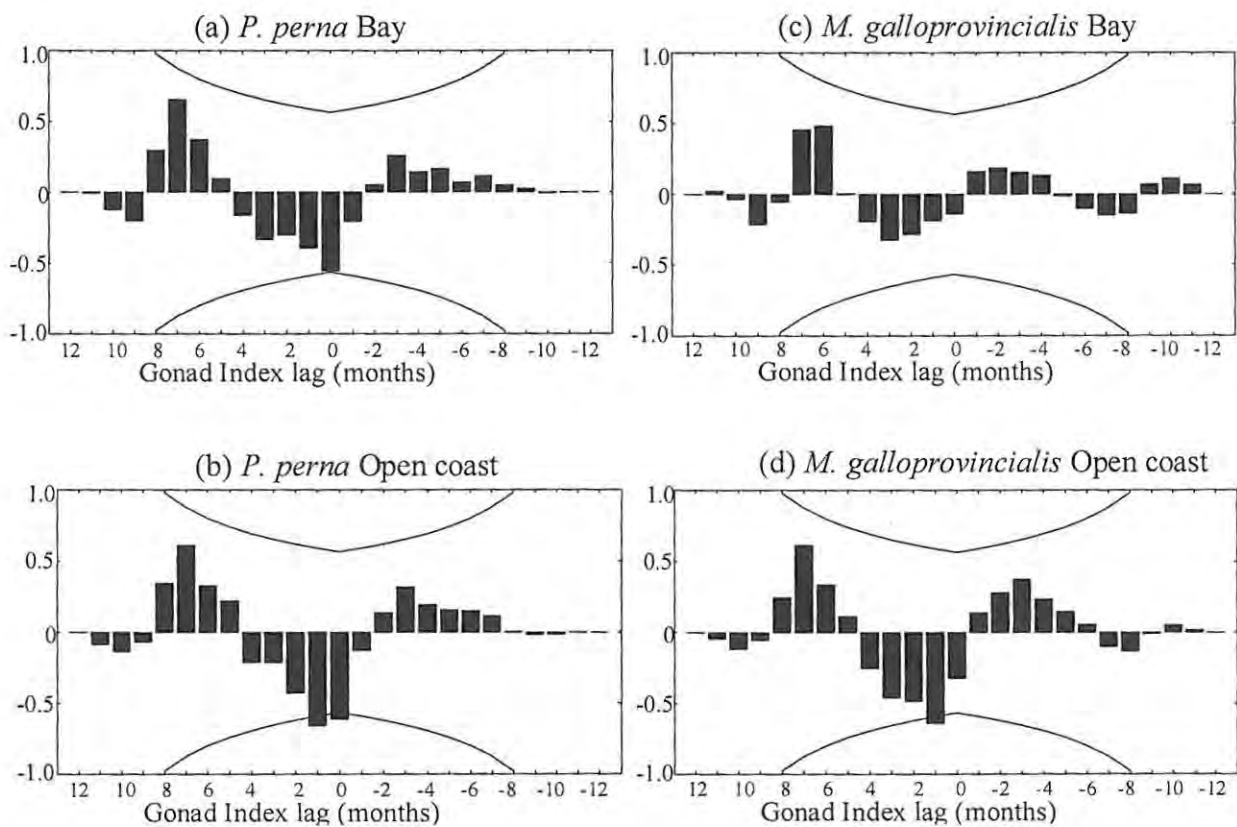
Fig. 2.9

Cross-correlation analyses of monthly mean attachment strength with monthly mean gonad index for (a) *P. perna* in bays, (b) *P. perna* open coast and (c) *M. galloprovincialis* in bays, (d) *M. galloprovincialis* open coast. Bars are correlation coefficients; curved lines are approximate 95% confidences levels for the significance of each correlation.
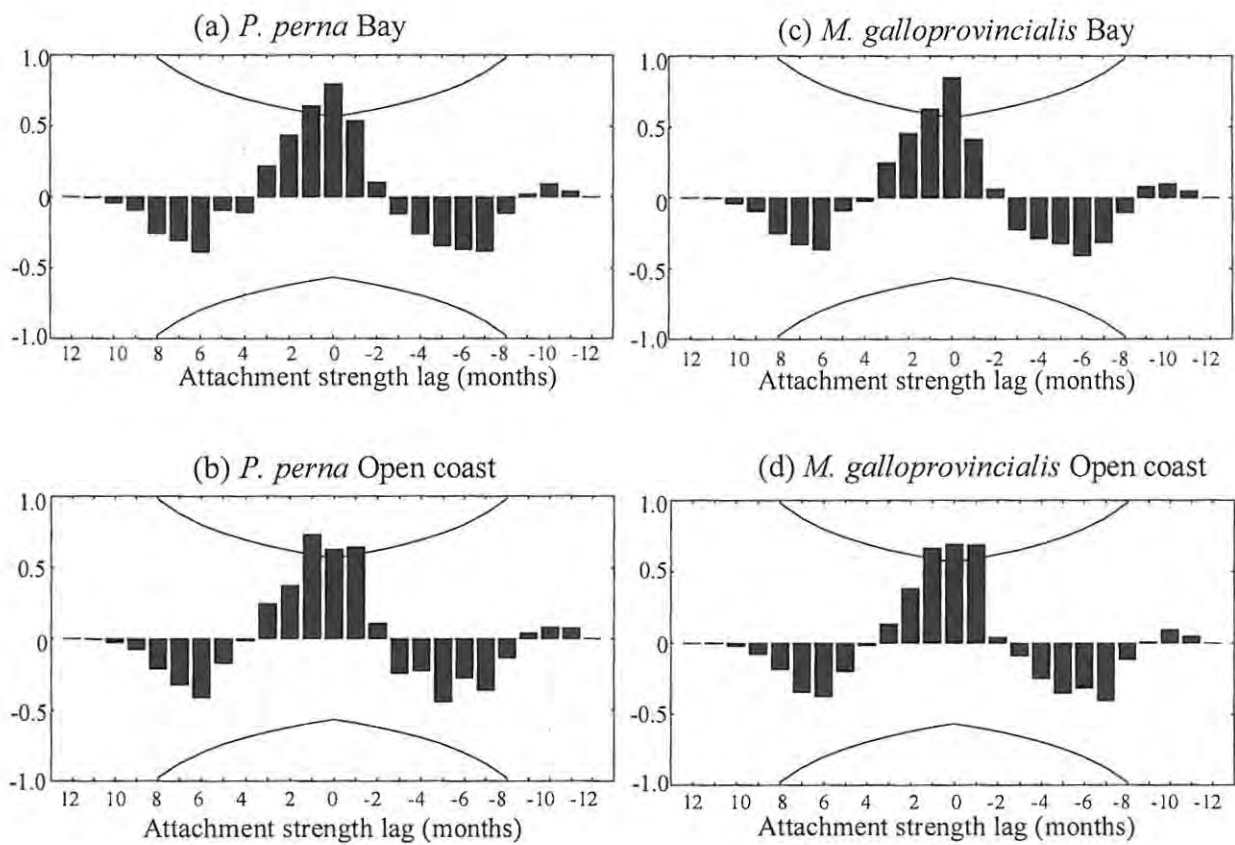
Fig. 2.10

Cross-correlation analyses of monthly mean attachment strength with monthly mean wave force for (a) *P. perna* in bays, (b) *P. perna* open coast and (c) *M. galloprovincialis* in bays, (d) *M. galloprovincialis* open coast. Bars are correlation coefficients; curved lines are approximate 95% confidences levels for the significance of each correlation.
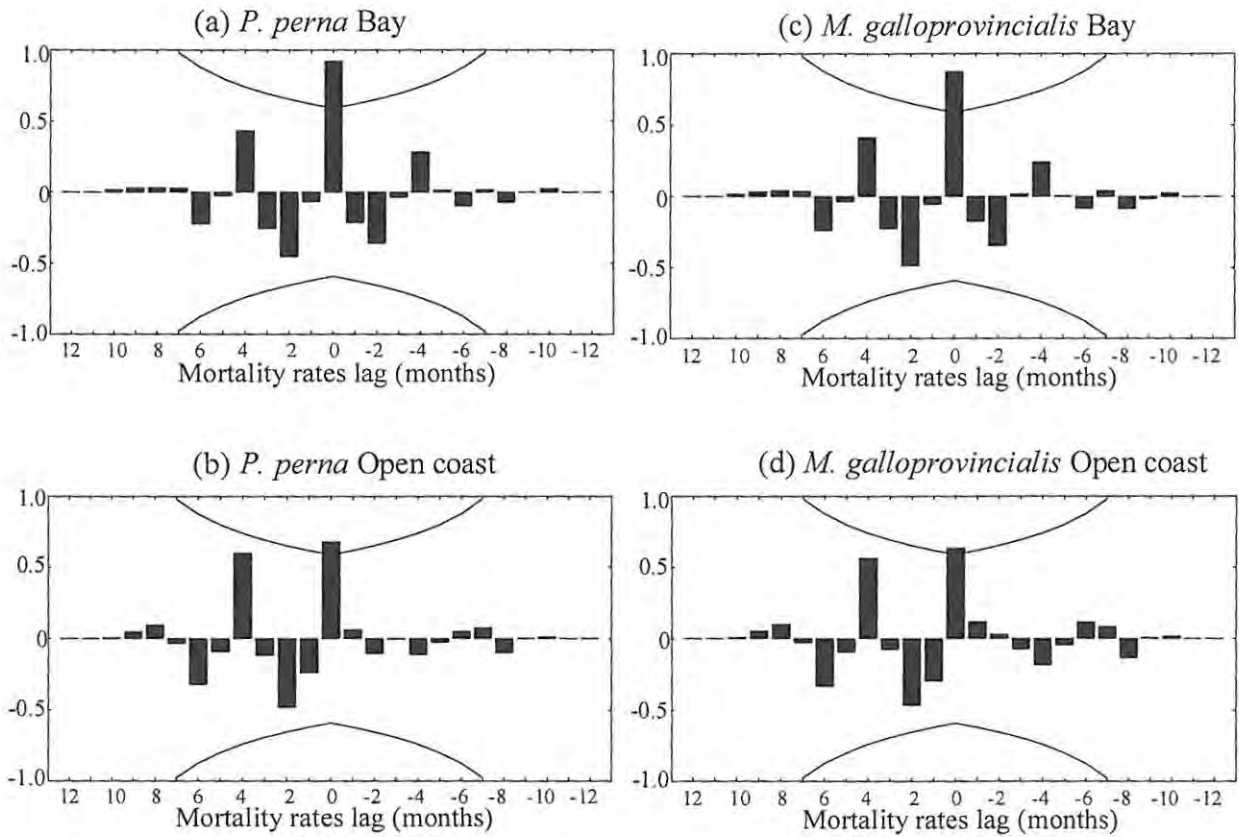
Fig. 2.11

Cross-correlation analyses of monthly mean mortality rates with monthly mean sand depth for (a) *P. perna* in bays, (b) *P. perna* open coast and (c) *M. galloprovincialis* in bays, (d) *M. galloprovincialis* open coast. Bars are correlation coefficients; curved lines are approximate 95% confidences levels for the significance of each correlation.
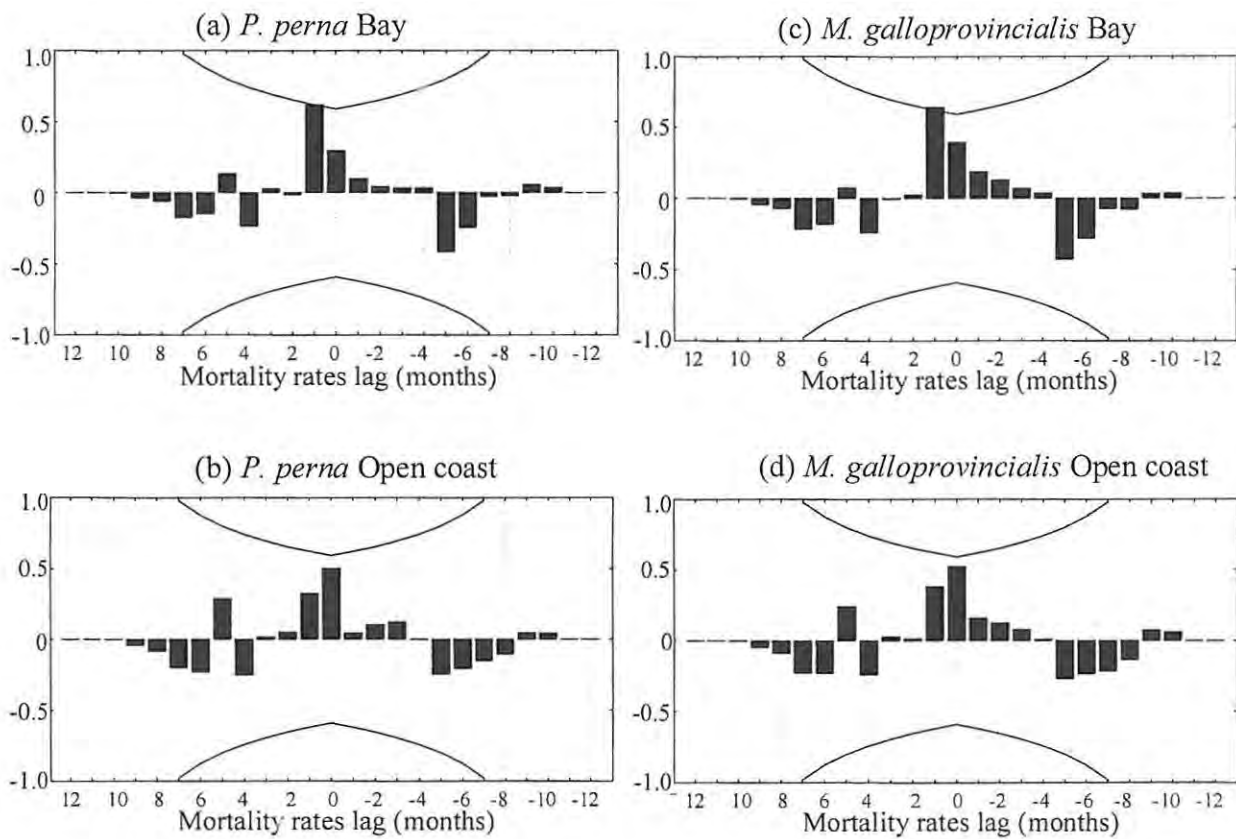
Fig. 2.12

Cross-correlation analyses of monthly mean mortality rates with wave force for (a) *P. perna* in bays, (b) *P. perna* open coast and (c) *M. galloprovincialis* in bays, (d) *M. galloprovincialis* open coast. Bars are correlation coefficients; curved lines are approximate 95% confidences levels for the significance of each correlation.

Table 2.6 Summary of the cross-correlation results. n.s. = non-significant; + = significant positive correlation; – = significant negative correlation.

	Bay	Open coast	lag (months)
Attachment strength / GI	n.s.	–	0; ±1
Attachment strength / wave force	+	+	0; ±1
Sand depth / mortality	+	+	0
Wave force / mortality	+	n.s.	+1

DISCUSSION

The results obtained over one year of study stress the importance of variation in the physical environment at different temporal and spatial scales. Wave and sand stress fluctuated seasonally and differed between bay and open coast habitats, affecting mortality rates and the physiological performances of *Mytilus galloprovincialis* and *Perna perna*.

Attachment strength showed strong temporal variation, generally decreasing in summer and increasing in winter, and was strongly positively correlated (with little lag) with seasonal fluctuations in wave force. Attachment strength also varied spatially, being higher on the open coast than at bay sites for both species, in parallel to the degree of wave action. While this appears to be yet another manifestation of a mussel's ability to sense and respond to its flow environment, similar to the way in which mussels respond to spatial variation in flow, note that previous studies have found that mussels are not always precise in their response. For example, Carrington (2002a) showed that *M. edulis* on Rhode Island shores is slow to build up tenacity following summer, perhaps due to energetic constraints on thread production during the reproductive season. Consequently, these mussels are particularly susceptible to dislodgment events at the onset of the storm season. Waves of equivalent magnitude arriving later in the storm season pose less of a dislodgment risk because attachment strength steadily increases.

My results show that, overall, *P. perna* had significantly higher attachment strength than *M. galloprovincialis*. Over one year, the two species had a major spawning event in summer. At bay sites, there was no correlation between gonad index and attachment strength for either species. In contrast, on the open coast, gonad index was negatively correlated at +1 and zero (only for *P. perna*) lag with attachment strength showing that,

42

for both species, attachment strength was high one month after or during the same month (*P. perna*) that GI was low. Alternatively, high gonad index coincided with or preceded (*P. perna*) low attachment strength. Note that the correlation refers only to attachment strength and gonad index, and does not take into consideration other energy demanding factors (e. g. shell growth and heat stress protein expression; Griffiths and King 1979; see Chapter 3) which could have a seasonal influence on either. Thus, major spawning events occurred during periods of low wave action when limited investment was required in attachment strength. In addition, the minor spawning for *M. galloprovincialis* coincided with periods of intense wave action and very high attachment strength. Hence, it seems that channelling more energy into attachment strength limits gonad tissue development indirectly supporting the concept of balancing reproduction against attachment strength (Carrington 2002a,b; Zardi et al. 2007b).

In South Africa, different spawning periods are reported in different regions and in different years for *P. perna* and *M. galloprovincialis* (Berry 1978, van Erkom Schurink and Griffiths 1991; Zardi et al 2007b). However these studies have reported that *M. galloprovincialis* has a higher reproductive output than *P. perna* (van Erkom Schurink and Griffiths 1991, Harris et al. 1998; Zardi et al. 2007b). Interestingly, my results only partially confirm these findings. While at bay habitats maximum GI *M. galloprovincialis* was 64% higher than for *P. perna*, on the open coast maximum values (i. e. January) for GI were not significantly different between the two species. This suggests that, on the open coast, both species invest more energy in increasing their attachment to rocks, but, while the reproductive output of *M. galloprovincialis* is negatively affected, *P. perna* is able to withstand this higher energy demand without altering gonad production.

43

Despite wave induced hydrodynamic stress playing a crucial role in regulating mortality rates of intertidal organisms, the correlation between wave action and mortality rates of *P. perna* and *M. galloprovincialis* was very weak in bay habitats and non-significant on the open coast. Probably, this was due to the masking effect of sand stress, which was particularly high during periods of low wave action (February). It is also possible that mussels on the open coast adjust to the more intense and frequent hydrodynamic stress typical of this habitat giving them higher resistance to sudden increases in wave action during storms. Hydrodynamic stress experienced by intertidal organisms depends also on shell shape (Denny 1995). Previous studies on the west coast of South Africa showed that the shell of *M. galloprovincialis* tended to be lower and narrower at exposed sites, perhaps reducing the effect of hydrodynamic forces (Steffani and Branch 2003). It is possible that intraspecific differences in shell shape allow mussel populations on the open coast to reduce the higher hydrodynamic stress. Nevertheless, our data showed that mortality rates are higher on the open coast. In addition, previous studies showed that *M. galloprovincialis* shells are 22% wider than *P. perna* shells for a given shell length (Zardi et al. 2006). Consequently, *M. galloprovincialis* has a significantly bigger minor shell planform area (Ap,min, minimum exposed shell area) than *P. perna*; consequently the lift hydrodynamic forces acting on Ap,min for *M. galloprovincialis* are approximately 22% higher than for *P. perna* for a 4.5 cm shell length exacerbating the vulnerability of *M. galloprovincialis* to wave action.

Sand fluctuation and wave action are greater in bays and on the open coast respectively. Cross-correlations between morality rates and sand elevation showed a high positive correlation between the two at bay sites, indicating sand inundation as a major stress disturbing intertidal mussel populations living in bays. Nevertheless, waves were higher

44

on the open coast than at bay sites, suggesting that waves negatively affect more mussels on the open coast than does sand. My results show that sand and wave stress operate out of phase over different temporal scales and sand inundation and burial are sudden events leading to mass mortality of the two species in both habitats. Wave stress is more prolonged over the year and is usually characterized by a relatively gradual seasonal increase. Consequently, in contrast to sand stress, mussels have the potential to adapt to higher wave action by increasing their attachment to the substratum. This could explain why mortality is less well correlated to wave force than to sand stress.

The greater effect of waves on mussel populations on the open coast could also increase the population turnover rates at this habitat, freeing more space on the rocks for new colonizers and allowing more frequent and intense gene pool replenishment (see Chapter 4).

Disturbance, defined as "any relatively discrete event in time that disrupts ecosystem, community, or population structure and changes resources, substrate availability, or the physical environment" (White and Pickett 1985), plays a major role in community dynamics (Sousa 1984; White and Pickett 1985; White and Jentsch 2001). Acting on different temporal and spatial scales, disturbance favours species heterogeneity, creates a mosaic of structures, modifies interactions between organisms and affects competition outcomes (Paine and Levin 1981; White and Pickett 1985; Chesson and Huntly 1997; McQuaid and Lindsay 2005; Erlandson et al. 2006; Rius and McQuaid 2006; Zardi et al. in press).

In densely colonised environments, such as the rocky intertidal zone, subtidal shores, salt marshes and tropical rain forests, the availability and utilization of space by sessile species regulate the dynamics and the organization of the community (Dayton 1979;

Bertness and Ellison 1987; Airoldi 1998). In these habitats, loss of biomass due to disturbance is the primary mechanism that creates open space, increasing opportunities for recruitment and expansion of sedentary organisms (Sousa 1984; Pickett and White 1985; Erlandsson et al. 2006). My results show that wave induced hydrodynamic stress and the scouring and suffocating effects associated with sand inundation and burial can be responsible for very high mortality rates; consequently they can generate large gaps of bare space, increasing the availability of primary substratum. To become a successful invader, an introduced species must have some advantage over an indigenous species, at least at particular times or places, or in a certain life-history trait, such as colonising ability (Shea and Chesson 2002; Hasting at al. 2005). A high reproductive output, together with fast growth and the capacity to colonise free space very rapidly, makes *M. galloprovincialis* a very strong competitor in terms of re-colonization of free space (Erlandsson et al. 2006). However, disturbance often prevents local competitive exclusion by dominant competitors because species usually exhibit trade-offs between competitive ability and colonization ability or competitive ability and stress tolerance (Wilson 1984; Petraitis et al. 1989; van Erkom Schurink and Griffiths 1991; Chesson and Huntly 1997). *M. galloprovincialis* has lower attachment strength than *P. perna*, consequently it suffers greater wave-induced mortality, particularly during winter and on the open coast where hydrodynamic stress is higher. In addition, my results show that the reproductive output in winter of *M. galloprovincialis* is significantly lower on the open coast than in bays, and that in general, on the open coast, GI values for the two species are not significantly different.

These results suggest that, at exposed sites, *M. galloprovincialis* low GI, together with the high wave stress of this habitat, will have a negative effect on the ability of the

invasive species to colonize free space and on post-settlement mortality, making this species a weaker competitor.

CHAPTER 3

THE EFFECTS OF HEAT STRESS AND DESICCATION ON INVASIVE (*Mytilus galloprovincialis*) AND INDIGENOUS (*Perna perna*) MUSSELS
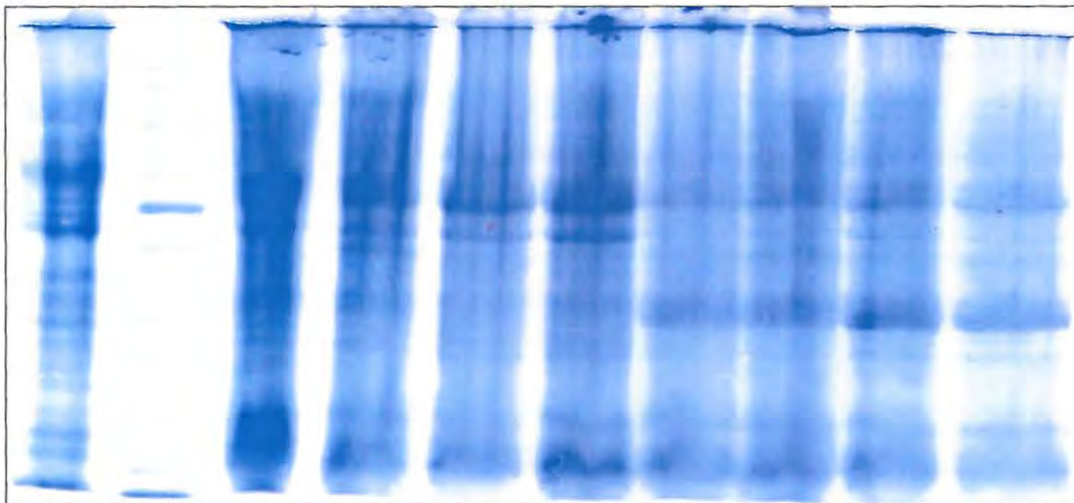
INTRODUCTION

Heat shock proteins (Hsps) are molecular chaperones involved in "house-keeping" functions in the cell, including the prevention of aggregation of damaged proteins, transportation, folding and unfolding, assembly and disassembly of multi-structured units, and the degradation of misfolded or aggregated proteins (Parsell and Lindquist 1993; Jolly et al. 1999; Gregersen et al. 2001). Several families of Hsps have been identified and named according to their molecular weight (kDa). In many organisms, Hsp70 is considered to be the major Hsp family consisting of solely inducible (hsp70), inducible and constitutive, and solely constitutive proteins (hsc70; Boshoff et al. 2004). Heat shock genes are highly conserved and show low between-species variation in the coding regions. Among the inducible hsp70, one of the most conserved Hsps, amino acid similarity between *Drosophila melanogaster*, *Escherichia coli* and *Homo sapiens* is around 70% (Lindquist 1986). Heat shock genes are found in all organisms (although not all species) from bacteria to plants and mammals. The low variation in HSP genes and their nearly universal presence suggest evolutionary importance and a role in the protection of cells during and after stress (Feder and Hofmann 1999).

