Genetics and ecosystem effects of the invasive mussel *Semimytilus algosus*, on the West Coast of South Africa

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“Unless someone like you cares a whole awful lot,

Nothing is going to get better. It's not.”

Dr. Seuss, *The Lorax*

This thesis is dedicated to Zirk and Tiaan du Toit without whose support this work would not have been possible

Declaration

I, Susanna Catharina Franzina Zeeman, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. I authorise the University to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

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Table of Contents

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Abstract

Invasive species can radically affect community composition and ecosystem processes, and human traffic has accelerated their spread. On the South African coast, 86 invasive species have been recorded, although until recently only the Mediterranean mussel *Mytilus galloprovincialis* and the barnacle *Balanus glandula* have had significant ecosystem effects on a large geographic scale. *Semimytilus algosus* was first detected at Elands Bay in 2009 and by 2010 it occupied 500 km of the West Coast of South Africa from Groenriviermond to Bloubergstrand. Given the fact that it is an ecosystem engineer forming dense beds, it is likely to have marked community effects. My study aimed to determine: (A) the identity, genetic composition and origin of the population, and (B) potential interactions between *M. galloprovincialis* and *S. algosus* and their effects on community composition. I conducted surveys to assess the abundance and zonation of *S. algosus*, analysed its competitive abilities by comparing its life-history strategies to other mussel species on the West Coast, and conducted field experiments to examine survival and interactions between *S. algosus* and *M. galloprovincialis* at different shore heights. I confirmed the identity of this species and found that the South African population has comparable genetic variation to the Chilean and Namibian populations. It spread to South Africa, seemingly through larval dispersal from Namibia, and now ranges from Groenriviermond in the north to Hout Bay in the south, spreading southward. It is most abundant on the low shore, with *M. galloprovincialis* on the mid shore. Mixed beds co-exist in the transition zone. Biodiversity in *S. algosus* beds is similar to that in *M. galloprovincialis* beds. High recruitment rates of *S. algosus* on the low shore, and its high genetic variation at all sites, enable it to colonise rock quickly and become established in new areas. The competitive ability of *Semimytilus algosus* is strongly related to shore height. It cannot survive on the high shore due to intolerance to desiccation. On the low shore its high recruitment rate offsets its high mortality due to wave action and predation.

Chapter 1

General introduction, aims and overview

1.1 Introduction

This thesis focuses on the bisexual mussel *Semimytilus algosus*, which occurs naturally on the West Coast of South America (Fig. 1.1), but now occupies extensive areas of Namibia, and recently invaded South Africa (Fig. 1.2).

Vermeij (1996) defines invasion as: “the geographical expansion of a species into an area not previously occupied by that species”, but most authors regard alien species as being invasive only if they have significant effects on indigenous species and communities. Invasive species have spread widely around the globe and many are having significant effects on community composition and ecosystem processes (Ruiz et al. 1997, Grosholz 2002, Hooper et al. 2005). Species ranges are naturally dynamic (Lodge 1993), and changes in abiotic features of marine ecosystems, such as sea surface temperatures due to climate change facilitated range shifts for many species (Hawkins et al. 2008). However human-mediated spread is much faster than natural range expansion (Sorte et al. 2010) and has accelerated the spread of organisms over the last century (Ruiz et al. 2000; Bax et al. 2003). Human mediation includes transport in the ballast water of ships (Williams et al. 1988; Carlton & Geller 1993), hull fouling and aquaculture (Minchin 1996). Ballast water is the most common vector for the spread of alien species, with aquaculture considered to be the second most common vector (McQuaid & Arenas 2009). Benthic organisms can spread as adults attached to the hulls of ships or as larvae in the ballast water (McQuaid & Arenas 2009). In South Africa, Haupt et al. (2010) found that the importation of oysters for mariculture is the most likely vector for the spread of four new invasive species from Namibia, the black sea urchin *Tetrapygus niger*, the European flat oyster *Ostrea edulis*, Montagu's crab *Xantho incisus*, and the brachiopod *Discinisca tenuis*. The geographical range of an introduced species can further expand in its new environment through asexual processes or larval dispersal (Branch & Steffani 2004, Mead et al. 2011).

