HABITAT SEGREGATION IN COMPETING SPECIES OF INTERTIDAL MUSSELS IN SOUTH AFRICA

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By

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Abstract

Mytilus galloprovincialis is invasive on rocky shores on the west coast of South Africa where it has become the dominant intertidal mussel. The success of this species on the west coast and its superior competitive abilities, have led to concern that it may become invasive on the south coast at the expense of the indigenous mussel *Perna perna.* On shores where these species co-occur, there appears to be habitat segregation among zones occupied by mussels. *M.galloprovincialis* dominates the high-shore and *P.perna* the low-shore, with a mixed zone at mid-shore level. This study examined the factors responsible for these differences in distribution and abundance. The study was conducted in Plettenberg Bay and Tsitsikamma (70km apart) on the south coast of South Africa. Each site included two randomly selected locations (300-400m apart). A third mussel species, *Choromytilus meridionalis,* is found in large numbers at the sand/rock interface at one location in Plettenberg Bay. Aspects of settlement, recruitment, growth and mortality of juvenile and adult mussels were examined at different tidal heights at each site. Quantitative analysis of mussel population structure at these sites supported the initial observation of vertical habitat segregation. Post-larvae were identified to species and this was confirmed using hinge morphology and mitochondrial DNA analysis. Size at settlement was determined for each species to differentiate between primary and secondary settlement. Adult distribution of *C.meridionalis* was primarily determined by settlement, which was highly selective in this species. Settlement, recruitment and growth of *P.perna* decreased with increasing tidal height, while post-settlement mortality and adult mortality increased higher upshore. Thus all aspects of *P.perna's* life history contribute to the adult distribution of this species. Presumably, the abundance of *P.perna* on the high-shore is initially limited by recruitment while those that survive remain prone to elimination throughout adulthood. *M.galloprovincialis* displayed the same patterns of settlement and recruitment as *P.perna.*

However, post-settlement mortality in this species was consistently low in the low and high zones. Juvenile growth also decreased upshore, suggesting that *M.galloprovincialis* may be able to maintain high densities on the high-shore through the persistence of successive settlements of slow-growing individuals. The low cover of *M.galloprovincialis* on the low-shore appeared to be determined by adult interactions. *M.galloprovincialis* experienced significantly higher adult mortality rates than *P.perna* in this zone. There were seasonal variations in the competitive advantages enjoyed by each species through growth, recruitment or mortality on the low-shore. In summer, *P.perna* had higher recruitment rates, faster growth and lower mortality rates, while *M.galloprovincialis* had slightly higher recruitment rates and faster growth rates in winter. *P.perna* is a warm water species while *M.galloprovincialis* thrives on the cold-temperate west coast of South Africa. Therefore both species appear to be at the edge of their optimal temperature regimes on the south coast, which may explain the seasonal advantages of each. Nevertheless, *P.perna* has maintained spatial dominance on the low-shore suggesting that it may ultimately be the winner in competition between these species. *M.galloprovincialis* appears to have a refuge from competition with *P.perna* on the high-shore due to its greater tolerance of desiccation stress, while being competitively excluded from the low-shore. Warm water temperatures coupled with poor recruitment rates at most sites may limit the success of *M.galloprovincialis* on this coast.

Contents

Abstract…… i
Acknowledgements……vi

Chapter 1: General introduction

1.1 Introduction …….. 1
1.2 Hypotheses and aims ……... 14
1.3 Study sites ……...…….15

Chapter 2: Patterns of distribution and abundance of *Perna perna, Mytilus galloprovincialis* and *Choromytilus meridionalis* in Plettenberg Bay and Tsitsikamma

2.1 Introduction…… 21
2.2 Materials and methods…….. 22
Percentage cover…… 22
Density…… 23
Analyses……... 24
2.3 Results…… 25
Population structure in 2001…… 25
1. Percentage cover…….. 25
2. Size structure and density…… 30
3. Recruit density and adult/recruit correlations…….. 36
4. Maximum lengths…… 39
Population structure in 2004…… 41
1. Percentage cover…….. 41
2. Size structure and density…… 45
2.4 Discussion……. 51

Chapter 3: Identification of mussel post-larvae using shell morphology and mitochondrial DNA analysis

3.1 Introduction……..…..... 59
3.2 Materials and methods……...…… 62
Morphological identification…… 62
SEM and hinge structure..…… 64
DNA analysis……... 64
1. DNA extractions……. 64
2. PCR amplification and sequencing…… 65
PCR amplification…….. 65
Sequencing…… 66
Species-specific primer design…….. 69
Specific PCR conditions and post-larval identification……. 69
Size at settlement……. 70
3.3 Results…… 71
Morphological identification..……. 71
Hinge structure…..……81
DNA analysis……. 87

Size at settlement…….. 89
3.4 Discussion…… 92

Chapter 4: Spatial and temporal variations in settlement and recruitment

4.1 Introduction…… 97
4.2 Materials and methods……. 100
Settlement…….. 101
Recruitment…… 102
Analyses……. 103
1. Settlement……. 103
2. Recruitment…….. 104
Wind correlations……... 105
4.3 Results…….. 106
Settlement…….. 106
1. April 2001…… 106
2. March 2003…….. 119
3. Size-dependent settlement in 2003…….. 129
Recruitment…… 134
1. Monthly recruitment rates…… 134
2. Temporal variation in the size of recruits…… 145
Wind correlations…….. 149
1. April 2001…… 149
2. March 2003…….. 149
3. Recruitment…….. 152
4.4 Discussion…… 152

Chapter 5: Juvenile growth and post-settlement mortality

5.1 Introduction…….. 164
5.2 Materials and methods……. 167
Juvenile growth…….. 167
Post-settlement mortality……... 169
Analyses……. 172
1. Juvenile growth…… 172
2. Post-settlement mortality……. 172
5.3 Results…….. 173
Juvenile growth…….. 173
Post-settlement mortality……... 180
5.4 Discussion…… 191

Chapter 6: Growth and mortality in adult *Perna perna* and *Mytilus galloprovincialis*

6.1 Introduction……..……...…… 200
6.2 Materials and methods……...…….. 203
Growth…… 203
Mortality……. 204
Analyses……..207
1. Growth…….…. 207

2. Mortality…….... 208
6.3 Results……...……... 208
Growth in 2001……...……... 208
Growth in 2003…….... 217
Mortality……. 228
6.4 Discussion……...……. 237

Chapter 7: General discussion……..……. 246

References……. 251

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Chapter 1

General introduction

1.1 Introduction

Humans have facilitated the spread of non-indigenous species for centuries, and the constant expansion of trade and global transport has led to an increase in the number and rates of biological invasions (Carlton 1987; Everett 2000; Mack *et al.* 2000; Kolar and Lodge 2001). Although the economic impact of invasions has long been recognised, particularly for agricultural systems, it is only in the last half of the past century that the ecological impact has been considered important (Everett 2000). In marine systems, transport of organisms for aquaculture and on ships, either as fouling species or in ballast water tanks, have been the primary mechanisms for marine introductions (Carlton 1987; Moyle 1991; Carlton and Geller 1993; Occhipinti-Ambrogi 2001). Carlton and Geller (1993) state that marine invasions are of such magnitude that they may be leading to profound ecological changes in the ocean. Invasive species can have a dramatic impact on natural communities by replacing or reducing the numbers of native ones (Elton 1958), changing ecosystem structure and function (Occhipinti-Ambrogi 2001), and even destroying habitat (Mack *et al.* 2000). Consequently, the global transfer of species across the world's oceans and the threat this poses to the biodiversity of the sea, particularly coastal systems, has become an issue of increasing concern (Occhipinti-Ambrogi 2001).

Introduced organisms, now more popularly referred to as non-indigenous species, become invasive following the establishment, spread and persistence of populations in a new environment (Everett 2000; Mack *et al.* 2000; Kolar and Lodge 2001). Although biological invasions are occurring at increasing rates, relative to the number of species that disperse, few manage to establish themselves (Holdgate 1986). An analysis of British introductions led to the conclusion that only 10% of the species introduced become established, and that only 10% of those become invasive (Holdgate 1986; Williamson and Brown 1986). Elton

(1958) noted that any colonising organisms would find themselves entering a highly complex community of different populations. Furthermore, the structure of any community varies in space and time in response to physical and biological factors (Menge and Sutherland 1987). Thus there are a number of interacting variables that may determine the success or failure of non-indigenous species becoming invasive.

Environmental factors may play an important role in invasion biology. Suitability of climate (Holdgate 1986; Swincer 1986) and habitat (Swincer 1986; Williamson and Fitter 1996) can be important, and a common characteristic found in successful invaders is the ability to tolerate a wide range of variability in the two (Swincer 1986). Another factor is the order, timing and intensity of physical disturbances. Severe disturbances at the time of arrival can be detrimental to the invading population (Mack *et al.* 2000). On the other hand, disturbances characteristically create spare or new resources to be exploited, often through the removal of indigenous species, and so often lead to successful establishment (Fox and Fox 1986; Hobbs 1989). Thus a small immigrant population could persist or perish due to a "lottery-like array" of environmental forces (Mack *et al.* 2000). Recruitment over protracted periods and in large numbers will therefore increase the chances of success, simply because the process is repeated under differing conditions of weather and competitor and predator density (Crawley 1986). Hence, high fecundity (Holdgate 1986; Hockey and van Erkom Schurink 1992; Williamson and Fitter 1996) and high recruitment rates (Williamson and Fitter 1996; Levine 2000; Kolar and Lodge 2001) are common characteristics of invasive species.

The state of recipient communities may influence invasion success (Ehrlich 1989), and several theories explore the possibility that some communities may be more vulnerable to

invasion than others. Heterogeneity in community structure may provide refuges, which permit recruitment into a community despite intense predation, competition and/or physical stresses (Crawley 1986). Environmental stress gradients are universal in all habitat types (Menge and Sutherland 1987) and it has been suggested that some communities may be more susceptible to invasion at particular zones along an environmental gradient (Fox and Fox 1986). This is likely to be influenced by the wide physiological tolerances possessed by many invading species.

There is a long-recognised connection between disturbed habitats, especially those simplified by man, and biological invasions (Elton 1958; Crawley 1986). Elton (1958) suggested that relatively simple communities are more vulnerable to invasion than species-rich ones, which are generally ecologically stable and therefore able to offer "ecological resistance". That species-rich communities are more resistant than simpler ones has been a common thread found in several reviews of biological invasions (Crawley 1986; Fox and Fox 1986; Holdgate 1986). Levine (2000) also found from natural experiments with plant communities that increased diversity enhanced resistance to invasion. In contrast, the "vacant niche hypothesis" proposed by Simberloff (1986) on insect introductions, suggests that the probability of a non-indigenous species becoming established depends on its habitat requirements and the availability of suitable habitat, and only secondarily on what other species are found there. Johnson and Carlton (1996) attribute the dramatically successful invasion of zebra mussels, *Dreissena polymorpha,* in North America, partly to the availability of an unexploited niche. However, this hypothesis is not well supported (Herbold and Moyle 1986).

Interspecific interactions, which include predation, parasitism and competition, may influence the abundances of colonising organisms. Escape from native predators or parasites may allow non-indigenous species to thrive in a new environment (Huffaker *et al.* 1971; Calvo-Ugarteburu and McQuaid 1998a, 1998b). Competitive ability may determine the outcome of interactions with indigenous species and invasive species often exhibit rapid growth rates (Holdgate 1986; Pimm 1989; Hockey and van Erkom Schurink 1992). Competition may be of particular importance amongst ecologically similar species with similar habitat requirements.

Competition can be defined as the negative effects one organism has upon another by consuming, or controlling access to, a limited resource (Keddy 1989). There are two basic mechanisms of competition, namely: exploitative competition, which occurs indirectly through the reduction of available resources, and interference competition, which involves direct interactions among species (Keddy 1989; Morin 1999). Regardless of the mechanism, competition can either be reciprocal, where the effects of both species are equal, or asymmetric, where one species exerts stronger per capita effects than the other (Keddy 1989; Morin 1999). When competition is asymmetric, it usually leads to competitive dominance of one species over another, often resulting in the exclusion of the weaker species (Keddy 1989).

A number of theories address the issue of competition and coexistence in communities. According to the competitive exclusion principle, if two species occupy the same ecological niche (i.e. the position of a species in a community based on its precise food, spatial and habitat requirements (de Bach 1966)), the stronger competitor will displace the weaker species, which will become extinct (Hardin 1960). Thus, complete competitors may

only coexist if they become ecologically different, and this may be achieved through resource partitioning (Schoener 1974). A slight variation on this is given by de Bach (1966) who states that "competitive displacement" occurs either when one species displaces another ecologically homologous species, or when it prevents such a species from successfully colonising all, or part, of its habitat. He adds that displacement occurs in varying degrees in nature so that actual exclusion is rare, although the process may be operating continuously. Several studies have shown how non-indigenous species have successfully invaded new habitats by outcompeting ecologically similar species that were previously established there (Holway 1999; Bøhn and Amundsen 2001; d'Antonio *et al.* 2001). In most of these studies, competitive displacement resulted in a significant reduction in the abundance and/or distribution of native species, rather than exclusion, as suggested by de Bach (1966). In contrast, MacNeil *et al.* (2001) have illustrated coexistence among native and introduced freshwater amphipods, which appears to be facilitated by spatial and temporal habitat heterogeneity.

There are several theories of coexistence that do not depend on ecological differentiation. Ives (1991) proposed that intraspecific aggregation or clumping might explain coexistence in patchy communities. If different species tend to aggregate in different patches then interspecific competition is reduced, and species are better able to coexist. Minor environmental fluctuations might allow nearly equivalent species to persist indefinitely (Keddy 1989). This is related to Hutchinson's theory, which suggests that species may coexist because environmental variability continually changes the competitive advantages enjoyed by each species (Harger 1972b). Harger (1972b) found support for this hypothesis in mixed species assemblages of the intertidal mussels *Mytilus galloprovincialis* (reported as *Mytilus edulis,* McDonald and Koehn 1988) and *Mytilus californianus.* Similarly, Paine

(1984) found that grazers introduced competitive uncertainty among macroalgae in a rocky intertidal community, generating high levels of reversals where losers overgrow winners and thus promote coexistence. In intertidal systems, coexistence may be a consequence of the renewal of space generated by disturbances, and hence disequilibrium conditions (Sousa 1979). The length of time that species will coexist then depends on the frequency and intensity of disturbances (Connell 1978), the relative rates of recruitment into disturbed patches and mortality (Sousa 1979). Finally, coexistence may occur if recruitment fluctuates between species in response to environmental conditions and adult mortality is relatively low, so that populations persist even when recruitment is poor (Warner and Chesson 1985).

The Mediterranean mussel, *M.galloprovincialis,* provides an excellent example of the global scale of marine introductions. This species has successfully colonised Hong Kong (Lee and Morton 1985), Japan (Wilkins *et al.* 1983), Korea (McDonald *et al.* 1990), Australia (McDonald *et al.* 1991), the west coast of North America (McDonald and Koehn 1988) and South Africa (Grant and Cherry 1985; Griffiths *et al.* 1992), probably through transport by ships (Branch and Steffani 2004). It therefore appears to be a highly successful invasive species. On South African shores the impact of invasion by *M.galloprovincialis* on intertidal communities and its interactions with indigenous species has received considerable attention (van Erkom Schurink and Griffiths 1990; van Erkom Schurink and Griffiths 1991; Griffiths *et al.* 1992; Hockey and van Erkom Schurink 1992; Calvo-Ugarteburu and McQuaid 1998a, 1998b; Steffani and Branch 2003).

Mytilus galloprovincialis was introduced on the west coast of South Africa in the late 1970's or early 1980's (Grant and Cherry 1985; Griffiths *et al.* 1992), and is now the

dominant intertidal mussel species from Cape Point to southern Namibia (Griffiths *et al.* 1992). Before its arrival, the mid to lower intertidal zone was largely occupied by the indigenous west coast mussel *Aulacomya ater* and the limpets *Scutellastra granularis* and *Scutellastra argenvillei* (Griffiths *et al.* 1992; Steffani and Branch 2003; Branch and Steffani 2004). Hockey and van Erkom Schurink (1992) found that *A.ater* grows more slowly and is less desiccation-tolerant than *M.galloprovincialis,* and is apparently unsuccessful in competition for primary rock space with this species. Consequently, *M.galloprovinciailis* has become dominant intertidally at the expense of *A.ater*, which is now mostly found at the lowest tidal levels and subtidally (van Erkom Schurink and Griffiths 1990; Griffiths *et al.* 1992; Hockey and van Erkom Schurink 1992). *M.galloprovincialis* is abundant in the mid and high zones (van Erkom Schurink and Griffiths 1990; Hockey and van Erkom Schurink 1992), which has resulted in both the vertical range and centre of gravity of the mussel beds being moved considerably upshore, as well as an increased overall mussel standing stock in these zones (Griffiths *et al.* 1992). Furthermore, it apparently exerts some competitive dominance over adult *S.granularis* and *S.argenvillei*, which become spatially contained and eventually eliminated by encroaching mussels (Griffiths *et al.* 1992; Hockey and van Erkom Schurink 1992; Steffani and Branch 2003). However, *M.galloprovincialis* mussel beds also provide a preferable settlement and recruitment site compared to *A.ater* beds, resulting in an increase in the density of *S.granularis* recruits. Thus the distribution and size structure of the limpet populations has changed (Hockey and van Erkom Schurink 1992). The situation is less favourable for *S.argenvillei,* which maintains much higher densities on primary rock space than on mussel beds. These limpets are also unable to reach the size at which they become sexually mature on mussel beds leading to low reproductive output from these populations (Branch and Steffani 2004).

There are four species of mussels that are abundant on South African shores: three predominantly west coast species, *M.galloprovincialis*, *A.ater* and *Choromytilus meridionalis,* which occurs subtidally and on the low intertidal at sand/rock interfaces or in silted areas (Hockey and van Erkom Schurink 1992), and *Perna perna* on the south and east coasts (van Erkom Schurink and Griffiths 1990). Comparative studies have shown that, relative to the three indigenous mussels, *M.galloprovincialis* exhibits several of the characteristics of an aggressive invasive species discussed, namely: a rapid growth rate, high fecundity, resistance to desiccation (van Erkom Schurink and Griffiths 1991; Hockey and van Erkom Schurink 1992; van Erkom Schurink and Griffiths 1993), and resistance to parasites (Calvo-Ugarteburu and McQuaid 1998a). It also appears to be a strong competitor for primary rock space. This species has spread onto the south coast of South Africa. It is unclear whether this resulted from a range expansion from the west coast or an independent introduction near Port Elizabeth (Fig. 1.1a; p.20) for mariculture purposes (McQuaid and Phillips 2000; Branch and Steffani 2004). In 1992, the proportion of *M.galloprovincialis* on the southern cape coast was estimated to be only 1% of overall mussel standing stock (Griffiths *et al.* 1992), with small numbers occurring as far as East London (van Erkom Schurink and Griffiths 1990). However, a recent survey showed that, although it still occurs in relatively low densities along this coastline, it has established and become abundant at certain sites such as Plettenberg Bay and Jeffreys Bay (Fig. 1.1a).

The brown mussel, *P.perna*, is the dominant mussel species along the south and east coasts, occurring as far as central Mozambique (Berry 1978). It is virtually absent from the central and southern parts of the west coast and reappears as the dominant mussel in northern Namibia (van Erkom Schurink and Griffiths 1990). It is essentially a mussel of wave-exposed shores and reaches its maximum abundance in the mid-lower intertidal (Berry

1978; van Erkom Schurink and Griffiths 1990). There are several factors that contribute to the concern that *M.galloprovincialis* may become invasive at the expense of *P.perna.*

Firstly, *P.perna* is heavily exploited on the south and east coasts, through recreational harvesting (Crawford and Bower 1983; van Erkom Schurink and Griffiths 1990; Tomalin 1995) and/or for subsistence purposes (Siegfried *et al.* 1985; Griffiths and Branch 1997). Studies in the Transkei (shaded area in Fig. 1.1a) have shown that recovery rates of over-exploited mussel beds are generally poor, indicating that *P.perna* is not very resilient to exploitation (Dye *et al.* 1997). Lasiak and Barnard (1995) found recruitment levels to be consistently lower than in other mytilids. Also, significantly lower recruitment intensities on the south and east coasts, compared to the west coast, coupled with low stock sizes, emphasise the vulnerability of this mussel to exploitation (Harris *et al.* 1998). Lambert and Steinke (1986) found that disturbances in *P.perna* beds resulted in those patches being dominated by a coralline turf. Similarly, Dye *et al.* (1997) concluded that even in marine reserves this species is easily displaced, particularly by coralline algae. Thus *P.perna* does not appear to be a competitive dominant (Lambert and Steinke 1986). In contrast, the competitive dominance exhibited by *M.galloprovincialis* on the west coast and its highly invasive attributes, suggest it may be more resilient to exploitation than *P.perna* (Hockey and van Erkom Schurink 1992).

Secondly, in South Africa *P.perna* is commonly infected with trematodes while *M.galloprovincialis* is not (Calvo-Ugarteburu and McQuaid 1998a). The two most common of these trematodes, *Proctoeces* metacercariae and bucephalid sporocysts, have been shown respectively to reduce growth rates in smaller mussels and to castrate larger female mussels, effectively removing them from the breeding population (Calvo-Ugarteburu and

McQuaid 1998b). These effects would be expected to reduce the competitive ability of infected mussels.

Thirdly, in a comparison of growth rates in Port Elizabeth, *M.galloprovincialis* grew considerably faster than *P.perna* (van Erkom Schurink and Griffiths 1993). Petraitis (1995) emphasises the importance of growth in maintaining spatial dominance on rocky shores. As mussels grow they compete for space (Griffiths and Hockey 1987), therefore a faster growth rate is advantageous among competing species (Harger 1972b; Barkai and Branch 1989; Hockey and van Erkom Schurink 1992). Also, it has been shown that *M.galloprovincialis* has a greater reproductive output than *P.perna*, although these studies were conducted at False Bay, which is at the edge of *P.perna's* geographic range (van Erkom Schurink and Griffiths 1991; Hockey and van Erkom Schurink 1992).

In combination, these factors could potentially afford *M.galloprovincialis* a competitive advantage over *P.perna.* This idea is reinforced by a study on populations of the same two species on the North African coast near Algiers (Abada-boudjema and Dauvin 1995). At one of the study sites, *P.perna* was initially the dominant mussel, but declined from 71% to 30% of the total mussel population over the 5-year study period when *M.galloprovincialis* became dominant. This decline was attributed to heavier exploitation of *P.perna* due to its larger sizes combined with consistently higher recruitment rates for *M.galloprovincialis*. It is therefore not unlikely that a similar outcome may result from interactions between these species on the south coast.

However, there are a number of fundamental differences between the south and west coasts of South Africa, both in terms of the oceanography of these regions and the communities

established there (Bustamante *et al.* 1997; Harris *et al.* 1998). The south coast is warm-temperate as opposed to the cold-temperate west coast (Brown and Jarman 1978). Recruitment rates of mussels are at least two orders of magnitude lower on the south coast than on the west coast, which may be related to the differing temperatures, physical oceanography or the species of mussels that predominate (Harris *et al.* 1998). Branch and Steffani (2004) suggested that low species richness on the west coast might have been a factor influencing the success of *M.galloprovincialis.* Species richness is greater on the south coast than the west coast (Bustamante *et al.* 1997), which may suggest that communities there would be more able to resist invasion by *M.galloprovincialis.* Furthermore, Hockey and van Erkom Schurink (1992) stated that since *P.perna* and *M.galloprovincialis* are more similar in their tolerances to desiccation and siltation, and both grow rapidly, any interaction between them intertidally is likely to be more evenly balanced than that between *M.galloprovincialis* and *A.ater* on the west coast.

Mytilus galloprovincialis is now a prominent feature of rocky shores in Plettenberg Bay on the south coast, where *P.perna* was previously the dominant mussel in the mid to low intertidal zones (Wooldridge 1988). There are three species of mussel that co-occur on these shores, the third being *C.meridionalis*. Although their vertical distributions do overlap, there appears to be some habitat segregation, with *M.galloprovincialis* being more abundant on the high shore and mixing with *P.perna* on the mid shore, while *P.perna* is generally more abundant on the low shore. *C.meridionalis* is found in large numbers at the sand/rock interface. Van Erkom Schurink and Griffiths (1993) made similar observations in False Bay where, in mixed beds, *M.galloprovincialis* tends to occur at the highest levels, followed by *P.perna* and then *C.meridionalis.* Present observations therefore suggest that *P.perna* may be excluding *M.galloprovincialis* from low-shore areas. It was also noted that

in the Tsitsikamma National Park (Fig. 1.1b; p.20), about 70km east of Plettenberg Bay, numbers of *M.galloprovincialis* remain relatively low, as along much of this coastline (Rius, 2005). At this site, mussels are most abundant on the mid-shore occurring only in scattered patches in the low and high zones. Nevertheless, a similar zonation pattern was evident although *M.galloprovincialis* appears to be largely confined to the high-shore. Thus two sites separated by only 70km support very different densities of *M.galloprovincialis.* Inclusion of both sites in this study may therefore provide information on the strength of interspecific interactions where *M.galloprovincialis* is abundant and where it is scarce. This would also potentially provide information on why some sites are more vulnerable to invasion by *M.galloprovincialis* than others.

The importance of interspecific interactions such as competition and predation and physical factors such as wave exposure and desiccation in determining the distribution and abundance of intertidal organisms has been widely documented (Connell 1961; Seed 1969b; Dayton 1971; Harger 1972b; Kennedy 1976; Suchanek 1978; Paine 1984; Griffiths and Hockey 1987; Barkai and Branch 1989; Menge *et al.* 1994; Bustamante *et al.* 1997; Petraitis 1998; McQuaid *et al.* 2000; Harley and Helmuth 2003). It has been established that these factors may also play a significant role in the success of biological invasions. Indeed, it has been noted that invasions may provide unique opportunities for assessing the roles of biotic interactions in community structure (Diamond and Case 1986 cited in Holway 1999).

The structure of intertidal communities is not, however, solely determined by physical and biological interactions. It has been increasingly realised that variations in settlement and recruitment may be important in determining the patterns of distribution and abundance of

adult populations (Denley and Underwood 1979; Underwood and Denley 1984; Connell 1985; Gaines and Roughgarden 1985; Bushek 1988; Davis 1988; Raimondi 1990; Menge 1991; Hunt and Scheibling 1998). Differential settlement of invertebrate larvae with respect to substratum and tidal height has been well documented (Barnett *et al.* 1979; Denley and Underwood 1979; Grosberg 1981; Young and Chia 1981; Petersen 1984; Bushek 1988; Raimondi 1988; Hurlburt 1991; Morse 1991; Petraitis 1991; Minchinton and Scheibling 1993; Davis and Moreno 1995; Nielsen and Franz 1995; Cáceres-Martínez and Figueras 1997). Organisms may settle preferentially among adult conspecifics (Barnett and Crisp 1979; Barnett *et al.* 1979; Bushek 1988; Raimondi 1988; Nielsen and Franz 1995), or avoid settling on or near competitive dominants (Grosberg 1981; Young and Chia 1981; Petersen 1984). For example, *M.edulis* larvae avoided competitively superior *M.californianus* adults and settled preferentially on algal patches, thereby avoiding interspecific competition and subsequent mortality (Petersen 1984).

However, settlement and recruitment rates are often poorly correlated due to variations in post-settlement mortality. Thus, post-settlement mortality is an additional factor that may have a significant influence on the structure of intertidal communities (Connell 1985; Hurlburt 1991; Minchinton and Scheibling 1993; Roegner and Mann 1995; Gosselin and Qian 1997; Hunt and Scheibling 1997; Osman and Whitlatch 2004). Gosselin and Qian (1997) reviewed the mortality of juveniles in benthic marine invertebrates and found that levels of early mortality across a variety of taxa frequently exceeded 90%. Furthermore, mortality was usually greatest within the first few days after settlement and tended to decrease with increasing age or size. Growth rates in early juveniles may therefore be important, individuals that grow more slowly remain vulnerable for longer thus reducing

their chances of survival (Gosselin and Qian 1997; Robles 1997; Osman and Whitlatch 2004).

The different processes that determine patterns of adult distribution and abundance are not independent and it has been suggested that levels of recruitment may determine the occurrence or strength of interspecific interactions (Underwood and Denley 1984; Roughgarden *et al.* 1988; Wootton 1993; Connolly and Roughgarden 1998). For example, variations in the settlement of potential competitors may have a major influence on the prevalence of competition (Underwood and Denley 1984). Also, the relative importance of different processes may differ at different spatial scales (Underwood and Chapman 1996), interspecifc interactions often becoming more important at local scales. Thus ecological communities may be considered as multiple response vectors, which are regulated by a variety of factors that interact at different spatial and temporal scales (Menge and Sutherland 1987). Understanding patterns in a given community therefore requires analysis of a wide range of variables influencing that community. This study therefore aims to determine what factors may be responsible for the observed differences in spatial distribution of *P.perna* and *M.galloprovincialis,* with implications for the impact of the invasion of *M.galloprovincialis* on *P.perna* populations.

1.2 Hypotheses and aims

Three general hypotheses may be invoked to explain the vertical distributions of these species:

1. *M.galloprovincialis* and *P.perna* settle differentially on the shore.
2. These species settle haphazardly but adults occur in certain zones because of zone-dependent differential post-settlement mortality.

3. *M.galloprovincialis* is able to exploit the higher levels of the shore where *P.perna* is scarce due to its increased tolerance to aerial exposure, while being competitively excluded from the low-shore by *P.perna.*

Studies were conducted at two sites, namely Plettenberg Bay and the Tsitsikamma National Park. Two locations were randomly chosen at each site as replicates. Within each location, the shore was divided into three zones or tidal heights. Specifically the aims of this study were the following:

1. to describe quantitatively the patterns of distribution and abundance of each species with respect to tidal height, location and site.
2. to monitor spatial and temporal variation in daily settlement of each species. This required morphological and genetic identification of early post-larval and juvenile stages of the three mussel species.
3. to monitor spatial and temporal variation in recruitment between months and seasons.
4. to determine whether there were species-specific differences in post-settlement mortality and juvenile growth with respect to tidal height, location and site.
5. to determine whether growth and mortality rates of adult *M.galloprovincialis* and *P.perna* differed among sites, locations or zones and if these patterns varied seasonally.

1.3 Study sites

Tides around the coast of South Africa are semi-diurnal, with tidal ranges of 2-2.5m over spring tides and 1m over neap tides (Field and Griffiths 1991). The south coast is classified

as warm-temperate (Brown and Jarman 1978), with mean monthly sea surface temperatures of 15°C in winter and 22°C in summer (Field and Griffiths 1991). The south and east coasts of South Africa are influenced by the Agulhas Current which flows in a south westerly direction down the coast. South of East London (Fig. 1.1a) the continental shelf begins to widen, deflecting the current off shore (Shannon 1989). As the shelf broadens, meanderings from the Agulhas current become more common. An important feature of the south coast is the formation of shear-edge eddies over the continental shelf (Lutjeharms 1981; Lutjeharms *et al.* 2003). The clockwise movement of water associated with these eddies results in nearshore currents that flow in the opposite direction to the Agulhas Current (Branch and Branch 1981; Shannon 1989). The region is also frequently subjected to discontinuous events of localised, wind-induced upwelling, which often results in sharp declines in sea temperature during summer (Schumann *et al.* 1982).

The two study sites, Plettenberg Bay (34°05'S; 23°19'E) and Storms River Mouth (33°1'S; 23°53'E) in the Tsitsikamma National Park, are located approximately 70km apart on the south coast of South Africa (Figs.1.1a-b). The chosen locations within each site are separated by a linear distance of 300-400m (Fig. 1.1c). Locations were divided into three vertical zones based on the distributions of mussels. Low-shore generally corresponded to the first 0.5-1.0m above low water mark at spring tide (LWS) and the lower limit of the mussel bed. This was followed by the mid-shore, which generally included the lower balanoid and upper balanoid regions. Above this zone was the high-shore, which also corresponded to the upper limit of the mussel bed. The high-shore zone in Plettenberg Bay was broader than in the Tsitsikamma National Park due to the abundance of *M.galloprovincialis* at this site.

Plettenberg Bay is one of a series of "half-heart", or log-spiral shaped bays found on the south coast (Branch and Branch 1981; Wooldridge 1988). It is characterised by a long stretch of beach that sweeps around the bay to the border of the Tsitsikamma National Park. Interspersed along the beach are patches of granite rocks, which are consequently considerably sand-swept. The two locations are situated on either side of a rocky outcrop near the middle of the bay at Lookout Rocks (Figs. 1.1 b-c). Location A will be referred to as Lookout Beach and location B as Beacon Isle. Both locations are exposed in terms of wave action, particularly to southeasterly swells, which are common on this coast. Two relatively small rivers open into the sea on either side of Lookout Rocks so that the two locations may occasionally be influenced by freshwater, but this effect will be minimal even after heavy rains.

The rocks at both locations are vertically inclined, but with areas of horizontal surface where most sampling was carried out. The lowest intertidal limit was initially at the sand-rock interface over spring tides. However, six months after sampling began (December 2000), a considerable amount of sand was swept away from Beacon Isle. As a result, the lowest limit at this location was then at LWS for the remainder of the study period. Incidentally, this removal coincided with an equally large deposit of sand on the low-shore at Lookout Beach, although this was only temporary.

The two locations in Plettenberg Bay do not conform to the general patterns of south coast zonation that have been described (Stephenson and Stephenson 1972; Branch and Branch 1981). Mussels and barnacles are the dominant space-occupiers. Mussel cover is generally more abundant in the lower zones and decreases towards the high-shore where barnacles (*Chthamalus dentatus*) are particularly abundant. Barnacles are also present in relatively

large numbers on the mid-shore, but do not occupy much primary space on the low-shore. However, large settlements were occasionally observed as epibionts on mussels in this zone. Other common organisms include algae (*Ulva sp.*), polychaetes and a variety of small limpets, particularly *Scutellastra granularis*. There are also a number of organisms that prey on mussels, such as whelks (*Burnupena spp.*), dogwhelks (*Nucella spp.*), shore crabs, gulls and Oyster Catchers. The mussel *C.meridionalis* is abundant on the low-shore at Lookout Beach but not at Beacon Isle.

The Storms River Mouth area is located virtually in the centre of the Tsitsikamma National Park (Fig. 1.1b), which covers a 220km stretch of open coast and extends 5km offshore (Robinson and de Graaff 1994). This site is referred to as Tsitsikamma. The majority of the coastline within the park is formed by sandstone rocks and is virtually beachless (Robinson and de Graaff 1994). Upwelling off the Tsitsikamma coast is the most intense and extensive found on the south coast, creating a region of high primary productivity. Caused by easterly winds, upwelling events are produced on the south side of rocky promontories (such as at Jeffreys Bay, Fig. 1.1a) and upwelled water tends to drift westwards (Schumann *et al.* 1982). As a result, upwelling is usually more intense in Tsitsikamma than in Plettenberg Bay, and depending on the strength and duration of easterly winds may not influence Plettenberg Bay at all.

The two locations are situated within a slight embayment and may therefore also receive some protection from southwesterly winds. Location C is referred to as Sandbaai and location D as Driftwood Bay. Both were considered as moderately exposed to exposed in terms of wave action based on the presence of a well-developed *Scutellastra cochlear* (previously *Patella cochlear*) belt on the low-shore. This limpet reaches greatest densities

under moderate wave action but tends to be replaced by mussels where wave action is severe (Branch 1985). These locations may be periodically influenced by freshwater input from the Storms River.

The rocks at both locations are more gently inclined than in Plettenberg Bay. The locations are situated on stretches of rock that project into the sea, and consequently border on relatively deep sea, so that the lowest intertidal limit is at LWS. Despite the presence of a small beach at Sandbaai, these rocks are not sand influenced at all. Unlike Plettenberg Bay, the general patterns of community structure at this site are characteristic of south coast rocky shores (Branch and Branch 1981). The limpet *S.cochlear* and encrusting coralline algae dominate the low-shore with mussels occurring in isolated patches. Branching red algae (*Plocamium sp.*) and articulated coralline algae often form large growths as epibionts on these mussel patches. On the mid-shore, mussels are the dominant space occupiers forming a relatively continuous bed. Interspersed are patches of algae (including *Gelidium pristoides*) and also the barnacle *Octomeris angulosa*. In the high zone, mussels are again found in isolated patches, amongst patches of algae and clumps of *O.angulosa.* Volcano barnacles (*Tetraclita serrata*) are also scattered throughout the upper mussel levels. The most abundant organism in this zone is the gastropod snail, *Littorina africana*, which extends its distribution virtually to the landward edge of these rocks. Other common organisms are Dwarf cushion stars (*Patiriella exigua*), winkles (*Oxystele spp.*) and limpets (particularly *S.granularis*). Organisms that may feed on mussels include whelks (*Burnupena spp.*), dogwhelks, shore crabs, octopus and starfish. The mussel *C.meridionalis* does not occur at this site.

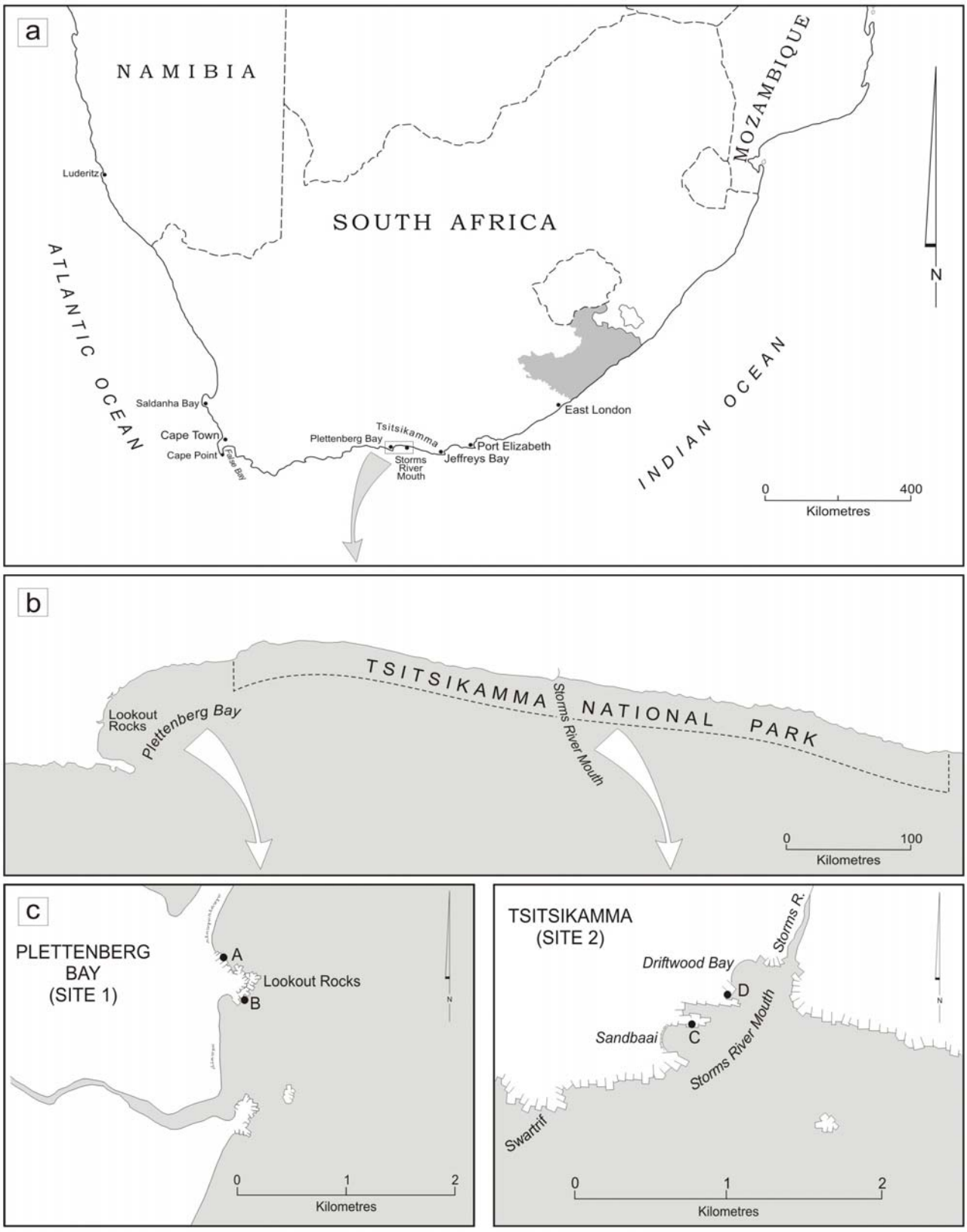


Figure 1.1a-c: a) Position of study sites on the south coast of South Africa; b) coastal topography of Plettenberg Bay and Tsitsikamma; c) position of locations within each site.

Chapter 2

Patterns of distribution and abundance of *Perna perna*, *Mytilus galloprovincialis* and *Choromytilus meridionalis* in Plettenberg Bay and Tsitsikamma

2.1 Introduction

The patterns of distribution and abundance of organisms vary both spatially and temporally in response to a variety of physical and biological factors (Menge and Sutherland 1987). Identifying and explaining these patterns has been the focus of community ecology for the past few decades. However, before any attempt at explanation can be made, it is necessary to describe quantitatively the patterns observed i.e. to determine the accuracy of initial observations using proper sampling methods (Connell 1974; Underwood and Chapman 1996; Underwood *et al.* 2000).

One of the most widely studied scales of spatial variation in community structure is that associated with vertical height in rocky intertidal habitats (Lewis 1964; Dayton 1971; Stephenson and Stephenson 1972; Underwood 1981; Menge 1991; Bustamante *et al.* 1997; Menconi *et al.* 1999). This is because intertidal systems encompass a range of environmental conditions from fully aquatic to fully terrestrial over a relatively small and easily observed scale of a few meters (Underwood 2000; Harley and Helmuth 2003). Rocky shores also support a rich variety of sessile and mobile organisms, which often occur in different zones along this environmental gradient (Menconi *et al.* 1999).

Community structure may also vary horizontally at a range of spatial scales, from local or within-site variation to differences between geographic regions (Bustamante *et al.* 1997; Connolly and Roughgarden 1998; Archambault and Bourget 1999; Menconi *et al.* 1999; Menge *et al.* 1999; McKindsey and Bourget 2000). Frequently, variability in communities at smaller scales cannot be fully explained without incorporating the processes that may be operating at larger scales (Menge *et al.* 1999). Documenting the scales at which abundances

of organisms differ therefore focuses attention on the relative importance of the different processes that determine these patterns (Underwood and Chapman 1996).

The aim of this chapter is to describe the spatial patterns of distribution and abundance of mussels at locations in Plettenberg Bay and Tsitsikamma. Towards the end of the entire study period it was noted that *M.galloprovincialis* appeared to have increased in abundance on the low-shore in Plettenberg Bay and the mid-shore in Tsitsikamma. To examine whether this was true the analysis was repeated in 2004. *C.meridionalis* is also found in large numbers on the low-shore at one location in Plettenberg Bay and has therefore been included.

2.2 Materials and methods

The study sites are described in detail in Chapter 1. Samples were initially taken over a single spring tide in April 2001 virtually a year after the study began. Unfortunately, there was no opportunity to do this earlier due to limited time over spring tides during which project sampling was being done. Ideally this analysis should have been undertaken at the start of the study, however the results appear to support initial observations suggesting that species abundance had not changed dramatically during this time. Samples were taken again in February 2004, nearly three years later. The same method of sampling was used. Several aspects of population structure were examined, including cover, density, maximum length and recruit/adult correlations.

Percentage cover

Percentage cover of *P.perna*, *M.galloprovincialis* and *C.meridionalis* was measured at each location in Plettenberg Bay and Tsitsikamma. Ten quadrats (25×25cm) were placed

randomly on the rocks in each zone and the percentage cover of each species was estimated visually. Any space without mussels was classified as "other" as this space was either bare or occupied by other organisms which differed depending on the zone and the location. Results from individual quadrats within each zone were plotted to give an indication of the patchiness of mussel cover.

Density

Mussel density was measured by placing three quadrats (25×25cm) in areas of 100% mussel cover in each zone at each location. All the mussels within each quadrat were removed. In the first year all mussels ≥2mm were identified to species and their lengths measured to the nearest millimeter using Vernier calipers. Mussels of each species were arranged into 10mm size groups and those 2-9.9mm in length were classified as recruits.

Due to time constraints, individual mussels were not measured in 2004 and recruits were also excluded. As a result, cover and density were the only population parameters measured in this year. Mussels of each species were sorted into size groups (only measured when necessary) and then counted. Densities were converted to density per m^2. For comparative purposes only the density of mussels ≥10mm were analysed for both years. Mussels 10-29mm may be considered as subadults and those ≥30mm as adults (Lawrie and McQuaid 2001). This classification is supported by the fact that *P.perna* and *M.galloprovincialis* appear to become sexually mature at sizes of 20-30mm (unpub. data). Therefore for adult/recruit correlations, adult density only included mussels ≥30mm.

Analyses

It is clear from the graphs of percentage cover and average density that the distribution of *C.meridionalis* was restricted to the low-shore at Lookout Beach in Plettenberg Bay (Figs. 2.1 a-f; p.26 and Figs. 2.2 a-f; p.27). It was therefore excluded from statistical analyses with *P.perna* and *M.galloprovincialis*. However, low-shore cover and density at Lookout Beach were compared between all three species using One-way Analysis of Variance (ANOVA) with species as a fixed factor. The abundance of these species was also compared between years using a factorial ANOVA with year as a random factor.

All aspects of population structure (cover, density, recruit density and maximum lengths) were analysed using a mixed model ANOVA with a combination of nested and factorial factors (Statistica 6.0, Advanced General Linear Model). There were four main factors, namely site, location, zone and species. Site, zone and species were fixed and were orthogonal to each other (terminology from Menconi *et al.* 1999), while location was random and nested within site, and was orthogonal to zone and species. For the between-year analyses, year was included as a fifth factor which was random and orthogonal to all other factors. Significant results were examined using Newman-Keuls multiple range tests ($\alpha = 0.05$). Variations in the maximum sizes of mussels were analysed using the maximum lengths of the ten largest individuals (quadrats pooled). The relationship between recruit and adult density was investigated using correlation analysis. The maximum length and percentage cover data did not require transformation. However, the density data were significantly heterogeneous with non-normal distributions, and sample sizes were small (n=3). Data were therefore transformed to the square root (Underwood 1997). All post-hoc results for density were plotted using transformed data.

2.3 Results

Population structure in 2001

1. Percentage cover

Figures 2.1 and 2.2 a-f (p.26-27) show the percentage cover of mussels in each zone at locations in Plettenberg Bay and Tsitsikamma respectively. General mussel cover appears to have been greater in Plettenberg Bay than in Tsitsikamma. Mussel cover was also quite patchy with successive quadrats sometimes differing by as much as 80% in cover. On the low and high-shores in Tsitsikamma, cover was generally low as mussels only occur in isolated clumps or patches in these zones. Cover of *C.meridionalis* was greater than that of *P.perna* and *M.galloprovincialis* on the low-shore at Lookout Beach in Plettenberg Bay and this difference was found to be significant ($F=4.95$; $p=0.01$). However, it was not apparent in the upper zones at this location or at Beacon Isle, and was absent from Tsitsikamma. It is also clear that cover of the *P.perna* and *M.galloprovincialis* was very low on the low-shore at Lookout Beach compared to Beacon Isle, which probably reflects the presence of *C.meridionalis.*

Results of the analysis of percentage cover are given in Table 2.1 (p.28). The difference in mussel cover between sites was significant ($p<0.05$), with greater mussel cover in Plettenberg Bay. Differences in the vertical zonation patterns of these species were also apparent, as there was a significant interaction between zone and species ($p=0.02$). Cover of *P.perna* was greatest on the mid-shore and was significantly lower on the high-shore than in the lower zones (Fig. 2.3; p.28). *M.galloprovincialis* on the other hand, had a significantly greater cover in the upper two zones than on the low-shore. Within zones, cover of *P.perna* was significantly greater than that of *M.galloprovincialis* on the low-

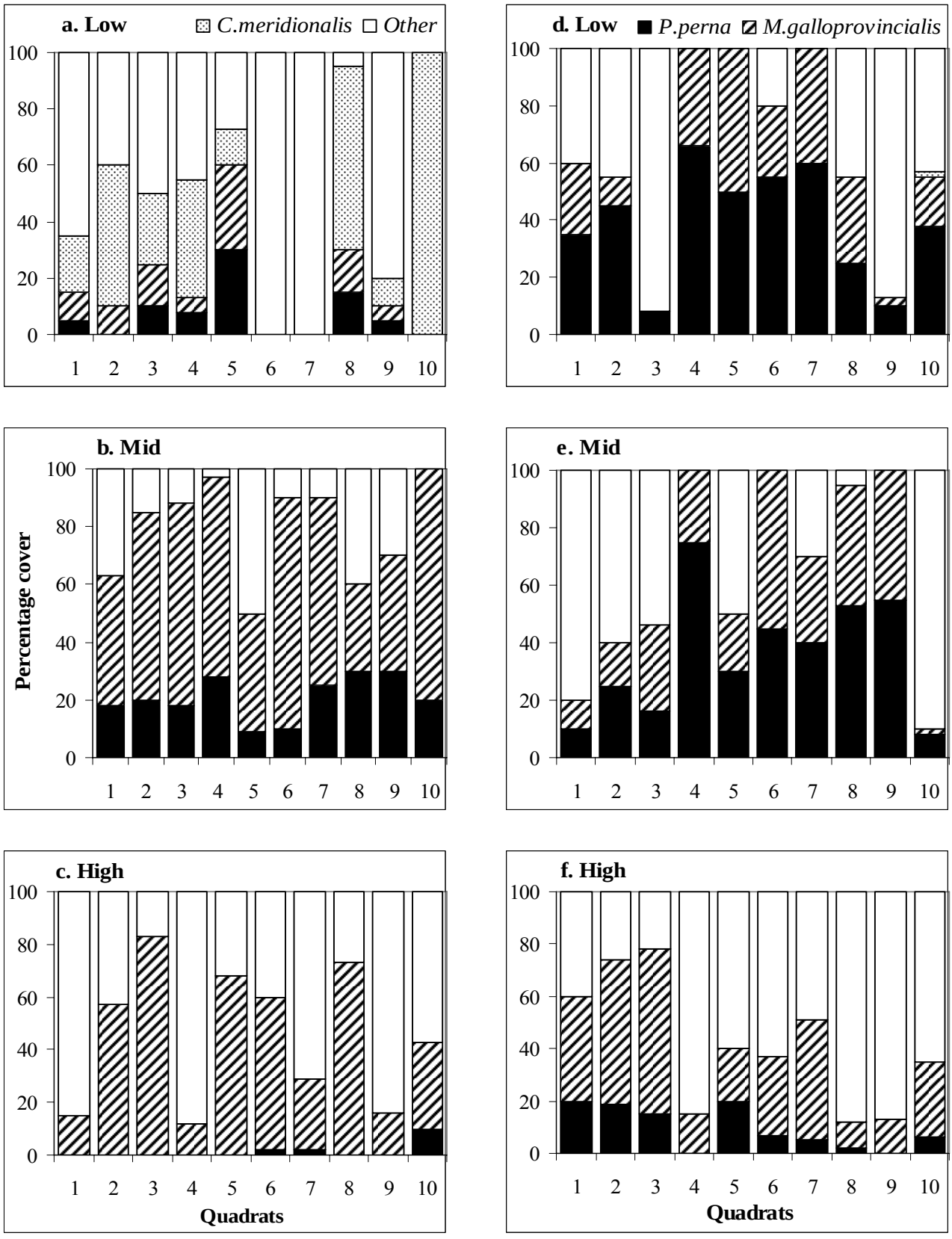


Figure 2.1 a-f: Percentage cover of *P.perna*, *M.galloprovincialis* and *C.meridionalis* in different zones at Lookout Beach (a-c) and Beacon Isle (d-f) in Plettenberg Bay in 2001

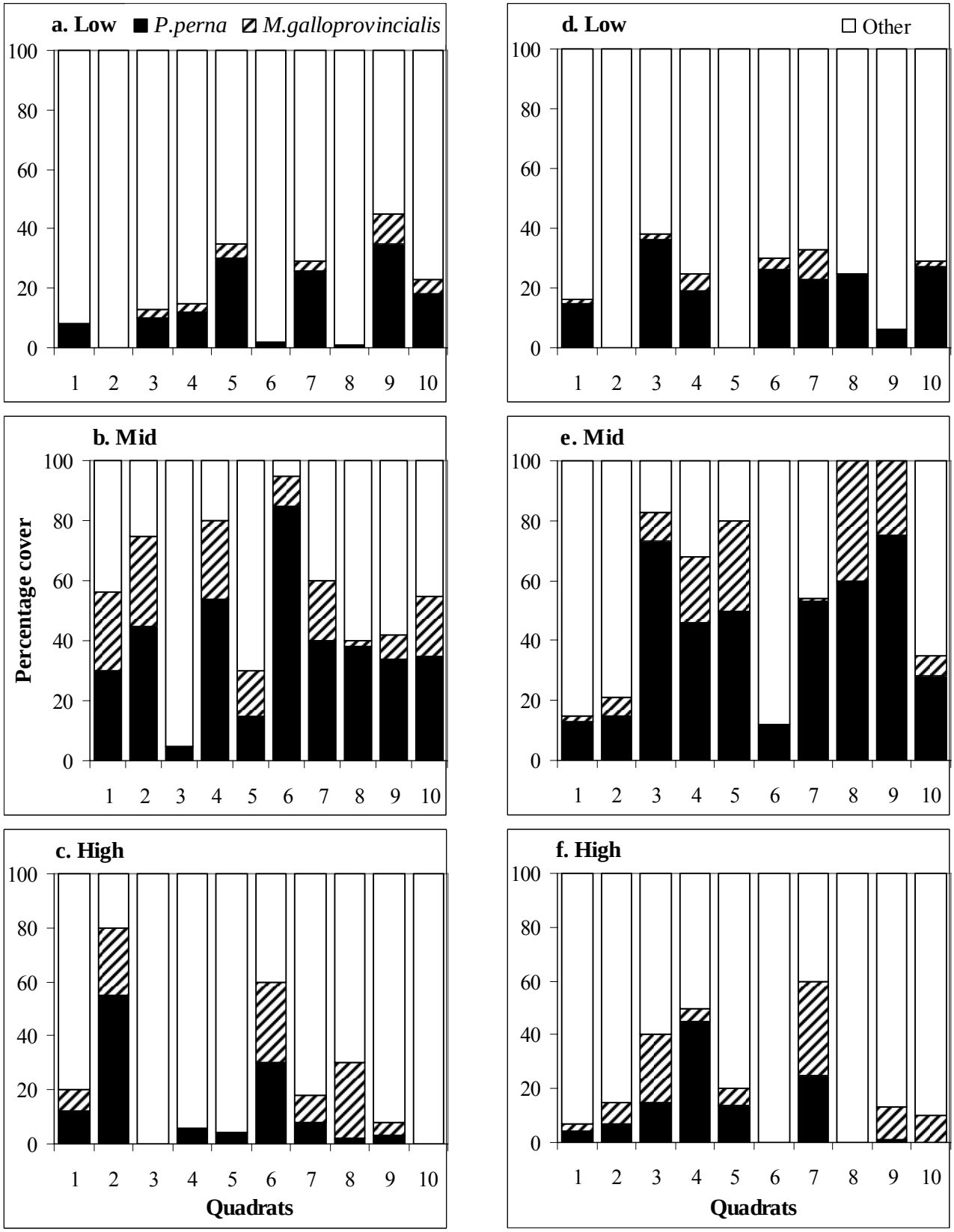


Figure 2.2 a-f: Percentage cover of *P.perna* and *M.galloprovincialis* in different zones at Sandbaai (a-c) and Driftwood Bay (d-f) in Tsitsikamma in 2001

Table 2.1: Results of mixed model ANOVA on the percentage cover of the mussels *P.perna* and *M.galloprovincialis* in 2001. Significant effects are marked with an asterisk (Df = degrees of freedom)

	Effect	**Df**	**MS**	**F**	**p**
site*	Fixed	1	5645.4	19.328	0.048*
zone	Fixed	2	7036.3	5.096	0.079
species	Fixed	1	1.1	0.000	0.987
site×zone	Fixed	2	46.5	0.034	0.967
site×species	Fixed	1	10666.7	3.621	0.197
zone×species*	Fixed	2	3822.8	11.786	0.021*
site×zone×species	Fixed	2	1618.9	4.991	0.082
location (site)	Random	2	292.1	0.073	0.931
location (site)×zone	Random	4	1380.7	4.257	0.095
location (site)×species*	Random	2	2945.8	9.082	0.033*
location (site)×zone×species	Random	4	324.3	1.398	0.236
Error		216	232.0		

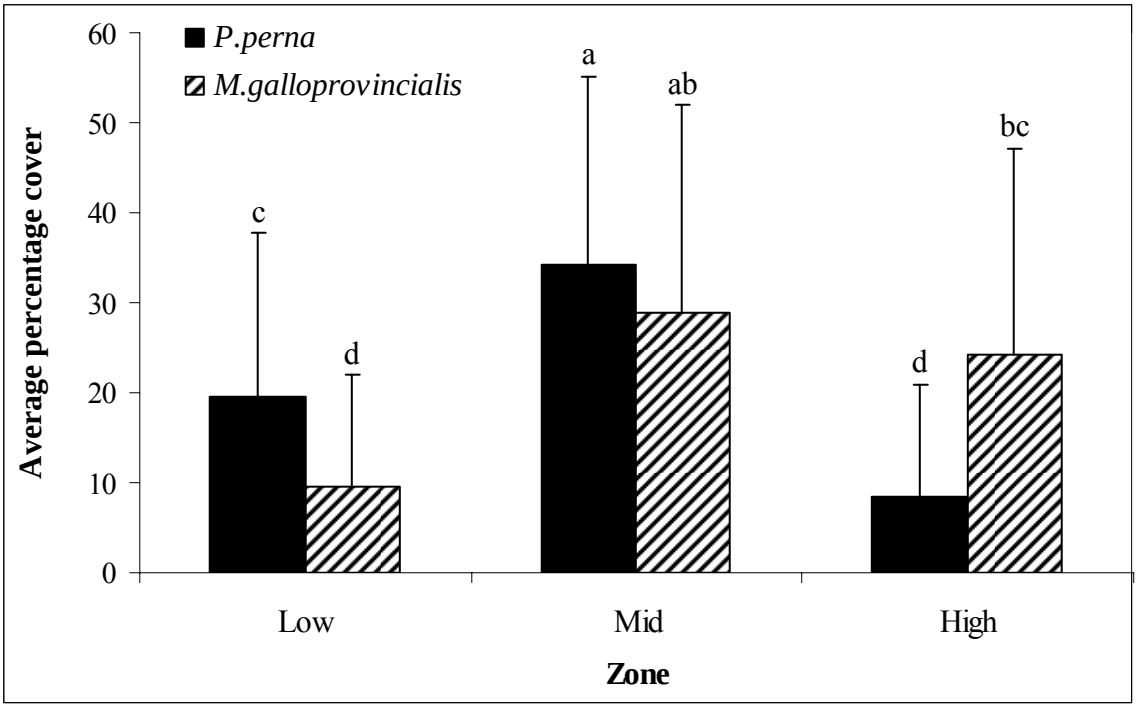


Figure 2.3: Post-hoc comparison of the interaction between zone and species on percentage cover in 2001. Similar letters indicate homogeneous groups. Error bars represent standard deviations

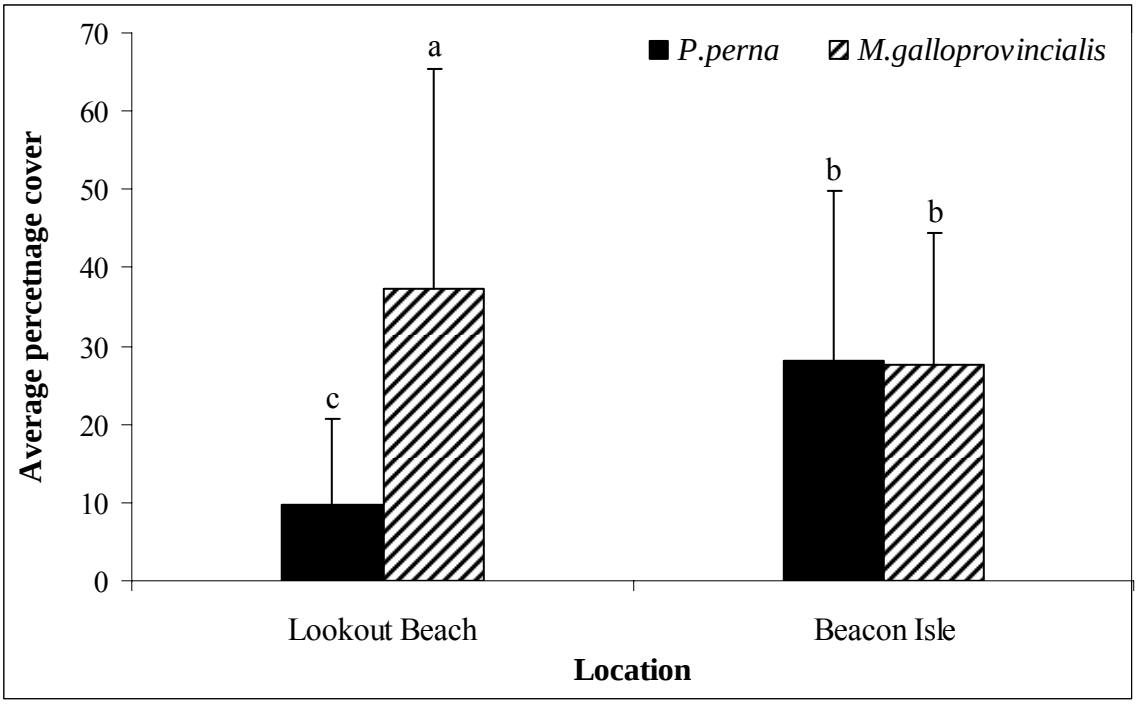


Figure 2.4: Post-hoc results of the interaction between location (site) and species on percentage cover in Plettenberg Bay in 2001. Error bars represent standard deviations

shore, while cover of *M.galloprovincialis* was significantly greater on the high-shore. There was no significant difference between species on the mid-shore. It should be noted, that cover of *P.perna* was much greater than *M.galloprovincialis* in this zone in Tsitsikamma while the opposite pattern occurred at Lookout Beach. In fact the only location where cover of the two species was similar was on the mid-shore at Beacon Isle (average covers of 36% and 27% for *P.perna* and *M.galloprovincialis* respectively).

Cover of the two species also varied between locations within sites as indicated by the significant interaction between location (site) and species ($p=0.03$). Post-hoc comparison revealed differences between locations in Plettenberg Bay (Fig. 2.4; p.29). Cover of *M.galloprovincialis* was significantly greater at Lookout Beach than at Beacon Isle, while the opposite was true for *P.perna.* However, while cover of *P.perna* was significantly lower than that of *M.galloprovincialis* at Lookout Beach, both species had similar cover at Beacon Isle. In Tsitsikamma, *P.perna* had significantly greater cover than *M.galloprovincialis* at both locations with no differences in the cover of each species between locations.

2. Size structure and density

The size distribution of mussels in Plettenberg Bay was unimodal irrespective of zone or species (Fig. 2.5 a-f; p.31). There was a peak in the average density of mussels in the smallest size group after which density progressively decreased with increasing size. In Tsitsikamma, however, there was no evidence of a negative relationship between density and size (Fig. 2.6 a-f; p.32). The size distribution of *P.perna* was also unimodal at this site, but the peak occurred amongst adult mussels 30-50mm in length. This trend was only obvious for *M.galloprovincialis* on the mid-shore at Sandbaai and the high-shore at

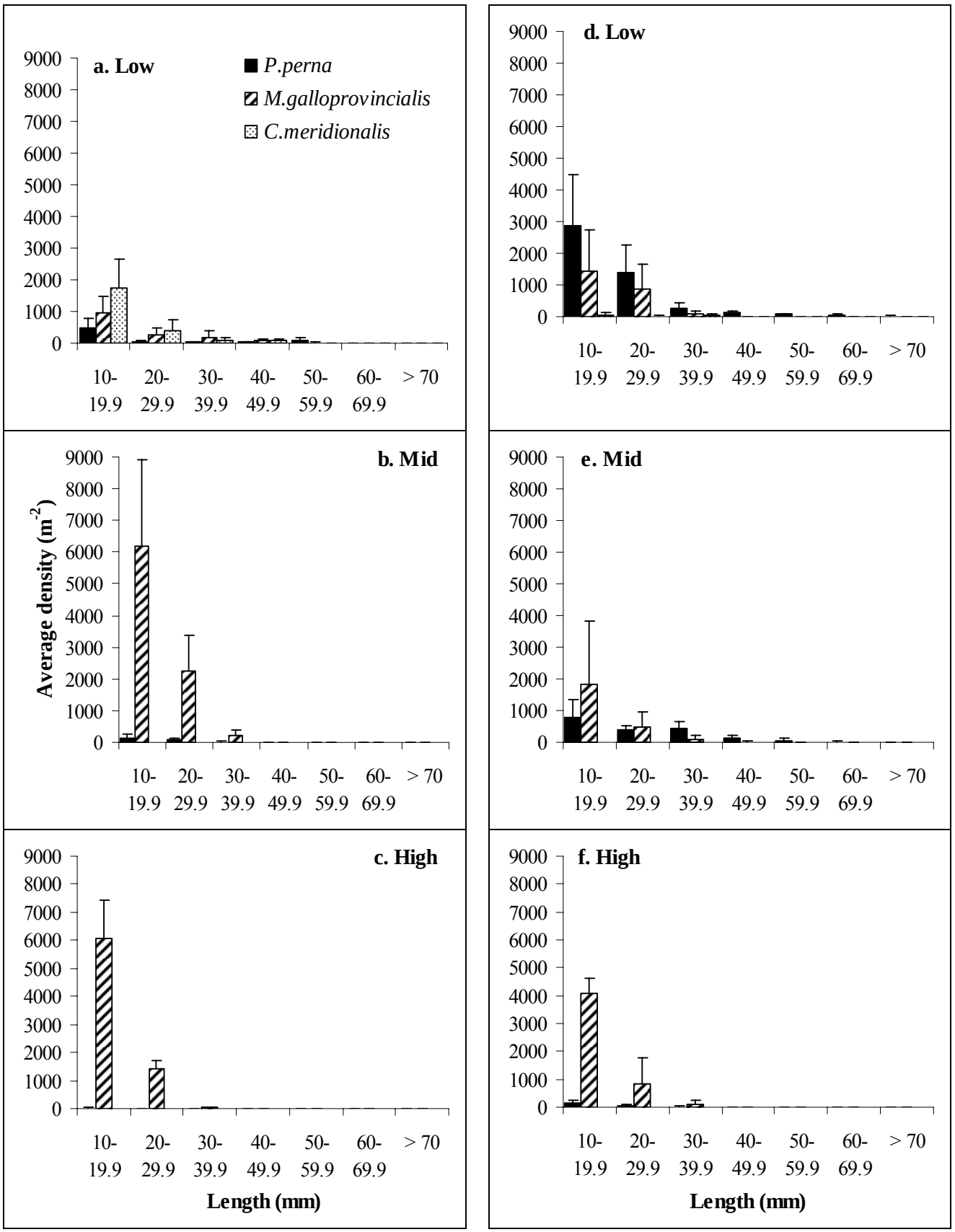


Figure 2.5 a-f: Size distributions of *P.perna, M.galloprovincialis* and *C.meridionalis* in different zones at Lookout Beach (a-c) and Beacon Isle (d-f) in Plettenberg Bay in 2001. Error bars represent standard deviations

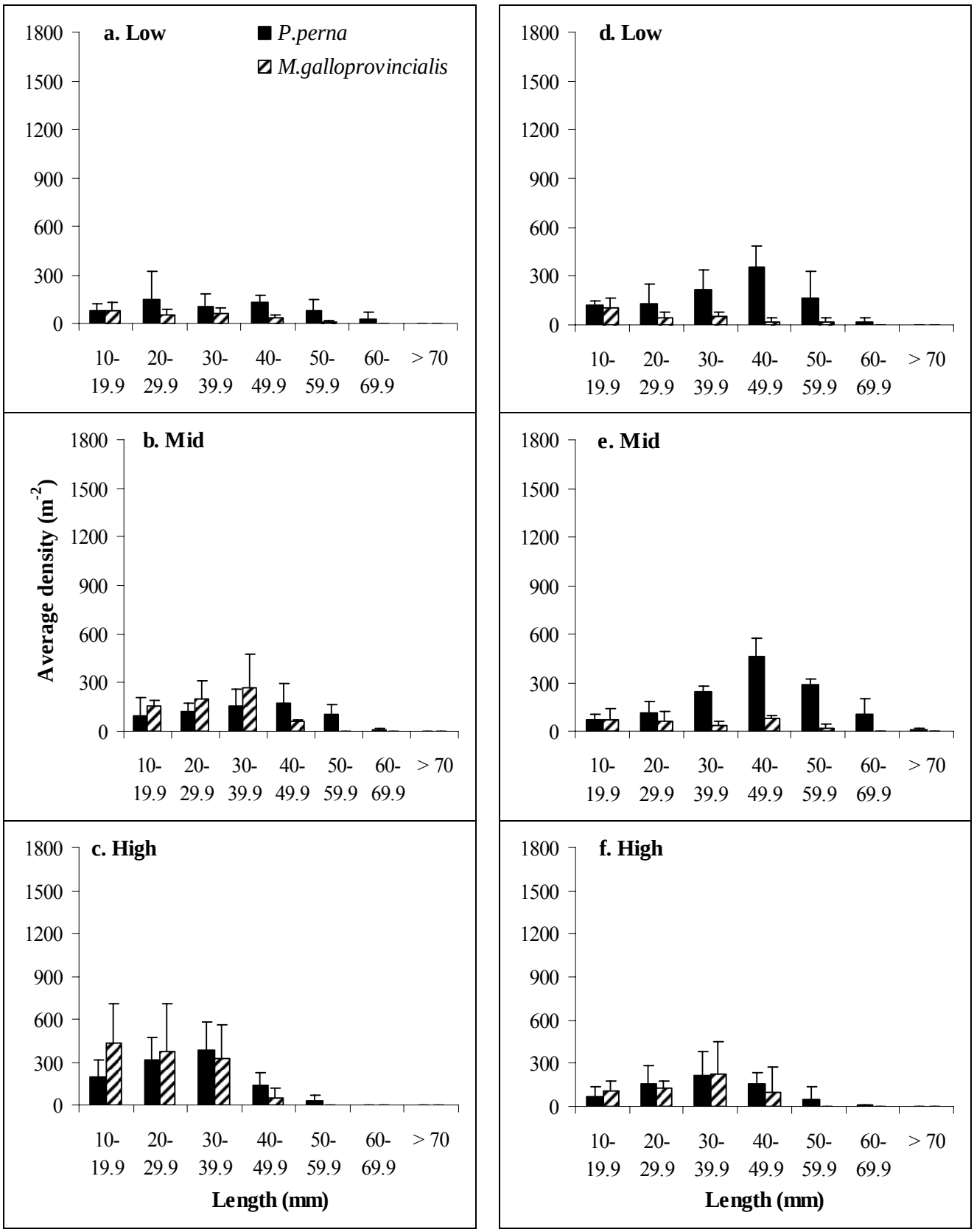


Figure 2.6 a-f: Size distributions of *P.perna* and *M.galloprovincialis* in different zones at Sandbaai (a-c) and Driftwood Bay (d-f) in Tsitsikamma in 2001. Note that the y-axis values are lower than those for Plettenberg Bay. Error bars represent standard deviations

Driftwood Bay where there was a slight peak in the density of mussels 30-40mm in length. On the high-shore at Sandbaai where density was greatest, there was a peak amongst the smaller size groups and where density was very low there was no peak at all. It should be noted that when recruits were included as a size group, the distribution of *P.perna* at this site became bimodal due to the appearance of a second, similar-sized peak amongst recruits. All distributions of *M.galloprovincialis* became unimodal with a peak in recruit density. The size distribution in Plettenberg Bay remained unimodal but the peak was then further to the left amongst recruits.