The rocky intertidal zone is regularly covered and uncovered by the movement of tides, subjecting intertidal organisms to a transition from aquatic to terrestrial conditions. Consequently this habitat is characterized by steep gradients in temperature, desiccation and oxygen availability (Newell 1979; Zandee et al. 1986), which affects the distribution and vertical zonation of intertidal communities (Wethey 1983; Menconi et al. 1999). Several studies have shown that life in the high intertidal involves adaptation responses such as increased thermal resistance (Sokolova et al. 2000), heat stability of key metabolic enzymes (Sokolova and Pörtner 2001), stress-induced expression of Hsps

(Tomanek and Somero 2000), increased extracorporeal water storage, reduced evaporation and increased radiative heat loss (McQuaid and Scherman 1988; Britton and McMahon 1990; Sokolova et al. 2000).

During air exposure, intertidal bivalves will either close their valves and undergo anaerobic metabolism, or exhibit alternate closure and opening (gaping) of the shell, allowing the maintenance of aerobic respiration (Widdows et al. 1979; Famme and Kofoed 1980). The first behaviour leads to reduced evaporative water loss at the cost of inefficient exploitation of organic energy; gaping is more compatible with optimal functioning of the metabolic machinery, but increases levels of water loss and the risk of desiccation (Marsden and Weatherhead 1998). Usually bivalves do not gape continually but alternate gaping with periods of valve closure. Periodic gaping may renew O_2 concentration and minimise evaporative water loss, but at the same time it pushes water out of the mantle cavity, increasing dehydration (McMahon 1983). Despite the increase in water loss, periodic gaping does not appear to have significant influence on body temperature through evaporative cooling for species examined to date (Bayne et al. 1976; Fitzhenry et al. 2004). In South Africa, the indigenous *Perna perna* relies on aerobic metabolism, which implies a certain degree of shell gaping (Marshall and McQuaid 1989; Calvo-Ugarteburu and McQuaid 1998), while *Mytilus spp.* are usually non-gaping species that undergo anaerobic respiration during valve closure (Widdows et al. 1979).

While submerged, an ectothermic invertebrate is likely to have a body temperature fairly similar to that of the surrounding water. However, during low tide several environmental factors such as air temperature, wind speed, solar radiation and relative humidity, can affect body temperature, which can be different from the temperature of

49

the surroundings (Bell 1995; Helmuth 1998, 1999). Temperature extremes experienced by intertidal organism during low tide can far exceed those experienced during submersion (Helmuth 1998). Natural populations of *Mytilus trossulus* experience maximal body temperatures during periods of tidal emersion (Hofmann and Somero 1995). The temperatures at which Hsps are induced occur almost exclusively during low tide (Roberts et al. 1997; Tomanek and Somero 1999; Helmuth and Hofmann 2001) and mass mortalities due to thermal stress are mainly the result of temperature extremes experienced during low tide (Suckanek 1978; Tsuchiya 1983; Williams and Morritt 1995). The heat-shock response is subjected to complex cell regulation (Ali et al. 1998; Morimoto 1998). At temperatures near the upper limits of thermal tolerance, Hsps are the major proteins synthesized (Morimoto et al. 1994). The length and severity of thermal stress can influence Hsp gene activation (Lindquist 1986) and the stability of Hsp70 mRNA varies as a function of temperature (Petersen and Lindquist 1990). In addition, once Hsps are synthesised, like other proteins, they also decay and their half-life is affected by cellular thermal conditions. This complex regulation indicates that Hsps expression is both sensitive to and affected by temperature. Moreover, there is evidence that expression of Hsps is not the only factor involved in the development of thermal tolerance at the cellular level (Chapple et al. 1998). Differential utilization of metabolic pathways, changes in protein turnover and membrane composition play an important role in the thermal resistance of organisms (Prosser 1986; Crete and Landry 1990; Rouse et al. 1994). Glycosylation (attachment of oligosaccharides) of the endoplasmatic reticulum and post-translational modification of proteins reduce the sensitivity of proteins to thermal damage (Kern et al. 1992; Roccheri et al. 1995).

Mytilus galloprovincialis and *P. perna* seem to have reached a distributional equilibrium through partial habitat segregation (Bownes and McQuaid 2006), but variations in time and space of the physical environment, including temperature, are known to play a crucial role in the dynamics of co-existence between indigenous and invasive species (Moyle and Light 1996; Mack et al. 2000). The upper tidal level limit of mussel distribution can be set by thermal stress and desiccation during emersion time (Tsuchiya 1983; Iwasaki 1995). Mussel body temperatures are consistently higher in the upper portion of mussel beds compared to the lower, with greater mortality rates in high intertidal areas (Tsuchiya 1983).

The aims of this chapter are (a) to investigate the separate effects of desiccation and thermal stress on the invasive *M. galloprovincialis* and indigenous *P. perna*, and their roles in the vertical zonation of the two species; (b) to determine meso-scale (10s of kilometres), topographically linked variation in Hsp expression in field. Here I hypothesise that (1) the two species show different behaviour during low tide, leading to a higher desiccation risk for the gaping species *P. perna*; (2) living at higher tidal heights, *M. galloprovincialis* shows higher resistance to heat stress; (3) Hsp expression level will fluctuate seasonally; (4) topographically different habitats will subject mussels to different thermal stresses resulting in different levels of Hsp expression.

MATERIALS AND METHODS

Field experiments

Micro-scale comparison between different tidal heights

Specimens were collected from Plettenberg Bay (Fig. 2.1) in November 2006 during low tide when the tidal level was at the lowest point for the day of collection. In the field, gill lamellae were dissected from *Mytilus galloprovincialis* from the high and mid mussel zones, and from *Perna perna* from the mid and low mussel zones (n = 4 each species, each zone) in sun-exposed areas with 100% mussel cover.

Seasonal meso-scale comparison between bays and open coast

Because of the extremely low Hsp expression levels in *P. perna*, in order to detect possible differences between open coast and bay habitat, this comparison was performed only on *M. galloprovincialis*. Specimens were collected from two bays (Plettenberg Bay, Algoa Bay) and at two open coast sites (Robberg, Cape Recife; Fig. 2.1). Each site had two locations 200m apart at which gill lamellae were taken from three *M. galloprovincialis* collected haphazardly in the mid-mussel zone in sun-exposed areas with 100% mussel cover. Collections were made during low tide, when the tidal level was at the lowest point for the day of collection, in four seasons: December 2006 (summer), May 2007 (autumn), July 2007 (winter) and September 2007 (spring).

Protein extraction

In the field, approximately 100mg of gill tissue collected was homogenized in 350µl of SDS/PAGE sampling buffer (0.5M Tris-HCl, pH = 6.8, 10% glycerol, 10% SDS, 5% β-mercaptoethanol, 0.05% bromophenol blue). The homogenate was heated at 100°C for four minutes and returned to the laboratory in a cooler box for further processing. In the

laboratory, samples were centrifuged at 12 000g for 15 minutes at room temperature and the resulting supernatant was used for electrophoretic analysis.

<u>Electrophoresis, immunodetection, and chemiluminescent autoradiography</u>

Proteins were separated by SDS/PAGE [15% (w/v) gel; Laemmli 1970] and transferred to nitrocellulose [Towbin et al. 1979; purchased from Amersham Pharmacia Biotech (Piscataway, NJ, U. S. A.)]. A sample of a mouse fibroblast cell line (NIH 3T3) crude extract, expressing hsc70 was included on each gel as a positive control and as an internal standard to allow comparison of multiple Western analyses. Proteins were extracted from equivalent gill tissue amounts for the two mussel species, resulting in equivalent protein levels being analysed. Proteins were revealed by chemiluminescent-based immunodetection (ECL Advance Western Blotting detection kit, Amersham) and digitally captured using a chemidoc (Biorad). The mouse monoclonal primary antibody [product no. SPA-820, purchased from StressGen Biotechnologis Corp. (Victoria, BC, Canada)] specific for hsc70 and hsp70 was diluted 1:5000 in Tris-buffered saline blocking solution [50mMl Tris/HCl (pH 7.5)/150mM NaCl/1% (w/v) non-fat milk powder]. The horseradish peroxidase-conjugated anti-mouse IgG secondary antibody (NA931, Amersham) was used at 1:5000 in the same blocking solution.

<u>Image analysis and quantification of expression of heat-shock proteins</u>

Digitised images were analysed with image analysis software (ImageJ Processing and Analysis; Abramoff et al. 2004) to quantify band intensities. All digital images were captured at a range of exposures, and only those images that were captured within the linear phase of the exposure were analysed for variation in Hsps levels. Separate Western blots were treated and exposed identically. For the microscale comparison between different tidal heights, variation in Hsps was analysed using Kruskal-Wallis

53

tests with species as the main effect (zones pooled) and, within the same species, with zone as the independent factor. For the seasonal meso-scale comparison between bays and the open coast, data were analyzed with a 2-way ANOVA with habitat (bay or open coast) and season as fixed factors. Daily average air temperatures, recorded at Algoa Bay (33° 58'S, 25° 36'E) by the South African Weather Service, were used to determine mean monthly values. Mean monthly air temperature was correlated with bay and open coast Hsp expression level (n = 48 for bays and open coast).

Laboratory experiments

Mussels (4 - 5cm) of both species were collected from the mid mussel zone in Plettenberg Bay (Fig. 2.1). Before the experiments, all individuals were acclimated in oxygenated seawater for 48h in a controlled environment chamber at 19°C under a 12:12h light: dark regime.

During analyses, Shapiro's and Levene's test was 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.

Gaping behaviour

Mussels (n = 10 for each temperature) of each species were exposed to air for 6h in a controlled environment chamber at 17°C or 37°C. Percentage of gaping mussels and number of valve movements (per 6h) were noted by continuous visual observation, without the width of the gape being taken into account.

Water loss

Ten individuals of each species were exposed to air for 6h in a controlled environment chamber at 37°C (constant humidity 60%). Individuals were weighed (precision 0.01g) at the beginning of the experiment and every 20 minutes to record loss of water. At the end of the experiment, each animal was wet weighted and then dried at 60°C to constant weight (including shell valves) and the dry weight (tissue plus shell) was measured. The values obtained were used to calculate the percentage water loss. After square root transformation, data were analysed using repeated measures ANOVA with species as a fixed factor.

Desiccation experiment

Mussels (n = 15 each species) were exposed to air in a controlled environment chamber at 17°C and 60% humidity with a hygrometer. Mortality was checked every 12h for 13 days and the experiment was repeated four times. Data were analysed for significant differences between the two species using repeated measures ANOVA with species as a fixed factor.

Heat stress experiment

Two temperature baths were set at 20°C (± 0.1°C). In each water bath, mussels were placed in four plastic containers containing seawater at 20°C (n = 15 of each species in each container). The temperature in the baths was raised at a rate of 1°C every two minutes to 27°C in one bath and 37°C in the other. Water temperature in the plastic containers was monitored and was equal to the water bath temperature.

Once the two temperatures had been reached, mussel mortality was checked every five minutes. Mortality was assessed as failure to close the valves when disturbed. Mortality

rates at 27°C and 37°C were analysed separately using repeated measures ANOVA with species as a fixed factor.

RESULTS

Field experiments

Micro-scale comparison between different tidal heights

Levels of the 70-kDa molecular chaperones, hsp70, and hsc70 in mussel gill tissue were significantly higher in *Mytilus galloprovincialis* than in *Perna perna* ($p < 0.001$; Fig. 3.1), but, within species, they did not differ significantly as a function of tidal height ($p = 0.56$ and $p = 0.25$ for *M. galloprovincialis* and *P. perna* respectively; see Fig. 3.1 for an example of Western analysis).

Seasonal meso-scale comparison between bays and open coast

Levels of the 70-kDa molecular chaperones, Hsp70 (hsp70 and hsc70) in mussel gill tissue were significantly higher on the open coast than in bays and varied significantly among season (Table 3.1; Fig. 3.2). Levels of protein expression were significantly correlated with monthly mean air temperature in bays only ($n = 48$; $R^2 = 0.4059$, $p > 0.05$ for open coast; $y = 0.1937x - 3.6347$ $R^2 = 0.5843$, $p < 0.05$ for bays).

Table 3.1 Results of a 2-way ANOVA applied to Hsp70 optical density with time and habitat as fixed factors

Source of variation	df	MS	F	p
Habitat	1	0.9	9.94	<0.01
Time	3	2.83	31.08	<0.0001
Habitat x Time	3	0.02	0.27	0.84
Error	88	0.09		
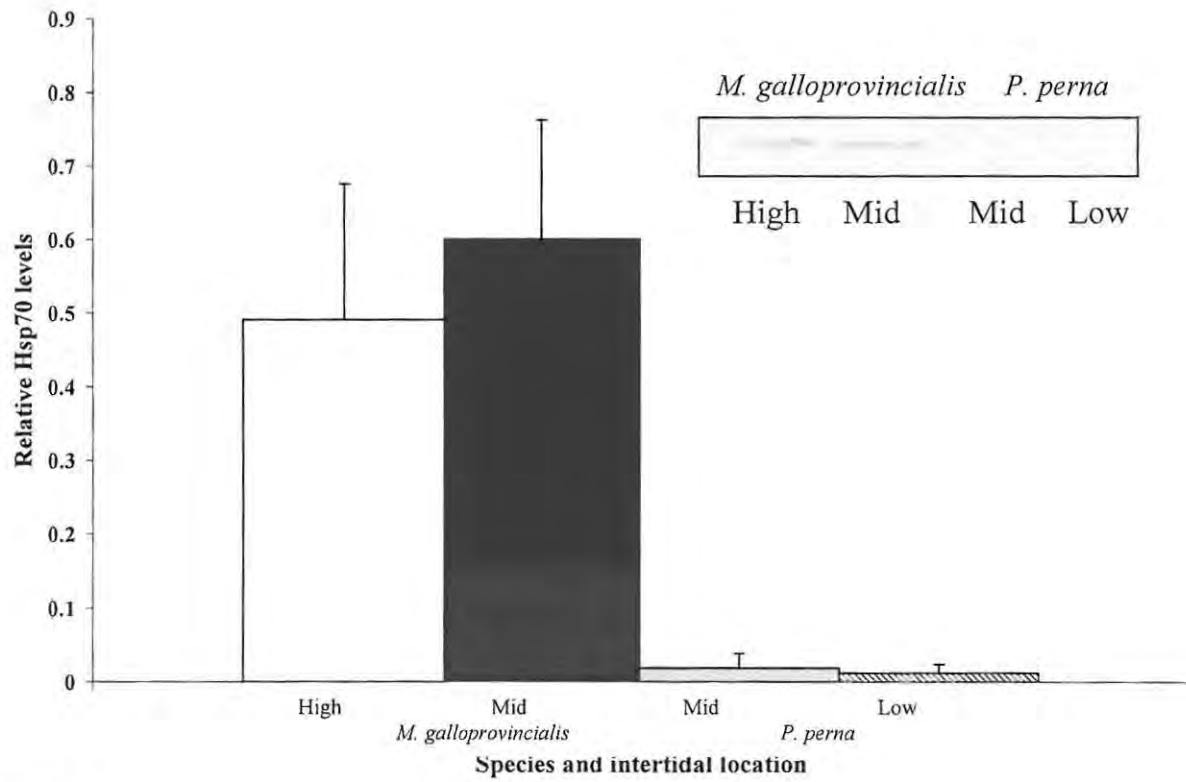
Fig. 3.1

Mean optical density quantification of Hsp70 (+SD) for four individuals of each species, each tidal height, and an example of Western-blot analysis.
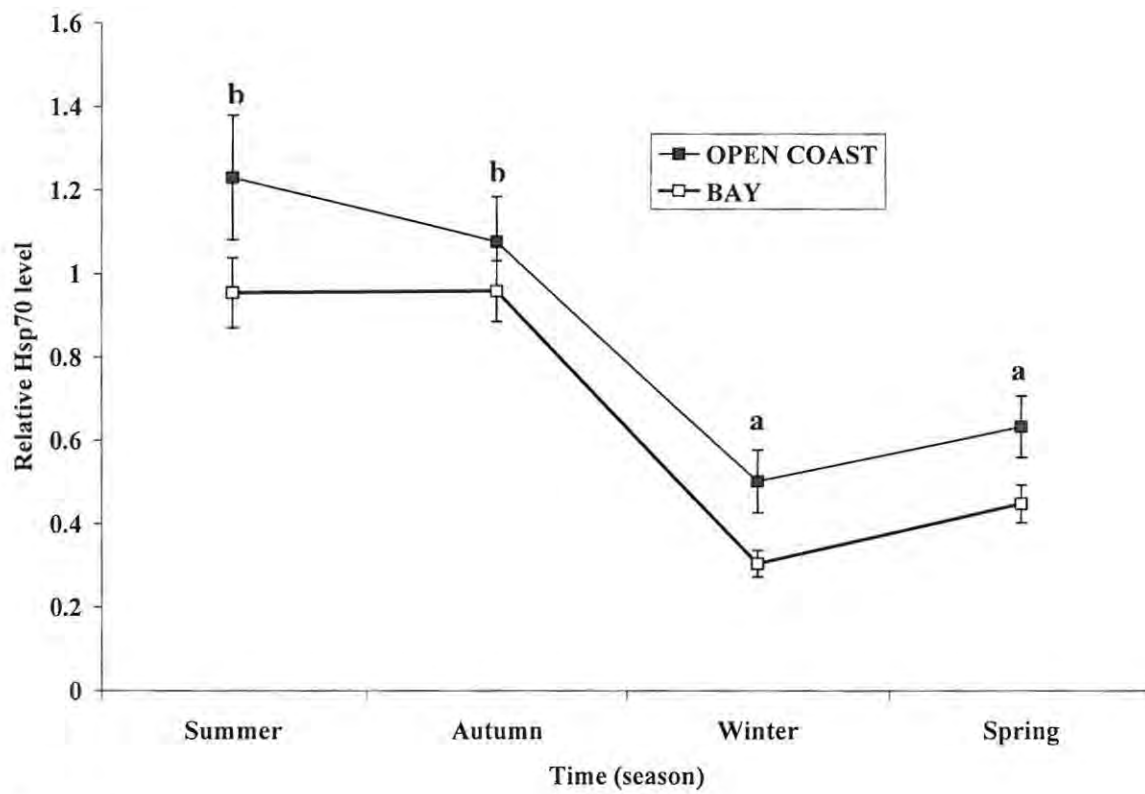
Fig.3.2

Mean optical density quantification of Hsp70 (±SE) for 12 individuals from each habitat, each season. Letters indicate homogenous group (Tukey's test for time, $p < 0.001$).

Laboratory experiments

Gaping behaviour

M. galloprovincialis did not shown gaping behaviour at either temperature. *P. perna* exhibited gaping at both temperatures, with an increase in ventilation movements (number of gapes per hour) and number of gaping individuals at the higher temperature (37°C). While the behaviour was observed for only the first two hours at 17°C, 40% of mussels kept gaping until the fifth hour at 37°C. Ventilation movements decreased with time at both temperatures (Table 3.2).

Water loss

Repeated measures ANOVA showed that water loss rates at 37°C were higher for *P. perna* than for *M. galloprovincialis*. There was, however, a significant (arcsine transformed, $p < 0.0001$; Table 3.3, Fig. 3.3a) species-time interaction, reflecting the steeper rate of increase in water loss over time for *P. perna*. Percentage of water loss increased every hour. After six hours, *P. perna* and *M. galloprovincialis* had lost an average of 47% and 16% of total body water respectively.

Desiccation experiment

Under desiccation stress, mortality rates were higher for *P. perna* than for *M. galloprovincialis* (arcsine transformed, $p < 0.001$; Table 3.4, Fig. 3.3b), with a time-species interaction due to the first day (Tukey's test, $p = 0.32$). *P. perna* and *M. galloprovincialis* began to die after one (1.7%) and four days (3.3%) respectively. Lethal time for 50% mortality (L_T50) was reached after four days for *P. perna* and 12 days for *M. galloprovincialis*.

Heat stress experiment

When subjected to heat stress at 27°C, *P. perna* and *M. galloprovincialis* began to die after 40 minutes (1.7%) and after 20 minutes (5%) respectively, reaching $L_T 50$ after 2h

and 20 minutes for *M. galloprovincialis* and after 3h and 20 minutes for *P. perna*. At 27°C, there was a significant (arcsine transformed, $p < 0.0001$; Table 3.5, Fig. 3.4a) species-time interaction, mainly due to the first five measurements when, in contrast to the following times, mortality rates between the two species were not significantly different (Tukey's test, $p = 1$ at 20, 40 and 60 minutes, $p = 0.99$ at 80 minutes and $p = 0.06$ at 100 minutes). In general, more *M. galloprovincialis* died than *P. perna* at 27°C ($p < 0.0001$), but mortality was not significantly different between the two species at 37°C ($p = 0.66$; Table 3.6, Fig. 3.4b).

Table 3.2 Gaping behaviour of *P. perna* at 17 and 37°C

| Time (h) | 17°C | | 37°C | |
	% gaping individuals	Ventilation movements (h⁻¹)	% gaping individuals	Ventilation movements (h⁻¹)
1	100	3.2 (±0.6)	100	3.1 (± 0.7)
2	90	1.3 (±0.9)	90	1.1 (±0.6)
3	0	0	90	1.3 (±0.7)
4	0	0	90	1 (±0.5)
5	0	0	40	0.4 (±0.5)
6	0	0	0	0

Table 3.3 Results of a repeated measure ANOVA applied to water loss with species as a fixed factor

Source of variation	df	MS	F	p
Species	1	1.7	42.34	<0.0001
Error	18	0.04		
Time	5	0.25	115.87	<0.0001
Time x Species	5	0.01	5.70	<0.001
Error	90	0.002		

Table 3.4 Results of a repeated measure ANOVA applied to desiccation experiment with species as a fixed factor

Source of variation	df	MS	F	p
Species	1	16.31	1276.31	<0.0001
Error	6	0.01		
Time	12	1.18	216.02	<0.0001
Time x Species	12	0.35	63.71	<0.0001
Error	72	0.005		

Table 3.5 Results of a repeated measure ANOVA applied to heat stress experiment at 27°C with species as a fixed factor

Source of variation	df	MS	F	p
Species	1	4.39	95.91	<0.0001
Error	6	0.04		
Time	9	1.42	176.26	<0.0001
Time x Species	9	0.38	47.65	<0.0001
Error	54	0.008		

Table 3.6 Results of a repeated measure ANOVA applied to heat stress experiment at 37°C with species as a fixed factor

Source of variation	df	MS	F	p
Species	1	0.07	0.22	0.66
Error	6	0.32		
Time	5	1.52	34.49	<0.0001
Time x Species	5	0.02	0.48	0.79
Error	30	0.04		

(a)

(b)