Invasive species may constitute one of the biggest threats to biodiversity (Bax et al. 2003, Hooper et al. 2005), although Briggs (2007) disagrees, as invasive species can have both negative and positive impacts on the host ecosystem, with the potential to create novel ecosystems (Hobbs et al. 2009). In marine ecosystems local species rarely become extinct due to invasions, but their ecological ranges and abundance are often restricted (Vermeij 1996; Bownes & McQuaid 2006). For example, in South Africa the indigenous mussel *Aulacomya atra* appears to be displaced by *Mytilus galloprovincialis* , and another indigenous mussel, *Choromytilus meridionalis*, is largely restricted to silted or subtidal areas where *M. galloprovincialis* is rare (Steffani & Branch 2003). Robinson and Griffiths (2002) found that in the Langebaan Lagoon on the West Coast of South Africa *M. galloprovincialis* caused a shift in fauna, from a sandbank-community to one more normally associated with a rocky-shore community, with negative impacts on the food chain upon which migrant waders are dependent. On the other hand, on rocky shores, *M. galloprovincialis* has increased habitat complexity and thus enhanced biodiversity (Robinson et al. 2007a), and improved food supplies for the African Black Oystercatcher *Haematopus moquini* (Hockey & Van Erkom Schurink 1992; Coleman & Hockey 2008) and the whelk *Trochia cingulata* (Branch & Steffani 2004). Similarly several other invasive species have been reported to have positive effects on biodiversity (Crooks & Khim 1999; Castilla et al. 2005), primary productivity (Ruesink et al. 2006) and served as exploitable resources (Castilla et al. 2005).

Few non-indigenous species survive transport and settlement in new environments to reproduce. Even if they achieve reproduction, immigrant communities may only survive for a few generations before local extinction. Nevertheless, a small number of non-indigenous species do become invasive (Mack et al. 2000), and there are several reasons for the success of these invaders.

Some communities are more susceptible to invasion than others. The biotic resistance hypothesis states that more diverse communities are more stable and less likely to be invaded, probably due to more complete use of resources by such communities. However, Britton-Simmons (2006) and Arenas et al. (2006) have concluded that invasion resistance depends more on the type of functional groups found in a community. Ricciardi (2001) found that the Great Lakes in USA, despite having a diverse community, have been invaded frequently. Moreover, some invaders may facilitate invasion by other species, for example by modifying habitat to better suit another invader. This could cause invasions to snowball, as per the invasional meltdown hypothesis by (Simberloff & Von Holle 1999). Disturbance can decrease the invasion resistance of a community, as it frees space and resources for invaders (Shea & Chesson 2002). Availability of empty niches, a super abundance of food and lack of efficient competitors, predators and parasites in invaded areas will also facilitate the spread of aliens (McQuaid & Arenas 2009).

Along different lines, high genetic variability can increase an invader's fitness for rapid range expansion and persistence in invaded areas (Stepien et al. 2005; Roman 2006; Rius et al. 2008). When high numbers of individuals of an invading species are introduced, this will increase the genetic variability of the population, as will multiple introductions (Ficetola et al. 2008). Facon et al. (2008) also reported that multiple introductions increase an invader's potential to adapt to its environment.

However, whether an organism becomes invasive is also highly dependent on the abiotic characteristics of the invaded area (Darling et al. 2008, McQuaid & Arenas 2009). For example, the green crab *Carcinus maenas* became an aggressive invader in the sheltered waters of the North American and Tasmanian coasts, with a rapid geographical expansion, but it failed to spread along the more wave-exposed coast of South Africa and remained largely restricted to the sheltered Table Bay harbour (Le Roux et al. 1990; Robinson et al. 2005; Hampton and Griffiths 2007). Physiological processes are also known to mediate competition between mussels. For example, although *M. galloprovincialis* outcompetes *Perna perna* in sheltered bays through faster recolonisation due to its greater recruitment rate, the opposite is true on wave-exposed shores where it is more easily dislodged (Erlandsson et al. 2006).