The density patterns of *C.meridionalis* in Plettenberg Bay mirrored those observed for percentage cover. This species was abundant on the low-shore at Lookout Beach but was absent from the upper zones at this location, while some smaller mussels were found in very low numbers on the low-shore only at Beacon Isle. No significant differences were found between the density of the three species in this zone at Lookout Beach ($F=2.23$; $p=0.19$). Analysis of the densities of *P.perna* and *M.galloprovincialis* revealed that there was a significant difference in density between sites (Table 2.2; p.34; $p=0.01$), with average densities of $2923m^{-2}$ and $696m^{-2}$ in Plettenberg Bay and Tsitsikamma respectively. Density also varied with height on the shore with a different pattern between species ($p=0.03$). Post-hoc comparison showed that the density of *P.perna* decreased with increasing height on the shore, and was significantly greater on the low-shore than on the high-shore (Fig. 2.7; p.34). *M.galloprovincialis* displayed the opposite pattern as density increased with height on the shore, and was significantly greater in the upper zones than on the low-shore. Density of *M.galloprovincialis* was also significantly greater than that of *P.perna* in the upper two zones, but was slightly lower on the low-shore although this difference was not significant.

Table 2.2: Results of mixed model ANOVA on the density of *P.perna* and *M.galloprovincialis* in 2001

	Effect	**Df**	**MS**	**F**	**p**
site*	Fixed	1	7324.83	78.543	0.012*
zone	Fixed	2	137.82	0.2377	0.799
species	Fixed	1	3534.79	1.5365	0.341
site×zone	Fixed	2	110.94	0.1913	0.833
site×species	Fixed	1	8332.16	3.6218	0.197
zone×species*	Fixed	2	2816.21	10.0911	0.027*
site×zone×species	Fixed	2	1504.72	5.3918	0.073
location (site)	Random	2	93.26	0.0358	0.965
location (site)×zone	Random	4	579.93	2.078	0.248
location (site)×species*	Random	2	2300.58	8.2435	0.038*
location (site)×zone×species	Random	4	279.08	2.263	0.076
Error		48	123.32		

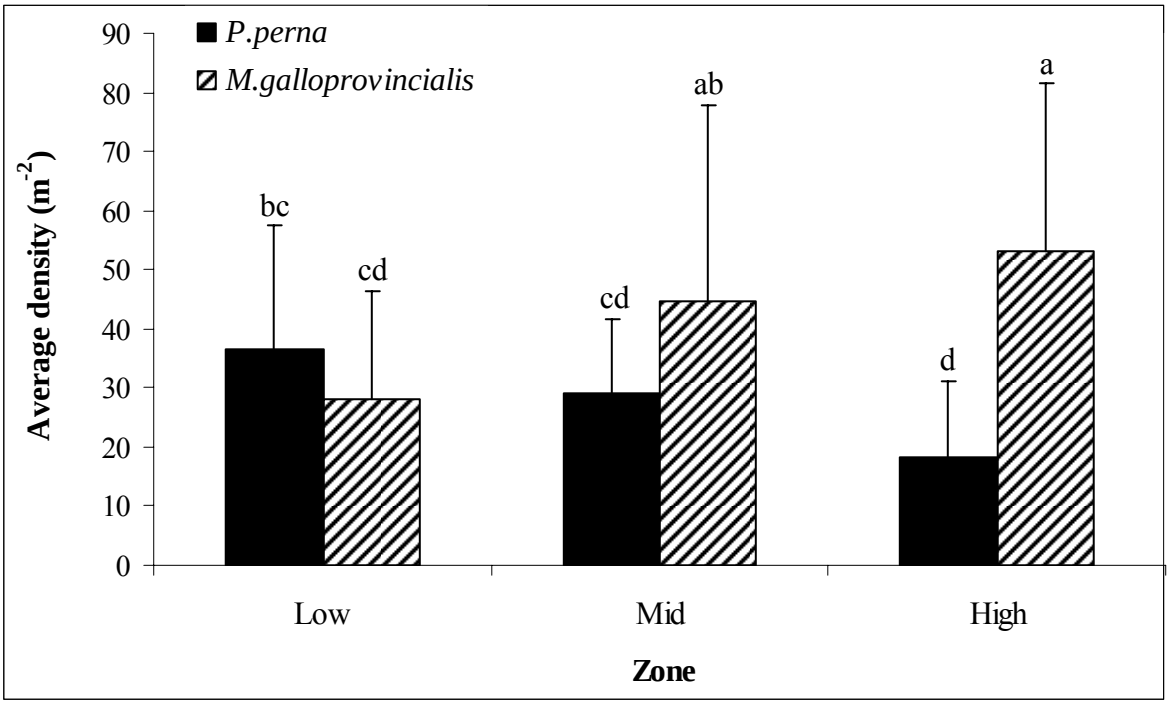


Figure 2.7: Post-hoc comparison of the interaction between zone and species on mussel density in 2001. Error bars represent standard deviations

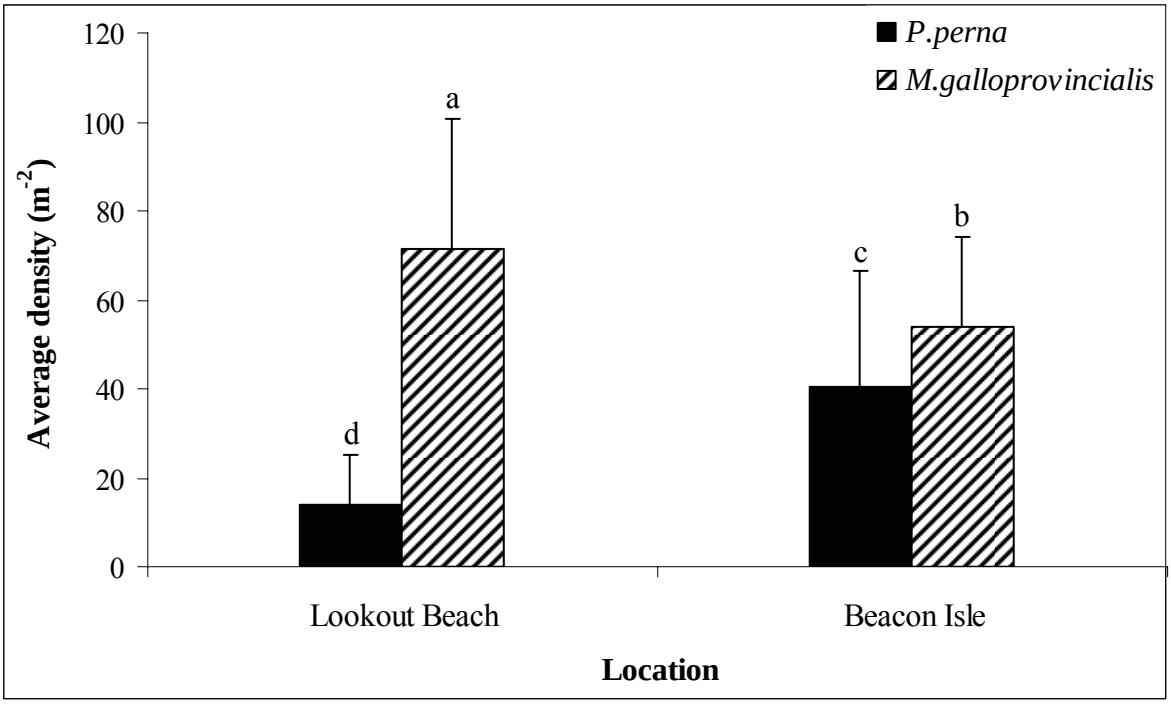


Figure 2.8: Post-hoc results of the interaction between location (site) and species on the density of mussels at locations in Plettenberg Bay in 2001. Error bars represent standard deviations

As with cover, the densities of these species also varied between locations within sites ($p=0.04$). Post-hoc comparison revealed that there were no differences in the density of *P.perna* and *M.galloprovincialis* in Tsitsikamma regardless of location. However, in Plettenberg Bay the density of *M.galloprovincialis* was significantly greater at Lookout Beach than at Beacon Isle, while the opposite was true for *P.perna* (Fig. 2.8; p.35). *M.galloprovincialis* also had a significantly greater density than *P.perna* at both locations.

3. Recruit density and adult/recruit correlations

The patterns that emerged from the analysis of recruit density were similar to those observed for the density of mussels ≥10mm (Table 2.3; pg.37). Site had a highly significant effect ($p<0.01$), and average recruit density in Plettenberg Bay was more than six times that in Tsitsikamma ($2636m^{-2}$ and $362m^{-2}$ respectively). Recruit density of *P.perna* and *M.galloprovincialis* varied significantly with height on the shore ($p=0.02$) and this pattern was similar between sites and locations. Density of *M.galloprovincialis* recruits increased with increasing tidal height and was significantly greater on the high-shore than in the lower zones, while the density of *P.perna* recruits decreased with increasing tidal height and was significantly greater on the low-shore than on the high-shore (Fig. 2.9; p.37). There were, however, no significant differences in recruit density between species in the lower zones, while *M.galloprovincialis* had a significantly greater density of recruits than *P.perna* on the high-shore.

The relationship between recruit and adult density at different tidal heights was examined at each site (Table 2.4; p.38). Significant positive correlations were found between adults and recruits of *M.galloprovincialis* on the low and mid-shore in Tsitsikamma. Correlations were also significant for *P.perna* on the high-shore at both sites and on the low-shore in

Table 2.3: Results of mixed model ANOVA on the density of recruits (2-9.9mm) of *P.perna* and *M.galloprovincialis* in 2001

	Effect	**Df**	**MS**	**F**	**p**
site	Fixed	1	13831.4	106.244	0.009*
zone	Fixed	2	347.22	0.588	0.597
species	Fixed	1	2844.76	4.845	0.159
site×zone	Fixed	2	31.55	0.053	0.949
site×species	Fixed	1	1617.27	2.754	0.239
zone×species	Fixed	2	2540.75	11.622	0.022*
site×zone×species	Fixed	2	1462.90	6.692	0.053
location (site)	Random	2	130.18	0.136	0.878
location (site)×zone	Random	4	590.72	2.702	0.179
location (site)×species	Random	2	587.14	2.686	0.182
location (site)×zone×species	Random	4	218.61	1.959	0.116
Error		48	111.59		

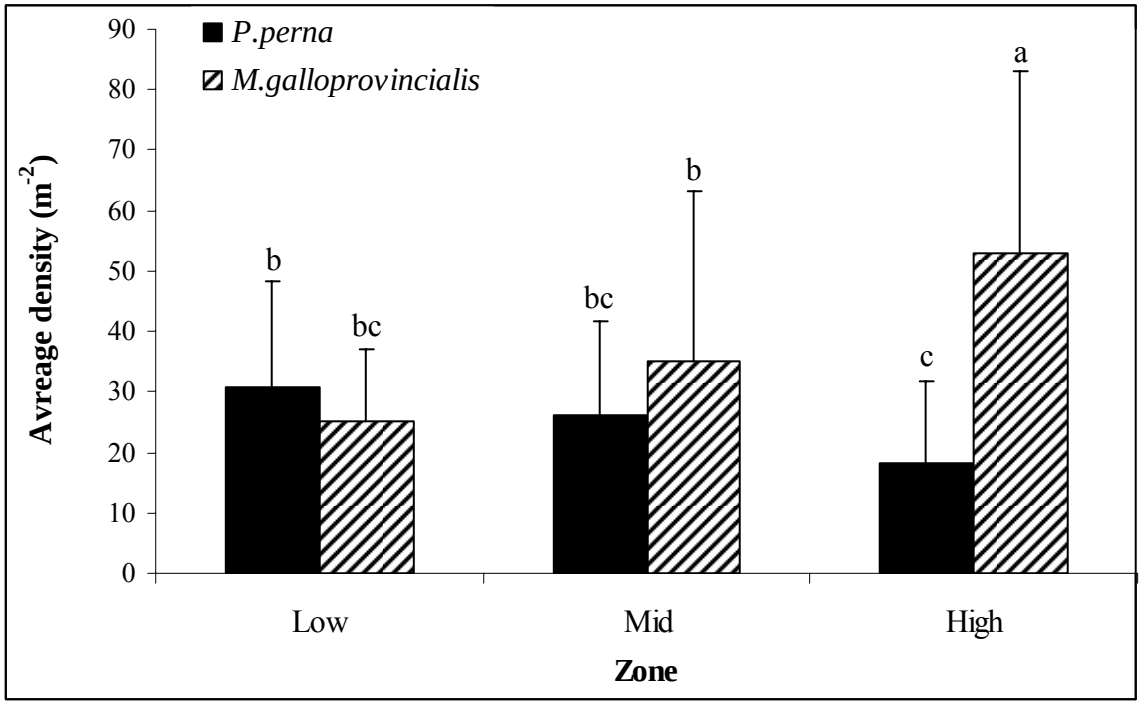


Figure 2.9: Post-hoc results of the interaction between zone and species on the density of recruits in 2001. Error bars represent standard deviations

Table 2.4: Pearsons Product Moment Correlations between recruit and adult densities of *P.perna* and *M.galloprovincialis* in each zone at each site (locations pooled; n = 6). Correlations that were significant after Bonferroni correction are marked with an asterisk

Species	**Zone**	**Site**	
		Plettenberg Bay	**Tsitsikamma**
P. perna	Low	r = 0.43 p = 0.39	r = 0.98* p < 0.001
	Mid	r = -0.28 p = 0.59	r = 0.22 p = 0.68
	High	r = 0.99* p < 0.001	r = 0.87 p = 0.03
M. galloprovincialis	Low	r = -0.14 p = 0.79	r = 0.9 p = 0.01
	Mid	r = 0.18 p = 0.73	r = 0.85 p = 0.03
	High	r = 0.49 p = 0.32	r = 0.66 p = 0.16

Table 2.5: Results of mixed model ANOVA on the maximum lengths of *P.perna* and *M.galloprovincialis* in 2001

	Effect	**Df**	**MS**	**F**	**p**
site	Fixed	1	7752.1	4.89	0.16
zone*	Fixed	2	5357.7	12.86	0.02*
species	Fixed	1	4690.5	2.74	0.24
site×zone	Fixed	2	2811.5	6.75	0.05
site×species	Fixed	1	1470.1	0.86	0.45
zone×species*	Fixed	2	2304.4	26.00	0.01*
site×zone×species*	Fixed	2	1103	12.45	0.02*
location (site)	Random	2	1585.6	0.78	0.54
location (site)×zone	Random	4	416.6	4.70	0.08
location (site)×species*	Random	2	1714.6	19.35	0.01*
location (site)×zone×species*	Random	4	88.6	5.83	<0.001*
Error		216	15.2		

Tsitsikamma. These results indicate that where adult density is low, recruit density is low and that within mixed populations this effect may even be species-specific.

4. Maximum lengths

Analysis of the mean maximum lengths of *P.perna* and *M.galloprovincialis* (Table 2.5; p.38) showed that there was a significant interaction between site, zone and species ($p=0.02$) and also a highly significant interaction between location (site), zone and species ($p<0.001$). In Plettenberg Bay mean maximum length (MML) of *P.perna* decreased significantly with increasing tidal height at both locations (Fig. 2.10a; p.40), with a MML of only 8mm on the high-shore at Lookout Beach, indicating a scarcity of adults in this zone. MML of *M.galloprovincialis* also decreased with increasing tidal height at Lookout Beach, but not at Beacon Isle where MML was greatest on the mid-shore (Fig. 2.10b; p.40). Both species attained greater maximum sizes at Beacon Isle than at Lookout Beach with the exception of *M.galloprovincialis* on the low-shore where MML of this species was significantly greater at Lookout Beach than at Beacon Isle. *P.perna* attained significantly larger sizes than *M.galloprovincialis* on the low-shore, while the opposite was true on the high-shore and this was irrespective of location. However, on the mid-shore MML of *P.perna* was greater at Beacon Isle while MML of *M.galloprovincialis* was greater at Lookout Beach.

In Tsitsikamma, MML of *P.perna* decreased with increasing tidal height at Sandbaai, with a significant difference between the low and high-shores (Fig. 2.11a; p.40). However, there was no significant difference in MML of *M.galloprovincialis* between zones at this location (Fig. 2.11b; p.40). At Driftwood Bay, MML of both species was significantly greater on the mid- shore than in the other two zones. Maximum size of *P.perna* was also significantly

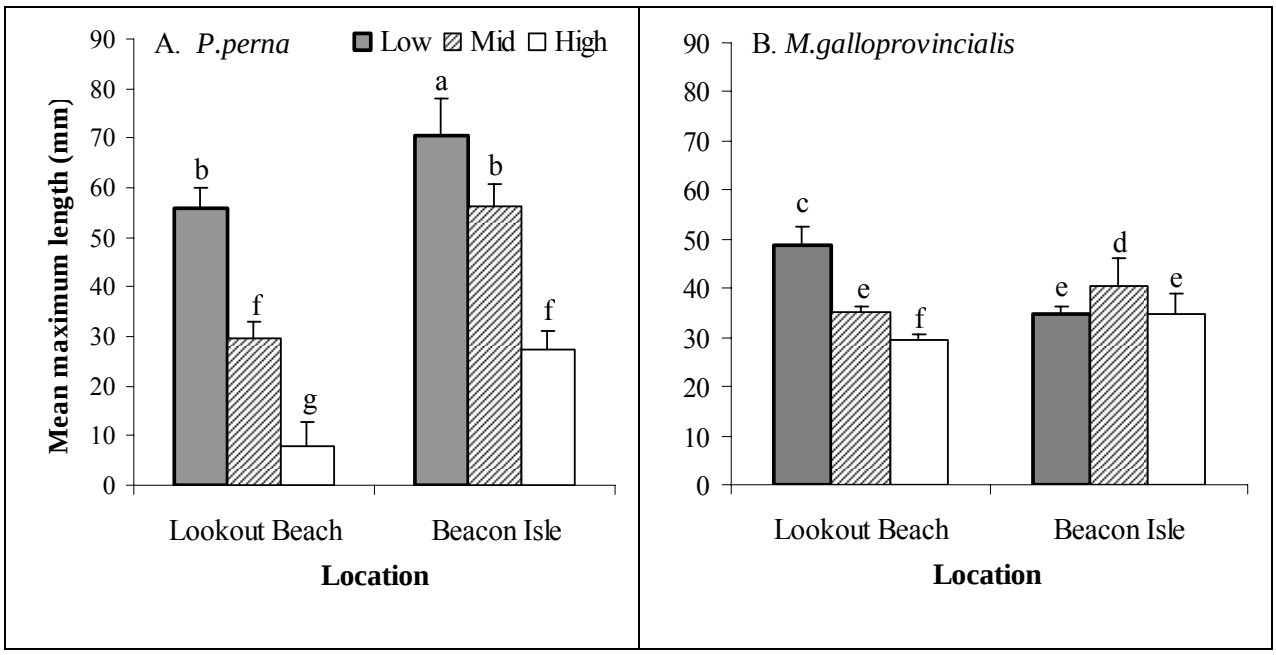


Figure 2.10a-b: Post-hoc results of the interaction between location (site), zone and species on the maximum lengths of *P.perna* (a) and *M.galloprovincialis* (b) in Plettenberg Bay. Letters indicating homogeneous groups apply to both graphs (a and b). Error bars represent standard deviations

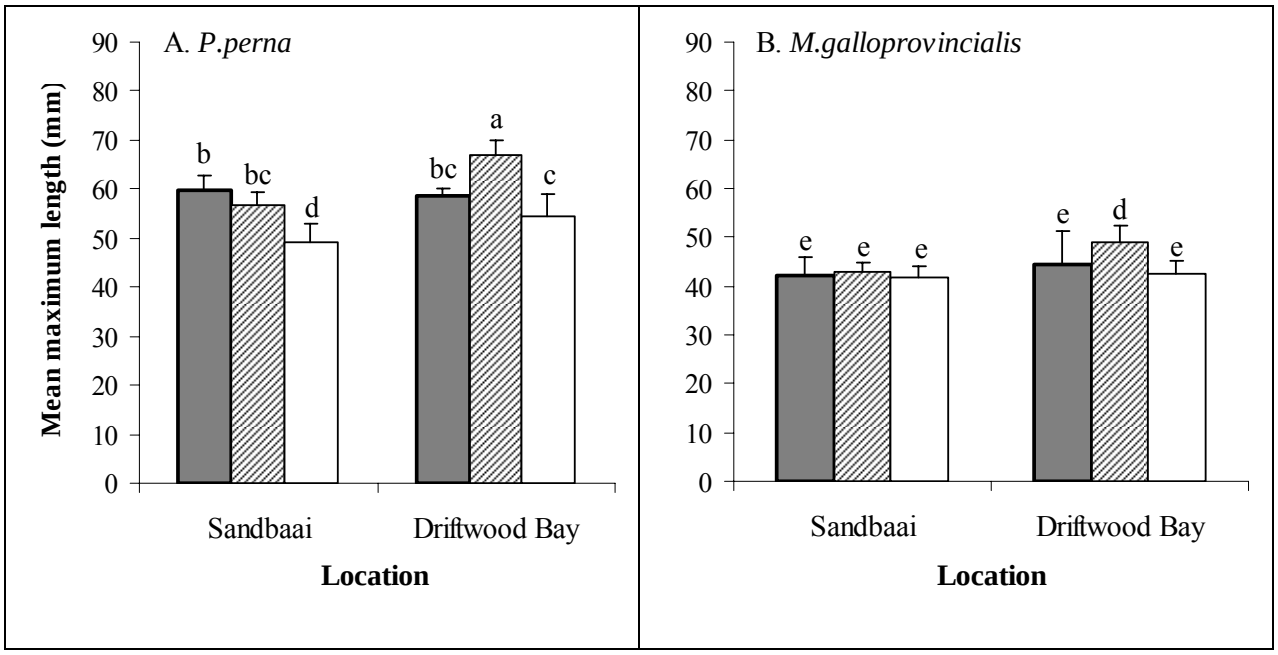


Figure 2.11a-b: Post-hoc results of the interaction between location (site), zone and species on the maximum lengths of *P.perna* (a) and *M.galloprovincialis* (b) in Tsitsikamma. Letters indicating homogeneous groups apply to both graphs (a and b). Error bars represent standard deviations

greater than *M.galloprovincialis* at this site, regardless of zone or location. Post-hoc results of the interaction between site, zone and species have not been presented. Noteworthy differences were that both species reached significantly greater maximum sizes in the upper two zones in Tsitsikamma than in Plettenberg Bay. On the low-shore, however, MML of *P.perna* was greater in Plettenberg Bay while there was no difference in MML of *M.galloprovincialis* between sites in this zone.

Population structure in 2004

1. Percentage cover

Comparison of the graphs of percentage cover in 2004 (Figs. 2.12-2.13 a-f; p.42-43) with those from 2001 revealed a considerable decrease in the cover of *C.meridionalis* on the low-shore at Lookout Beach, where cover of *P.perna* and *M.galloprovincialis* had increased substantially. These differences in cover were significant based on post-hoc results of the significant interaction between year and species ($F=9.9$; $p<0.001$). Total mussel cover appears to have increased at all locations and was thus generally less patchy. In particular, cover of *M.galloprovincialis* appeared to have increased on the mid-shore at both sites, and on the high-shore in Plettenberg Bay.

Analysis of the percentage cover of *P.perna* and *M.galloprovincialis* in 2004 revealed a very similar pattern of interspecific differences in vertical zonation as seen by the highly significant interaction between zone and species (Table 2.6; p.44; $p=0.001$). Cover of *P.perna* was significantly lower on the high-shore than on the low or mid-shore (Fig. 2.14; p.44). Low- shore cover of *P.perna* had increased with no change on the mid-shore. Cover of *M.galloprovincialis* had increased by ±30% in all three zones, with the same pattern of

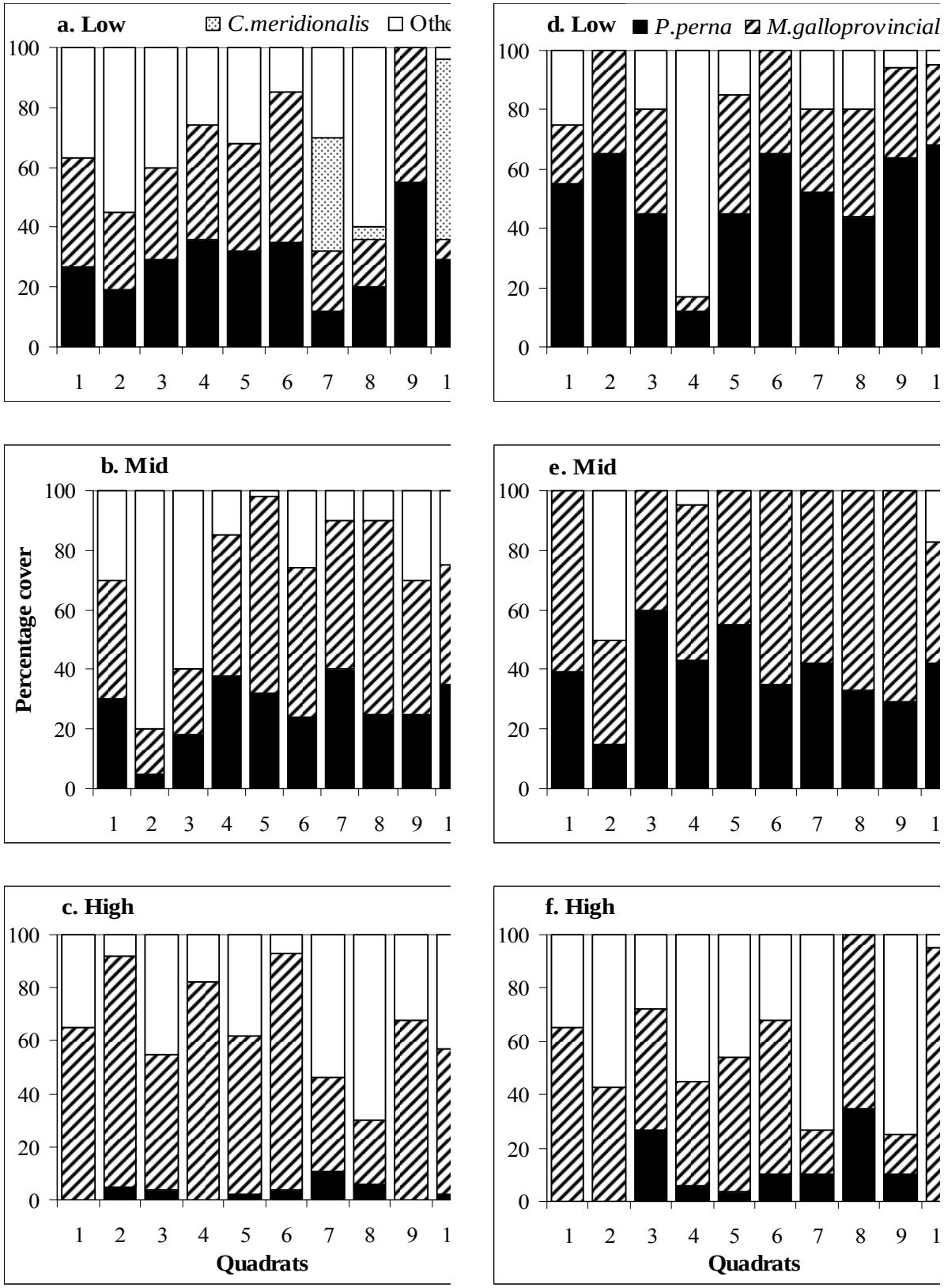


Figure 2.12 a-f: Percentage cover of *P.perna, M.galloprovincialis* and *C.meridionalis* in different zones at locations in Plettenberg Bay in 2004

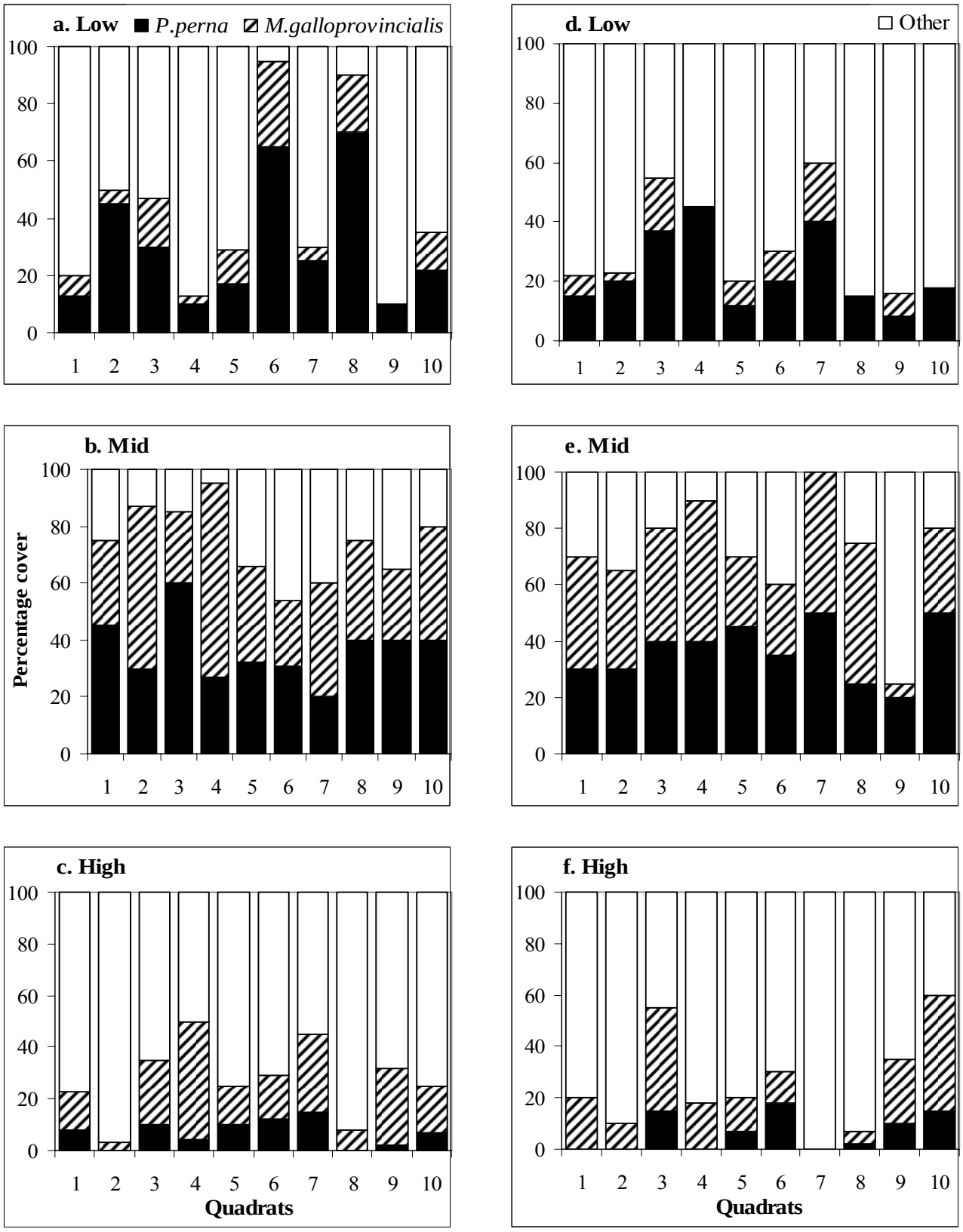


Figure 2.13 a-f: Percentage cover of *P.perna* and *M.galloprovincialis* in different zones at locations in Tsitsikamma in 2004

Table 2.6: Results of mixed model ANOVA on percentage cover of *P.perna* and *M.galloprovincialis* in 2004

	Effect	**Df**	**MS**	**F**	**p**
site	Fixed	1	10388.5	15.49	0.059
zone*	Fixed	2	5863.8	17.59	0.01*
species	Fixed	1	3960.9	4.63	0.164
site×zone	Fixed	2	1108.3	3.32	0.141
site×species	Fixed	1	5635.7	6.59	0.124
zone×species*	Fixed	2	10083.1	59.92	0.001*
site×zone×species	Fixed	2	1081.9	6.43	0.056
location (site)	Random	2	670.6	0.66	0.588
location (site)×zone	Random	4	333.4	1.98	0.262
location (site)×species	Random	2	855.2	5.08	0.080
location (site)×zone×species	Random	4	168.3	0.91	0.460
Error		216	185.3		

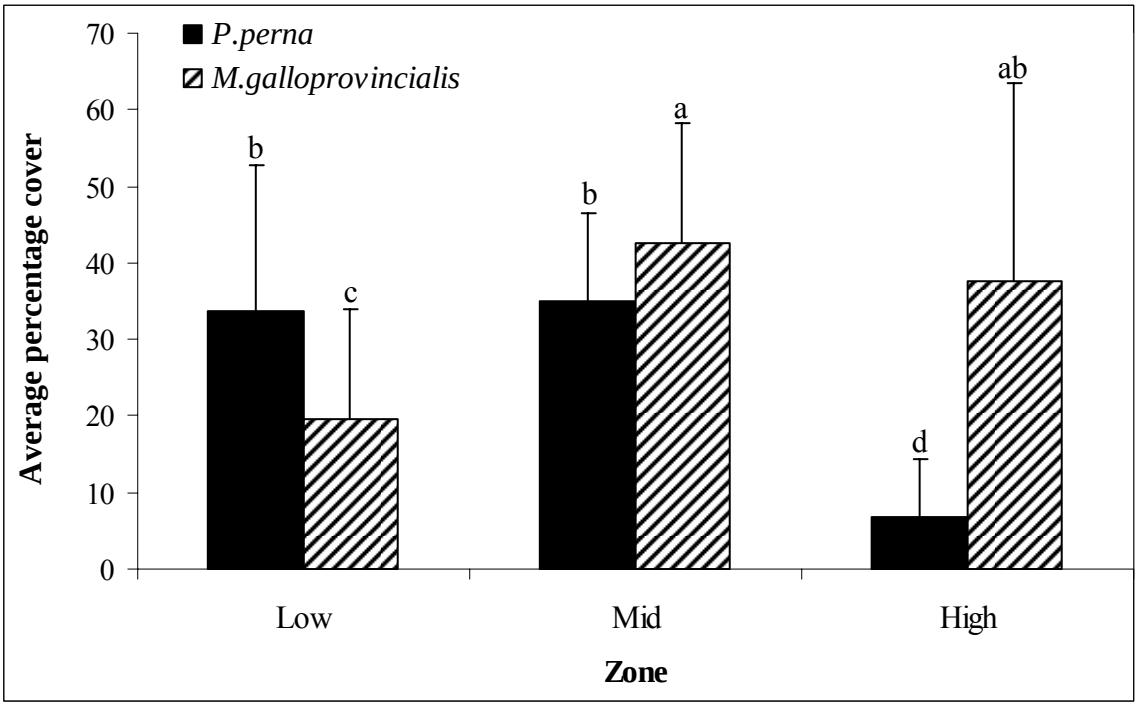


Figure 2.14: Post-hoc results of the interaction between zone and species on the percentage cover of mussels in 2004. Error bars represent standard deviations

significantly greater cover in the upper zones than on the low-shore. As a result, cover of *P.perna* was still significantly greater than *M.galloprovincialis* on the low-shore, with the opposite trend occurring on the high-shore, while on the mid-shore cover of *M.galloprovincialis* was now slightly greater than that of *P.perna.* This shift in dominance on the mid-shore was not due to a decrease in the cover of *P.perna*, but rather an increase in the cover of *M.galloprovincialis,* particularly in Tsitsikamma. An analysis comparing the change in percentage cover of each species between years revealed the difference between years was not significant and there were also no significant interactions with year ($F=5.78$; $p=0.45$). The lack of significant effects in this analysis is possibly due to the large number of variables involved.

2. Size structure and density

The size distributions of mussels in Plettenberg Bay still generally showed a negative relationship between density and size with the highest peak in the smaller size groups (Fig. 2.15 a-f; p.46). However, on the low-shore at Beacon Isle there was a shift in the size group at which peak density occurred. The distribution of *P.perna* became bimodal with peaks in the smallest size group and in mussels 40-50mm in length, while density of *M.galloprovincialis* peaked in mussels 30-40mm in length. In Tsitsikamma distributions were also still unimodal but there was again a general shift in peak density to the right on the length axis (Fig. 2.16 a-f; p.47). With the exception of the high zones, density of *P.perna* peaked in mussels 50-70mm in length, while density of *M.galloprovincialis* peaked at 40-50mm. A notable difference from 2001 was the appearance of a negative size/density relationship for both species on the high-shore, particularly for *M.galloprovincialis*, so that high-shore trends at this site were now more similar to those observed in Plettenberg Bay. It is also clear that density of *M.galloprovincialis* had

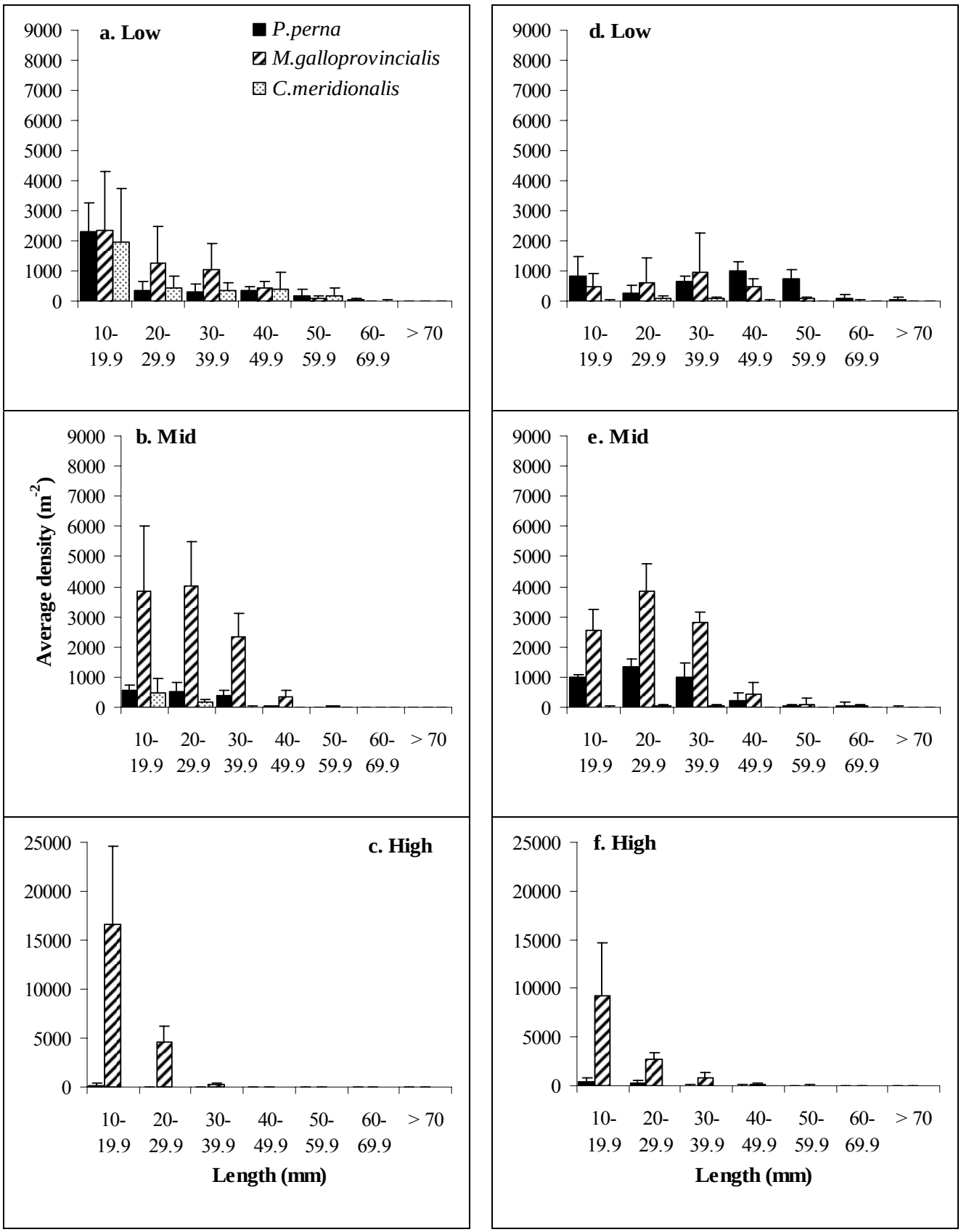


Figure 2.15 a-f: Size distributions of *P.perna, M.galloprovincialis* and *C.meridionalis* in different zones at Lookout Beach (a-c) and Beacon Isle (d-f) in Plettenberg Bay in 2004. Note the difference in y-axis values. Error bars represent standard deviations

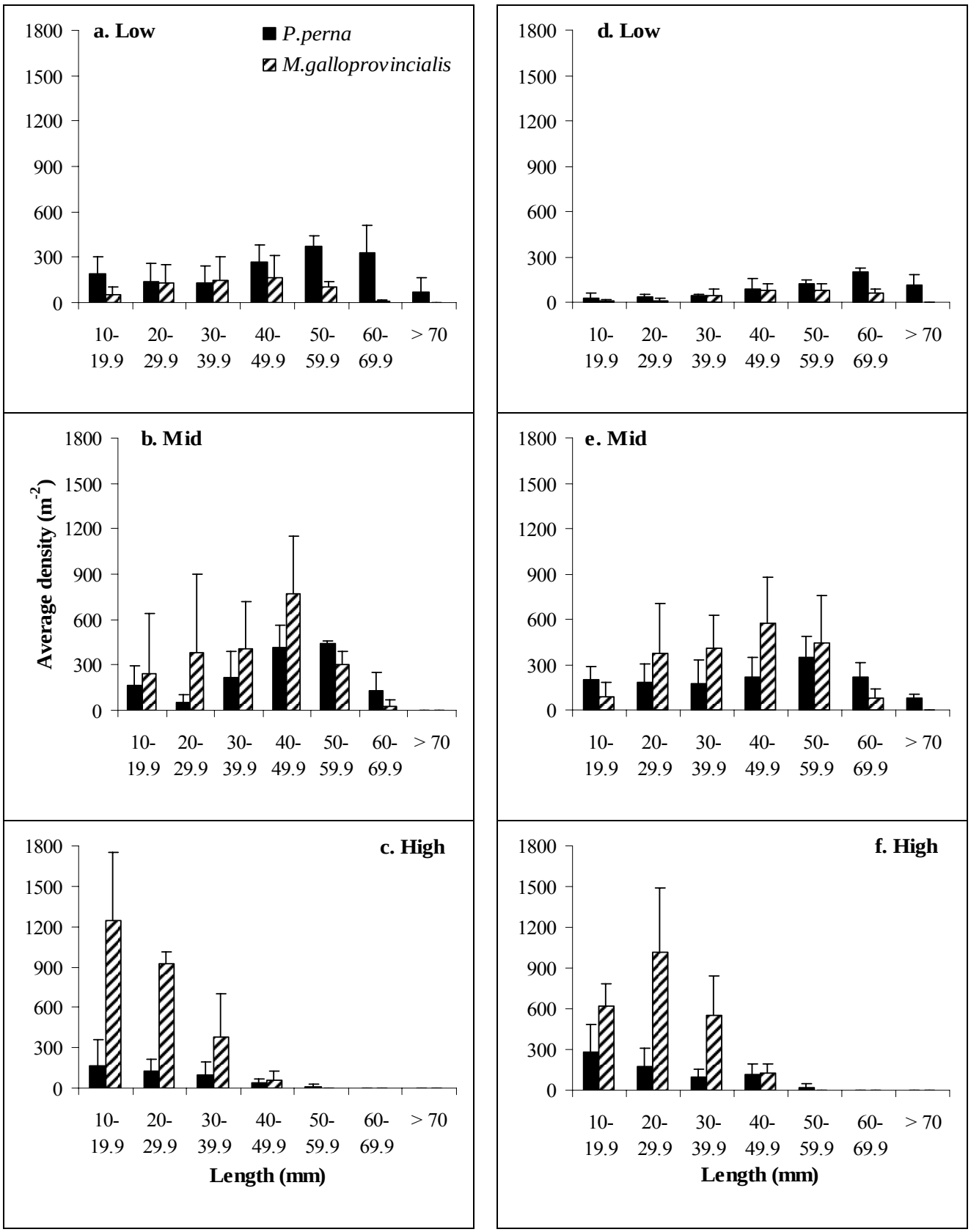


Figure 2.16 a-f: Size distributions of *P.perna* and *M.galloprovincialis* in different zones at Sandbaai (a-c) and Driftwood Bay (d-f) in Tsitsikamma in 2004. Note that the y-axis values are lower than those in Plettenberg Bay. Error bars represent standard deviations

increased substantially at both sites, but particularly in the upper zones in Plettenberg Bay.

Patterns of density and cover of *C.meridionalis* were very similar in 2001, however this was no longer the case. While the cover of this species on the low-shore at Lookout Beach had decreased quite substantially, density was still high. Furthermore, smaller mussels were found in low numbers on the mid-shore at this location, where they were previously absent, and were also present in very low numbers on both low and mid-shores at Beacon Isle. ANOVA revealed that the density of these species on the low-shore at Lookout Beach had not increased significantly between years ($F=7.76$; $p=0.1$).

Results of the analysis of the densities of *P.perna* and *M.galloprovincialis* in 2004 are given in Table 2.7 (p.49). Density of these two species still showed variations with tidal height, with different densities between sites ($p<0.01$). The zonation patterns of each species were similar at the two sites (Fig. 2.17; p.49). Density of *P.perna* was more similar in the lower zones than in 2001, and decreased on the high-shore, although the difference was only significant in Plettenberg Bay. Density of *M.galloprovincialis* increased significantly with increasing tidal height in Plettenberg Bay, however in Tsitsikamma, density of this species only differed significantly between the low and high-shores. *M.galloprovincialis* also had a significantly greater density in Plettenberg Bay than in Tsitsikamma in all zones. Low-shore density of *P.perna* was significantly greater in Plettenberg Bay but with no difference between sites in the upper zones.

There was also a significant interaction between location (site) and species ($p=0.02$). As in 2001, *M.galloprovincialis* had a significantly greater density at Lookout Beach than at Beacon Isle in 2004 and density of this species was still significantly greater than that of

Table 2.7: Results of mixed model ANOVA on the densities of *P.perna* and *M.galloprovincialis* in 2004

	Effect	Df	MS	F	p
site*	Fixed	1	21423.2	281.141	0.004*
zone*	Fixed	2	1665.7	7.081	0.049*
species	Fixed	1	16169.2	15.889	0.058
site×zone	Fixed	2	40.3	0.172	0.848
site×species	Fixed	1	9132.6	8.975	0.096
zone×species*	Fixed	2	8607.2	96.765	0.000*
site×zone×species*	Fixed	2	2022.0	22.732	0.007*
location (site)	Random	2	76.2	0.065	0.938
location (site)×zone	Random	4	235.2	2.644	0.185
location (site)×species*	Random	2	1017.6	11.440	0.022*
location (site)×zone×species	Random	4	88.9	0.385	0.818
Error		48	231.2		

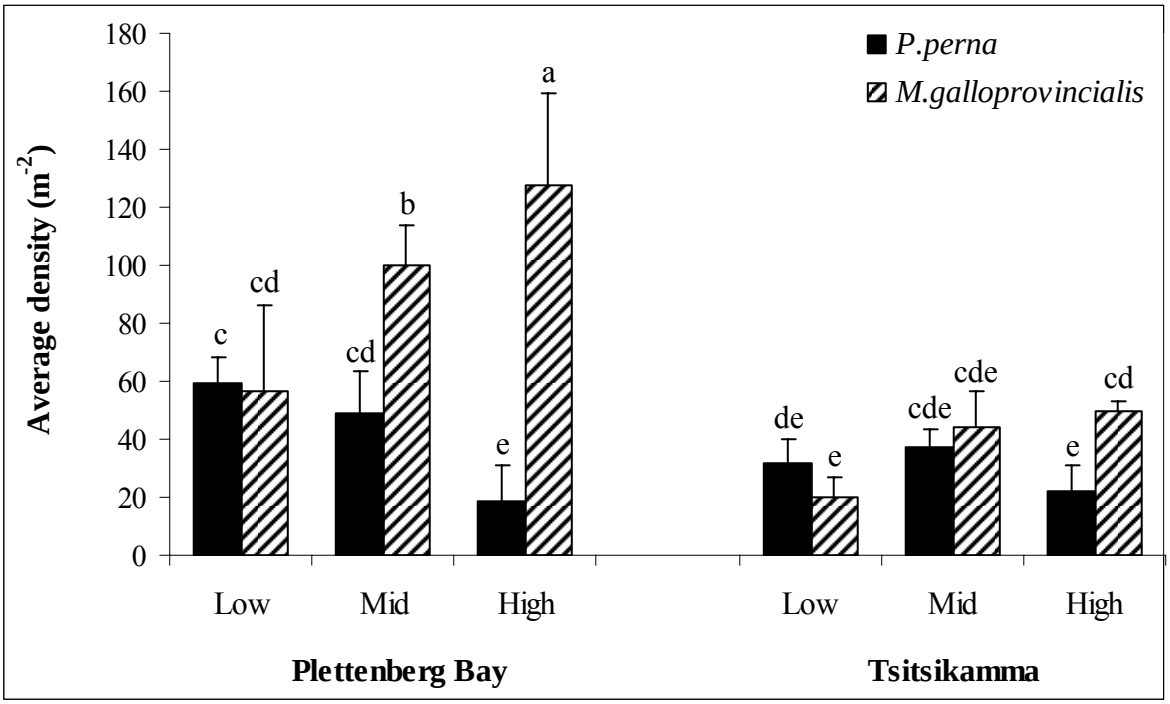


Figure 2.17: Results of post-hoc comparison of the interaction between site, zone and species on mussel density in 2004. Error bars represent standard deviations

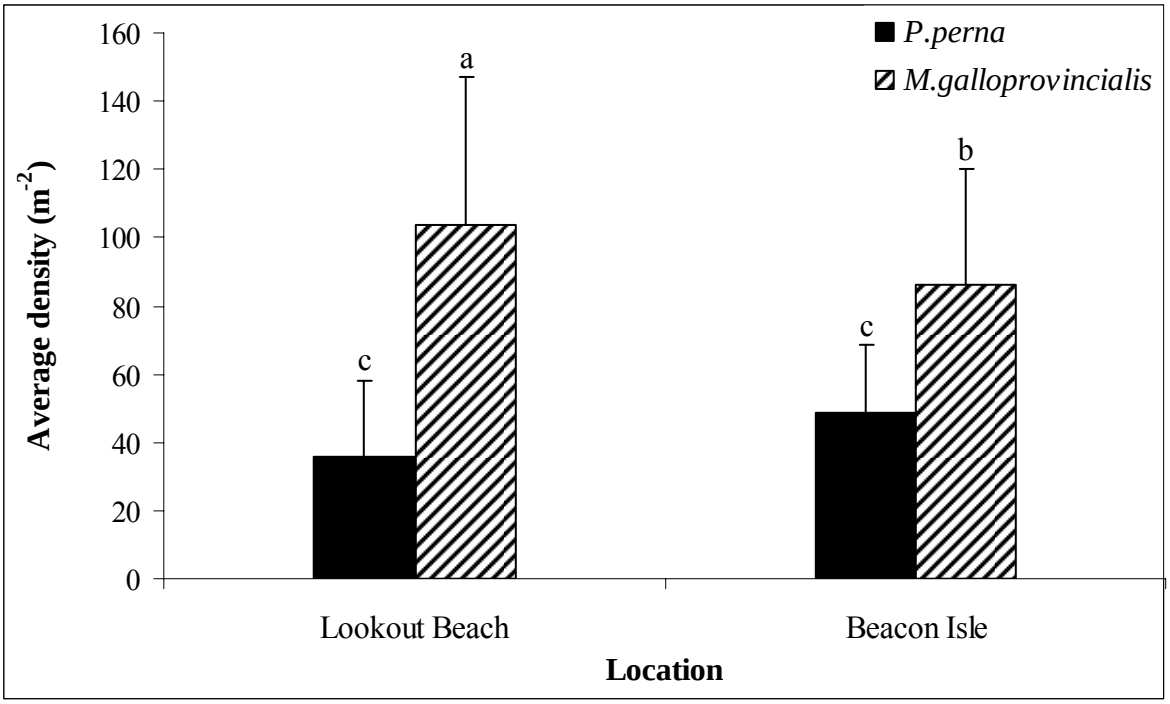


Figure 2.18: Post-hoc results of the interaction between location (site) and species on the density of mussels at locations in Plettenberg Bay in 2004. Error bars represent standard deviations

P.perna at both locations (Fig. 2.18; p.50). However, due to an increase in density of *P.perna* at Lookout Beach, the difference between locations for this species was no longer significant. As in 2001, there were no differences in the density of either species in Tsitsikamma, regardless of location. Although average densities (*P.perna* and *M.galloprovincialis*) had increased by 46% and 52% in Plettenberg Bay and Tsitsikamma respectively, the difference between years was not significant ($F=2.63$; $p=0.3$). There were also no significant interactions with year.

2.4 Discussion

The patterns of distribution and abundance of the mussels *Perna perna* and *Mytilus galloprovincialis* in 2001 were generally consistent with initial observations. There were significant differences in abundance at all spatial scales considered. In particular, there was evidence of vertical habitat segregation, especially between the two extremes of tidal elevation. Zone also affected all other aspects of population structure including recruit density, recruit/adult correlations and the mean maximum length of each species.

On the south coast of South Africa, *P.perna* typically forms large, continuous beds from low to mid-shore levels, and decreases in abundance higher on the shore (McQuaid *et al.* 2000). Where it is largely excluded from low-shore areas by dense bands of the limpet *Scutellastra cochlear* and encrusting algae, as in Tsitsikamma, it reaches maximum abundance on the mid-shore (Stephenson and Stephenson 1972; van Erkom Schurink and Griffiths 1990). On the other hand, *M.galloprovincialis* is frequently more abundant at mid to high-shore levels on the west coast (van Erkom Schurink and Griffiths 1990; Griffiths *et al.* 1992; Hockey and van Erkom Schurink 1992), although it is capable of dominating low-shore communities on exposed shores (Bustamante *et al.* 1997). In addition, density of

P.perna typically decreases further upshore (McQuaid *et al.* 2000), while *M.galloprovincialis* density has been found to increase at higher shore levels (Leeb 1995). The same vertical zonation patterns of these species were found in Plettenberg Bay and Tsitsikamma in 2001. This suggests that invasion of *M.galloprovincialis* at these sites had not dramatically altered the distribution and abundance of *P.perna.*

There were also significant variations at larger spatial scales of location and site, although the vertical zonation patterns were independent of these differences. *M.galloprovincialis* was generally more abundant than *P.perna* at Lookout Beach in Plettenberg Bay, but not at Beacon Isle. This may reflect the presence of *C.meridionalis*, which dominated the low-shore at Lookout Beach in 2001. On the other hand this species is absent from Beacon Isle where *P.perna* dominates the low-shore and the overall abundance of this species and *M.galloprovincialis* was more similar.

General mussel abundance was also significantly greater in Plettenberg Bay than in Tsitsikamma. Recruitment has been found to be an important factor contributing to large-scale differences in community structure (Roughgarden *et al.* 1988; Connolly and Roughgarden 1998; Archambault and Bourget 1999). Recruit density of *P.perna* and *M.galloprovincialis* was nearly six times greater in Plettenberg Bay than in Tsitsikamma in 2001, suggesting that recruitment may be an important factor structuring these communities.

There were also interspecific differences in recruit density with height on the shore. *P.perna* and *M.galloprovincialis* recruited in similar densities in the lower zones suggesting that post-recruitment processes such as competition and mortality may be more important

in structuring adult populations on the low-shore. However, recruitment of *M.galloprovincialis* increased on the high-shore, while recruitment of *P.perna* decreased significantly in this zone. This suggests that there may be differential settlement on the high-shore, or alternatively that there is differential post-settlement mortality i.e. either *P.perna* does not readily settle in this zone or it experiences significantly higher post-settlement mortality. There are two alternative scenarios for *M.galloprovincialis.* One is that it settles in greater numbers on the high-shore than in the lower zones. However, settlement is often lower on the high-shore due to shorter immersion times (Cáceres-Martínez and Figueras 1997) and growth is slower (Seed 1969b). Therefore the higher recruit density in this zone could reflect the cumulative effect of successive settlements of slower-growing individuals.

Significant positive correlations were found between recruits and adults of *P.perna* on the high-shore in Plettenberg Bay and the low-shore in Tstisikamma. Generally, strong positive correlations were found where adult densities were low and this was also species-specific. Connell (1985) and Menge (1991) also found that recruit density was positively correlated with adult density when recruitment was low. This has been attributed to density-dependent mortality at high recruitment levels (Connell 1985). The fact that this correlation may be species-specific in mixed populations suggests that larvae may settle preferentially near adult conspecifics when their densities are low.

Differences between the patterns shown by density and cover are generally related to mussel size. As suggested, cover is estimated as the amount of space occupied by a particular organism within a given area. Since larger animals occupy more space, cover is greater for larger-sized organisms. Thus, cover of *P.perna* was greater than

M.galloprovincialis on the low-shore, but densities tended to be similar in this zone. This was reflected in the mean maximum length of mussels in this zone, where *M.galloprovincialis* was significantly smaller than *P.perna.*

Differences in mortality and growth can influence size (McQuaid *et al.* 2000). High mortalities can reduce longevity leading to a rapid population turnover (Seed 1969b), which in turn reduces the maximum size that can be attained by such populations. Differential mortality rates may therefore be responsible for the smaller size of *M.galloprovincialis* in low-shore areas and in Tsitsikamma. This could potentially be a result of interference competition by *P.perna.* Also, in Plettenberg Bay *M.galloprovincialis* was significantly larger than *P.perna* on the high-shore, particularly at Lookout Beach. *P.perna* is less tolerant of desiccation than *M.galloprovincialis* and experiences higher mortality rates at increased levels of aerial exposure (Hockey and van Erkom Schurink 1992; Marshall and McQuaid 1993). Therefore high mortality rates and/or significantly reduced growth rates may explain the significantly smaller size of *P.perna* on the high-shore at this site.

In Plettenberg Bay, there was generally an inverse relationship between mean maximum size and increasing tidal height. This is commonly observed in intertidal mussels (Seed 1969b; Griffiths and Buffenstein 1981; McQuaid *et al.* 2000) and is considered to be a result of reduced feeding time in high-shore populations, which consequently grow more slowly (Seed 1969b; Griffiths 1981). However, in Tsitsikamma there was comparatively little difference in maximum sizes between zones, although *P.perna* tended to be slightly smaller on the high-shore. The high-shore at this site corresponded more or less to the upper limit of *P.perna,* therefore the level of the shore at which samples were taken may have been lower than in Plettenberg Bay where *M.galloprovincialis* forms a comparatively

broad zone on the high-shore. Thus it is probable that the reduction in feeding time and/or level of desiccation stress is not as pronounced for high-shore populations in Tsitsikamma.

C.meridionalis was restricted to the low-shore at Lookout Beach in Plettenberg Bay where it was initially the dominant mussel species. Intertidally, this species characteristically occupies low-shore rock surfaces subject to sand-scour or siltation (van Erkom Schurink and Griffiths 1990; Hockey and van Erkom Schurink 1992), and is able to survive burial under sand for months (Marshall and McQuaid 1993; pers. obs.). The low-shore at Lookout Beach is considerably sand-swept and was periodically buried in sand during the course of the study and therefore provides a suitable habitat for this species.

The upper limit of *C.meridionalis* was also distinct, as both adults and recruits were absent from the upper zones at Lookout Beach and Beacon Isle. *C.meridionalis* has been shown to experience reduced growth rates and higher mortality rates than *P.perna* and *M.galloprovincialis* at increasing levels of aerial exposure (Griffiths 1981; Griffiths and Buffenstein 1981; van Erkom Schurink and Griffiths 1993). However, Griffiths and Buffenstein (1981) found that *C.meridionalis* was able to maintain a positive energy balance at 50% exposure levels, and Marshall and McQuaid (1993) actually found that this species was more tolerant of aerial exposure than *P.perna* in the laboratory. It has therefore been suggested that temperature may be an important factor controlling the upper limit of this species, rather than intolerance to aerial exposure (Griffiths and Hockey 1987; Marshall and McQuaid 1993). Air temperatures on the south coast of South Africa can rise well above water temperature and often increase with increasing height on the shore (Marshall and McQuaid 1993). Nevertheless, the constant association of intertidal *C.meridionalis* with regular and often prolonged inundation of sand or silt, which

presumably provides protection from the physical stresses associated with intertidal existence, suggests that factors such as desiccation and/or high temperatures are possible causes of the upper limit of this species. Other factors such as initial settlement, post-settlement mortality and recruitment of *C.meridionalis* were measured in this study and should therefore provide some insight into the importance of these factors in structuring adult distributions of this species.

The distribution of *C.meridionalis* in 2004 was not dramatically different from 2001, however, there were some important differences in the structure of the low-shore community at Lookout Beach as a whole. Both cover and density showed similar patterns in 2001 and *C.meridionalis* was dominant in this area. However, in 2004, patterns of density and cover were very different. While cover of this species had decreased considerably, density had increased indicating that it was still prevalent at this location.

The discrepancy observed between cover and density of this species was due to extensive multilayering of the mussel bed. When destructive samples were taken to measure density, it was noted that *C.meridionalis* occupied the bottom layer of the bed, where large amounts of sand had accumulated, effectively burying, or at least partially burying most individuals. Both *P.perna* and *M.galloprovincialis* occupied the top layers, which were sand scoured but free from accumulative burial. Where the multilayer was thinner, *C.meridionalis* was often exposed and so contributed slightly to mussel cover. During the first year of study when sites were intensively sampled (May 2000-July2001), the low-shore at Lookout Beach was periodically buried in sand, for up to two months on one occasion. Both *P.perna* and *M.galloprovincialis* are less tolerant of sand stress than *C.meridionalis* (Hockey and van Erkom Schurink 1992; Marshall and McQuaid 1993), which may have prevented these

species from sustaining large numbers in this zone. When sampling was resumed in February 2003, the low-shore at this location was exposed and remained free from sand burial throughout the year until at least February 2004. This extended period free from sand burial may have allowed *P.perna* and *M.galloprovincialis* to penetrate this zone, but due to build up of sand in the bottom layers also allowed *C.meridionalis* to persist. On a visit to the study sites two months later (April 2004) it was discovered that the low-shore had been completely buried again as far as mid-shore level. The dynamics of low-shore mussel populations at Lookout Beach therefore appear to be highly variable with species abundances constantly mediated by the effects of sand inundation.

Both cover and density of *P.perna* and *M.galloprovincialis* had increased in 2004. In particular, the density of *M.galloprovincialis* increased substantially on the high-shore in Plettenberg Bay. Furthermore, while the density of this species was significantly greater in Plettenberg Bay than Tsitsikamma across all zones, the differences in *P.perna* density between sites was only significant on the low-shore. The vertical patterns of zonation were similar to those observed in 2001.

In summary, there was habitat segregation at the scale of vertical shore height with *P.perna* dominating the low-shore and *M.galloprovincialis* the high-shore. After three years this pattern of zonation was still strongly evident. Thus *P.perna* had maintained spatial dominance on the low-shore despite increasing densities of *M.galloprovincialis.* The vertical distributions of these species may therefore be a stable state in mixed communities where the two species are able to co-exist by means of habitat segregation. This is not unlikely since similar zonation patterns between these two species have been observed on the southwest coast of South Africa (van

Erkom Schurink and Griffiths 1990), and also on the Atlantic coast of Morocco where their distributions overlap (Id Halla *et al.* 1995). Interestingly, this pattern has also been observed between *M.galloprovincialis* and other *Perna* species. Lee and Morton (1985) mention that *M.galloprovincialis* thrives in the high intertidal zone in Hong Kong where the green mussel *P.viridis* dominates the intertidal. In New Zealand, *Perna canaliculus* is more abundant on the low-shore while *M.galloprovincialis* increases in abundance farther upshore (Kennedy 1976).

Aspects of settlement, post-settlement mortality, recruitment and post-recruitment processes such as growth and mortality were investigated in this study to determine what factors are important in structuring the adult distribution patterns of these species. This could also provide insight as to whether these species are truly coexisting or if invasion by *M.galloprovincialis* is still in process. The fact that *M.galloprovincialis* had become abundant in Tsitsikamma by the end of the study implies that this site was susceptible to invasion by this species, but that where recruitment is low this process may be slow.

Chapter 3

Identification of mussel post-larvae using shell morphology and mitochondrial DNA analysis

3.1: Introduction

Ecological studies of the early life history stages of marine bivalves are frequently hindered by the difficulty of distinguishing morphologically between the larvae and post-larvae of sympatric species. Traditionally, larvae have been identified by examination of the hinge morphology using scanning electron microscopy (SEM), which has proven to be a reliable tool for species-level identification of bivalves (Le Pennec 1980; Fuller and Lutz 1989). However, this method is laborious and impractical for processing large numbers of samples (Hare *et al.* 2000). In more recent years, increased awareness of the importance of pre-recruitment processes in structuring marine invertebrate communities has led to the development of several molecular identification techniques (Garland and Zimmer 2002).

Despite the range of techniques available, microscopic identification remains the most popular method for distinguishing bivalve species (Garland and Zimmer 2002). It is the most cost-effective and also the fastest method of quantification, which requires the separation of individual organisms (e.g. measurement of settlement and recruitment rates). Bivalve larvae are particularly difficult, if not impossible, to distinguish morphologically (Le Pennec 1980). For animals of this size molecular techniques are probably the most efficient method of choice. However, post-larvae are larger, more developed and may have features that are diagnostic for different species. Discrimination of post larval stages using shell morphology provides a valuable tool for studies in larval ecology (Martel *et al.* 2000). Potential problems associated with this method are that it is subjective and identification may be questionable as a result of phenotypic plasticity, due to environmental conditions, which is often observed among closely related species (Garland and Zimmer 2002). Martel *et al.* (1999; 2000) have used several morphological criteria by which two congeneric species of sympatric mussels could be identified both as early post larvae and juveniles. An

important element in these studies was that morphology-based identification was confirmed using genomic DNA analysis, which removed any subjectivity or uncertainty.

Although examination of hinge structure is impractical for identification purposes, as a method of confirmation it is less costly and time consuming than molecular techniques. The hinge structure of larval and post-larval stages has been described for several different mussel species (de Schweinitz and Lutz 1976; Lutz and Hidu 1979; Fuller and Lutz 1989; Martel *et al.* 1995; Bellolio *et al.* 1996; Galinou-Mitsoudi and Sinis 1997; Comtet *et al.* 2000), including *Perna perna* (Siddall 1980) and *Mytilus galloprovincialis* (Le Pennec and Masson 1976). No information is available on *Choromytilus meridionalis.* However, the shell and hinge development of *C.chorus* has been described (Ramorino and Campos 1983 cited in Fuller and Lutz 1989; Bellolio *et al.* 1996). In mussels of the family Mytilidae, variations in the number and type of provincular and lateral teeth are characteristics that facilitate distinction among similar species (Fuller and Lutz 1989). One characteristic that separates *P.perna* post-larvae from *M.galloprovincialis* and *C.meridionalis* is the presence of primary lateral hinge teeth on the dorsal shell margin, which are absent in the genus *Mytilus* (Siddall 1980) and in *C.chorus* (Ramorino and Campos 1983 cited in Fuller and Lutz 1989), and therefore possibly *C.meridionalis* as well. These teeth are formed during or shortly after metamorphosis and may therefore be used to distinguish newly settled *P.perna* post-larvae.