Fig. 3.3

Laboratory experiment results for *P. perna* and *M. galloprovincialis*. (a) mean cumulative percentage water loss for 10 individuals of each species (+SD) when exposed to air at 37°C, (b) mean cumulative percentage mortality for four replicates (+SD) when exposed to desiccation (60% humidity).
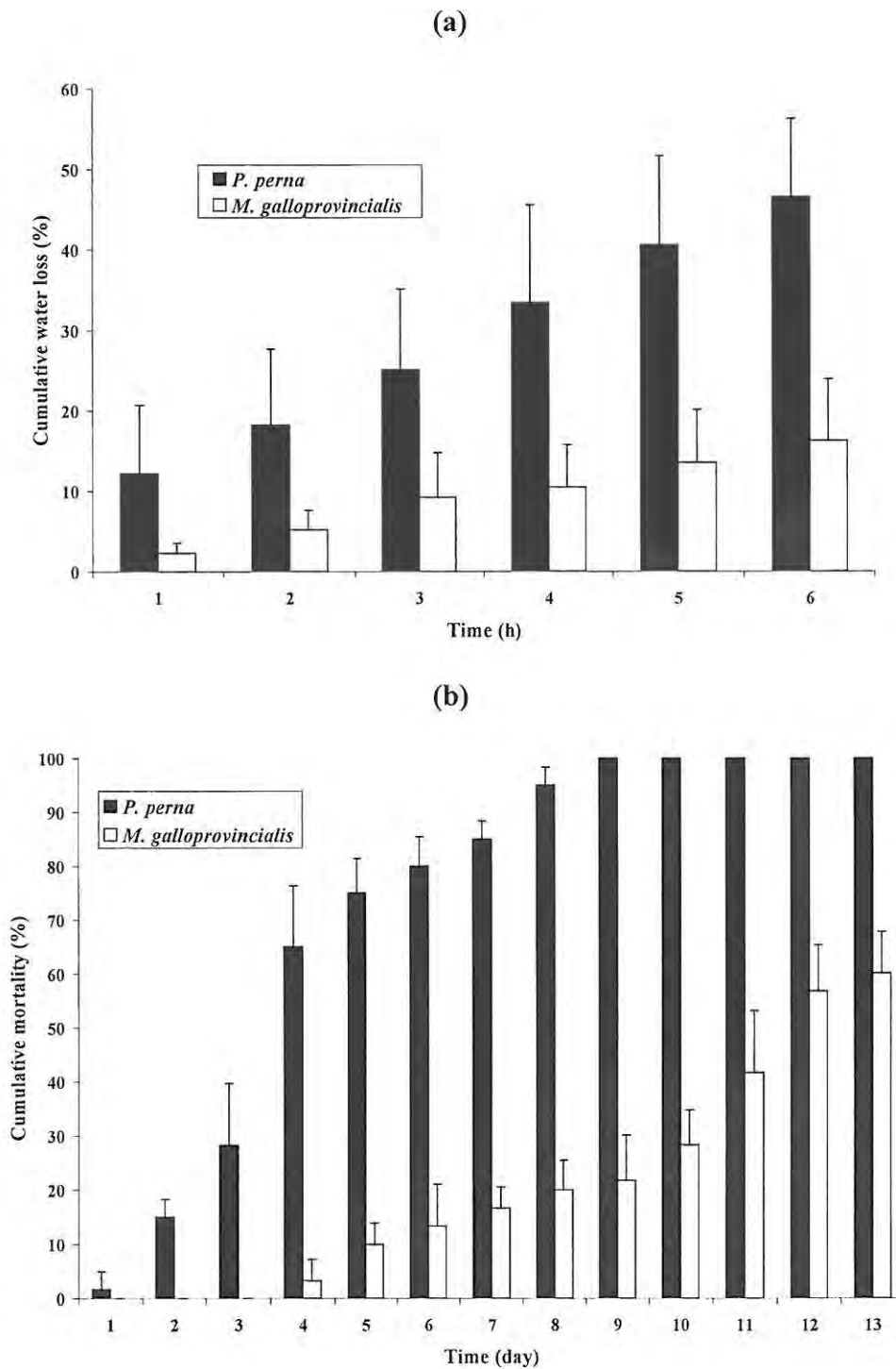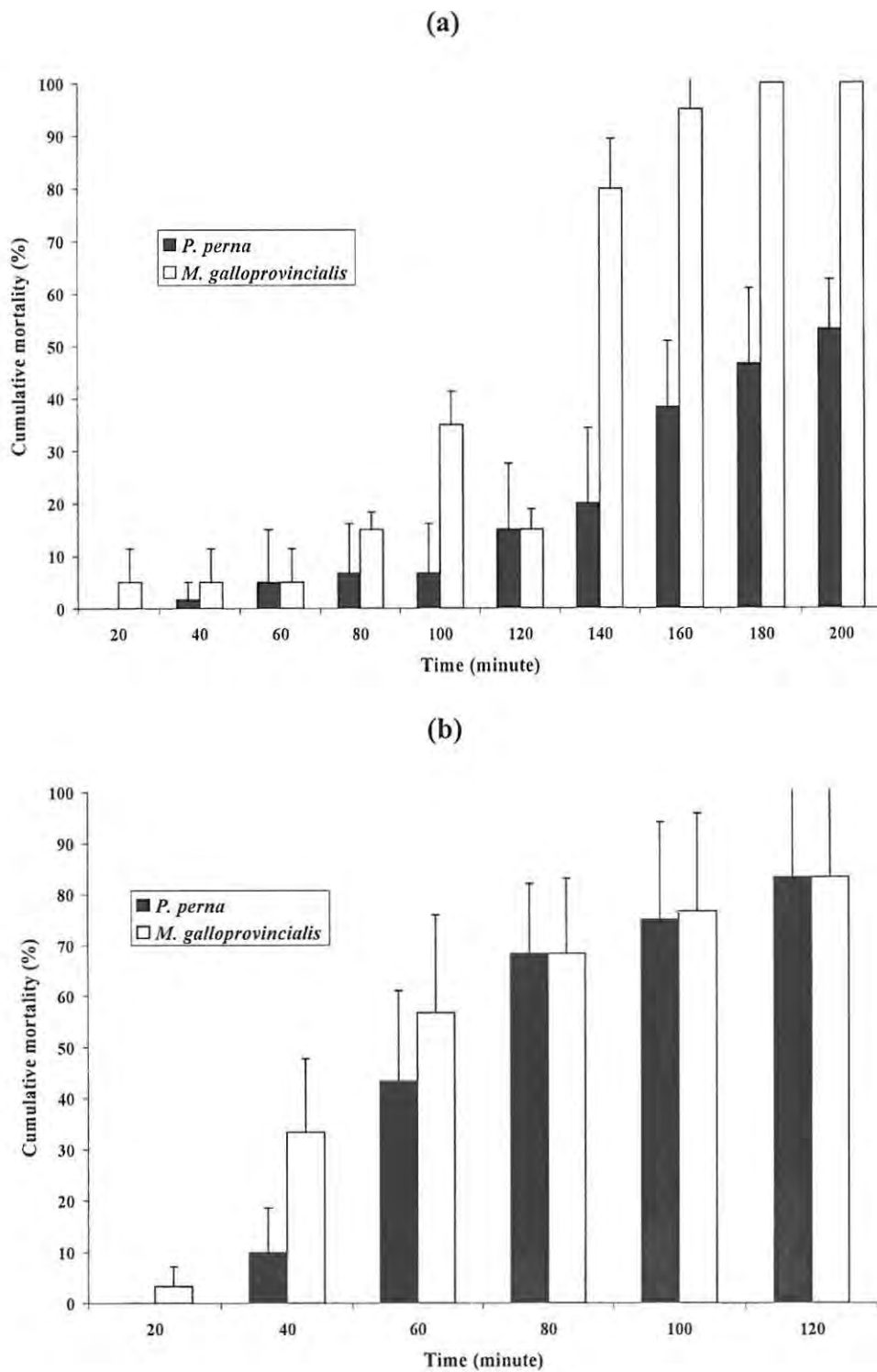
(a)

(b)

Fig. 3.4

Laboratory experiment results for *P. perna* and *M. galloprovincialis*. (a) mean cumulative percentage mortality for four replicates (+SD) when exposed to heat stress at 27°C, (b) mean cumulative percentage mortality for four replicates (+SD) when exposed to heat stress at 37°C.

DISCUSSION

Although heat and desiccation stress often co-occur, my results show that they play different and defined roles in regulating vertical zonation of these two species. Contrary to my expectations, the species that is less well adapted physiologically to coping with heat stress maintains dominance in a habitat where such stress is high. Both mortality under heat stress and Hps70 measurements showed *Perna perna* to be more tolerant of high temperatures than the invasive *Mytilus galloprovincialis*. However, its gaping behaviour exposes *P. perna* to the risk of desiccation and may be important in limiting its ability to colonise the upper mussel zone. *P. perna* is more sensitive than *M. galloprovincialis* to anoxic conditions (Zardi et al. 2006a), which probably forces it to rely on aerobic metabolism during air exposure. Periodic gaping of the valves of mussels allows an O_2 gradient across the gills and the mantle wall, but has no significant influence on body temperature through evaporative cooling (Bayne et al. 1976; Fitzhenry et al. 2004). When aerially exposed, *P. perna* has significantly higher water loss than the non-gaping species, and this is probably due to both evaporation and active expulsion of water during valve closure (Bayne et al. 1976; pers. obser.). Note that the environmental chambers did not have any air flow, so that the rate of evaporative water loss in the field would be much greater. The upper limits of intertidal zonation are generally influenced by environmental gradients and it has been suggested that the upper vertical limits of sessile intertidal organisms are inversely correlated with temperature (Lubchenco et al. 1993; Mathieson et al. 1998; but see Davenport and Davenport 2005). The domination of the high shore by *M. galloprovincialis* appears to come at the price of thermal stress, as shown by the high levels of Hsp70. Although the indigenous species is more resistant to heat stress, it is not able to colonise the *Mytilus*

zone because of its physiological and behavioural limitations in terms of desiccation. This is supported by translocation/competition experiments (McQuaid, unpub. data), and implies that *M. galloprovincialis* occupies a niche/zone not available to *P. perna*.

Mainly due to increasing greenhouse gas concentrations, global air and sea surface temperatures have risen by 0.4 - 0.8°C in the past century and this trend is expected to accelerate in the current century (IPCC 2001). The structure and function of ecosystems worldwide will be dramatically affected by the predicted climate change (Dukes and Mooney 1999; Carlton 2001; Walther et al. 2002). A broad range of organisms have already been affected by recent climate change and communities are undergoing re-assemblage (Hughes 2000; Wuethrich 2000; Walther et al. 2002), but whether global warming will increase the success of biological invaders is still an open question. There are indications that climate change will favour the establishment and the spread of invasive species, in particular facilitating the poleward spread of species characteristic of warmer temperature regimes (Southward et al. 1995; Holbrook et al. 1997; Sagarin et al. 1999; Stachowicz et al. 2002). However, it is likely that invasive species that are already well established in temperate regions, but near their tolerance limits, will be negatively affected by increasing temperatures and related environmental changes in the same way as indigenous species coexisting in the same habitat.

Living at the margins of both the terrestrial and the marine environment, many intertidal organisms are expected to display strong responses to climate change (Somero 2002). If the upper zonation limit of an intertidal species is a consequence of high body temperatures in air, then we should observe a downward shift in the upper limit of zonation when terrestrial temperatures increase (Wethey 1983; Harley and Helmuth 2003). In addition, changes in temperature can alter species co-existence equilibria and

69

modify species distributions (Stachowicz et al. 2002; Schneider and Helmuth 2007). Consequently, the intertidal ecosystem can be used as a model system to predict the effects of climate change on species geographic and local distribution as climatic condition approach or exceed species' physiological limits of tolerance.

Ongoing experiments along the whole South African coastline show that, during summer, the temperature of artificial mussels made of resin and placed in mussel beds can peak to 40-45°C (unpub. data). Despite the general assumption that climate shift will favour alien species, thus increasing the impact of invasions on ecosystems (Dukes and Mooney 1999; Stachowicz et al. 2002), I show here that in this case increasing temperature will have a greater negative effect of on the invasive than on the co-existing indigenous species. In fact, global warming is likely to decrease the upper distribution limit of the invasive species with a reduction of total mussel biomass, and intertidal biomass including organisms living within mussel beds.

Other environmental changes induced by global warming are likely to affect intertidal assemblages. Sea level will rise and, depending on the topography of the coast, the effect will range from an upward relocation of communities to a complete displacement of the available rocky substratum (IPCC 2001). A rise in sea level could increase the erosion of coastal dunes and mobilisation of sand and, in combination with storms and other oceanographic conditions, this could result in changes in sand and sediment transport (Shackleton et al. 1996; IPCC 2001; Bromirski et al. 2003). The weaker attachment strength of *M. galloprovincialis* and its higher mortality during winter storms (Zardi et al. 2006b) suggest that the invasive species will be negatively affected by higher hydrodynamic stress. However, *M. galloprovincialis* has a higher re-colonization rate, which allows it to out compete *P. perna* in more sheltered areas (such

70

as bays) that are periodically disturbed by storms (Erlandsson et al. 2006). Moreover, although *P. perna* occupies the lower mussel zone, it is less resistant to sand stress than *M. galloprovincialis* (Zardi et al. 2006a). Consequently alterations of seasonal sand fluctuation and increased storms events could have dramatic effects on the distribution of these two co-existing species (Zardi et al. 2006a).

The success of an invasive species depends on finding a time or place where it is competitively superior to resident species (Chesson et al. 2001). The west and south coasts of South Africa offer the invasive *M. galloprovincialis* different niche opportunities, by offering different combinations of physical factors (e.g. sea temperature, frequency of sand inundation) and biological factors (food resources and competitors). On the south coast, where *P. perna* is present, the European mussel colonizes the upper mussel zone. In contrast, on the west coast of South Africa, where *P. perna* is absent because of the cool-temperate conditions, *M. galloprovincialis* extends its distribution down the shore to the sub-littoral fringe (Bustamante et al. 1998). The presence of *P. perna* has obliged the invasive species to colonize a less favourable niche, the high shore, where temperature and desiccation are harsher because *P. perna* outcompetes *M. galloprovincialis* on low shore (Rius and McQuaid 2006). On the south-east coast, it seems that *M. galloprovincialis* has reached its biogeographic limit where there is a shift from warm-temperate to subtropical conditions (Emanuel et al. 1992; Robinson et al. 2005). This may be reinforced by the fact that the Agulhas Current flows very close to the coast in the region of the break between the two biogeographic regions and may be responsible for limiting transport of mussel larvae from the south to the east coast (Lutjeharms 2004; Zardi et al. 2007a). Fossil evidence and distributional surveys after short term temperature changes (e. g. ENSO events)

71

show that biogeographic range shifts are associated with climate change in marine environments (Fields et al. 1993; Keister et al. 2005). During the unusually warm summer of 1982/83 (probably connected with the strong El Nino event in the Pacific; Schumann et al. 1985), the species that showed increasing or decreasing abundances along the South African coast were at the limits of their ranges, while populations of species within their optimum range displayed no change (Branch 1984). Recent distribution surveys (Robinson et al. 2005), ongoing oceanographic studies and transplant experiments strongly indicate that *M. galloprovincialis* has reached its eastern distributional limit. My results, together with these studies, suggest that global warming could not only reduce the local distribution (i.e. vertical zonation) of *M. galloprovincialis*, but could also push its eastern biogeographic limit to the cooler western regions.

Temperature seems to be particularly important in controlling mussel gametogenesis and spawning (e.g. Wilson 1987; Seed and Suchanek 1992). Zardi et al. (2007b) showed that *M. galloprovincialis* spawning events along this coast took place within a critical temperature range (16.4 - 19.5°C) while *P. perna* spawned at the highest (about 22°C) and the lowest (about 15°C) temperatures recorded during an 18 month survey. It has recently been shown that the rate of larval development is strongly influenced by water temperature (O'Connors et al. 2007). Thus, long-term temperature increases could affect the timing of spawning, dispersal, and patterns of recruitment of these two species. Apart from water temperature, ocean flows themselves can generate distributional patterns. Gaylord and Gaines (2000) suggest that simple, common flow fields often observed in association with biogeographic boundaries worldwide may have the potential to constrain a species' geographic range, even when suitable habitat is

abundant outside that range. Model predictions suggest that these boundaries can function as one- or two-way barriers to range expansion and may be differentially permeable.

Only a few studies have examined the influence of environmental temperature on endogenous levels of stress proteins in animals in nature. Together these studies highlight the complexity of examining an environmentally induced gene expression event in organisms in a natural population. Chapple et al. (1998) found that levels of Hsp70 proteins in *M. edulis* varied seasonally and were positively correlated with seasonal changes in environmental temperature. Hofmann and Somero (1995) found that Hsp expression levels of *M. trossulus* varied seasonally with *in situ* body temperature and as a function of average habitat temperature. Seasonal temperature fluctuations are also correlated to expression levels of other stress proteins (e. g. Hsp90; Diet and Somero 1992). In contrast, in other studies patterns and values of Hsp70 expression varied significantly across seasons, but had no link to seasonal temperature (Encomio and Chu 2005). In the subtidal mussel *Modiolus modiolus*, a negative correlation between concentration of Hsp70 and temperature was observed, with expression levels of Hsp70 being greater in winter than in summer (Lesser and Kruse 2004).

My results show that expression levels of Hsp70 fluctuated seasonally at all sites and were about two-fold higher in summer and autumn than in winter and spring. Summer and autumn temperatures were very similar and were higher than winter and spring. Hsp70 variations mirrored air temperature fluctuations, with higher differences between summer/autumn and winter/spring. However the correlation between levels of Hsp70 and air temperature was significant only for bays. Although air temperature does not

necessarily directly influence mussel body temperature (Helmuth 1998), these data give important insights about major thermal seasonal fluctuations.

Interestingly, levels of Hsp70 were significantly higher on the open coast than at bay habitat. Other physiological stressors may be responsible for this difference. Wind speed, solar radiation, and relative humidity can affect body temperature (Johnson 1975; Bell 1995; Helmuth 1998, 1999). Hypoxic and desiccation stress may contribute to the influence of temperature on HSP induction (Feder and Hofmann 1999). Wind speed seems to be higher on the open coast (pers. obser.) and consequent wind-induced desiccation is a likely candidate as an effect on levels of Hsp70 on the open coast. However, other experiments are definitely needed to test this hypothesis.

Hawkins (1991) has estimated the cost of protein synthesis to constitute 20 - 25% of the energy budget of the mussel *Mytilus edulis*. This cost represents an additional energy burden because stress proteins do not directly contribute to increase in growth or reproduction, and because under stress condition Hsps may be synthesized preferentially, so that other proteins critical for the normal functioning of the organism are either synthesized at reduced rates or not synthesized at all. Furthermore, the function of stress proteins may require considerable ATP turnover; refolding of a protein may consume an excess of 100 ATP molecules (Creighton 1991; Martin et al. 1991; Parsell and Lindquist 1993). This high energy demand could affect other physiological performances. For *M. galloprovincialis*, high Hsps expression could represent a competitive disadvantage, particularly on the open coast (see also Chapter 6) because of the need to repair endolithic infested shells.

In conclusion, my results support the first hypothesis but reject the second. The gaping, indigenous *P. perna* faces higher desiccation risk than the non-gaping species, *M.*

galloprovincialis. Contrary to my expectation, the species that is less tolerant to heat stress maintains dominance in a habitat where thermal stress is higher. In this context, heat and desiccation stress have different roles, with the former regulating species distribution at macro-scales (i.e. along the coast of South Africa) and the latter playing a crucial role in the vertical zonation of the two competing species. As I hypothesised, Hsps expression varied seasonally and topographically. However, the finding that mussels in open coast habitats exhibited higher levels of expression than those at bay sites was unexpected. My results suggest that increasing temperatures will have a greater negative affect on *M. galloprovincialis*, a species that is one of the most successful marine invaders worldwide (Lowe et al. 2000) and certainly the most successful invasive marine species in South Africa (Robinson et al. 2005), decreasing its distribution at both local (i.e. vertical zonation), meso- (i.e. open coast vs bays) and macro-scales (biogeographic regions).

CHAPTER 4

MESOSCALE, TOPOGRAPHICALLY DRIVEN GENETIC

STRUCTURE IN INDIGENOUS (*PERNA PERNA*) MUSSELS
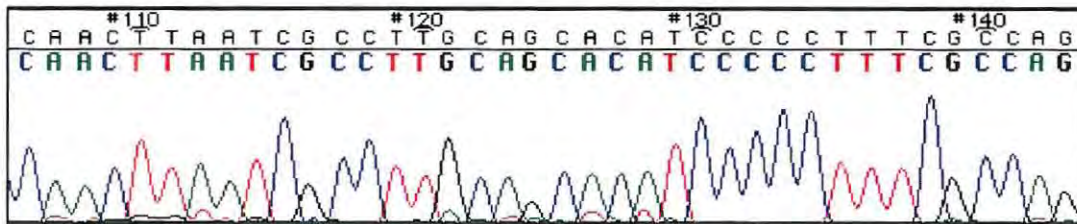
INTRODUCTION

A key role of genetic studies in ecology lies in discerning scales of propagule dispersal through the elucidation of population connectivity. The quantification of larval dispersal distances and larval origins is still an open question in marine ecology. In particular, in sessile or sedentary marine organisms, the dispersal of early life stages is critical to their population dynamics (Sale 1991; Caley et al. 1996; Todd 1998). 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). An analysis of either the dispersal process or of the resultant population structure is needed to quantify levels of dispersal (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; Todd et al. 1998; Lambert et al. 2003). Genetic differentiation can also occur among populations of marine organisms due to local differences in selection (Johnson and Black 1984; Hedgecock 1986; Bertness and Gaines 1993). 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).

In the 1960s, population biologists demonstrated that electrophoretic surveys of protein variation could be used to estimate levels of genetic variation within a species and to describe population structure (Harris 1966; Lewontin and Hubby 1966; Johnson et al. 1966). Innis et al. (1990) developed the polymerase chain reaction (PCR), allowing the analysis of DNA sequences. 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 that are 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 wide variety 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 (mtDNA) have become widely used tools in molecular phylogeny and phylogeography (Avise 2000). mtDNA data from marine invertebrates have frequently identified population genetic differentiation, whereas allozyme studies have often revealed little population subdivision. 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* (Quesada et al. 1995), and oysters, *Crassostrea virginica* (Reeb and Avise 1990; Karl and Avise 1992). These discrepancies can be explained by 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). Several characteristics that the animal mitochondrial genome exhibits make mithochondrial genes ideal 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 and Jarrell 1993), which sometimes exhibit substitution rates that are roughly similar between invertebrate mitochondrial and nuclear genomes (e.g. Vawter and Brown 1986; Sharp and Li 1989; Lynch and 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. In the families Mytilidae (sea mussels) and Unionidae (freshwater mussels) the mtDNA transmission system is exceptional because it possesses two mtDNA lineages, one transmitted through the egg, the F (female) lineage, and one transmitted through the sperm, the M (male) lineage (Skibinski et al. 1994a, 1994b; Zouros et al. 1994a, 1994b; Liu et al. 1996). This phenomenon is known as doubly uniparental inheritance (DUI; Zouros et al. 1994a). M and F DNA sequences normally differ by as much as 20% (Hoeh et al. 1997). Sperm mtDNA is delivered into the oocyte and eliminated within the first 24h after fertilization in females (Sutherland et al. 1998) but 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. 1994b; Steward et al. 1995; Garido-Ramos et al. 1998; Passamonti and Scali 2001).

The pelagic larval duration and behaviour and movement of water masses are critical features when assessing and quantifying the potential for larval dispersal among populations. *Perna perna* and *M. galloprovincialis* have high dispersal potential; they 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. Larvae are often considered passive particles, mainly transported by currents (Scheltema 1986; Shanks 1995) because their swimming abilities are very limited and they are considered to have only a very limited influence on their actual dispersal. However, Shanks and Brink (2005) have recently shown that the effect of upwelling and downwelling on larval distributions varies with larval behavior and vertical distribution, and some taxa do not behave as passive particles and are not swept offshore by upwelling or onshore by downwelling. On the south coast of South Africa, wind is the main forcing function regulating the dispersal of larvae in shallow, nearshore waters (Schumann 1981; McQuaid and Phillips 2000).

Coastal heterogeneity can potentially enhance or limit the dispersal of larvae through its effect on the direction and speed of flow of the dispersing medium and thus can alter patterns of population connectivity, population dynamics and population structure in sedentary marine organisms (Gaines and Bertness 1992; McKindsey and Bourget 2000; McCulloch and Shanks 2003; Shanks et al. 2003).

Bays are a common topographic feature of marine coastlines, and can act as retention zones. Some studies have observed higher settlement and recruitment patterns in bays than surrounding areas (Gaines and Bertness 1992; Wing et al. 1995; Archambault and Bourget 1999; McQuaid and Phillips 2006) and others have reported increased

zooplankton abundance in fronts and eddies generated by headlands associated with bays (Rankin et al. 1994; Graham and Largier 1997; Wing et al. 1998). Indirect evidence suggests that species with planktonic larvae could also show genetic differences between bay and nearby open coast populations (Stauber 1950; Loosanoff and Nomejka 1951; Bertness and Gaines 1993). Defining the degree of gene flow between bay and open coastal habitats can help to explore mesoscale migration of larvae and consequently give new insight into the dynamics of marine populations.

Bay morphology influences oceanographic features not only by retaining water, thus changing circulation patterns and consequently affecting larval dispersal (Roughan et al. 2005), but also by creating different selective environments from those of the open coast. In bays, populations are often subjected to high thermal stress, different food concentrations, lower salinities and lower wave action compared to open coast sites (Ricketts and Calvin 1968; Castilla et al. 2002; Largier 2004). Environmental conditions prevailing in different regions could exert strong selection on the physiological tolerance and performance of adult mussel populations leading to genetic structuring of bay and open coast populations.

In this study, I test the hypothesis that the influence of coastal topography on larval dispersal and selective regime produces a discernible and predictable effect on the genetic structure of mussel populations. I sampled 90 individuals of the intertidal mussel *P. perna* from populations living in three bays and at three open coast sites along 600km of the South African shoreline and sequenced 400 base pairs of the mitochondrial cytochrome oxidase I gene. As expected of a recent invader, *M. galloprovincialis* is characterised by low haplotype diversity and a lack of genetic

structure over its entire biogeographic distribution within South Africa (Zardi et al. 2007a), and consequently it was not considered in this study.

MATERIALS AND METHODS

Sampling, DNA extraction, amplification and sequencing

Populations of *Perna perna* were sampled at three location inside bays (Plettenberg Bay, Jeffreys Bay, Algoa Bay) and three on the open coast (Cape St Francis, Cape Recife, Kenton-On-Sea; n = 15 for each population; Fig. 4.1). Previous studies, based on mtDNA, show a strong genetic cline on the south-east coast, leading to the formation of two genetic lineages (Zardi et al. 2007a). All populations examined in this study belonged to the same genetic lineage. 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 in mussels (DUI; Zouros et al. 1994a), 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 LCOI1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-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 2μl of genomic DNA extractions, 0.4μM of each primer, 5μl of Qiagen PCR buffer, 200μM of each dNTP, and 2.5U of Taq DNA polymerase (Qiagen). The PCR cycling profile comprised an initial denaturation step at 94°C for 2min, 35 cycles of denaturation at 94°C for 60s, annealing at 54°C for 60s, extension at 72°C for 90s, and a final extension at 72°C for 5min. PCR products from each individual were purified with a Qiaquick gel extraction kit (Qiagen) and cycle sequenced in both 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 an ABI 3100 genetic analyzer.

Genetic data analysis

Sequence data (400 bp in length) from 90 *Perna perna* individuals were analysed using the program IM (Hey and Nielsen 2004), which estimates migration rates between two populations by taking into account their effective populations sizes and time of divergence under the coalescent model (Kingman 1982). One group contained all of the IN populations and the other all OUT populations. I specified the HKY model (Hasegawa et al. 1985) with an inheritance scalar of 0.25 for mitochondrial DNA. After a number of exploratory runs to determine suitable upper bounds for each model parameter, I used the following search strategy: -b500000 –q1100 –q2200 –qa50 –qu1 – t2.5 –m15 –m210 –fg –n20 –g10.01 –g22 –k20 (population1: IN; population 2: OUT). To ensure consistency of results, 10 independent runs with random starting seeds and at least 2 million genealogical steps were performed. The final estimates of the migration rates were calculated with the run with the highest effective sample sizes. Migration rate estimates were converted to number of female migrants per generation by multiplying each population's mean migration rate parameter (M) by its mean effective population size parameter (θ).

ARLEQUIN 3.1 (Excoffier et al. 2005) was used to calculate Φ_{st} values (sequence divergence among haplotypes; Excoffier et al. 1992) among IN and OUT populations using pairwise differences among sequences.

RESULTS

Sampling, DNA extraction, amplification and sequencing

A total of 33 haplotypes were identified, 26 of which were private (i.e. present in a single population only; Fig. 4.1). Populations within bays (referred to as IN) had greater numbers of haplotypes and a higher endemism index than populations on the open coast (referred to as OUT; Table 4.1). Haplotype 1 was the most common and, together with haplotype 2, was present at all locations. The number of shared haplotypes (shared index; Table 4.1) was higher among OUT than IN populations; only three haplotypes were shared between IN and OUT populations.