On the South African coast, at least 86 invasive species are known (Griffiths et al. 2009, Mead et al. 2011a & b) although until recently only the Mediterranean mussel *Mytilus galloprovincialis* has had significant ecosystem effects over a large geographic scale (Branch & Steffani 2004, Robinson et al. 2005). *Mytilus galloprovincialis* occurs from the North Atlantic coast to the British Isles as well as occurring in the Mediterranean Sea but is commonly referred to as the Mediterranean mussel (Branch & Steffani 2004). Arrival of the barnacle *Balanus glandula* has now added another species with similar invasive characteristics (Laird & Griffiths 2008)

The mussel *Semimytilus algosus* was first detected on South African shores at Elands Bay in 2009. De Greef et al. (2013) undertook a preliminary investigation, which reported the current geographic range of *S. algosus*, its abundance and intertidal distribution. However, no further studies have been conducted on this mussel in South Africa.

This species is a member of the family *Mytilidae* and naturally occurs along the West Coast of South America from Ecuador to Chile, an area known as the Warm-Temperate Southwestern Pacific (Carranza et al. 2009a). It was discovered in Namibia in 1931 by Lamy (1931) under the name *Modiola pseudocapensis* (Mead et al. 2011a). During 1968 and 1969 the State Museum, Windhoek and the South African Museum undertook an expedition to survey the intertidal fauna of Namibia, and they reported that *S. algosus* occurred only north of Swakopmund at that time (Kensley & Penrith 1970).

Semimytilus algosus currently occupies 500 km of the West Coast of South Africa from the mouth of the Groenrivier to Bloubergstrand (De Greef et al. 2013), but its arrival there seems recent, as it was not recorded on surveys in the 1990s and early 2000s (Bustamante & Branch 1996a; Xavier et al. 2007). It is not known whether the South African population is a new introduction from Chile or whether it is the result of natural range expansion by the Namibian population.

1.2 Overall aims of the thesis

My study aims, first, to verify the identity of the invasive mussel in southern Africa by conducting a study of the genetic structure of *Semimytilus algosus* in its native region in comparison with populations of what are putatively the same species in Namibia and South Africa. My study also examines the extent of the effects *S. algosus* will have on West Coast rocky-shore ecosystem by (1) conducting surveys to assess the abundance, zonation and tolerance to wave exposure of *S. algosus*; (2) analysing the competitive capabilities of *S. algosus* through an assessment of its life-history strategies such as growth, reproduction and settlement rates, and its shell and byssus strength in relation to other mussels species that co-occur with it, and (3) investigating the survival and interactions between *S. algosus* and *M. galloprovincialis* at different shore heights through field experiments. My study also evaluates phylogeographical relationships between populations in Chile, Namibia and South Africa, to establish whether the colonization of South Africa was an independent introduction through human mediated spread from Chile or Namibia, or a natural range expansion through larval dispersal from Namibia.

1.3 Overview of thesis

This thesis comprises seven chapters:

Chapter 1: General introduction, aims and overview

Chapter 2: Molecular identification of *Semimytilus algosus*, *Aulacomya atra* and an unknown invasive mussel from Lüderitz in comparisons of southern African and Chilean populations

Chapter 3: Field surveys of *Semimytilus algosus* in South Africa: abundance, zonation and tolerance to wave exposure

Chapter 4: Comparisons of life-history strategies of *S. algosus* and three other mussel species on the West Coast of South Africa

Chapter 5: Field experiments assessing survival, growth, condition, recruitment and interactions between *Semimytilus algosus* and *Mytilus galloprovincialis* at different shore heights

Chapter 6: Phylogeography of the invasive mussel *Semimytilus algosus* in South Africa relative to natural populations in Chile and Namibia

Chapter 7: Synthesis and conclusions

Chapter 2

Molecular identification of *Semimytilus algosus*, *Aulacomya atra* and an unknown invasive mussel from Lüderitz in comparisons of southern African and Chilean populations