The initial intention of this chapter was to confirm microscopic identifications of post-larvae by examining the hinge structure of *P.perna, M.galloprovincialis* and *C.meridionalis.* While *P.perna* was easily distinguished, there were no immediately obvious differences in the hinge morphologies of the latter species. Therefore confirmation

using molecular methods was required. Immunological techniques have been used successfully to distinguish bivalve larvae (Paugam *et al.* 2000; Abalde *et al.* 2003). However, there are two major drawbacks to this method (Garland and Zimmer 2002). Firstly, changes in protein conformation are fairly common and may lead to greater variability in the antibody binding response, and secondly, larval proteins may not differ sufficiently for use as species-specific markers. Genetic techniques, which involve the amplification of DNA using Polymerase Chain Reaction (PCR), have the advantage of a higher degree of specificity (Garland and Zimmer 2002) and sensitivity, so that only small amounts of DNA are required (Rocha-Olivares 1998). Several different methods have been used for species-level identification in bivalves, including randomly amplified polymorphic DNA (RAPD) analysis (André *et al.* 1999; Klinbunga *et al.* 2000; Rego *et al.* 2002), restriction fragment length polymorphisms (Bell and Grassle 1998) and microsatellite markers (Morgan and Rogers 2001). Martel *et al.* (1999; 2000) confirmed identifications by comparing post-larval sequences with sequences from adult mussels of each species.

A simpler approach involves a single-step PCR assay in which species-specific primers amplify a section of DNA that is unique to the target species (Rocha-Olivares 1998; Hare *et al.* 2000). Examination of the PCR products using gel electrophoresis reveals the presence or absence of target species bands. Thus identifications can be confirmed or rejected without the need for additional steps, such as digestion with restriction enzymes or sequencing (Hare *et al.* 2000). Mitochondrial DNA has been found to be useful for discrimination at the species level (Garland and Zimmer 2002). In particular, the mitochondrial cytochrome oxidase I (COI) gene has been used successfully to distinguish several bivalve species, and marine bivalves are apparently highly variable at this locus (Hare *et al.* 2000). Thus, for the purposes of this study, species-specific primers were

designed using the cytochrome oxidase I (COI) gene from the mitochondrial DNA of female mussels (Wares and Cunningham 2001).

Mussels may enter a secondary dispersal phase after settlement (Hunt and Scheibling 1997). Bayne (1964) defined settlement following this post-settlement dispersal phase as secondary settlement, and the initial attachment of larvae as primary settlement. Secondary dispersal is generally facilitated by the production of mucus threads that allow post-larvae to remain suspended in the water column following detachment and this has been termed "bysso-pelagic migration" or "byssus drifting" (Sigurdsson *et al.* 1976; Lane *et al.* 1985). Thus, in settlement studies it is important to differentiate between primary and secondary settlement as the processes influencing the distribution and abundance of larvae and post-larvae may differ. In order to do so it was necessary to determine the size of newly settled post-larvae of the study species.

The aims of this chapter are therefore three-fold: firstly, to distinguish early post-larvae and juveniles of the study species using morphological characters; secondly, to confirm species identifications using SEM and PCR-based genomic analysis; and thirdly, to differentiate between primary and secondary settlers by determining the size at which the larvae of each species settles.

3.2 Materials and methods

Morphological identification

Post-larvae were collected from locations in Plettenberg Bay, where all three species are found, and Tsitsikamma where *C.meridionalis* does not occur. No recruits of this species were found in Tsitsikamma (Chapter 2), thus it is probable that few or no settlers would be

present in samples from this site. Additional specimens were also obtained from sites northwest of Port Elizabeth where *M.galloprovincialis* and *C.meridionalis* are rare, so that the majority of settlers were expected to be *P.perna;* and from mussel ropes in Saldanha Bay on the west coast of South Africa where *P.perna* is absent. Samples were processed as described in Chapter 4 and settlers were preserved in 70% alcohol. Post-larvae of a range of sizes from each site were carefully examined under a dissecting microscope (50x). External shell characteristics such as colour, shape or texture were noted. Morphometric measurements included measurements of length, shell height (the longest distance between the dorsal and ventral shell margins) and shell width (as seen from a dorsal view). There appeared to be differences in the shape and size of the umbo, therefore in smaller post-larvae (<600μm) the width of the umbo (on the dorsoventral axis) was also measured. Martel *et al.* (2000) also noted that the larger provincular hinge teeth in mussel post-larvae are visible externally on the hinge line as two red spots. The distance between the centres of these spots, called the PLT distance, was found to be a useful distinguishing feature. Measurements of PLT distance were therefore included. All measurements were calculated as a ratio of the total shell length (measured as the longest distance from the umbo/anterior hinge to the furthest posterior tip). In larger juveniles (>600μm) neither the umbo length nor the PLT distance could be measured. An additional measurement for larger juveniles was the Dorsal Apex Ratio (DAR) (Martel *et al.* 1999), which describes the position of the dorsal apex in relation to total shell length. All measurements were done using an ocular micrometer fitted to a dissecting microscope.

Morphometric data were analysed using Principal Component Analysis (PCA) (Statistica 6.1), which is a multivariate technique that essentially combines the variables into different

components that explain the variation in the data set. Thus the variation in the data, and hence the discriminatory power, is increased by examining the variables in combination rather than individually. Since different criteria were used for small (<600μm) and large juveniles (>600μm) these two groups were analysed separately. A simple discriminant function analysis was also performed for each data set to determine the percentage of individuals that could be correctly classified using these criteria.

SEM and hinge structure

Preserved specimens of post-larval shells of varying lengths that had been tentatively identified to species were immersed in a 1-2% sodium hypochlorite solution to dissolve tissue and separate the valves (Siddall 1980). Disarticulated valves were rinsed with distilled water and prepared for SEM. Specific characters that were examined included: the number and size of provincular teeth, the presence/absence of primary lateral teeth, the number of secondary lateral and dysodont teeth and the size at which these teeth develop (terminology from Siddall 1980).

DNA analysis

1. DNA extractions

DNA from adult mussels was extracted using a phenol-chloroform extraction procedure adapted from Doyle and Doyle (1990) and Stothard and Rollinson (1996) (Seaman 2002). Small sections of fresh gonad tissue (±2mm³) were ground in a mortar and pestle and mixed with 600μl extraction buffer (100mM Tris-HCl (pH 8); 1.4M NaCl; 20mM EDTA; 2% CTAB) with 2-3 drops β-mercaptoethanol. Following incubation at 60°C for 1-2hrs, 600μl

phenol-chloroform-isoamylalcohol (25:24:1) was added, the solution vortexed and then centrifuged for 10mins at 10000rpm. Using the supernatant this step was repeated with 600µl chloroform-isoamylalcohol (24:1). The supernatant was then added to a solution of 600µl cold absolute ethanol, 25µl 3M ammonium acetate and 49µl TE buffer and incubated overnight at -5°C. DNA was collected by centrifugation for 10mins. The pellet was gently rinsed in 600µl isopropanol, centrifuged for 5mins if necessary, air-dried and then resuspended in 70-80µl sterile water.

Post-larval DNA was extracted using a slight modification of the method of Gilg and Hilbish (2000). Individual post-larvae were digested with 2-10µl proteinase K and 10µl lysis buffer (10mM Tris-HCl (pH 7.5); 1mM EDTA) for 3hrs at 55°C. Post-larvae >500µm were punctured using a sterile needle, and for mussels >1.0mm, the whole body was removed from the shell and only the tissue was used. After digestion, samples were placed in boiling water for 5mins to denature the proteinase K. Preserved specimens were rinsed in sterile water before DNA extraction. A chelex extraction method was also used. Individual larvae were placed in a solution of 100µl 5% chelex and 100µl sterile water. The samples were vortexed, microfuged for a few seconds and then boiled for 15mins. The supernatant was removed and used as the DNA template.

2. PCR amplification and sequencing

PCR amplification

All PCR amplifications were performed using either a Corbett PC-960 Gradient Thermal Cycler or a ThermoHybaid PCR Sprint Temperature Cycling System. The reaction mixture,

at a final volume of 50µl, contained the following: 5µl 10x Bioline NH_4 dilution buffer; 3µl 50mM $MgCl_2$; 8mM dNTPs; 3% primers; 2µl DNA template; 1 unit BioTaq (Bioline Taq Polymerase). Reaction conditions were optimised for each set of primers (e.g. changing the Magnesium concentration, annealing temperature or number of cycles) and these have been summarised in Table 3.1 (p.67). PCR products were run on a 1% agarose gel containing ethidium bromide and observed using a UV transilluminator.

Sequencing

Sequences from known adults of *P.perna* and *M.galloprovincialis* that were collected from various sites along the south coast of South Africa were obtained from a colleague working on the population genetics of these species (Zardi, in prep.). Adult *C.meridionalis* were collected for sequencing from Plettenberg Bay and also Saldanha Bay on the west coast. Unfortunately no specimens from other localities on the south coast could be found. The DNA of female mussels was extracted using the method described and amplified using the mitochondrial COI gene primers LCO 1490 and HCO 2198 developed by Folmer *et al.* (1994) (Wares and Cunningham 2001). Primer sequences are given in Table 3.2 (p.68). PCR products were cleaned using the QIAGEN QIAquick purification kit. The cleaned product was sequenced using the ABI Prism BigDye Terminator v3.1 Ready Reaction Cycle sequencing kit (Applied Biosystematics) with the mtCOI primers. Sequences of *C.meridionalis* were edited using Sequencher (3.1) and then aligned using Clustal W. Nucleotide substitutions were confirmed using Sequencher. Unfortunately only one useful sequence was obtained for *C.meridionalis* from Saldanha Bay with an additional ten sequences from Plettenberg Bay.

Table 3.1: Reaction conditions for standard PCR amplification using the universal MtCOI primers, cycle sequencing and Touchdown PCR conditions for targeting *P.perna* and *M.galloprovincialis* products

PCR	T (ºC)	Time	Cycles
Standard PCR	95	45 secs	
	42	45 secs	
	72	2.30 mins	35
Cycle sequencing	96	30 secs	
	50	15 secs	
	60	4 mins	30
Touchdown PCR	95	45 secs	
	65	1 min	
	72	3 mins	10
	95	45 secs	
	60	1 min	
	72	3 mins	10
	95	45 secs	
	55	1 min	
	72	3 mins	15

Table 3.2: General and specific primer sequences used in PCR reactions (F=forward primer and R=reverse primer sequence)

Primer	Sequence
LCO 1490	GGTCAACAAATCATAAAGATATTGG
HCO 2198	TAAACTTCAGGGTGACCAAAAAATCA
Perna-F	AGATTATATAACGTGGTGGTG
Perna-R	TCCCCCTTTTATCTTAACTGT
Mytilus-F	GGTCTGAGGAGGTTTGTTC
Mytilus-R	CCGCGGATAAATTATATCTTT
Choro-F	TGAATACATGTATAATGTAA
Choro-R	GCTCCTAATAAGGACCTC

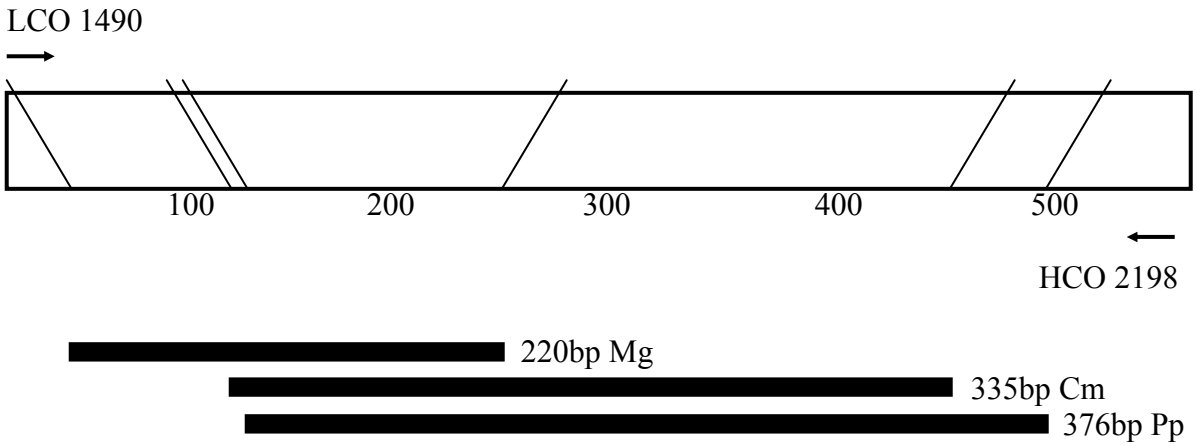


Figure 3.1: Map of the MtCOI gene fragment showing the positions of the species-specific primers. Primers were designed to amplify different sized fragments for each species (below). Mg = *M.galloprovincialis;* Cm = *C.meridionalis;* Pp = *P.perna*

Species-specific primer design

Sequences of all three species were aligned and compared using Clustal W. Primers were designed manually by searching for regions with several nucleotide differences between the three species while ensuring that each region was conserved within the target species. Initially only specific forward primers were designed. However, occasionally non-specific PCR products were obtained with a success rate of 52%-77% for the different species primers. Thus species-specific reverse primers were also designed to amplify different sized products for each species. The primer sequences are given in Table 3.2. Fig. 3.1 (p.68) illustrates the position of the primers on the MtCOI gene fragment and the size of the PCR products for the specific primers.

Specific PCR conditions and post-larval identification

Species-specific primers were initially tested with adult DNA extracts to determine the optimum reaction conditions. PCR reactions with each adult extract were performed using all three species primers at a range of Magnesium concentrations (1-5mM) and annealing temperatures. A positive (successful) result was obtained when the extracts of each species only amplified with their respective primers. A negative result occurred when extracts of one species amplified with the primers of another species. When this happened, the PCR products always produced much fainter, different-sized bands to those obtained with the target species primers. The addition of cosolvents such as DMSO (Dimethylsulfoxide) may improve the specificity of PCR amplifications (Varadaraj and Skinner 1994; Van Dessel *et al.* 2003). Thus 1µl 2% DMSO was added to the reaction mixture. The addition of DMSO improved the brightness and clarity of target species bands, but did not remove non-specific bands. The optimum reaction conditions were obtained using Touchdown PCR (Don *et al.* 1991). Reaction conditions for *P.perna* and *M.galloprovincialis* are given in Table 3.1

(p.67). The conditions for *C.meridionalis*-specific primers were the same, but with a starting temperature of 55ºC. For confirmation of the identification of post-larvae, all PCRs were run with an adult DNA extract of the target species and water as a negative control. Final sample sizes were 11 for *M.galloprovincialis,* 36 for *C.meridionalis* and 15 for *P.perna.*

Size at settlement

Bayne (1976) and Martel *et al.* (1995) provide detailed descriptions of shell morphology and development in bivalves. The larval shell is called the prodissoconch and it is composed of two distinct regions: prodissoconch I (PI), which is secreted early in development (D-shaped or straight-hinged stage) and prodissoconch II (PII), which continues to grow until larvae are fully developed and physiologically competent to settle. Following settlement and metamorphosis post-larvae begin to secrete the adult shell form or the dissoconch (D). There is usually a clear demarcation between the larval and the adult shell regions, which is visible under a dissecting microscope and enables the size at settlement to be determined (Martel *et al.* 1995).

The primary purpose of this analysis was to determine the size range at which larvae settle so that these could be grouped into a single size category representing primary settlers. Thus a frequency distribution of the size at settlement of all measured post-larvae was plotted for each species. Size at settlement in mussels may vary both spatially and temporally and also between species (Martel *et al* 2001; Phillips and Gaines 2002). Thus specimens from all zones at both locations in Plettenberg Bay and Tsitsikamma were measured. No settlers of *C.meridionalis* were found in Tsitsikamma and very few from

Beacon Isle in Plettenberg Bay, thus these measurements are mostly based on specimens from a single location.

3.3 Results

Morphological identification

Examination of early post-larval and juvenile shells revealed a diagnostic colour marking on the shells of ***P.perna.*** This mark was in the shape of a red arc at the tips of the umbo on each valve, which could be clearly viewed by tilting the umbo region of the shell upwards at an angle of 45-80° (Fig. 3.2; p.72). There also tends to be a reddish colouring to the entire anterior hinge region (Fig. 3.3a; p.73). These two features remain visible in shells up to ±1.0mm long. Another notable feature of the early post-larval *P.perna* shell is the shape, size and position of the umbo. This species has a narrow, well-rounded umbo that is very obvious even in the smallest specimens. The umbo is also positioned almost centrally on the anterior hinge line so that the shell is bilaterally symmetrical in appearance. At sizes >1.0mm, additional red markings begin to appear towards the posterior edge of the juvenile shell (Fig. 3.3e). These eventually form a pattern of triangular-shaped marks, almost arranged in rows to form a tessellated pattern that covers most of the juvenile shell (Fig. 3.3f).

The earliest post-larval shell of *M.galloprovincialis* is not characterised by any distinguishing colour markings, but is generally cream-coloured with a tinge of brown, particularly at the shell margins (Fig. 3.4a; p.74). An important distinguishing feature is the shape of the post-larval shell which is elongated on the ventral margin so that the umbo is positioned closer to the dorsal margin, making the shell asymmetrical. The umbo in this

Figure 3.2: Dorsal view of a *P.perna* post-larva placed at an angle to show the red arc at the tip of the umbo, a marking which is diagnostic for this species

a. 333µm (32x)

b. 382µm (32x)

c. 451µm (32x)

d. 686µm (32x)

e. 1.31mm (20x)

f. 5.2mm (6.3x)

Figure 3.3a-f: Post-larval development of *P.perna*

a. 333µm (32x)

b. 392µm (32x)

c. 500µm (32x)

d. 765µm (32x)

e. 1.31mm (32x)

f. 5.2mm (6.3x)

Figure 3.4a-f: Post-larval development of *M.galloprovincialis*

a. 333µm (32x)

b. 373µm (32x)

c. 451µm (32x)

d. 765µm (32x)

e. 1.35mm (25x)

f. 5.28mm (6.3x)

Figure 3.5a-f: Post-larval development of *C.meridionalis*

species is also wider and flatter than that of *P.perna.* Dissoconch growth is highly skewed towards the postero-dorsal margin (Fig. 3.4 b-d). The dissoconch also tends to be blue in colour. In larger juveniles, shells range from blue with shades of brown to blue/black in colour. At sizes >600µm *M.galloprovincialis* shells often appear to be "hairy". These hairs may be few or numerous, and are generally towards the posterior region of the shell (Fig. 3.4 d-e).

Like *M.galloprovincialis,* the early post-larvae of *C.meridionalis* are not distinguished by any specific colour markings. Shells tend to be a very pale cream, with an often whitish/semi-transparent appearance. Pigmentation of the provincular hinge (as described by Martel *et al.* 2000) tends to be quite marked in this species, more so than in *M.galloprovincialis,* which frequently exhibits no obvious hinge pigmentation. Although there is frequently a slight elongation on the ventral shell margin, this is never as pronounced as in *M.galloprovincialis* (Fig. 3.5a; p.75). The umbo is wider and rounder than that of *M.galloprovincialis*. Dissoconch growth in *C.meridionalis* is initially fairly straight compared to the other two species so that the longest distance is usually on a vertical axis from umbo to tip (Fig. 3.5 a-b). Although shell colouring in this species is similar to *M.galloprovincialis,* the shell surface tends to be smooth and no hair was ever observed on juvenile shells. In larger juveniles, the ventral anterior hinge is also markedly less prominent than in the other two species (Figs. 3.3-3.5 d-e), so that the umbo is the most anterior point on the shell.

Initial results of PCA revealed a high degree of overlap in the morphometrics of *P.perna* with the other two species. Since the shell markings on post-larval and juvenile shells in this species are diagnostic, the presence/absence of red markings was included as a

variable, with rankings of either 1 (present) or 0 (absent). Results of the PCA on the shell characteristics of post-larvae <600μm are shown in Table 3.3 (p.78). Principal component 1 (PC1) accounted for 43% of the variation in shell morphology. All the variables associated with size contributed to this component. PC2 was largely concerned with the presence/absence of the red markings, which accounted for 25% of the variation. A further 17% was explained by PC3, which could be attributed to variation in the shell width:length ratio. Since the first two principal components explained the greatest portion of variation in shell morphology between species, these two components were plotted (Fig. 3.6; p.79). *P.perna* came out as a separate group due to the presence of the shell markings. There was a high degree of overlap between *M.galloprovincialis* and *C.meridionalis,* suggesting that shell morphometrics do not readily distinguish the post-larvae of these species. Discriminant function analysis revealed that 79% and 71% of *M.galloprovincialis* and *C.meridionalis* post-larvae respectively, could be correctly classified using these criteria and that the ratio of shell width:length was the only significant variable of discrimination (Wilks' Lambda=0.8; p=0.001).

The PCA on data for juvenile mussels (>600μm) produced three important principal components (Table 3.3). PC1 explained 47% of the variation in shell morphology and the variables associated with size contributed significantly to this component. PC2 and PC3 accounted for 25% and 21% of the variation respectively. The presence/absence of red markings was the major contributing variable to PC2 and the dorsal apex ratio (DAR) to PC3. Results were plotted using PC1 and PC2 (Fig. 3.7; p.80). Again, *P.perna* was clearly separated from the other two species due to its diagnostic markings. In contrast to the smaller mussels, the shell morphometrics of juvenile *M.galloprovincialis* and *C.meridionalis* resulted in distinct groupings of the two species. Discriminant function

Table 3.3: Results of Principal Component Analysis on the shell characters of post-larval and juvenile mussels, showing the Eigenvectors (the relative contributions of individual variables to each principal component), Eigenvalues and the percentage total variance explained by each component

Post-larvae (<600μm)					
Variables	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Width	-0.341	0.07	0.937	0.016	-0.019
Height	-0.508	0.417	-0.197	-0.472	0.554
PLT distance	-0.558	-0.073	-0.209	0.787	0.139
Umbo width	-0.558	-0.289	-0.189	-0.356	-0.665
Red mark	0.039	0.856	-0.062	0.176	-0.481
Eigenvalue	2.135	1.235	0.848	0.459	0.323
% total variance	42.701	24.706	16.968	9.173	6.453
Juveniles (>600μm)					
Variables	**PC1**	**PC2**	**PC3**	**PC4**	
Width	-0.873	-0.078	0.322	0.357	
Height	-0.905	0.075	0.182	-0.377	
DAR	-0.515	-0.212	-0.829	0.0425	
Red mark	-0.112	0.977	-0.168	0.067	
Eigenvalue	1.86	1.011	0.853	0.276	
% total variance	46.5	25.285	21.328	6.888	

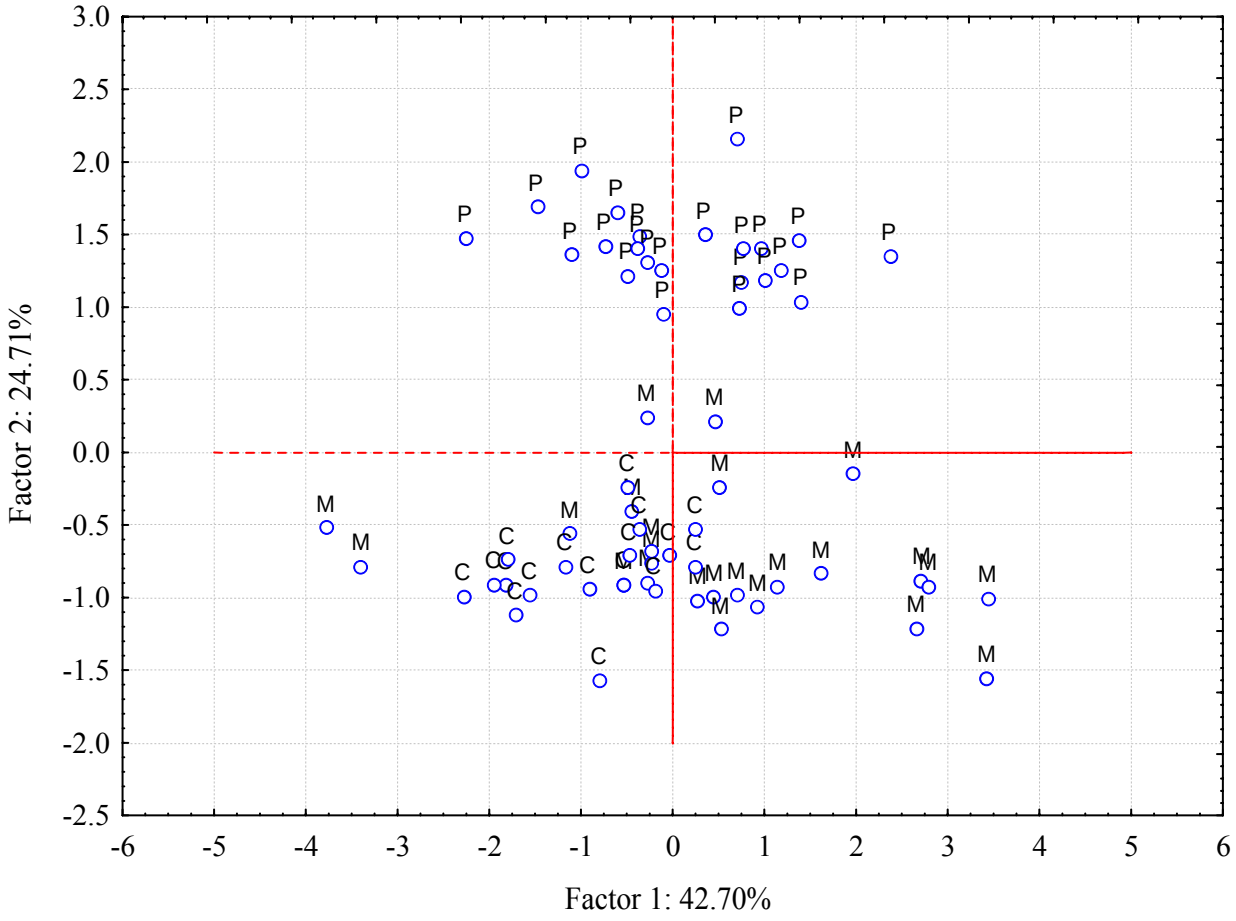


Figure 3.6: Plot of PC1 and PC2 from Principal Component Analysis of post-larval shell morphologies of the three mussel species. PC1 concerns the variables associated with size, while red markings was the main contributing factor to PC2

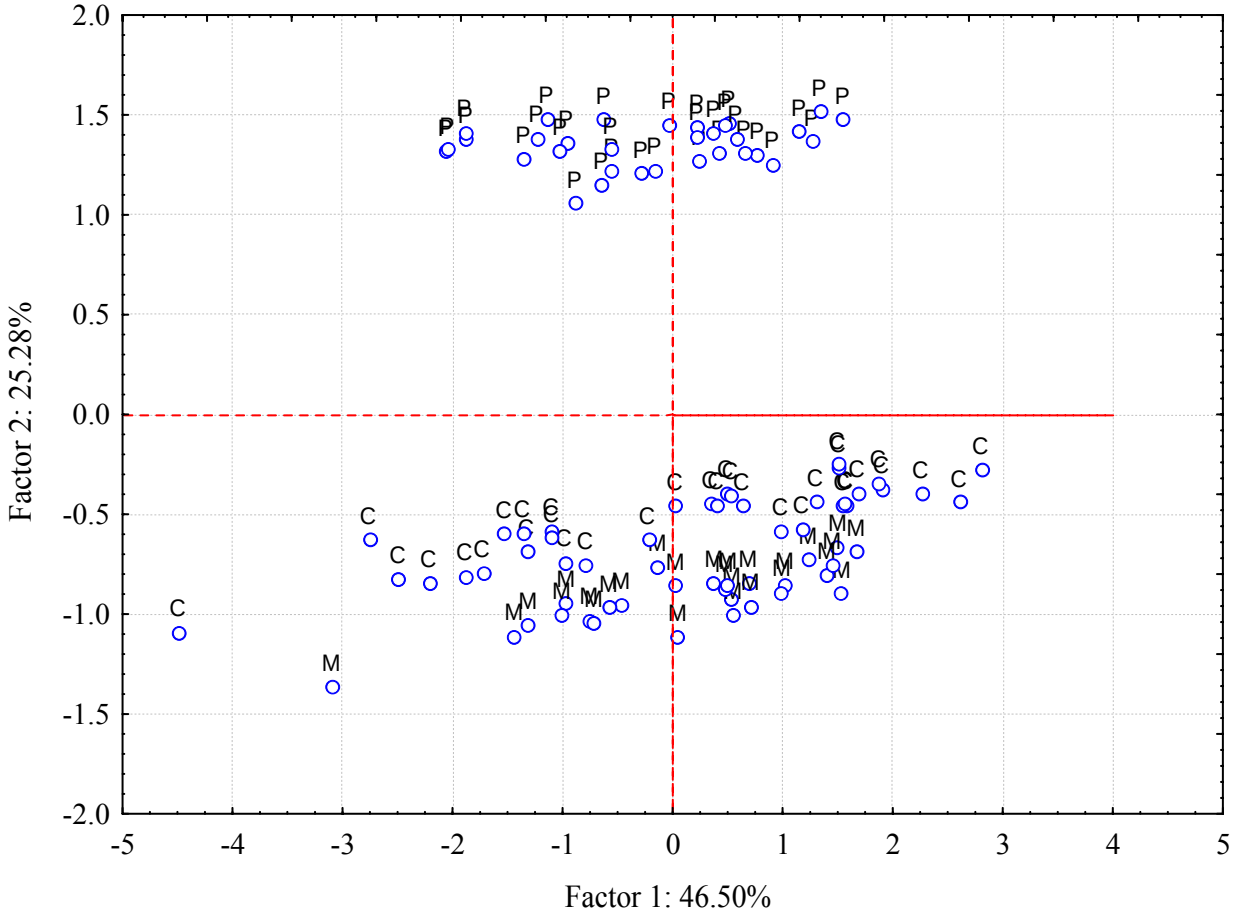


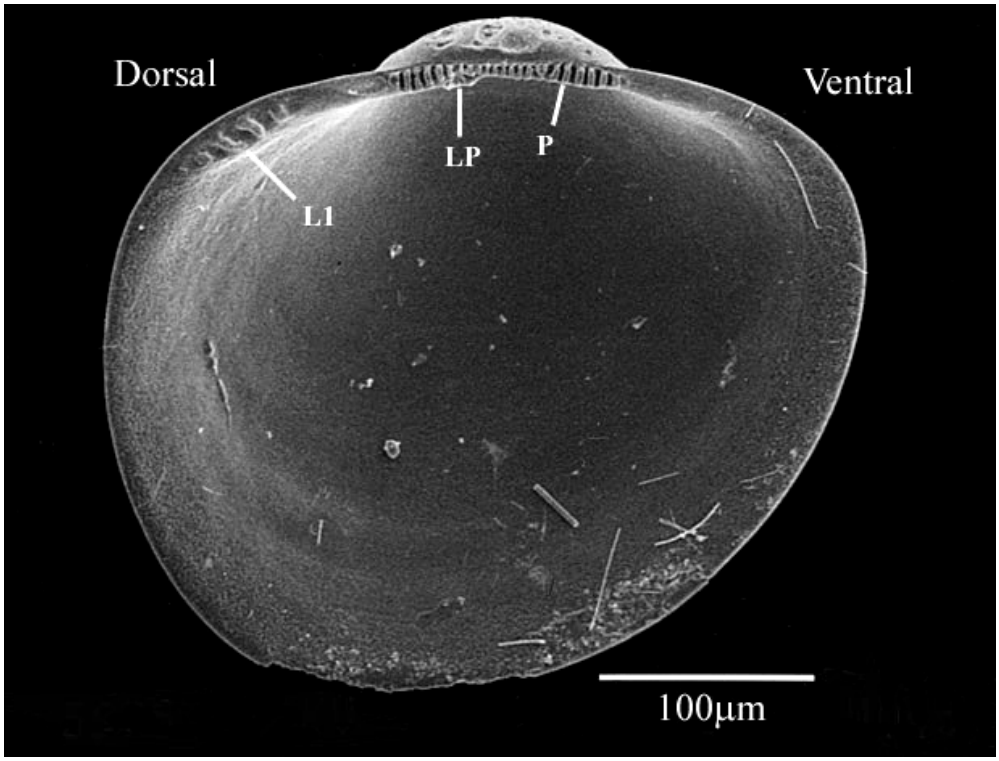
Figure 3.7: Plot of PC1 and PC2 from Principal Component Analysis of the shell morphologies of juvenile mussels. PC1 was largely determined by variables associated with size, while red markings was the main contributing variable to PC2

analysis correctly classified all measured individuals to species (i.e. 100%). In this case, the height:length ratio and DAR were the significant discriminatory variables. *C.meridionalis* has a greater shell height:length ratio than *M.galloprovincialis.*

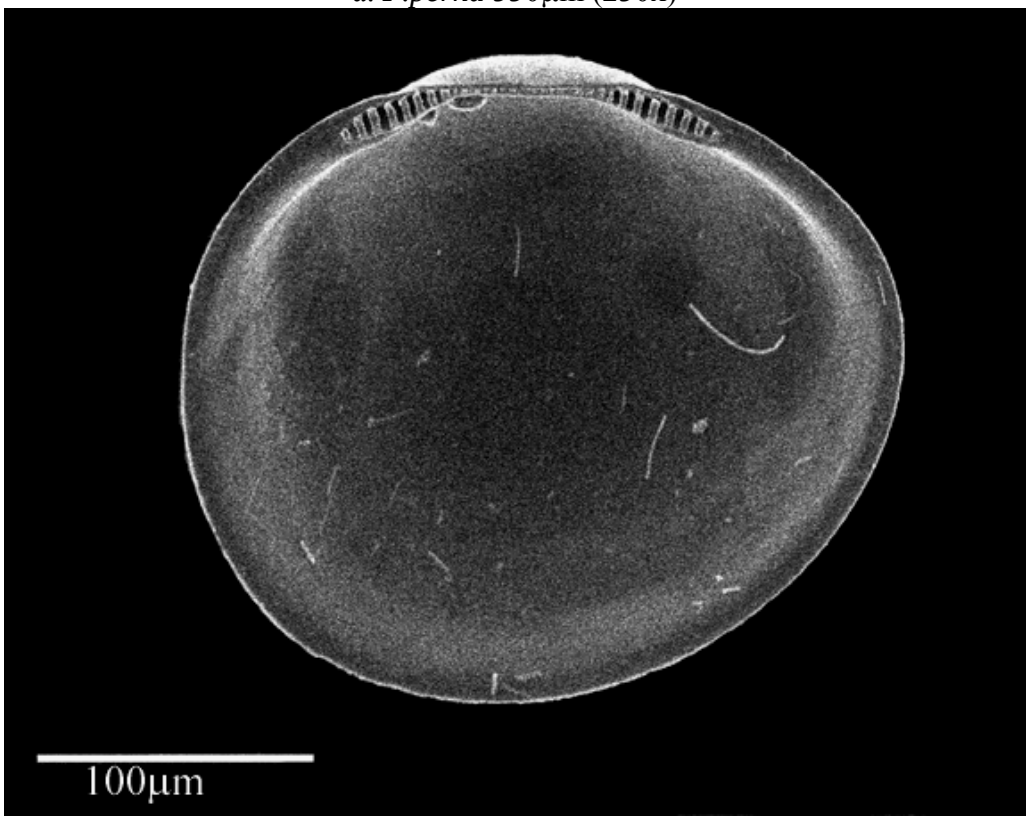
Hinge structure

Identification of *P.perna* was confirmed by the presence of primary lateral teeth (L1) on the dorsal shell margin (Fig. 3.8a; p.82), which were absent in tentatively identified *M.galloprovincialis* and *C.meridionalis (*Fig. 3.8b-c; p.82-83). These teeth increase in number with increasing size from 2-3 teeth in early post-larvae to ±10 in juveniles 600-700μm in length. Provincular teeth (P) were always clearly visible and ranged from 22-24 in number regardless of size. Secondary lateral teeth (L2) and dysodont teeth (D) start appearing on shells 450-500μm long (Fig. 3.9a; p.26). Secondary lateral teeth develop on the dorsal shell margin posterior to the primary laterals and dysodonts develop on the ventral shell margin. This species generally has 2 dysodont teeth. The secondary ligament pit (LP2) forms immediately posterior to the provincular teeth on the dorsal hinge. This ligament appears fairly early in development (±370μm) but was generally much clearer and more frequently observed between 500-600μm. It continues to grow and begins to obscure the primary lateral teeth in juveniles >700μm and eventually the secondary lateral teeth in animals >1.0mm. The dysodont teeth are the only teeth that remain distinctly visible in adult mussels (Siddall 1980).

The provinculum in *M.galloprovincialis* appears to be longer along the dorso-ventral axis than in *P.perna* (which is probably reflected in the shorter umbo of this species) (Fig. 3.8b). This was confirmed by the number of provincular teeth, which ranged from 30-36 in

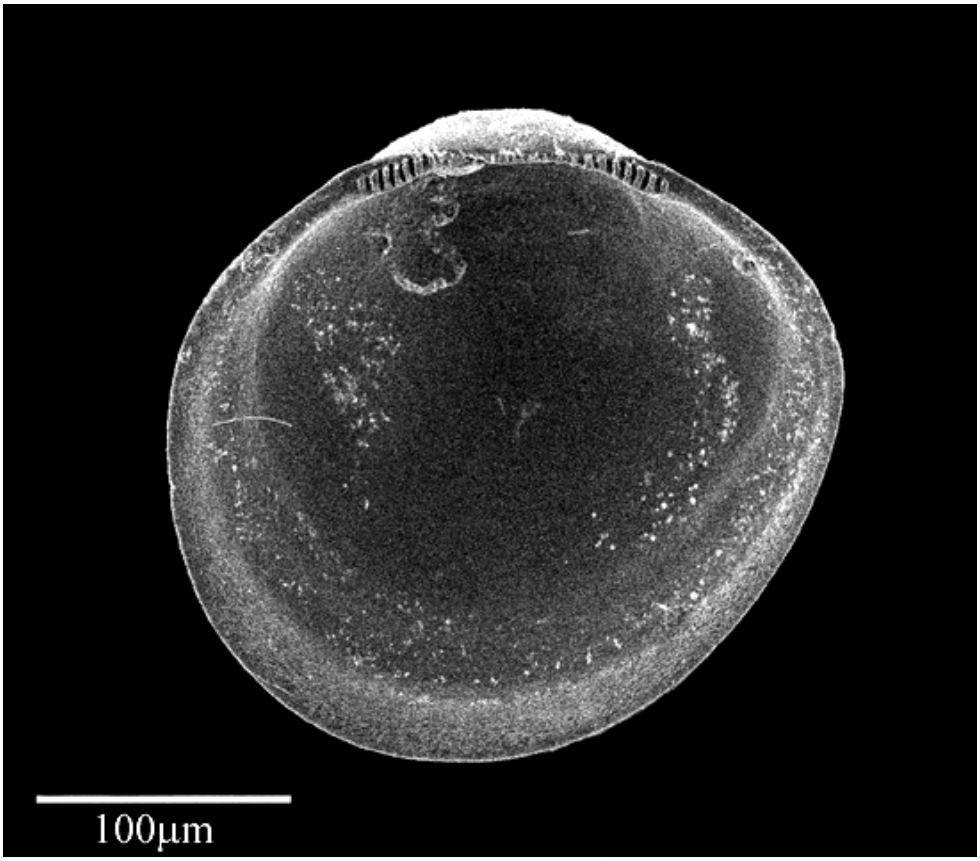


a. *P.perna* 330µm (230x)

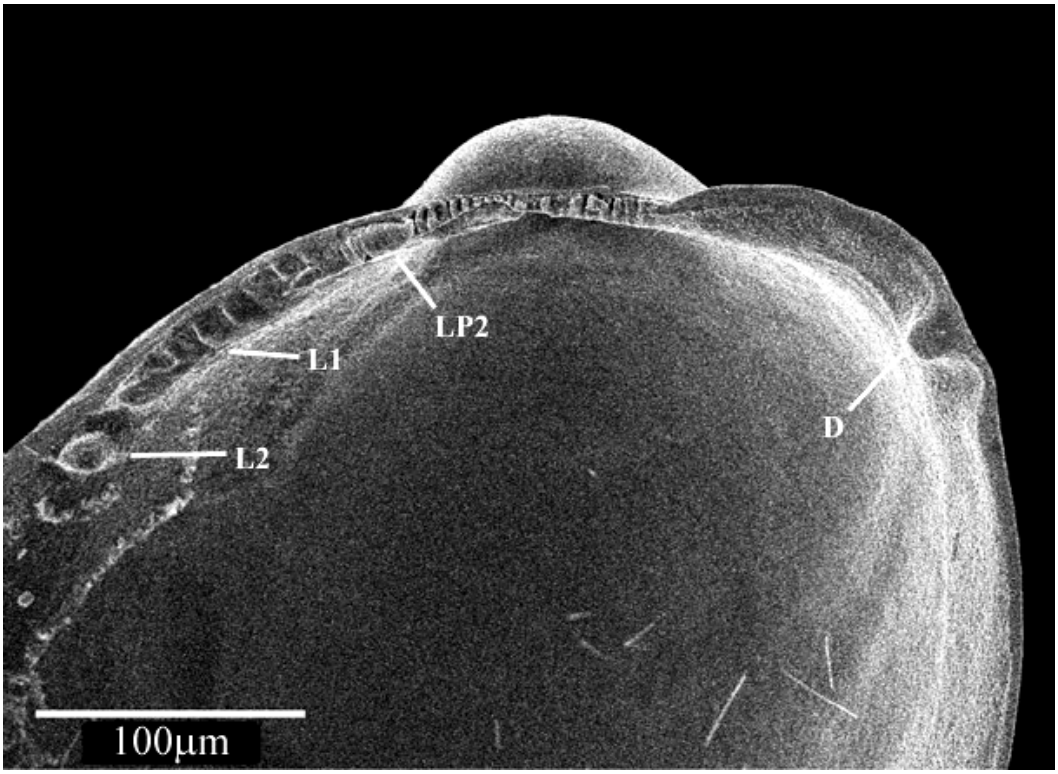


b. *M.galloprovincialis* 275µm (270x)

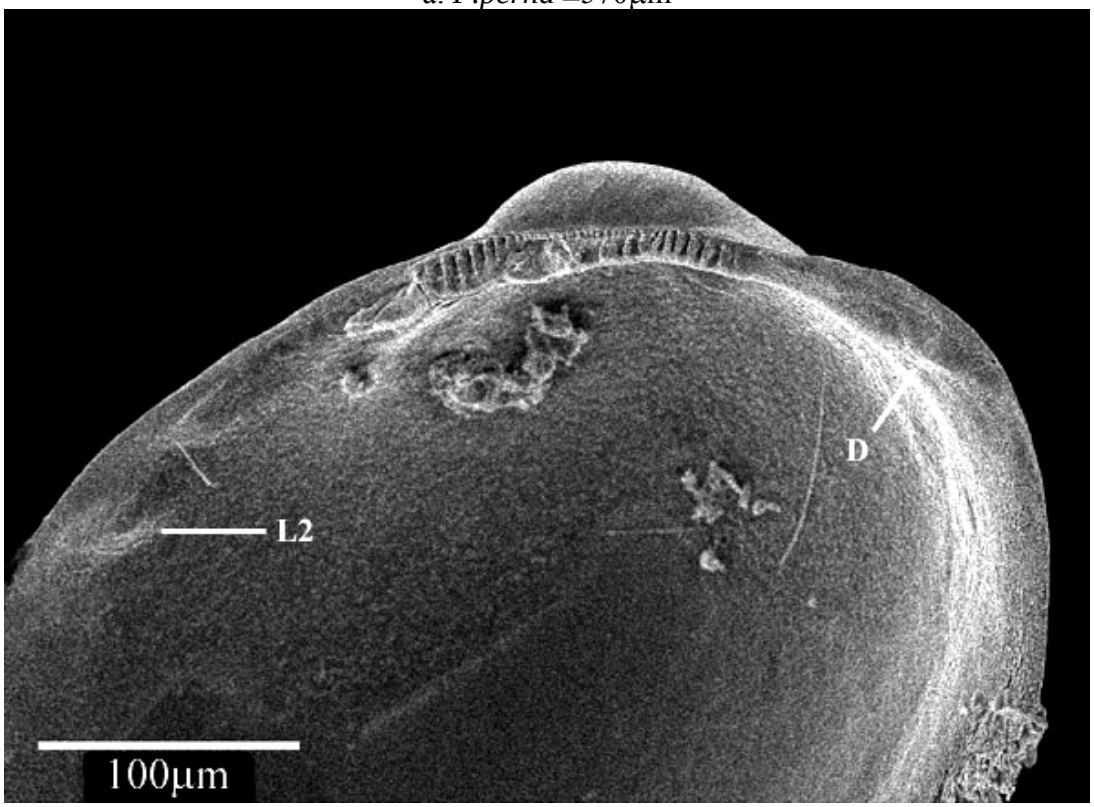
Figure 3.8a-c: Hinge structure of the left valves of a) *P.perna,* b) *M.galloprovincialis* and c) *C.meridionalis* where L1 = primary lateral teeth; LP = the ligament pit; P = provincular teeth.



c. *C.meridionalis* 275µm (270x)

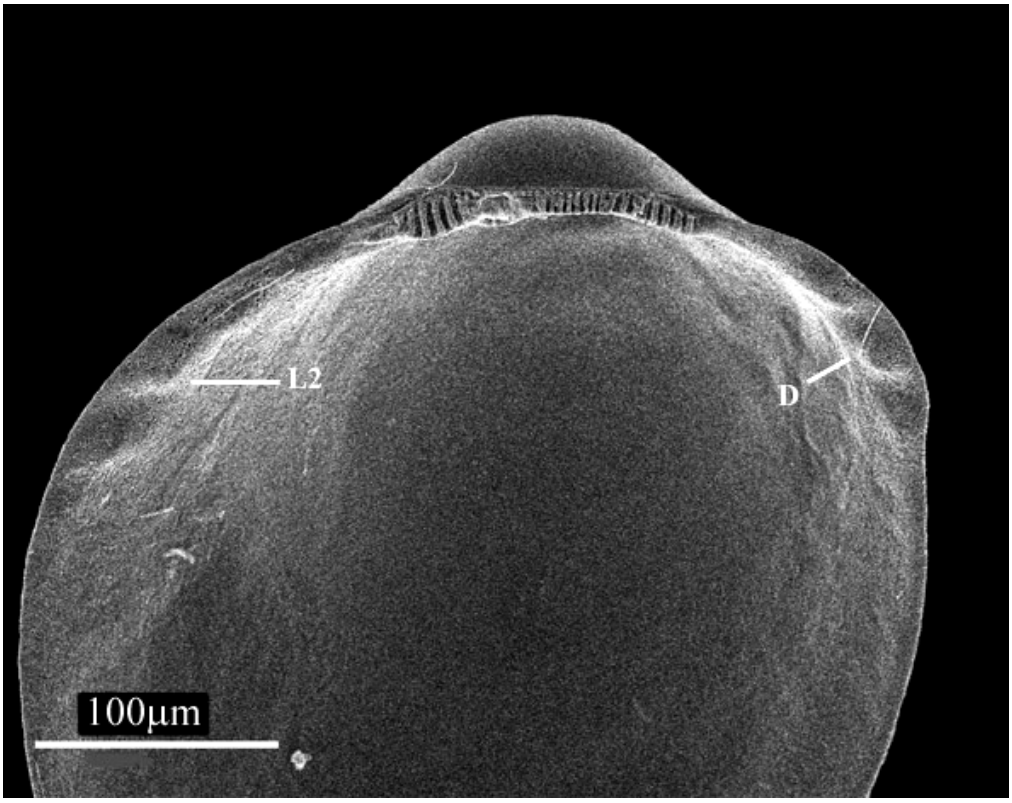


a. *P.perna* ±570µm



b. *M.galloprovincialis* 549µm (250x)

Figure 3.9a-c: Hinge structure of the left valves of a) *P.perna,* b) *M.galloprovincialis* and c) *C.meridionalis* where L2 = secondary lateral teeth; D = dysodont teeth and LP2 = secondary ligament pit



c. *C.meridionalis* 471µm (250x)

M.galloprovincialis and this number did not appear to increase with size. The inner provinculum is also narrower than in *P.perna.* As with *P.perna,* the secondary laterals and dysodont teeth start appearing at 450-500µm (although occasionally development of at least one set of teeth only started in shells 550-650µm long) (Fig. 3.9b; p.84). The secondary laterals (2-3 in number) eventually become obscured by growth of the secondary ligament, but were still observed in some specimens up to 1.8mm long. This species generally has 3 dysodont teeth. The second tooth appears soon after the first one, however the third tooth only develops in larger juveniles (>750µm).

There were no obvious differences in the hinge structure of small post-larvae identified as *C.meridionalis* and *M.galloprovincialis.* The width of the inner provincular teeth is similar in the two species (Fig. 3.8c; p.83). However, it seems that the length of the provinculum may be slightly shorter in *C.meridionalis.* There also appeared to be fewer large outer provincular teeth than in *M.galloprovincialis* although this number was variable in both species. The total number of provincular teeth could not be accurately counted in *C.meridionalis* specimens, but there appear to be 28-32 teeth in early post-larvae. Secondary lateral and dysodont teeth start developing earlier in this species, and first become visible in post-larvae 350-400µm long. In post-larvae 450-500µm these teeth are fairly well developed (Fig. 3.9c; p.85). This species may have 2-3 dysodont teeth. The secondary lateral teeth increase in number as the mussel grows. At sizes of ±2.0mm long, there are 4-5 secondary laterals that are still clearly visible and are also visible under a dissecting microscope. The early development of the secondary lateral and dysodont teeth in *C.meridionalis* appears to be the main hinge feature that may distinguish post-larvae

>350μm from *M.galloprovincialis.* In larger specimens the number of dysodont and particularly secondary lateral teeth may also be useful distinguishing features.

DNA analysis

The success of the primers in terms of specificity varied. The primers designed to target *C.meridionalis* were specific, as these primers never amplified DNA from post-larvae identified as either *P.perna* or *M.galloprovincialis* (Table 3.4; p.88). The primers specific to *P.perna* never amplified with *M.galloprovincialis* post-larvae. However, tests with post-larvae identified as *C.meridionalis* occasionally produced faint bands of a different size to that expected for *P.perna*. The primers designed to target *M.galloprovincialis* were the least successful. These primers amplified DNA from post-larvae of all three species. Non-specific bands were generally either of a different size to that expected for *M.galloprovincialis* specimens or multiple bands were obtained. PCR reactions in which no products were obtained were considered to be due to either failure during the extraction procedure or possibly primer mismatch.

The identification of *M.galloprovincialis* post-larvae was confirmed using PCR as all specimens amplified with their specific primers only (Table 3.4). 87% of the PCR reactions with *P.perna* post-larvae produced successful positive identifications. Two individuals (13%) produced non-specific bands with *M.galloprovincialis*-specific primers only and a few individuals produced bands with both sets of primers. The results for *C.meridionalis* were highly variable. Only 50% of PCR reactions with putative *C.meridionalis* post-larval extracts amplified with the target species primers, as well as one or both of the primers specific to *P.perna* and *M.galloprovincialis.* Positive amplifications were obtained for post-

Table 3.4: Summary of results of PCR reactions with species-specific primers and individual post-larvae of each species, where Pp = *P.perna;* Mg = *M.galloprovincialis* and Cm = *C.meridionalis*. Note that there is some overlap where individuals produced bands with more than one set of primers. Failures are reactions in which no products were obtained.

	Pp primers	**Mg primers**	**Cm primers**	**Failures**
Pp post-larva	87%	40%	0	0
Mg post-larva	0	82%	0	18%
Cm post-larva	42%	58%	50%	14%

larvae ≥300μm in length. Fig. 3.10 (p.90) shows successful results of PCR reactions for all three species. Of the five individuals <300μm identified as *C.meridionalis,* two failed to amplify with any primers, one amplified with *P.perna*-specific primers and two amplified with *M.galloprovincialis*-specific primers. It was also interesting that even larger juveniles (600μm-2.0mm) identified as *C.meridionalis* produced spurious bands. Thus the identification of *C.meridionalis* was not confidently confirmed using mitochondrial DNA (MtDNA) analysis. Possible reasons for complications in this analysis and the merits of the morphological characters described for this species will be discussed.

Size at settlement

Fig. 3.11 (p.91) plots the size frequency distributions of newly settled post-larvae of *P.perna, M.galloprovincialis* and *C.meridionalis.* The majority of settlers were <330μm in length, with a maximum size of 340μm for *P.perna. P.perna* and *M.galloprovincialis* had very similar size frequency distributions, generally ranging from 270-320μm, with the largest percentage of larvae settling at ±290μm. *C.meridionalis* appeared to settle at slightly smaller sizes ranging from 260-310μm with the largest percentages at 260μm and 280μm, although this was based on a comparatively small sample size and on specimens collected from a single location. Based on the observed distributions, primary settlers will be classified as post-larvae of <340μm in length and all mussels of ≥340μm as secondary settlers.

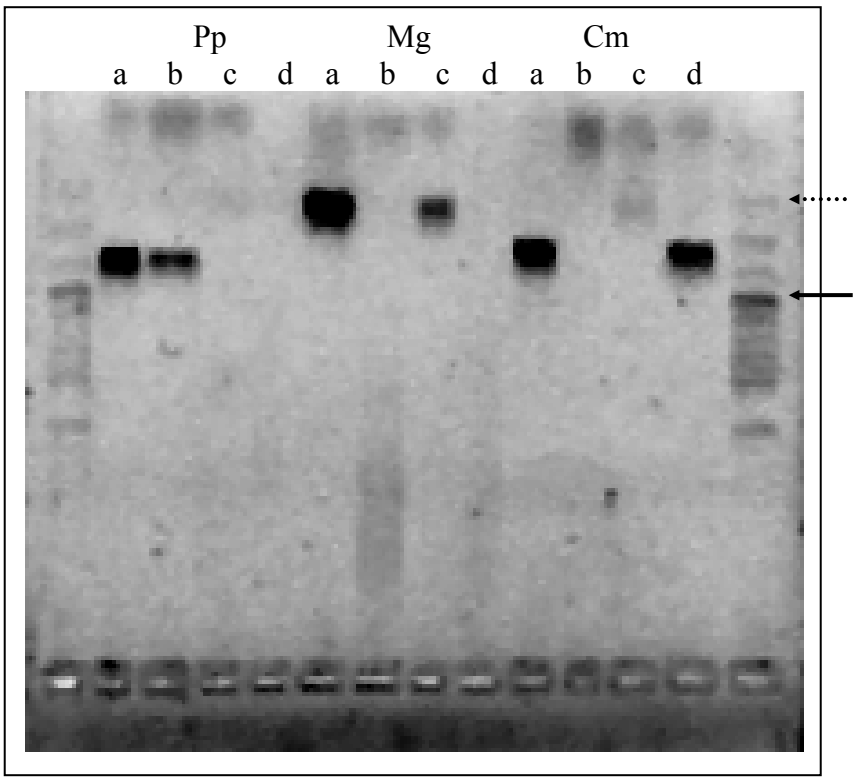


Figure 3.10: Results of PCR amplifications of specific primers with adults and post-larvae of all three species, where Pp = *P.perna;* Mg = *M.galloprovincialis* and Cm =*C.meridionalis;* a = adult extracts of each species amplified with their respective primers; b-d = extracts of individual post-larvae of *P.perna* (390µm), *M.galloprovincialis* (317µm) and *C.meridionalis* (1.86mm) amplified with *P.perna*-specific primers (b); *M.galloprovincialis*-specific primers (c) and *C.meridionalis*-specific primers (d). The dashed arrow indicates the 200bp marker and the solid arrow indicates the 500bp marker

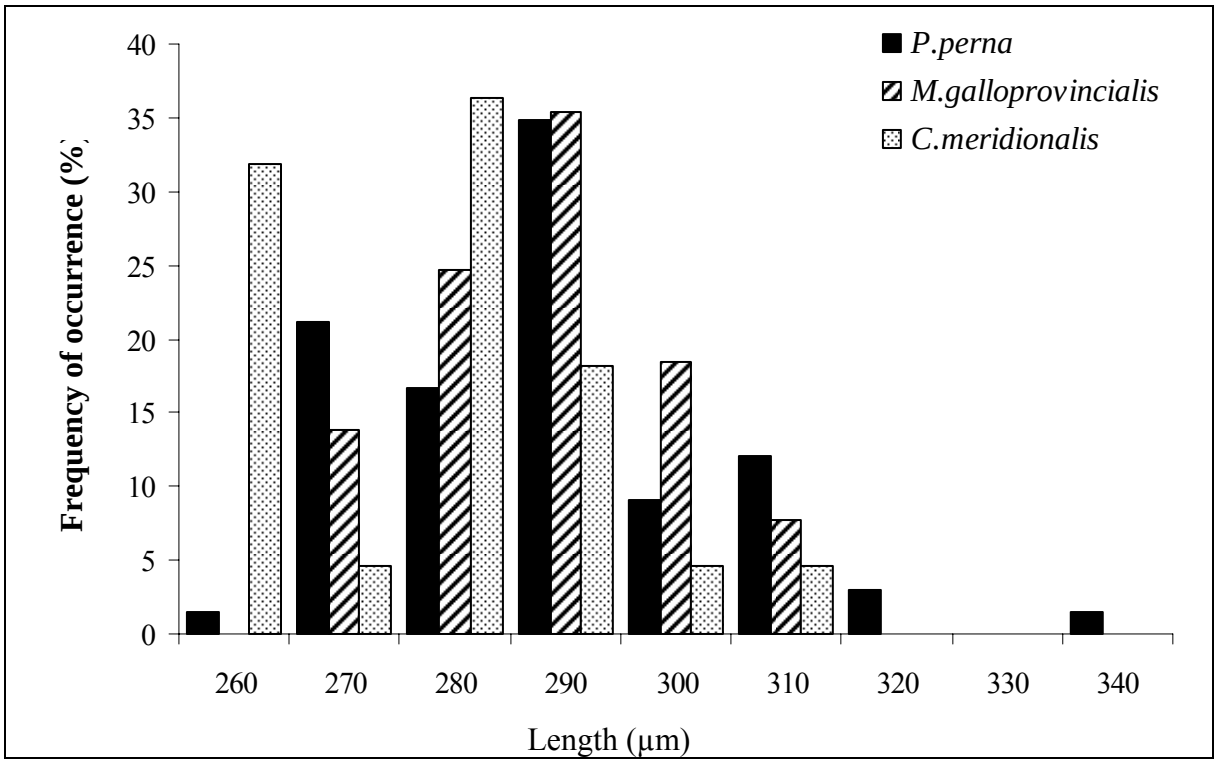


Figure 3.11: Size frequency distributions of newly settled post-larvae. *n*=66; 65 and 28 for *P.perna, M.galloprovincialis* and *C.meridionalis* respectively

3.4 Discussion

Early post-larvae and juveniles of *Perna perna* were easily differentiated using diagnostic shell characters that could be observed under a dissecting microscope. These characters include: a red mark at the tip of each umbo; a reddish hinge region; a narrow but well-rounded umbo centrally positioned on the anterior hinge; and red triangular-shaped patterns on the juvenile shell. Siddall (1980) has described these triangular or "zigzag" markings in young *Perna spp.* Identification of this species was confirmed by examination of the hinge morphology under SEM. Several characteristics were found to distinguish *P.perna* from the other two species. All post-larval specimens identified as *P.perna* had primary lateral teeth on the dorsal hinge structure, a feature that has previously been found to be useful in distinguishing members of the genus *Perna* from *Mytilus* (Siddall 1980). Primary lateral teeth are also absent in *Choromytilus chorus* post-larvae (Ramorino and Campos 1983 cited in Fuller and Lutz 1989) and in *C.meridionalis* and therefore may also be used to distinguish the genera *Perna* and *Choromytilus*. The size of the provinculum and the number of provincular teeth could also be used to distinguish this species. Further confirmation of the identification of this species was obtained using mitochondrial DNA (MtDNA) analysis.

Early post-larvae of *M.galloprovincialis* do not possess any diagnostic shell markings, but could be differentiated using characters based on shape. These include: an elongated ventral shell margin and a wide, flat umbo positioned near the dorsal margin on the anterior hinge. Increased growth along the ventral margin of early post-larvae has been described for several Mytilid species including *M.edulis* (Fuller and Lutz 1989) and *M.galloprovincialis* (Le Pennec and Masson 1976). The dissoconch in this species is usually bluish in colour, which may distinguish this species from *P.perna,* but not *C.meridionalis,* which has similar

colouring. A notable feature is the direction of dissoconch growth, which is skewed towards the postero-dorsal margin. An additional characteristic that is diagnostic in larger juveniles of this species (>500μm) is the presence of "hair" on the dissoconch, or adult shell. These hairs were also observed in published photographs of the shells of three *Mytilus spp.* (including *M.galloprovincialis*) (Martel *et al.* 1999), although the authors do not mention this feature. Examination of the hinge morphology showed considerable differences to the hinge structure of *P.perna,* but not necessarily early post-larval *C.meridionalis*. However, morphological identifications of this species were positively confirmed with MtDNA analysis.

The early post-larval shell of *C.meridionalis* does not have any specific diagnostic characteristics, but several features may distinguish this species from *M.galloprovincialis.* These include: a wider, well-rounded umbo that is more centrally positioned on the anterior hinge; marked pigmentation on the provincular hinge, which is often barely visible in *M.galloprovincialis* shells (see Martel *et al.* 2000); and a rounded or oval shape with considerably less elongation on the ventral margin. Dissoconch growth is initially virtually straight at the posterior tip. Analysis of shell morphometrics revealed that smaller post-larvae (<600μm) could not be completely segregated using the measured criteria, but discriminant function analysis was able to group 79% and 71% of *M.galloprovincialis* and *C.meridionalis* post-larvae correctly, largely based on the width:length ratio, which was greater in *C.meridionalis.* On the other hand, analysis of juvenile (>600μm) shell morphometrics of these species resulted in completely separate groupings. This was confirmed with discriminant function analysis which correctly classified all measured

specimens based on the height:length ratio, which was greater in *C.meridionalis,* and the dorsal apex ratio (see Martel *et al.* 1999), which was smaller in this species.

The hinge morphology of this species did not show any obvious differences to *M.galloprovincialis* in small post-larvae. The number of provincular teeth and hence the length of the provinculum appeared to be slightly smaller than in *M.galloprovincialis.* However, this species could be distinguished by the early development of secondary lateral and dysodont teeth in post-larvae of 350-400μm compared to 450-600μm in *P.perna* and *M.galloprovincialis*. Siddall (1980) documented a similar size for the development of these teeth in *P.perna,* and this has also been noted for *M.edulis* and *M.galloprovincialis* (Le Pennec 1980; Fuller and Lutz 1989). The number of dysodont and secondary lateral teeth also varied between species.

The identification of *C.meridionalis* post-larvae was tentatively confirmed using MtDNA analysis at sizes ≥300μm. At sizes below this, no amplifications were obtained with the primers specific to *C.meridionalis,* although results were only obtained from three specimens of this size. Nevertheless, positive confirmations and negative results were obtained for this species even in specimens ±2.0mm in length. There are two possible explanations for the production of spurious bands in PCR amplifications other than misidentification. One possibility is contamination of the post-larval shells (Wood *et al.* 2003). The mucus threads produced by mussel post-larvae during secondary dispersal may also play a role in settlement by becoming ensnared on substrata (de Blok and Tan-Maas 1977; Cáceres-Martínez *et al.* 1994). Mucus threads also readily adhere to surfaces in the laboratory, including other post-larvae, which often form clumps held together by these

threads (Cácerez-Martínez *et al.* 1994). Thus, there is a possibility that traces of mucus threads could be present on the shells of field-collected post-larvae. Contamination of tissue from other organisms or microorganisms is also possible.

An additional and more likely possibility is that the primers were not specific enough. The number of sequences that were available for *P.perna* and *M.galloprovincialis* was far greater than the eleven sequences for *C.meridionalis,* which in addition, were from specimens collected at only two localities. Thus it is possible that there was more haplotype variation at what was considered to be a conserved region in the DNA sequences of *C.meridionalis*. To improve these primers, additional sequences of *C.meridionalis* need to be obtained from across its distribution range.

It is clear that there may be confusion in the identity of early post-larvae of *M.galloprovincialis* and *C.meridionalis* (<300µm). While several morphological characteristics of early *C.meridionalis* were highlighted, none of them can be considered to be definitively diagnostic. An additional concern was the similarity in the hinge morphologies of small post-larvae of these species. Perhaps the most convincing evidence confirming the identification of *C.meridionalis* comes from the patterns of settlement and recruitment. It will be seen in Chapter 4 that post-larvae identified as *C.meridionalis* settled primarily within the narrow distribution range occupied by adults in Plettenberg Bay. Furthermore, no post-larvae identified as *C.meridionalis* were ever observed settling in Tsitsikamma, where adults of this species do not occur. Thus at present it will be assumed that the identifications of all *C.meridionalis* post-larvae are correct. However, further confirmation using MtDNA analysis is required, particularly of the smallest post-larvae.

Examination of the size at settlement of these species revealed that the post-larvae of *P.perna* and *M.galloprovincialis* settled at sizes of 270-320µm. A similar size range of primary settlers has been reported for *M.galloprovincialis* (HRS-Brenko 1973; Phillips 2002; Phillips and Gaines 2002). *C.meridionalis* settled within a slightly smaller size range than the other two species (260-310µm), although this was based on a comparatively small sample size (*n*=22). This is also considerably smaller than the size range of 295-365µm previously reported for this species, both in laboratory-reared individuals and in the field (du Plessis 1977). Since the size at settlement of this species was only measured using larger individuals (350-500µm), the size range obtained in the present study is unlikely to be due to misidentification. Size at settlement in mussels may vary both spatially and temporally (Martel *et al* 2001; Phillips and Gaines 2002). A preliminary analysis comparing the size at settlement of *P.perna* and *M.galloprovincialis* between locations and sites revealed that both species settled at significantly smaller sizes at Lookout Beach (276µm ±0.01) than at Beacon Isle or locations in Tsitsikamma (295-299µm ±0.01) (unpub. data). There was also no difference in the average size at settlement between all three species at Lookout Beach. Thus the smaller size at settlement obtained for *C.meridionalis* in this study may reflect differences in the physical environment between coasts. Based on the size range at which *P.perna, M.galloprovincialis* and *C.meridionalis* settle, primary settlers will be classified as post-larvae <340µm in length, and secondary settlers as post-larvae ≥340µm in length.

Chapter 4

Spatial and temporal variations in settlement and recruitment

4.1 Introduction

There is increasing evidence to suggest that variations in the settlement and recruitment rates of sessile marine invertebrates are frequently the cause of variations in adult community structure and dynamics (Denley and Underwood 1979; Gaines and Roughgarden 1985; Bushek 1988; Davis 1988; Raimondi 1988; Roughgarden *et al.* 1988). Settlement may be defined as the process of initial attachment of larvae to the substratum (Keough and Downes 1982). Thus in marine invertebrates with a planktonic life history stage, settlement rates will be partly dependent on the supply and delivery of competent larvae to the shore. Several studies have found that settlement is closely correlated to the availability of larvae in the near shore water column (Gaines *et al.* 1985; Bertness *et al.* 1996; Jeffery and Underwood 2000; Chícaro and Chícaro 2001; Bellgrove *et al.* 2004). However, settlement includes an additional component which is that of larval behaviour and habitat selection.

Larval supply will be influenced by the timing and intensity of spawning events, which may vary between different geographic populations and with reproductive season (Seed 1969a; van Erkom Schurink and Griffiths 1991). Temporal variations in settlement may be closely linked to the timing of spawning events (Chipperfield 1953; Seed 1969a; Hrs-Brenko 1973; Berry 1978; King *et al.* 1989). However, spawning in adult populations is also often uncoupled from local settlement (Dare 1976; van Erkom Schurink and Griffiths 1991; Snodden and Roberts 1997). This may be due to larval mortality in the plankton (Connell 1985; Chícaro and Chícaro 2000; Qui *et al.* 2002) or larval dispersal (van Erkom Schurink and Griffiths 1991; Chícaro and Chícaro 2000). There are several factors that may influence the dispersal or retention of larvae and their delivery onto the shore. Large-scale oceanographic processes such as upwelling may transport larvae offshore, while subsequent

relaxation events or downwelling move larvae back onshore (Roughgarden *et al.* 1988; Connolly and Roughgarden 1998). On the other hand, upwelling near convex coastlines can result in retention of larvae near the shore (Graham and Largier 1997). Hydrodynamic conditions associated with shoreline topography (Thiébaut *et al.* 1994; Archambault and Bourget 1999) and tidal cycles (Chícaro and Chícaro 2001) may result in variations in local larval supply. Wind-driven currents may also be important both as an agent of dispersal (McQuaid and Phillips 2000) and in the onshore transport of larvae (Bertness *et al.* 1996; Young *et al.* 1996; Jeffery and Underwood 2000).

Thus large-scale processes are largely responsible for the movement of larvae in the plankton and their subsequent delivery to nearshore sites. However, once in the nearshore water column, small-scale processes may modify settlement patterns. For example, settlement often decreases with increasing tidal height and it has been suggested that this may be caused by reduced immersion time further up shore (Chipperfield 1953; Denley and Underwood 1979; Cáceres-Martínez and Figueras 1997). In addition, the zonation or vertical distribution of larvae in the water column may result in zonation at settlement (Bushek 1988; Miron *et al.* 1995). Hydrodynamic forces such as turbulence as a result of substrate heterogeneity may enhance or inhibit settlement (Abelson and Denny 1997; Snodden and Roberts 1997) and predation by adults has been known to significantly reduce the availability of larvae for settlement (Navarrete and Wieters 2000). Several studies have also found that settlement is directly related to the availability of free space and that once free space becomes limited, settlement is reduced (Dare 1976; Gaines and Roughgarden 1985; Minchinton and Scheibling 1993a; Osman and Whitlatch 1995a, 1995b).

Habitat selection in marine invertebrates has been widely documented (Barnett *et al.* 1979; Raimondi 1988; Morse 1991; Minchinton and Scheibling 1993a; Osman and Whitlatch

1995a, 1995b; Lemire and Bourget 1996; Zhao *et al.* 2003). Substratum selection may occur in response to chemical cues associated with adult conspecifics, natural biofilms or other organisms (Raimondi 1988; Morse 1991; Osman and Whitlatch 1995b; Zhao *et al.* 2003), small-scale substratum heterogeneities (Lemire and Bourget 1996) or local flow regimes (Bushek 1988; Abelson and Denny 1997). For example, Raimondi (1988) found that the barnacle *Chthamalus anisopoma* actively settled in response to cues associated with organisms living within its tidal range. Therefore vertical zonation patterns may be caused by selective settlement. Larvae have also been known to avoid settling near competitive dominants (Young and Chia 1981; Grosberg 1981; Peterson 1984). Differential settlement may therefore serve as an important mechanism by which species are able to avoid detrimental post-settlement interactions (Bushek 1988).