Genetic data analysis

Analyses of gene flow using the program IM revealed asymmetrical migration rates that were approximately 17 times as high from IN to OUT as in the opposite direction (Fig. 4.2 for examples of posterior probability plots).

Although there was no significant genetic structure among IN populations and among OUT populations, the amount of genetic structure among the IN populations was nonetheless distinctly higher (Table 4.1).
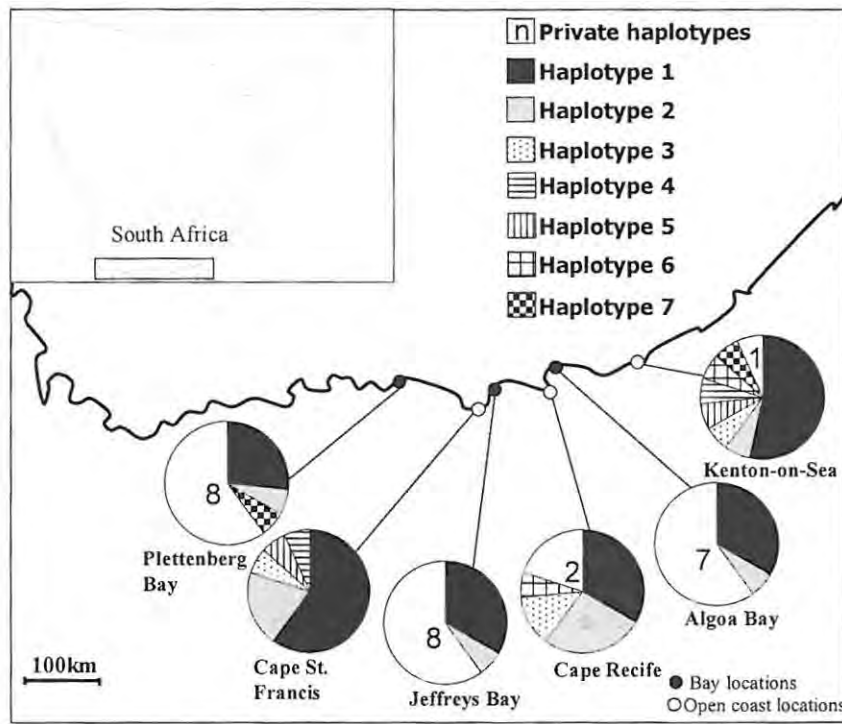
Fig. 4.1

P. perna haplotype frequencies at each sample location.

Table 4.1

Φ_{st} values, number of haplotypes, endemism index and shared index at the locations sampled. Endemism index was calculated as E = e/n, where e and n are the numbers of putatively endemic haplotypes and the total number of haplotypes detected in each sample, respectively. Shared index was calculated as S = s/n-e, where e and n are the numbers of putatively endemic haplotypes and the total number of haplotypes detected in each sample respectively, while s is the number of non-endemic haplotypes shared by at least at two locations.

	Φ_{st}	Location	No. of haplotypes	Endemism index	Shared index
IN	0.032 (p = 0.06)	Plettenberg Bay	11	0.73	0.29
		Jeffreys Bay	10	0.8	
		Algoa Bay	9	0.78	
OUT	0.008 (p = 0.34)	Cape St. Francis	5	0	0.86
		Cape Recife	6	0.33	
		Kenton-on Sea	8	0.12	

(Overall shared index: 0.43)

Fig. 4.2

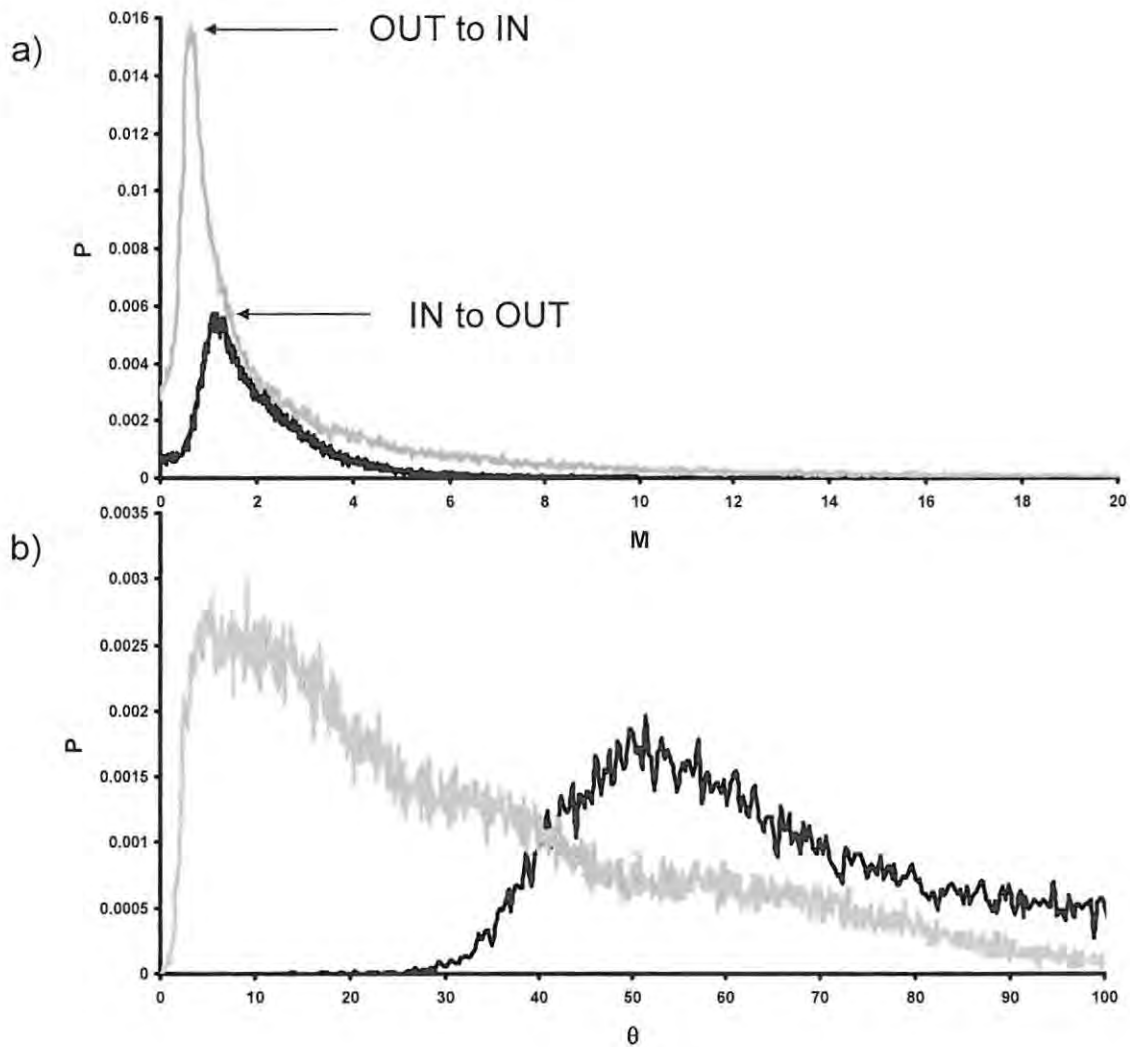Migration rates and effective population sizes of *P. perna* populations in bays (black) and on the open coast (grey); a) Pairwise estimates of gene flow and b) effective population sizes scaled to neutral evolutionary rate using mitochondrial COI sequence data. Marginal posterior probabilities are depicted on the y-axis. M = population's mean migration; θ = mean effective population size parameter.

DISCUSSION

My results show that strong haplotype divergence can develop between IN and OUT mussel populations at mesoscale distances (i.e. distances of tens of kilometers, see Fig. 4.1). Coastline topography can have an effect on both oceanographic features and the degree of environmental stress, consequently influencing the degree of larval dispersal, selection and adult population dynamics.

The sheltering effect of within bay habitats subjects mussels to a less stressful environment leading to lower mortality rates (see Chapter 2). This is in accordance with a higher number of adult mussels in bays than at the open coast sites (von der Meden, unpublished data). Growth and mortality rates are much higher for mussels on more exposed shores, while percentage cover declines, suggesting much higher turnover rates in populations exposed to strong wave action (McQuaid and Lindsay 2000; Hammond and Griffiths 2004). During 12 months, monthly mortality rates of *Perna perna* and *Mytilus galloprovincialis* fluctuated in time but were significantly higher on the open coast than inside bays (see Chapter 2). At a genetic level, greater fluctuation in population sizes may result in the elimination of rare haplotypes. The high wave action experienced on the open coast (see Chapter 2) subjects intertidal communities to frequent mass mortality, generating free space that can be colonized *en mass* by larvae in the water column, which comprise a subset of available haplotype diversity. The freeing of space makes primary substrata available for settlers that potentially arrive from different locations, allowing more frequent and intense gene pool turnover. Genetic differentiation and the shared index showed a high degree of gene flow among OUT populations, while each of the IN populations were quite distinct. The higher gene flow from IN towards OUT populations than *vice versa* seems to contrast with the

retention concept. Larvae do leave bays and colonize the opens coast, but the high mortality rates of adult mussels cause more frequent genetic turnover and consequently a lower endemism index for OUT populations. Good quality habitats with natality rates higher than mortality rates can be designated as sources and low quality habitats with a demographic deficit as sinks, requiring migration in order to persist (Dias 1996). Adult mussels in bays could act as sources with higher spawning (McQuaid and Phillips 2006), and consequently higher larvae output, compared to the open coast. OUT populations act as sinks where larvae from IN populations are recruited into the free space generated by a more severe selective environment.

Clearly topography is an important consideration when studying marine connectivity and my results prove the importance of a careful choice of sampling sites for marine population genetics. Mitochondrial DNA sequences for *P. perna* indicate a strong phylogeographic break on the east coast of South Africa, north of East London, leading to the formation of an eastern and western lineage (Zardi et al. 2007a). No significant isolation by distance was found within lineages. The authors' do not comment about a possible effect of embayment on the population genetic structure, however it is evident that a higher number of private haplotypes is always associated with, and characteristic of, bay populations. Therefore, it is important to stress that population genetic studies should also consider the possible effect of mesoscale topography.

Topography (i. e. open coasts vs bays) has effects on both population genetics, through the creation of different selective environments, and on recolonization patterns. That topography has such predictable effects on genetic structure is remarkable and suggests that populations in bays and on the open coast may have quite different potentials for responding to long-term climate change and/or stochastic local extinctions associated

with environmental events. For example, changes in atmospheric circulation due to global warming might also change storm frequency; an increase in the frequency of winter storms has already been observed in coastal oceans (Bromirski et al. 2003), and the trend is expected to continue (IPCC 2001). This could lead to a higher impact of storms on mussel populations, and consequently a higher mortality, on the open coast than in bays.

CHAPTER 5

BEHAVIOURAL RESPONSES OF INVASIVE (*MYTILUS GALLOPROVINCIALIS*) AND INDIGENOUS (*PERNA PERNA*) MUSSELS EXPOSED TO RISK OF PREDATION AND TO HYDRODYNAMIC STRESS
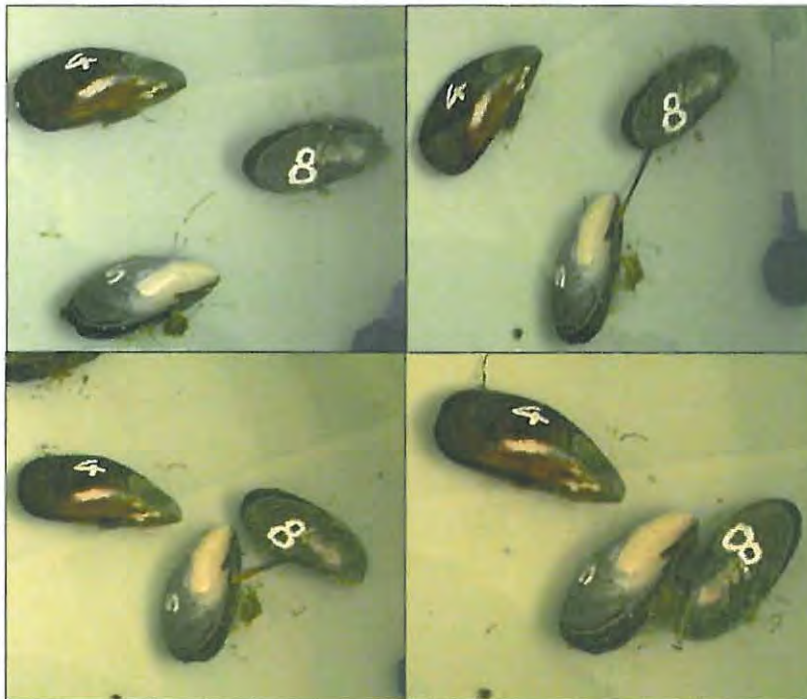
INTRODUCTION

The interaction between an invasive prey species and indigenous predators can play a crucial role in the success of the invader (Settle and Wilson 1990; Torchin et al. 1996; Mack et al. 2000). In particular, when predators are at low abundances or are less effective against new species, escape opportunities arise (Settle and Wilson 1990; Torchin et al. 1996). However, a naïve invader might not be well defended against generalist enemies, in which case escape opportunities are reduced (Mack 1996). Prey respond to predators through physiological, morphological and behavioural adaptations (Vermeij 1987; Endler 1986; Caro and Castilla 2004) and, in sessile and sedentary marine organisms, group living is a common behavioural response to risk of predation (e. g. Reimer and Tedengren 1997; Côté and Jelnikar 1999). When exposed to water-borne predator effluent in the laboratory, mussels form more, larger clumps more rapidly than mussels under control conditions (Côté and Jelnikar 1999). In nature, mussels clump together to form large, dense beds and are often successful primary space holders in the intertidal habitat (Seed and Suchanek 1992). Several studies have shown that adult mussels are able to move over short distances, making a mussel bed a dynamic structure composed of constantly re-arranging individuals (e. g. Paine and Levine 1981; Schneider et al. 2005). Rocky shores often exhibit high levels of environmental heterogeneity and consequently movements over even small distances could greatly influence rates of growth and mortality if they change the location of a mussel within a patch or result in movement to a new patch (Hunt and Scheibling 2002). Wave forces can vary by orders of magnitude over scales of centimetres to meters due to the interaction of breaking waves with the rocky shore (Denny et al. 2003, 2004). Mussels living in the centre of a bed are more difficult for predators to catch and hold than individuals living at the bed edge or solitary mussels

(Petraitis 1987; Svane and Ompi 1993) and are subjected to lower rates of predation (Okamura 1986). Mussels anchor themselves by means of byssus threads and under natural conditions they produce many byssal threads that may be attached to each other as well as to the substratum. As a result, more force is required to dislodge individuals in the middle of a bed than either those at the edge, or solitary animals (Zardi et al. 2006a). Movement can be ecologically very important in particular when differences occur between co-existing species competing for space and resources (Schneider et al. 2005).

The invasion of the South African coast by the European mussel *Mytilus galloprovincialis* has major consequences for community structure and strongly affects the faunal assemblages found within mussel beds; moreover it extends the vertical distribution of mussel beds further upshore, increasing intertidal biomass and consequently food sources for predators (van Erkom Schurink and Griffiths 1990, Hammond and Griffiths 2004). On South African rocky shores, predators have much weaker effects on prey populations than in other parts of the world (Castilla et al. 1994; Bustamente and Branch 1996), but one of the most important predators is the rock lobster *Jasus lalandii. J. lalandii* is the most important commercial lobster in southern Africa and, in South Africa, it co-occurs with both *Perna perna* and *M. galloprovincialis* (Griffiths and Seiderer 1980; personal observation). Studies of *J. lalandii* stomach contents have shown that it feeds mainly on mussels, although sea urchins, algae, polychaetes and crustaceans are also taken (Newman and Pollock 1974; Pollock 1978). In areas where it is abundant, *J. lalandii* is capable of eliminating standing stocks of prey species (e. g. the mussels *Aulacomya ater* and *Choromytilus meridionalis* on the west coast; Griffiths and Seiderer 1980).

93

The aim of this chapter is to investigate clumping behaviour of the invasive *M. galloprovincialis* and the indigenous *P. perna* when subjected to predatory risk as indicated by water-borne cues from the generalist predator *J. lalandii* and damaged conspecifics, the latter being a signal of a nearby attack by a crushing predator. It has been shown that, among mussels, cues from damaged conspecifics induce increased byssus production (Cheung et al. 2004) and thicker, and mechanically stronger shells (Leonard et al. 1999). Here I tested the following hypotheses: (1) the interaction between the European *M. galloprovincialis* and the South African rock lobster is recent and therefore the invasive mussel will exhibit a less pronounced reaction to this predator than will the indigenous *P. perna*; (2) the difference between the two mussel species will be less pronounced when subjected to the general risk of predation simulated by the presence of damaged conspecifics, rather than to the specific risk indicated by effluent from the South African predator. Moreover, movement of the two species was investigated in the field to compare mussel behaviour in sheltered bays and at more exposed sites on the open coast. Here, I tested the hypotheses that (1) due to higher hydrodynamic stress at open coast sites, the two species continuously adjust their position looking for a sheltered, more secure, arrangement (2) mussels at the centre of a clump will move less than mussels at the edge of clumps where individuals are less constrained; (3) as previous studies have shown that *P. perna* has a higher attachment strength (through a higher number of byssal threads) than *M. galloprovincialis* (Zardi et al. 2006b), the indigenous species will move less, adopting a less dynamic strategy.

MATERIALS AND METHODS

Mussels exposed to the risk of predation

Mussel collection

Adult mussels (4 - 5cm shell length) of both species were collected from a rocky shore in Plettenberg Bay. Before each laboratory experiment, all individuals were acclimated in oxygenated seawater for 48 hours. All experiments were run in a controlled environment chamber at 19°C under a 12:12h light: dark regime. Salinity was maintained at 35‰. Experimental aquaria were aerated and the water was replaced daily. Before the start of each experiment, byssus threads were carefully cut with a pair of scissors to separate the mussels from each other and individual mussels were numbered using white correction fluid.

Predator effluent and damaged conspecifics

Two days before the start of the experiment, two rock lobsters (*Jasus lalandii*, cephalothorax length approximately 15cm) collected at Plettenberg Bay were placed individually in tanks containing 50l of seawater and not fed. Air was bubbled via an air stone and a biological filter was placed in each of the tanks. After 48h the seawater from these aquaria was used as effluent for experiments involving lobster effluent. In the damaged conspecifics treatment, four damaged mussels were equally spaced around the edge of round plastic containers (35cm diameter) for the duration of the experiment; for the mixed species groups, we used two damaged individuals of each species. Mussels used for this purpose were 4 - 5cm long; they were damaged by cutting the adductor muscle and placing them in the containers with the shell valves open.

Clumping behaviour and crawling distance

Experimental treatments were administered to each of three species combinations in round plastic containers (35cm diameter) containing 5l of oxygenated seawater. Mussels were arranged in a grid. Two containers each held 16 mussels from a single species (one for each species studied) and one held a mixed group of 16 mussels (eight of each species arranged alternately). Each species treatment was replicated three times (three containers). Two experiments were carried out using different individual mussels.

In the first experiment, mussels were placed in plastic tanks containing control seawater or lobster effluent and subjected to one of four experimental treatments: (1) mussels in control seawater arranged in a grid 1.5cm apart, (2) mussels in control seawater 4.5cm apart, (3) mussels in lobster effluent sea water 1.5cm apart, (4) mussels in lobster effluent sea water 4.5cm apart. The two distances were such that the extended mussel foot could cover the near but not the far distance.

The second experiment was run with the following treatments: (1) mussels in control seawater 1.5cm apart, (2) mussels with damaged conspecifics 1.5cm apart. The position of each mussel was recorded after 1, 3, 6, 12, 18, 24 hours with digital photographs and clumps were defined as two or more mussels attached to each other by one or more byssal threads. Clumping behaviour was quantified as number of mussels forming clumps and crawling distance was recorded for the first three hours of each experiment.

For experiment 1 (lobster effluent), data on the numbers of mussels forming clumps after 24 hours were analysed using 3-way ANOVA, with distance, treatment and species as fixed factors. Experiment 2 (damaged conspecifics) was run using one distance only, and data on the numbers of mussels forming clumps after 24 hours

were analysed using 2-way ANOVA, with species and treatment as fixed factors. Significant effects were examined using Tukey post-hoc tests.

Data on the total distances crawled by mussels in the first three hours of experiment 1 failed the requirements of Shapiro's test and Levene's test. Data were analysed using Kruskal-Wallis tests to investigate the effects of species (*P. perna* or *M. galloprovincialis*) and treatment (lobster effluent or control) in separate analyses. Data on crawling distances in experiment 2 failed the requirements of Shapiro's test but Levene's test showed homogeneity of the data. Parametric analysis was used on the assumption that ANOVA is relatively robust to the effects of non-normality (Zar 1999), therefore data were analysed using 2-way ANOVA with species (*P. perna* or *M. galloprovincialis*) and treatment (damaged conspecifics or control) as fixed factors.

Foot measurements

Shell lengths of *M. galloprovincialis* and *P. perna* individuals (n = 30 for each species), covering a wide size range (3.1 to 6.1cm), were determined after any encrusting organisms had been removed. These mussels were then opened by carefully slicing through the adductor muscle and the maximum width of the fully contracted foot was measured. This is proportional to, but more accurately measured than, maximum foot length (Seed and Richardson 1999). 1-way ANCOVA was used to evaluate the effects of species (fixed factor) and shell length (co-variate) on the maximum width of the contracted foot.

Mussels exposed to hydrodymanic stress

Mussel movement measurements

Mussel movement was measured in two bays (Plettenberg Bay, Algoa Bay; Fig. 2.1) and two open coast locations (Robberg, Cape Recife). Each location had two sites

200m apart at which digital pictures of 12 fixed quadrats (20 x 20cm) were taken monthly for six months. In each quadrat, six individuals of each species were selected (identified from shell shape and colouring) from mussels in the centre of mussel beds and another six from mussels living at the edge of the bed. For each mussel, distance moved was estimated every month as displacement between consecutive photos.

Data on the total distances crawled by mussels in six months failed the requirements of Shapiro's test and Levene's test. Data were analysed using Kruskal-Wallis tests to investigate the effects of species (*P. perna* or *M. galloprovincialis*), position in the bed (centre or edge for each species separately), and habitat (bay or open coast for each species separately) in separate analyses. Moreover, mean movement of each species in bays and on the open coast was cross-correlated with mussel mortality rate (see Chapter 2) with movement as the lagged variable and a lag interval of one month.

RESULTS

Mussels exposed to the risk of predation

Clumping behaviour

Perna perna clumping behaviour was significantly greater when exposed to predator effluent or damaged conspecifics, while *Mytilus galloprovincialis* showed significantly more clumping when exposed to damaged conspecifics, but not lobster effluent.

Experiment 1 (lobster effluent).

There were significant effects of all three factors with significant treatment x distance and treatment x species interactions (Table 5.1, Fig. 5.1). All species showed greater clumping when separated by 1.5cm than when separated by 4.5cm, and none showed a treatment effect when separated by 4.5cm (Tukey test, $p = 1$). The percentage of mussels forming clumps differed significantly among species (Tukey test, $p < 0.05$ in the order *M. galloprovincialis* > mixed groups > *P. perna*), but when separated by 1.5cm, only *P. perna* had a significant treatment effect (Tukey test, $p < 0.05$). As clumping behaviour was more pronounced with spacing of 1.5cm, the greater distance was not investigated further.

Experiment 2 (damaged conspecifics).

Both factors had significant effects, with no significant interaction (Table 5.2, Fig. 5.2). A significantly higher percentage of *M. galloprovincialis* than *P. perna* formed clumps (Tukey test, $p < 0.05$) but mixed groups did not differ significantly from either of the other two species ($p = 0.1$ with *M. galloprovincialis* and $p = 0.8$ with *P. perna*).

Crawling distance

Experiment 1 (lobster effluent).

Neither species showed a significant treatment effect (Kruskal-Wallis test, p = 0.3 and

p = 0.4 for *P. perna* and *M. galloprovincialis* respectively), but there was a significant

difference between species when subjected to the treatment (Kruskal-Wallis test, p <

0.01; Fig. 5.3a).

Experiment 2 (damaged conspecifics).

There was a significant species x treatment interaction, with no significant difference

between species under control conditions (Tukey test, p = 0.8), while *M.*

galloprovincialis crawled farther than *P. perna* when exposed to effluent from

damaged conspecifics (ANOVA, p < 0.001, Table 5.3; Fig. 5.3b). Again, there was no

significant treatment effect for either species (p = 0.8).

Foot measurements

The slopes of regressions for shell length against foot width for the two species were

homogenous (t = 8.86, df = 50). 1-way ANCOVA showed that, over the entire range

of shell lengths, the foot was significantly wider in *P. perna* than *M. galloprovincialis*

(p < 0.001, Table 5.4; Fig. 5.4).