2.1 Introduction

Molecular tools for the identification of mussels

The family *Mytilidae*, which includes genera such as *Perna*, *Modiolus*, *Aulacomya*, *Choromytilus*, *Mytilus* and *Semimytilus*, dates from the Devonian (Soot-Ryen 1969), and has an almost world-wide distribution, occurring in both temperate and warm oceans (Gosling 1992; Santaclara et al. 2006). These mussels play important roles in the functioning of intertidal communities (Petraitis 1995; Tokeshi & Romero 1995). They are also harvested from the shore and are an economically important part of aquaculture in many parts of the world (Navarro et al. 1991; Kaiser et al. 1998; Naylor et al. 2000). Early studies of the taxonomy of *Mytilidae* were based on shell morphology and morphometrics (Gosling 1992). However, mussels are known to exhibit high morphological plasticity influenced by environmental factors such as water temperature (Seed 1968). Thus, distinguishing similar species on morphology alone is difficult. A comparison of gene sequences can, however, shed light on their identity (Blair et al. 2006; Derycke et al. 2008), and there have been several molecular studies dealing with the molecular identification of species (Toro 1998; Rego et al. 2002; Wood et al. 2003; Santaclara et al. 2006) For example, Wood et al. (2007) investigated the phylogeny of the genus *Perna* using nuclear (ITS1&2) and mitochondrial (COI) sequences, and confirmed that it is monophyletic. There was strong support for three clades: *P. viridis* (Linnaeus 1758), *P. perna* (Linnaeus, 1758), *P. canalicula* (Gmelin, 1791), confirming their status as separate species. Mitochondrial and nuclear markers were also used to define the genetic structure of populations of the Floridian scorched mussel *Brachidontes exustus* (Linnaeus, 1758), revealing two distinct sister clades, the Gulf of Mexico and Atlantic clades, and placed the origin of the species in the Pliocene (Lee & Foighil 2004). The *Mytilus edulis* complex is distributed globally and consists of three distinct but closely related species: *M. edulis* Linnaeus, 1758, *M. galloprovincialis* Lamarck, 1819, and *M. trossulus* Gould, 1850 (Gosling 1994). No record of fossils older than 2 000 000 years are available (Seed 1976) so the complex is relatively new, with *M. edulis* the ancestral form and *M. galloprovincialis* evolving during the Pleistocene. *Mytilus trossulus* is reported to be a zoogeographic remnant of a previously widely distributed mussel, with an age of approximately 1-2 mya (Gosling 1994). Toro (1998) used morphology as well as nuclear (ITS and GLU-5) and mitochondrial (COIII) markers to study the taxonomic status of the Chilean blue mussel *Mytilus chilensis* Hupé, 1854, and confirmed that it is a subspecies of *M. edulis* known as *M. edulis platensis* d'Orbigny, 1842.

Mitochondrial DNA has been widely used in phylogenetic studies as it evolves more quickly than nuclear DNA and thus it is better suited to detect recent divergences between closely related species (Brown et al. 1979; Moore 1995). Several papers have, however, criticized the usefulness of mitochondrial DNA in reaching conclusions on species delimitation (Edwards et al. 2005), divergence times, population size and growth and gene flow (Edwards & Beerli 2000), citing the fact that mtDNA does not evolve neutrally but has an inconsistent evolutionary rate (Bazin et al. 2006). This intermittent adaptive evolution of mtDNA could lead to homogenisation due to hybrid introgression (leading to low interspecific divergence), or yield balancing selection that leads to high intraspecific divergence (Bazin et al. 2006; Galtier et al. 2009). However, reviews in which the phylogenetic estimates from mtDNA and microsatellite markers were compared in avian studies (Zink & Barrowclough 2008), and mtDNA and nuclear marker discordance in animals (Toews & Brelsford 2012), found only low levels of incongruence between the different methods used. Lukoschek et al. (2008) in their study of olive sea snakes (*Aipysurus laevis*) also found congruent genetic divergence values between microsatellite and mtDNA analyses. More specifically the mitochondrial gene cytochrome oxidase I (COI) has proven valuable in identifying species and also has some other positives, such as universal primers (DeSalle & Birstein 1996, Wood et al. 2007). Cytochrome oxidase I (COI) is therefore widely used in barcoding. For example, Boyer et al. (2011) evaluated the use of nuclear (ND1) and mtDNA (COI) in barcoding and found that both loci gave good results for species identification of unionid mussel larvae. Layton et al. (2014) reported great success in identifying mussel species through barcoding of the COI gene. In the family *Mytilidae*, 79 species (out of around 340 known species) have been barcoded according to the marine barcode of life project (http://www.marinebarcoding.org/progress/region/1/class-order_name/bivalvia-Mytiloida).