Recruitment refers to the number of settlers that have survived after a certain period of time. It therefore includes settlement and a defined period of post-settlement mortality (Keough and Downes 1982; Connell 1985). However, defining settlement and recruitment is more problematic for mussels as they may enter a secondary dispersal phase after settlement (Hunt and Scheibling 1997). This process of detachment and re-attachment in mussels may also occur many times before juveniles permanently enter the population (Bayne 1964). Secondary settlers can by definition be called recruits since they have settled and survived for a period of time. In this study, the distinction will be made based on sampling frequency (Hunt and Scheibling 1997). All mussels collected on a daily basis will be referred to as settlers (primary or secondary) and all those collected at monthly intervals as recruits.

In Chapter 2 it was determined that density of *Mytilus galloprovincialis* increased with increasing tidal height, while *Perna perna* showed the opposite trend. There were also location-specific and site-specific differences in patterns of adult distribution and abundance. It was hypothesised that these differences may be caused by differential settlement. The aim of this chapter is to determine whether there is species-specific differential settlement and/or recruitment with respect to tidal height, or at larger spatial scales of location and site, and whether these patterns vary temporally. Furthermore, I examine whether temporal variation in settlement and recruitment is associated with daily or monthly wind patterns.

Secondary settlement has been found to be important in determining the distribution and abundance of adult mussel populations (Buchanan and Babcock 1997; Hunt and Scheibling 1998). Since secondary dispersal is often an active process that is facilitated by byssus drifting (Sigurdsson *et al.* 1976; de Blok and Tan-Maas 1977; Lane *et al.* 1985), subsequent settlement may also be selective at any time during this dispersal phase. For example, Buchanan and Babcock (1997) found that substratum preferences in juvenile *Perna canaliculis* changed as a function of size and age. An additional aim is therefore to examine whether differential settlement of the study species is size-dependent.

4.2 Materials and methods

Both settlers and recruits were collected using plastic filamentous pot scourers or scouring pads. All pads were initially soaked in sea water for a day to remove any chemical traces that could influence settlement. Pads were attached to screws that had been drilled into the rocks. For daily collections, these were attached using a series of washers and cable ties and

for more secure attachment the screws were passed through the pads with a flat metal disc above to hold them down. Six screws were placed in each zone, generally 0.5-1.0m apart.

Settlement

Settlement was measured over a month from the 27 March to the 26 April 2001. Pads were collected on the same day from each zone at locations in Plettenberg Bay and Tsitsikamma. Recruitment pads were also collected monthly. Therefore of the six pads placed in each zone, three were replaced daily and the remaining three were collected at the end of the month. On two occasions pads could not be collected for a few days over neap tide. These samples were excluded as the numbers would reflect recruitment rather than settlement. Thus only pads that had been out for a period of 24 hours were analysed. Settlement was also measured over a twelve day period from the 17-28 March 2003. Settlement in April 2001 and monthly recruitment in Tsitsikamma were generally very low, so the experiment in 2003 was only done at Lookout Beach and Beacon Isle in Plettenberg Bay. Six pads were placed in each zone and at each location and were collected and replaced daily at low tide. Due to rough seas, Beacon Isle was only sampled until 24 March.

Scouring pads were frozen immediately after collection. In the laboratory settlers were removed by vigorously shaking the pad in water which was then poured through two sets of sieves of 1mm and 0.15mm mesh size. This process was repeated three or four times to ensure that all mussels were removed. Juveniles were collected off the larger sieve with a pair of tweezers and measured under a dissecting microscope using a micrometer. Smaller mussels were then washed out of the 0.15mm sieve into a petri dish. The dish was examined under the microscope and settlers were transferred to a piece of filter paper using a pipette. Post-larvae were subsequently identified using the morphological characteristics

described in Chapter 3, and measured under the dissecting microscope. All samples were then stored in 70% alcohol.

Mussels were distinguished as being either "dead" or "alive" at the time of collection for the purpose of measuring post-settlement mortality. Mussels that had no tissue remaining in the shell were classified as dead. For settlement, both dead and live mussels were counted, assuming that all mussels were alive upon arrival.

Primary settlers were categorised as mussels <0.34mm in length (Chapter 3). All mussels ≥0.34mm were considered to be secondary settlers. Primary and secondary settlement were analysed separately. To examine whether there was size-dependent differential settlement amongst secondary settlers these were further divided into the following size categories: 0.34-0.59mm; 0.6-1.49mm; 1.5-3.0mm; >3.0mm.

Recruitment

Scouring pads were collected monthly from June 2000-July 2001 at Lookout Beach and Beacon Isle in Plettenberg Bay. Recruitment was also measured from August 2000 at Sandbaai and from October 2000 at Driftwood Bay in Tsitsikamma. On some occasions pads were collected at two-week intervals and on one occasion after six weeks. If certain samples could not be collected on a specific occasion the number of recruits in each pad was averaged for the two time periods over which they were in the field. Data were grouped into months and then standardised by converting the number of recruits to recruitment in 30 days.

Recruitment pads were processed in the same way as the settlement samples. However, samples with exceptionally high numbers were subsampled using a plankton splitter. Mussels collected in each sieve were subsampled separately. Generally only the smaller mussels (<1.0mm) had to be subsampled. The plankton splitter is not considered to be ideal for larger, heavier organisms which as a result of their weight may not be distributed randomly. This method was therefore tested by measuring the number of each species in the different size categories from a whole sample and then subsampling it. In addition, numbers from two subsamples of the same sample were counted and compared. In both cases the number of recruits in the different size categories from the samples and/or subsamples was sufficiently similar to proceed with this method. Recruits were also distinguished as either dead or alive upon collection and only numbers of live mussels were plotted and analysed.

Recruits were divided into the following size categories: 0.34-0.59mm; 0.6-1.49mm; 1.5-3.0mm; >3.0-5.0mm; >5.0mm. Primary settlers were excluded as these were assumed to have settled within 24hrs of collection and were therefore not considered to be recruits. Due to the large numbers that were frequently found in pads, mussels were placed directly into size groups (under the microscope) and only measured when necessary. To examine whether there was temporal variation in the size of recruits, graphs were plotted using the average size of recruits each month. Since mussels were not individually measured, the mean size of each size category was used.

Analyses

1. Settlement

Primary and secondary settlement of *P.perna, M.galloprovincialis* and *Choromytilus meridionalis* were plotted using the average number of settlers per pad on each date. In 2001, more than 80% of the dates had a sample size of three replicates in Tsitsikamma. Since settlement was so low at this site, the data were not analysed. At locations in Plettenberg Bay, sample sizes were too low on most days (n = 1-3) to use date as a factor in the analysis. Data were therefore analysed using ANCOVA (Statistica 6.1, Advanced General Linear Models). Using daily tidal amplitudes as a covariate, it was possible to pool dates, solving the problem of poor sample sizes, while taking into account the variability associated with date. This method also provides additional information on whether tidal amplitude has an effect on settlement.

In 2003, more than 90% of the dates had sample sizes between four and six at both locations. The data from this year could have been analysed with date as a factor. However, I decided that including tidal amplitude as a covariate would be more informative than the effect of date. The data were therefore analysed using ANCOVA with location, zone and species as factors. To determine whether there was differential settlement in different size classes, size was included as a fixed factor and examined at each location separately. Analyses on settlement of *C.meridionalis* were performed separately. Post-hoc comparisons were made using Newman-Keuls multiple range tests. All graphs showing post-hoc comparison results were plotted with Standard Errors. Data did not require transformation.

2. Recruitment

Recruitment data were grouped into seasons. Since samples were only collected at both locations in Tsitsikamma from October onwards, seasons were defined as follows: October-November (spring), December-February (summer), March-May (autumn) and June-July

(winter). A mixed model ANOVA was performed with season, site, location, zone and species as factors, where location was random and nested within site. Recruitment of *C.meridionalis* was analysed separately. This species exhibited large recruitment peaks in winter 2000 (June-August) with little recruitment in the winter of 2001. Therefore data from the winter months in 2000 were used in the seasonal analysis.

Wind correlations

Records of wind data were obtained from a weather station just off shore of Tsitsikamma (South African Weather Services, George Airport). Wind directions and velocities were recorded hourly. The coastline in this region is south-facing and wind directions were grouped according to those directions that would cause surface currents to flow either onshore, offshore, alongshore in an easterly direction (easterly) or alongshore in a westerly direction (westerly). In the shallower waters of bays, current direction is usually aligned with wind direction due to frictional effects (Schumann *et al.* 1982; Goschen and Schumann 1988). For Plettenberg Bay, winds were grouped in the following manner: east winds (easterly); west winds (westerly); east-south-east to west-south-west winds (onshore); west-north-west to east-north-east winds (offshore). No correlations were performed for Tsitsikamma, where both settlement and recruitment rates were very low.

For daily samples, only data for the twelve hours before pads were collected were considered. The number of hours for which the winds in each category blew was counted and the average velocity over that time was calculated for each day. The duration and velocity of winds were correlated with the average daily settlement of mussels from the low-shore only for both years. Settlement patterns differed between primary and secondary

settlers, species and locations. Therefore correlations were performed for each separately. In April 2001, only data from the 5 April onwards were used.

For recruitment data, the number of hours for which winds blew in each month was recorded. In order to be able to correlate wind data with the monthly recruitment rates, data were standardised to a period of 30 days. Wind velocities did not need to be standardised. Average recruit density for each month was calculated for each location by pooling zones. All correlations were performed using Pearson's Product-Moment Correlation.

4.3 Results

Settlement

1. April 2001

Figs. 4.1-4.2 a-c (p.107-108) illustrate the patterns of primary settlement of *P.perna*, *M.galloprovincialis* and *C.meridionalis* at Lookout Beach and Beacon Isle respectively. Primary settlement of *P.perna* was very low and generally consisted of single individuals arriving randomly with respect to zone and date. Settlement of *M.galloprovincialis* varied considerably among dates. A major peak was observed at both locations on the low-shore on the day after new moon spring tide, however there was very little settlement on the following full moon spring tide. Generally it appears that settlement was both higher and more frequent at Beacon Isle than at Lookout Beach. In addition, it seemed that when settlement was high, it was greatest on the low-shore. However, when settlement was low there was generally little difference among zones. Primary settlement of *C.meridionalis* was very low and appeared to be slightly greater on the low-shore at Lookout Beach.

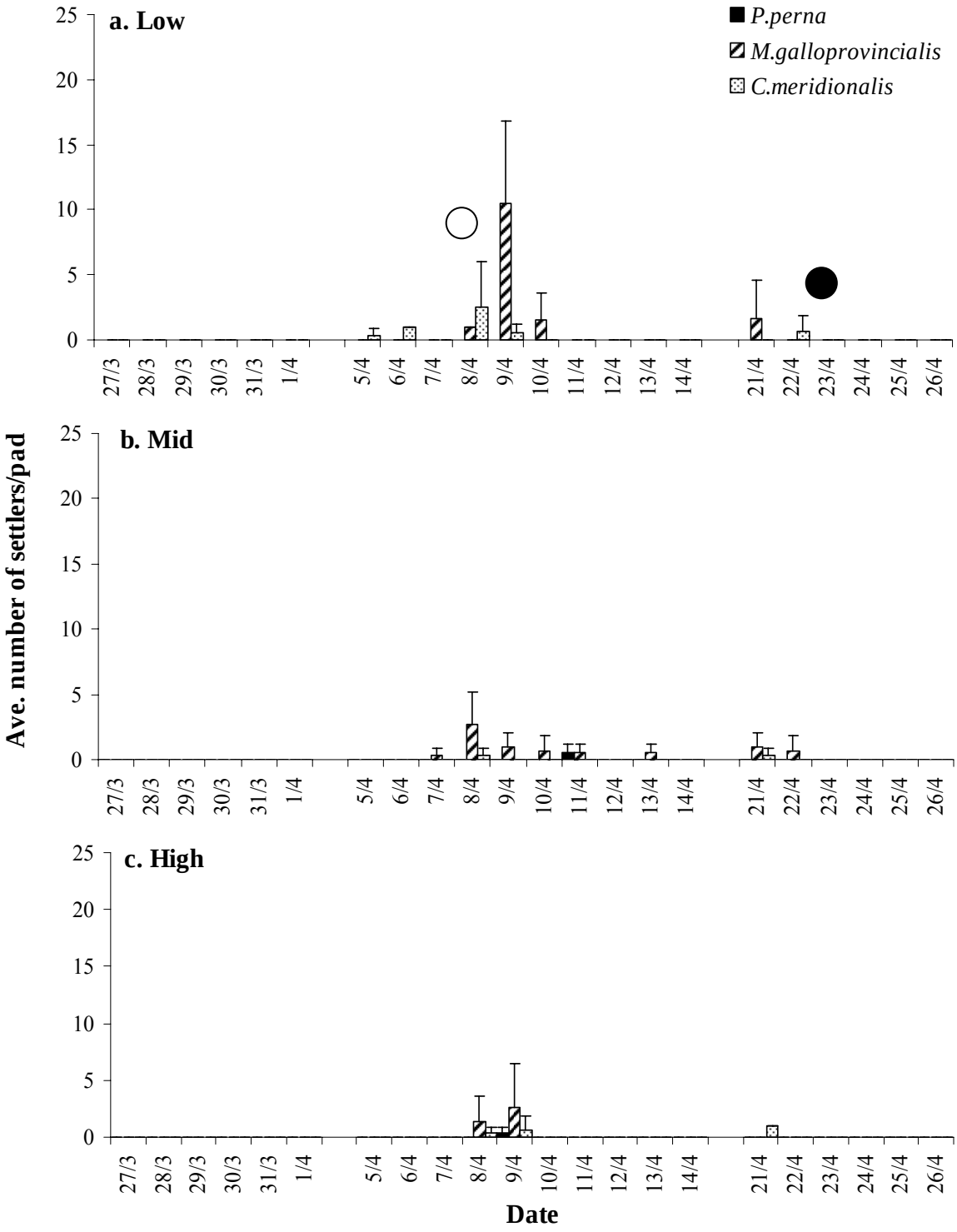


Figure 4.1 a-c: Primary settlement of *P.perna*, *M.galloprovincialis* and *C.meridionalis* at different tidal heights at Lookout Beach, Plettenberg Bay, in April 2001. Gaps indicate days when samples could not be collected over neap tides. Circles indicate spring tides at new moon (open) and full moon (filled). Error bars represent standard deviations. Lack of standard deviations indicates a sample size of one.

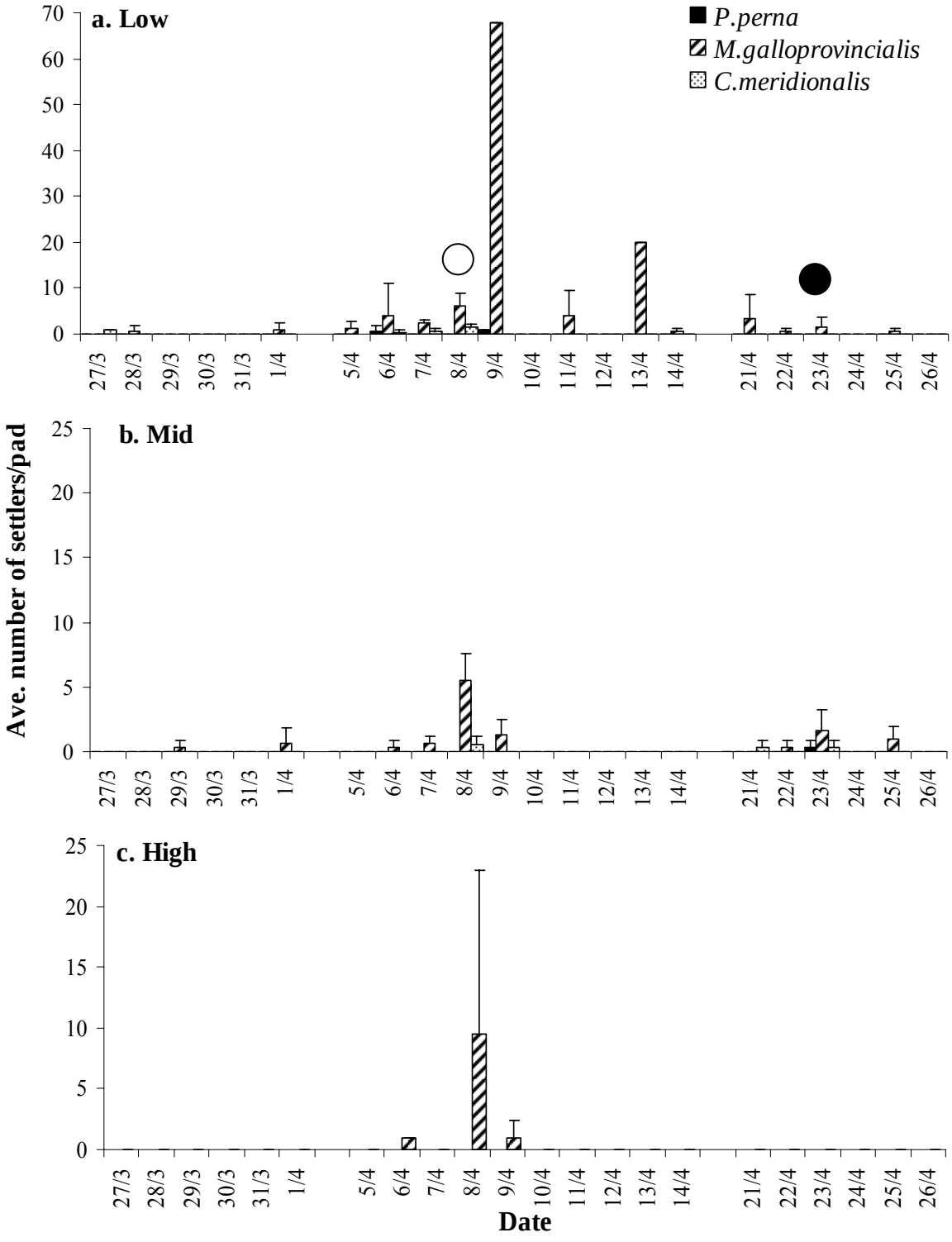


Figure 4.2 a-c: Primary settlement of *P.perna*, *M.galloprovincialis* and *C.meridionalis* at different tidal heights at Beacon Isle, Plettenberg Bay, in April 2001. Note that the y-axis values are higher than at Lookout Beach. Error bars represent standard deviations.

Patterns of secondary settlement generally differed considerably between the two locations (Figs. 4.3-4.5 a-c; p.110-112). Settlement at Lookout Beach appeared to be more sporadic compared to Beacon Isle which experienced a fairly continuous trickle of secondary settlers throughout the sampling period. While there was a fairly large peak in settlement of all three species on the new moon spring tide at Lookout Beach, there was no obvious peak at Beacon Isle. *M.galloprovincialis* and *C.meridionalis* also exhibited a second peak around the following spring tide at Lookout Beach which suggests a possible correlation between the tidal cycles and settlement. The fact that peak settlement of these species also appeared to be synchronised suggests that settlement rates are influenced by the same processes and/or that they tend to aggregate in the water column. Peak settlement of *C.meridionalis* was also much greater than that of *P.perna* or *M.galloprovincialis* at Lookout Beach, but appeared to be location-specific with comparatively little settlement at Beacon Isle. Although peaks in both primary and secondary settlement appeared to be associated with spring tides, these peaks were not synchronised, being a day apart.

Settlement of *P.perna* and *M.galloprovincialis* in Tsitsikamma was very low and sporadic (Figs. 4.6-4.7 a-c; p.113-114). Settlers of both species appeared to arrive randomly with respect to date, zone and location. In other words, there was no relationship with tidal cycle. There did not appear to be any trend in the abundance of settlers with zone. It also seemed that overall settlement was slightly greater at Driftwood Bay than at Sandbaai. There was no primary settlement at this site and total numbers of secondary settlers were substantially lower than in Plettenberg Bay. There was no settlement of *C.meridionalis* at this site.

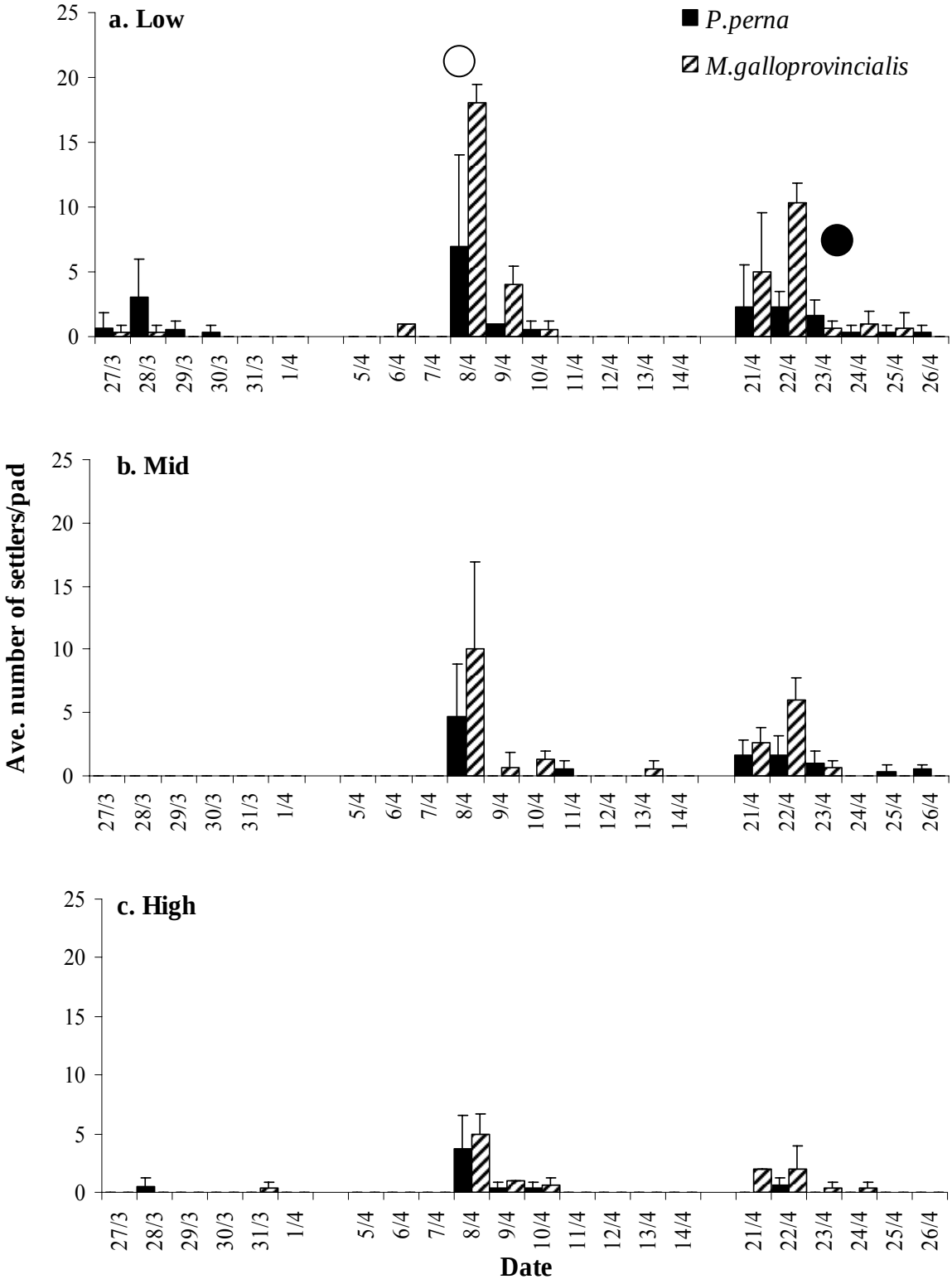


Figure 4.3 a-c: Secondary settlement of *P.perna* and *M.galloprovincialis* at different tidal heights at Lookout Beach in April 2001. Error bars represent standard deviations

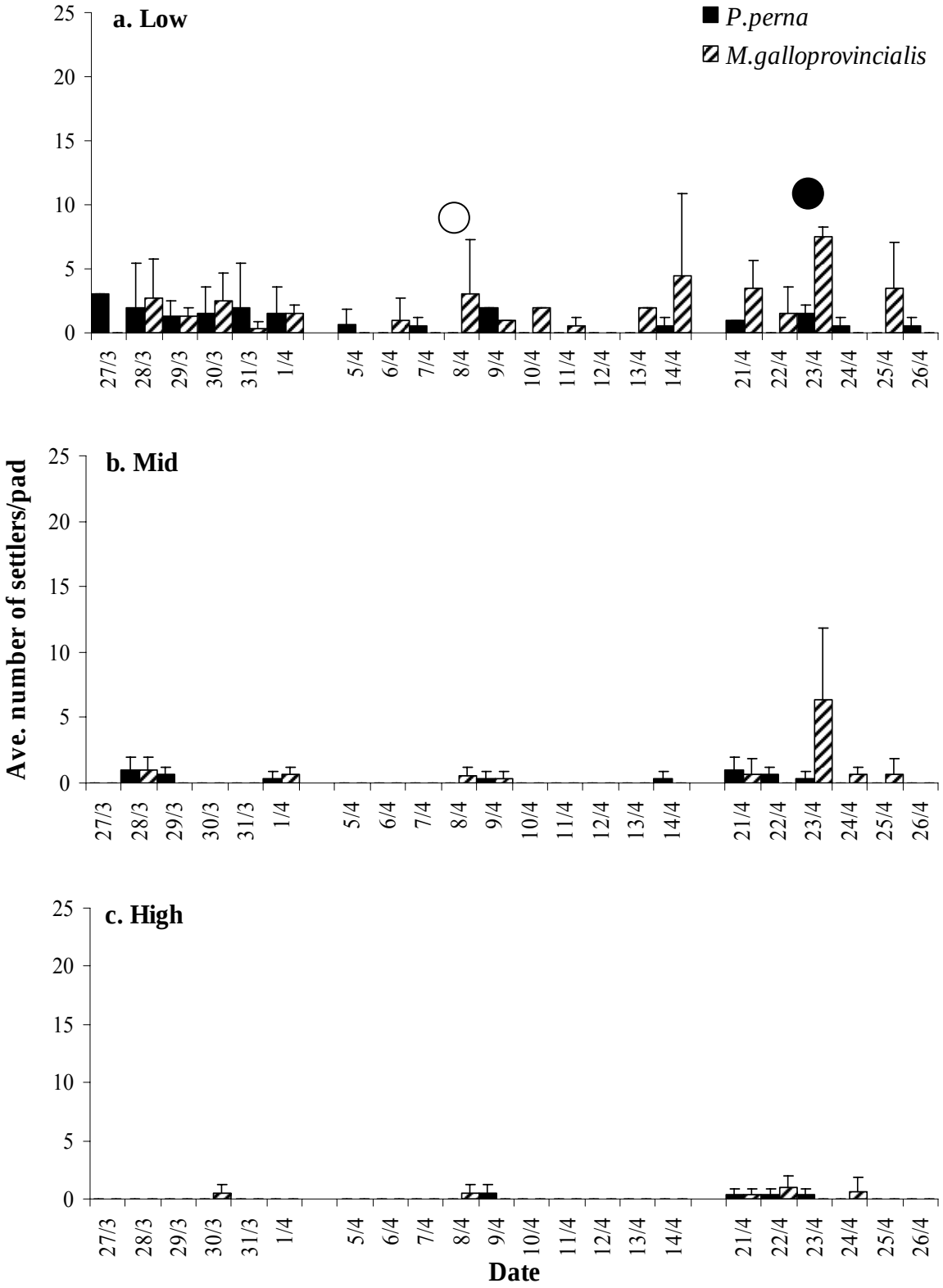


Figure 4.4 a-c: Secondary settlement of *P.perna* and *M.galloprovincialis* at different tidal heights at Beacon Isle in April 2001. Error bars represent standard deviations

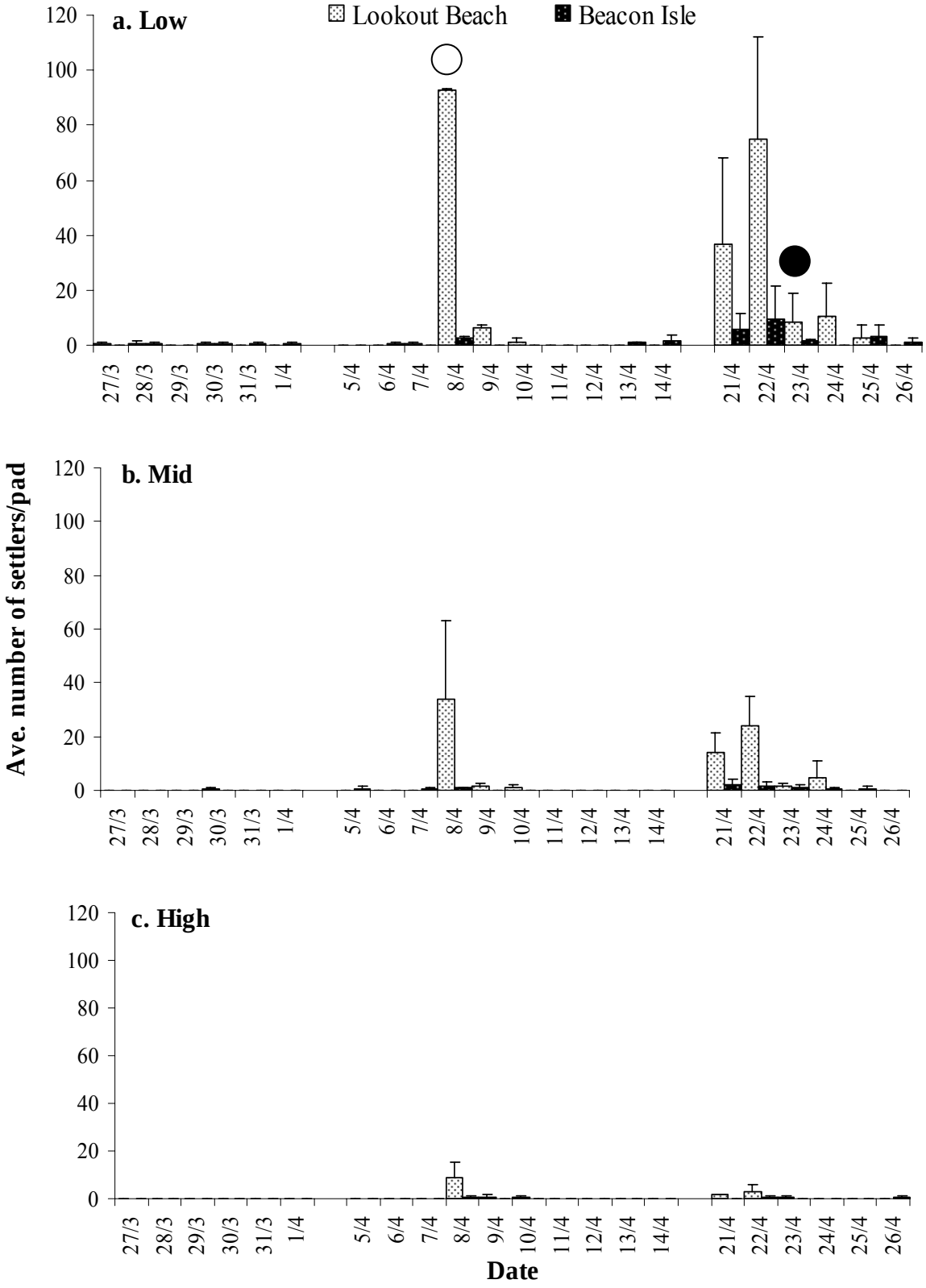


Figure 4.5 a-c: Secondary settlement of *C.meridionalis* at different tidal heights at Lookout Beach and Beacon Isle in April 2001. Note that the y-axis values are higher on the low-shore. Error bars represent standard deviations

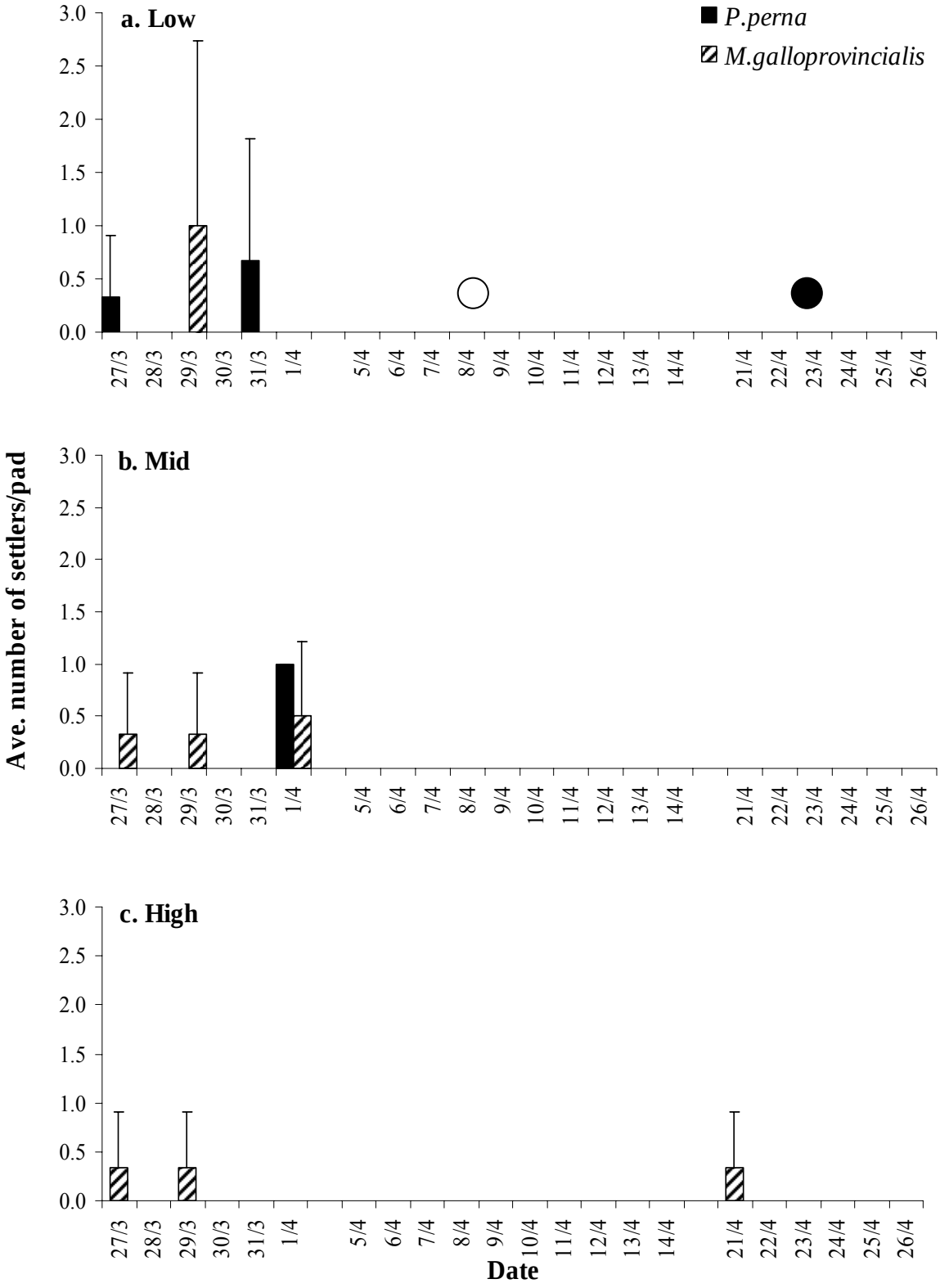


Figure 4.6 a-c: Secondary settlement of *P.perna* and *M.galloprovincialis* at different tidal heights at Sandbaai, Tsitsikamma, in April 2001. Note the difference in y-axis values in comparison to Plettenberg Bay. Error bars represent standard deviations

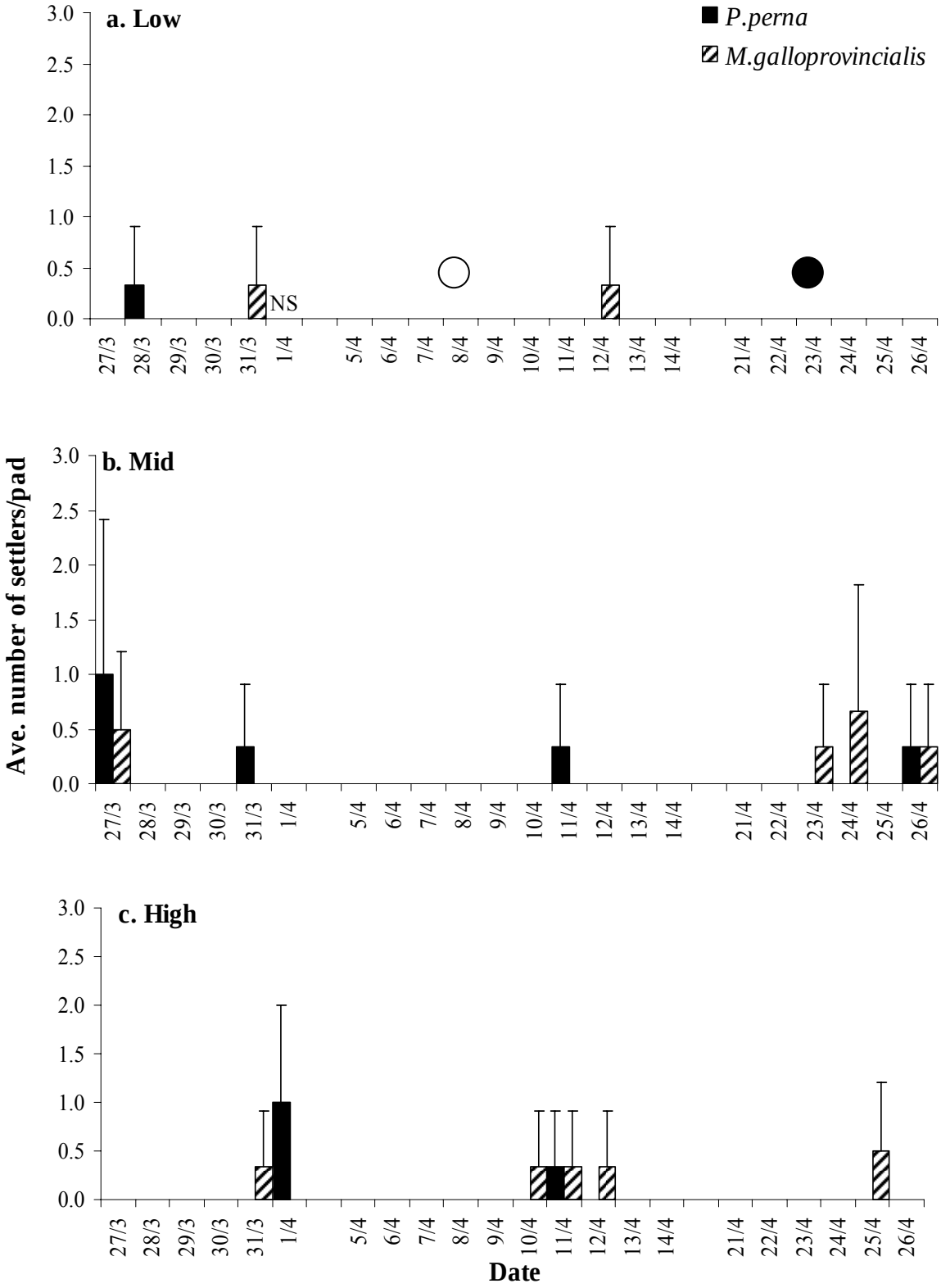


Figure 4.7 a-c: Secondary settlement of *P.perna* and *M.galloprovincialis* at different tidal heights at Driftwood Bay, Tsitsikamma, in April 2001. "NS" denotes days when no samples were recovered. Error bars represent standard deviations

For the analysis of primary settlement in Plettenberg Bay, only days from the 5 April onwards were included as there was virtually no settlement before this. Since there was so little primary settlement of *P.perna* and *C.meridionalis,* only data for *M.galloprovincialis* were analysed. There was a significant interaction between location and zone ($F=3.34$; $p=0.04$; $df=2$). The difference between locations was only significant on the low-shore and settlement was higher at Beacon Isle (Fig. 4.8; p.116). At this location, primary settlement of *M.galloprovincialis* was also significantly greater on the low-shore than in the other zones. Settlement tended to decrease with increasing tidal height at Lookout Beach but with no significant differences between zones. There was a significant relationship between primary settlement of *M.galloprovincialis* and tidal amplitude ($p<0.05$).

All dates were included in the analysis of secondary settlement of *P.perna* and *M.galloprovincialis.* There were significant interactions between location and species and between zone and species ($p=0.01$) (Table 4.1; p.117). Secondary settlement of *M.galloprovincialis* was significantly greater than that of *P.perna* at Lookout Beach but with no difference between species at Beacon Isle (Fig. 4.9; p.118). In contrast to primary settlement, secondary settlement of *M.galloprovincialis* was significantly greater at Lookout Beach than at Beacon Isle, while secondary settlement of *P.perna* did not differ between locations. *M.galloprovincialis* also settled in significantly greater numbers than *P.perna* on the low-shore but not in the upper zones, and it seems that the difference between species decreased further up the shore so that settlement rates were low and similar on the high-shore (Fig. 4.10; p.118). Secondary settlement of *M.galloprovincialis* was greater on the low-shore than in the upper zones and settlement of *P.perna* was significantly greater on the low-shore compared to the high-shore. Results also suggest that differences between variables (zones, species etc.) were usually only observed when

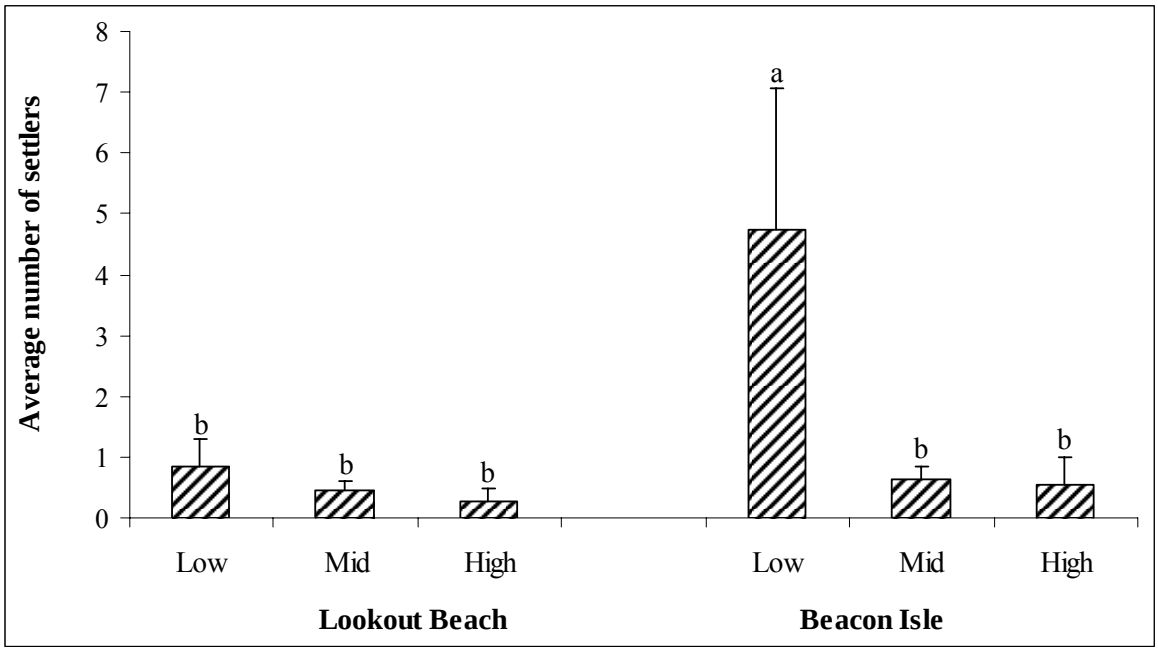


Figure 4.8: Post-hoc comparison of the interaction between location and zone on primary settlement of *M.galloprovincialis* in April 2001. Similar letters indicate homogeneous groups. Error bars represent standard errors

Table 4.1: Three-way ANCOVA on secondary settlement of *P.perna* and *M.galloprovincialis* in different zones at locations in Plettenberg Bay in April 2001

	Effect	**Df**	**MS**	**F**	**p**
tidal amplitude*	Fixed	1	71.05	19.146	<0.001
location	Random	1	11.62	5.319	0.168
zone*	Fixed	2	67.55	108.620	0.01*
species	Fixed	1	39.32	24.426	0.127
location×zone	Random	2	0.62	14.186	0.066
location×species*	Random	1	1.61	25.799	0.01*
zone×species*	Fixed	2	6.46	147.773	0.01*
location×zone×species	Random	2	0.04	0.012	0.988
Error		651	3.71		

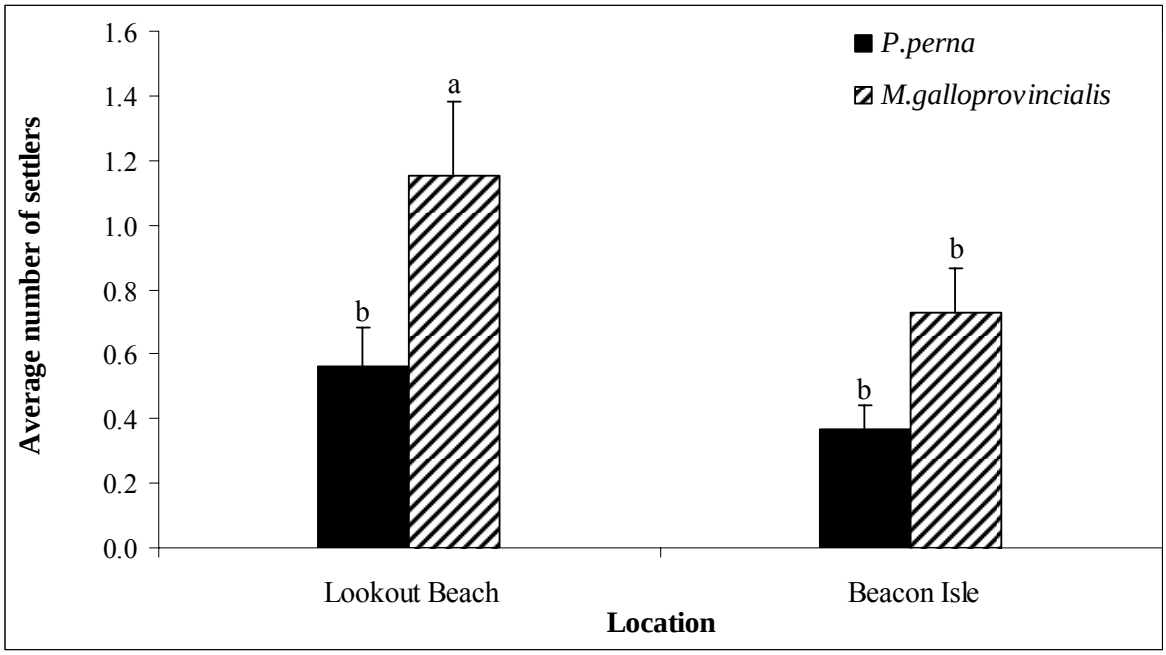


Figure 4.9: Results of post-hoc comparison of the interaction between location and species on secondary settlement of *P.perna* and *M.galloprovincialis* in Plettenberg Bay in April 2001. Error bars represent standard errors

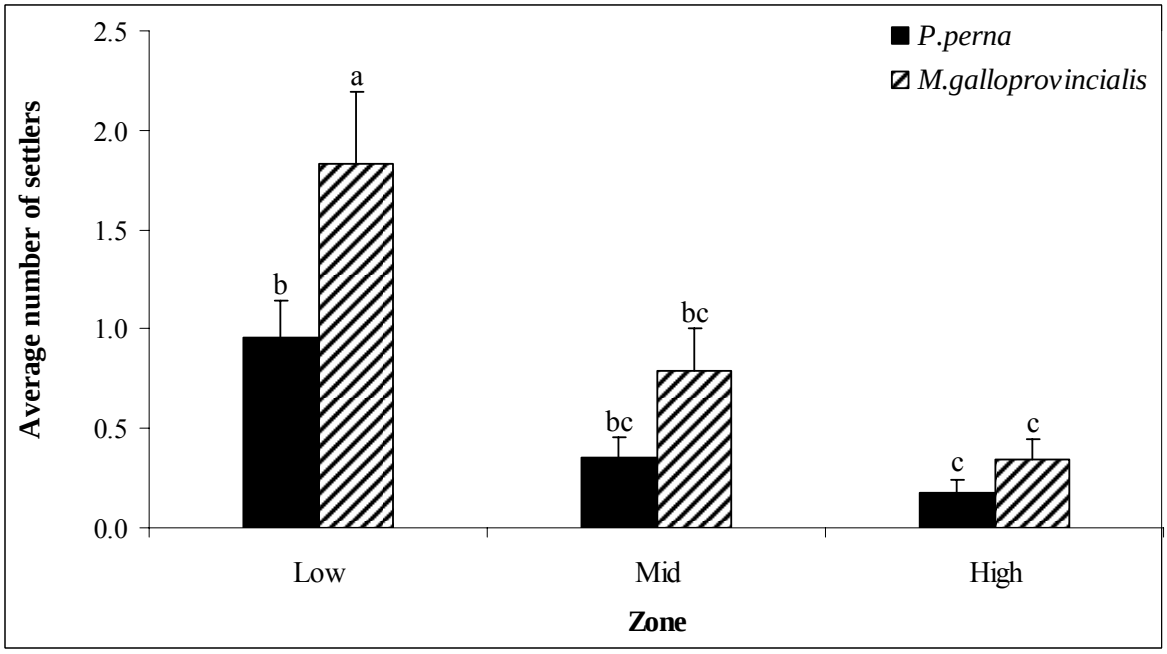


Figure 4.10: Post-hoc comparison of the interaction between zone and species on secondary settlement of *P.perna* and *M.galloprovincialis* in Plettenberg Bay, April 2001. Error bars represent standard errors

settlement was high. The effect of tidal amplitude was highly significant ($p<0.001$).

In the analysis of secondary settlement of *C.meridionalis* only data from the 5 April onwards were included as there was virtually no settlement before this. There was no significant effect of either location or zone, however, there was a significant interaction between them ($F=5.18$; $p=0.01$). The difference between locations was only significant on the low-shore and secondary settlement was greater at Lookout Beach than at Beacon Isle (Fig. 4.11; p.120). At Lookout Beach, low-shore settlement was also significantly greater than in the upper zones, while there was no difference between zones at Beacon Isle. Settlement of this species was significantly correlated with tidal amplitude ($p=0.06$)

2. March 2003

Primary settlement was generally very low at both locations (Figs. 4.12-4.13; p.121-122), but there was a clear shift in the prevalence of each species with time. *P.perna* was the dominant settler for the first five days, after which settlement of *M.galloprovincialis* increased. Settlement of *M.galloprovincialis* peaked three days after neap tide. In contrast to primary settlement of this species in 2001, this peak was also observed in the upper zones although in smaller numbers. Primary settlement of *C.meridionalis* also occurred primarily in the second week at Lookout Beach, but no settlement occurred at Beacon Isle. No peaks were observed for this species or *P.perna* and there was no apparent relationship between the tidal cycles and settlement.

Patterns of secondary settlement differed considerably between the two locations (Figs. 4.14-4.15; p.123-124). Furthermore there was no synchrony between primary and secondary settlement. As in 2001, secondary settlement of all three species peaked at the

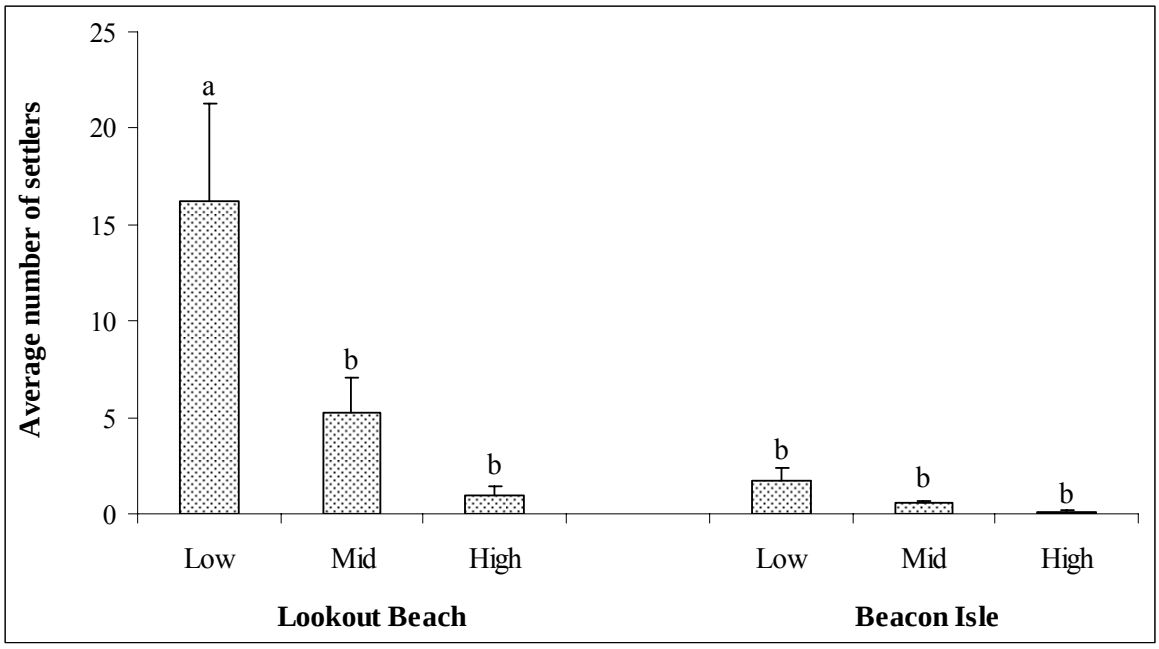


Figure 4.11: Post-hoc comparison of the interaction between location and zone on secondary settlement of *C.meridionalis* in Plettenberg Bay in April 2001. Error bars represent standard errors

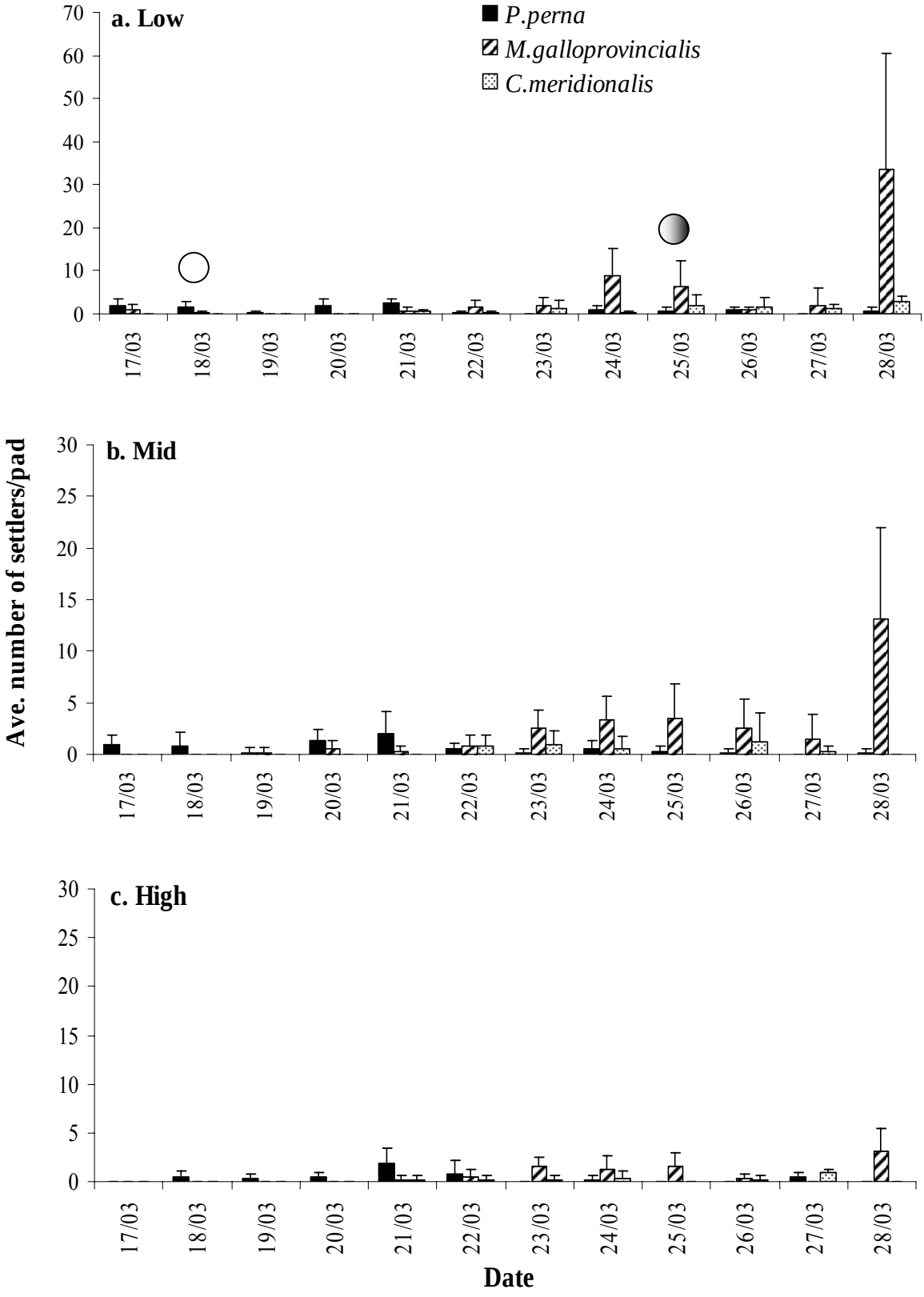


Figure 4.12 a-c: Primary settlement of *P.perna*, *M.galloprovincialis* and *C.meridionalis* in different zones at Lookout Beach, Plettenberg Bay, in March 2003. Circles indicate spring tide at new moon (open) and neap tide (shaded). Error bars represent standard deviations

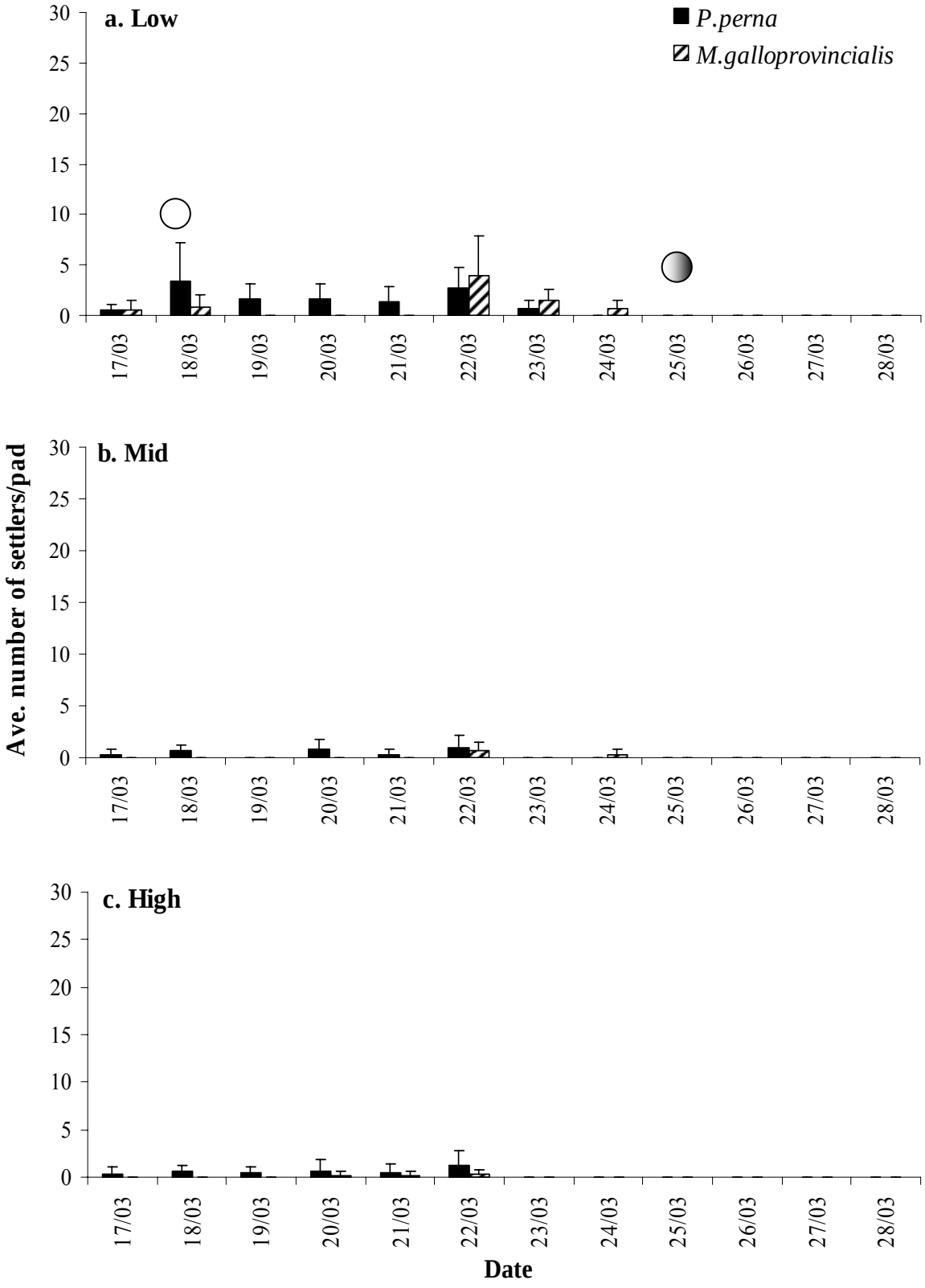


Figure 4.13 a-c: Primary settlement of *P.perna* and *M.galloprovincialis* at different tidal heights at Beacon Isle, Plettenberg Bay, in March 2003. Note that the y-axis values are lower than at Lookout Beach. Error bars represent standard deviations

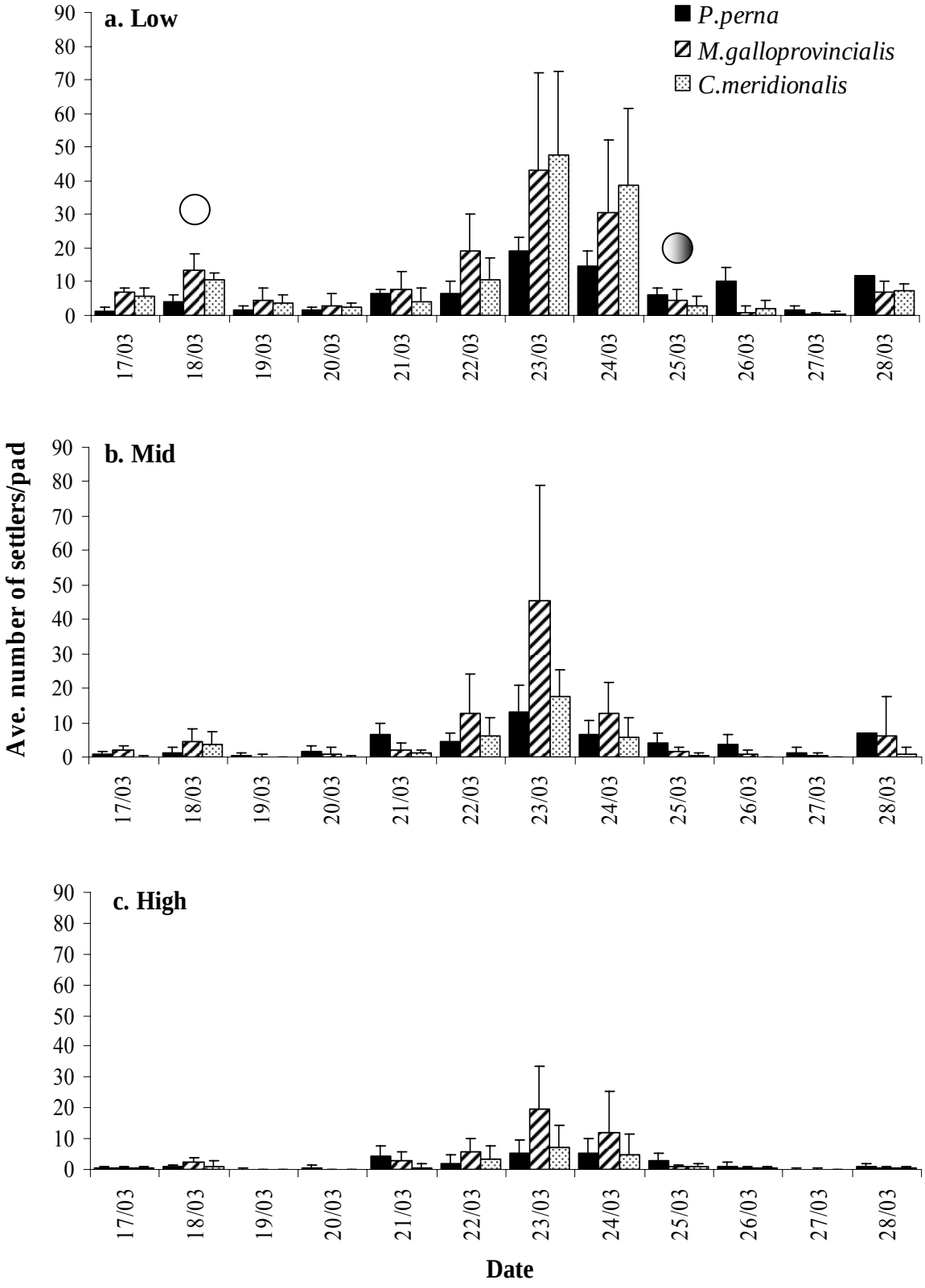


Figure 4.14 a-c: Secondary settlement of *P.perna*, *M.galloprovincialis* and *C.meridionalis* at different tidal heights at Lookout Beach in March 2003. Error bars represent standard deviations

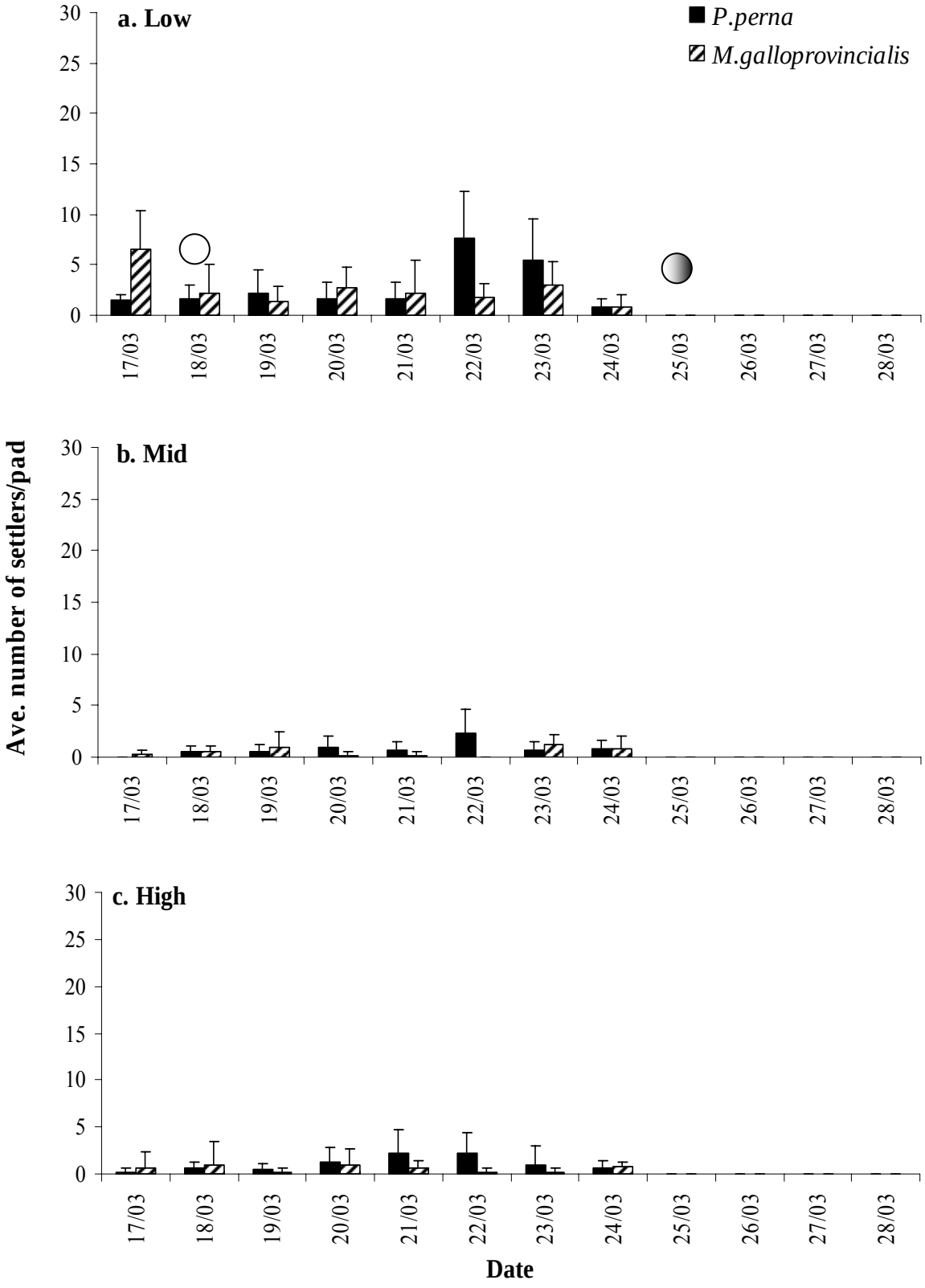


Figure 4.15 a-c: Secondary settlement of *P.perna* and *M.galloprovincialis* at different tidal heights at Beacon Isle in March 2003. Note that the y-axis values are lower than at Lookout Beach. Error bars represent standard deviations

same time at Lookout Beach. However, this peak occurred two days before neap tide rather than over the spring tide. This peak was also not apparent at Beacon Isle where settlement was comparatively low. Secondary settlement of *C.meridionalis* was restricted to Lookout Beach, with no settlement at Beacon Isle. In contrast to primary settlement, there was a tendency for secondary settlers of *P.perna* to be more abundant in the second week. This possibly reflects growth of primary settlers that settled within the first week and then relocated.