Table 5.1 Results of a 3-way ANOVA applied to number of mussels forming clumps after 24h in experiment 1 (lobster effluent) with treatment, species and distance as fixed factors

Source of variation	df	MS	F	p
Treatment	1	17.36	6.44	0.02
Species	2	48.03	17.82	<0.0001
Distance	1	650.25	241.33	<0.0001
Treatment x Species	2	11.86	4.4	0.02
Treatment x Distance	1	17.36	6.44	0.02
Species x Distance	2	4.75	1.76	0.19
Treatment x Species x Distance	2	5.03	1.87	0.18
Error	24	2.69		

Table 5.2 Results of a 2-way ANOVA applied to number of mussels forming clumps after 24h in experiment 2 (damaged conspecifics) with treatment and species as fixed factors

Source of variation	df	MS	F	p
Treatment	1	76.06	40.26	<0.0001
Species	2	7.72	4.09	0.04
Treatment x Species	2	1.06	0.56	0.59
Error	12	1.89		

Table 5.3 Results of a 2-way ANOVA applied to crawling distance in experiment 2 (damaged conspecifics) with treatment and species as fixed factors

Source of variation	df	MS	F	p
Treatment	1	8.8	0.08	0.7764
Species	1	1696.43	15.45	<0.001
Treatment x Species	1	836.31	7.61	<0.01
Error	188	109.77		

Table 5.4 Results of a 1-way ANCOVA applied to foot width with species as a fixed factor and shell length as the co-variate

Source of variation	df	MS	F	p
Length (co-variate)	1	26.39	88.81	< 0.0001
Species (fixed factor)	1	4.47	15.05	< 0.0001
Error	49	29		

1.5 cm

a) *M. galloprovincialis*

b) *P. perna*

c) Mixed groups

4.5 cm

d) *M. galloprovincialis*

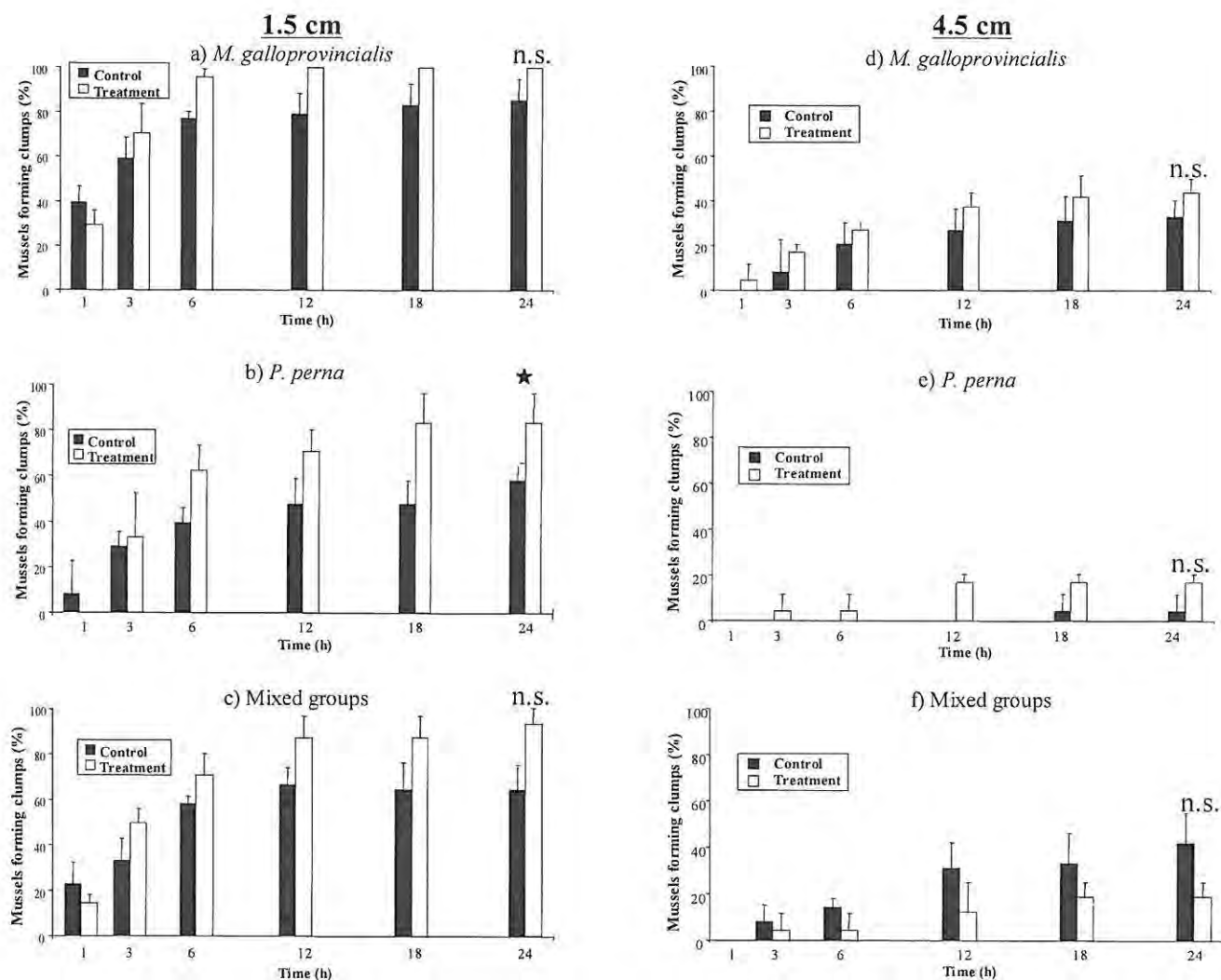e) *P. perna*

f) Mixed groups

Fig. 5.1

Percentage (+SD) of mussels forming clumps for mussels held in lobster effluent and in control water. (a) *M. galloprovincialis*, (b) *P. perna* and (c) mixed species groups for mussels at a distance of 1.5cm and (d) *M. galloprovincialis*, (e) *P. perna* and (f) mixed species groups for mussels at a distance of 4.5cm. Results of Tukey test post-hoc comparisons of control vs. treatments at 24h are shown (n.s. = non-significant at p > 0.05; ★ = significant at p < 0.001).
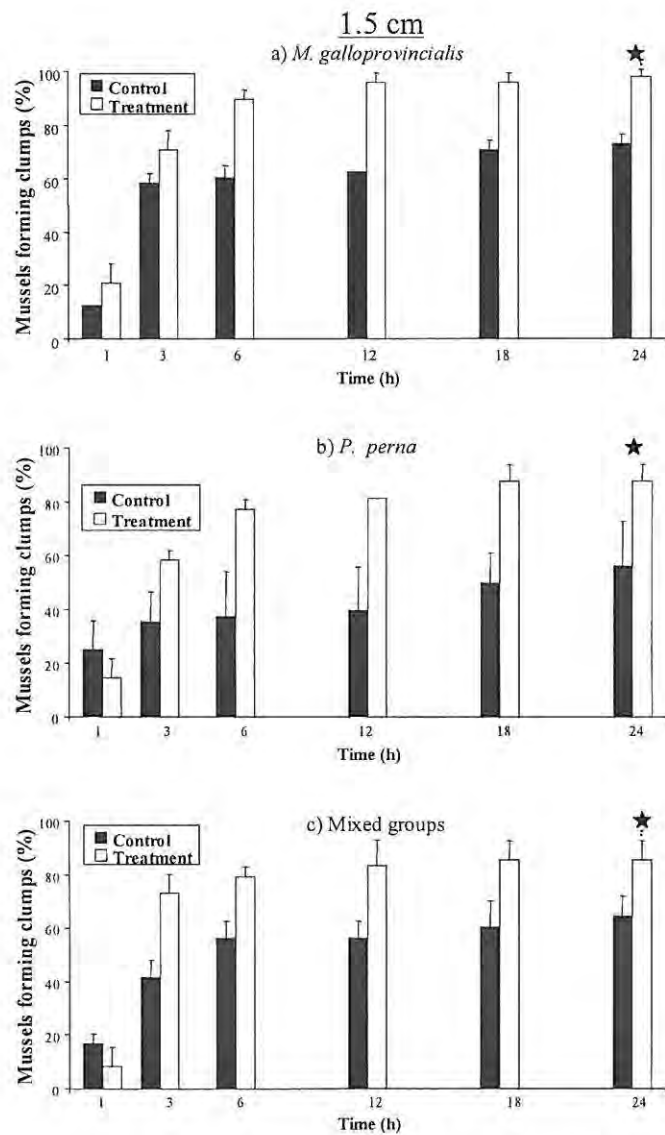
Fig. 5.2

Percentage (+SD) of mussels forming clumps for mussels in presence of damaged conspecifics and in control water. (a) *M. galloprovincialis*, (b) *P. perna* and (c) mixed species groups. Results of Tukey test post-hoc comparisons of control vs. treatments at 24h are shown (n.s. = non-significant at $p > 0.05$;★ = significant at $p < 0.001$).
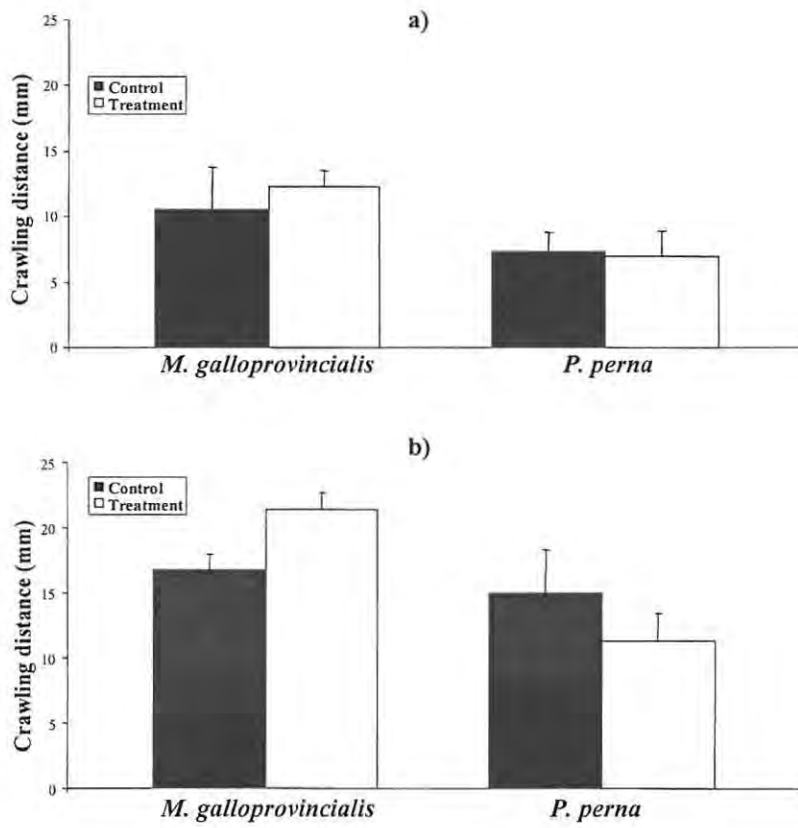
Fig. 5.3

Mean crawling distance (+SD) in the first three hours of the experiment for *M. galloprovincialis* and *P. perna* held in control water and exposed to (a) lobster effluent, (b) the presence of damaged conspecifics.
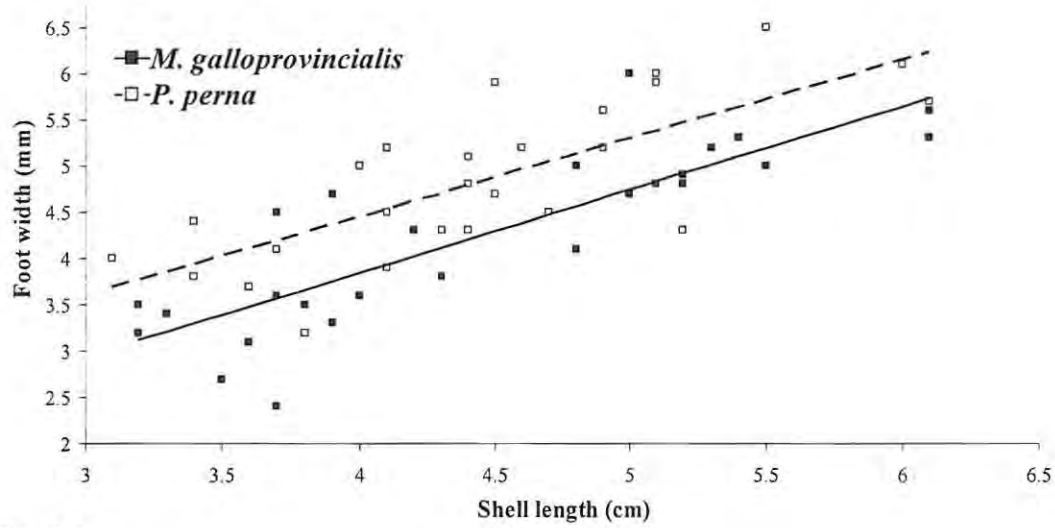
Fig. 5.4

Relationship between shell length and maximum foot width for *M. galloprovincialis* (n = 30, R^2 = 0.7034, y = 0.9012x + 0.2387, p < 0.001) and *P. perna* (n = 30, R^2 = 0.5724, y = 0.8502x + 1.0525, p < 0.001).

Mussels exposed to hydrodynamic stress

Mussel movement measurement

The total distances crawled by mussels in six months was significantly higher for *M. galloprovincialis* than for *P. perna* (Kruskal-Wallis test, $p < 0.0001$; Fig. 5.5a,b). At bay locations *M. galloprovincialis* crawled 75% and 56% more than *P. perna* for individuals living at the edge and in the centre of the bed respectively, while at open coast locations *M. galloprovincialis* crawled 65% and 61% more than *P. perna* for individuals living at the edge and in the centre of the bed respectively. Generally, mussels at the edges of beds moved farther than mussels within beds (Kruskal-Wallis test, $p < 0.0001$), the only exception was for *P. perna* on the open coast, where this trend was reversed, with mussels in the centre moving more than those at the edges of beds (Kruskal-Wallis test, $p < 0.01$). All individuals at bay locations crawled farther than mussels on the open coast, regardless of their position in the bed.

Crawling distance varied monthly, showing a similar pattern for both species. There was a rapid increase in crawling distance in March (early autumn) and a decrease in the following months (Fig. 5.6a,b). The highest positive correlations, between movement and mortality rates was observed when movement lagged 1 month behind mortality for both species at both habitats (lag = -1; Fig. 5.7a,b,c,d).

a) *M. galloprovincialis*
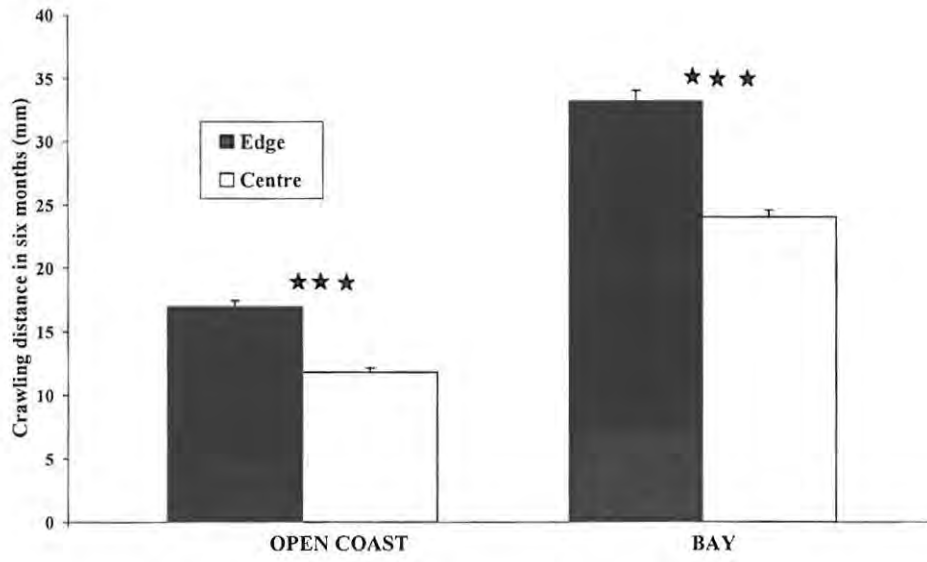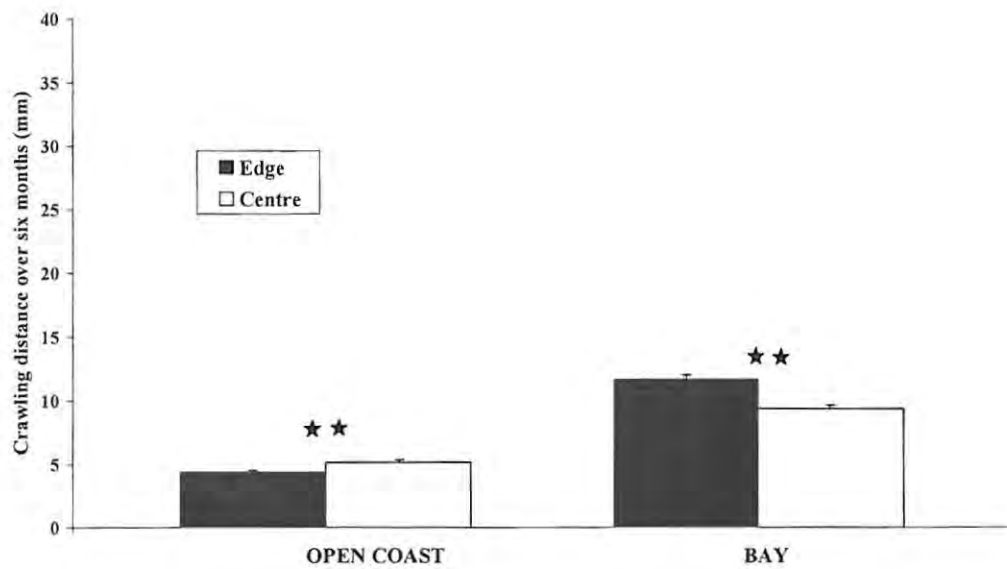
b) *P. perna*

Fig. 5.5

Mean crawling distance (+SD) after six months for *M. galloprovincialis* and *P. perna* within and at the edge of a mussel bed in bays and on the open coast. ★★ = significant difference at p < 0.01; ★★★ = significant differences at p < 0.001.

a) Bay
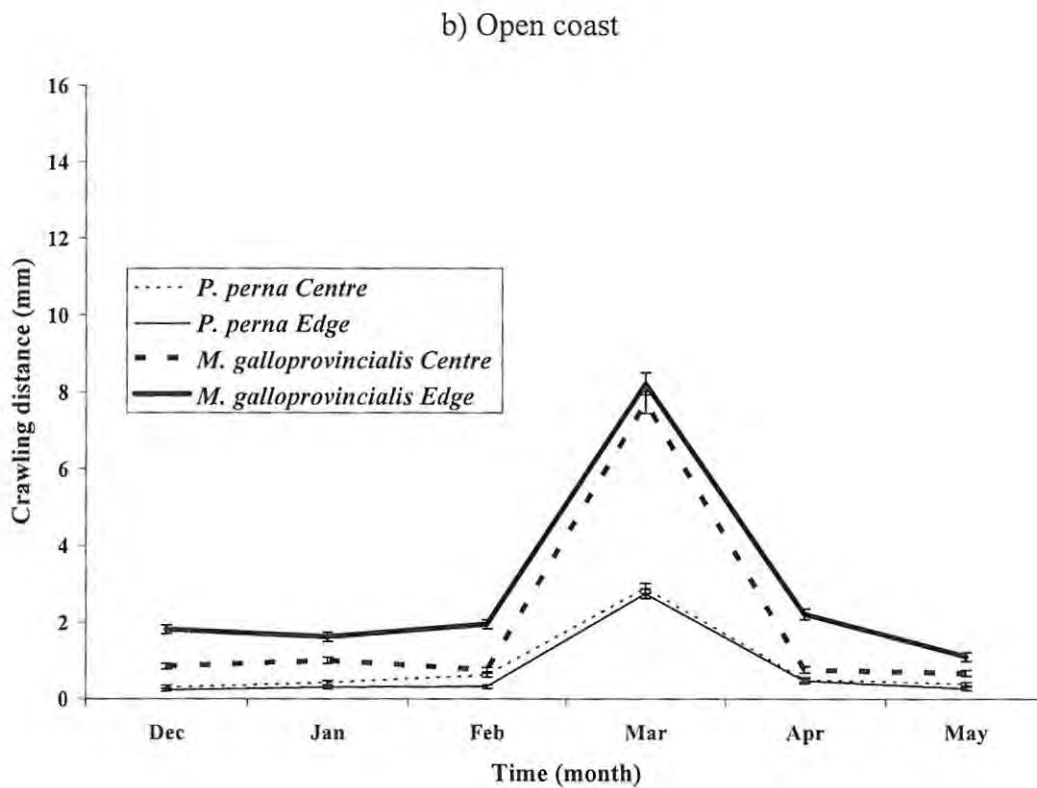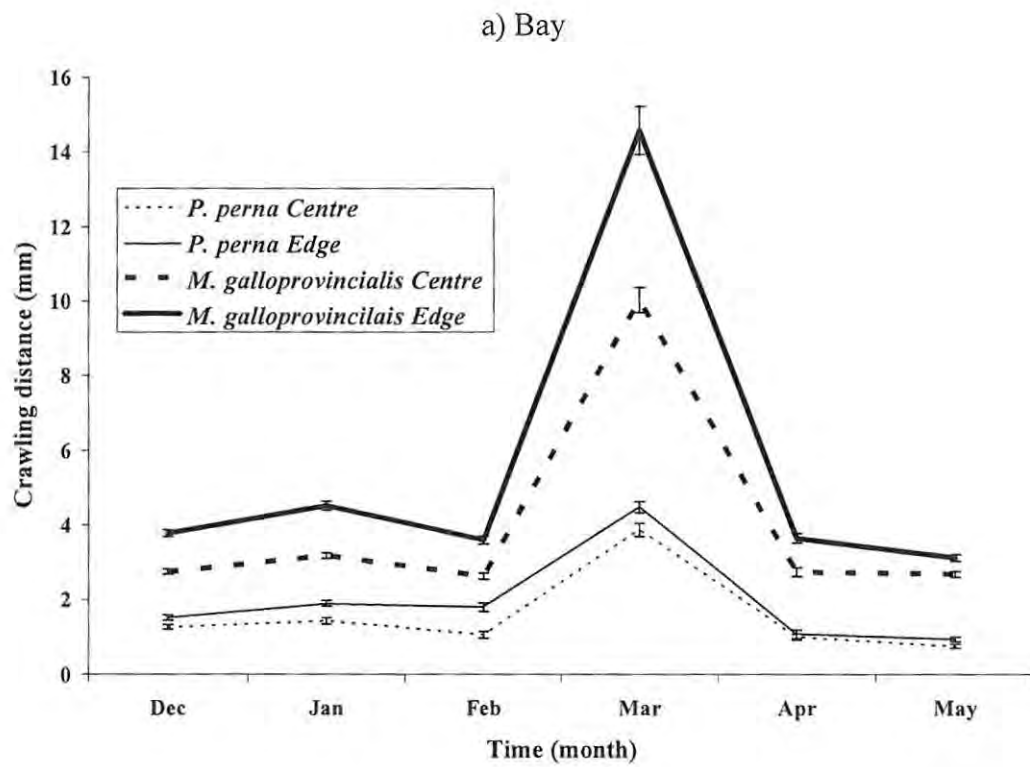
b) Open coast

Fig. 5.6

Mean monthly crawling distance (± SD) for *M. galloprovincialis* and *P. perna* within
and at the edge of a mussel bed (a) in bays and (b) on the open coast.
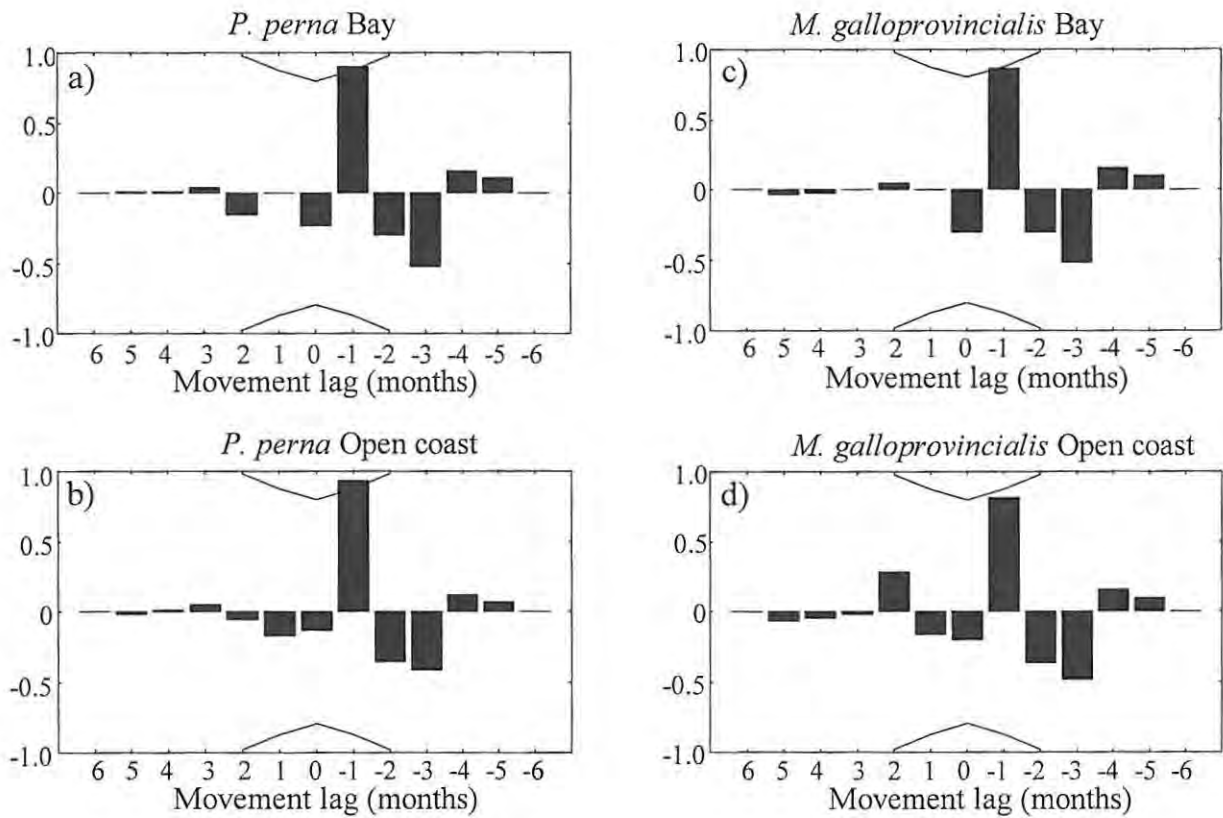
Fig. 5.7

Cross-correlation analyses of monthly mean movement with mortality rates for (a) *P. perna* in bays, (b) *P. perna* open coast and (c) *M. galloprovincialis* in bays, (d) *M. galloprovincialis* open coast. Bars are correlation coefficients; curved lines are approximate 95% confidences levels for the significance of each correlation.