Sequence divergence is one of the tools used as a criterion to delimit species boundaries (Avise & Zink 1988; Hebert et al. 2003; Layton et al. 2014), and often clearly delineates species because interspecific divergence is much larger than intraspecific divergence (Moore 1995). Lefébure et al. (2006) also found that molecular divergence and taxonomy are correlated. Johns & Avise (1998) calculated mean genetic distances (p) for published sequences of the mitochondrial cytochrome b (cytb) for vertebrate species. They found that birds generally had lower p than mammals and reptiles, and concluded that the difference between genetic differences between species and within species depends on taxonomic group. Rates of evolution differs between species which in turn, affects sequence divergence values (Arbogast et al. 2002).

Study species

Four mytilid species occur abundantly along the South African West Coast. Two are indigenous: the ribbed mussel *Aulacomya atra* (Molina, 1782) - which until recently was named *A. ater* - and the black mussel *Choromytilus meridionalis* (Krauss, 1848). The other two are alien: the Mediterranean mussel *Mytilus galloprovincialis* (Lamarck 1819) and the bisexual mussel *Semimytilus algosus* (Gould, 1850). Interestingly *A. atra* and *S. algosus* are also both considered indigenous to South America (Carranza et al. 2009a).

Semimytilus algosus is a small mussel that grows to a size of 40 mm, has an elongated dark brown shell and forms dense monospecific beds (Kensley & Penrith 1970, Tokeshi & Romero 1995) (Fig. 2.1). It was first thought to be confined to the west coast of South America (Seed 1976), but was documented in Namibia in 1969 (Kensley & Penrith 1970), although it was first recorded there when Lamy (1931) described it as a new species under the name of *S. pseudocapensis* (now synonymised with *S. algosus*). Since then, it has become established over large portions of the West Coast of South Africa (De Greef et al. 2013), where it covers a 500-km stretch of coast, from Brand se Baai in the north to Bloubergstrand in the south. Recently, it has spread into the warmer waters of False Bay (T. Robinson, pers. comm.). The identity of the mussel in South Africa does, however, need verification, and a central focus of this portion of my thesis was to use molecular methods to determine its identity by comparing COI gene sequences from three populations (Chile, Namibia and South Africa) to assess whether they are conspecific. Differences do exist in shell morphology between the two regions (Fig. 2.2). My analysis is restricted exploring their genetic similarity, but I recognise that for a full resolution of their relationships, genetics and morphology need to be integrated.

Aulacomya atra, the ribbed mussel, is considered native to both South America and South Africa (Branch et al. 2010; Bouchet & Gofas 2011). In South Africa it is found largely on the West Coast (Hockey & Van Erkom Schurink 1992), and in South America it ranges from the Buenos Aires province in Argentina to Peru in the Pacific Ocean (Carranza et al. 2009a).

Aulacomya atra grows to a size of 90 mm and has distinctive ridges that run the length of the shell (Branch et al. 2010) (Fig. 2.3). No molecular studies have been conducted comparing populations of *A. atra* in southern Africa with those from South America, and I also used molecular means to assess whether or not populations from Chile, and South Africa are conspecific. *Aulacomya maoriana* (Iredale, 1915), which occurs in New Zealand, is regarded as a subspecies of *A. atra* by some (Dell 1964), but is granted specific status by others (Beu 2004). Consequently, I derived genetic data for it from Genbank, and added it to my analyses to provide better insight into the evolutionary history of this species with disjunct distributions.

Whilst searching for *S. algosus* at Diaz Point near Lüderitz, Namibia, specimens of a mussel thought to be *S. algosus* were collected, but upon closer inspection did not appear to be any of the known species found at Lüderitz. Accordingly, I included them in my genetic analyses to resolve their identity.

This chapter of my thesis was therefore designed to establish the following: (a) The identity of the suspected alien mussel found in South Africa in 2009 and thought to be *S. algosus*; (b) whether *Aulacomya atra* in South Africa is the same species as that named *A. atra* in South America, and (c) the identity of the unknown mussels found at Lüderitz. This was achieved by molecular studies comparing the mitochondrial gene cytochrome oxidase I (COI) of these mussels, incorporating existing data in Genbank.