In the analysis of primary settlement of *P.perna* and *M.galloprovincialis* between locations, no main effects or interactions were significant. Thus primary settlement in the first eight days did not differ between species, zones or locations. Analysis of primary settlement at Lookout Beach over twelve days revealed that both zone and species had significant effects and there was also an interaction between them ($F=4.1$; $p=0.02$). Post-hoc comparison revealed that settlement of both species decreased with increasing tidal height (Fig. 4.16; p.126). Primary settlement of *P.perna* did not differ significantly between zones, while *M.galloprovincialis* settled in significantly greater numbers on the low-shore than in the upper zones. Settlement of *M.galloprovincialis* was significantly greater than that of *P.perna* on the low-shore with no difference between species in the upper zones. Again, it is clear that the difference between species decreases further up shore so that there was very little difference in high-shore settlement. The correlation between primary settlement and tidal amplitude was significant ($p<0.01$).

Surprisingly, there were also no significant main effects or interactions in the analysis of secondary settlement of *P.perna* and *M.galloprovincialis* between locations and zones, although settlement was clearly greater at Lookout Beach than at Beacon Isle. The analysis

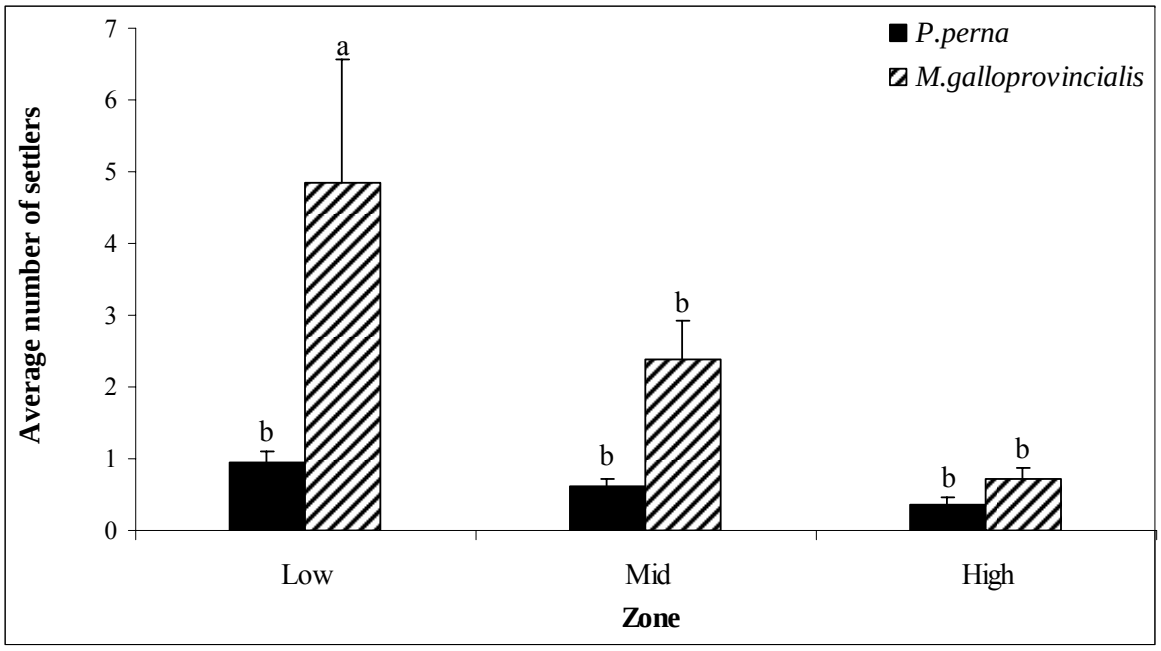


Figure 4.16: Post-hoc comparison results of the interaction between zone and species on primary settlement of *P.perna* and *M.galloprovincialis* at Lookout Beach in March 2003. Error bars represent standard errors

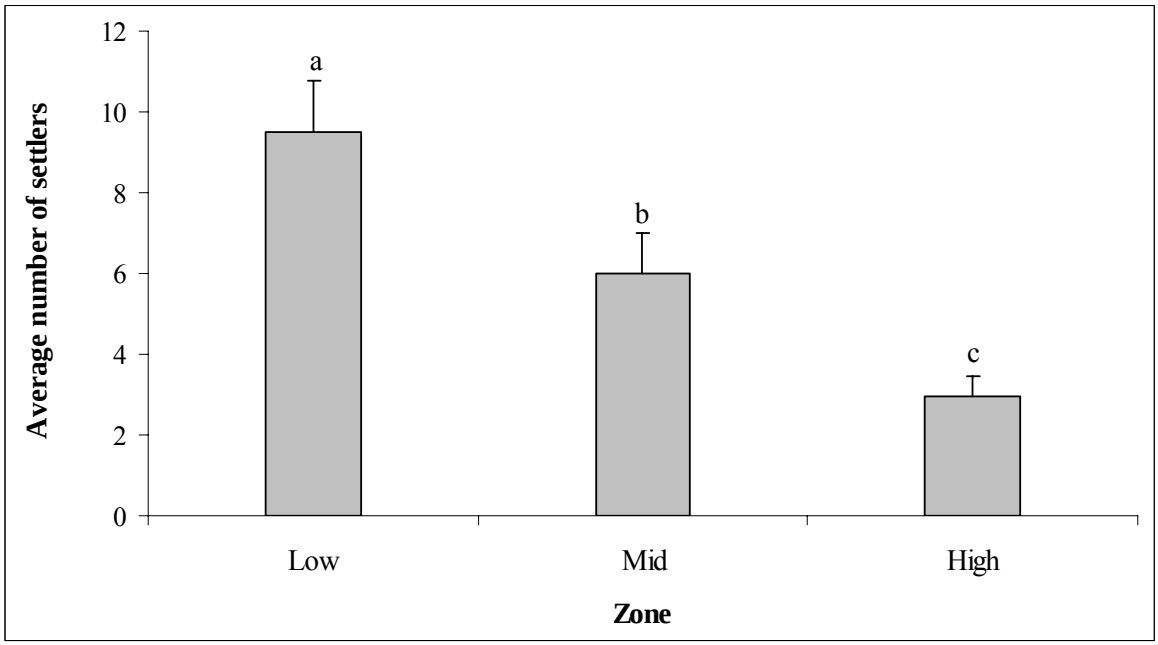


Figure 4.17: The effect of zone on secondary settlement of mussels (*P.perna* and *M.galloprovincialis*) at Lookout Beach in March 2003. Error bars represent standard errors

of secondary settlement at Lookout Beach alone revealed that zone and species had highly significant effects but there was no interaction between them ($F=11.65$; $p<0.001$ and $F=9.38$; $p=0.002$ respectively). Secondary settlement of both species decreased significantly with increasing tidal height (Fig. 4.17; p.126) and *M.galloprovincialis* settled in significantly greater numbers than *P.perna.* There was no relationship between tidal amplitude and secondary settlement on this occasion ($p=0.14$).

The analyses of both primary and secondary settlement of *C.meridionalis* at Lookout Beach revealed that zone had a highly significant effect ($F=8.01$; $p<0.001$ and $F=16.05$; $p<0.001$ for primary and secondary settlement respectively). Settlement of this species was significantly greater on the low-shore than on the mid or high-shore (Fig. 4.18; p.128). Surprisingly, secondary settlement of *C.meridionalis* at Lookout Beach was strongly correlated with tidal amplitude, even though patterns of settlement were similar to that of the other species.

Summary:

The patterns of primary settlement and secondary settlement of these species differed slightly between 2001 and 2003. For example, primary settlement of *M.galloprovincialis* was greater at Beacon Isle in 2001 but showed a distinct peak only at Lookout Beach in 2003. Settlement in 2001 also peaked around spring tides while settlement in 2003 peaked around neap tide. However, there were also many similarities in patterns of settlement on these two occasions. Primary settlement of *M.galloprovincialis* was much greater than that of either *C.meridionalis* or *P.perna* on both occasions, which probably reflects the time of year at which samples were taken. In contrast, secondary settlers of all three species were abundant on both occasions. Unlike primary settlement, secondary settlement was

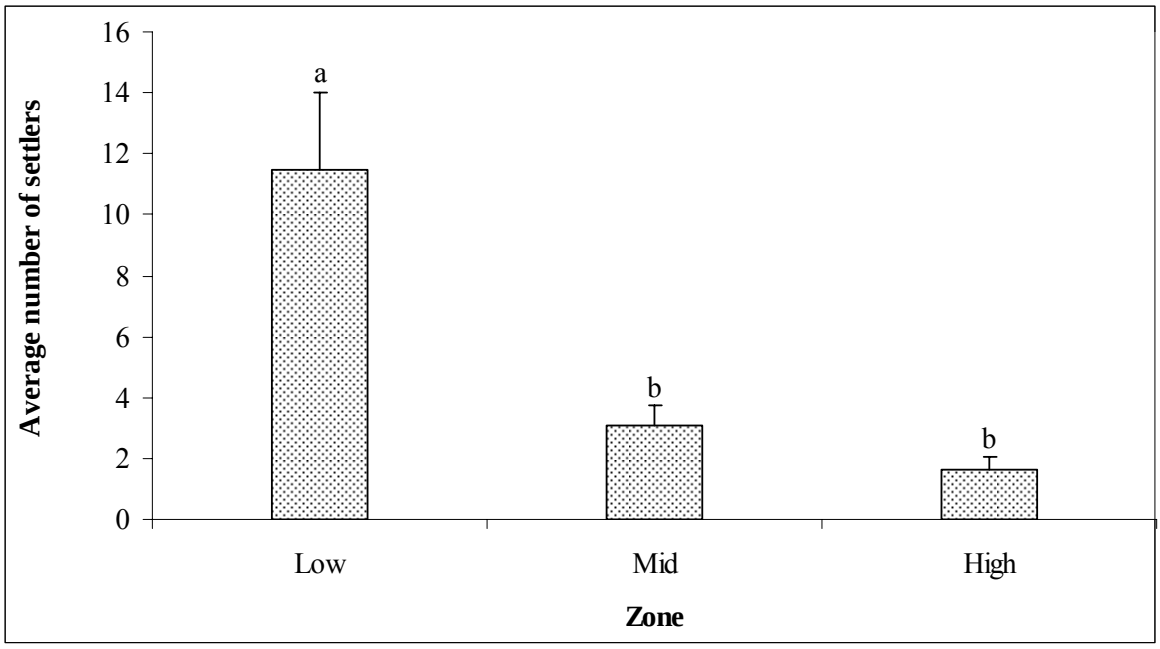


Figure 4.18: Post-hoc comparison of the effect of zone on secondary settlement of *C.meridionalis* at Lookout Beach in March 2003. Error bars represent standard errors

synchronized among species. Both primary and secondary settlement decreased up the shore. Primary settlement tended to be concentrated on the low-shore while secondary settlement of *M.galloprovincialis* and *P.perna* decreased more gradually with increasing tidal height.

These results suggest that differential settlement with respect to zone may contribute to the distribution patterns of *P.perna* and *C.meridionalis.* The fact that settlement of *C.meridionalis* was strongly location-specific suggests that settlement may be selective in this species, specifically for the low-shore at Lookout Beach. Nevertheless, there was some settlement of this species in the upper zones, thus preferential settlement may not be the only factor determining its distribution. In contrast, the settlement patterns of *M.galloprovincialis* do not reflect its distribution. This species does not settle preferentially on the high-shore and furthermore there were no species-specific differences in settlement rate in this zone. *M.galloprovincialis* also settled in significantly greater numbers on the low-shore than in the upper zones. There also appeared to be a relationship with tidal amplitude and settlement.

3. Size-dependent settlement in 2003

Analysis of the effect of size on secondary settlement at Lookout Beach revealed that zone, species and size all had highly significant effects, and there was also a significant interaction between them (p=0.01; Table 4.2; p.130). The results from the post-hoc comparison of this interaction were quite interesting. The most immediate observation is that settlement decreased with increasing tidal height regardless of size or species (Fig. 4.19; p.130). However, there were significant differences in the size of settlers between species. *P.perna* was most abundant in the smallest size class (0.34-0.59) which appears to

Table 4.2: Three-way ANCOVA on the size of secondary settlers of *P.perna* and *M.galloprovincialis* in different zones at Lookout Beach in March 2003

	Df	MS	F	p
tidal amplitude*	1	58.0191	4.49325	0.03*
zone*	2	304.5180	23.58320	<0.001*
species*	1	245.0418	18.97711	<0.001*
size*	3	349.1590	27.04040	<0.001*
zone×species	2	17.7318	1.37323	0.254
zone×size*	6	35.9277	2.78240	0.01*
species×size*	3	493.3222	38.20502	<0.001*
zone×species×size*	6	38.4661	2.97898	0.01*
Error	1471	12.9125		

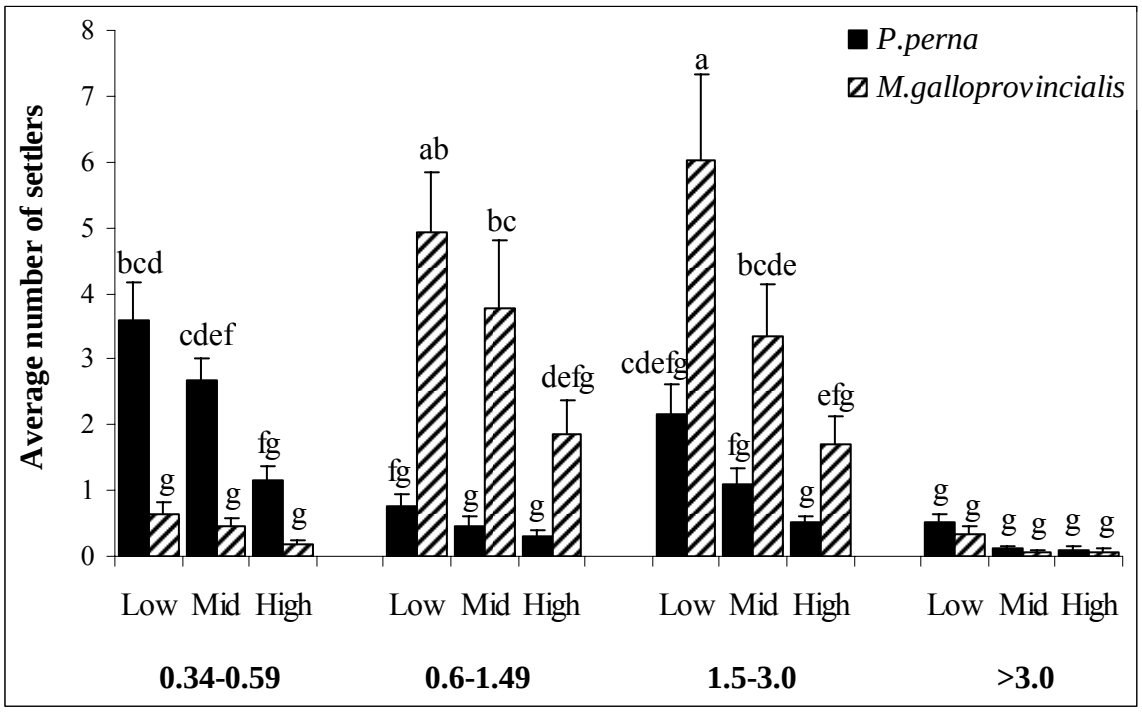


Figure 4.19: Post-hoc comparison of the interaction between zone, species and size on secondary settlement of *P.perna* and *M.galloprovincialis* at Lookout Beach in March 2003. Error bars represent standard errors

confirm the suggestion that these were the primary settlers that arrived within the first week and then relocated. This also implies that primary settlers may only remain attached for a few days before detaching and re-entering the water column. In contrast there were very few secondary settlers of *M.galloprovincialis* in this size class and the majority were 0.6-3.0mm in length. There were also very few settlers >3.0mm of both species and these appeared to occur primarily on the low-shore, although there were no significant differences between zones.

Results from the analysis at Beacon Isle revealed that zone, species and size all had significant effects and there was a significant interaction between them ($p<0.001$; Table 4.3; p.132). Settlement was largely confined to the low-shore regardless of species or size (Fig. 4.20; p.132). However, small *P.perna* settled in significantly greater numbers in the upper zones than either species in the larger size classes. The ratio of the two species in the different size groups was the same as at Lookout Beach suggesting that there was no difference in the size frequency distributions between the two locations. Again there were very few settlers >3.0mm, with the majority occurring on the low-shore, although the difference between zones was not significant.

The analysis of size of *C.meridionalis* at Lookout Beach gave the same results, with significant effects of zone and species and a significant interaction between them ($F=7.93$; $p<0.001$). The majority of settlers were 0.6-3.0mm in length, which were largely confined to the low-shore (Fig. 4.21; p.133). There were very few settlers in the smallest and largest size classes with no significant differences between zones.

Table 4.3: Three-way ANCOVA on the size of secondary settlers of *P.perna* and *M.galloprovincialis* in different zones at Beacon Isle in March 2003

	Df	MS	F	p
tidal amplitude	1	0.010	0.012	0.913
zone*	2	29.421	35.042	<0.001*
species*	1	3.478	4.142	0.04*
size*	3	17.878	21.293	<0.001*
zone×species	2	0.010	0.012	0.988
zone×size*	6	2.350	2.798	0.01*
species×size*	3	41.436	49.352	<0.001*
zone×species×size*	6	7.804	9.295	<0.001*
Error	1047	0.840		

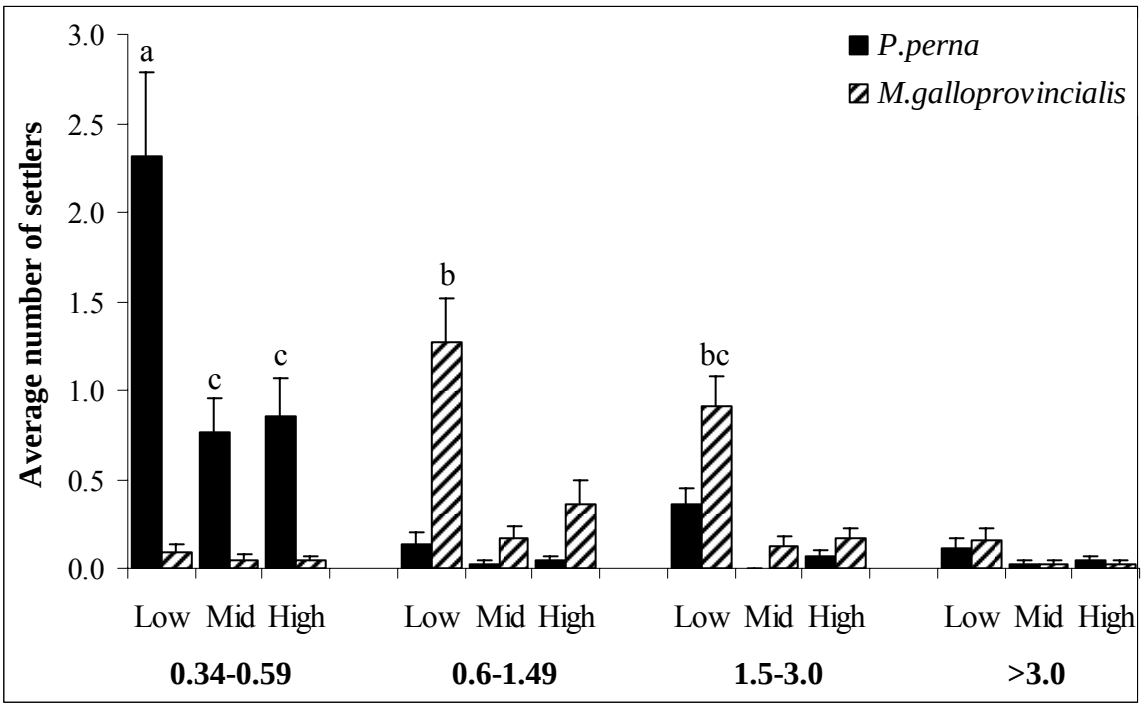


Figure 4.20: Post-hoc comparison of the interaction between zone, species and size on secondary settlement of *P.perna* and *M.galloprovincialis* at Beacon Isle in March 2003. Note that all bars without letters were significantly different from those with letters but not from each other. Error bars represent standard errors

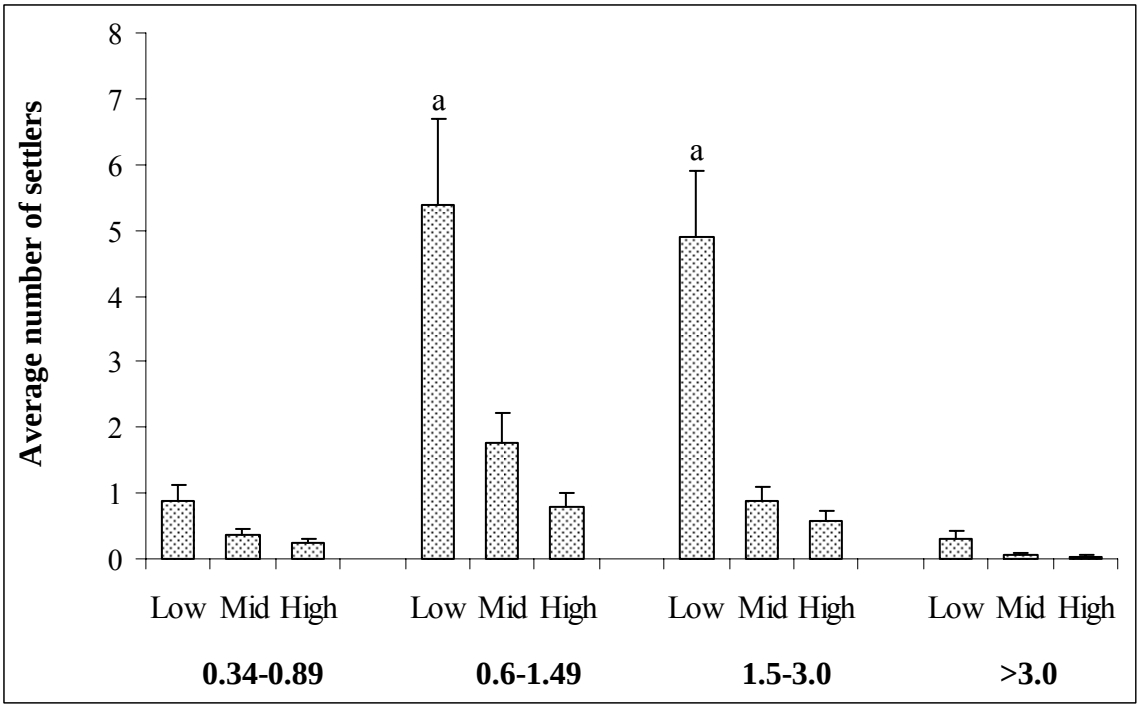


Figure 4.21: Post-hoc comparison results of the interaction between zone and size on secondary settlement of *C.meridionalis* at Lookout Beach in March 2003. Note that all bars without letters were significantly different from those with letters but not from each other. Error bars represent standard errors

Unfortunately, not all species were well represented in each size group. Nevertheless, the effect of zone on secondary settlement at each location was also observed for each species in their dominant size groups. The evidence therefore does suggest that there is no size-dependent differential settlement with respect to zone. There were also very few settlers of any species >3.0mm. This was also found in the data for settlement in 2001 when mussels in this size class generally made up <10% of secondary settlement. This may suggest that 3mm is generally the size at which the majority of mussels stop detaching and reattaching.

Recruitment

1. Monthly recruitment rates

The graphs of monthly recruitment in Plettenberg Bay revealed very different patterns of recruitment in the three species (Figs. 4.22-4.23; p.135-136). At Lookout Beach recruitment of *C.meridionalis* peaked primarily over winter/spring, with large peaks in June, August, October and April. There was very little recruitment in summer. Peak recruitment of this species was also markedly higher than either *P.perna* or *M.galloprovincialis* at this location, but very little recruitment occurred at Beacon Isle. Recruitment of *P.perna* showed virtually the opposite pattern, peaking in spring and summer. This was particularly evident at Beacon Isle, where the majority of recruitment occurred between November and February. In contrast, *M.galloprovincialis* appeared to have more or less continuous recruitment throughout the year with no major peaks, particularly at Beacon Isle. However, there was a period between July and September 2000 where there was very little recruitment of either *P.perna* or *M.galloprovincialis.* Recruitment of all species decreased considerably up the shore and was very low on the high-shore.

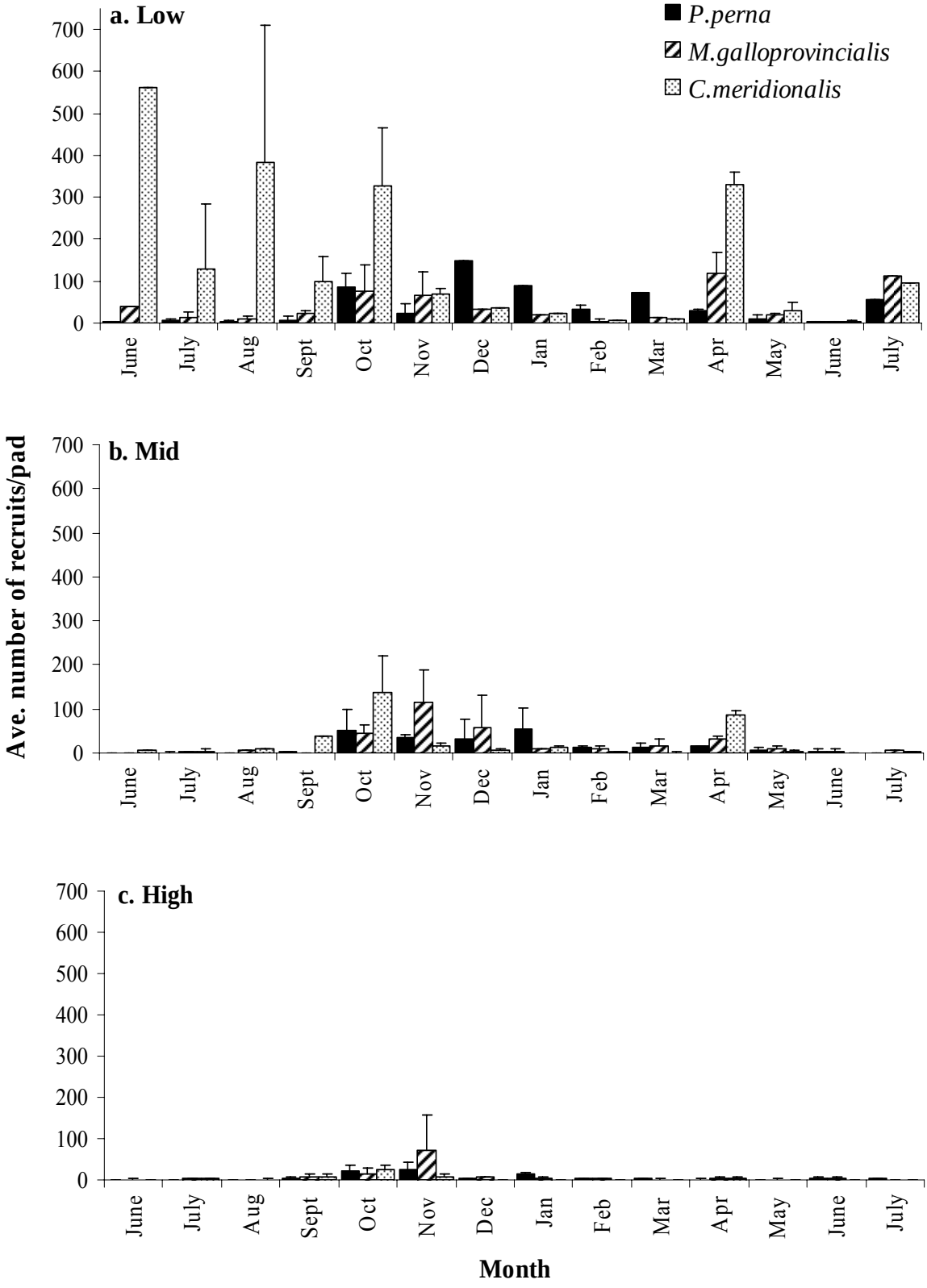


Figure 4.22 a-c: Monthly recruitment of *P.perna*, *M.galloprovincialis* and *C.meridionalis* at different tidal heights at Lookout Beach in Plettenberg Bay from June 2000 to July 2001. Lack of standard deviations indicates a sample size of one

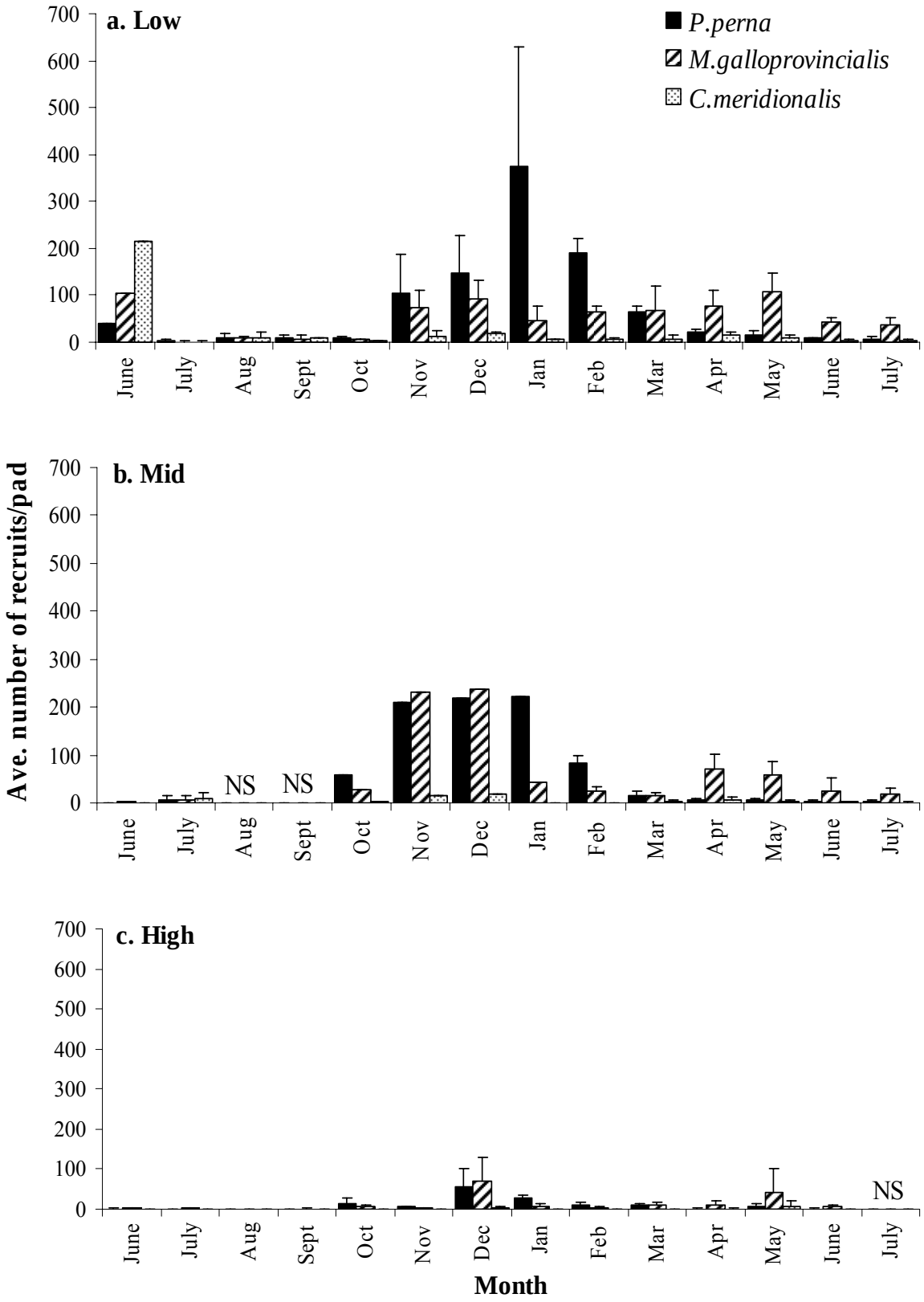


Figure 4.23 a-c: Monthly recruitment of *P.perna*, *M.galloprovincialis* and *C.meridionalis* at different tidal heights at Beacon Isle in Plettenberg Bay from June 2000 to July 2001. "NS" denotes months when no samples were recovered. Error bars represent standard deviations

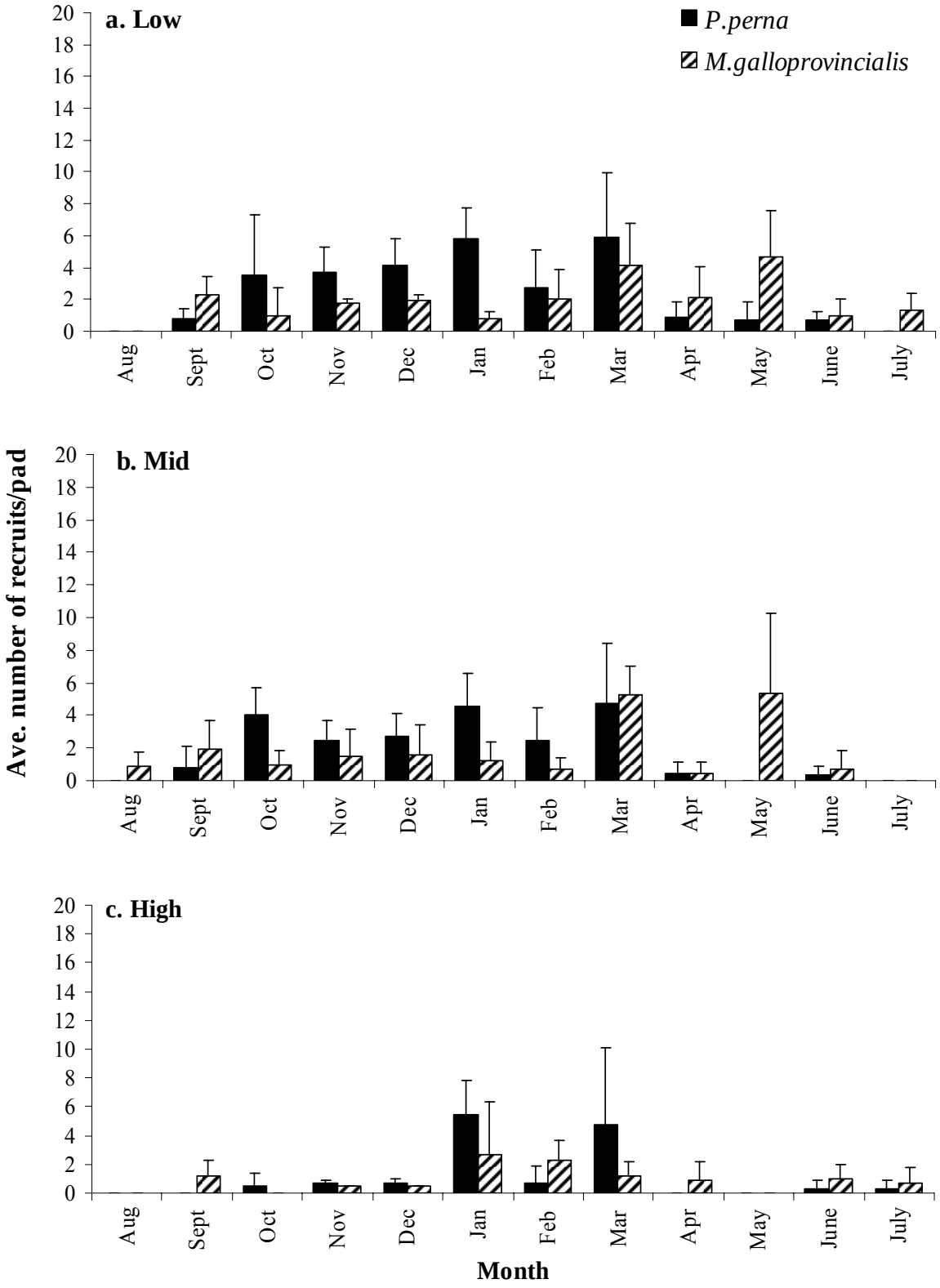


Figure 4.24 a-c: Monthly recruitment of *P.perna* and *M.galloprovincialis* at different tidal heights at Sandbaai in Tsitsikamma from August 2000 to July 2001. Note the difference in y-axis values to those in Plettenberg Bay. Error bars represent standard deviations

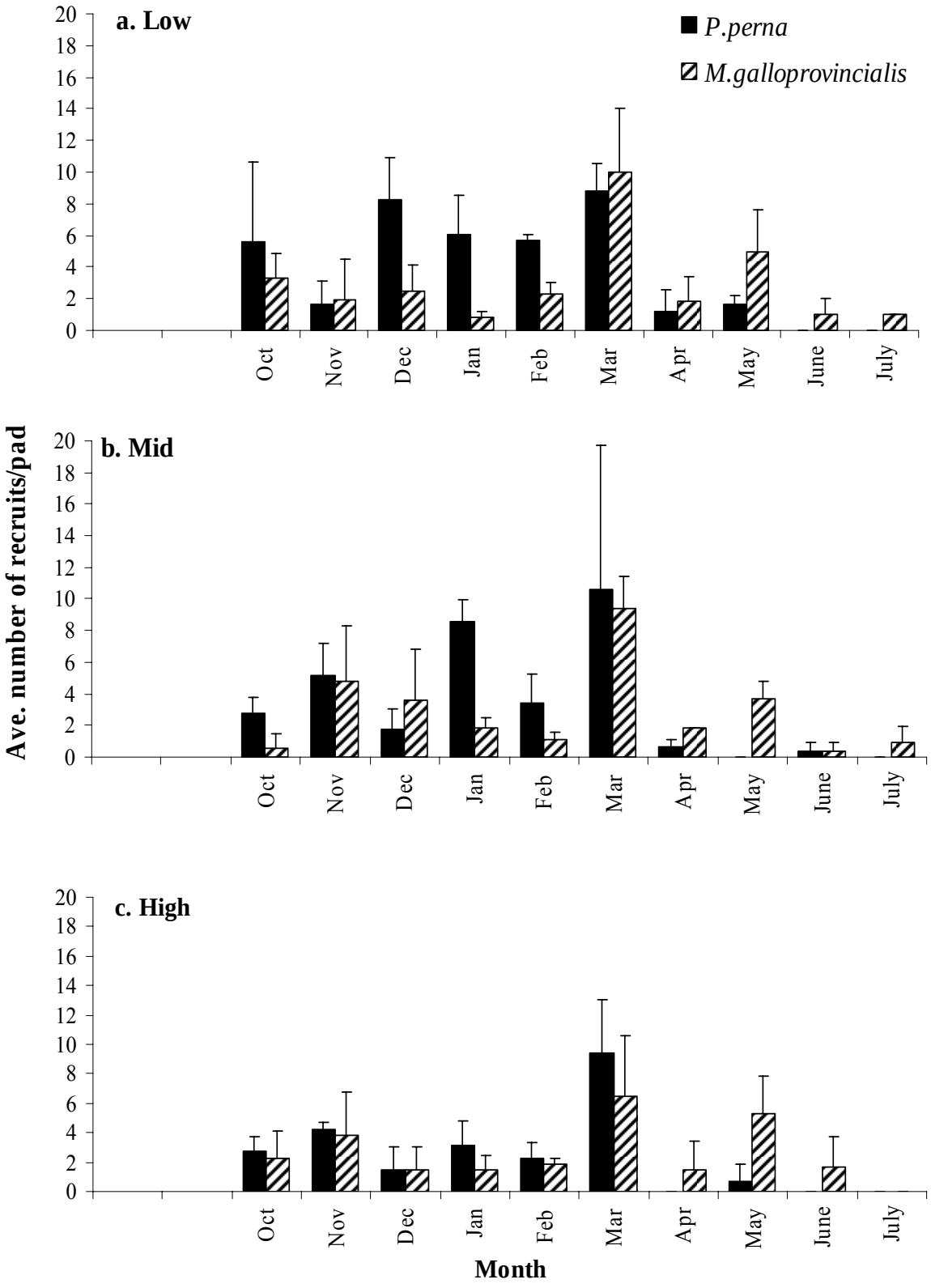


Figure 4.25 a-c: Monthly recruitment of *P.perna* and *M.galloprovincialis* at different tidal heights at Driftwood Bay in Tsitsikamma from October 2000 to July 2001. Error bars represent standard deviations

Although the density of recruits was much lower in Tsitsikamma, the seasonal patterns of recruitment of *P.perna* and *M.galloprovincialis* were similar to those observed in Plettenberg Bay, though there were no obvious peaks (Figs. 4.24-4.25; p.137-138). *P.perna* was most abundant in the spring and summer months with an additional peak in March and very little recruitment in winter. Recruitment of *M.galloprovincialis* was more consistent throughout the year, with minor peaks in March and May (autumn). Recruitment in the lower zones appeared to be slightly greater than on the high-shore.

The analysis of recruitment of *P.perna* and *M.galloprovincialis* gave a significant interaction between season, site, zone and species ($p=0.01$; Table 4.4; p.140). Recruitment of both species was significantly greater in Plettenberg Bay than in Tsitsikamma in the lower zones regardless of season (Fig. 4.26 a-b; p.141). However, high-shore recruitment did not differ between sites. Although there were no significant differences between seasons and zones in Tsitsikamma, the patterns observed were remarkably similar to those in Plettenberg Bay. Recruitment of both species was generally lowest in winter. *P.perna* recruits were prevalent at both sites in the summer months while *M.galloprovincialis* recruits were more abundant in autumn and winter. In Plettenberg Bay, recruitment generally decreased with increasing tidal height. This was particularly apparent for *P.perna* in summer when recruitment decreased significantly in the upper zones. Recruitment of *P.perna* in summer was also significantly greater than for *M.galloprovincialis* but only on the low-shore. In fact, regardless of season there were no differences in recruitment between species in the upper zones. Low-shore recruitment of *M.galloprovincialis* was also significantly greater than for *P.perna* in autumn. As suggested, there was generally little difference in recruitment of *M.galloprovincialis* between seasons, while low-shore recruitment of *P.perna* was significantly greater in summer than any other season.

Table 4.4: Mixed model ANOVA examining the effect of site, season, location, zone and species on recruitment of *P.perna* and *M.galloprovincialis*

	Effect	Df	MS	F	p
season	Fixed	3	20850.4	2.387	0.167
site	Fixed	1	200792.2	7.935	0.106
zone	Fixed	2	32273.9	6.463	0.055
species	Fixed	1	577.5	0.405	0.588
season×site	Fixed	3	19261.1	2.205	0.188
season×zone	Fixed	6	6631.0	2.296	0.103
site×zone	Fixed	2	30006.5	6.009	0.062
season×species	Fixed	3	13363.8	3.741	0.079
site×species	Fixed	1	406.3	0.285	0.646
zone×species	Fixed	2	1420.4	2.228	0.218
season×site×zone	Fixed	6	6396.7	2.215	0.113
season×site×species	Fixed	3	11436.4	3.201	0.104
season×zone×species*	Fixed	6	4508.3	5.340	0.01*
site×zone×species	Fixed	2	1305.1	2.047	0.239
season×site×zone×species*	Fixed	6	4068.3	4.819	0.01*
location (site)	Random	2	25750.8	2.869	0.182
location (site)×season	Random	6	8888.8	1.559	0.248
location (site)×zone	Random	4	5069.5	1.907	0.198
location (site)×species	Random	2	1434.6	0.429	0.673
location (site)×season×zone*	Random	12	2929.9	3.480	0.02*
location (site)×season×species*	Random	6	3624.0	4.302	0.02*
location (site)×zone×species	Random	4	631.8	0.748	0.577
location (site)×season×zone×species	Random	12	842.0	0.889	0.558
Error		575	947.0		

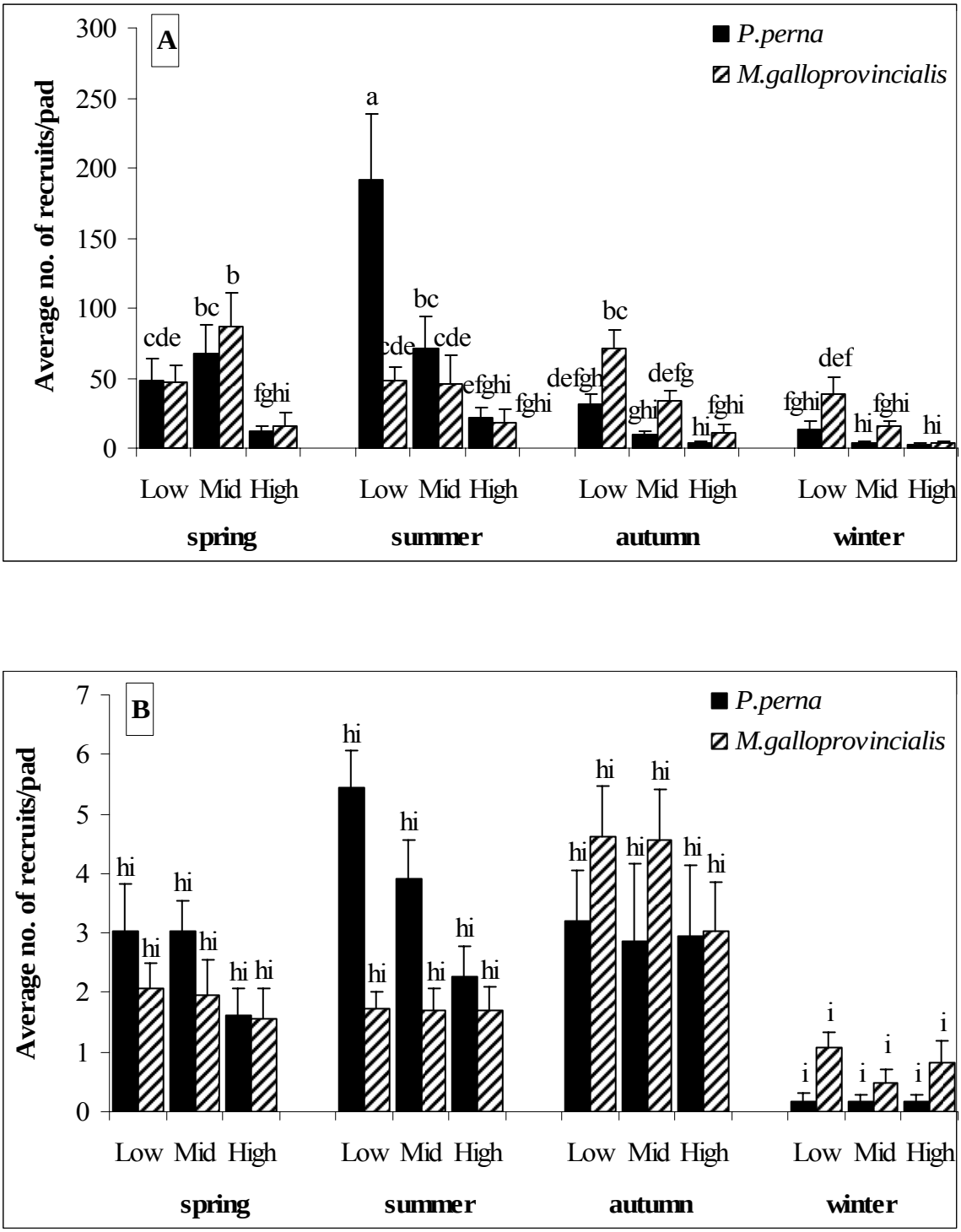


Figure 4.26 a-b: Post-hoc comparison of the interaction between site, season, zone and species on recruitment of the mussels *P.perna* and *M.galloprovincialis* in a) Plettenberg Bay and b) Tsitsikamma. Similar letters indicate homogeneous groups. Error bars represent standard errors

There were also significant interactions between location (site), season and species (p=0.02) and location (site), season and zone (p=0.02). Since I was primarily interested in species-specific differences in recruitment, the latter interaction has not been considered. Post-hoc comparisons revealed that there were no significant differences in recruitment between locations in Tsitsikamma. In Plettenberg Bay, there were no differences in recruitment of *P.perna* and *M.galloprovincialis* at Lookout Beach regardless of season (Fig. 4.27; p.143). Furthermore, there were no differences in recruitment of *P.perna* between seasons, while *M.galloprovincialis* recruited in significantly greater numbers in spring than any other season. However, there were seasonal differences in recruitment between species at Beacon Isle. Recruitment of *P.perna* was significantly greater than that of *M.galloprovincialis* in summer while recruitment of *M.galloprovincialis* was significantly greater in autumn with no difference between species in spring and winter. *P.perna* also recruited in significantly greater numbers in summer at Beacon Isle than at Lookout Beach, or any other season at both locations. There was generally little difference in recruitment of *M.galloprovincialis* between seasons, although recruitment was lowest in winter. Recruitment of this species was also significantly greater at Beacon Isle than at Lookout Beach in summer and autumn.

In the analysis of recruitment of *C.meridionalis* in Plettenberg Bay, no main effects were significant, however there was a significant interaction between location and zone (p=0.03; Table 4.5; p.144). Recruitment of *C.meridionalis* was significantly greater on the low-shore at Lookout Beach than in the upper zones (Fig. 4.28; p.144). Low-shore recruitment was also significantly greater at this location than at Beacon Isle where there were no differences between zones.

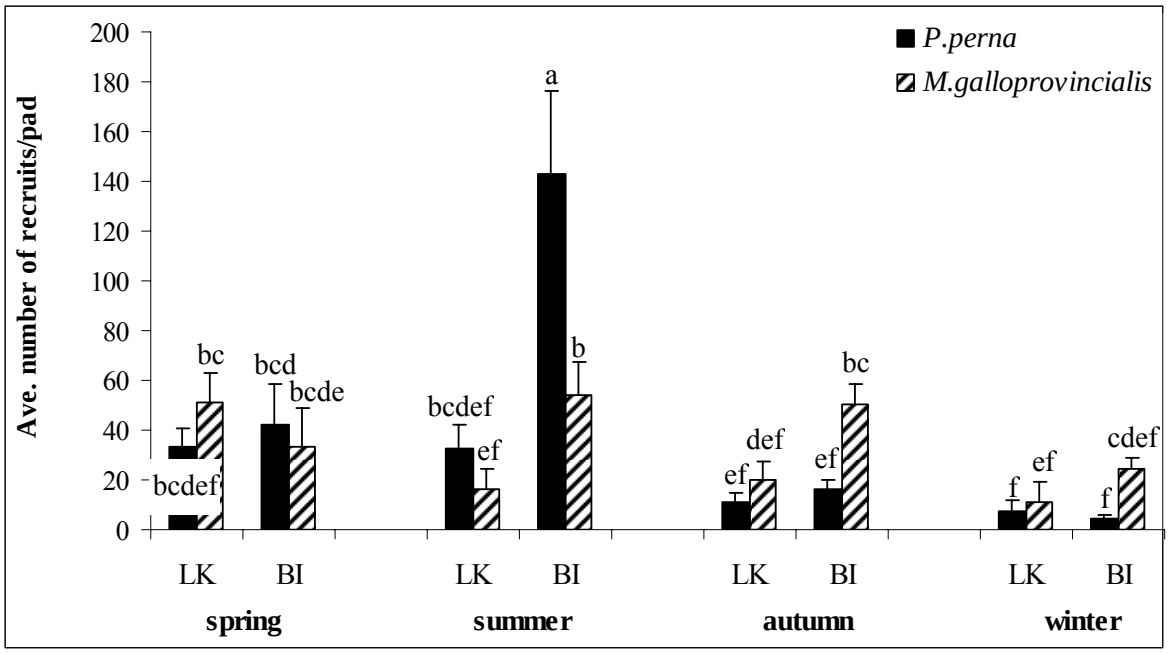


Figure 4.27: Post-hoc comparison results of the interaction between location, season and species on recruitment of *P.perna* and *M.galloprovincialis* in Plettenberg Bay. Error bars represent standard errors

Table 4.5: Three-way ANOVA on recruitment of *C.meridionalis* between seasons, locations and zones in Plettenberg Bay.

	Effect	Df	MS	F	p
season	Fixed	3	21756.0	1.464	0.381
location	Random	1	115613.0	1.477	0.343
zone	Fixed	2	115454.8	1.516	0.397
season×location	Random	3	14862.4	1.255	0.370
season×zone	Fixed	6	20288.5	1.699	0.268
location×zone*	Random	2	76140.2	6.444	0.03*
season×location×zone	Random	6	11940.9	2.164	0.050
Error		134	5518.4		

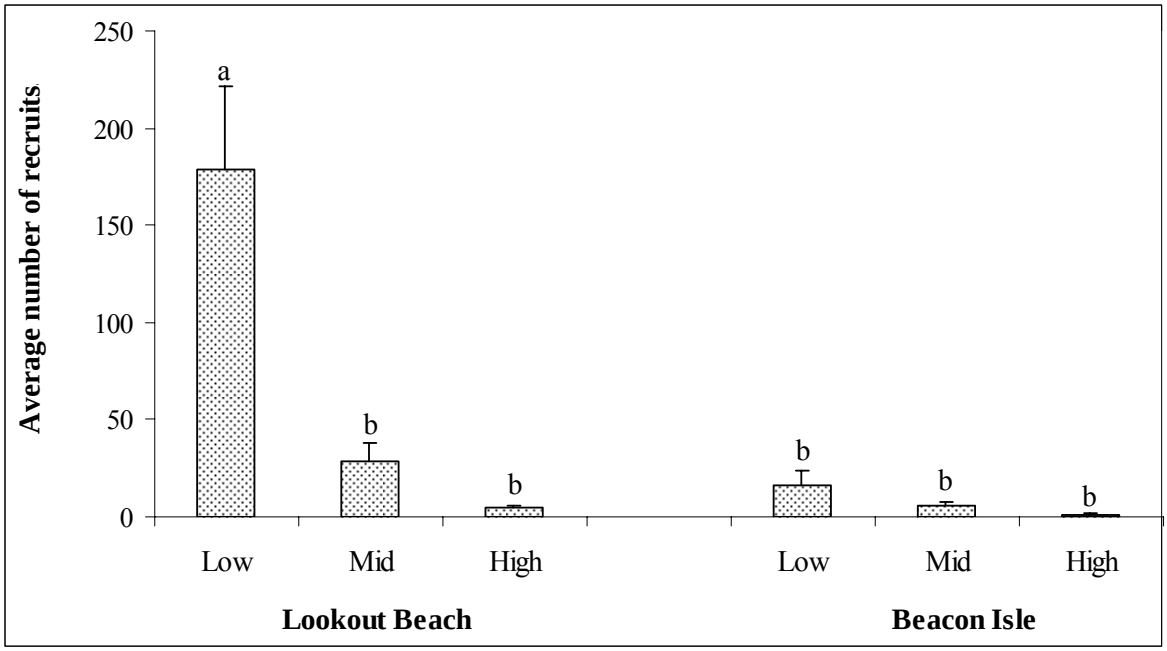
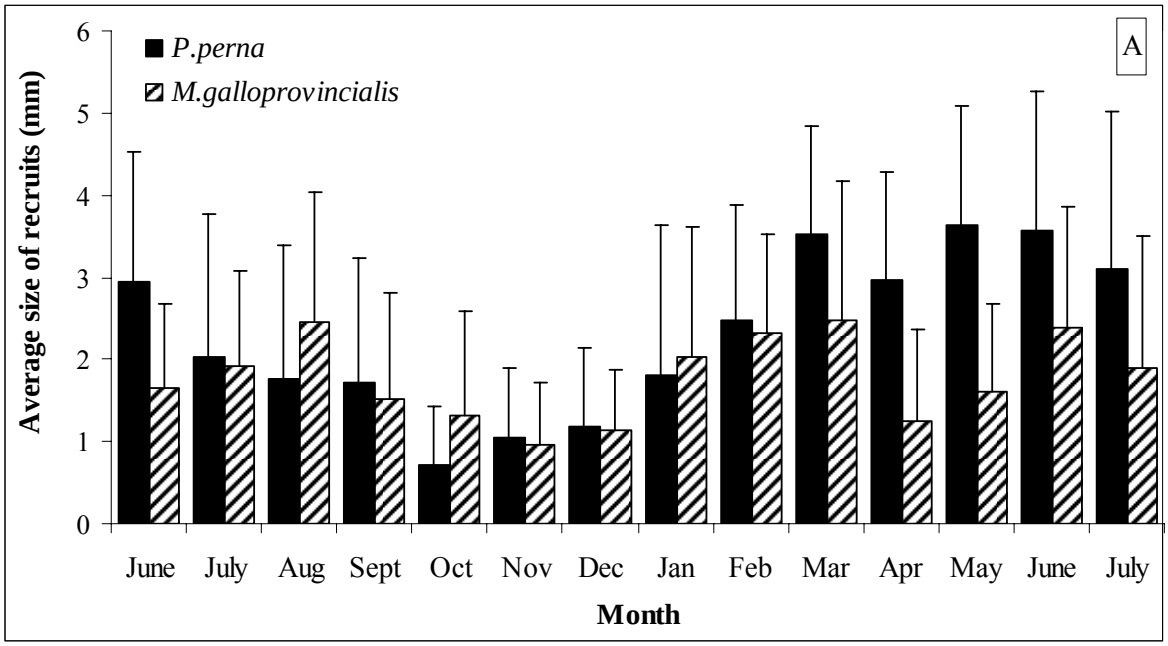


Figure 4.28: Post-hoc comparison of the interaction between location and zone on recruitment of *C.meridionalis* in Plettenberg Bay. Error bars represent standard errors

2. Temporal variation in the size of recruits

Graphs of the average size of recruits were initially plotted for each location separately. However, it was discovered that the temporal pattern was the same for locations within each site. Locations were therefore pooled and graphs were plotted for each site separately (Figs. 4.29 a-b; p.146). Average size of recruits of both *P.perna* and *M.galloprovincialis* was lowest between September and January at both sites. This possibly indicates that primary settlement of these species occurred primarily during these months. Examination of several samples that were collected daily between October and November (see Chapter 3) also revealed that the majority of mussels settling at this time were either primary settlers or early secondary settlers (<0.59mm). In Plettenberg Bay this period generally coincided with peak recruitment of these species, particularly for *P.perna.* Average size of *P.perna* recruits then increased progressively, suggesting that there was only one settlement peak during the sample period. Since the availability of primary settlers is linked to spawning, it also suggests that there was only one spawning peak for this species. Size of *M.galloprovincialis,* however, increased progressively until April when it decreased again owing to a peak in primary settlement at this time. Thus, it seems that *M.galloprovincialis* exhibited two settlement peaks and possibly two spawning peaks during the year.

It is interesting to note that, although *M.galloprovincialis* must have had a settlement peak at least in the early summer months, recruitment of this species was very low at that time. This either suggests that settlement of *M.galloprovincialis* was low, or that post-settlement mortality was high. Also, the fact that peak recruitment generally coincided with peak settlement suggests that input through primary settlement may be more important than subsequent input through secondary settlement in determining adult populations, particularly for *P.perna.* For *M.galloprovincialis* at Beacon Isle where recruitment was



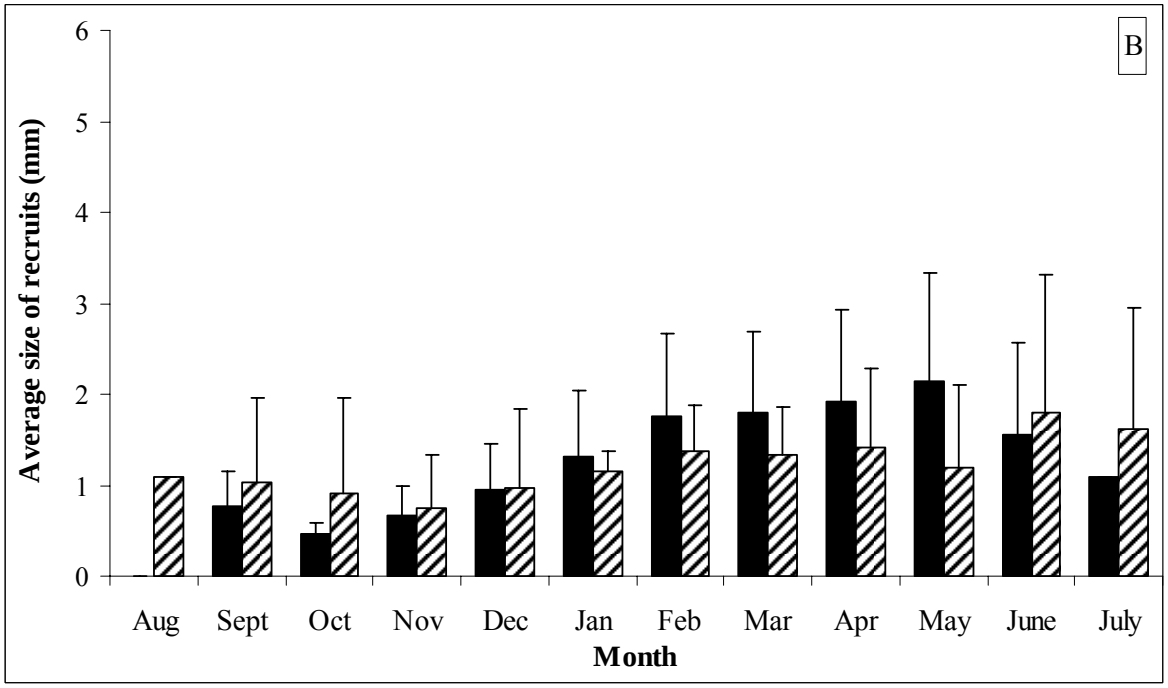


Figure 4.29 a-b: Temporal variation in the average size of recruits of *P.perna* and *M.galloprovincialis* in Plettenberg Bay (a), and Tsitsikamma (b). Error bars represent standard deviations

relatively consistent from October onwards, primary and secondary settlement may be equally important. This may not be surprising since, if this species has more than one settlement peak in a year, then secondary settlers are available throughout the year as well. However, for *P.perna* with a single settlement peak, as time goes by and settlers grow, the number of secondary settlers available should become significantly less in view of the fact that they appear to stop dispersing at a size of about 3mm.

In Tsitsikamma, average size of both species increased progressively after December. There was no evidence of a settlement peak for *M.galloprovincialis* in April at this site, and this was in fact confirmed from the 2001 settlement data which showed that there were no settlers of either species <0.6mm. In addition, there was no obvious relationship between the size of recruits and recruitment peaks. This suggests that at Tsitsikamma, where settlement and recruitment rates are low, both primary and secondary settlements are equally important in maintaining adult populations.

C.meridionalis displayed a similar result to that of *P.perna* and *M.galloprovincialis* in Plettenberg Bay, with the smallest size of recruits coinciding with peak recruitment of this species in June, September and October (Fig. 4.30; p.148). This may suggest that this species exhibited two major settlement peaks and possibly two spawning peaks, or alternatively a single extended spawning period. In August, the average size of recruits was larger and was probably mostly secondary settlers. This may suggest that secondary settlement in this species is occasionally a synchronous event, with a large number detaching and reattaching within the same time period.

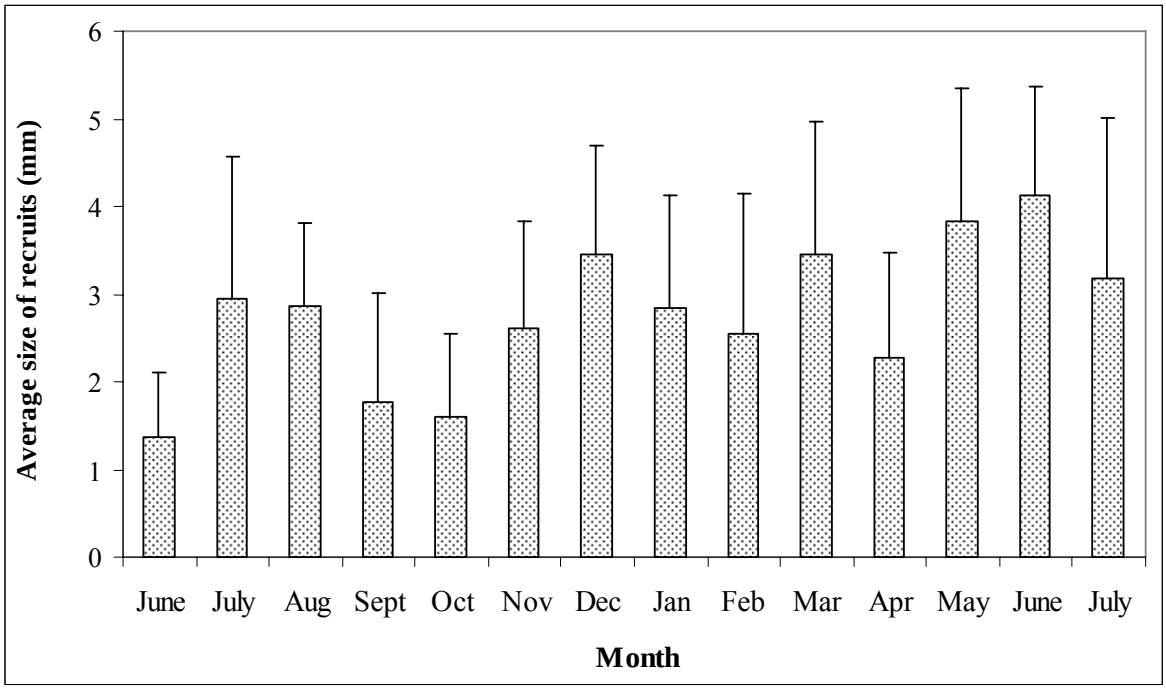


Figure 4.30: Temporal variation in the average size of recruits of *C.meridionalis* in Plettenberg Bay. Error bars represent standard deviations

Wind correlations

1. April 2001

Primary settlement of *M.galloprovincialis* was not significantly correlated with any wind direction or velocity at either location. For secondary settlement there were a number of significant correlations although all were generally quite poor (Table 4.6; p.150). Settlement of all three species was positively correlated with the duration of onshore winds at Lookout Beach, but this correlation was only significant for *C.meridionalis* at Beacon Isle. There were also positive correlations with the velocity of onshore winds that varied between locations. Secondary settlement of *M.galloprovincialis* and *C.meridionalis* was also negatively correlated with the duration of offshore winds at Lookout Beach, while *P.perna* was negatively correlated with the velocity of these winds. Secondary settlement of *M.galloprovincialis* was also positively correlated with the duration of westerly winds at Beacon Isle.

2. March 2003

There were no significant correlations between winds and primary settlement of any species. Secondary settlement of *P.perna* at Lookout Beach was positively correlated with the duration of onshore winds ($r=0.6$; $p=0.04$; Table 4.7; p.151). No other correlations were significant. Fig. 4.31 (p.151) plots the duration of onshore and offshore winds over the twelve day sampling period. It is clear that onshore winds were prevailing when settlement was high. The influence of winds may only be important when larvae or post-larvae are present in the water column. Therefore the lack of settlement when onshore winds were still frequent may merely reflect a lack of available settlers. When the correlations were redone only using data up to the 24 March there were very strong positive correlations with the

Table 4.6: Daily wind correlations with the average number of secondary settlers of *P.perna*, *M.galloprovincialis* and *C.meridionalis* on the low-shore at Lookout Beach and Beacon Isle in April 2001. Significant correlations have been highlighted

	Hours onshore	**Velocity onshore**	**Hours offshore**	**Velocity offshore**	**Hours westerly**	**Velocity westerly**
Lookout Beach						
P.perna	**r=0.52** **p=0.04**	**r=0.5** **p=0.047**	r=-0.48 p=0.06	**r=-0.55** **p=0.03**	r=0.03 p=0.9	r=0.46 p=0.07
M.galloprovincialis	**r=0.62** **p=0.01**	r=0.48 p=0.06	**r=-0.51** **p=0.045**	r=-0.47 p=0.07	r=-0.03 p=0.9	r=0.48 p=0.06
C.meridionalis	**r=0.63** **p=0.01**	r=0.46 p=0.07	**r=-0.5** **p=0.05**	r=-0.4 p=0.12	r=-0.02 p=0.1	r=0.47 p=0.07
Beacon Isle						
P.perna	r=0.02 p=0.94	r=0.13 p=0.63	r=0.01 p=.96	r=0.28 p=0.29	r=0.1 p=0.84	r=0.24 p=0.38
M.galloprovincialis	r=0.29 p=0.28	**r=0.57** **p=0.02**	r=-0.34 p=0.2	r=-0.22 p=0.42	**r=0.51** **p=0.045**	r=0.37 p=0.15
C.meridionalis	**r=0.59** **p=0.02**	**r=0.5** **p=0.049**	r=-0.47 p=0.07	r=-0.01 p=0.98	r=0.24 p=0.36	r=0.49 p=0.05

Table 4.7: Daily wind correlations with the average number of secondary settlers of *P.perna*, *M.galloprovincialis* and *C.meridionalis* on the low-shore at Lookout Beach and Beacon Isle in March 2003

Lookout Beach	Hours onshore	Velocity onshore	Hours offshore	Velocity offshore	Hours easterly	Velocity easterly
P.perna	**r=0.6**	r=0.3	r=-0.54	r=-0.3	r=-0.16	r=-0.34
	p=0.04	p=0.34	p=0.07	p=0.35	p=0.62	p=0.27
M.galloprovincialis	r=0.42	r=0.38	r=-0.32	r=0.01	r=-0.14	r=-0.17
	p=0.17	p=0.23	p=0.32	p=0.98	p=0.66	p=0.61
C.meridionalis	r=0.42	r=0.35	r=-0.3	r=-0.01	r=-0.2	r=-0.21
	p=0.18	p=0.27	p=0.34	p=0.98	p=0.54	p=0.51

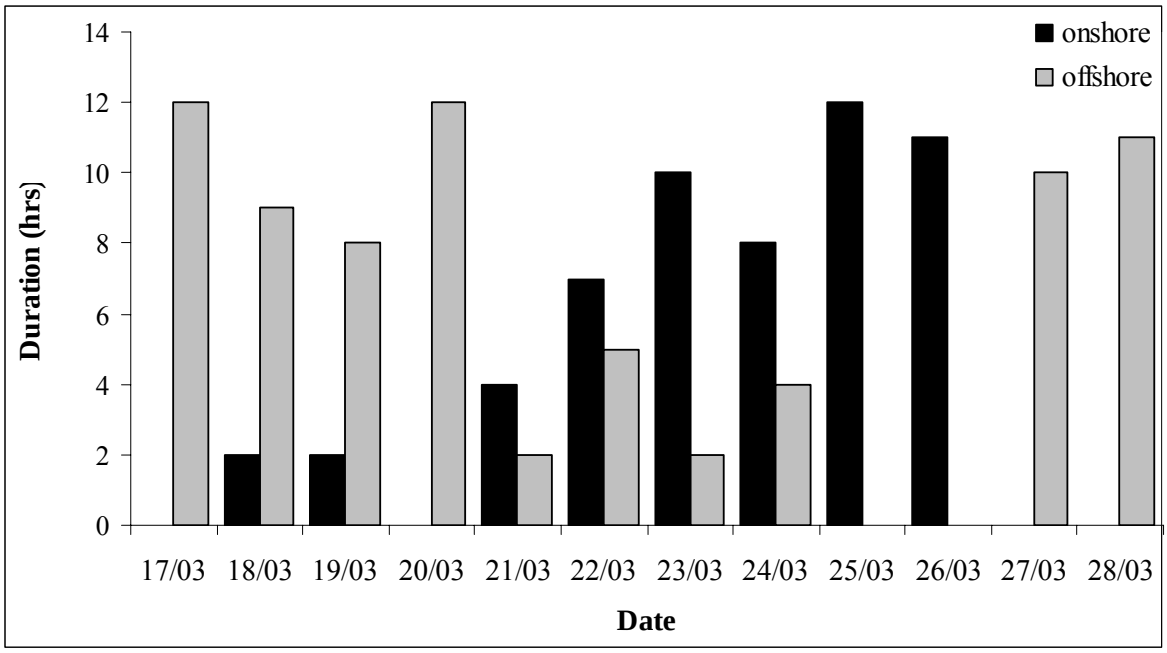


Figure 4.31: Duration of onshore and offshore winds in March 2003

duration of onshore winds (r>0.85) for all species and secondary settlement of *P.perna* was also negatively correlated with the duration of offshore winds (r>-0.77).