DISCUSSION

In contrast to previous laboratory investigations of *Mytilus edulis* (Côté and Jelnikar 1999), my results show that the initial distance of separation among mussels has a significant effect on clumping behaviour; here, both mussel species clumped significantly more when the distance among individuals could be covered by the extended foot. This suggests that the detection of another mussel or of a solid object encourages clumping. Some studies have suggested that mussel clumps are a result of random movement (e. g. Urya et al. 1996). However, other studies indicate chemotaxis as a promoter of aggregation and recently it has been shown that tripeptides are the molecules involved in mussel communication (de Vooys 2003). Here, I suggest that mussels respond to both chemical stimuli and tactile cues.

In the laboratory, *Mytilus galloprovincialis* tended to crawl farther than *Perna perna* under treatment conditions and generally aggregated more in all laboratory experiments, underlining its greater mobility. Mussels move by extending the muscular foot between the valves, attaching it and then contracting it, to pull themselves forward. Measurements of the fully contracted foot excluded the possibility that morphological differences are responsible for behavioural differences. *P. perna* has a larger foot, but *M. galloprovincialis* shows a stronger effect of treatment on crawling distance (significant species x treatment interaction). This suggests that responses to chemical cues, induced by the presence of nearby conspecifics, may be enhanced in the invasive species. *P. perna* has higher byssal attachment strength than *M. galloprovincialis* and consequently is more tolerant to hydrodynamic stress (Zardi et al. 2006b), but this is balanced by lower reproductive output than in *M. galloprovincialis* (van Erkom Schurink and Griffiths 1991). Together with my results, this points to an evolutionary strategy in *P. perna* that

emphasises safety from wave action. While the indigenous species invests more in byssal production, the invasive species adopts a more dynamic strategy looking for aggregation or a safer arrangement. Field experiments confirm this suggestion. In general, over six months, *M. galloprovincialis* moved significantly more than *P. perna*. Previous studies on mussel movement (Hunt and Scheibling 2002) and on colonization and patch dynamics (Hunt and Scheibling 1998, 2001; Schneider et al. 2005) showed that post larval dispersal can play a crucial role in the dynamics and structure of intertidal mussel beds. Due to the extreme spatial and temporal heterogeneity in environmental conditions of rocky intertidal habitats, even the small scale movements observed during my field experiment are likely to subject mussels to different micro-environmental conditions (Helmuth and Denny 2003) and to be important in regulating population dynamics. It has been suggested that differences in movement between *M. edulis* and *M. galloprovincialis* could account for observed species-specific differences in mussel survival (Schneider et al. 2005). In my study, the more dynamic strategy adopted by *M. galloprovincialis*, together with a lower attachment strength than *P. perna* (Zardi et al. 2006b; Chapter 2), could explain the higher mortality rates experienced by *M. galloprovincialis* during a 12 month survey (Chapter 2).

For both species, total movement over six months was higher at bay locations than open coast locations, possibly because bays tend to experience less wave action and the lower hydrodynamic stress experienced at sheltered sites allows mussels to be less strongly attached to the substratum (Chapter 2). For bay and open coast *M. galloprovincialis* and for *P. perna* in bays, total mussel movement over six months was higher for mussels at the edge of a bed than for mussels deep within a bed. This can be explained by a lower availability of free space within mussel beds and by the

fact that mussels inside patches are bound to the substratum not only by their own attachment, but also their neighbours' byssal threads.

Unexpectedly, on the open coast, *P. perna* within beds moved significantly more than individuals at the edges of beds. The magnitude of these differences was minute (< 1mm), suggesting that this is result is of minor ecological importance.

Movement rates of both species varied seasonally, and were exceptionally high in March 2007. In February 2007, the very high mortality rates experienced by mussel populations due to sand inundation and burial (Chapter 2) generated gaps within mussel beds. The availability of free space, the diminished attachment to neighbours, and the necessity of finding safer arrangements through clumping are likely explanations for the high movement rates observed. This is also supported by the cross-correlation between monthly mean movement and mortality rates, where high mortality was followed by high movement rates. High mortality increases the availability of free space and decreases binding to surrounding mussels, consequently giving a greater freedom of, and perhaps need for, movement.

In the mixed groups of the laboratory experiments, there was no species-specific attraction. The proportions of individuals involved in clumps were the same for the two species. The threat of predation did not enhance the speed of mussel locomotion. I thus exclude the possibility that the higher number of clumped mussels under treatment conditions is a consequence of enhanced crawling and random movement and conclude that chemical attraction between mussels is involved.

An invader may not be affected by specialist natural enemies in the invaded community and could achieve great advantage because it loses its own specialist natural enemies (Moyle and Light 1996). Generalist natural enemies of the invaded community, however, will affect the invader depending on their ability to attack

113

(Keane and Crawley 2002). A naïve invader might not be well defended against these enemies, in which case they should suffer higher predation rates than non-naïve, indigenous prey (Mack 1996). Intraspecific behavioural and morphological comparisons have been made between individuals living in predator-free environments and individuals living in areas where predation pressure is high. For example, Geller (1982) showed that gastropods from areas with high predation pressure due to crabs responded strongly to chemical cues released by the crabs, while gastropods from sites where crabs were absent did not react. In addition, mussels exhibit inducible shell thickening when exposed to water borne cues from the predator shore crab *Hemigrapsus sanguineus*, while naïve mussel population in sites where this predator is absent do not respond (Freeman and Byers 2006). Similarly, crab and starfish effluent induced a weaker response in predator-free Baltic Sea mussels than in individuals from the North Sea (Reimer and Harms-Ringdahl 2001). Caro and Castilla (2004) proposed that the distribution and abundance of predators in the field explains inter-population shell thickness differences in the mussel *Semimytilus algosus*. As *M. galloprovincialis* invaded South Africa only in the late 1970s, I initially hypothesised that its response to risk of predation by an indigenous predator would be relatively limited. My results confirmed this hypothesis. Clumping behaviour of the invasive *M. galloprovincialis* was not influenced by the risk of rock lobster predation, while rock lobster effluent had a significant effect on the indigenous species. Given that the invasion of the European mussel is recent (Robinson et al. 2005), it is possible that the invasive species does not perceive the rock lobster as a predator. However, both species reacted to damaged conspecifics and the difference between the species was less pronounced when exposed to this general risk of predation, again supporting my initial hypothesis.

The European mussel has several properties that favour its spread on the South African coast, including resistance to gonad parasites, desiccation and sand stress (van Erkom Schurink and Griffiths 1993; Calvo-Ugarteburu and McQuaid 1998, Zardi et al. 2006a). Local predators can play a crucial role in regulating the interaction between invasive and indigenous prey, but given the generally low levels of predation pressure in South Africa (Bustamante and Branch 1996), the selective impact of predation probably does not drastically affect competition between these species on this coast. However, higher hydrodynamic stress experienced on the open coast and at the edge of a mussel bed, represents a disadvantage for the more dynamic and less strongly attached invasive species, and could consequently play a crucial role in setting patterns of co-existence between *M. galloprovincialis* and *P. perna*.

CHAPTER 6

THE EFFECTS OF ENDOLITHS ON INVASIVE (*MYTILUS GALLOPROVINCIALIS*) AND INDIGENOUS (*PERNA PERNA*) MUSSELS
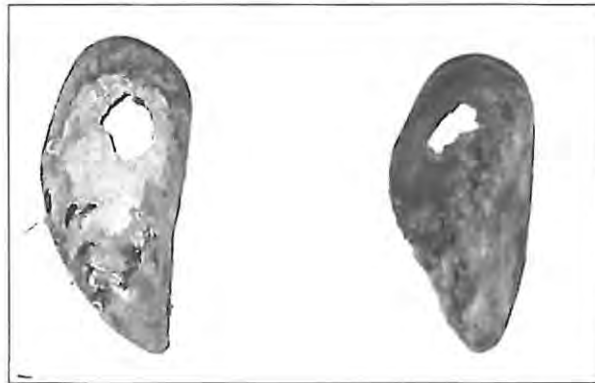
INTRODUCTION

Microbial euendoliths (true endoliths) occupy a distinct ecological niche inside hard mineral substrates, primarily carbonates and phosphates, in terrestrial, freshwater and marine environments (Golubic et al. 2005). They have a diameter less than 100μm and can be separated into phototrophic (cyanobacteria and algae) and heterotrophic (mainly bacteria and fungi) species (Golubic et al. 1981). In the boring process, $CaCO_3$ can be dissolved by secreted acidic substances or complexing agents such as extracellular polymers; however, the boring mechanism of endolithic cyanobacteria is not yet fully understood. (Le Campion-Alsumard et al. 1996).

Microbial endoliths participate in many geologically and ecologically significant processes, including the production of fine grain sediment, bioerosion of limestone and other calcareous substrates such as shells, and the skeletons of living or dead animals and plants (Torunski 1979; Schneider and Torunski 1983; Golubic and Shneider 2003; Pantazidou et al. 2006). Through their activity, they are able to inflict damage on their hosts (Kaehler and McQuaid 1999; Bentis et al. 2000), to contribute to primary production on coral reefs (Larkum et al. 2003), and to attract grazing by gastropods, echinoderms and fish in combined bioerosion activity (Tribollet et al. 2002).

The limiting factor for the distribution of photoautotrophic species is light and this is reflected in the reduction of species diversity with increasing depth of water (Golubic et al. 1975; May et al. 1982). Cyanobacteria prefer supra- and intertidal habitats (Radtke et al. 1996), shallow waters are inhabited by all known endolithic taxa in more or less equal proportions (May et al. 1982; Perry and Macdonald 2002), and where light gets scarce, low light specialists among the phototrophs plus heterotrophs populate the dysphotic benthos (Bentis et al. 2000; Golubic et al. 2005). Only heterotrophic endoliths

117

are able to colonise the aphotic benthic zones of the ocean (Golubic 1993; Freiwald et al. 1997).

However, due to the three-dimensional structure of reef frameworks, light is not a constant factor at each depth (Vermeij and Bak 2002). Gektidis et al. (2007) conclude that shading has a large impact on the intertidal endolithic community, a low impact on the upper-shallow water community, and no visible impact on the lower shallow-water community. Large shaded areas are common in the intertidal environment and are a consequence of corrosion and hydraulic power, creating wave-cut platforms (Lewis 1996). The shaded intertidal community is characterised by the absence of approximately 60% of the cyanobacteria species and its composition is comparable to an upper shallow-water community (Gektidis et al. 2007).

Cyanobacterial endoliths are common on South African shores, their incidence is highly variable (about 23 to 95%) and they may locally infest up to 100% of large mid-shore mussels (Webb and Korrubel 1994; Kaehler 1999). Small mussels are usually free from endoliths and become infested only once the periostracum is damaged (Kaehler 1999). In larger mussels, endolithic infestation can be responsible for 50% of total mortality and for sub-lethal effects. Infested mussels have reduced shell strength and, to repair the damage caused by the endoliths, must increase the rate of shell thickening. However, this higher rate of increase in shell thickness is not enough to compensate for endolithic degradation (Kaehler et al. 1999). Shell repair is an energetically demanding process that can alter the energetic budgeting of an organism and can reduce the energy available for reproduction and growth (Geller 1990; Ambariyanto and Seed 1991).

The incidence of infested shells varies significantly over different spatial scales. Mussel populations at exposed sites are more infested than those at sheltered sites; at smaller

scales, the infestation incidence is greater at higher tidal levels than on the low shore (Kaehler 1999). The majority of shells on the low shore have an intact periostracum, while at higher tidal heights the outer shell layer is often heavily abraded. Several studies indicate that the removal of the periostracum is a prerequisite to endolithic infestation (Raghukumar et al. 1991; Webb and Korrûbel 1994; Kaehler 1999). Greater damage of this layer on the high shore may be related to higher hydrodynamic and wind stress experienced on the high shore (Kaehler 1999).

In this study I quantified the degree of infestation of *Mytilus galloprovincialis* and *Perna perna* and I then tested the following hypotheses: (1) the impact and incidence of infestation on *M. galloprovincialis* and *P. perna* populations will be higher on the open coast than within bays because of the higher hydrodynamic stress; (2) at smaller scales (meters), the incidence of infested shells will be lower at shaded areas, because low light exposure will limit the development of phototrophic endoliths; (3) endolithic infestation has a negative effect on shell strength and on other energetically demanding physiological processes with detectable effects on condition index and attachment strength.

MATERIALS AND METHODS

Incidence of endoliths

Meso-scale comparison between bay and open coast

The incidence of endoliths was measured in two bays (Plettenberg Bay, Algoa Bay) and at two open coast location (Robberg, Cape Recife; Fig. 2.1). Each location had two sites 200m apart at which three quadrats (15 x 15cm) were haphazardly placed in the mid-mussel zone in sun-exposed areas with a 100% mussel cover. All mussels inside each quadrat were brought to the laboratory and separated by species. Mussels were individually measured (shell length) and separated into 10mm size classes. Infestation severity was assessed subjectively by placing them into five categories, depending on degree of infestation: Group A, shells with clean, intact periostracum and distinct outer lines; Group B, shells with central portion of surface eroding, outer striations on periostracum becoming indistinct; Group C, shells with erosion spreading past central portion, grooves and pits appearing on the shell surface; Group D, shells heavily pitted and becoming deformed, outer striations on periostracum almost completely absent; Group E, shells extremely pitted, deformed and brittle, eventually holed (Fig. 6.1). The proportion of infested shells (i. e. all the mussels from group B to group E) was analysed with a 2-way ANOVA with habitat (bay or open coast) and species as fixed factors.

Micro-scale comparison between shaded and non-shaded sites

The incidence of endoliths was measured at Robberg at two sites 200m apart. At each site, six quadrats (15 x 15cm) were haphazardly placed in the mid-mussel zone in areas with 100% mussel cover in shaded and non-shaded areas (three quadrats each area) with the same wave exposure (i. e. same tidal height, lack of protecting structures in front of

the area, same orientation towards the incoming waves, same shoreline angle) to avoid different scouring effects of waves between the two types of areas. Shaded areas were classified as cool sites that were estimated to be exposed to solar radiation for < 25% of the day and, during high sun intensity (i. e. spring sunny day, 10a.m. to 4p.m.), experiencing PAR of 0 - 0.3µmol $m^{-2}s^{-1}$. Non-shaded or sun-exposed areas were classified as surfaces with limited shading that are likely to be exposed to solar radiation > 60% of the day and, during high sun intensity (as above), experienced PAR higher than 0.13µmol $m^{-2}s^{-1}$. The PAR values were estimated with an Integrating Quantum/Radiometer/Photometer (Li-188B). Mussels inside each quadrat were brought to the laboratory and separated by species. Mussels were then individually measured (shell length), separated into 10mm size classes and infestation severity was assessed subjectively by placing them into the five categories described above (Fig. 6.1). The proportion of infested shells (i. e. those in groups B to E) was analysed with a 2-way ANOVA with shade (shaded and non-shaded) and species as fixed factors.

Sub-lethal effects of endolithic infestation

Adult mussels of both species belonging to Groups A and D (shell length 4 - 5cm) were collected at Robberg and brought to the laboratory to be processed.

Shell strength

Clean (Group A) and infested (Group D) mussel shells (n = 50, for each group species, one valve per individual) were tested for strength. The compressive force required to crack the shell was measured using a mechanical, recording spring scale (Chatillon-N.Y.-U.S.A.-MODELIN-25). One valve for each mussel was placed horizontal on a plane surface and gradually loaded at the site of the adductor muscle (over an area of 25mm^2). As the compressive force in Newtons required to break the shell was applied

over a small area, it was taken only as a relative and not absolute estimate of shell strength. The data failed the requirements for parametric analysis (Shapiro's and Levene's test) and were analysed using Kruskal-Wallis tests, with species as the main effect (i. e. pooled groups) and, within the same species, with endolith infestation (group A or D) as the independent factor.

Shell thickness

Clean shells (i. e. Group A; n = 20 each species) were embedded in resin and sectioned longitudinally along the anterior-posterior axis using a diamond saw. An industrial rotating sander with different grades of sanding discs was used to smooth the cut surface before polishing to a blemish-free surface with Brasso household metal polish. Shells were observed under a dissecting microscope (50X) and the widths of the whole shell and of the periostracum above the adductor muscle were measured to the nearest 0.01mm using an ocular eyepiece. The data were analysed with 1-way ANOVA with species as a fixed factor for the shell width and for the periostracum separately.

Condition index

The wet body was dissected from the valves (n = 50 for each species, each group) and dried at 60°C to a constant weight. Samples were weighed to the nearest 0.001g and the condition index (CI) was calculated using the following equation from Davenport and Chen (1987):

CI = dry flesh weight (mg) / dry shell weight (mg)

This measurement was performed in July (2007) and repeated in September (2007) to observe the effects of endolith infestation at different stages of the reproductive cycle. The data, even after transformation, failed the requirements for parametric analysis (Shapiro's and Levene's test) and were analysed with Kruskal-Wallis tests (Sokal and

Rohlf 1995) to assess the effect of endolith infestation (group A or D) on CI for each species and each month separately.

<u>Attachment strength</u>

Mytilus galloprovincialis and *Perna perna* individuals (n = 15 for each species, each infestation group) were tested *in situ* for attachment strength (see Chapter 2 for methods). Data were analysed using a 1-way ANOVA for each species separately, with the presence of endoliths (group A or D) as a fixed factor.

Lethal effects of endolithic infestation

Mussel mortality was investigated by clearing five 1m x 1m quadrats of all dead (gaping widely during low tide) mussels. Quadrats were placed haphazardly at Robberg over 100m of coast in sun-exposed areas with a 100% mussel cover in the mid-mussel zone. In order to ensure that only recent mortalities were included in the survey, only shells with a shiny inner nacreous layer were used. The insides of mussel shells become heavily fouled within a month after death and all such shells were discarded. Endoliths cause a distinctive discoloration, dissolution and finally fracturing of the shell around the site of adductor muscle attachment (Kaehler 1999). All shells exhibiting these fracture holes were assumed to have died from endolithic activity. Data were analysed using a Kruskal-Wallis test with species as the main factor and percentage mussel mortality attributed to endolithic activity as the dependent factor.
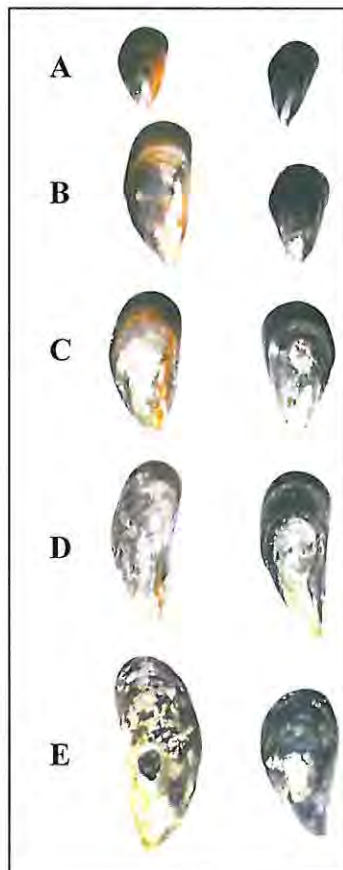
Fig. 6.1

Examples of shells at varying stages of endolith infestation. Group A, shells with clean, intact periostracum and distinct outer lines; Group B, shells with central portion of surface eroding, outer striation on periostracum becoming indistinct; Group C, shells with erosion spreading past central portion, grooves and pits appearing on the shell surface; Group D, shells heavily pitted and becoming deformed, outer striation on periostracum almost completely absent; Group E, shells extremely pitted, deformed and brittle, eventually holed.

RESULTS

Incidence of endoliths

Meso-scale comparison between bays and open coast

There was a significant species x habitat interaction, with no significant differences between *Mytilus galloprovincialis* at bay habitat and *Perna perna* on the open coast (Tukey's test, p = 0.14). At both bay and open coast habitats, *M. galloprovincialis* had a significantly (ANOVA, p < 0.0001; Table 6.1; Fig. 6.2) higher proportion of infested shells than *P. perna*, while, within species, significantly (p < 0.0001) more mussels on the open coast had endoliths than at bay habitats. *M. galloprovincialis* was 48.5% and 38.2% more infested than *P. perna*, on the open coast and at bay habitats respectively.

Infestation increased with shell length and was more severe on the open coast than at bay habitats for both species (Fig. 6.3a,b; Fig. 6.4a,b).

Initial infestation (Group B, see Fig. 6.1) of *P. perna* occurred in mussels 10 - 20 and 20 - 30mm in length, at open coast and bay habitats respectively. By the time they had reached 50 - 60mm in length, 100% of *P. perna* on the open coast exhibited endolith-induced erosion and most of the shells were heavily or extremely pitted (Groups C and D). In bays, about 25% of the 50 - 60mm shells were not infested, while the other shells exhibited Group C and D deformation. None of the *P. perna* shells examined in this experiment were holed because of endolithic infestation.

Initial infestation of the invasive species was visible in shells smaller than 1cm. Most of the *M. galloprovincialis* 40 - 50mm in shell length had Group C or D deformation (Fig. 6.4a,b). On the open coast, endolith-induced shell fractures (Group E) occurred in mussel size class 30 to 40mm and above; once grown to the maximum size, more than 20% exhibited holes related to endolithic infestation.

Micro-scale comparison between shaded and non-shaded sites

At shaded and non-shaded sites, *M. galloprovincialis* had a significantly higher proportion of infested shells than *P. perna* collected at the same sites (ANOVA, p < 0.0001; Table 6.2), while significantly (p < 0.0001) more mussels at non-shaded sites had endoliths than in shaded areas. *M. galloprovincialis* mussels were 32.5% and 37% more infested than *P. perna*, at shaded and non-shaded sites respectively (Fig. 6.5). Infestation increased with shell length and was dramatically more severe at non-shaded than at shaded sites for both species (Fig. 6.6a,b).

Initial infestation (Group B) of *P. perna* occurred in mussels 10 - 20 and 30 - 40mm in length, at non-shaded and shaded sites respectively. By the time they had reached 40 - 50mm in length, 100% of *P. perna* at non-shaded sites exhibited endolith-induced erosion and at 50 - 60mm more than 50% of the shells were heavily or extremely pitted (Group D and E). At shaded sites, more than 40% of the 50 - 60mm in length shells was infested, while the other shells exhibited only Group B deformation.

In the invasive species, Group B and C deformation was visible in shells smaller than 1cm at non-shaded sites, while, when shaded, initial infestation (Group B) occurred only in mussels longer than 10mm. Non-infested mussels (i.e. Group A) were not recorded for sizes bigger than 40 and 20mm at shaded and non-shaded sites respectively. At non-shaded sites, endolith-induced shell fractures (Group E) occurred in size classes 30 - 40mm and above; once grown to the maximum size, more than 50% exhibited holes related to endolithic infestation.

Table 6.1

Results of a 2-way ANOVA applied to the proportion of infested shells with habitat (open coast or bays) and species as fixed factors

Source of variation	df	MS	F	p
Species	1	5.11	106.38	<0.0001
Habitat	1	2.48	51.66	<0.0001
Species x Habitat	1	0.39	8.13	<0.01
Error	44	0.05		

Table 6.2

Results of a 2-way ANOVA applied to the proportion of infested shells with shade (shaded or non-shaded) and species as fixed factors

Source of variation	df	MS	F	p
Species	1	15576.21	899.72	<0.0001
Shade	1	7260.6	419.39	<0.0001
Species x Shade	1	31.21	1.803	0.19
Error	20	17.31		

Fig. 6.2

Mean (+SD) percentage of *M. galloprovincialis* and *P. perna* shells that exhibited damage induced by endolithic infestation, at in bay and open coast habitats. Letters indicate homogenous group (Tukey's test, p < 0.05).

Fig. 6.3

Proportions of *P. perna* shells exhibiting different degrees of infestation severity (Groups A - E, see Fig. 6.1). Infestation severity was determined in the mid-mussel zone at (a) bay and (b) open coast habitats for shells grouped into 10mm size classes.