2.2 Methods and materials

2.2.1 Sampling:

To determine the identity of southern African populations putatively identified as *Semimytilus algosus*, mussels were collected at Melkbosstrand (S33°43′2015″ E18°26′3347″) on the West Coast of South Africa, at Pelican Point jetty, Langstrand (S22°48′41.4″ E14°32′32.8″) in Namibia, and at ECIM sur (S33°30′0834″ W71°38′0588″) at the research station Estación Costera de Investigaciones Marinas, in central Chile, and fixed in 98% alcohol on collection. I used morphological characteristics to distinguish between *S. algosus* and *Mytilus galloprovincialis* in southern Africa (Fig. 2.2). *Semimytilus algosus* has an elongated shell and is smooth and brownish-pink in colour; whereas *M. galloprovincialis* is blue-black in colour with a broad shell, wider at the base. The internal adductor muscle attachment differs between the two species, with the anterior byssus retractor muscle of *S. algosus* being characteristically divided into two parts (Kensley and Penrith 1970).

To establish whether specimens of *Aulacomya atra* from Chile and South Africa are the same species, mussels were collected at Melkbosstrand (S33°43′2015″ E18°26′3347″) on the West Coast of South Africa and at ECIM sur (S33°30′0834″ W71°38′0588″) in central Chile, and fixed in 98% ethanol on collection. The species are easily distinguishable from other mussels on these shores due to their ribbed shells. Three specimens of the unknown mussel were collected at Lüderitz (S26°38′42.24″ E15°05′45.62″), Namibia. Sampling sites are indicated on Fig. 1.1 and Fig. 1.2.

2.2.2 DNA extraction, PCR and sequencing:

Tissue from the mussel gonad was dissected and DNA was extracted using the Nucleospin extraction kit (Macherey-Nagel), following the manufacturer's protocol. PCR amplification of the 5' end of the mitochondrial DNA cytochrome oxidase I gene (COI) used a species-specific forward primer for *S. algosus* (SemMytF1; GGA ATA TGA AGA GGT TTG GTT GGA G; this study) and the universal reverse primer, HCO2198 (Folmer *et al*. 1994). Universal primers jgLCO1490 and jgHCO2198 (Geller et al. 2013) were used for *A. atra*. The unknown mytilid DNA was amplified using the universal primers LCO1490 and HCO2198 developed by Folmer et al. (1994). PCR amplifications were performed in a 25µl solution containing 2µl DNA, 13.4µl distilled water, 2.5 µl Qiagen PCR buffer, 2.5µl dNTPS, 2 µl Magnesium, 1.25 µl of each primer and 0.1 µl Taq DNA polymerase (Qiagen). The PCR cycle comprised one initial denaturation step at 93°C for 3 minutes, 35 cycles of denaturation at 93°C for 30 seconds, annealing for 30 seconds at 52°C for *S. algosus*, 48°C for *A. atra* and 42°C for the unknown mussel, extension at 72°C for one minute and final extension at 72°C for 5 minutes. PCR products were visually checked on 1% agarose gels stained with ethidium bromide. Clear bands were then gel purified using a Gel Extraction kit (Qiagen), and then sequenced. Sequences were generated using BigDye terminators (Applied Biosystems) and run on an ABI3100 automated sequencer. Sequences for all species were aligned by eye in BioEdit (Hall, 1999) and, using EMBOSS transeq, were translated to ensure no stop-codons were present, to prevent the inclusion of pseudogenes in the analyses. In all cases where data from Genbank were incorporated, I assumed identifications were correct as per the information provided in GenBank. A list of all reference samples obtained from Genbank, with accession numbers and references appears in Table 2.1. Sequences available in the Barcode of Life data base duplicated those in Genbank, so I relied on the latter source.

2.2.3 Genetic and statistical analyses:

Semimytilus algosus identification

Five sequences of 400 base pairs (bp), from each of the three regions were compared. To put my results into context, *Brachidontes rodriguezii* (d'Orbigny, 1842), as well as *Mytilus galloprovincialis* sequences, were used as outgroups for the analyses. *Brachidontes rodriguezii* is known to belong to a sister genus, and *M. galloprovincialis* is a sister clade to *S. algosus* (Santaclara et al. 2006). The uncorrected pairwise distance between these sequences were calculated using MEGA v.6.06 (Tamura et al. 2013). This allowed me to compare the inter- and intraspecies sequence divergences for the different mytilid species to resolve whether the populations from South Africa, Namibia and Chile were conspecific.