3. Recruitment

Monthly recruitment of *P.perna* was significantly correlated with both the frequency of onshore and easterly winds at both locations in Plettenberg Bay (Table 4.8; p.153). This was probably due to the large summer recruitment of this species when onshore and easterly winds are most frequent and offshore winds are less frequent (Fig. 4.32; p.153). Recruitment of *M.galloprovincialis* was also correlated with the duration of easterly winds at Beacon Isle. Recruitment of *C.meridionalis* appeared to be unaffected by seasonal wind patterns.

4.4 Discussion

There was considerable spatial and temporal variation in both settlement and recruitment. The two most important scales of variation in terms of the distribution and abundance of *Perna perna* and *Mytilus galloprovincialis* were at the level of zone and site. Settlement and recruitment of both species decreased with increasing tidal height. In other words, *M.galloprovincialis* did not settle differentially on the high-shore. Similar settlement patterns with zone have been observed for *M.galloprovincialis* in the Ria de Vigo, Spain (Cáceres-Martínez and Figueras 1997) and for *P.perna* at other sites along the south coast of South Africa (Lukac and McQuaid, unpub. data).

Primary settlement of *M.galloprovincialis* was generally concentrated on the low-shore. The process of metamorphosis is physically stressful and may result in large mortalities of newly settled larvae (Gosselin and Qian 1996; García-Esquivel *et al.* 2001). Thus, it is

Table 4.8: Monthly wind correlations with the average number of settlers per month of *P.perna*, *M.galloprovincialis* and *C.meridionalis* at Lookout Beach and Beacon Isle (zones pooled)

	Hours onshore	**Velocity onshore**	**Hours offshore**	**Velocity offshore**	**Hours easterly**	**Velocity easterly**
Lookout Beach						
P.perna	**r=0.72** **p=0.004**	r=0.16 p=0.6	r=-0.52 p=0.06	r=0.35 p=0.22	**r=0.78** **p=0.001**	**r=0.67** **p=0.01**
M.galloprovincialis	r=0.37 p=0.2	r=-0.1 p=0.7	r=-0.4 p=0.15	r=-0.1 p=0.8	r=0.43 p=0.13	r=0.41 p=0.15
C.meridionalis	r=-0.02 p=0.95	r=-0.2 p=0.49	r=0.24 p=0.4	r=-0.37 p=0.19	r=-0.23 p=0.43	r=-0.1 p=0.84
Beacon Isle						
P.perna	**r=0.61** **p=0.02**	r=0.12 p=0.69	r=-0.42 p=0.14	r=0.22 p=0.45	**r=0.61** **p=0.02**	r=0.39 p=0.17
M.galloprovincialis	r=0.32 p=0.26	r=-0.1 p=0.77	r=-0.41 p=0.15	r=0.2 p=0.49	**r=0.57** **p=0.04**	r=0.24 p=0.4
C.meridionalis	r=-0.19 p=0.52	r=-0.33 p=0.25	r=0.03 p=0.92	r=0.28 p=0.34	r=0.1 p=0.76	r=-0.26 p=0.36

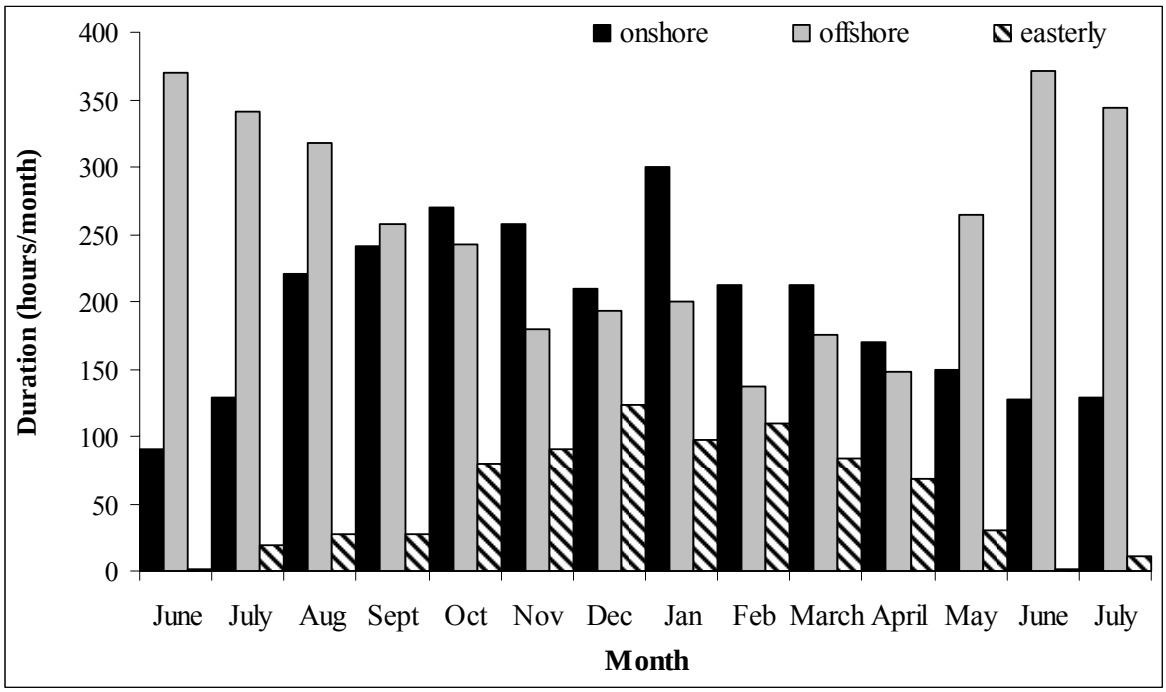


Figure 4.32: Monthly duration of onshore, offshore and easterly winds in Plettenberg Bay between June 2000 and July 2001.

possible that larvae selectively settled in this zone where conditions are more favourable. Alternatively this may reflect the distribution of larvae in the water column. McQuaid and Phillips (2000) found that mussel larvae off the south coast of South Africa were generally homogeneously distributed throughout the water column, but occasionally were concentrated near the surface or near the bottom.

In contrast to primary settlement, secondary settlement was synchronous among all three species, which may suggest that post-larvae aggregate in the water column. In the laboratory, *M.galloprovincialis* post-larvae usually remained where they settled unless they were disturbed (Cáceres-Martínez *et al.* 1994). Therefore disturbances or unfavourable conditions may result in synchronous dispersal of post-larvae of different species in the disturbed habitat. The production of mucus threads for secondary dispersal may also lead to clumping in the water column if post-larvae become ensnared in these threads (see Cáceres-Martínez *et al.* 1994). Secondary settlement in *P.perna* and *M.galloprovincialis* decreased progressively with increasing tidal height. This settlement pattern generally reflects the duration of immersion time at each shore level, and also suggests that post-larvae were homogeneously distributed in the water column (Miron *et al.* 1995). If secondary settlement was merely a consequence of immersion time then it may also suggest that settlement in *P.perna* was not "selective" for the low-shore. Nevertheless, differential settlement in this species with respect to zone may be responsible for adult distributions.

There was no evidence to suggest that spatial patterns of secondary settlement in *P.perna* and *M.galloprovincialis* varied as a function of size. However, the size of secondary settlers did differ between species. The majority of *P.perna* settlers were small (0.34-0.59mm), while the majority of *M.galloprovincialis* settlers were larger (0.6-3.0mm). The size of

secondary settlers will be determined by an array of different factors e.g. reproductive cycle and larval or juvenile growth rates. There was also a tendency for larger mussels (>1.5mm) of both species to be more abundant on the low-shore than in the upper zones. The sinking velocity of byssus drifting mussels increases linearly with increasing shell length (Lane *et al.* 1985; Buchanan and Babcock 1997). This difference in sinking velocity among different sized settlers may contribute to the vertical homogeneity of post-larvae in the water column, and may also explain the distribution of larger mussels on the shore. Cáceres-Martínez and Figueras (1997) also found that larger *M.galloprovincialis* post-larvae were most abundant at low-shore levels.

Daily settlement rates were measured in 2001 and 2003 and on both occasions post-larvae >3.0mm were very rare. This may suggest that this is the size at which the majority of post-larvae stop dispersing, which is in agreement with studies on *M.edulis* for which byssus drifting occurred primarily in post-larvae <2.5mm in length (Sigurdsson *et al.* 1976; de Blok and Tan-Maas 1977; Lane *et al.* 1985). Buchanan and Babcock (1997) observed post-larvae of *P.canaliculus* using byssus drifting at sizes of up to 6mm. However, results from this study suggest that this is not common. It is not known whether post-larvae continue to grow in the water column or if they need to be attached to grow. If the latter is true, it is reasonable to assume that after a certain time growth may become more important than the advantages gained by relocating.

There was very little primary settlement of either *P.perna* or *C.meridionalis* when daily settlement was measured, however, there were distinct peaks in primary settlement of *M.galloprovincialis*. This probably reflects the time of year at which samples were collected (March/April) and differences in the reproductive seasons among species. All

three species have been recorded to have two spawning seasons in summer and again in autumn/winter in South Africa, although the exact timing of these events may be variable (van Erkom Schurink and Griffiths 1991). Water temperature can have a significant influence on reproductive cycles in mussels (Seed and Suchanek 1992). *M.galloprovincialis* and *C.meridionalis* may be considered as cold-water species, while *P.perna* is a warm-water species (Berry 1978). Therefore their reproductive cycles in the warm-temperate waters of the south coast may vary.

Settlement and recruitment of *C.meridionalis* were largely restricted to the low-shore and were very location-specific. For secondary settlers this was also irrespective of size. Thus this species appeared to settle selectively on the low-shore at Lookout Beach i.e. within the narrow zone occupied by adults. Differential settlement may therefore be an important determinant of the adult distribution of this species. Griffiths (1981) found that recruitment of *C.meridionalis* in False Bay on the south-west coast of South Africa was restricted to subtidal and low-shore areas, but that secondary settlers migrated further upshore due to overcrowding. It should be noted though that the high-shore in her study represented the upper limit of the *C.meridionalis* mussel bed at 0.85m above LWS, which probably corresponds to lower mid-shore at this location. There was some settlement and recruitment of *C.meridionalis* above this zone in Plettenberg Bay, but in significantly lower numbers. Therefore, although differential settlement of this species may be of primary importance in determining adult community structure, post-settlement mortality and/or post-recruitment processes may also contribute to the virtual absence of this species from higher shore levels and from Beacon Isle.

There was considerable temporal variation in the daily settlement patterns of primary and secondary settlement. In 2001, both primary and secondary settlement peaked a day apart over spring tides. However, in 2003, settlement peaked around neap tide with little settlement occurring over spring tide (however, tides were not replicated). Jeffery and Underwood (2000) found a correlation between spring tides and settlement of barnacle larvae, however, this was also always associated with onshore winds. Similarly, Porri (2003) found that settlement of *P.perna* tended to be greatest around spring tides, but also appeared to be correlated with the frequency of onshore winds. In the present study, when settlement peaked, onshore winds were prevalent, although correlations were not necessarily significant. This was because onshore winds often blew on days when settlement was low. Naturally, any potential association between settlement and wind-driven currents or tidal cycles might only be observed if there are mussels available in the water column. While there were differences between years in the timing of settlement in terms of tide, settlement was correlated with onshore winds in both years. Wind patterns may therefore be a better predictor of settlement than tidal cycles in this study. Bertness *et al.* (1996) found that both spatial and temporal variations in settlement of the acorn barnacle, *Semibalanus balanoides,* were strongly linked to the delivery of larvae by wind-driven currents. However, Whethey (1985) found an inconsistent relationship with wind direction and settlement of the same species and suggested that other factors such as turbulence or larval behaviour may frequently override the effects of wind patterns.

Recruitment of *P.perna* peaked in the spring and summer months at both sites with an exceptionally high peak during summer at Beacon Isle, and very little recruitment in winter. Studies on the south-east and east coasts of South Africa found that recruitment of *P.perna* peaked in winter and spring (Berry 1978; Lasiak and Barnard 1995). However, Lindsay

(1998) recorded the highest recruitment of this species on the south coast between summer and autumn and a similar result was obtained in Tsitsikamma (Crawford and Bower 1983). The seasonal recruitment of *P.perna* observed in this study therefore appears to be consistent with previous reports for this coast. Results from these studies also suggest that there may be a latitudinal gradient in seasonal reproduction and recruitment patterns between the south and east coasts. Based on the average size of recruits, there appeared to be only one major primary settlement peak during the year between spring and early summer. The presence of primary settlers at this time was confirmed through daily samples taken between October and November. Thus in Plettenberg Bay, primary settlement may be more important than secondary settlement in contributing to adult stocks, while in Tsitsikamma where recruitment of larger mussels continued through autumn, both primary and secondary settlement may be equally important.

In contrast, *M.galloprovincialis* exhibited more continuous recruitment throughout the year, though slight peaks were observed in spring and autumn at both sites with an additional peak in winter in Plettenberg Bay. Average size of recruits over the year showed two peaks of smaller mussels occurring in spring and early summer and again in autumn, which coincides with the relatively large primary settlement of this species observed in April 2001. On the west coast of South Africa, *M.galloprovincialis* settled on mussel culture ropes mainly over the summer/autumn period, particularly in April (van Erkom Schurink and Griffiths 1991). Cáceres-Martínez *et al.* (1997) also found that recruitment of *M.galloprovincialis* in Spain occurred throughout the year but with peaks in spring and early autumn. In the Bahía de Todos Santos, Mexico, recruitment of this species occurred throughout the year but with a peak in late autumn and winter (Ramírez and Cáceres-Martínez 1999). The patterns of recruitment observed in this study therefore appear to be

typical for this species. Due to the more or less consistent recruitment of *M.galloprovincialis* over the year, both primary and secondary settlement may be equally important in contributing to adult abundances.

Recruitment of *C.meridionalis* showed strikingly different seasonal patterns to that of *P.perna* in Plettenberg Bay. The largest recruitment peaks were observed in the winter months in 2000, with additional peaks in spring and autumn and a smaller peak the following winter (2001). Virtually no recruitment was observed in the summer months. Average size of recruits of this species was small during all peak recruitment months except August when recruits were larger and probably reflect a significant secondary settlement during the month. Du Plessis (1977) recorded recruitment peaks of this species on the west coast primarily between late spring and early autumn, and recruitment was generally very low during winter. One possible explanation for the differences in seasonal recruitment of this species on the two coasts could be water temperature. Warmer water temperatures on the south coast during summer could potentially influence the survival of new recruits. An additional aspect may be that of seasonal patterns of sand inundation. At Lookout Beach, sand inundation occurred primarily in summer and early autumn. McQuaid and Dower (1990) also noted that rocky shores on the south coast of South Africa accumulate sand deposits during summer and that these are abruptly removed by winter storms. For *C.meridionalis*, which settles selectively on the low-shore, it might be preferable to settle primarily in the months when sand inundation is lowest and adult beds are exposed. Sand influence may also be responsible for the comparatively lower recruitment rates of *P.perna* and *M.galloprovincialis* at this location compared to Beacon Isle, as these species are less tolerant of sand inundation than *C.meridionalis* (Hockey and van Erkom Schurink 1992; Marshall and McQuaid 1993).

Both settlement and recruitment rates were significantly higher in Plettenberg Bay than in Tsitsikamma. Regional variations in recruitment may therefore be the cause of variations in adult abundances at these sites. Several studies have found that bays may act as larval retention sites (Thiébaut *et al.* 1994; Archambault and Bourget 1999; Helson and Gardner 2004). Retention may be facilitated by local hydrodynamic forces caused by tidal processes and wind patterns, or by large-scale processes such as the presence of eddies or upwelling (Thiébaut *et al.* 1994; Young *et al.* 1996; Graham and Largier 1997; Young *et al.* 1998; Chícharo and Chícharo 2001).

The oceanography of the south coast was described in Chapter 1 (p.16). Shear-edge eddies frequently form over the continental shelf and become trapped in the region southwest of Port Elizabeth (Lutjeharms 1981; Lutjeharms *et al.* 2003). In a modelling study, Lutjeharms *et al.* (2003) revealed the presence of a small anticyclone shorewards of such an eddy and directly south of the Plettenberg Bay-Tsitsikamma region. Although this appeared to be a permanent feature of their time-series simulations, it was unclear whether this was merely an artifact of the boundary conditions of the model (Lutjeharms *et al.* 2003). If this anticyclonic circulation does, in fact, exist it may be an important mechanism causing retention of larvae within the bay as the nearshore edge would drive water into the bay before recirculating it. It is interesting to note that the presence of such anticlockwise circulations near bays has been mentioned in other studies (Dare 1976; Young *et al.* 1998), and Dare (1976) also suggested this might be a feature contributing to larval retention in Morecambe Bay, England.

Upwelling and the presence of an "upwelling shadow" has been advocated as a potential cause for retention of planktonic organisms in Monterey Bay, California (Graham and

Largier 1997). Briefly, upwelling originates north of the bay and moves south following the isobaths, curving slightly into the bay. A front develops between the warmer waters within the bay and the cooler upwelled waters just offshore. This front enhances the tendency for some of this warm water to remain within a cyclonic circulation in the bay, which in turn facilitates the retention of planktonic organisms. The authors also suggest that upwelling shadows are not restricted to semi-enclosed systems but rather are associated with convex coastlines downstream of capes or headlands. In the region of Tsitsikamma, wind-induced upwelling originates on the south side of Cape St. Francis (near Jeffreys Bay; Fig. 1.1a) and moves west along the coast towards Plettenberg Bay. In Tsitsikamma, the 100m isobath is close inshore and this is consequently a region of intense upwelling. West of Tsitsikamma the isobath begins to veer offshore before reaching Plettenberg Bay. Consequently, upwelling moving westwards would theoretically remain offshore of Plettenberg Bay and could potentially create the situation described by Graham and Largier (1997). The fact that recruitment of *P.perna* was significantly correlated with the frequency of East winds which cause upwelling adds credance to this suggestion. However, it must be noted that the occurrence of Easterly winds does not necessarily cause upwelling, as it largely depends on the duration and intensity of these winds (Schumann *et al.* 1982). In addition, if upwelling did occur it may not reach as far west as Plettenberg Bay.

Upwelling may also play a very different role in Tsitsikamma. Wind-induced upwelling occurs frequently along the Tsitsikamma coast in the summer and autumn months. These events are often very rapid and intense leading to sudden, sharp drops in sea temperature (Schumann *et al.* 1982). Studies by Roughgarden *et al.* (1988) and Connolly and Roughgarden (1998) have found that upwelling transports larvae offshore leading to poor recruitment rates at nearshore sites. Thus it is possible that low recruitment rates in

Tsitsikamma are a result of frequent upwelling at this site. It is also possible that sudden drops in temperature may influence larval mortality.

Seasonal wind patterns appeared to influence recruitment of *P.perna* but not *M.galloprovincialis* and *C.meridionalis.* Both onshore and easterly winds which drive water into the bay were most frequent between spring and autumn when offshore winds are less frequent. Peak settlement of *P.perna* occurred at this time thus seasonal wind patterns may be important in determining recruitment success of this species. However, recruitment of *C.meridionalis* was unaffected by wind patterns in Plettenberg Bay, and this species actually exhibited large peaks in winter when offshore winds were most common. Recruitment of *M.galloprovincialis* was more consistent throughout the year, and therefore also appeared to be unaffected by wind patterns. Since settlement of all three species was correlated with onshore winds, it is possible that daily wind patterns are better predictors of settlement than seasonal wind patterns.

In summary both settlement and recruitment of all three species varied significantly at all spatial and temporal scales considered. Settlement and recruitment were significantly greater in Plettenberg Bay than in Tsitsikamma. Thus the differences in adult abundance between sites appear to be largely the result of differential recruitment rates. Variations in recruitment rates may determine the occurrence or strength of interspecific interactions between sites (Underwood and Denley 1984; Connolly and Roughgarden 1998). This suggests that competition should be more important in Plettenberg Bay than in Tsitsikamma.

Within Plettenberg Bay, settlement and recruitment rates of *P.perna* and *C.meridionalis* appeared to be important in determining adult distribution and abundance of these species, both at the scale of vertical shore height and between locations (100s of meters). Differences in recruitment of *M.galloprovincialis* between locations also reflected adult densities of this species. However, patterns of either settlement or recruitment could not account for the large densities of this species in the upper intertidal zones. It therefore seems likely that the abundance of *M.galloprovincialis* on the high-shore is a result of successive settlements of slow-growing individuals. This also implies that mortality is low so that populations may persist. Both *P.perna* and *C.meridionalis* are scarce on the high-shore, particularly at Lookout Beach. Since settlement and recruitment rates did not differ between species in this zone, it is also suggested that these species should experience significantly greater mortality than *M.galloprovincialis* on the high-shore.

Chapter 5

Juvenile growth and post-settlement mortality

5.1 Introduction

The relationship between settlement and recruitment is often obscured by patterns of post-settlement mortality, which may vary both spatially and temporally in response to a variety of physical and biological factors (Connell 1961; Denley and Underwood 1979; Keough and Downes 1982; Hurlburt 1991; Minchinton and Scheibling 1993; Roegner and Mann 1995; Hunt and Scheibling 1997; Jarrett 2000; Osman and Whitlatch 2004).

Physical stresses such as wave dislodgement, sedimentation or desiccation have been found to influence the spatial distribution of recruits (Connell 1961; Seed 1969b; Foster 1971; Denley and Underwood 1979; Iwasaki 1995; Roegner and Mann 1995). Grazers can cause significant post-settlement mortality by "accidentally" ingesting or bulldozing invertebrate settlers (Dayton 1971; Hunt and Scheibling 1997). For example, on South African shores, grazing by the limpet *Scutellastra cochlear* generally excludes both algae and mussels from low-shore areas where it is abundant (Stephenson and Stephenson 1972; Branch 1985). Established organisms may cause mortality either directly by overgrowing, crushing or undercutting early settlers (Connell 1961; Denley and Underwood 1979; Grosberg 1981; Osman and Whitlatch 1995a) or indirectly, for example, overtopping in corals may increase juvenile mortality in the understory (Baird and Hughes 2000).

The importance of predation as a source of early mortality has been well documented (Keough and Downes 1982; Moreno 1995; Ray and Stoner 1995; Moksnes *et al.* 1998; Chan and Williams 2003; Bishop *et al.* 2005). Osman and Whitlatch (2004) found that recruitment of several sessile invertebrates was directly controlled by predation on early juvenile stages, leading to consistent large-scale spatial differences in community structure

and composition. Competition for space amongst settlers may result in density-dependent post-settlement mortality (Connell 1961; Connell 1985; Griffiths and Hockey 1987). However, this is likely to become more important as recruits grow and come into contact with one another, and may therefore be a more important source of early post-recruitment mortality (Hunt and Scheibling 1997). Density-dependent mortality may also occur due to increased levels of predation when settlement is high (Gaines and Roughgarden 1985). However, gregarious behaviour at settlement may increase the chances of survival leading to inverse density-dependent mortality (Holm 1990).

Mortality of juvenile intertidal invertebrates is often determined within the first few days after settlement (Gosselin and Qian 1996; Gosselin and Qian 1997). This is a critical period of survival for settling larvae due to the physiological stresses associated with metamorphosis (García-Esquivel *et al.* 2001) and the transition from pelagic to benthic habitats that are subject to very different environmental conditions (Gosselin and Qian 1996). The condition of larvae may influence their ability to survive this transition phase (Gosselin and Qian 1997; Phillips 2002). Gosselin and Qian (1997) found that following this brief initial period of high mortality, mortality rates decreased sharply with increasing age before levelling off, and this was evident across a variety of different invertebrate taxa and habitats. They suggested that the unifying element was a vulnerability to mortality which decreases as a function of age or size.

Size may influence vulnerability to grazing, predation, physical stresses such as desiccation and dislodgement, and the outcomes of competitive interactions (Connell 1961; Dayton 1971; Foster 1971; Seed and Brown 1978; Moreno 1995; Moksnes *et al.* 1998; Osman and Whitlatch 2004). Differences in growth rates of early juveniles may therefore determine

their ability to escape mortality and hence their chances of survival (Gosselin and Qian 1997; Robles 1997; Jarrett 2000). Early post-larval and juvenile growth rates may vary both spatially and temporally (Connell 1961; Seed 1969b; Roegner and Mann 1995; Bartol *et al.* 1999; Jarrett 2000). Food supply and larval history may also influence the performance of early juveniles (Phillips 2002).

Several studies have investigated the relative importance of settlement versus pos-settlement mortality on subsequent recruitment and adult distributions (Denley and Underwood 1979; Connell 1985; Gaines and Roughgarden 1985; Whethey 1985; Davis 1988; Hurlburt 1991; Osman and Whitlatch 1995b; Hunt and Scheibling 1997; Appelbaum *et al.* 2002). The outcome of these studies is that initial settlement may be more important in some cases, while early mortality may be more important in others, and that the relative importance of each may vary among different species within the same community (Hurlburt 1991; Appelbaum *et al.* 2002).

In the previous chapter, it was determined that settlement may be a contributing factor to the zonation patterns of *Perna perna* and *Choromytilus meridionalis,* but not *Mytilus galloprovincialis*. It was hypothesised that the increased density of *M.galloprovincialis* on the high-shore may be a result of successive settlements of slow-growing individuals which experience low mortality rates. On the other hand, mortality of *P.perna* and *C.meridionalis* would have to be greater in this zone in order to account for the differences in density and cover of these species which were not reflected in settlement.

This study examines patterns of post-settlement mortality and early juvenile growth of mussels at different tidal heights and between locations. Due to consistently low settlement

rates in Tsitsikamma, these experiments were only conducted in Plettenberg Bay. However, some information on variations of mortality at the level of site (mesoscale) was inferred from monthly recruitment data. It should also be noted that post-settlement mortality in this study refers to mortality of recently settled mussels, including both primary and secondary settlers.

5.2 Materials and methods

Juvenile growth

Growth of juvenile mussels was measured *in situ* using the fluorochrome growth marker calcein. This has proven to be a reliable method for measuring shell growth in juvenile mussels (Kaehler and McQuaid 1999) and juvenile gastropods (Moran 2000) with no apparent effects on survivorship or growth.

This experiment was initially a process of trial and error, which involved several attempts before meaningful results were obtained. The first experiment was launched in March 2001 where two pads were placed in each zone at Lookout Beach and Beacon Isle for a period of 24-48 hours to collect settlers. After this time, pads were removed from the rocks and were immersed in a container filled with a calcein-sea water solution ($125mg.l^{-1}$) for 1-2hrs. Pads were placed in individually marked bags with large holes to allow complete penetration of the calcein solution. After immersion, pads were replaced in their original positions on the shore and left for a period of two weeks. Samples were immediately frozen after collection. In the laboratory juveniles were extracted in the usual manner and were measured individually under the light microscope fitted with an ocular micrometer. Once measured, each mussel was then examined under an Olympus flourescence microscope for the presence of calcein marks. Growth was measured as the distance between the growing edge

of the shell and the calcein mark. The success rate for this experiment varied between 0-16% for the different samples.

The experiment was repeated again in July 2001. The same procedure was followed, however, six pads were marked in each zone and at each location. These pads were left out for one month. Since growth may be reduced during winter (Seed 1969b; Dare 1976), it was necessary to leave the samples out for longer so that reasonable growth increments could be obtained. No mussels with calcein marks were recovered from these samples. There are several possible reasons why these two experiments may have failed, which include: a lack of initial settlement; death or emigration of marked individuals; calcein concentrations were too low or mussels did not take up the calcein. Separating these factors as potential causes is virtually impossible. However, the large number of recruits present in the July samples compared to the March samples suggests that some settlement probably did occur in the two days prior to immersion in this experiment, and that the length of time that pads were in the field may have been an important factor. This is especially true for mussels which may detach and reattach many times after settlement (Bayne 1964). Therefore, the longer samples are in the field, the greater the risk of losing marked individuals to mortality or emigration.

Growth was measured again from 4-16 March 2003. Six pads were placed in each zone for a period of 48hrs. Marking success in juvenile mussels using calcein increases with increasing concentration and immersion time (Eads and Layzer 2002), however, mortality may also increase when concentrations are too high (Moran 2000). The calcein concentration was therefore increased to 200mg.l^{-1} to ensure that low concentrations were not the cause of poor recovery rates. Pads were immersed in the calcein-sea water solution

for two hours before being replaced and were collected two weeks later. Recovery rates of marked recruits from this experiment were very good on the low-shore, particularly at Lookout Beach, but were poor on the mid-shore and no results were obtained for the high-shore. Low settlement rates on the high-shore may have been the main reason for poor recovery rates in this zone. Since high-shore growth was still an important factor that was missing, the experiment was repeated again from 27 October – 9 November 2003. Eighteen pads were placed on the low-shore to collect settlers. Following immersion for two hours in a 200mg.l^{-1} calcein solution, six pads were replaced on the low-shore and the rest were transplanted into the upper zones. Samples were collected after two weeks. This experiment proved to be the most successful and results were obtained for all shore levels.

Post-settlement mortality

Post-settlement mortality was measured on two consecutive occasions that covered a period of six days over a spring and a neap tide respectively. Twelve scouring pads were placed in each zone at each location in Plettenberg Bay. Within each zone, pads were attached in pairs to six different nails spaced approximately 0.5-1.0m apart. One pad was replaced daily while the other was left until the end of each sample period, resulting in six replicates of each per zone. The daily settlement and six-day recruitment pads were attached to the same nail in an attempt to minimise the variation in rates of settlement between the two. Mortality for each pair was calculated by subtracting the total number of live recruits in the 6-day pads at the end of the sample period from the cumulative daily settlement that occurred during that time. Mortality was then expressed as a percentage of the cumulative daily settlement. The same procedure and the same time interval were used on both sampling occasions. However, due to rough working conditions during neap tide at Beacon Isle, no mortality data were obtained for the second week from this location.

Scouring pads were frozen immediately after collection. Samples were processed using the methods described in Chapter 4. In all samples mussels were distinguished as either "dead" or "alive" upon collection, however, this distinction was not always straightforward. This was because many mussels often had varying amounts of tissue left in the shell. This could have been due either to recent death in the field or possibly due to some aspect that caused the tissue to shrivel or dehydrate after collection. Therefore only mussels that had no evidence of tissue left in the shell were classified as dead. In this case mortality may have been underestimated. Another problem was that once the mussels were frozen, the valves tended to loosen slightly and gape. As a result, it occasionally happened that the whole body was dislodged from the shell during the washing process. Since there was no way of determining their origin, the empty shells of these mussels had to be considered as dead animals.

A potential problem associated with this method is that one cannot separate actual mortality from emigration of mussels from the recruitment pads. Daily mortality rates were therefore calculated by expressing mortality as the proportion of dead mussels to the total number of mussels that settled. This method assumes that all mussels that died remained attached to the pads for 24hrs. The numbers of dead mussels were much higher in the recruitment samples than the daily samples, which suggests that dead mussels accumulated on these pads over the sampling period and may therefore remain attached for several days after death. Therefore this assumption seems reasonable. Estimating mortality in this way poses an additional problem which is related to the number of mussels in each sample. A single settler that dies represents 100% mortality. Thus mortality was only estimated where a minimum of ten individuals of each species were present. This restricted the analysis to samples collected from the 22-24 March when settlement was high.

To determine whether there was a relationship between size and mortality, mortality was calculated as the proportion of dead mussels to the total number of mussels in each size group. The numbers of mussels in the largest size group (>3.0mm) were too low to be analysed, therefore the following size categories were examined: <0.34, 0.34-0.59, 0.6-1.49 and 1.5-3.0mm. Size-specific mortality rates were calculated from recruitment samples that had been out for six days over neap tide at Lookout Beach. Using the criterion of a minimum of ten individuals precluded the use of daily or spring tide recruitment samples for this analysis. In addition, each species was not well represented in all size groups. Examination of the data revealed that the pattern that emerged was evident for each species when size comparisons could be made. Species were therefore pooled for the analysis. There are two potential problems associated with this approach. Firstly, mussels may have grown out of the size groups in which they settled during this time. Estimated daily growth rates from Figs. 5.1-5.2 (p.174-175) revealed that growth in 1-6 days would generally be minimal. Although it is possible that mussels closer to the limit of each size group may have grown out, it may be assumed that the majority would still represent the size categories in which they settled. Secondly, some dead mussels may have been washed off the pads and mortality may have been underestimated. However, since mussels may remain attached to the pads for a few days after death, it is possible that the majority would still be accounted for.

The percentage mortality of mussels was estimated from monthly recruitment pads. In this case mortality will most certainly be underestimated. It is highly unlikely that all mussels that settled and died over a period of a month would remain attached to the pads. This analysis was performed primarily to determine whether there was evidence of site-specific

or temporal differences in mortality rates. However, the results may only be considered as suggestive.

Analyses

1. Juvenile growth

Low-shore growth of all three species in March was highly variable with no obvious trend with size ($r<0.45$) (Fig. 5.1 a-c; p.174). On the mid-shore, growth tended to increase with increasing size, however this relationship was still weak and was not significant ($r=0.57$ and 0.55 for *P.perna* and *M.galloprovincialis* respectively; $p>0.05$). Data for all size classes from this month were therefore pooled and analysed using a factorial ANOVA with location, zone or species as factors. Juvenile growth in November showed strong positive relationships between initial length and growth which were generally significant (Fig. 5.2 a-f; p.175). November data were therefore analysed using ANCOVA with initial length as a covariate. Data did not require transformation.

2. Post-settlement mortality

Some recruitment pads were lost during each week resulting in different sample sizes for each zone (n=3-6). ANOVAs were performed with unbalanced sample sizes to make use of all available information. Mortality was analysed using Factorial ANOVA with either location (random), zone or species as factors. To examine whether mortality varied temporally, tide was included as a fixed factor. It should be noted though that tide was not replicated therefore any tidal effects should be interpreted with caution. Mortality of *C.meridionalis* was only analysed using samples from Lookout Beach due to a lack of settlement at Beacon Isle. Data did not require transformation.

Daily mortality rates were not analysed as these were generally very low (<7.0%) and could only be measured over a three day period. Size-specific mortality was analysed using zone and size as fixed factors. Recruit mortality was initially plotted for the lower zones for each month. Very few data were available for the high-shore, which was excluded. There was generally little difference in patterns or rates of mortality between the low and mid-shores. Also, since sample sizes in some months were very poor (n=1), data from these two zones were pooled. Mortality rates in Tsitsikamma could only be calculated for the months November to March. Thus only these months were compared between sites using a mixed model ANOVA with month, site, location and species as factors. Month and location were random and location was nested in site.

5.3 Results

Juvenile growth

Juvenile growth rates from March 2003 are shown in Figs. 5.1 a-c (p.174). Data were only obtained for the low and mid-shore at Lookout Beach and the low-shore at Beacon Isle. Juvenile growth of *P.perna* and *M.galloprovincialis* in March was compared between zones at Lookout Beach. There was a significant effect of zone (F=7.0; p=0.01) but not of species, and there was no interaction between them. Both species grew significantly faster on the low-shore than on the mid-shore. Juvenile growth of all three species was also compared on the low-shore at this location. There was a highly significant effect of species (F=10.2; p<0.001) and *C.meridionalis* grew significantly more slowly than *P.perna* and *M.galloprovincialis.* Growth of juvenile *M.galloprovincialis* did not differ significantly between locations (F=0.02; p=0.9). It should be noted, though, that due to the large variability in growth during this month, these results should be considered cautiously.

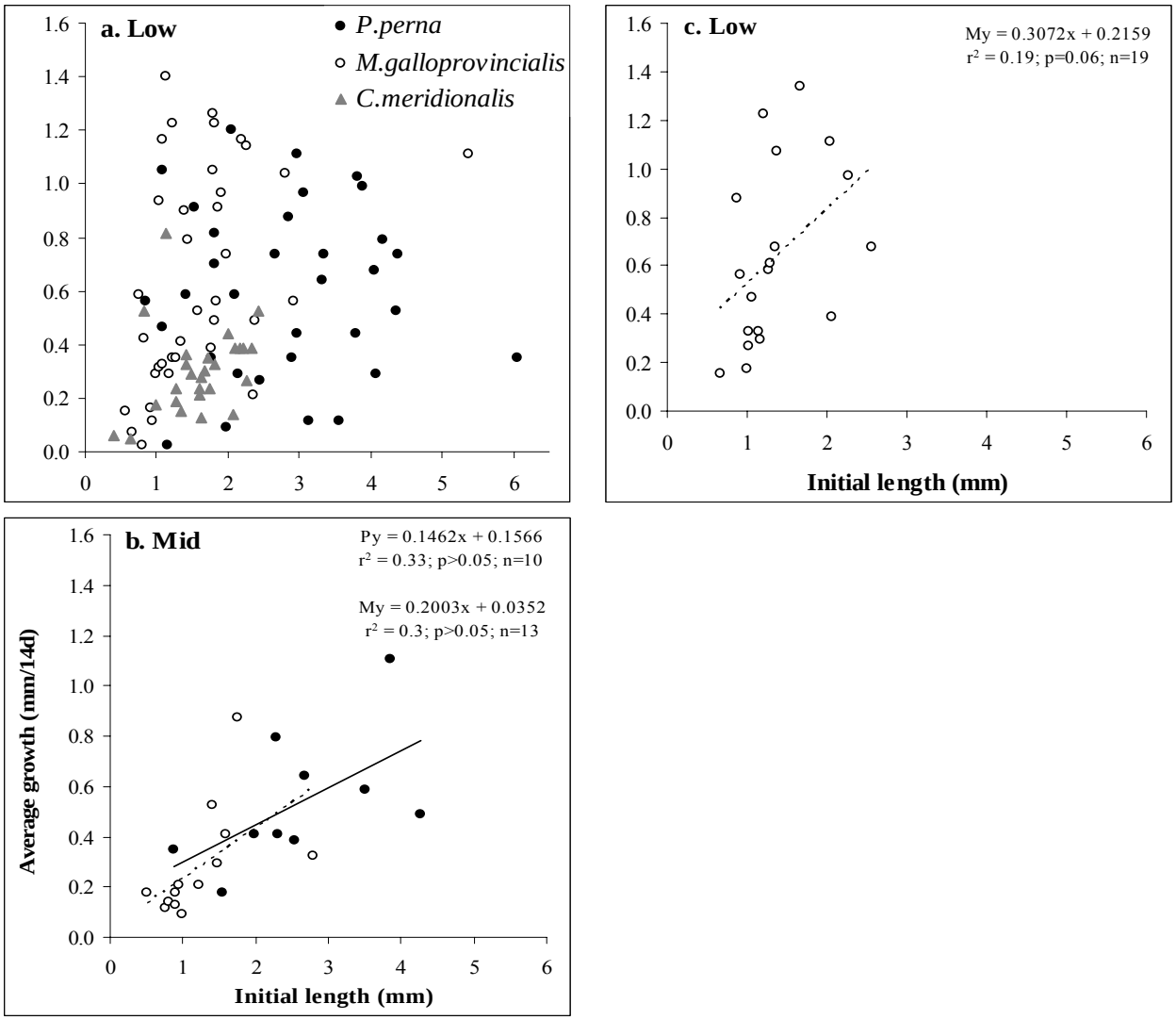


Figure 5.1 a-c: Juvenile growth of mussels at Lookout Beach (a-b), and Beacon Isle (c) in March 2003. Solid trend lines apply to *P.perna* and dashed trend lines to *M.galloprovincialis*. Equations and correlation coefficients have been given where Py = *P.perna* and My = *M.galloprovincialis*

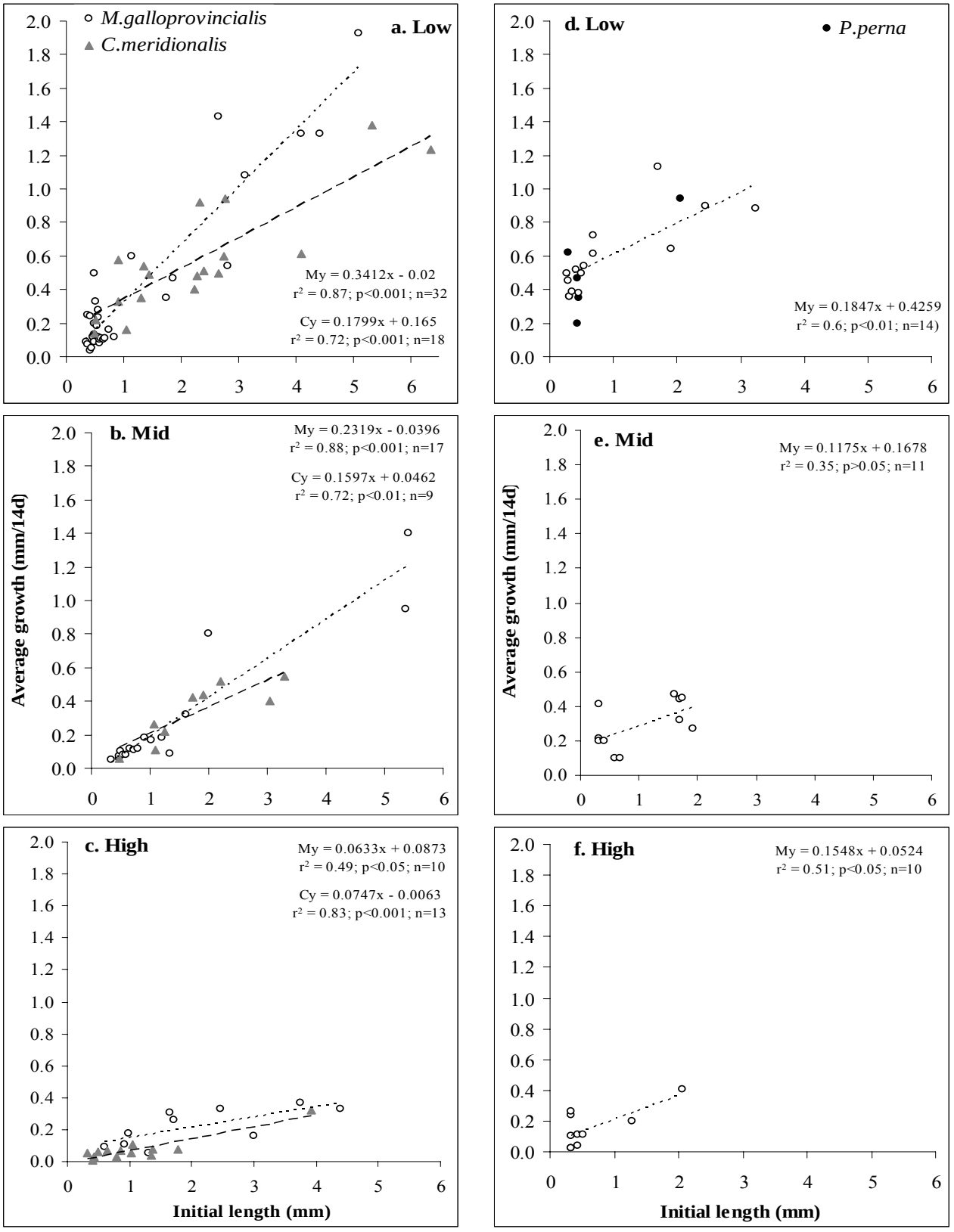


Figure 5.2 a-f: Juvenile growth of mussels at different tidal heights at Lookout Beach (a-c) and Beacon Isle (d-f), Plettenberg Bay, in November in 2003. Trend lines with long dashes apply to *C.meridionalis*. Cy = *C.meridionalis*

Growth rates from Lookout Beach and Beacon Isle in November 2003 are shown in Figs. 5.2 a-f (p.175). There was a very clear trend for growth to increase with increasing size and this relationship was best described by a linear model. However, this trend was less obvious on the high-shore where growth of larger juveniles was slower. Unfortunately there was very little settlement of *P.perna* at this time so only a few data points were obtained for this species on the low-shore at Beacon Isle. These were plotted to show that there appeared to be little difference in growth between *P.perna* and *M.galloprovincialis* on the low-shore at this location.

An analysis comparing growth of *M.galloprovincialis* between locations and zones in November gave no significant main effects or interactions. There appeared to be an effect of zone on growth therefore separate analyses were performed at each location. At Beacon Isle, there was a highly significant effect of zone on growth of *M.galloprovincialis* ($F=35.99$; $p<0.001$) and growth decreased significantly with increasing tidal height (Fig. 5.3; p.177). Since data were also available for *C.meridionalis* at Lookout Beach, growth of these species was compared between zones. Zone had a highly significant effect ($p<0.001$; Table 5.1; p.178) but there was no effect of species or interaction between them. As at Beacon Isle, growth decreased significantly with increasing tidal height (Fig. 5.4; p.178).

There is generally little information available on early post-larval growth in mussels, therefore the average growth rates of mussels 280-600µm in length from different tidal heights at Lookout Beach and Beacon Isle in November 2003 were calculated (Table 5.2; p.179). Although there were too few data to analyse there appeared to be a strong location effect that was observed at all tidal heights. Furthermore, post-larval growth also decreased with increasing tidal height at both locations.

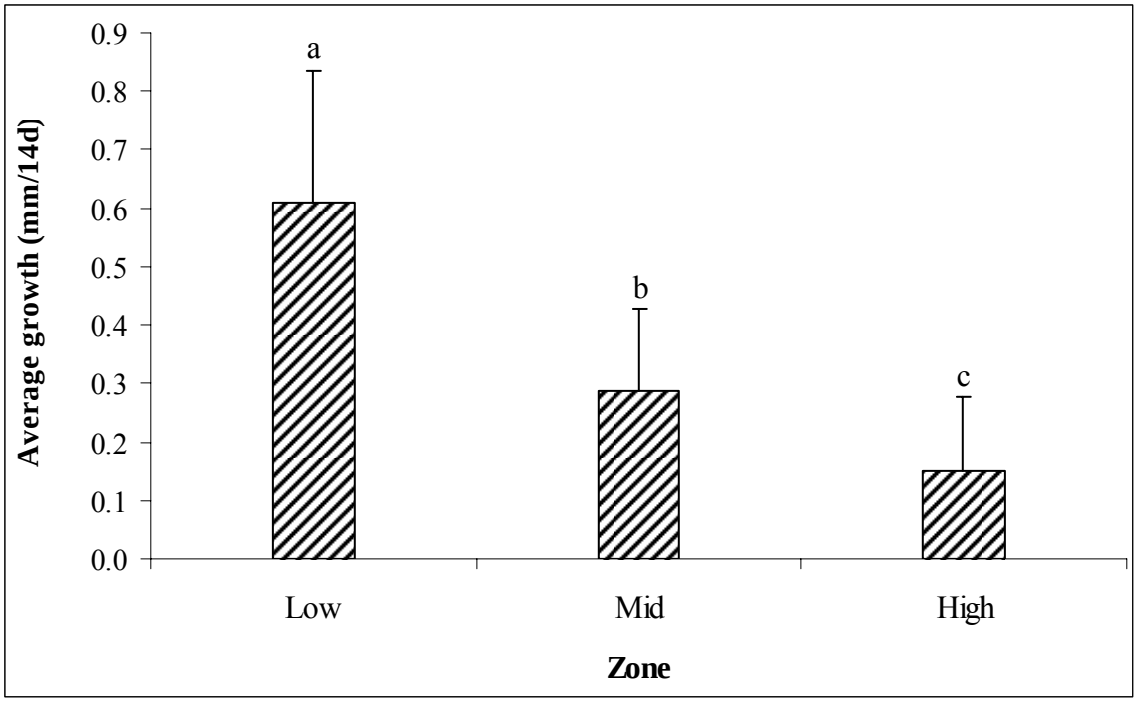


Figure 5.3: Post-hoc comparison of the effect of zone on juvenile growth of *M.galloprovincialis* at Beacon Isle in November 2003. Similar letters indicate homogeneous groups. Error bars represent standard deviations

Table 5.1: Two-way ANCOVA examining the effects of zone on juvenile growth of *M.galloprovincialis* and *C.meridionalis* at Lookout Beach in November 2003

	Df	MS	F	p
length	1	8.619	232.296	<0.001
zone*	2	0.712	19.197	<0.001*
species	1	0.002	0.042	0.838
zone×species	2	0.034	0.916	0.404
Error	92	0.037		

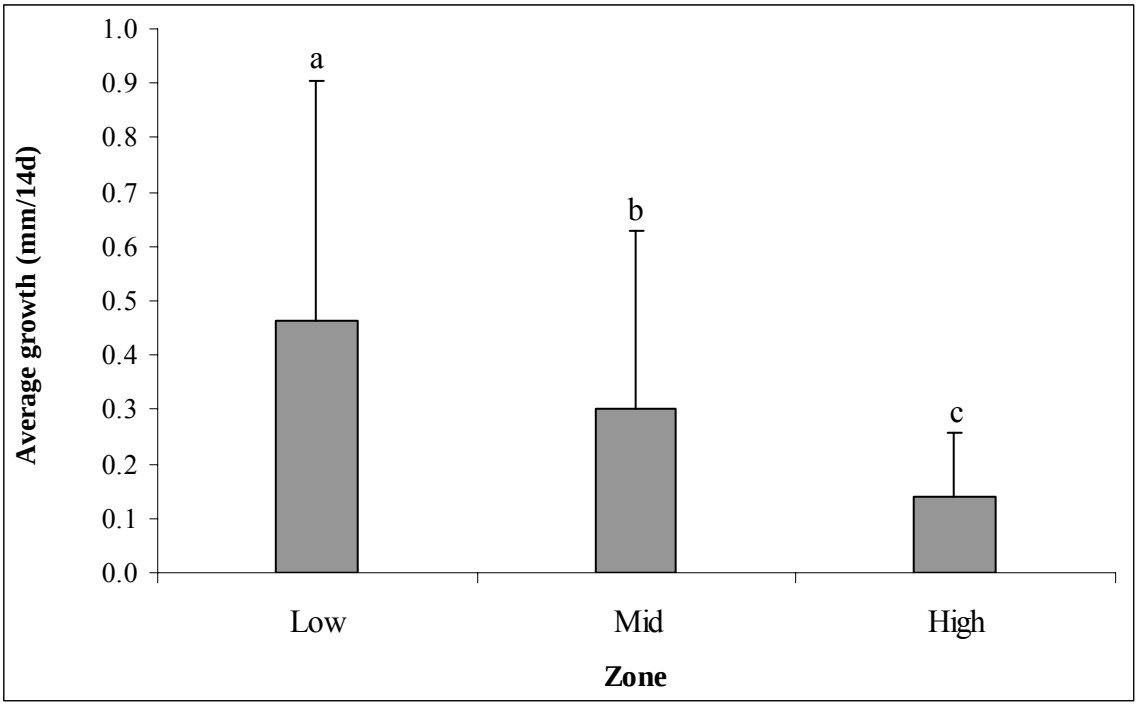


Figure 5.4: Post-hoc comparison of the effect of zone on juvenile growth of mussels (*M.galloprovincialis* and *C.meridionalis*) at Lookout Beach in November 2003. Error bars represent standard deviations

Table 5.2: Average daily growth rates of early post-larval mussels (0.28-0.59mm) of *P.perna* (P), *M.galloprovincialis* (M) and *C.meridionalis* (C) at different tidal heights in November 2003

Zone	**Species**	**Lookout Beach** Growth (µm/day)	**Beacon Isle** Growth (µm/day)
Low	P	--	28.6 ± 11.1
	M	12 ± 8.4	32.5 ± 5.0
	C	13 ± 4.0	--
Mid	M	5.1 ± 1.37	16 ± 8.1
	C	--	--
High	M	--	8.0 ± 6.6
	C	2.7 ± 1.58	--

Post-settlement mortality

Analysis of post-settlement mortality of *P.perna* and *M.galloprovincialis* between zones and locations over spring tide revealed that there were no significant main effects or interactions. In other words, mortality did not differ between locations ($F=2.78$; $p=0.3$), nor were there any zone or species interactions with location. When each location was examined separately, there was a significant effect of zone ($F=3.85$; $p=0.04$) at Lookout Beach, but no effect of species. Early mortality was significantly greater in the upper zones than on the low-shore. The interaction between zone and species was not quite significant ($F= 3.17$; $p=0.06$), however, further examination revealed zone-specific differences in mortality between species that were considered to be biologically significant. These results are presented in Fig. 5.5 (p.181). Mortality of *P.perna* and *M.galloprovincialis* was similar in the two lower zones and mortality of both species was significantly higher on the mid-shore than on the low-shore. However, this pattern changed sharply on the high-shore. While mortality of *P.perna* continued to increase upshore, mortality of *M.galloprovincialis* decreased. The result was that post-settlement mortality of *P.perna* was markedly higher than *M.galloprovincialis* on the high-shore (65% and 17% respectively).

Mortality at Beacon Isle over spring tide did not differ significantly between zones or species, and there was no interaction between them ($F=0.99$; $p=0.4$). Mortality was generally very low at this location, possibly due to lower overall settlement rates than at Lookout Beach. There was also considerable variation between samples largely because a number of samples had no mortalities. For example, low-shore mortality of *M.galloprovincialis* was 75% in one sample and 0% in the remaining samples. This was because the number of recruits was greater than the total number of cumulative settlers.

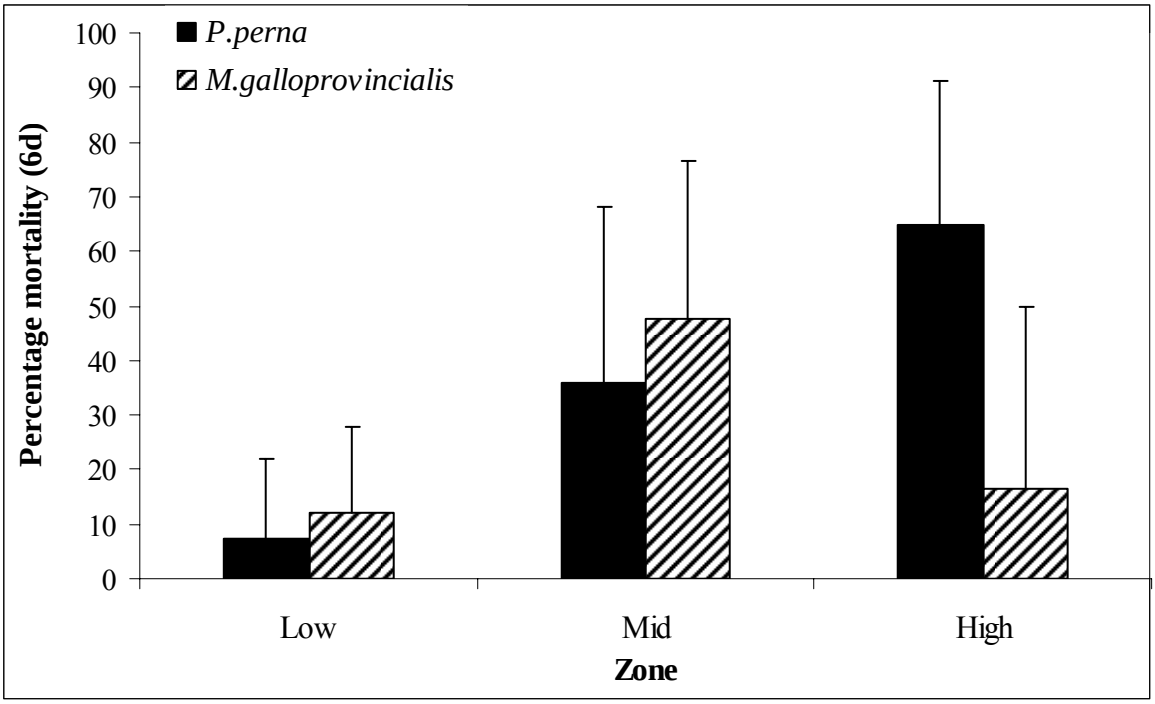


Figure 5.5: Average post-settlement mortality of *P.perna* and *M.galloprovincialis* at different tidal heights over spring tide at Lookout Beach. Error bars represent standard errors

This may suggest that the presence of established settlers or recruits enhanced settlement to these pads, particularly when settlement is low.

Results of the analysis on mortality over neap tide at Lookout Beach revealed that there was a significant effect of zone ($F=4.06$; $p=0.03$) but not species and no interaction between them. Post-hoc comparison revealed that mortality of both species was significantly greater on the mid-shore than either the low or high-shore (Fig. 5.6; p.183). Standard deviations were very large which was probably also due to enhanced settlement on some recruitment pads resulting in no mortality. Nevertheless, it seems that *P.perna* exhibited different patterns of high-shore mortality between tides, with 65% mortality over spring tide compared to only 6% over neap tide. On the other hand, *M.galloprovincialis* displayed the same mortality pattern with zone on both occasions. Mortality was significantly lower over neap than spring tide in all zones ($F=7.16$; $p=0.01$) and there was no interaction of tide with either zone or species.

Analysis of post-settlement mortality of *C.meridionalis* at Lookout Beach over spring tide revealed that zone did not have a significant effect ($F=3.07$; $p=0.1$). This was surprising in view of the average mortalities in each zone. These results were again thought to be biologically significant considering the distribution of this species and have therefore been presented in Fig. 5.7 (p.184). Mortality of this species was greater in the upper zones than on the low-shore. Mortality over neap tide was also not significantly different between zones ($F=2.4$; $p=0.1$). However, *C.meridionalis* displayed the same differences in high-shore mortality between tides as *P.perna* with a reduction in mortality from 64% over spring tide to 29% over neap tide (Fig. 5.7). A comparison of mortality between tides

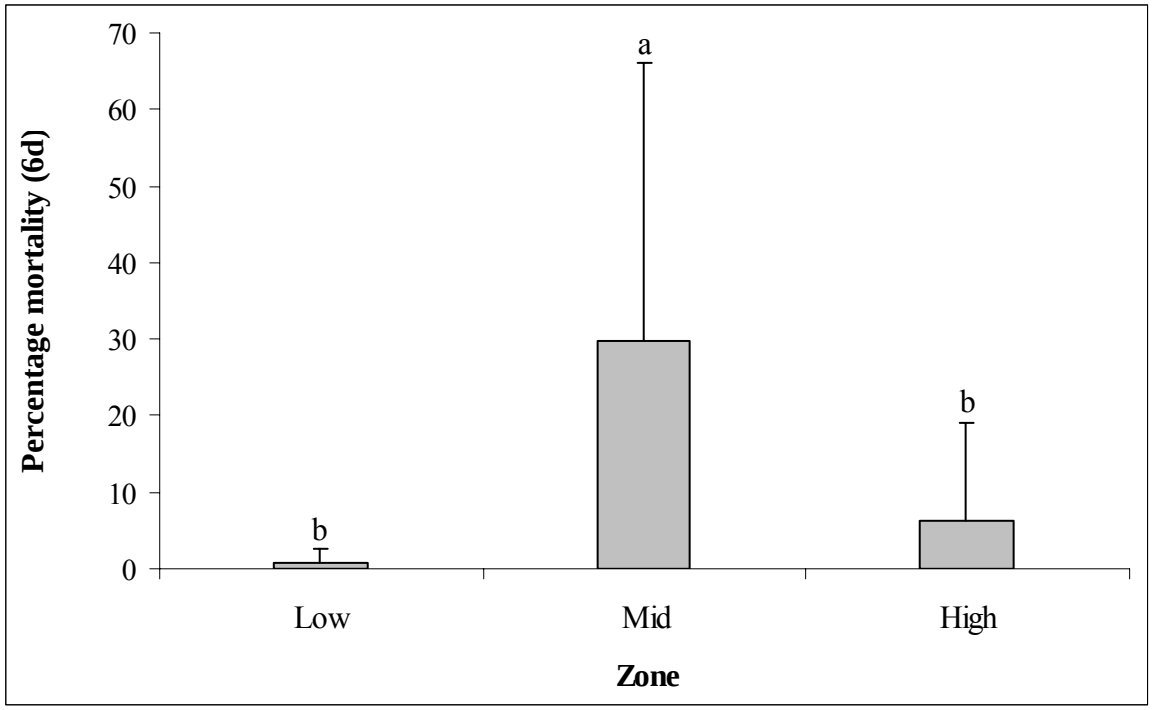


Figure 5.6: The effect of zone on post-settlement mortality of mussels (*P.perna* and *M.galloprovincialis*) over a neap tide at Lookout Beach. Error bars represent standard errors

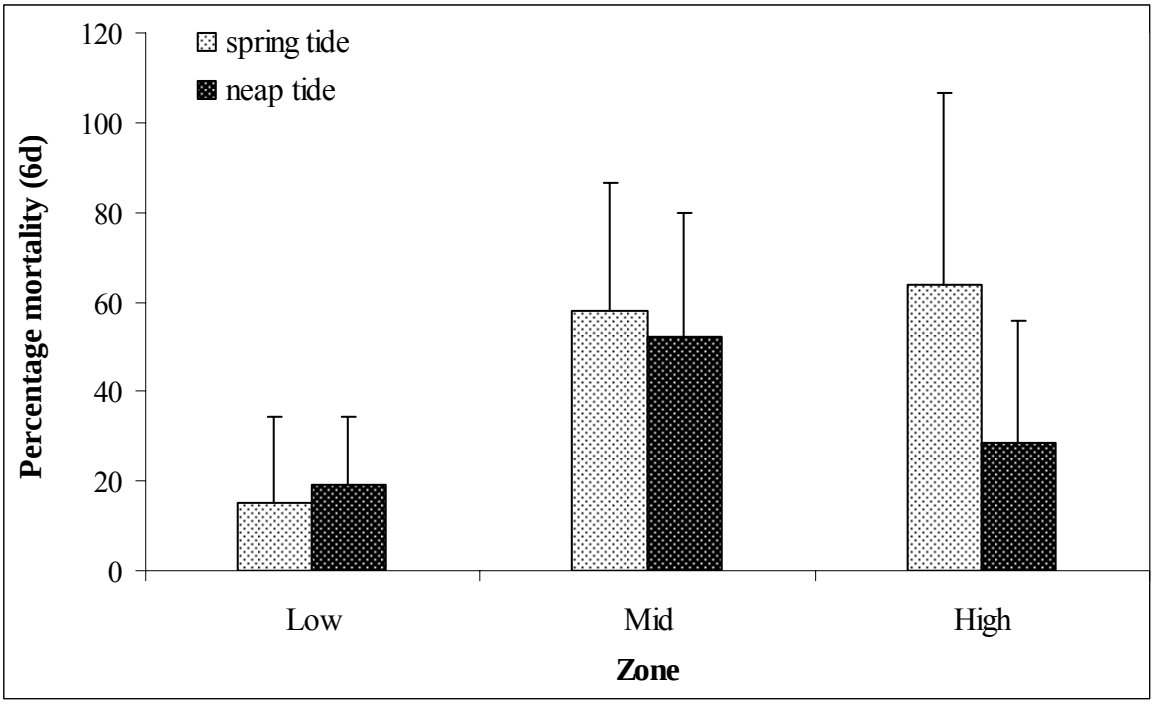


Figure 5.7: Post-settlement mortality of *C.meridionalis* at different tidal heights at Lookout Beach over a spring and a neap tide. Error bars represent standard errors

revealed that mortality of this species did not differ significantly between tidal cycles ($F=1.38$; $p=0.3$).

In summary, all three species had low mortality rates on the low-shore regardless of tide, and significantly higher mid-shore mortalities that were also independent of tide. However, on the high-shore, mortality of *P.perna* and *C.meridionalis* appeared to be affected by tidal cycle. Mortality of both species increased on the high-shore during spring tide, particularly for *P.perna,* while mortality decreased relative to the mid-shore during neap tide. In contrast high-shore mortality of *M.galloprovincialis* was low and independent of tide. It was also interesting that mid-shore mortality differed from both low and high-shore mortality regardless of tide, which may suggest that the factors controlling post-settlement mortality in this zone are different from those in either the low or high zones, and that these factors are not influenced by tidal cycle or species.

Average daily mortality rates from the 22-24 March at Lookout Beach generally never exceeded 7% regardless of species or zone, with the exception of the mid-shore where mortality of *P.perna* was 13%. Examination of daily data from the remaining period revealed that mortality was generally very low even when settlement was low. However, there was one notable exception. On the 28 March there was a relatively large primary settlement of *M.galloprovincialis* and mortality for these settlers was 44% on the low-shore and 23% on the mid-shore. Since the majority of mussels settling on 22-24 March were 0.6-3.0mm in length this may suggest that size plays a role. In other words, larger mussels may be less vulnerable to mortality at least within a period of one day. Analysis of mortality from six-day samples at Lookout Beach, revealed that size had a highly significant effect ($F=30.07$; $p<0.001$). There was no effect of zone or any interaction between size and zone.

Post-hoc comparison revealed that mortality decreased with increasing size (Fig. 5.8; p.187). Mortality amongst primary settlers was significantly higher than early secondary settlers (0.34-0.59) and subsequently decreased significantly in mussels >0.6mm in length.

Monthly recruit mortality rates were plotted for Lookout Beach and Beacon Isle in Plettenberg Bay (Fig. 5.9 a-b; p.188). Sample sizes for *C.meridionalis* were generally too small at Beacon Isle and were therefore not included. At Lookout Beach, mortality of all three species was fairly low and consistent between July and October but increased considerably in November. Mortality decreased after this month and was generally consistent throughout the remaining months. A similar, but far more striking pattern was observed at Beacon Isle. However, the high November mortality continued through to January before dropping sharply in February. This pattern was most pronounced for *P.perna* but less so for *M.galloprovincialis* which experienced higher mortalities than *P.perna* in the first few months.