Fig. 6.4

Proportions of *M. galloprovincialis* shells exhibiting different degrees of infestation severity (Groups A - E, see Fig. 6.1). Infestation severity was determined in the mid-mussel zone at (a) bay and (b) open coast habitats for shells grouped into 10mm size classes.
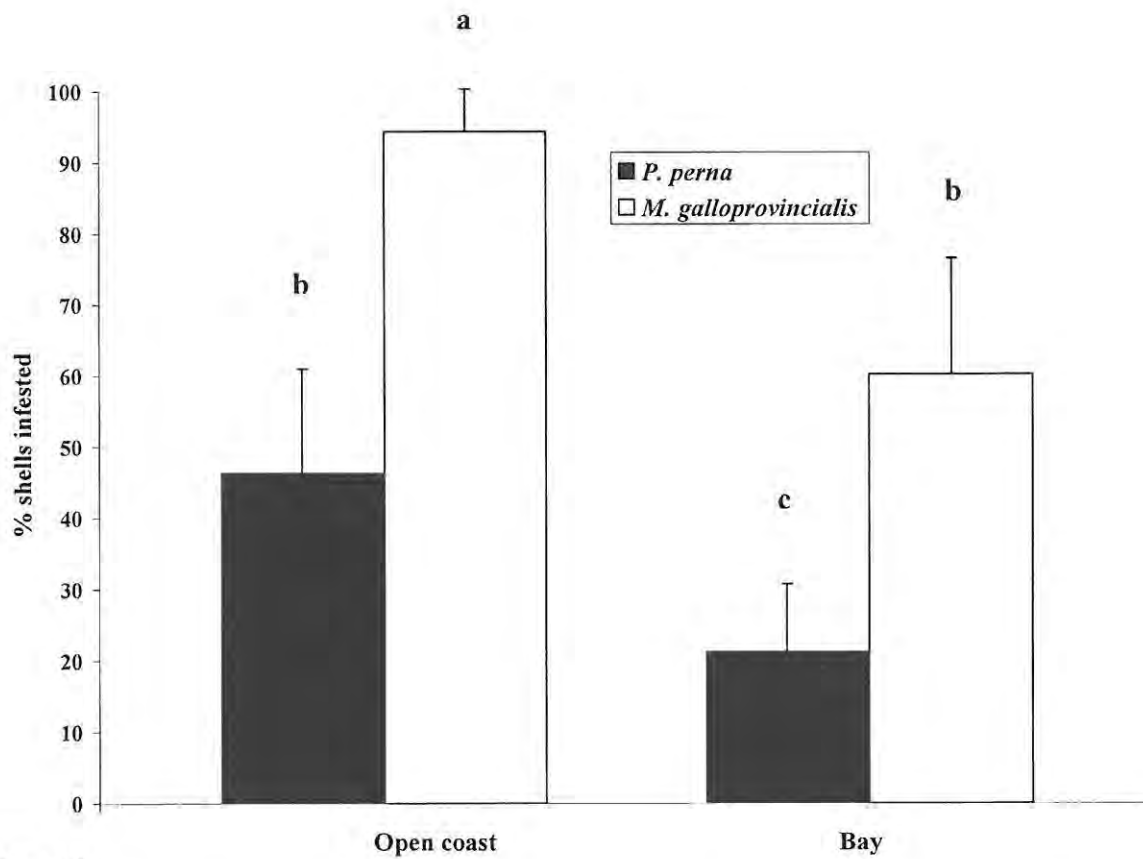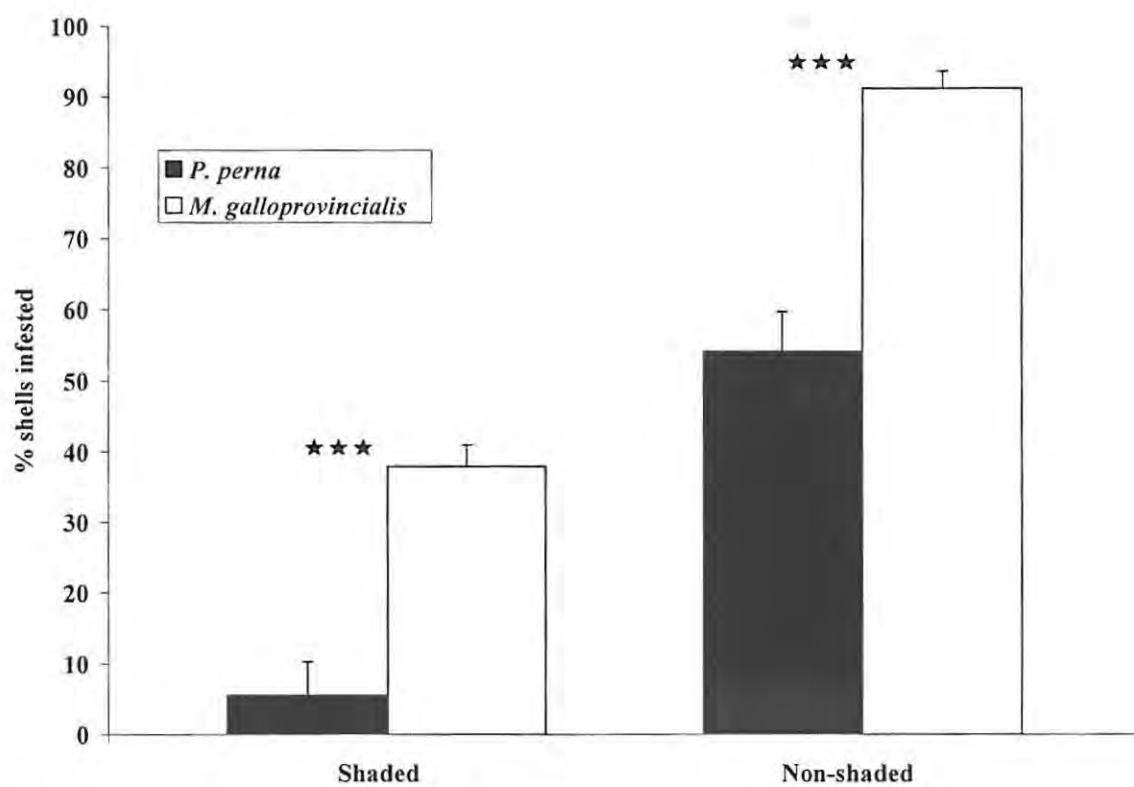
Fig. 6.5

Mean (+SD) percentage of *M. galloprovincialis* and *P. perna* shells that exhibited damage induced by endolithic infestation, at shaded and non-shaded sites. ★★★ = significant differences at p < 0.001.
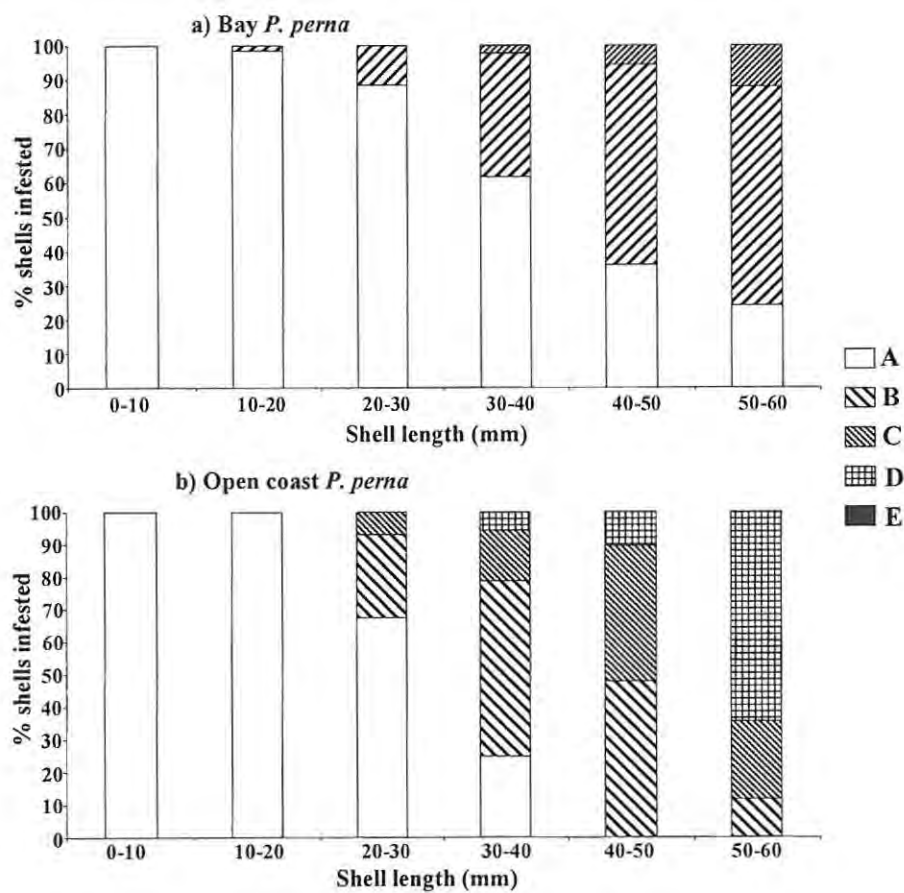
Fig. 6.6

Proportions of *P. perna* shells exhibiting different degrees of infestation severity (Groups A - E, see Fig. 6.1). Infestation severity was determined in the mid-mussel zone at (a) shaded and (b) non-shaded sites for shells grouped into 10mm size classes.
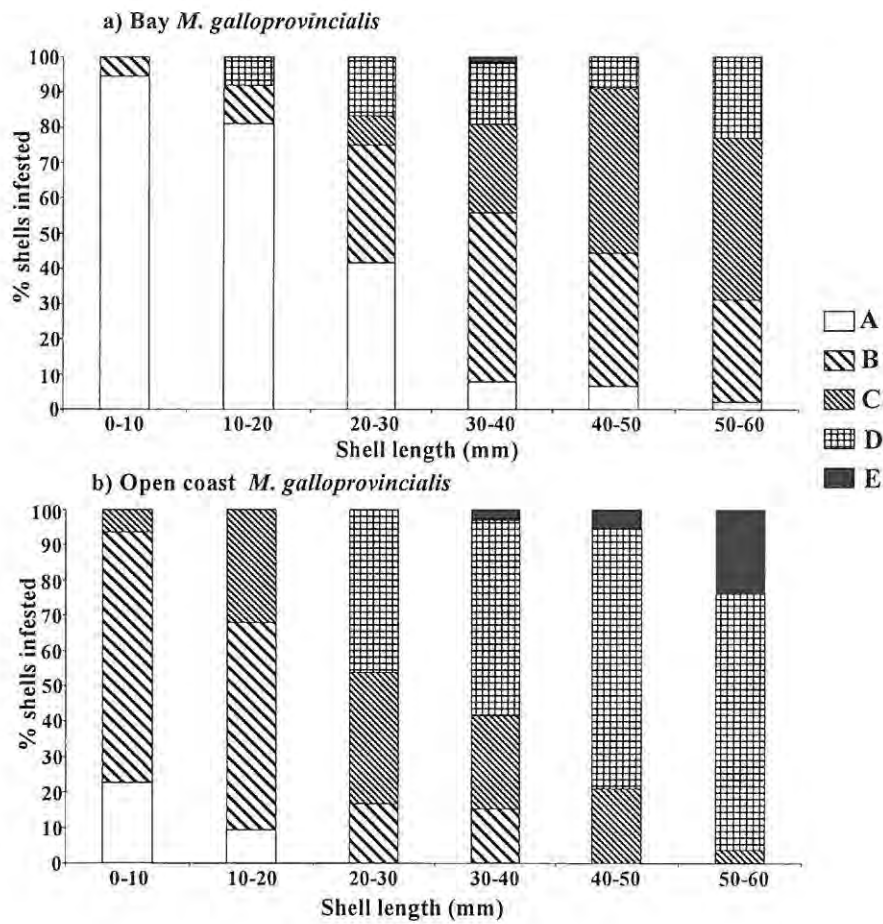
Fig. 6.7

Proportions of *M. galloprovincialis* shells exhibiting different degrees of infestation severity (Groups A - E, see Fig. 6.1). Infestation severity was determined in the mid-mussel zone at (a) shaded and (b) non-shaded sites for shells grouped into 10mm size classes.
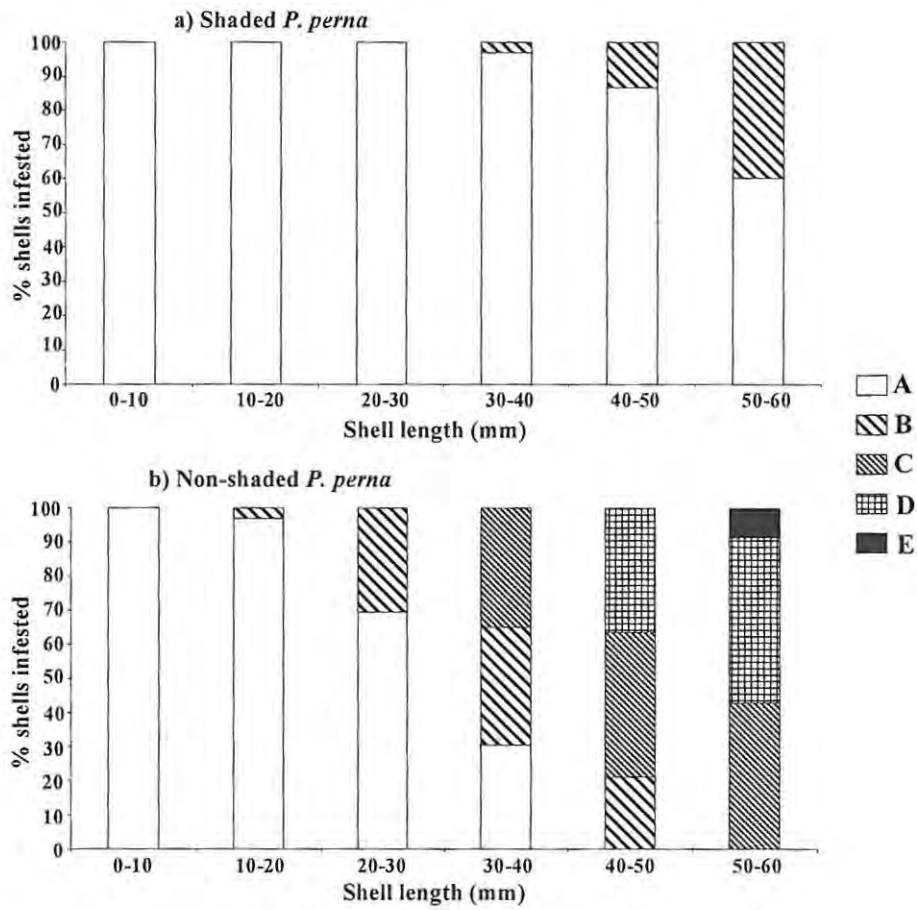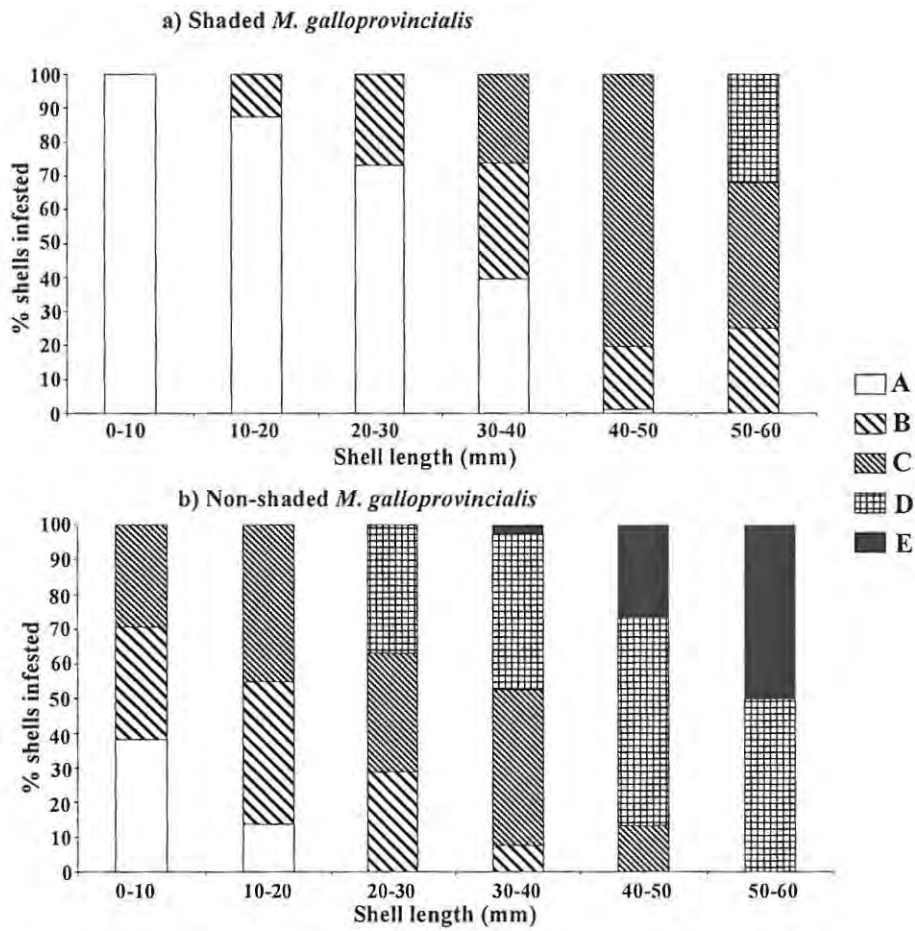
Sub-lethal effects of endolithic infestation

Shell strength

P. perna shell strength was significantly higher (p < 0.0001, Kruskal-Wallis; Fig. 6.8) than that of *M. galloprovincialis*. Within the same species, there was a significant effect of endoliths (p < 0.0001, Kruskal-Wallis), with the shell being more robust when not infested.

Shell thickness

Both periostracum (p < 0.0001, 1-way ANOVA; Table 6.4a; Fig. 6.9) and whole shell (p < 0.0001, 1-way ANOVA; Table 6.4b) were significantly thicker for *P. perna* than for *M. galloprovincialis*. On average, the periostracum and shell of *M. galloprovincialis* were 34.7% and 37.6% respectively thinner than in *P. perna*.

Condition index

Both *M. galloprovincialis* and *P. perna* mussels collected in July and September showed significant effects of endolith infestation on condition (p < 0.05 and p < 0.0001 for *P. perna* and *M. galloprovincialis* respectively, Kruskal-Wallis; Fig. 6.10a,b). The difference between CI of infested (Group D) and clean (Group A) mussels was higher for *M. galloprovincialis* (on average 23%) than for *P. perna* (9.4%).

Attachment strength

When infested, both species had a lower attachment strength than clean mussels (1-way ANOVA; p < 0.001 and p < 0.0001 for *P. perna* and *M. galloprovincialis* respectively; Table 6.5a,b). On average, endolithic colonization reduced attachment strength by 19.9% in *P. perna* and 36.5% in *M. galloprovincialis* (Fig. 6.11).

Table 6.4 Results of a 1-way ANOVA applied to the thickness of (a) the periostracum, and (b) the whole shell, with species as a fixed factor.

a)

Source of variation	df	MS	F	p
Species	1	51.3	144.18	<0.0001
Error	38	0.36		

b)

Source of variation	df	MS	F	p
Species	1	8.93	88.15	<0.0001
Error	38	0.1		

Table 6.5 Results of a 1-way ANOVA applied to attachment strength with effect of endoliths (Group A or D) as fixed factor for (a) *P. perna*, and (b) *M. galloprovincialis*.

a)

Source of variation	df	MS	F	p
Effect of endoliths	1	9.52	13.58	<0.001
Error	28	0.70		

b)

Source of variation	df	MS	F	p
Effect of endoliths	1	9.75	41.18	<0.0001
Error	28	0.24		
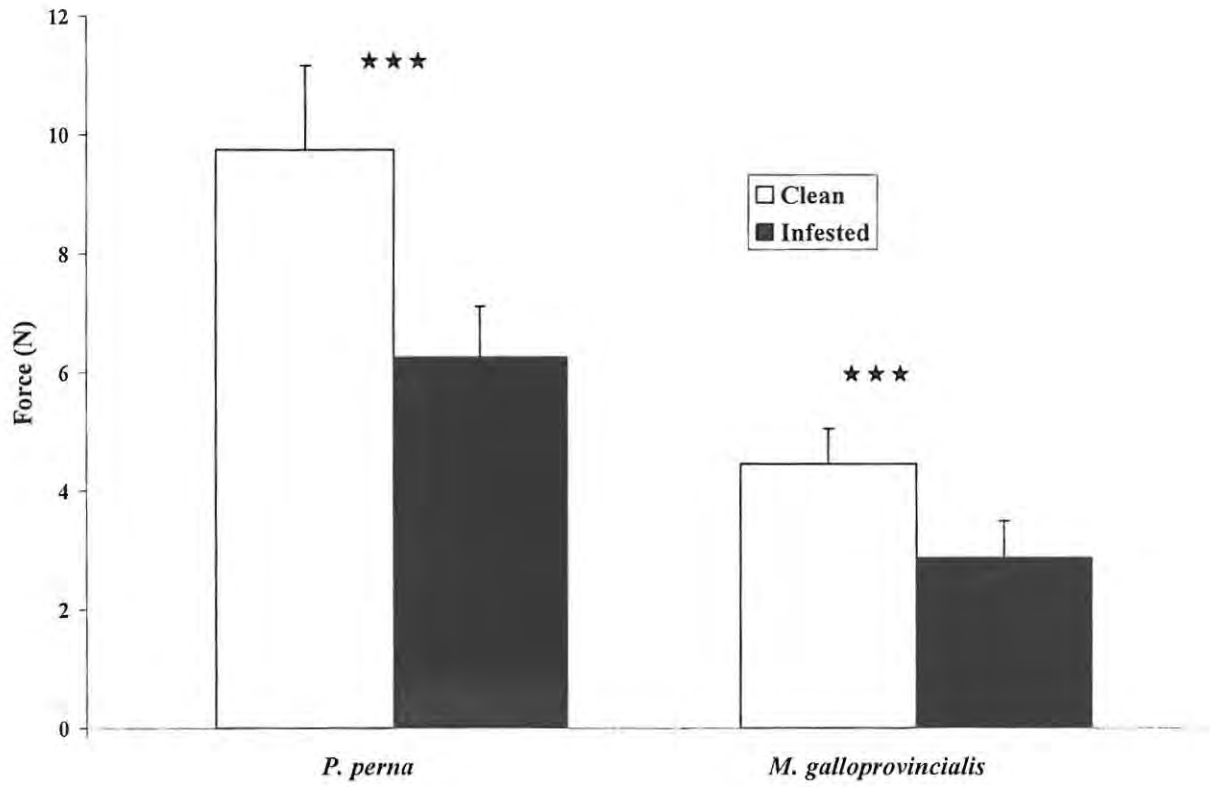
Fig. 6.8

Mean (+SD) force required to break shells of infested (Group D) and clean (Group A) mussels for *M. galloprovincialis* and *P. perna*. ★ ★ ★ = significant differences at p < 0.001.
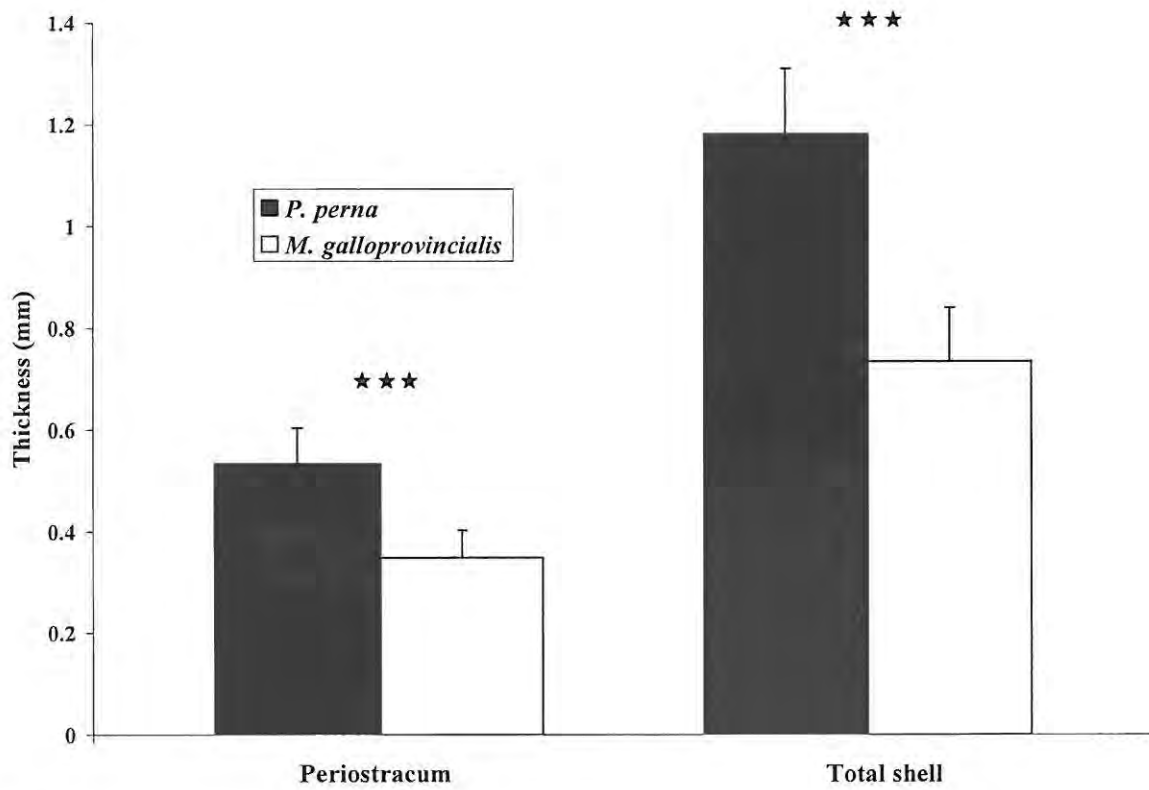
Fig. 6.9

Mean (+SD) periostracum and total thickenss shell of infested (Group D) and clean (Group A) mussels for *M. galloprovincialis* and *P. perna*.★ ★ ★= significant differences at p < 0.001.