Identification of Aulacomya atra

Five (315bp) sequences of *A. atra* from each population (Chile and South Africa), were compared with each other, and the uncorrected pairwise distance between these sequences were calculated using MEGA v.6.06. *Aulacomya maoriana* sequences obtained from Genbank were also included in these analyses as some regard it as a subspecies of *A. atra* (Dell 1966), so its incorporation throws light on its taxonomic status. *Mytilus galloprovincialis* was used as an outgroup to establish a baseline for inter- and intra-specific sequence divergence.

Identification of the unknown mussel

Sequences of six known species of mytilid mussels from Genbank were compared with three (315bp) sequences of the unknown mussels sampled at Lüderitz. MEGA v.6.06 was used to calculate sequence divergence. Genbank sequences of *M. edulis* were used in the comparison, as a BLAST (http://blast.ncbi.nlm.nih.gov) search conducted on the unknown mussel sequences indicated that these unknown mussels might have been *M. edulis*. *Mytilus trossulus* and *M. galloprovincialis* were added to the comparison, as they are part of the *M. edulis* complex. *Mytilus galloprovincialis* is also known to occur widely in Namibia and South Africa and was also included. *Mytilus californianus, A. atra* and *Perna perna* were used as outgroups. *Semimytilus algosus* was added to the analyses to confirm that these unknown mussels were not *S. algosus*. A neighbor-joining tree was calculated, incorporating the abovementioned sequences, using MEGA7. The evolutionary distances were computed using the Tajima-Nei method. Branch support was evaluated using non-parametric bootstrap analysis based on a 1000 bootstrap replicates (Felsenstein 1985). Maximum-parsimony was also calculated, to test the results of the neighbor-joining tree, using MEGA7 utilising a Subtree-Pruning-Regrafting (SPR) algorithm with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates).

The analysis involved 49 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 293 positions in the final dataset. Branch support was evaluated using non-parametric bootstrap analysis based on a 1000 bootstrap replicates.

2.3 Results

Semimytilus algosus identification

Comparison of the COI gene sequences from the three populations of *S. algosus* showed that there was an average dissimilarity of 0.5% between South Africa and Chile, 0.6% between South Africa and Namibia and 0.7% between Chile and Namibia. Divergences between *S.*

algosus and the Genbank sequences for *M. galloprovincialis* and *B. rodriguezii* were much higher, with values ranging from 24.5% to 41.1% (Table 2.2).

Aulacomya atra identification

The average dissimilarity between COI gene sequences for specimens from Chile and South Africa was 2.9% (Table 2.3). Sequence divergence within populations were 0.4% for the South African population and 0.7% for the Chilean population (Table 2.4). Divergences of *A. atra* from the Genbank sequences for *A. maoriana* and *M. galloprovincialis* were larger, spanning 17.9-18.7% and 49.5-49.9% respectively, and *A. maoriana* exhibited an even greater distance from *M. galloprovincialis* (Table 2.3).

Identification of the unknown mussel

Some general patterns emerged from the neighbor-joining tree with the unknown mussel (Lüderitz) falling within the main clade (Fig. 2.4). (a) The *M. edulis* complex formed the main clade and included the three Lüderitz sequences as well as the *M. edulis* and *M. galloprovincialis* sequences, with 94% bootstrap support (BP). (b) The *M. trossulus* sequences formed a separate clade but was closely related to the main cluster (73% BP). (c) The *S. algosus* sequences fell in a separate clade supported by 100% BP. (d) *Perna perna* fell within a separate clade with 98% bootstrap support, as did *Aulacomya atra* (100%BP) and *M. californianus* (100%BP). The maximum parsimony tree mirrored the main findings of the neighbor-joining tree (Bootstrap support shown in Fig. 2.4 underneath branches).