Analysis of monthly recruit mortality rates between sites revealed that site did not have a significant effect (Table 5.3; p.189), nor were there any significant interactions with site. However, month had a significant effect and there was an interaction between month and location (site) ($p=0.004$). There were significant differences in mortality between locations in Plettenberg Bay in December and January, where mortality was greater at Beacon Isle (Fig. 5.10a, p.190). At Beacon Isle, mortality was significantly higher between November and January than in February or March, while at Lookout Beach there was generally little difference in mortality between months, with the exception of November when mortality was greatest. In contrast there were no location-specific differences in mortality in Tsitsikamma (Fig. 5.10b) regardless of month. Mortality rates at Beacon Isle and in

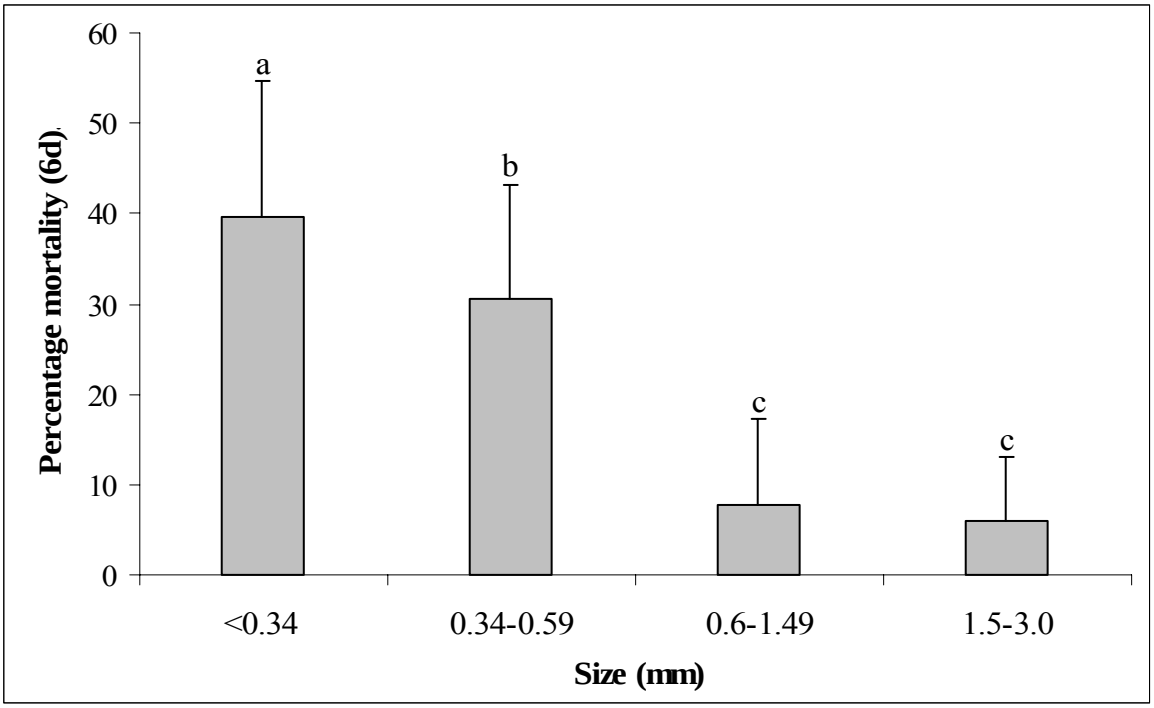


Figure 5.8: Post-hoc comparison of the effect of size on post-settlement mortality of mussels over neap tide at Lookout Beach. Error bars represent standard errors

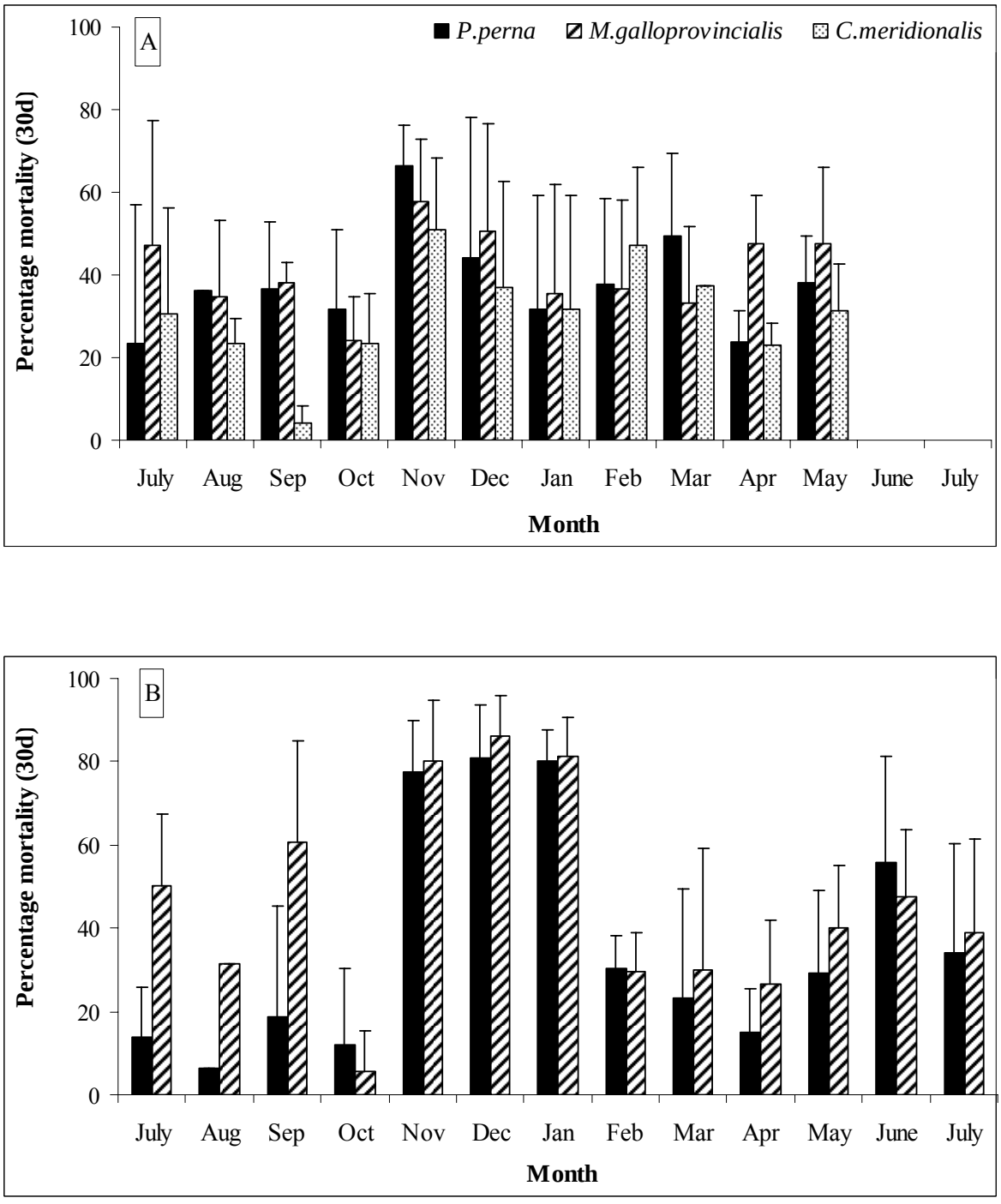
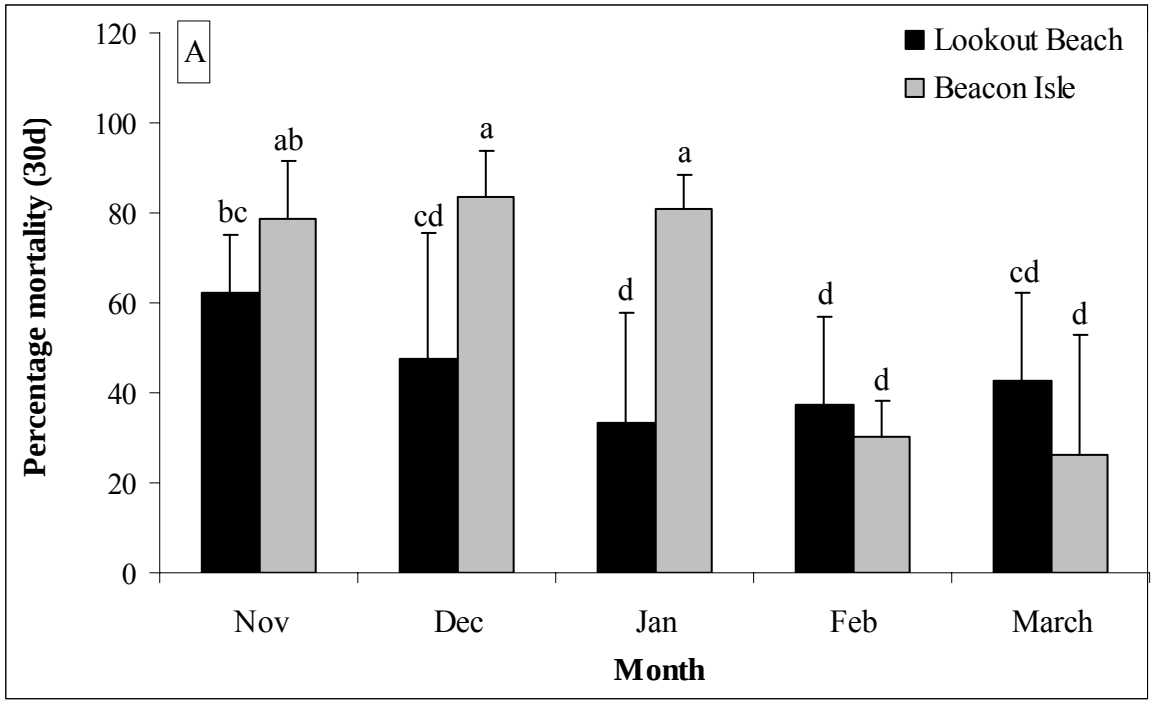


Figure 5.9 a-b: Average monthly mortality rates of mussel recruits at a) Lookout Beach and b) Beacon Isle in Plettenberg Bay from July 2000-July 2001 (low and mid zones pooled). Error bars represent standard deviations

Table 5.3: Mixed model ANOVA examining the effects of month, site, location and species on recruit mortality of mussels in Tsitsikamma and Plettenberg Bay between November and March 2001

	Effect	Df	MS	F	p
month*	Random	4	11386.1	21.76	0.001*
site	Fixed	1	9067.8	5.73	0.365
species	Fixed	1	73.8	0.14	0.738
month×site	Random	4	308.6	0.22	0.917
month×species	Random	4	290.8	3.82	0.111
site×species	Fixed	1	81.2	0.27	0.699
month×site×species	Random	4	76.1	0.38	0.818
location (site)	Random	2	2815.8	1.60	0.260
month×location (site)×species	Random	8	198.4	0.72	0.670
location (site)×species	Random	2	429.6	2.15	0.176
month×location (site)*	Random	8	1551.3	7.82	0.004*
Error		148	273.8		



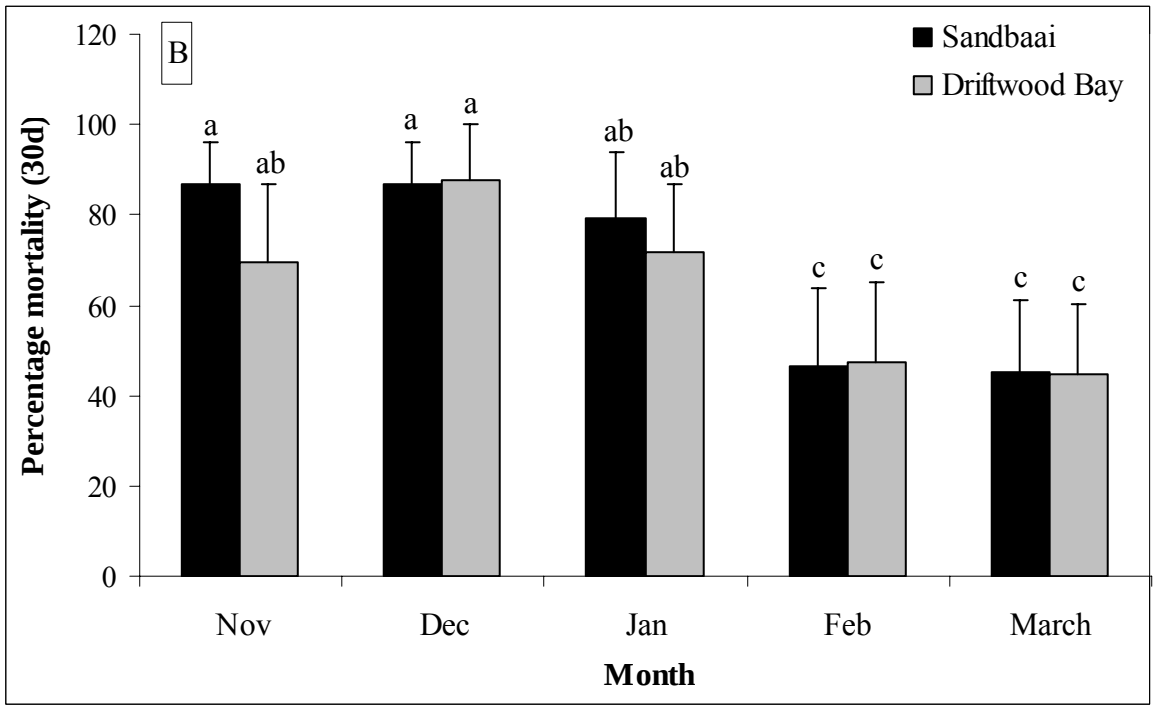


Figure 5.10 a-b: Results of the post-hoc comparison of the interaction between month and location in a) Plettenberg Bay, and b) Tsitsikamma. Error bars represent standard deviations

Tsitsikamma were very similar, with very high mortalities of ±80% between November and January which dropped to 30-50% in February and March. Considering that the total number of mussels recorded during the months when mortality was high was nearly 40× greater at Beacon Isle than in Tsitsikamma, such similar mortality rates would have significantly different consequences for recruitment at these locations. Thus high mortality rates still produced a few hundred successful recruits at Beacon Isle (see Chapter 4), while in Tsitsikamma this resulted in <12 mussels per month recruiting to the population.

5.4 Discussion:

Post-settlement mortality of mussels in Plettenberg Bay appeared to differ between tidal cycles. However, tide only affected high-shore mortality and only in certain species. Post-settlement mortality of *Perna perna* and *Choromytilus meridionalis* increased with increasing tidal height during spring tide, but not during neap tide when high-shore mortality was significantly reduced. In contrast, high-shore mortality of *Mytilus galloprovincialis* was low and apparently unaffected by tidal cycle. Post-settlement mortality has been found to increase with increasing height on the shore in a variety of intertidal invertebrates, including barnacles (Connell 1961; Minchinton and Scheibling 1993a, 1993b; Chan and Williams 2003), oysters (Roegner and Mann 1995) and mussels (Tan 1975; Kennedy 1976; Iwasaki 1995). This is generally attributed to increased desiccation stress at higher levels of aerial exposure, which suggests that juvenile *M.galloprovincialis* may be more tolerant of desiccation than *P.perna* and *C.meridionalis.* These findings are supported by those of Kennedy (1976), who found that late juveniles (5-15mm) of *M.galloprovincialis* (reported as *M.edulis aoteanus,* see McDonald *et al.* 1991) were more resistant to desiccation than *P.canaliculus* in New Zealand. A similar result was

also obtained in adults of the three South African species studied here (Hockey and van Erkom Schurink 1992; van Erkom Schurink and Griffiths 1993).

High-shore mortality of *P.perna* and *C.meridionalis* was significantly greater over spring tide than neap tide and this may reflect differences in desiccation stress with tidal cycle. On several occasions when daily samples were collected, including this study, conditions over neap tide were often too rough to sample. Consequently, high-shore areas generally received considerably more wave splash during neap low tides than during spring low tides. In addition, air temperatures on this occasion were lower during neap tide due to overcast conditions on most days (average air temperatures of 20ºC and 18ºC for spring and neap tide respectively). In contrast, post-settlement mortality in the barnacle *Semibalanus balanoides* increased as a result of prolonged aerial exposure during neap tides that were coincident with a period of warm, calm weather (Connell 1961; Foster 1971). Thus interactions between environmental conditions and tidal cycle may be more important than tidal cycle alone in determining early mortality rates at upper shore levels. Alternatively, mortality may have been independent of tidal cycle and largely determined by environmental conditions. Since tidal cycle was not replicated in this study, the relative importance of these factors remains unclear and requires further investigation.

These results suggest that differential post-settlement mortality rates between species may be an important determinant of adult community structure on the high-shore in Plettenberg Bay. Species-specific differences in early mortality rates have been found to influence adult abundance at upper shore levels in barnacles (Connell 1961) and the mussels *Hormomya mutabilis* and *Septifer virgatus* in Japan (Iwasaki 1995). Although early mortality rates may be dependent on climatic conditions, consistently low mortalities in *M.galloprovincialis*

relative to *P.perna* and *C.meridionalis* should lead to comparatively higher abundances of this species on the high-shore. Furthermore, if vulnerability to desiccation persists with age in *P.perna* and *C.meridionalis*, then individuals that survive the early juvenile stage may remain prone to elimination whenever conditions become unfavourable.

Post-settlement mortality of all three species in the lower zones was very similar, with significantly greater mortalities on the mid-shore than on the low-shore where survival was generally very high (>80%). Low mortality rates on the low-shore may merely reflect the lower levels of desiccation stress in this zone. While desiccation may have contributed to higher mortality rates on the mid-shore (post-settlement mortality was slightly lower over neap tide), it was unlikely to be the main factor affecting survival in this zone as mortality was independent of tidal cycle or species.

Grazing and density-dependent mortality due to competition for space were not considered to be important factors limiting recruitment on artificial collectors. The carrying capacity of these collectors is close to 8500 juvenile mussels per pad (based on the total numbers that accumulated over a period of four months in the field). Post-settlement mortality in this experiment was measured over six days, and the total number of mussels at the end of this period did not exceed 300 per pad. Furthermore, minimal growth would have occurred during this time so that competition would be unlikely to occur even between two adjacent individuals (see Underwood and Denley 1984).

Wave dislodgement may be a source of early mortality, however the effects of wave action might be expected to be greater on the low-shore than on the mid-shore. It therefore seems that variations in predation intensity may be the most likely explanation for differential

mortality rates in the lower zones. Predators of juvenile mussels include whelks, crabs and other small gastropods (Moreno 1995; Hunt and Scheibling 1998). Juvenile mussels with frayed or chipped edges which are characteristic of crab predation (Seed 1969b) were observed. However, probably the most important predator of small mussels is whelks (Sousa 1984; Griffiths and Hockey 1987; Moreno 1995; Hunt and Scheibling 1998). Rough estimates of predator abundance were obtained from destructive samples of the mussel bed in May 2004. At Lookout Beach, dogwhelks (*Nucella spp.*) were nearly seven times more abundant on the mid-shore than on the low-shore. Therefore variations in predator density may have been responsible for the different mortality rates on the low and mid-shores.

Although dogwhelks may occur at all tidal levels (pers. obs.) they are usually more abundant at low-shore levels, particularly as adults (Connell 1961; Dayton 1971; Hughes *et al.* 1992). There are two possible reasons for the distribution of whelks at this location. One possibility is that sand scour on the low-shore may deter predators, particularly at Lookout Beach, although large numbers of the scavenging whelk (*Burnupena spp.*) were found on the low-shore. Another reason may be the more extensive multilayering of the mussel bed on the mid-shore than on the low-shore. Whelks are relatively intolerant of desiccation stress and increased levels of wave exposure, which reduce foraging efficiency (Hughes *et al.* 1992; Etter 1996). Davenport *et al.* (1998) found that whelks were frequently found in "hummocks" of mussels which provide a refuge from physical stresses and predation as well as an abundant food supply.

In this study, dogwhelks preyed on a wide range of sizes from mussels as small as 0.3mm (primary settlers) to the largest secondary settlers which were generally <5.0mm in length (pers. obs.). The size of drilled holes in juvenile mussels, which gives an indication of the

size of the whelk (Hughes and Dunkin 1984), was relatively small, particularly in the smallest mussels. Thus whelk recruits may be particularly important predators of small mussels. Indeed, Hunt and Scheibling (1998) found that recently recruited whelks may significantly limit recruitment in mussels.

Daily mortality rates of juveniles were generally very low and rarely exceeded 7% for any species. However the majority of mussels settling at this time were secondary settlers. On the occasion when a marked primary settlement of *M.galloprovincialis* occurred, mortality within 24hrs was 44% on the low-shore. Several studies have found that mortality of newly settled invertebrates is usually high within the first few days after settlement (Roegner and Mann 1995; Gosselin and Qian 1996; Gosselin and Qian 1997; Jarrett 2000; Osman and Whitlatch 2004). This is largely due to the physiological stress associated with a changing environment and the demands of metamorphosis (Gosselin and Qian 1996; García-Esquivel *et al.* 2001). After this initial period, mortality frequently decreases with increasing age or size (Gosselin and Qian 1997). When mortality rates were measured amongst different size groups of mussels, a similar pattern was found. There was a strong negative relationship between the size of settlers and mortality, with the highest mortality amongst primary settlers. Thus vulnerability to mortality in mussels appears to decrease with increasing size. It should be noted though, that mortality here refers to different sized mussels that settled at more or less the same time (primary and secondary settlers). Therefore it is not a measure of mortality against time since settlement, but rather size at settlement. It is quite possible that mortality rates of juveniles of all size classes would increase with time as they become exposed to different conditions/predators etc. For example, mortality of the barnacle *Chthamalus anisopoma* increased with time, becoming important only one or two months

after settlement (Raimondi 1990), while no relationship between mortality and time since settlement was found for the barnacle *Balanus glandula* (Gaines and Roughgarden 1985).

There was a remarkable similarity in the temporal patterns and rates of recruit mortality between Beacon Isle in Plettenberg Bay and locations in Tsitsikamma. There appeared to be a strong pattern of high summer mortalities of >80% between November and January, which dropped sharply in February and remained low and more consistent during the rest of the year. Such similar mortality rates between sites with vastly different settlement rates (Chapter 4) may have important consequences for recruitment. Thus high mortality rates still resulted in a few hundred successful recruits at Beacon Isle, while in Tsitsikamma this resulted in <12 mussels per month recruiting to the population. This pattern was less evident at Lookout Beach. Recruit mortality there was relatively low (<50%) and more or less consistent throughout the year, although a minor peak was observed in November.

Spatial and temporal variations in early mortality rates have been well documented (Connell 1985; Whethey 1985; Minchinton and Scheibling 1993a, 1993b; Jarrett 2000; Bishop *et al.* 2005). Seasonal variations in temperature (Roegner and Mann 1995) and the abundance of predators may influence recruitment rates (Bishop *et al.* 2005). Predators, including whelks, are usually more abundant during the spring and summer months (Seed 1969b; Hunt and Scheibling 2001). However, it is probably unlikely that predator densities would be so sharply defined between successive months and furthermore that high densities would be confined to a three-month period. Whelks were also abundant in May 2004 at which time recruit mortality had decreased significantly (pers. obs.) Thus, temperature probably contributed significantly to the high summer mortality at these sites.

Growth rates of juvenile mussels were measured *in situ* on two separate occasions in March and November 2003. Growth rates in March were highly variable, particularly on the low-shore, with no obvious relationship with size. The reasons for this large variability are unknown. All these mussels were secondary settlers and it is possible that different individuals had previously settled in either "good" or "bad" areas that may have affected their condition. If food was limited at this time, it is also possible that exploitative competition for food resources led to variable growth rates. In addition, individual genetics will differ resulting in different levels of fitness (Phillips 2002). Nevertheless, results from this experiment were generally supported by those in November. Growth rates from November may be considered to be more reliable as there was a strong positive relationship between initial length (0.28-6.0mm) and growth, which is characteristic of early post-larval and juvenile bivalves including oysters (Roegner and Mann 1995; García-Esquivel *et al.* 2001) and mussels (Bayne 1964).

Juvenile growth decreased significantly with increasing tidal height. There were generally no species-specific differences in growth regardless of zone and also no significant differences in growth between locations 300-400m apart. Seed (1969b) plotted growth rates in juvenile *M.edulis* >2.0mm in length at two tidal levels. Growth was slower on the mid-shore than on the low-shore. Juvenile oysters have also been found to grow significantly faster subtidally than in the intertidal zone (Roegner and Mann 1995; Bartol *et al.* 1999). Several studies have found that growth of subadult and adult mussels decreases with increasing aerial exposure (Seed 1969b; Griffiths 1981; van Erkom Schurink and Griffiths 1993). This may be due to reduced feeding time and increased physiological stress at higher shore levels (Griffiths and Griffiths 1987; Hockey and van Erkom Schurink 1992). Presumably the same factors influence growth in early juvenile mussels.

In contrast, Phillips (2002) found that increasing aerial exposure had no effect on the growth rates of early juvenile *M.galloprovincialis* (<2 weeks post-metamorphosis). It was suggested that the influence of tidal height on mussel growth may not become apparent for several weeks post-settlement. However, results from this study show that zone may, in fact, have a very strong effect on the growth of recently settled juvenile mussels (280-600µm in length). Growth of *M.galloprovincialis* at Beacon Isle decreased from 32.5µm/day on the low-shore to 8µm/day on the high-shore. There was also a strong location effect that was observed at all tidal heights. The average growth rate of *M.galloprovincialis* on the low-shore at Lookout Beach was only 12µm/day. Growth of *M.edulis* placed subtidally was 30.6µm/day for similar sized mussels (Bayne 1964), and de Blok and Geelen (1958) recorded growth rates of 38µm/day for the same species. Laboratory growth of newly settled *P.canaliculus* was ±21µm/day (Buchanan and Babcock 1997). Results from this study show that post-larval growth in the field may be highly variable. Furthermore, in light of the growth rates reported, it seems that growth at Lookout Beach was well below average. Environmental factors that could effect growth, such as water temperature, salinity or food availability (Seed 1976; Dare 1976; Kautsky 1982; Karayücel and Karayücel 2000) are unlikely to vary between locations 300-400m apart. Turbulence (Ackerman and Nishizaki 2004) may differ between locations, and sand scour is a significant factor influencing low-shore communities at Lookout Beach but not at Beacon Isle. It is possible that sandy waters may limit the availability of food or possibly the ability to filter and process food in mussels. Phillips (2002) also found that size at settlement significantly influenced growth rates in early juveniles. Mussels settling at larger sizes tended to grow faster as juveniles than mussels settling at smaller sizes. Size at settlement of *P.perna* and *M.galloprovincialis* was found to be significantly smaller at

Lookout Beach than at Beacon Isle (unpub. data). The difference in juvenile growth rates between locations may therefore reflect spatial variations in the size at which larvae settle.

In view of the low and variable settlement and recruitment rates reported for *M.galloprovincialis* on the high-shore, these results suggest that this species may be able to maintain high densities in this zone, due to the persistence (through low mortality rates) of cumulative settlements of slow-growing individuals. Furthermore, the abundance of *P.perna* and *C.meridionalis* in this zone may be limited by low settlement and recruitment rates of individuals that are unable to survive. There is also evidence to suggest that species-specific differential mortality rates may maintain these patterns throughout benthic life. In contrast, there were no differences in either juvenile growth or early post-settlement mortality between species in the lower zones. There was some indication that *M.galloprovincialis* may experience significantly higher mortality rates than *P.perna* in certain months at Beacon Isle. However, it seems that the abundance of *M.galloprovincialis* on the low-shore may be determined primarily by post-recruitment processes. Furthermore, while differential settlement rates may be the most important factor influencing mussel densities in Plettenberg Bay and Tsitsikamma, high early mortality rates may also have a significant impact on subsequent recruitment rates.

Chapter 6

Growth and mortality in adult *Perna perna* and *Mytilus galloprovincialis*

6.1 Introduction

Early studies of intertidal ecology emphasised the importance of physical stresses and biological interactions in determining the distribution and abundance of organisms on rocky shores (Connell 1961; Lewis 1964; Seed 1969b; Dayton 1971; Harger 1972b; Paine 1974; Suchanek 1978; Griffiths and Hockey 1987; Barkai and Branch 1989). However, due to a growing awareness of the role of pre-recruitment processes in community ecology, many of the generalisations about the processes structuring rocky shore communities that emerged from these studies have been reassessed (e.g. Underwood and Denley 1984; Menge and Sutherland 1987). In particular, it has been suggested that variations in recruitment may have a direct influence on post-recruitment interactions (Underwood and Denley 1984; Menge and Sutherland 1987; Connolly and Roughgarden 1998; Connolly *et al.* 2001). This theory suggests that when recruitment is high, interspecific interactions such as competition and predation become important, but when recruitment is low, populations are largely regulated by pre-recruitment processes. Furthermore the importance of these processes may differ at different spatial scales, interspecific interactions generally becoming more important at local scales (Underwood and Chapman 1996).

Amongst similar species, competition is of particular interest and may be an important mechanism by which an organism is able to usurp or maintain spatial dominance on rocky shores where space is frequently limited (Paine 1984; Connell 1985). In intertidal communities, interference competition by overgrowing, crushing or undercutting has been observed in barnacles (Connell 1961), mussels (Harger 1972b) and coralline algae (Paine 1984). Exploitative competition in sessile benthic invertebrates is most likely to occur in competition for food resources. Faster growth rates will allow those animals attaining larger

sizes more quickly to exploit food resources better than smaller, slower growing individuals (Wootton 1993). Regardless of the mechanism involved, it is clear that a fast growth rate is important in determining competitive dominance and often results in reduced fitness or higher mortality of slow growing individuals (Griffiths and Hockey 1987; Barkai and Branch 1989; Petraitis 1995).

Growth in intertidal animals may be influenced by a number of factors. Food availability is considered to be the most important factor in determining growth rates (Seed 1976). Growth may also vary with tidal height (Seed 1969b; Griffiths 1981; Griffiths and Griffiths 1987; Marsden and Weatherhead 1999; McQuaid and Lindsay 2000), wave exposure (McQuaid and Lindsay 2000; Steffani and Branch 2003; Ackerman and Nishizaki 2004) and density (Kautsky 1982; Peterson and Beal 1989; Alunno-bruscia *et al.* 2000), and also seasonally or annually as a result of fluctuations in temperature, reproductive cycle or food availability (Seed 1969b; Dare 1976; Kautsky 1982; Tomalin 1995; Grant 1996; Dekker and Beukema 1999). Other factors known to affect bivalve growth include salinity (Karayücel and Karayücel 2000; Westerbom *et al.* 2002), light (Seed 1969b), sedimentation (van Erkom Schurink and Griffiths 1993), the presence of fouling organisms such as barnacle epibionts (Buschbaum and Saier 2001), parasites (Calvo-Ugarteburu and McQuaid 1998b) and genetic variability (Brichette *et al.* 2001).

Variations in one or more of these factors may have different effects on the growth rates of different species and this has important consequences for competition between them. For example, Harger (1972b) showed that different levels of wave exposure modify the competitive advantages of the mussels *Mytilus galloprovincialis* and *Mytilus californianus* through differential growth and mortality rates.

Mortality may influence the outcome of interspecific interactions. For example, Paine (1984) found that grazers introduced competitive uncertainty in coralline algal assemblages leading to competitive reversals. Increased mortality of one species in a particular zone or habitat may allow other species to dominate. Thus differences in mortality may influence the distribution and persistence of different species in different zones (Sherwood and Petraitis 1998). Causes of mortality in the intertidal include biological factors such as predation (Seed 1969b; Paine 1974; Menge *et al.* 1994; Petraitis 1998), intraspecific competition (Griffiths and Hockey 1987), interspecific competition (Connell 1961; Dayton 1971; Barkai and Branch 1989) and grazing (Paine 1984; Sousa 1981) and physical factors such as desiccation and temperature (Kennedy 1976; Hockey and van Erkom Schurink 1992), sedimentation (Hockey and van Erkom Schurink 1992; Marshall and McQuaid 1993) and differences in wave exposure (Harger 1970b; Dayton 1971; McQuaid and Lindsay 2000). Mortality may also vary seasonally due to variations in the abundance of predators (Beal *et al.* 2001) and temperature (Thieltges *et al.* 2004)

In Chapter 4 it was determined that recruitment rates were significantly lower in Tsitsikamma than in Plettenberg Bay. Competition and mortality may therefore be less important in structuring mussel populations at this site than in Plettenberg Bay. Furthermore, pre-recruitment processes contributed substantially to the zonation patterns of *Perna perna* but not *M.galloprovincialis.* It was suggested that the lower distribution limit of adult *M.galloprovincialis* may be determined by post-recruitment interactions. To determine whether interactions between *P.perna* and *M.galloprovincialis* at the post-recruitment level influence their distribution patterns, mortality and growth of these species was measured at different spatial and temporal scales.

6.2 Materials and methods

Growth

Of the various methods that may be used to measure growth in bivalves, fluorochromes present an inexpensive, relatively non-invasive and rapid field technique (Kaehler and McQuaid 1999). Calcein has also been shown to produce long-lasting fluorescent shell marks with no apparent affect on survivorship or growth in subadult and adult *P.perna* (Kaehler and McQuaid 1999). Calcein was therefore initially used as a growth marker following the methods of Kaehler and McQuaid (1999).

Between April 2000 and July 2001, four *in situ* growth experiments were performed during autumn, spring, summer and winter to examine the possibility of seasonal differences in the growth of *P.perna* and *M.galloprovincialis.* Mussels were marked by inserting a syringe needle between the valves and injecting a calcein-sea water solution (125mg.l^{-1}) into the mantle cavity. The solution was injected until the cavity filled up and started to overflow (Kaehler and McQuaid 1999). A patch of approximately 80 mussels (±40 per species) was marked in each zone at each location in Plettenberg Bay and Tsitsikamma, covering as broad a range of mussel sizes as possible (generally 10-80mm in length). Unfortunately this was not always possible, for example on the high-shore at Lookout Beach *P.perna* is scarce and neither species exceeds 30mm in length. Mussels were marked over a single spring tide and collected after one month. In the laboratory, individual lengths were measured to the nearest mm using Vernier calipers. The shell valves of each mussel were marked individually. One valve was set in polyester resin, sectioned sagitally using a diamond saw, and then examined under an Olympus fluorescence microscope. Shell length increase was determined with a micrometer by measuring the distance between the green fluorescent mark and the growing tip (Kaehler and McQuaid 1999).

The recovery rate of marked mussels using this technique was poor. Very few samples were obtained for autumn and spring growths therefore these seasons were excluded. Due to the patchy results obtained using calcein, growth experiments were repeated using an alternative method in summer and winter of 2003. Mussels were marked in the field using small triangular paper tags (adapted from Millstein and O'Clair 2001). One valve of each mussel was dried using paper towelling. Bostik quick-drying superglue was applied to a tag, which was then placed with the longest point of the triangle at the tip of the growing edge of the valve. To prevent weathering or losses tags were glued over once in place. Recovery rates using this method were generally good. Sample sizes were occasionally quite small, particularly from high-shore zones because mussels frequently had no measurable growth. This may have been due to gluing the valves together or possibly due to stress caused while marking. Alternatively this may have been a real result as growth of mussels is generally much slower on the high-shore (Seed 1969b; McQuaid and Lindsay 2000). However, since this result was potentially due to experimental error, these mussels were excluded.

As in 2001, a patch of 80-100 mussels was marked in each zone. Due to poor sampling conditions only Driftwood Bay within the Tsitsikamma site was sampled. Mussels were collected after one month and growth was measured as the distance from the tip of the triangle to the tip of the valve (Fig. 6.1; p.205). Measurements were done under a light microscope using a micrometer.

Mortality

Mortality was only measured during summer when conditions high on the shore are expected to be harsher. Mortality may also be highly variable along a horizontal stretch of shore. For example, whelks (*Nucella spp.*) were often observed in aggregations on patches of mussels.

Figure 6.1: An example of a *P.perna* individual marked in the field using triangular tags, where g = growth after one month. The initial length of the mussel can be measured from the umbo to the tip of the triangle

Thus it was necessary to mark a number of different patches within each zone. Mortality was first measured in February 2003. However, this experiment was relatively unsuccessful, largely due to poor working conditions. Some results on mortality after one and two months were obtained from Beacon Isle in Plettenberg Bay and Driftwood Bay in Tsitsikamma. Due to poor sample sizes, missing data and a lack of replication within sites these results will be considered as preliminary.

The experiment was repeated between November 2003 and March 2004. I attempted to mark ten patches of twenty mussels (ten of each species) in each zone at each location. However, this was not always possible due to low numbers of *P.perna* in high-shore zones and of *M.galloprovincialis* on the low-shore in Tsitsikamma. Sample sizes therefore differed between zones and locations, with a minimum sample size of five. Generally mussels of 30-50mm in length were marked, however on the high-shore the maximum size of mussels is smaller and those marked were 20-40mm in length. The shell of each mussel was dried and then marked with a spot of yellow paint. Once the paint had hardened the spot was glued over using Bostik quick-drying superglue. Mortality was then measured on several occasions over a period of four months. At each sampling interval, the number of mussels remaining was counted and the percentage mortality calculated. It should be noted that high-shore patches in Plettenberg Bay were lost over the sampling period due to wave action, therefore sample sizes also varied over time. After two months there were also no marked patches visible on the low and mid-shore at this site. At Lookout Beach, this was primarily due to substantial overgrowth by algae so that markings were no longer visible. It should be noted that this followed the removal of a large amount of sand from this location during a storm and was not a common occurrence. Similarly, at Beacon Isle a dense settlement of barnacles covered the mussel bed to mid-shore level.

Analyses

1. Growth

The growth data were initially analysed using Analysis of Covariance (ANCOVA) (General Linear Model, Statistica 6.1). However, in some cases the assumption of homogeneous slopes was not met, and together with unbalanced sample sizes this was potentially problematic. An alternative method was to analyse a subset of the data using ANOVA, provided that growth within the chosen range showed no relationship with size. Since growth was greatest in smaller mussels and it is the variations in maximum growth that are likely to differ (Seed 1969b), analyses were performed using the growth rates of mussels within the size range of 10-40mm in length. Results from these analyses proved to be very similar to those from ANCOVA. ANCOVA is preferable as it incorporates all the growth measurements by accounting for the variation associated with length, therefore this method was used for the analyses.

Based on the experimental design of this study, it is not strictly feasible to compare individual locations from the two sites. This is primarily because inferences of site-specific differences would be questionable because of lack of replication within sites. However, due to the patchiness of the data no site comparisons using this design were possible. Thus growth was compared between locations from different sites using location as a random factor. Bearing in mind the problems with this approach, any differences between these locations should be considered as suggestive. Due to the patchiness of the data in 2001, several different ANCOVAs had to be performed with one or more of the following factors: season, zone and species as fixed factors and location as a random factor. The factors included in specific analyses will be mentioned with the results. Since growth from the two

years was measured using different methods, inter-annual variations could not be examined.

2. Mortality

For the second experiment in 2004, data sets were too fragmented to allow for repeated measures ANOVA. Mortality after four months could be analysed at both sites with a minimum sample size of three. Thus a four-way mixed model ANOVA was performed with location nested within site. Mortality after four months at locations in Tsitsikamma was analysed using a three-way factorial ANOVA with location, zone and species as factors. Data did not require transformation.

6.3 Results

Growth in 2001

Plots of initial length versus growth at Beacon Isle in Plettenberg Bay and Sandbaai in Tsitsikamma are shown in Figs. 6.2-6.4 a-f (p.209-211). No growth data were obtained from Lookout Beach. Generally there were clear patterns of decreasing growth with increasing size, although this often became less obvious when growth rates decreased e.g. on the high-shore, and for *P.perna* during winter. Both species also appeared to grow faster at Beacon Isle than at locations in Tsitsikamma, particularly in summer. It is also clear that the majority of the spatial/temporal variation in growth was found in smaller mussels (<40mm in length). There was an exception on the low-shore at Beacon Isle in summer where large *P.perna* (50- 70mm) grew unusually fast, with growth rates of 1.5-3.5mm per month. In comparison, similar sized mussels experienced minimal growth in winter (<0.3mm/month).

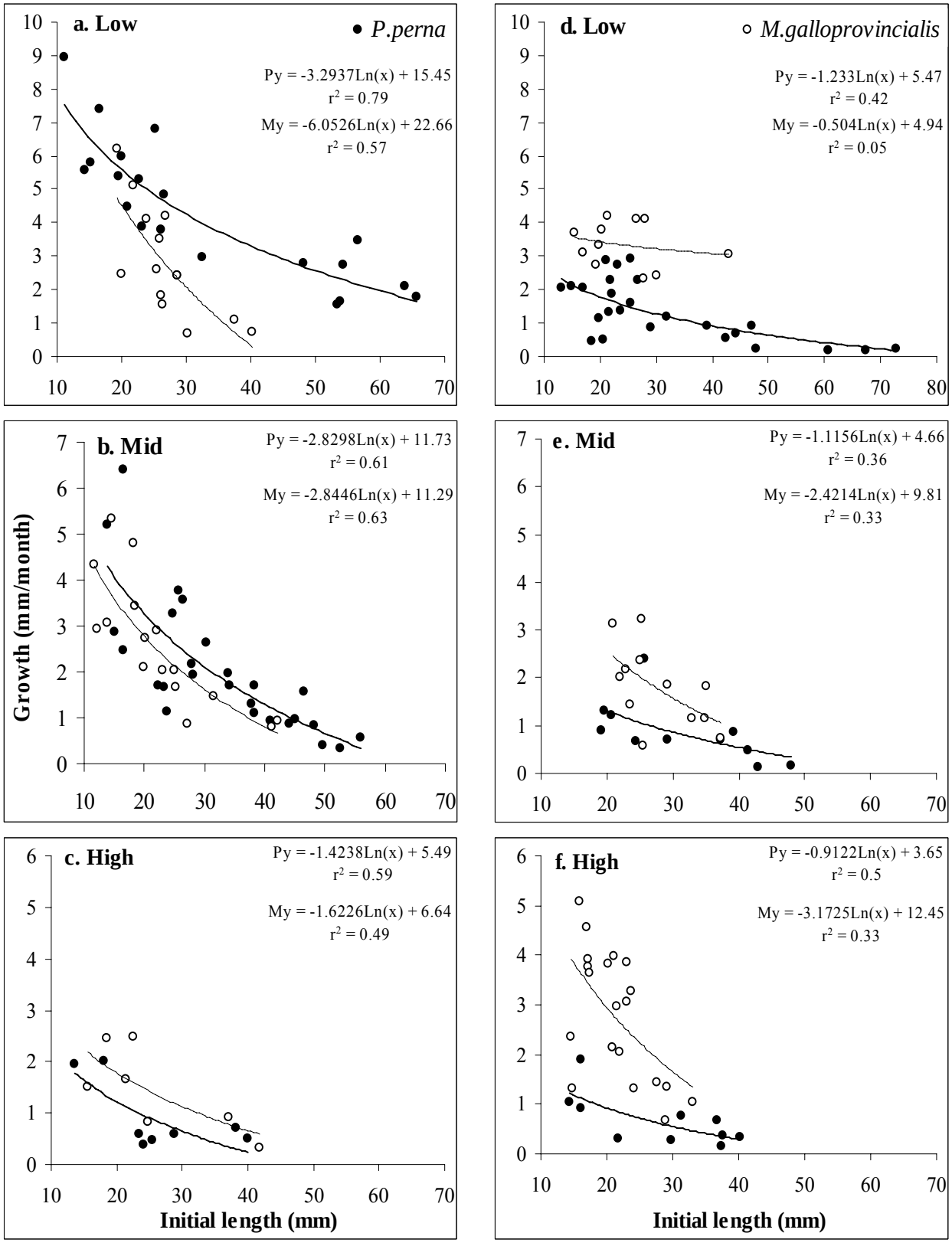


Figure 6.2 a-f: Growth curves of *P.perna* and *M.galloprovincialis* in different zones at Beacon Isle, Plettenberg Bay, in summer (a-c) and winter (d-f) 2001. Equations and correlation coefficients are given, where Py = *P.perna* and My = *M.galloprovincialis*

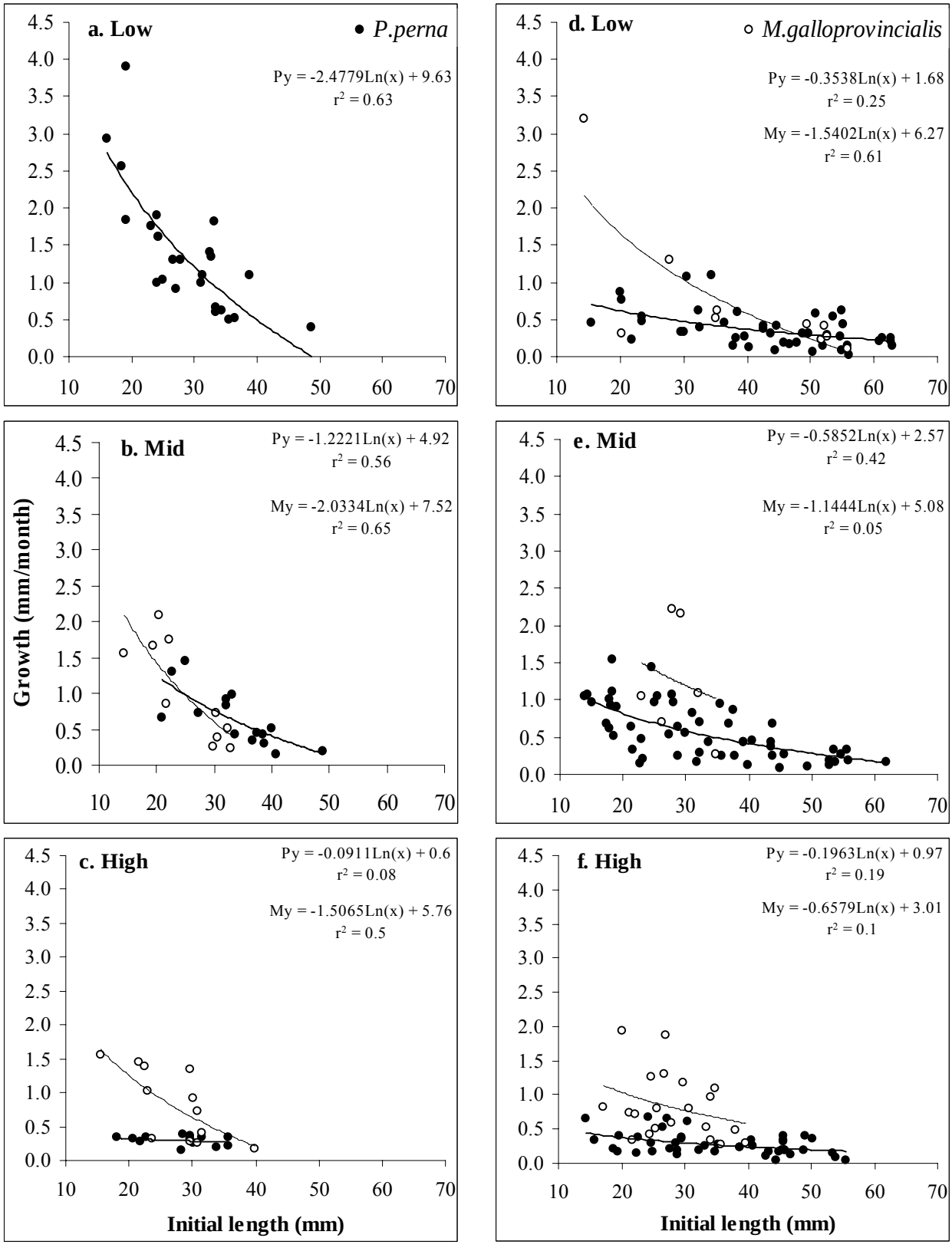


Figure 6.3 a-f: Growth curves of *P.perna* and *M.galloprovincialis* in different zones at Sandbaai, Tsitsikamma, in summer (a-c) and winter (d-f) 2001. Trendlines: solid = *P.perna;* dashed = *M.galloprovincialis.*

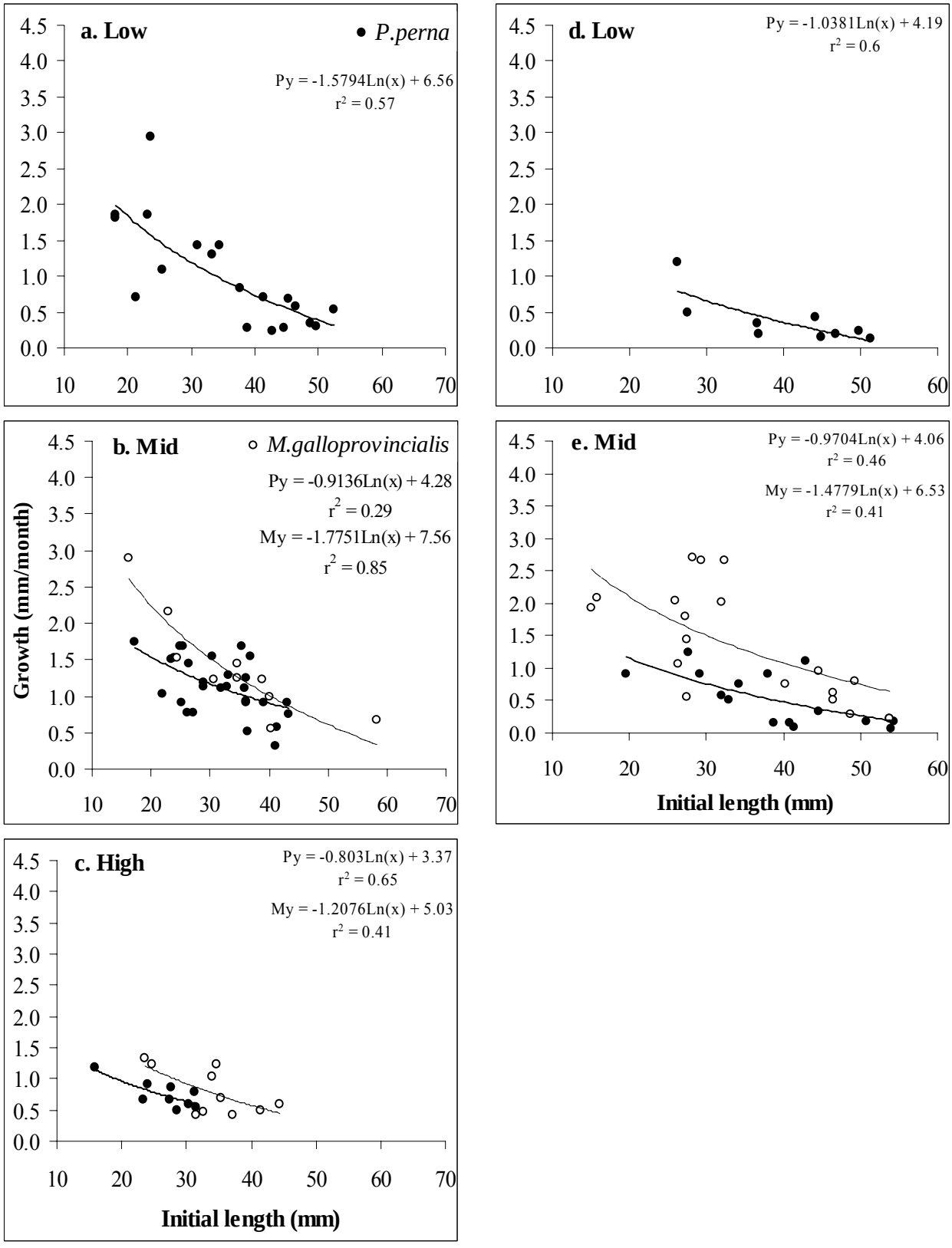


Figure 6.4 a-f: Growth curves of *P.perna* and *M.galloprovincialis* in different zones at Driftwood Bay, Tsitsikamma, in summer (a-c) and winter (d-e) 2001

Growth of *P.perna* and *M.galloprovincialis* at Beacon Isle was analysed with season, zone and species as factors (Table 6.1; p.213). All main effects and interactions were significant, with a highly significant interaction between all three factors ($p<0.001$). In summer, growth of *P.perna* decreased significantly with increasing tidal height (Fig. 6.5; p.213). *M.galloprovincialis* had similar growth rates on the low and mid-shores, however, growth decreased significantly on the high-shore. *P.perna* also grew significantly faster than *M.galloprovincialis* on the low-shore with no significant difference between species in the upper zones. Low and mid-shore growth of *P.perna* was significantly slower in winter than in summer, with no change on the high-shore. Thus although the same trend with zone was observed in winter, the differences in growth between zones for this species were no longer significant. In contrast, growth of *M.galloprovincialis* in the lower zones did not differ significantly between seasons, and this species actually grew significantly faster on the high-shore in winter than in summer. As a result, high-shore growth did not differ significantly from growth in the lower zones in winter. However, due to a slight reduction in winter growth on the mid-shore, growth in this zone was significantly slower than on the low-shore. Furthermore, growth of *M.galloprovincialis* in winter was significantly greater than that of *P.perna* on the low and high-shores.

At locations in Tsitsikamma, data were very patchy and several different analyses had to be performed. No data were obtained on growth of *M.galloprovincialis* on the low-shore in summer at both locations. Also, very few results were obtained from Driftwood Bay in winter and these were therefore excluded from the analyses.

A two-way ANCOVA was performed comparing growth of *P.perna* between zones and seasons at Sandbaai. The effects of season and zone were significant and there was a highly significant interaction between them ($p<0.001$; Table 6.2; p.215). Post-hoc results revealed

Table 6.1: Three-way ANCOVA examining the effect of season, zone and species on growth of *P.perna* and *M.galloprovincialis* at Beacon Isle, Plettenberg Bay, in 2001

	Df	MS	F	p
length	1	122.558	134.302	<0.001*
season	1	13.544	14.842	<0.001*
zone	2	41.688	45.683	<0.001*
species	1	4.180	4.580	0.03*
season×zone	2	10.239	11.220	<0.001*
season×species	1	31.867	34.921	<0.001*
zone×species	2	5.486	6.012	0.003*
season×zone×species	2	7.701	8.439	<0.001*
Error	167	0.913		

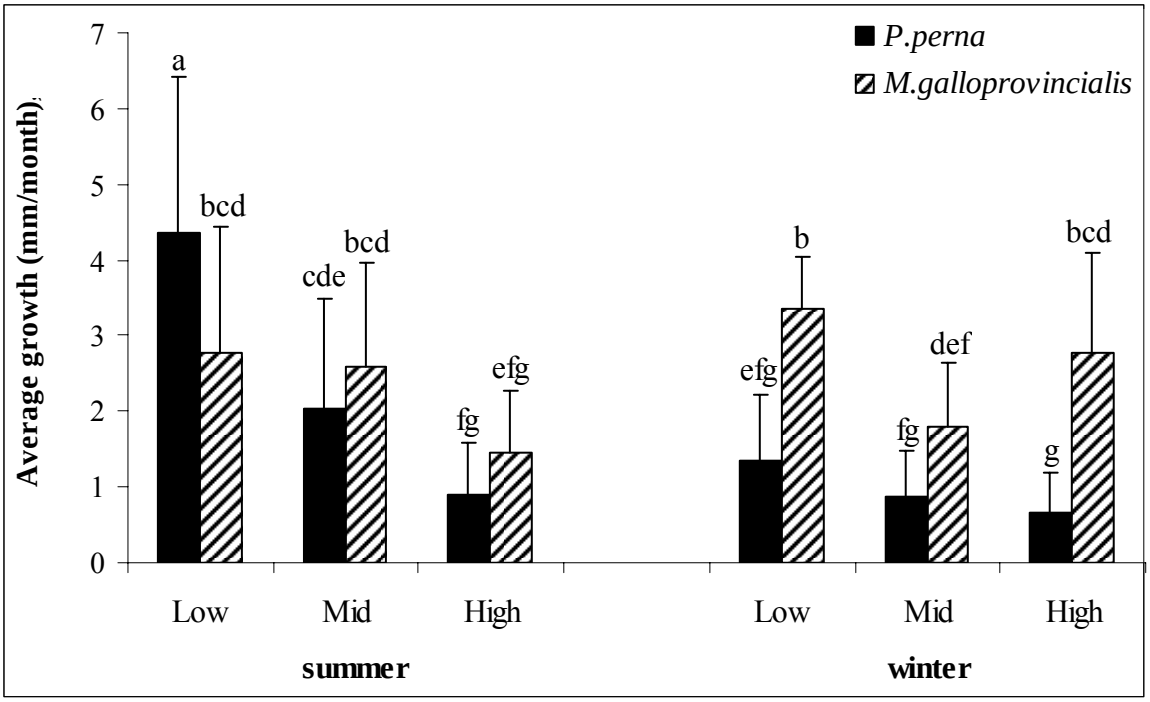


Figure 6.5: Results of post hoc comparison of the interaction between season, zone and species on the growth of mussels at Beacon Isle in 2001. Similar letters indicate homogeneous groups. Error bars represent standard deviations

that growth of *P.perna* decreased significantly with increasing tidal height in summer (Fig. 6.6; p.216). Low-shore growth was significantly slower in winter than summer, however there was no difference in growth between seasons in the upper zones. Furthermore, in winter, growth was significantly greater on the mid-shore than in the other two zones. A comparison of winter growth of both species at this location revealed a similar effect of zone on the growth of *M.galloprovincialis* as for *P.perna* in winter, however, growth of this species was significantly faster than *P.perna* in all zones (Table 6.2). When growth of *P.perna* and *M.galloprovincialis* was compared between seasons on the mid and high-shores at this location, it was found that the same difference between mid and high-shore growth was observed for both species regardless of season (Table 6.2). However, growth of *M.galloprovincialis* was significantly faster than *P.perna,* irrespective of either zone or season.

To summarise, at Sandbaai in Tsitsikamma, both species grew significantly faster on the mid-shore than the high-shore in both summer and winter, with no differences in growth between seasons. Low-shore growth of both species was also significantly slower than on the mid-shore in winter. In all comparisons between species, *M.galloprovincialis* grew significantly faster than *P.perna.* However, low-shore growth of *P.perna* was also significantly slower in winter than in summer, and in summer, growth of this species decreased with increasing tidal height.

Summer growth of *P.perna* was also compared between locations and zones at Sandbaai and Driftwood Bay in Tsitsikamma. There was no effect of either location or zone, however there was a significant interaction between them ($F=3.3$; $p=0.04$). At Driftwood Bay, growth was more similar between zones and was significantly faster on the mid-shore than

Table 6.2: Summary of statistics on growth of *P.perna* and *M.galloprovincialis* between seasons and zones at Sandbaai in Tsitsikamma, 2001

1. Seasonal growth of *P.perna*				
	Df	**MS**	**F**	**p**
length*	1	6.879	58.511	<0.001*
season*	1	2.157	18.349	<0.001*
zone*	2	5.320	45.247	<0.001*
season×zone*	2	2.820	23.990	<0.001*
Error	190	0.118		
2. Winter growth of *P.perna* and *M.galloprovincialis*				
	Df	**MS**	**F**	**p**
length*	1	5.867	50.108	<0.001*
zone*	2	0.945	8.071	<0.001*
species*	1	4.796	40.956	<0.001*
zone×species	2	0.144	1.229	0.295
Error	174	0.117		
3. Seasonal growth of *P.perna* and *M.galloprovincialis* on the mid and high-shore only				
	Df	**MS**	**F**	**p**
length*	1	5.658	47.657	<0.001*
Season	1	0.217	1.831	0.178
zone*	1	2.817	23.723	<0.001*
species*	1	4.944	41.639	<0.001*
season×zone	1	0.010	0.086	0.770
season×species	1	0.154	1.299	0.256
zone×species	1	0.022	0.184	0.669
season×zone×species	1	0.395	3.328	0.070
Error	167	0.119		

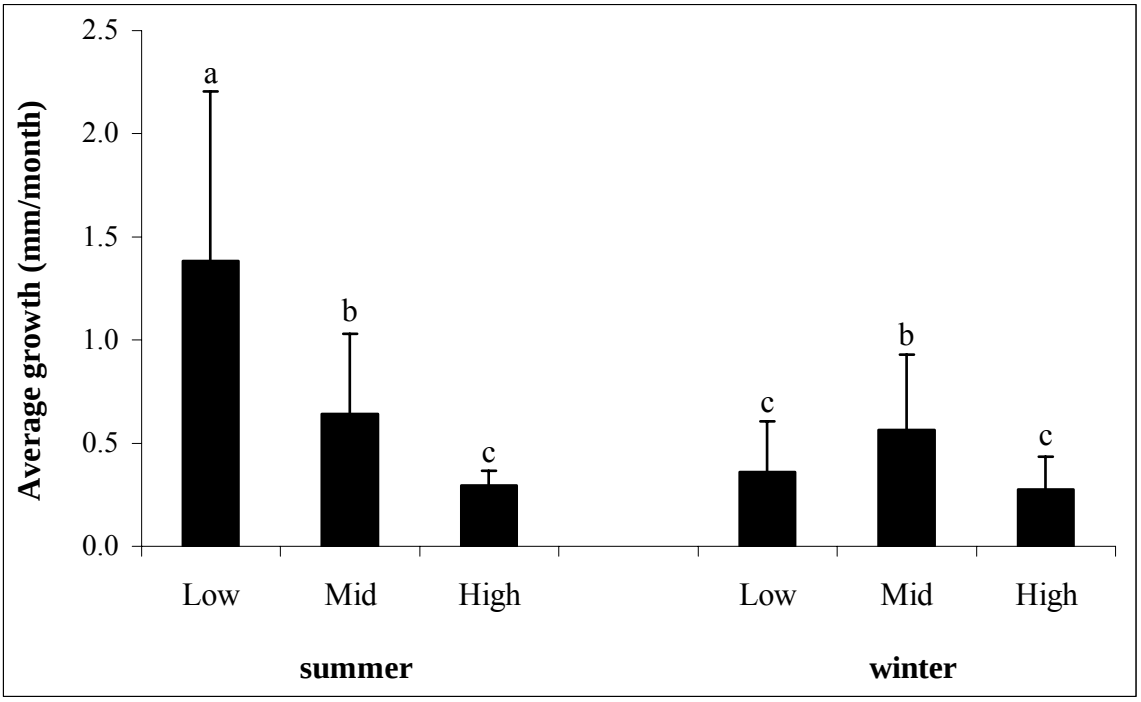


Figure 6.6: Post-hoc comparison of the interaction between season and zone on growth of *P.perna* at Sandbaai, Tsitsikamma, in 2001. Error bars represent standard deviations

on the high-shore. Low-shore growth was significantly faster at Sandbaai than at Driftwood Bay, while growth in the upper zones was significantly faster at Driftwood Bay.

To determine whether there were differences in growth of *P.perna* and *M.galloprovincialis* between locations from different sites, a three-way ANCOVA was performed with location, zone and species as factors. Growth was compared between the mid and high-shores in Beacon Isle, Sandbaai and Driftwood Bay in summer. Results revealed that there was no difference between locations ($F=4.57$; $p=0.18$) and there were no significant interactions with location. When growth was compared in all zones between Beacon Isle and Sandbaai in winter, there was a significant interaction between location, zone and species ($F=5.78$; $p<0.01$). *M.galloprovincialis* grew significantly faster at Beacon Isle in Plettenberg Bay than at Sandbaai in Tsitsikamma in all zones. *P.perna,* on the other hand, grew significantly faster at Beacon Isle on the low-shore with no difference between locations in the upper shore zones.

Growth in 2003

Plots of initial length versus growth at Lookout Beach, Beacon Isle and Driftwood Bay in summer and winter are shown in Figs. 6.7-6.9 a-f (p.218-220). No data were collected from Sandbaai in Tsitsikamma during this year. As in 2001, there was generally a negative relationship between size and growth in both species. This relationship was less obvious in winter at Beacon Isle where growth of both species was highly variable, particularly on the low and mid-shores (Fig. 6.8 d-f).

Growth was compared between locations in Plettenberg Bay using a four-way ANCOVA with season, location, zone and species as factors (Table 6.3; p.222). There were significant

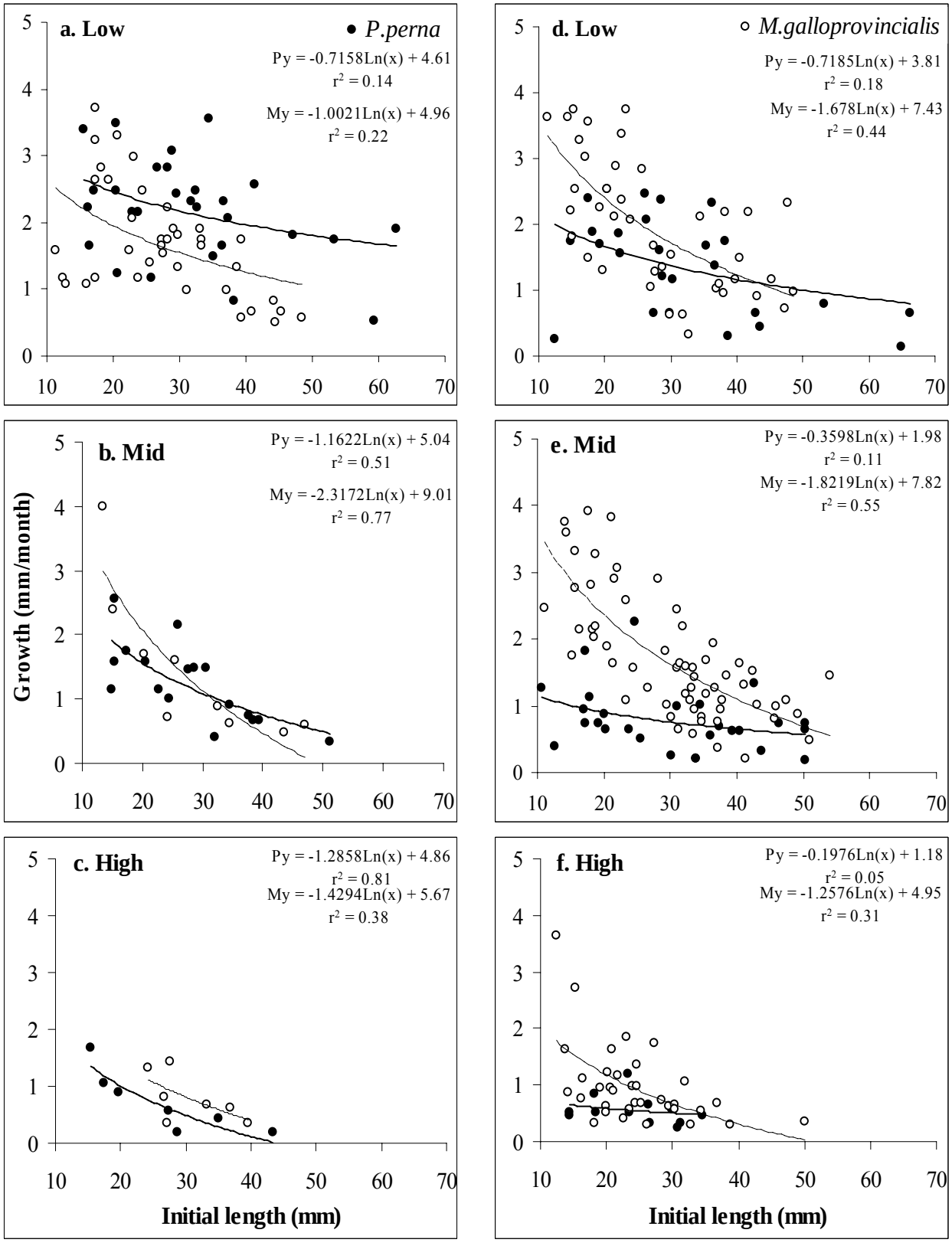


Figure 6.7 a-f: Growth curves of *P.perna* and *M.galloprovincialis* in different zones at Lookout Beach in Plettenberg Bay in summer (a-c) and winter (d-f) 2003

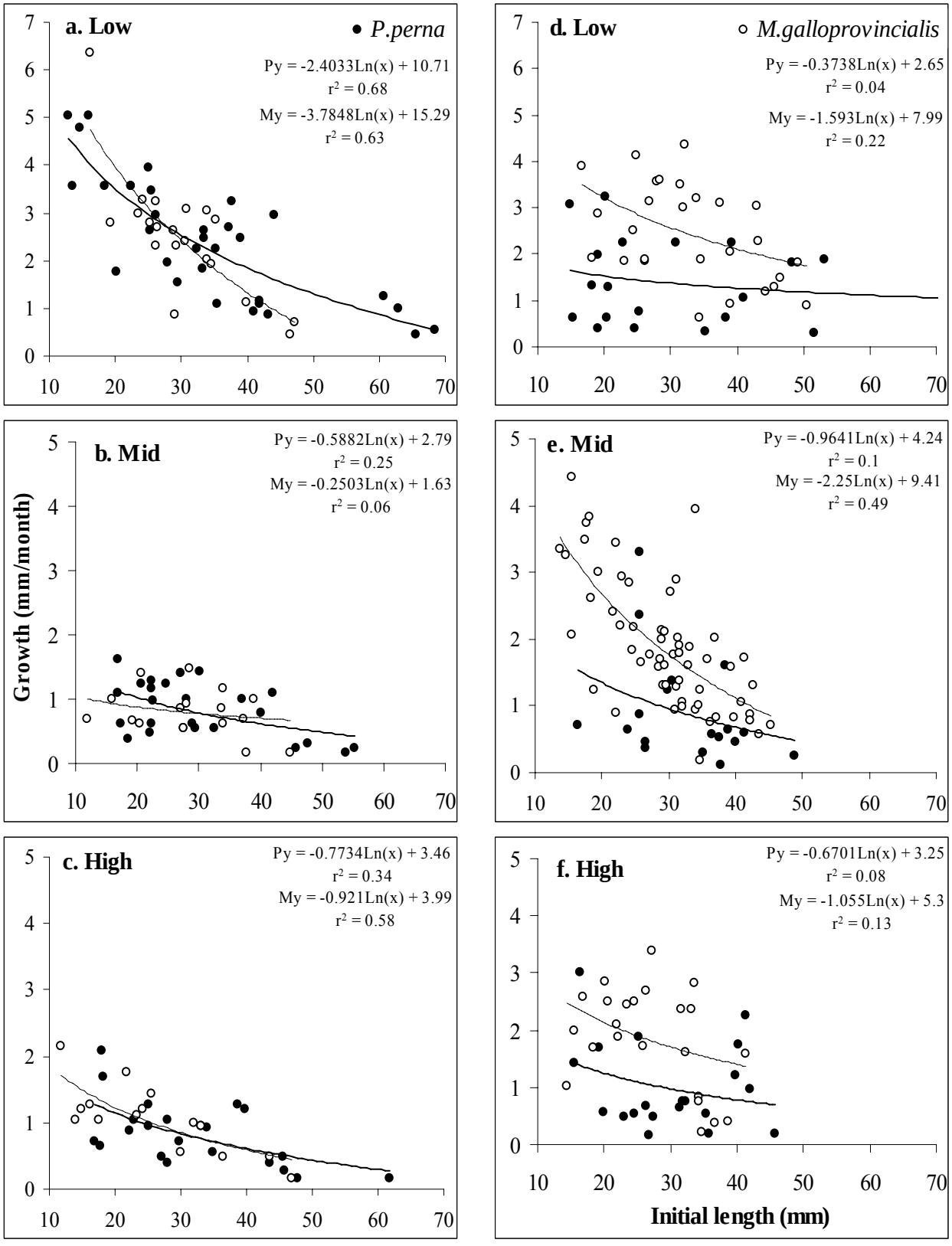


Figure 6.8 a-f: Growth curves of *P.perna* and *M.galloprovincialis* in different zones at Beacon Isle in Plettenberg Bay in summer (a-c) and winter (d-f) 2003

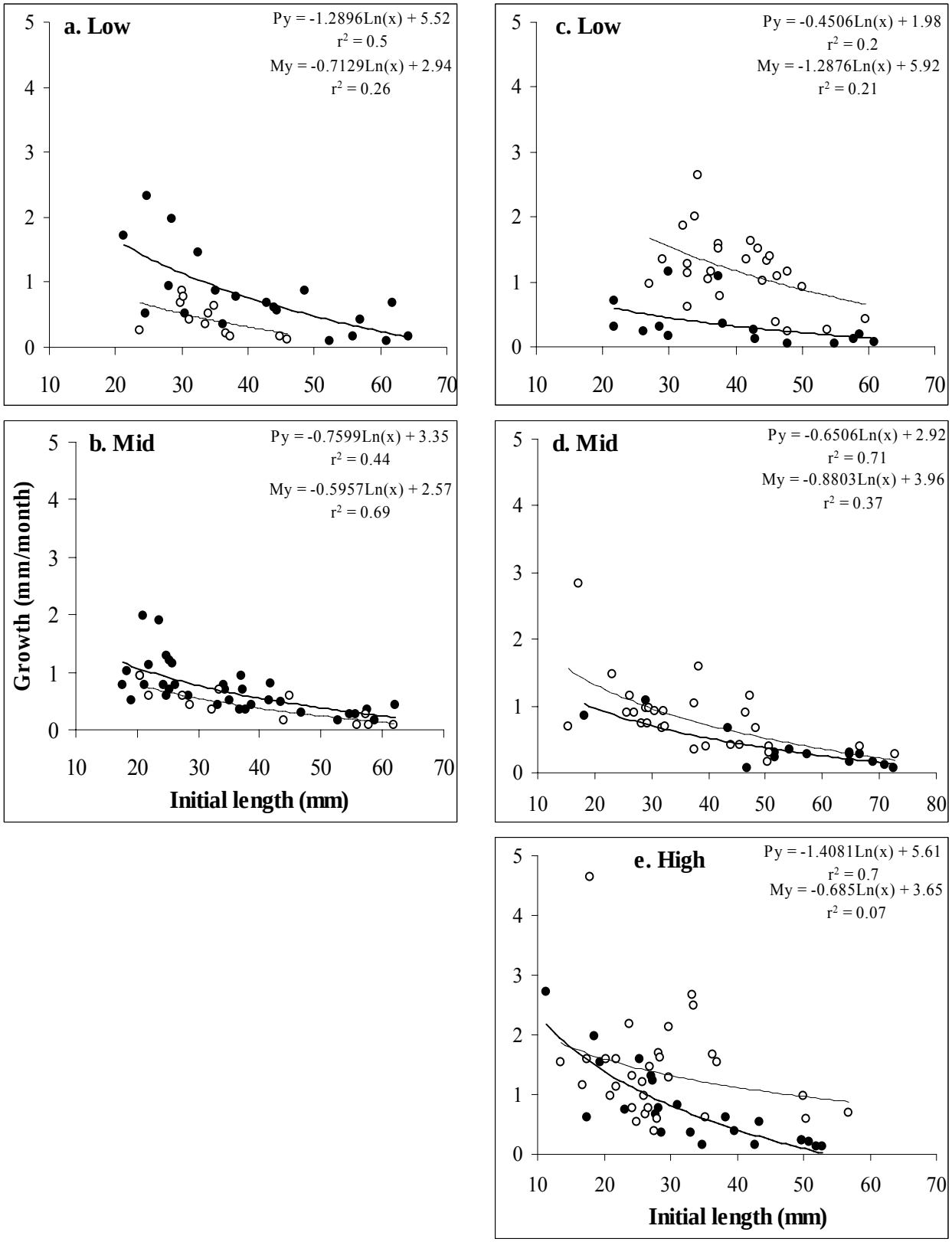


Figure 6.9 a-e: Growth curves of *P.perna* and *M.galloprovincialis* in different zones at Driftwood Bay in Tsitsikamma in summer (a-b) and winter (c-e) 2003.

interactions between season, location and zone (p=0.03) and between location, zone and species (p=0.03). In summer, growth of mussels at Lookout Beach decreased significantly with increasing tidal height (Fig. 6.10; p.223). However, at Beacon Isle growth decreased significantly from low to mid-shore but was similar in the upper shore zones. Mussel growth was also significantly greater on the low-shore at Beacon Isle than at Lookout Beach, while the opposite was true on the mid-shore. There was no difference in growth between locations on the high-shore. In winter, growth decreased with increasing tidal height at both locations with a significant difference between the low and high-shores. Growth was similar between locations in the lower zones but was significantly greater on the high-shore at Beacon Isle than at Lookout Beach. There was also no significant difference between seasons at Lookout Beach regardless of zone. On the other hand, low-shore growth at Beacon Isle was significantly slower in winter than in summer while mid and high-shore growth was significantly faster in winter.