(a) *M. galloprovincialis*
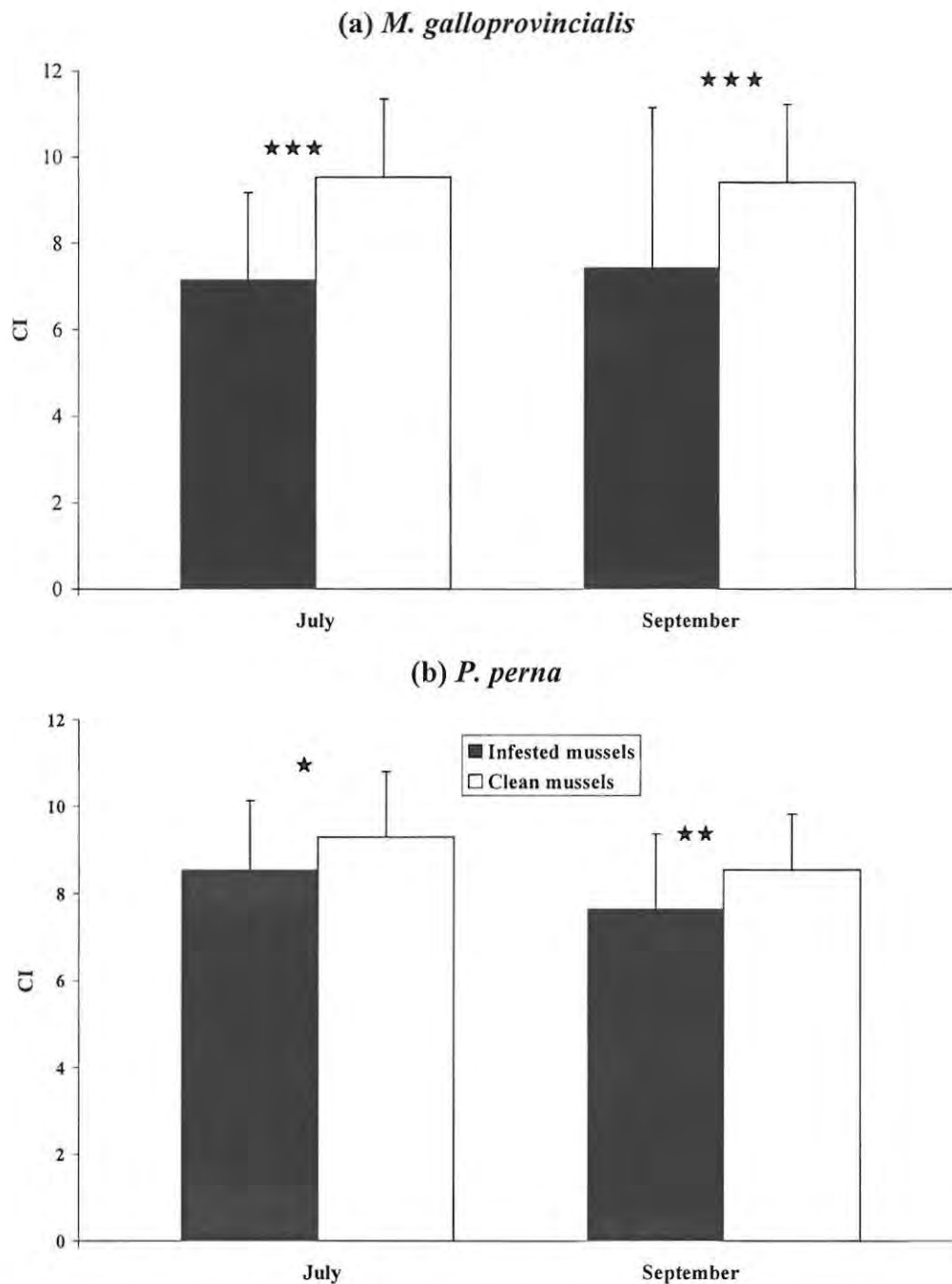
(b) *P. perna*

Fig. 6.10

Mean (+SD) condition index of infested (Group D) and clean (Group A) mussels during July and September 2007 for (a) *M. galloprovincialis* and (b) *P. perna.*★ = significant at p < 0.05;★★ = significant difference at p < 0.01; ★★★ = significant differences at p < 0.001.
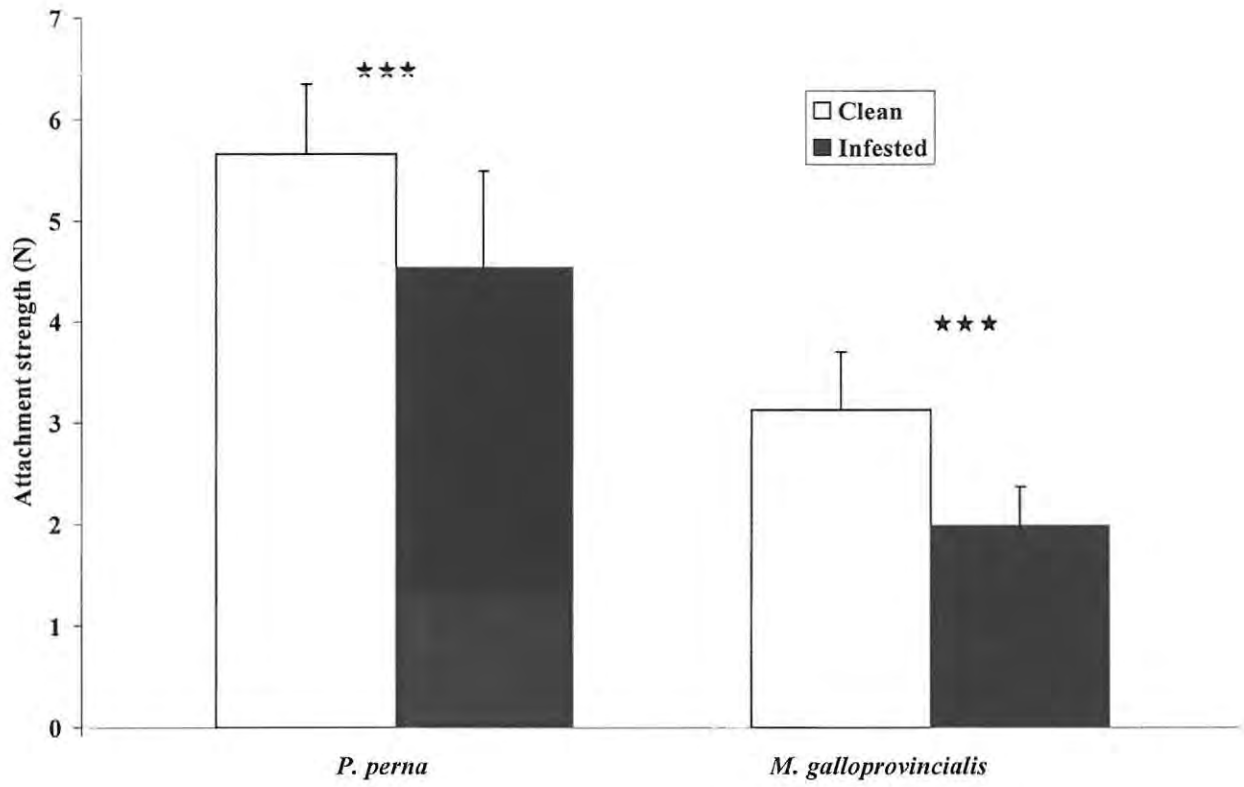
Fig. 6.11

Mean (+SD) attachment strength of infested (Group D) and clean (Group A) mussels. ★★★
= significant differences at p < 0.001.

Lethal effects of endolithic infestation

When comparing mean percentage mortality attributed to endolithic activity, *M. galloprovincialis* mortality rates were significantly higher than those of *P. perna* (Kruskal-Wallis, $p < 0.01$). A total of 190 recently dead mussels were collected from five 1 x 1m quadrats. Of these shells, 26.5% and 9% exhibited large endolith-induced probably lethal fracture holes for *M. galloprovincialis* and *P. perna* respectively (Fig. 6.12).
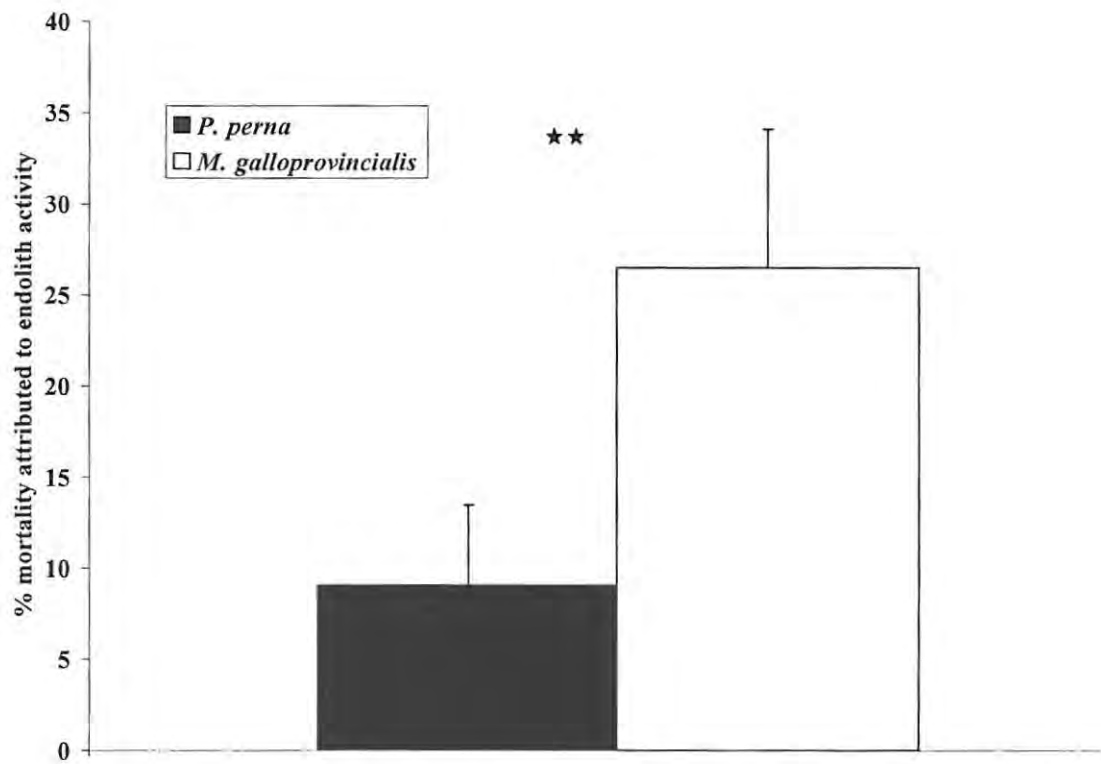
Fig. 6.12

Mean percentage of total mortality (+SD) attributed to endolith-induced fracture holes in *P. perna* and *M. galloprovincialis*. ★ ★ = significant at $p < 0.01$.

DISCUSSION

Mussel beds exhibited the highest incidence of infested shells (up to 70% of shells) on the open coast, while mussels within bays displayed reduced incidence (as low as 30%). Greater wave action on the open coast (see Chapter 2) compared to sites within bays may result in an increase in shell erosion both through contact between adjacent mussels and the abrasion of shells by sediment carried by more powerful waves. This increase in erosion may result in greater damage to the periostracum of shells and strongly influence the frequency of infestations along coastal exposure gradients.

The intertidal habitat is characterized by high environmental heterogeneity where tidal regimes, local patterns of wave splash and substratum inclination can overwhelm patterns of large-scale climate, leading to complex small scale mosaics of environmental gradients (Helmuth and Hofmann 2001). For example, shading can lead to temperature differences that are much greater than average differences observed over large geographic gradients (Helmuth 2002). Previous studies have shown that the incidence of infested mussels is not related to the type of substratum the mussels grow on (i. e. dune rock vs. sand stone), but there is a zone effect, with more mussels infested higher on the shore (Kaehler 1999). Spatial variation in rates of infestation may be linked to different tidal height, but light limitation may also be important. Since many micro-borers are photosynthetic (Golubic et al. 1975), a key factor in the development of species composition of endolithic assemblages is the degree of light exposure. Numerous studies have examined the bathymetric distribution of micro-borers (e. g. May et al. 1982; Glaub 1994; Vogel et al. 2000). However, it is surprising that only a few studies have examined between-site variations in the composition of micro-endolithic assemblages (Perry and Macdonald 2002; Gektidis et al. 2007). The depth

range of micro-endolithic assemblages is reduced in turbid waters compared to clear-water sites (Perry and Macdonald 2002). Moreover, when analysing micro-endolithic community patterns in experimental carbonate blocks at shaded and non-shaded intertidal habitats, a strong effect of shading is observed (Gektidis et al. 2007). Here, I demonstrate that shaded sites have a diminished infestation frequency compared to non-shaded sites, indicating an important role to micro-scale variation of sun exposure in the control of endolithic infestation. Even if the three most abundant cyanobacteria species infesting *Perna perna* shells in the South African rocky intertidal habitat (Kaehler 1999) can be found at depths of < 40m, their photosynthetic activity, and consequently their boring activity could be influenced by light and be higher at non-shaded intertidal sites. In addition, other biotic factors, such as differences in the abundance of macro-borers and grazers at shaded and sun-exposed sites, could also play a role (see Schneider and Le Campion-Alsumard 1999). For example, endolithic activity attracts grazing by gastropods and abrasion by grazing organisms stimulates endolithic cyanobacterial erosion (Schneider and Torunski 1983).

Overall, endolith colonisation occurred more in the invasive *Mytilus galloprovincialis* than the indigenous *P. perna*. The initial colonization of shells also varied between the two species, with endolith infestation never occurring in the smallest size classes of *P. perna* while *M. galloprovincialis* from 0 to 10mm in shell length exhibited endoliths. For the latter species, larger than the smallest size class, endoliths spread throughout the shell, causing progressive damage and, in some cases, resulted in localised shell disintegration, especially on the open coast.

A number of studies have remarked on the protective properties of the molluscan periostracum (e.g. Bottjer 1981; Kumar and Ayyakkannu 1991; Harper and Skelton

1993; Hook and Golubic 1993; Knauth-Koehler et al. 1996), and it has been suggested that the successful colonisation of shells by endoliths may be dependent on the prior removal or damage of this outer shell layer (Raghukumar et al. 1991; Webb and Korrubel 1994; Mao Che et al. 1996; Kaehler 1999). In the present study the invasive *M. galloprovincialis* was found to have a thinner periostracum than *P. perna*, leading to a lower protection against endolith colonization. The mollusc shell is composed of 95 - 99% crystalline calcite or aragonite (a form of calcium carbonate), however the fracture load it can bear can be up 3000 times that of pure aragonite (Currey 1977). Such great magnification of strength can be attributed to the microstructure of the shell, which can vary between species as well as within the same species (Chen et al. 2004). Thus, it is possible that the differences in shell robustness between *M. galloprovincialis* and *P. perna* are due not only to differences in shell thickness, but also to differences in shell microstructure.

Shell strength of both species was weakened by the presence of endoliths, but the invasive *M. galloprovincialis* has a thinner, weaker shell than *P. perna*, enhancing the negative effects of endolithic boring. In mussels, up to 25 to 50% of the total body energy can be engaged in shell deposition (Griffiths and King 1979; Gardner and Thomas 1987). A previous study suggested that *M. galloprovincialis* is likely to be a stronger competitor at exposed sites where density, growth and condition are highest (Steffani and Branch 2004). However, the higher endolithic infestation of the invasive species on the open coast shown here probably requires a higher energetic investment in shell construction and thickness than in the indigenous species, and could reduce its performance on the open coast, at least at sites where infestation rates are high.

Previous studies showed that the flesh mass of infested mussels is significantly lower than that of infested individuals (Kaehler and McQuaid 1999). However this difference was observed only during reproductive periods and was due solely to gonad mass. My results only partially confirm these findings. Differences in condition index between clean and infested mussels were observed not only during periods of high reproductive output, but also when the gonad mass was low. These results suggest that endolithic infestation limits not only the development of the reproductive tissues, but also negatively affects somatic tissue growth.

Mussels attach to the substratum by mean of a bunch of collagenous threads called the byssus (Waite 1992). The process of byssal thread production can be energetically expensive, forming 8 to 15% of a mussels' monthly energetic expenditure (Griffiths and King 1979). Infested mussels had significantly lower attachment strengths than clean mussels, suggesting that the need to repair the shell limits the energy available for the production of byssal threads. It is likely that the negative consequences of a trade-off between an intact shell and strong attachment strength will be particularly marked on the open coast, where shell scouring (leading to endolith attack) and the risk of dislodgment are both high.

In addition to these sub-lethal effects, the activity of photoendoliths can result in the mortality of a large number of adult mussels (Webb and Korrubel 1994; Kaehler and McQuaid 1999). My results indicate that endolithic activity contributed to mortality rates of both species. This experiment was carried out only at one site (Robberg), and given the high variability of endolithic infestation along the South African coastline, the results must be interpreted only in terms of a comparison between *P. perna* and *M. galloprovincialis*. Moreover, it is important to note that my data can overestimate the

contribution of endoliths to mussel mortality because they did not take into account the effects of predation (Smale and Buchan 1981).

M. galloprovincialis has several characteristics of a strong competitor and an opportunistic species, including resistance to the parasites that affect *P. perna* gonad tissue (Hockey and van Erkom Schurink 1992; Griffiths et al. 1992; Calvo-Ugarteburu and McQuaid 1998; Rius and McQuaid 2006). Here I show that endolith infestation of the shell occurs in both the invasive and in the indigenous species, but with higher incidences in *M. galloprovicncialis*, probably due to morphological differences between the shells, especially the periostracum. The open coast and sun-exposed situations are areas of enhanced of endolithic colonization, probably affecting the distribution and physiological performances of the two mussel species at meso- (10s of kilometers) and micro- (meters) scales. In particular, the invasive and competitive capacities of *M. galloprovincialis* will be limited at sites with high solar radiation and, at least on the south coast of South Africa, with strong hydrodynamic stress.

CHAPTER 7

GENERAL DISCUSSION

Organisms live in an ever-changing world. They inhabit environments that have many dimensions (temperature, precipitation etc) each of which can vary temporally and spatially (Levins 1968; Cohen 1996; Shea and Chesson 2002). The outcome of evolution under heterogeneous conditions mainly depends on the nature and scale of this environmental variation. Spatial diversity and temporal fluctuations can occur across large or small patches and over short and long time scales respectively (Levins 1968). Within these time scales, variation can occur on a regular temporal scale, such as daily and seasonal temperature changes, or randomly (Levins 1968). Both abiotic and biotic environmental factors can fluctuate. Abiotic fluctuations are represented by changes in climate and other physical factors, while biotic environment includes changes in food, prey/predator and parasite abundance and inter-specific competition (Meyers and Bull 2002). My results show that intertidal mussels experience, and respond to, spatial and temporal fluctuation of several biotic and abiotic stressors.

Depending on the intensity, the frequency and the suddenness of fluctuations, environmental changes can either preclude or limit the productivity and growth of a species assemblage or cause partial or total removal of organisms (Grime 1977). Short- or long-term stressful changes in environmental conditions, which impair or threaten to impair homeostasis, can trigger physiological and/or behavioural responses by an individual (Saplosky 1992; Broom and Johnsen 1993). However a sudden or higher stress event can have lethal consequences, and is known to affect space allocation, recruitment and subsequent inter-specific competition and species composition in marine benthic communities (e. g. Dayton 1971; Connel and Keough 1985; Sousa 2001). In hard substrate habitats, these disturbances play a determinant role in generating free space (Dayton 1971; Bertness and Ellison 1987; Holt et al. 1995),

consequently affecting settlement and patterns and rates of succession (Underwood 1998; Airoldi 1998; Sousa 2001; Erlandsson et al. 2006). This thesis shows that sand burial can be considered a major disturbing factor in mussel mortality. High temperature (Hutchinson and Williams 2001) and wave action (Erlandsson et al. 2006; Zardi et al. 2006b) can also be responsible for generating free space through mussel removal, but low intensity and/or slow seasonal increase can result in sub-lethal acclimation responses such as Hsps (heat-shock proteins) expression and increased attachment strength. Moreover endolithic infestation can also trigger both lethal (perforation of the shell) and sub-lethal responses (decreased condition index and attachment strength). All these stressful biotic and abiotic effects are known to initiate a cascade of physiological mechanisms that are potentially costly for metabolic processes and that cause a redirection of energetic resources (Buchanan 2000). Less stressful conditions in terms of wave action in bay habitats, allow mussels to maintain a low strength of attachment and invest more energy in gonad development. This suggestion is supported by the lower gonad production recorded in the more hydrodynamically stressful open coast and during winter when wave action is more severe. This trade-off between gonad production and attachment strength can also be enhanced by high Hsps demand and probably the need for greater shell deposition as a consequence of greater endolithic infestation on the open coast.

During 12 months, mussel mortality rates fluctuated in time but were higher on the open coast than inside bays. The freeing of space makes primary substrata available for settlers that potentially arrive from different locations. My results show a high degree of gene flow from bays towards open coast populations. High mussel mortality rates lead to high population turn over on the open coast, while the more stable bay habitat allows

149

a higher genetic diversity. Mussel populations on the open coast may be unable to maintain a positive growth without this input, acting as a sink population. Consequently they are maintained by migration from neighbouring bay populations. It is also possible that migration of larvae from such a stable source population results in an immigration of locally maladaptive alleles which can limit the establishment of locally adapted alleles. This too could reduce or prevent mussel population growth on the open coast (Kirkpatrick and Barton 1997; Lenormand 2002).

Mytilus galloprovincialis and *Perna perna* reacted to a changing environment by adapting their physiological and behavioural performances over different spatial and temporal scales. Their evolutionary strategies are indeed very different. *M. galloprovincialis* emphasises reproduction over attachment strength, and adopts a more dynamic approach, seeking aggregation or a safer arrangement as a mechanism of coping with hydrodynamic stress. In contrast, *P. perna* has higher attachment strength at the expense of lower reproductive output. Invasive species have been characterized as tolerant of environmental extremes with rapid growth, early maturity, short life spans, and elevated fecundity, allowing rapid population recovery after reductions by environmental extremes. Extensive resistance capacities offer little adaptive value to invasive, *r*-selected species, because population reductions occur in their unstable habitats regardless of degree of stress tolerance. In contrast, native species are often adapted to stable habitats where perturbation is infrequent. They are characterized by slow growth, extended life spans, and low effective fecundities, slowing population recoveries (*k*-selected), and have evolved extensive resistance adaptations to avoid extirpation during environmental extremes (Safriel and Ritte 1986; Safriel and Sasson-Frosting 1988; McMahon 2002; Erlandssson et al. 2006). Organisms with high

colonization rates following disturbances can be successful invaders, even if they are weaker competitors. The higher re-colonization rate and recovery of *M. galloprovincialis* following disturbance (Erlandssson et al. 2006) can be explained by its *r*-selected traits of high fecundity, recruitment and growth rates (van Erkom Schurink and Griffiths 1992, 1993; Harris et al. 1998; Branch and Steffani 2004). Bownes (2005) showed that settlement rates in the Plettenberg Bay area are generally lower and less inconsistent for *P. perna* than for *M. galloprovincialis*.

Here I analyse two very different habitats that can differently affect these two mussels species. In fact, it has been argued that some native environments, especially if they are 'extreme', can resist invaders (Daehler 2003), and thus an invader can often fail to establish (Mark 1996). Therefore it is important to examine the impact of different environments on the invasive species in relation to resident species. The open coast habitat reduces the *M. galloprovincialis r*-selected trait of high fecundity because of the need to channel energy elsewhere. Gonad development of the invasive species is affected by spatial topography while the indigenous species spawning rate is preserved. Therefore re-colonization rates of the two species could have different responses when comparing a relatively stable habitat, such as bays, and a more extreme habitat, such as the open coast. This indicates that the interaction between the two species is very habitat-specific. Temperature, waves and sand and biotic components such as parasite abundance prevent an optimal adaptation of the mussel to a single environment needing constant physiological, behavioural and genetic variation. *M. galloprovincialis* is a strong competitor in bays while, on the open coast, the harsher habitat and the lower reproductive output turns the evidence in favour of the indigenous species. As suggested by previous studies (Radford and Cousens 2000; Daehler 2003; Burns 2006; Richards et

al. 2006; Faush in press), my results underline the fact that invasive species can have varying degrees of success in establishing and proliferating, depending on environmental characteristics.

Climate operates over large geographic scales, however its effect can be modified at very much smaller scales by local and regional environmental variables (Helmuth and Hofmann 2001; Schneider and Helmuth 2007). It has been suggested that local patterns of wave splash can overwhelm the effects of large-scale climate in driving patterns of body temperature, leading to complex mosaics of temperature (Helmuth et al. 2006). Environmental stressors affect the distribution and the genetic structure of *M. galloprovincialis* and *P. perna* over large spatial scales. *P. perna* is abundant along the entire east and south coast of South Africa, but mitochondrial DNA sequences of *P. perna* indicate a strong phylogeographic break on the east coast, probably leading to two isolated stocks (Zardi et al. 2007a; unpubl. data). After spreading along the south coast, it seems that *M. galloprovincialis* has reached its biogeographic limit near East London (Fig. 1.1) on the east coast of South Africa (McQuaid and Philips 2000, Robinson et al. 2005), that is, in the area of phylogenetic discontinuity in *P. perna*. However the shift in environmental stressors (i.e. temperature and desiccation) between the south temperate and the east sub-tropical regions can also be experienced by the two species over smaller spatial scales in the intertidal habitat. My results show that *M. galloprovincialis*, though less tolerant than the indigenous species to high temperature, colonizes the upper mussel zone, but at the cost of high expression of Hsps. This suggests that temperature is probably a limiting factor in its invasion along the sub-tropical east coast. Ongoing experiments suggest that western lineage of *P. perna* is far less tolerant to high temperature and desiccation than the eastern lineage (unpubl. data).

Desiccation rather than temperature seems to drive the vertical zonation of the two species, preventing the colonization of the upper mussel zone by *P. perna*. Different degrees of hydrodynamic stress can also be experienced at different spatial scales. Previous studies observed gradients of wave action over meter scales in the intertidal habitat, increasing lower on the shore (Zardi et al. 2006b). My results show a higher wave action on the open coast than bays.

In conclusion, it is clear that there are inter- and intra-specific differences in responses to the environment that highlight the efforts of *M. galloprovincialis* and *P. perna* to find an equilibrium in resource allocation in order to balance survival and reproduction. Determining these differences is crucial to understanding patterns of coexistence between competing indigenous and invasive species (Davis et al. 2000; Mack et al. 2000). It is important to examine the response of invasive and indigenous species over a full range of environmental conditions and at different spatial and temporal scales (Steffani and Branch 2003a,b; Pranovi et al. 2006; Zardi et al. in press). By observing their responses across gradients, it is possible to identify characteristic differences in relative performance under a wide range of conditions and to compare the ecological plasticity of the two species. This approach can help us to interpret the current distribution of invasive and indigenous species and predict their potential distribution across a region (Sher and Hyatt 1999; Chesson 2000; Shea and Chesson 2002; Branch and Steffani 2004).

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