There were also interspecific differences in growth that varied between zones and locations but were generally independent of season. At Lookout Beach, growth of *P.perna* decreased significantly with increasing tidal height, while *M.galloprovincialis* had a similar growth rate in the lower zones with significantly slower growth on the high-shore (Fig. 6.11; p.223). *M.galloprovincialis* grew significantly faster than *P.perna* on the mid-shore at this location with no significant differences between species in the other two zones. At Beacon Isle both species grew significantly faster on the low-shore than in the upper shore zones. *M.galloprovincialis* also grew significantly faster than *P.perna* in all three zones at this location. Furthermore, growth of *P.perna* did not differ significantly between locations regardless of zone. However, *M.galloprovincialis* grew significantly faster on the low and

Table 6.3: Four-way ANCOVA examining the effects of season, location, zone and species on growth of *P.perna* and *M.galloprovincialis* at Lookout Beach and Beacon Isle in Plettenberg Bay in 2003

	Effect	Df	MS	F	p
length*	Fixed	1	124.253	243.388	<0.001*
season	Fixed	1	0.095	0.030	0.891
location	Random	1	9.376	2.677	0.558
zone	Fixed	2	61.015	10.407	0.088
species	Fixed	1	12.709	18.607	0.144
season×location	Random	1	3.178	0.838	0.435
season×zone	Fixed	2	6.559	2.044	0.328
location×zone	Random	2	5.893	1.089	0.423
season×species	Fixed	1	18.478	22.979	0.131
location×species	Random	1	0.683	0.235	0.663
zone×species	Fixed	2	0.983	0.430	0.699
season×location×zone*	Random	2	3.212	37.665	0.026*
season×location×species	Random	1	0.804	7.688	0.063
season×zone×species	Fixed	2	1.098	12.867	0.072
location×zone×species*	Random	2	2.280	26.323	0.034*
season×location×zone×species	Random	2	0.085	0.167	0.846
Error		581	0.511		

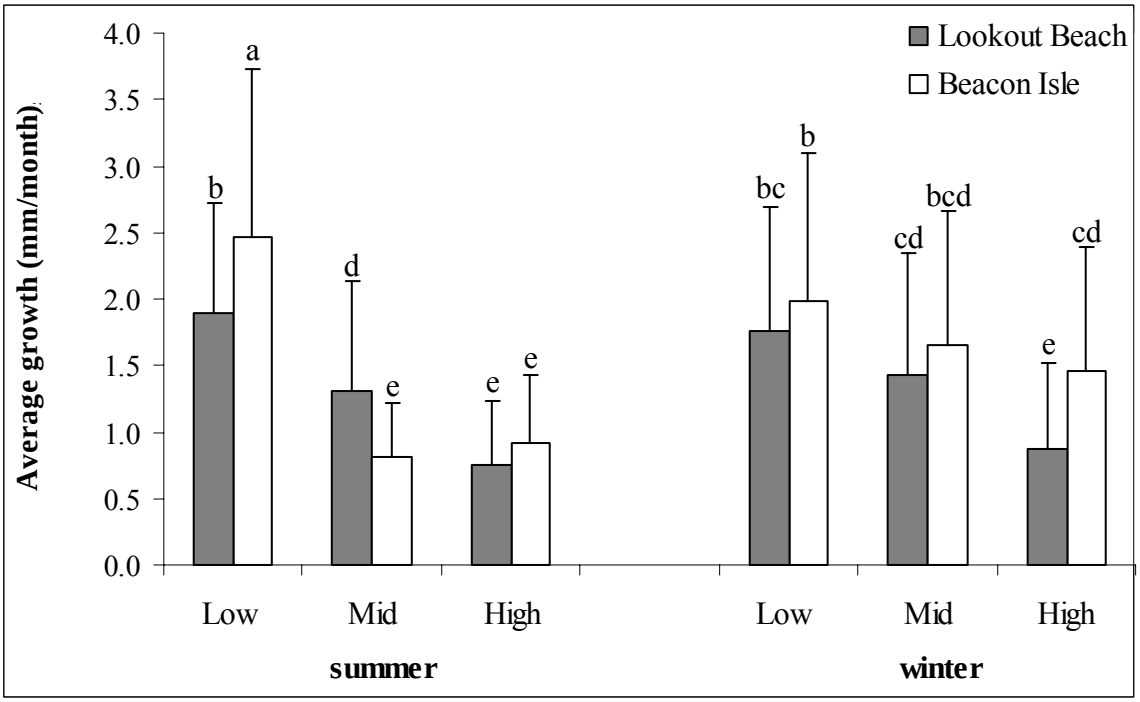


Figure 6.10: Post-hoc comparison of the interaction between season, location and zone on growth of mussels in Plettenberg Bay in 2003. Error bars represent standard deviations

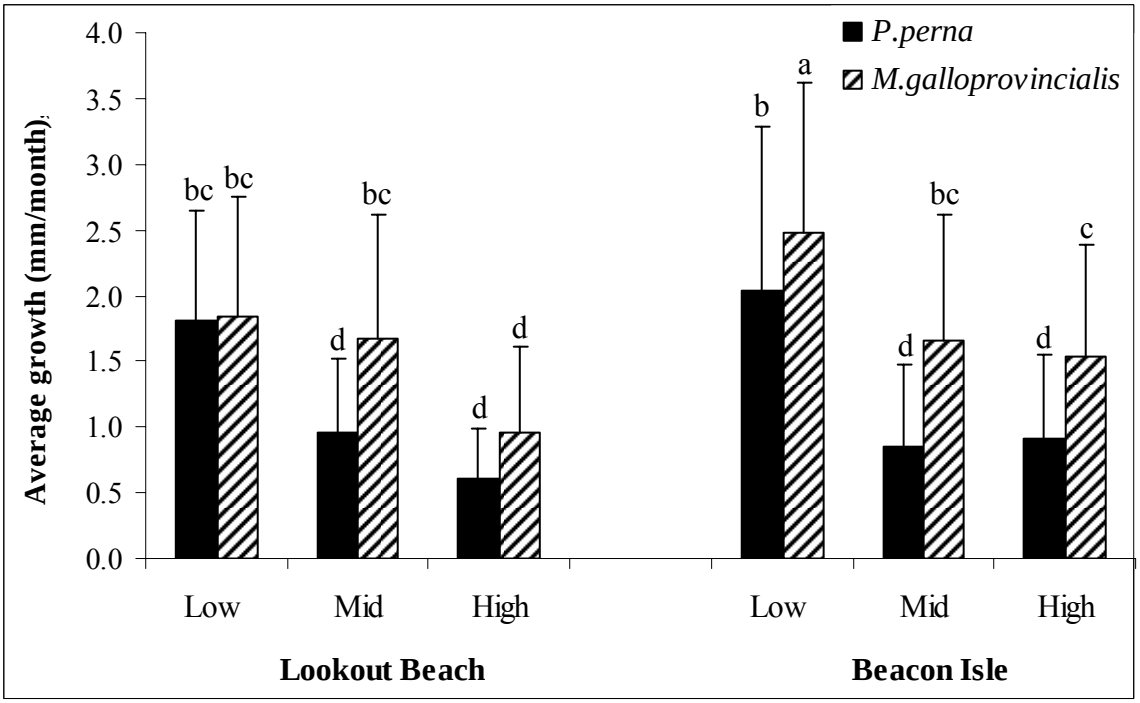


Figure 6.11: Post-hoc results of the interaction between location, zone and species on growth of *P.perna* and *M.galloprovincialis* in Plettenberg Bay in 2001. Error bars represent standard deviations

high-shore at Beacon Isle than at Lookout Beach, with no difference between locations on the mid-shore.

No data were obtained from the high-shore at Driftwood Bay in summer. Growth between seasons could therefore only be compared between the low and mid-shore (Table 6.4; p.225). There was a highly significant interaction between season, zone and species ($p<0.001$). In summer, *P.perna* grew significantly faster than *M.galloprovincialis* in both zones and there was no difference in the growth of either species between zones (Fig. 6.12; p.226). However, growth of *P.perna* decreased significantly in winter while *M.galloprovincialis* grew significantly faster in winter than in summer, and this was irrespective of zone. As a result, *M.galloprovincialis* grew significantly faster than *P.perna* in both zones in winter. Also while there was no difference in the growth of *P.perna* between zones, *M.galloprovincialis* grew significantly faster on the low-shore than on the mid-shore in this season.

An analysis comparing the growth of these species in all three zones in winter gave a significant interaction between zone and species ($p<0.01$; Table 6.4). Post-hoc comparison revealed that growth of *P.perna* was significantly greater on the high-shore than in the lower zones. *M.galloprovincialis* also grew significantly faster in this zone than on the mid-shore. Growth of *M.galloprovincialis* was also significantly greater than that of *P.perna* on the high-shore.

A three-way ANCOVA with location, zone and species as factors compared growth between Lookout Beach, Beacon Isle and Driftwood Bay on the low and mid-shore in summer. There was no effect of location ($F=0.91$; $p=0.53$) or any significant interactions

Table 6.4: Summary of statistics on growth of *P.perna* and *M.galloprovincialis* between seasons and zones at Driftwood Bay, Tsitsikamma, in 2003

1. Seasonal growth of *P.perna* and *M.galloprovincialis* on the low and mid-shore only				
	Df	**MS**	**F**	**p**
length	1	11.460	80.922	<0.001*
season	1	1.450	10.238	0.002*
zone	1	0.098	0.695	0.406
species	1	0.144	1.017	0.315
season×zone	1	0.002	0.012	0.915
season×species	1	5.765	40.706	<0.001*
zone×species	1	0.373	2.633	0.107
season×zone×species	1	2.358	16.648	<0.001*
Error	154	0.142		
2. Winter growth of *P.perna* and *M.galloprovincialis,* all zones				
	Df	**MS**	**F**	**p**
length	1	11.115	38.078	<0.001*
zone	2	0.136	0.467	0.628
species	1	6.382	21.863	<0.001*
zone×species	2	1.416	4.853	0.009*
Error	131	0.292		

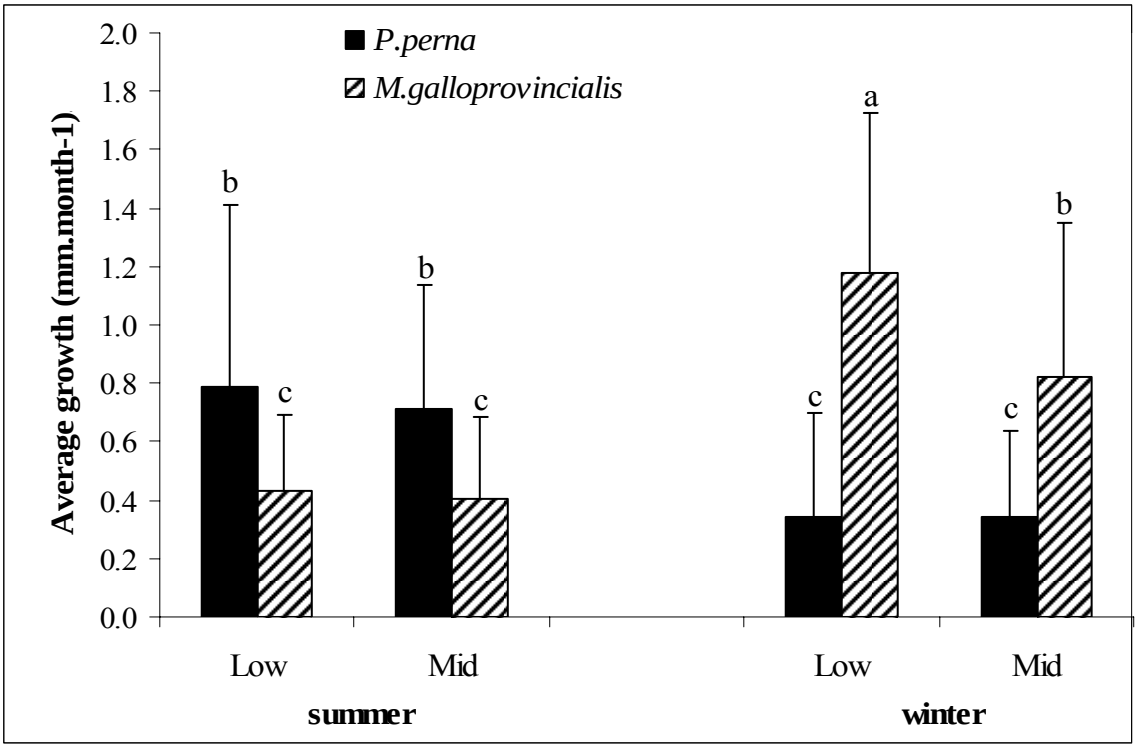


Figure 6.12: Post-hoc results of the interaction between season, zone and species on growth of mussels at Driftwood Bay in Tsitsikamma in 2003. Error bars represent standard deviations

with location. A similar comparison in all zones at these locations in winter gave a significant interaction between location, zone and species. Growth of *M.galloprovincialis* at both locations in Plettenberg Bay was significantly greater than at Driftwood Bay in Tsitsikamma in the lower zones but there was no significant difference between sites on the high-shore. The same was true for *P.perna* but the difference between sites was only significant on the low-shore.

Summary

Tidal height significantly affected the growth of these species, although the effects were variable between seasons. Growth of both species generally decreased higher up the shore in summer. However, growth of *P.perna* was significantly reduced in winter on the low-shore and sometimes on the mid-shore as well. High-shore growth of this species was low and unaffected by season. In contrast, growth of *M.galloprovincialis* was generally unaffected by season in the lower zones, although high-shore growth frequently increased in winter. As a result, *M.galloprovincialis* tended to grow significantly faster than *P.perna* in winter. In summer, these two species either exhibited similar growth rates on the low-shore, or *P.perna* grew significantly faster than *M.galloprovincialis.* Higher up the shore, *M.galloprovincialis* tended to grow faster than *P.perna,* although the difference was not always significant. The seasonal effects on the growth rates of these species were observed at locations within both sites. There were also variations between locations within sites. For example, in 2003 mussels tended to grow faster at Beacon Isle than at Lookout Beach, particularly on the low-shore. There was also evidence to suggest that there were site-specific differences in growth rates, with slower growth of mussels in Tsitsikamma.

Mortality

In the initial experiment carried out in February 2003, mortality was generally low within the first month at both locations (<20%), with the exception of *P.perna* on the mid-shore at Beacon Isle, which experienced 41% mortality. Mortality of both species remained low at Driftwood Bay in Tsitsikamma after two months, although *M.galloprovincialis* had a greater mortality rate than *P.perna* (average mortalities of 23% and 6% respectively in the upper zones). However, at Beacon Isle, high-shore mortality of *P.perna* had increased to 40% so that mortality in the upper zones was similar, while low-shore mortality remained low (<20%). Also, mortality of *M.galloprovincialis* had increased to 30-40% in all three zones. The net result was that mortality of *M.galloprovincialis* was 20% higher than *P.perna* on the low-shore, mortality of *P.perna* was 20% higher than *M.galloprovincialis* on the mid-shore, with no difference between species on the high-shore. Preliminary results from the initial experiment therefore suggest that mortality of *P.perna* will be significantly greater higher on the shore than on the low-shore and that *M.galloprovincialis* will experience significantly higher mortality rates than *P.perna* in this zone. There is also evidence to suggest that there may be site-specific differences in mortality. Furthermore, zone did not appear to influence mortality of *M.galloprovincialis.*

Data from the second experiment on the cumulative mortality of *P.perna* and *M.galloprovincialis* between November 2003 and March 2004 in Plettenberg Bay and Tsitsikamma have been plotted in Figs. 6.13-6.16. Mortality results in the lower zones in Plettenberg Bay were only obtained for the first two months. However, high-shore patches were followed throughout the sampling period. Cumulative mortality of *P.perna* and *M.galloprovincialis* in this zone showed slightly different trends at the two locations (Figs. 6.13-6.14; p.229). Mortality of *P.perna* was consistently higher than that of

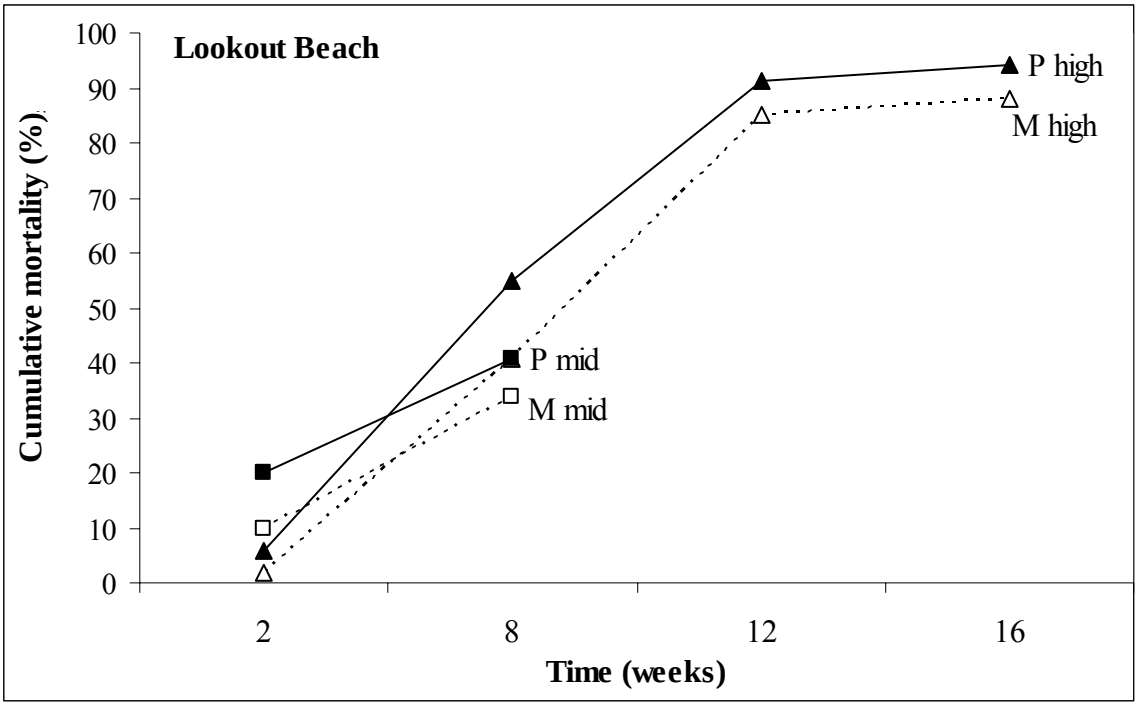


Figure 6.13: Average cumulative mortality of *P.perna* and *M.galloprovincialis* on the low (o), mid (□) and high-shores (Δ) at Lookout Beach in Plettenberg Bay 2004. Solid symbols represent *P.perna* and open symbols *M.galloprovincialis*

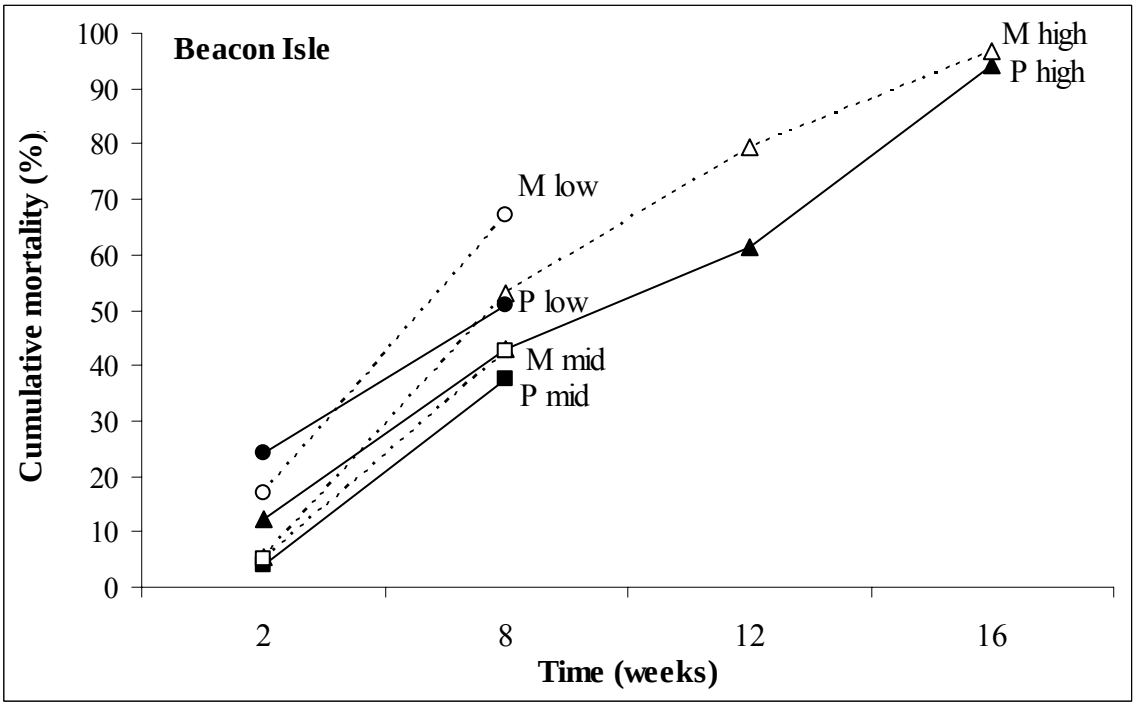


Figure 6.14: Average cumulative mortality of *P.perna* and *M.galloprovincialis* in different zones at Beacon Isle in Plettenberg Bay 2004. (*n* = 3-10)

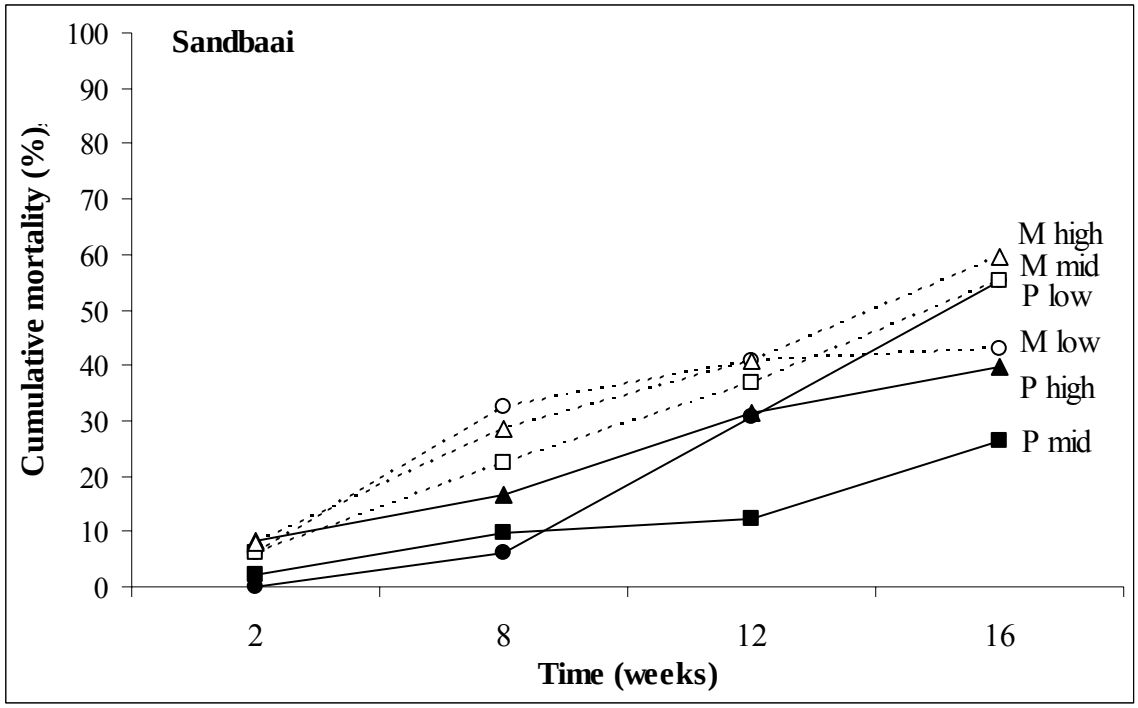


Figure 6.15: Average cumulative mortality of *P.perna* and *M.galloprovincialis* in different zones at Sandbaai in Tsitsikamma 2004

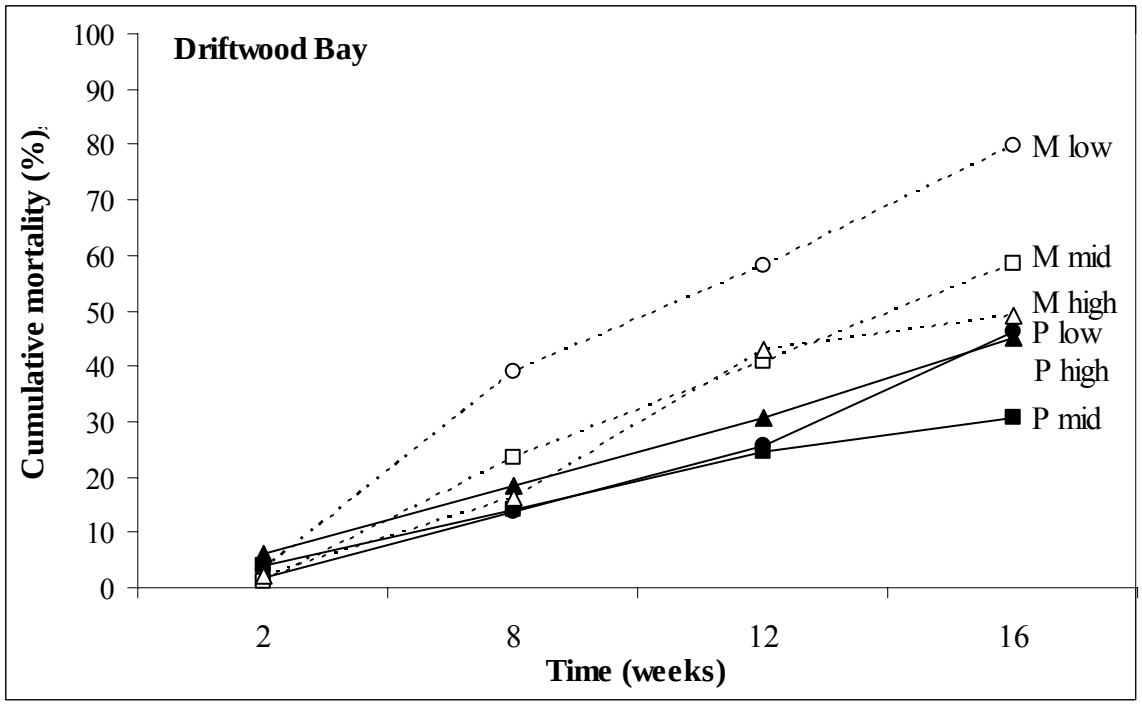


Figure 6.16: Average cumulative mortality of *P.perna* and *M.galloprovincialis* in different zones at Driftwood Bay in Tsitsikamma 2004. (*n*=5-10)

M.galloprovincialis at Lookout Beach while the opposite occurred at Beacon Isle. Interestingly mortality increased similarly for both species over time resulting in virtually parallel curves. The same differences between the two species at each location were observed in the lower zones.

In Tsitsikamma, mortality of *M.galloprovincialis* was generally higher than that of *P.perna* in all zones, particularly on the low-shore at Driftwood Bay (Figs. 6.15-6.16; p.230). However, on the low-shore at Sandbaai mortality of *P.perna* increased fairly dramatically after two months and was greater than that of *M.galloprovincialis* at four months. Comparing these graphs with those for Plettenberg Bay it seems that mortality is lower in Tsitsikamma. High-shore mortality was less than 60% at this site after four months compared to virtually 100% for mussels in Plettenberg Bay.

Results of the mixed-model ANOVA comparing mortality of mussels in Plettenberg Bay and Tsitsikamma after two months are given in Table 6.5 (p.232). There was a significant difference in mortality between sites ($p<0.05$) with higher mortalities in Plettenberg Bay than in Tsitsikamma. There were interspecific differences in mortality between zones, which were independent of both site and location, as seen by the significant interaction between zone and species ($p<0.01$). Mortality of *P.perna* tended to increase with increasing tidal height and was significantly greater on the high-shore than on the low and mid-shores (Fig. 6.17; p.232). Mortality of *M.galloprovincialis* was similar between zones but was significantly greater than mortality of *P.perna* on the low-shore. There were no differences between species in the upper zones.

Table 6.5: Mixed model ANOVA examining mortality of *P.perna* and *M.galloprovincialis* after two months in Plettenberg Bay and Tsitsikamma in 2004

	Effect	Df	MS	F	p
site*	Fixed	1	12962	19.109	0.046*
zone	Fixed	2	431	0.534	0.622
species	Fixed	1	2616.8	5.043	0.149
site×zone	Fixed	2	532.8	0.660	0.564
site×species	Fixed	1	642.6	1.239	0.378
zone×species*	Fixed	2	996	13.126	0.006*
site×zone×species	Fixed	2	16.2	0.214	0.813
location (site)	Random	2	692.1	0.546	0.608
location (site)×zone*	Random	4	836.1	13.167	0.014*
location (site)×species*	Random	2	526.8	7.184	0.027*
location (site)×zone×species	Random	4	63.5	0.213	0.931
Error		124	298.1		

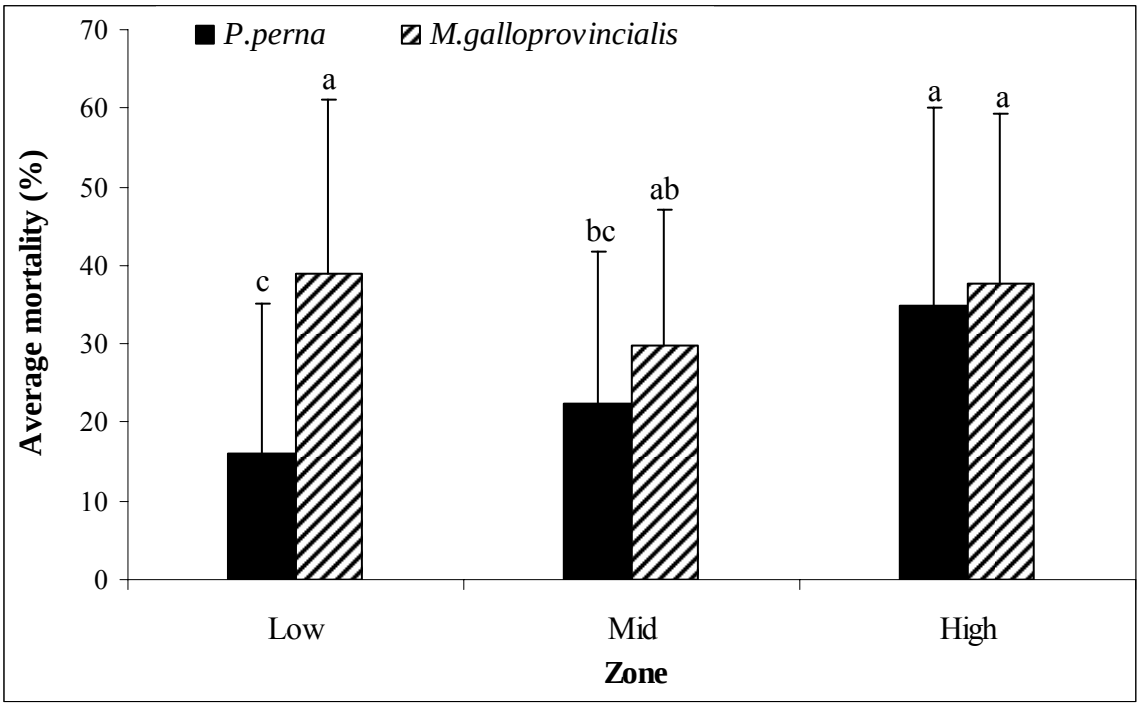


Figure 6.17: Results of post-hoc comparison of the interaction between zone and species on the mortality of mussels in 2004. Error bars represent standard deviations

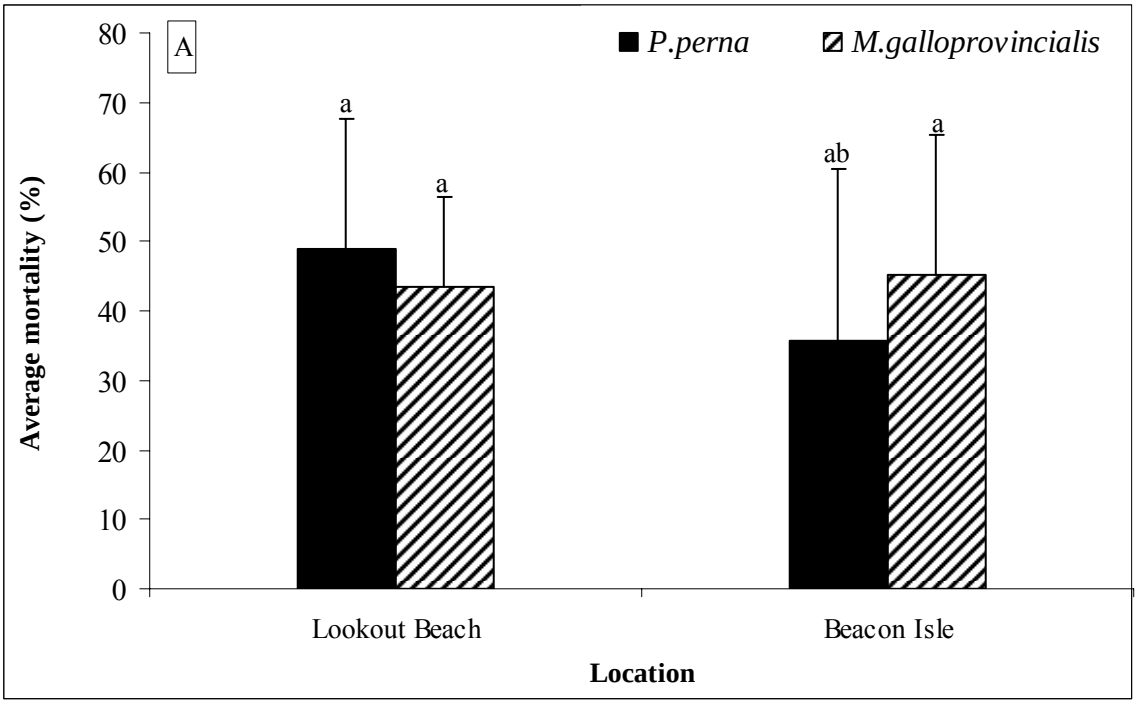
There were also species-specific differences in mortality between locations within each site ($p=0.03$). Post-hoc comparison revealed that there were no differences in mortality between species either within or between locations in Plettenberg Bay (Fig. 6.18a; p.234). However, in Tsitsikamma mortality of *M.galloprovincialis* was significantly greater than *P.perna* at Sandbaai (Fig. 6.18b). There were no differences in mortality of either species between locations within a site.

There was also a significant interaction between location (site) and zone ($p=0.01$). In Plettenberg Bay, mortality on the low-shore was significantly higher at Lookout Beach than at Beacon Isle with no differences between locations in the upper zones (Fig. 6.19; p.235). There were no significant differences in mortality between locations in Tsitsikamma.

Analysis of mortality after four months at locations in Tsitsikamma gave a significant interaction between location, zone and species ($p=0.02$; Table 6.6; p.236). Mortality of *P.perna* tended to be lowest on the mid-shore, and while mortality of *M.galloprovincialis* tended to increase with height on the shore at Sandbaai, the opposite trend was observed at Driftwood Bay (Fig. 6.20; p.236). However, these trends were not significant. At Driftwood Bay, mortality of *M.galloprovincialis* was significantly greater than that of *P.perna* in both the low and mid zones, with no difference between species on the high-shore. Low-shore mortality of *M.galloprovincialis* was also significantly greater at Driftwood Bay than at Sandbaai.

Summary

Mortality of *P.perna* was significantly greater higher up the shore than on the low-shore, while mortality of *M.galloprovincialis* was unaffected by zone. *M.galloprovincialis*



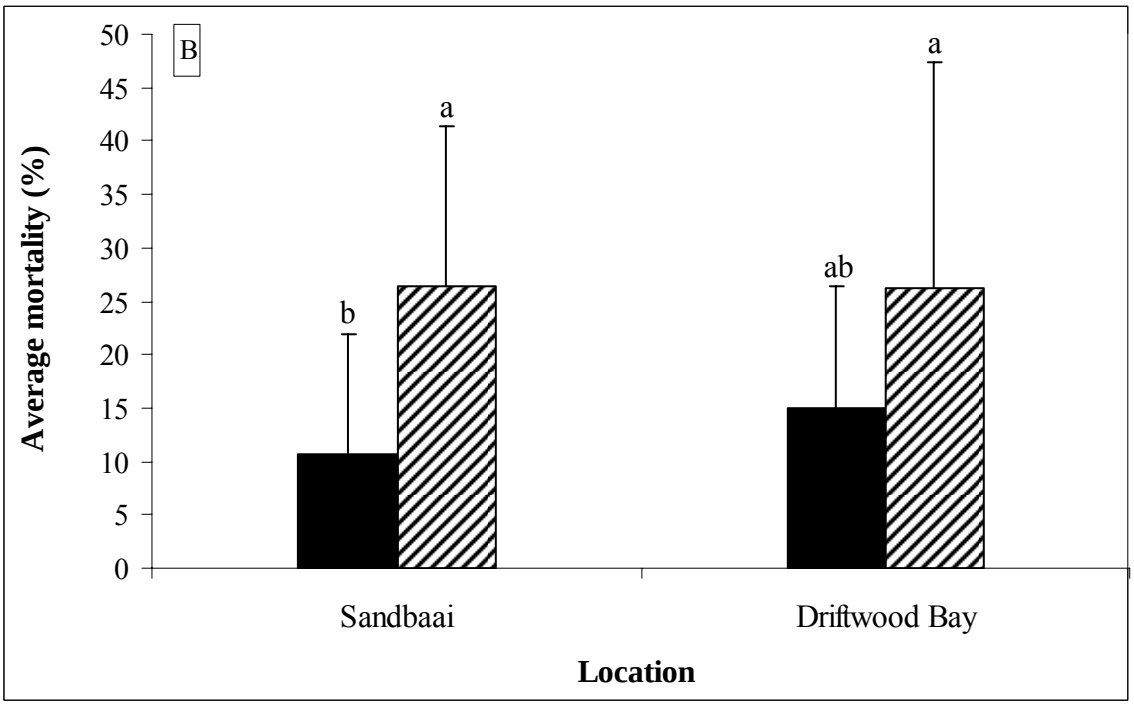


Figure 6.18 a-b: Post-hoc results of the interaction between location (site) and species on mortality of *P.perna* and *M.galloprovincialis* at locations in Plettenberg Bay (a) and Tsitsikamma (b) in 2004. Error bars represent standard deviations

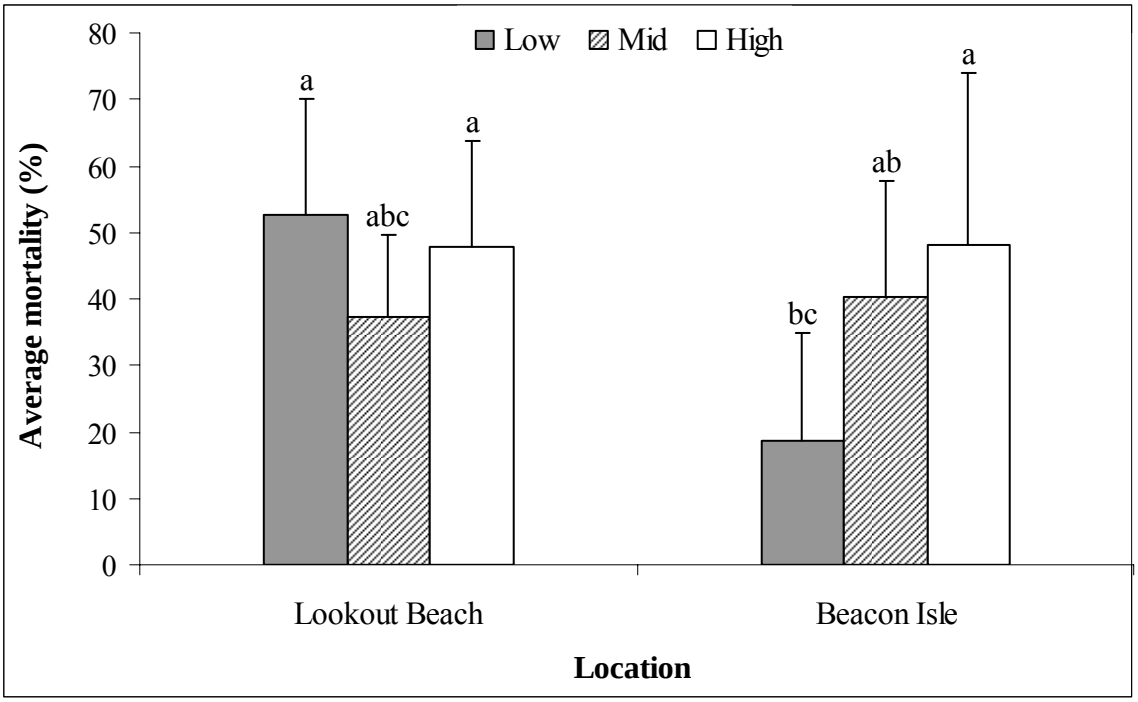


Figure 6.19: Post-hoc result of the interaction between location (site) and zone on mortality of mussels after two months at locations in Plettenberg Bay in 2004. Error bars represent standard deviations

Table 6.6: Three-way ANOVA examining the effects of location, zone and species on the mortality of mussels after four months in Tsitsikamma in summer 2004

	Effect	Df	MS	F	p
location	Random	1	59.1	0.178	0.912
zone	Fixed	2	936.7	1.191	0.456
species	Fixed	1	4349.1	6.395	0.24
location×zone	Random	2	786.5	0.694	0.59
location×species	Random	1	680	0.6	0.52
zone×species	Fixed	2	562.7	0.496	0.668
location×zone×species*	Random	2	1133.8	4.055	0.024*
Error		48	279.6		

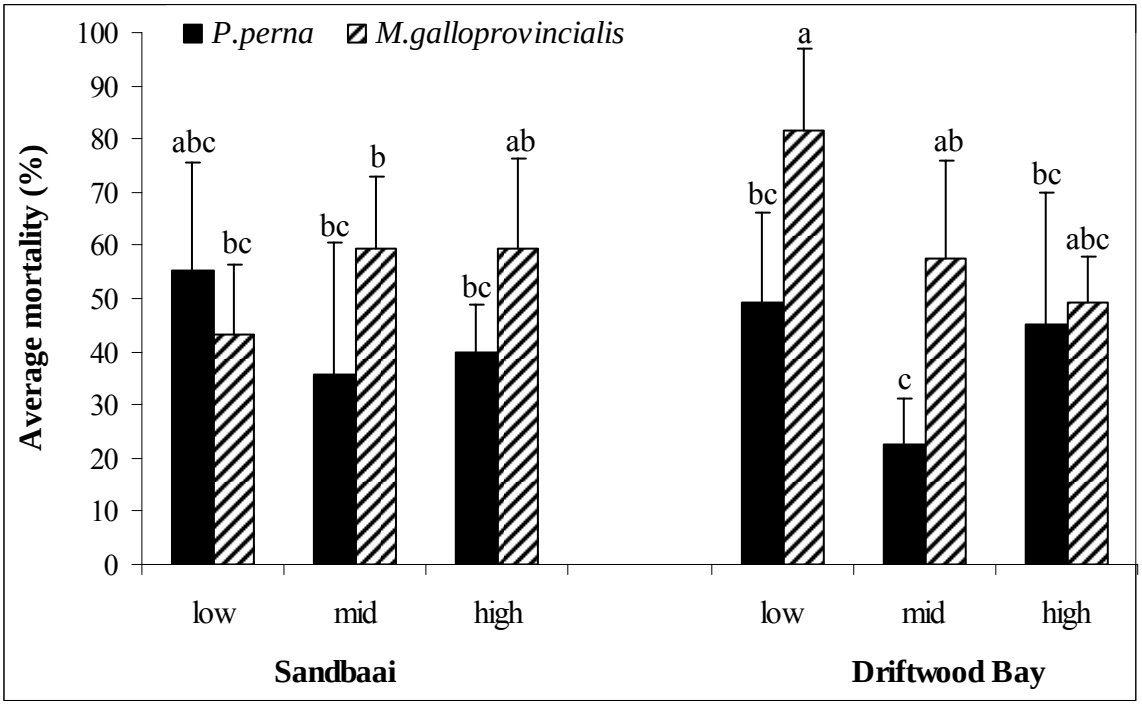


Figure 6.20: Results of post-hoc comparison on the interaction between location, zone and species on mortality of mussels after four months in Tsitsikamma in 2004. Error bars represent standard deviations

experienced a significantly higher mortality than *P.perna* on the low-shore, while mortality tended to be more similar on the high-shore. Mortality of *M.galloprovincialis* was also greater than *P.perna* in Tsitsikamma and average mortality of mussels at this site was lower than in Plettenberg Bay.

6.4 Discussion

Several methods may be used to measure growth in bivalves. These include the use of modal length frequency distributions, measurements of external or internal growth rings in the shell and measurements of marked mussels (Seed 1976). The most commonly used *in situ* marking methods include notch marking (McQuaid and Lindsay 2000; Steffani and Branch 2003), and caging or transplanting of individual mussels. Notch marking may cause growth checks in marked mussels and McQuaid and Lindsay (2000) found that this method produced the lowest growth estimates of the three methods used in their study. Similarly, caging and transplantation of animals often results in the animals being disturbed, or alternatively, flow regimes may be altered through caging which may lead to biased growth estimates. The use of the fluorochrome calcein for *in situ* marking of growth in mussels was found to be successful in the mussel *Perna perna* (Kaehler and McQuaid 1999). However, in this study the recovery rate of marked animals was generally poor, particularly for *Mytilus galloprovincialis.* This species appears to hold its valves together more tightly than *P.perna,* and was more difficult to inject so that valves were frequently damaged. Furthermore, severe growth checks were sometimes found in mussels at the point where calcein was absorbed into the shell, suggesting that marking using this method may have caused a growth disturbance.

Millstein and O'Clair (2001) marked mussels in the field using external plastic tags that were glued to the shell. This method produced a growth curve that compared very well with that obtained through growth ring analysis (Millstein and O'Clair 2001). External shell tagging may therefore provide a useful method for marking mussels *in situ* that minimises any disturbance that may cause growth to be underestimated. A slightly different tagging method was used in this study, which proved to be quite successful with greater recovery rates than obtained using calcein. No comparisons were made with other methods used to estimate growth. However, there may be some advantages of this method over others. With the exception of notch marking, most of the methods discussed may be very time consuming, either in the field or in the laboratory (e.g. acetate peel analysis) or both (e.g. cohort analysis and calcein marking). Tagging may also be relatively time consuming in the field depending on the number of parameters that are being measured, but this may be compensated for by the quick processing time in the laboratory.

Most analyses of growth performance in mussels and other bivalves involve the use of growth models such as the von Bertalanffy model to construct curves of shell length increase with age (e.g. Dare 1976; Anwar *et al.* 1990; Tomalin 1995; Lomovasky *et al.* 2002; Steffani and Branch 2003). While these models provide useful estimates of growth and longevity of bivalve populations they fail to take into account seasonal or annual variations in growth (Seed 1976). They also assume that growth is determinate and in many bivalves growth may not actually cease at any fixed adult size (Seed 1976). The potential advantage of tagging methods and the resultant length-growth curves is the ability to measure temporal changes in growth over a discrete period of time (Millstein and O'Clair 2001). Since it also provides descriptions of length-specific growth rates from a cross-

section of a population (Millstein and O'Clair 2001), it is possible to measure the specific response of different sized individuals to environmental conditions at any given time.

Growth in mussels decreases with increasing initial length (Kaehler and McQuaid 1999; McQuaid and Lindsay 2000; Millstein and O'Clair 2001). This has been attributed to a lower metabolic activity in older mussels (Seed 1969b) and it has also been suggested that decreased rates of water transport in larger animals reduce feeding efficiency (Jørgensen 1976). The same patterns were generally observed in *Perna perna* and *Mytilus galloprovincialis* in this study. However, this pattern often became less obvious at higher shore levels and during winter growth of *P.perna,* largely due to depressed growth rates of smaller, usually faster growing individuals. This is to be expected since where growth is slow, as it is in larger mussels, it is less likely to fluctuate in response to environmental conditions (Seed 1969b).

Growth of *P.perna* and *M.galloprovincialis* decreased higher up the shore in summer. In winter either the same pattern was observed or there was little effect of zone. Decreased growth rates with increasing tidal height have been observed in a number of intertidal bivalves (Seed 1969b; Griffiths 1981; van Erkom Schurink and Griffiths 1993; Vincent *et al.* 1994; Bartol *et al.* 1999; Marsden and Weatherhead 1999; Beal *et al.* 2001). This is generally considered to be due to a reduction in the available feeding time higher up the shore (Seed 1969b; Griffiths and Griffiths 1987; Marsden and Weatherhead 1999). This may also be due to physical stresses such as desiccation and high temperatures at higher shore levels, as more energy is required for metabolic maintenance, which may reduce the amount of energy available for growth (Bartol *et al.* 1999). Growth rates of *M.galloprovincialis* frequently increased on the high-shore in winter when temperatures

and desiccation stress are lower. However, high-shore growth of *P.perna* was consistently low and did not vary between seasons. van Erkom Schurink and Griffiths (1993) found that *M.galloprovincialis* was able to maintain faster growth rates than *P.perna* at increasing levels of aerial exposure, probably because it is more tolerant of desiccation stress than *P.perna* (Hockey and van Erkom Schurink 1992). These results suggest that physiological stress on the high-shore may be more important than food supply in limiting the growth of these species, although the latter undoubtedly plays a role.

One of the most striking outcomes of this study was the strong seasonal effect on growth of these two species. Both species exhibited similar growth rates in summer, however, growth of *P.perna* was significantly depressed in winter, particularly on the low-shore. On the other hand, growth of *M.galloprovincialis* was generally unaffected by season and consequently this species experienced a significantly faster growth rate than *P.perna* across the shore in winter. The effect of season was also observed at both sites and in both years that growth was measured. This is in agreement with results obtained for these mussels in Algiers, where *P.perna* exhibited strong seasonal differences in growth while *M.galloprovincialis* had more regular growth throughout the year (Abada-Boudjema and Dauvin 1995).

Many benthic invertebrates experience depressed growth rates in winter, which is generally attributed to a decrease in temperature or food supply (Seed 1969b; Dare 1976; Tomalin 1995; Grant 1996; Dekker and Beukema 1999; Beal *et al.* 2001; Wong and Cheung 2001) or to the reproductive cycle (Berry 1978; Bayne and Widdows 1978; Kautsky 1982; Cranford and Hill 1999). Seasonal variation in food supply is unlikely to be important since this would affect the growth of both species. In South Africa, both *P.perna* and

M.galloprovincialis may have a winter spawning event (van Erkom Schurink and Griffiths 1991). Furthermore, the majority of mussels in which growth rate was fastest and most variable in space and time were below the size at which these species become sexually mature (±30mm; unpub. data). Thus reproductive effort was not considered to be a factor limiting the growth of *P.perna* in winter.

P.perna is essentially a warm-water species that is widely distributed in tropical and subtropical regions of the Indian and Atlantic Oceans and also occurs in dense aggregations on rocky shores on the subtropical east coast of South Africa (Berry 1978). It is also absent from the cold-temperate west coast of South Africa (van Erkom Schurink and Griffiths 1990) which suggests that it is intolerant of low temperatures. The south coast is considered to be warm-temperate, but may experience winter temperatures comparable to those observed on the west coast (Field and Griffiths 1991). Thus temperature is the most likely factor causing seasonal variations in the growth rate of *P.perna*. *M.galloprovincialis,* on the other hand, has flourished following its invasion on the west coast, which implies that it is well adapted to the cold-temperate conditions there. Also, the fact that growth of this species sometimes increased in winter in this study suggests that winter on the south coast may be the optimal time of year for *M.galloprovincialis*. Thus it may be that both species are towards the limit of their optimal temperature ranges on the south coast, particularly *P.perna.*

Conclusions about variations in growth between sites were tenuous due to a lack of replication within sites. However, in both years that growth was measured, growth appeared to be slower in Tsitsikamma than in Plettenberg Bay, particularly in winter. Several studies have demonstrated that mussels experience significantly faster growth rates

on wave-exposed shores than on sheltered shores (Leeb 1995; McQuaid and Lindsay 2000; Steffani and Branch 2003). Locations in Plettenberg Bay are slightly more exposed to wave action than locations in Tsitsikamma (refer to Chapter 1). Archambault *et al.* (1999) also found that mussels within embayments grew faster than mussels outside bays, and this was related to differences in current velocity and phytoplankton concentration. Thus differences in wave exposure or food supply may account for the differences in growth rates between sites.

Mortality of *P.perna* in summer was generally greater higher up the shore than on the low-shore. However, this effect of zone was no longer apparent after four months at locations in Tsitsikamma, due to an increase in low-shore mortality. In contrast, mortality of *M.galloprovincialis* was not zone-dependent. *M.galloprovincialis* also experienced significantly greater mortality rates than *P.perna* on the low-shore, while mortality in the upper zones tended to be more similar.

Mortality after four months on the high-shore in Plettenberg Bay was virtually 100% for both species compared to 60% in Tsitsikamma. In Plettenberg Bay this was primarily due to removal of whole patches of mussels by wave action (pers. obs.). *M.galloprovincialis* forms densely packed multilayered beds in the upper zones at this site, which are typical of this species (Griffiths *et al.* 1992). Attachment in multilayered clumps is more tenuous so that mussels are more easily dislodged by wave action (Petraitis 1995). This is particularly evident on the high-shore where mussels are smaller and can be removed by hand with ease. Leeb (1995) found that attachment strength was weaker in smaller mussels (*M.galloprovincialis*) than in larger mussels, and consequently was weaker in high-shore than low-shore populations. Since *P.perna* occurs in these unstable clumps with

M.galloprovincialis, removal of whole patches would result in similar mortality rates between species. This effect of clump instability may also explain why mortality was generally higher in Plettenberg Bay than in Tsitsikamma where beds are monolayered.

Physiological intolerance of desiccation is considered to be an important factor limiting the upper distribution of intertidal mussels (Suchanek 1985). *P.perna* is less tolerant of desiccation stress than *M.galloprovincialis* (Hockey and van Erkom Schurink 1992). Thus it may be expected that background mortality (in the absence of large disturbances) would be greater for *P.perna* than *M.galloprovincialis* on the high-shore. However, mortality of these species was similar in this zone in Tsitsikamma where wave action is less severe. High-shore population structure at this site in 2004 closely resembled that in Plettenberg Bay where mussels were generally small (<30mm in length). It is possible that weaker attachment strength in smaller mussels may render these species equally vulnerable to mortality in this zone.

Attachment strength may also have been a factor contributing to the differences in mortality between *P.perna* and *M.galloprovincialis* on the low-shore at these sites. Several studies have shown how differential attachment strength can influence species distributions (Harger 1972b; Bell and Gosline 1997; Seed and Richardson 1999). *P.perna* has stronger attachment strength than *M.galloprovincialis* both as solitary mussels and in beds (Zardi, unpub. data). Thus *M.galloprovincialis* may be less tolerant of wave action than *P.perna* resulting in differential mortality rates in this zone. However, this species reaches its greatest abundance on exposed shores on the west coast of South Africa (Steffani and Branch 2003) and on the coast of Ireland (Gosling and McGrath 1990). This may suggest that *M.galloprovincialis* has greater attachment strength in colder waters. If this species is

near the edge of its optimal temperature regime on the south coast, then the physiological condition of mussels on this coast may be poorer than on the west coast.

Predation can be an important source of mortality on rocky shores (Seed 1969b; Dayton 1971; Menge *et al*. 1994; Petraitis 1998), but was not directly measured in this study. Birds, whelks and possibly crabs, appear to be the major predators of mussels in Plettenberg Bay. Starfish, which are important predators of mussels (Paine 1974; Menge *et al.* 1994), were never observed at this site. However, in Tsitsikamma where the communities are more diverse, and also extend subtidally, starfish and octopus were both observed in addition to whelks. It is possible that predation contributed to the relatively high mortalities of mussels on the low-shore at this site by the end of the study period. It is also possible that differential predation rates between species may have contributed to the higher mortality rate of *M.galloprovincialis*. This may be related to their attachment strength since *M.galloprovincialis* is easier to dislodge.

Manipulative competition experiments with these species on the low-shore on the south coast revealed that *P.perna* negatively affects the survival of *M.galloprovincialis,* while *M.galloprovincialis* does not influence the survival of *P.perna* (Rius 2005). Thus interspecific competition for space in which *P.perna* is dominant may also result in increased mortality of *M.galloprovincialis* on the low-shore.

Regardless of the mechanism involved, these results suggest that *P.perna* is able to maintain spatial dominance on the low-shore due to enhanced mortality rates of adult *M.galloprovincialis.* If interference competition is the primary cause of mortality in *M.galloprovincialis,* then *P.perna* may actively eliminate *M.galloprovincialis* from this

zone. Alternatively, *P.perna* may have a competitive advantage indirectly through its superior tolerance to wave exposure or to differential predation rates. It is also clear that enhanced mortality rates in adult *M.galloprovincialis* may be responsible for the smaller size of this species compared to *P.perna* in low-shore areas (Chapter 2).

Poor growth rates and high mortality rates may also contribute to the low abundance and significantly smaller size of *P.perna* on the high-shore at these sites. *M.galloprovincialis* exhibited the same growth and mortality rates as *P.perna* in this zone in summer. However, in winter, high-shore growth of this species increased to rates similar to that in the lower zones. Petraitis (1995) suggests that faster growth rates are able to compensate for mortality thereby allowing organisms to maintain spatial dominance. Thus *M.galloprovincialis* may be able to compensate for high summer mortalities on the high-shore due to a faster growth rate in winter.

The strength and direction of interspecific interactions will change depending on the relative rates of recruitment, growth and mortality (Sherwood and Petraitis 1998). This appears to be the case in low-shore interactions between these species. *P.perna* has a strong competitive advantage over *M.galloprovincialis* in summer. It has high summer recruitment rates, fast and sometimes superior growth rates and low mortality rates. On the other hand, *M.galloprovincialis* grows significantly faster than *P.perna* in winter and has slightly higher recruitment rates. At present it seems that the competitive advantages enjoyed by each species are reversed with the seasons. However, if the differential mortality rates of these species in summer are consistent throughout the year, then *P.perna* may ultimately be the winner in competition between these species on the low-shore. Seasonal variations in mortality rates between these species need to be measured.

Chapter 7

General discussion

Discussion

For decades now, community ecology has focussed on the study of patterns of community organisation and understanding the processes underlying these patterns. With the volumes of information available, it is increasingly evident that populations are regulated by an array of different factors operating at different times, at different spatial scales and at different stages in their life histories. The present study investigated the interaction between the indigenous mussel *Perna perna* and the invasive mussel, *Mytilus galloprovincialis* in Plettenberg Bay and Tsitsikamma on the south coast of South Africa.

Initial observations suggested that these species might be coexisting by means of vertical habitat segregation, with *M.galloprovincialis* occupying the highest levels and *P.perna* the lower levels with a zone of overlap on the mid-shore. Quantitative analysis of the patterns of distribution and abundance of these species supported initial observations. Furthermore, when this analysis was repeated three years later it was clear that the same vertical patterns were evident despite significant increases in the density of *M.galloprovincialis.*

Three hypotheses were originally proposed to explain the vertical distributions of *P.perna* and *M.galloprovincialis* in Plettenberg Bay and Tsitsikamma. The first hypothesis suggested that *P.perna* and *M.galloprovincialis* selectively settle at different heights on the shore. Settlement and recruitment of both *P.perna* and *M.galloprovincialis* decreased with increasing tidal height. Thus differential settlement may contribute to the population structure of *P.perna* at these sites but not *M.galloprovincialis.* An alternative hypothesis to that of differential settlement was that these species settle haphazardly on the shore, but occur in different zones because of zone-dependent differential post-settlement mortality. Post-settlement mortality appeared to be important in structuring high-shore populations,

but failed to account for the low adult abundance of *M.galloprovincialis* on the low-shore. Post-settlement mortality of *P.perna* increased higher up the shore and may therefore be important in limiting the abundance of this species on the high-shore. In contrast, *M.galloprovincialis* had low mortality rates on the high-shore. It was suggested that the high densities of this species in this zone may be a result of cumulative settlements of slow-growing individuals.

The third hypothesis proposed that the distribution of these mussels is determined by post-recruitment interactions such as competition and mortality. *M.galloprovincialis* may be excluded from the low-shore as adults due to significantly higher mortality rates than *P.perna* in this zone. Poor growth rates and high adult mortality rates on the high-shore may also contribute to the low abundance of *P.perna* in this zone. Thus the processes influencing the population structure of mussels at these sites varied in importance between species and among zones on the shore. Therefore, no single hypothesis could explain the distributions of these species, which emphasises the complex nature of communities and the processes influencing them.

M.galloprovincialis is widely distributed in the temperate zones of the world but is absent from the tropics (Hilbish *et al.* 2000). It also seems to be primarily a cold-temperate species although it is capable of existing in subtropical conditions such as Hong Kong (Lee and Morton 1985). *P.perna* on the other hand has a subtropical distribution and is generally absent from cold-temperate zones (Berry 1978). *P.perna* and *M.galloprovincialis* therefore both appear to be towards the limits of their optimal temperature regimes on the south coast of South Africa. As a result there appear to be seasonal variations in the competitive advantages enjoyed by each. This was evident in the interaction between these species on

the low-shore. *P.perna* has a strong competitive advantage over *M.galloprovincialis* in summer. It has higher summer recruitment rates, fast and sometimes superior growth rates and lower mortality rates. On the other hand, *M.galloprovincialis* grows significantly faster than *P.perna* in winter and has slightly higher recruitment rates. Mortality rates were not measured in winter. However, since *M.galloprovincialis* has weaker attachment strength than *P.perna* on this coast (Zardi, unpub. data) it may be particularly vulnerable during winter storms. Thus any advantage *M.galloprovincialis* might have gained on the low-shore in winter through rapid growth rates may be lost due to higher mortality rates in winter. Therefore, *P.perna* may ultimately be the winner in competition between these species on the low-shore as indicated by competition experiments between these species (Rius 2005).

Refuges of various kinds permit recruitment of non-indigenous species into communities by providing enemy-free or competitor-free space (Crawley 1986). *M.galloprovincialis* appears to have a refuge from competition with *P.perna* on the high-shore due to its enhanced tolerance of desiccation stress and its ability to establish large populations there. This appears to be the mechanism of invasion by *M.galloprovincialis* on this coast. Once established on the high-shore, populations begin to expand to lower shore levels. This is supported by long-term surveys of mussel populations along the south coast since 1994 (McQuaid, unpub. data) as well as this study. In 2001, *M.galloprovincialis* was initially largely confined to the high-shore in Tsitsikamma, but after three years had spread to the mid-shore. It also appears to display characteristics of a fugitive species on the low-shore. Fugitives are species that have a temporal refuge in competition from dominants (Connell 1985). *M.galloprovincialis* is able to recruit in large numbers in this zone and grow rapidly to sizes at which it can reproduce before high mortality in larger mussels sets in.

High fecundity and high recruitment rates have contributed significantly to the success of *M.galloprovincialis* on the west coast of South Africa (Branch and Steffani 2004). On the south coast, recruitment rates are generally low (Lasiak and Barnard 1995; Harris *et al.* 1998; Porri 2004). This may be due to differences in the physical oceanography between these coasts or to the different species that are found there (Harris *et al.* 1998). It is possible that *M.galloprovincialis* has a lower reproductive output in warmer waters compared to the west coast. Alternatively recruitment of any species with planktotrophic larvae may be limited by local hydronamics or mortality. Nevertheless, the process of invasion by *M.galloprovincialis* on the south coast may be slow or limited due to low recruitment rates. The corollary of this, is that high recruitment rates in Plettenberg Bay, possibly due to larval retention within the bay, has probably contributed to the success of *M.galloprovincialis* at this site.

The interactions between *P.perna* and *M.galloprovincialis* have not been examined where mussels are heavily exploited. Because *P.perna* reaches significantly larger sizes than *M.galloprovincialis* it may be targeted for harvesting and this may have consequences for competition between them. Tsitsikamma is a marine reserve and therefore protected from exploitation. Although Plettenberg Bay is not in a reserve, local residents and conservationists actively contribute to the protection of natural resources in the area. In addition, high recruitment rates in Plettenberg Bay are not characteristic of the majority of the coastline where recruitment and hence recovery rates are poor (Lasiak and Barnard 1995; Dye *et al.* 1997; Harris *et al.* 1998; Porri 2004).

At present colonisation by *M.galloprovincialis* on the south coast does not appear to have displaced or reduced the abundance of *P.perna.* Previous studies on communities in

Plettenberg Bay and Tsitsikamma before the arrival of *M.galloprovincialis* showed that *P.perna* was most abundant at low and mid-shore levels and was scarce on the high-shore (Crawford and Bower 1983; Wooldridge 1988). Thus *P.perna* has been able to maintain spatial dominance on the low-shore despite increasing densities of *M.galloprovincialis.* It is concluded that at present *P.perna* and *M.galloprovincialis* seem to be coexisting on south coast rocky shores by means of vertical habitat segregation.

It should be noted though, that the impacts of introduced species are frequently only appreciated by intensive and long-lasting studies after invasion in a new habitat. In some cases, especially at the beginning of spread, invasion by a non-indigenous species has initially been considered as a positive addition to the structural and functional diversity of recipient communities (Occhipinti-Ambrogi 2001). Long-term monitoring of populations of *M.galloprovincialis* is therefore necessary. Further studies are also required to determine what impact invasion by *M.galloprovincialis* might have had on other intertidal organisms, particularly on the mid and high-shores where it is most abundant.

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