2.4 Discussion

Mussels are known to show great phenotypic plasticity, influenced by the environment which they occupy (Seed 1968; Seed 1973), which is why identification of mussel species cannot be based on morphological characteristics alone (Toro 1998). The use of DNA sequences are known to be a powerful tool in establishing phylogenetic relationships and can thus be used as an additional tool to confirm the identity of an unknown species. Mitochondrial genes are often used for this purpose, as they are variable enough to allow for the differentiation between species (Terol et al. 2002). The mitochondrial gene COI typically gives a high interspecific divergence and low intraspecific divergence and is used in many studies to delineate species and explore species relationships (Sarver & Silberman 1998; Cognato 2007). Lefèbure et al. (2006) showed that the COI gene is suitable to identify different species, but they also recommended that at least two genes, one nuclear and one mitochondrial be used for species delimitation. DNA sequences are then analysed using distance methods to delineate species (Chapela et al. 2002; Terol et al. 2002; Cognato 2006). Quintero et al. (1998) concluded that genetic distance is useful in evaluating the similarity of an unknown DNA sequence to that of a known species. The thinking behind this is that the interspecific divergence will be an order of magnitude higher than the intraspecific divergence (Hung et al. 1999). For example, Hebert et al. (2004) suggested that a measure of ten times the within-species divergence be used as criterion to delimit species. However, Zou et al. (2011) found an overlap in inter-and intraspecific divergence in the COI gene, and attribute this to the fact that the group they considered, the Neogastropoda, includes many recently diverged species. They therefore cautioned against using a fixed distance-based threshold as suggested by Hebert et al. (2004). Initial studies using the distance method focused on vertebrate species and found that divergences of more than 2% indicated different species (Hebert et al. 2004). However, it is not possible to use a standardized percent molecular variation to predict species boundaries as subsequent studies showed that this approach correctly predicts only 45% of species boundaries (Cognato 2007). For example, bivalve species have been found to exhibit deep intraspecific divergence of up to 26.4% (Layton 2014). DeSalle et al. (2005) recommended that the cut-off percent divergence to delineate a species be revised on a group-to-group basis. Together with inter- and intraspecific divergence, phylogenetic trees are also used to illustrate species limits (Bonnaud et al. 1994; Terol et al. 2002; Zou et al. 2011). Bootstrap support is commonly used to test the significance of clusters in a phylogenetic tree (Felsenstein 1985). Bootstrap support greater than 70% is equal to a probability of 95% that the corresponding cluster is correct and represents a separate species (Felsenstein 1985; Hillis & Bull 1993).

Semimytilus algosus identification

The average interspecific dissimilarities were consistently high between specimens of *Semimytilus algosus* and *Mytilus galloprovincialis* as well as with *Brachidontes rodriguezii*, ranging between 24.5% and 41.1% (Table 2.2). By contrast, the divergence between southern African, Namibian and Chilean populations was less than 1%. This is a clear indication that the sequences from South Africa, Namibia and Chile represent the same species, namely *S. algosus*.

The COI gene is not always appropriate in studies of this kind as it evolves quickly and its substitution rate may vary both within and between species. This may result in an overlap between inter-and intraspecific divergence (Zou et al. 2011). However, in my study the sequence divergence results reported enough variability to reliably distinguish between different species. Pairwise distances of COI gene sequences clearly distinguished between *S. algosus* and its sister species (*B. rodriguezii*) as well as between it and the separate species *M. galloprovincialis*. This lends credibility to the intraspecific divergence which was an order of magnitude less than the interspecific divergence. These results also concurred with a study by Santaclara et al. (2006) with regards to the interspecies boundaries. The sequences I analysed were quite short (400bp). Nonetheless, there was enough variation in the sequences to distinguish between species, as discussed above. Several other studies were successful in identifying species with comparably short sequences. For example, Terol et al. (2002) successfully distinguished between species of tuna using sequences that were 271bp, and Hung et al. (1999) found evidence of a cryptic species within the nematode *Cylicostephanus minutus*, with sequences of 372bp and 216bp.

The focus of this study is the identification of the mussel putatively identified as *S. algosus*, and not a comprehensive phylogeny of the species. *Semimytilus algosus* has not been the subject of many molecular studies, making further comparisons difficult. Santaclara et al. (2006) constructed a neighbour-joining tree of nuclear genes, which included the genera *Perna*, *Mytilus*, *Semimytilus*, *Brachidontes*, *Aulacomya*, *Choromytilus*, and *Perumytilus*. They found that *Semimytilus algosus* was monophyletic (99% BP), and did not fall within the *Mytilus* clade. This pattern was reiterated in the Santaclara et al. (2007) study of the phylogeny of the mussel *Xenostrobus securis*. The neighbor-joining tree did correspond with their findings, showing a separate cluster for *S. algosus*.

Aulacomya atra identification

The low sequence divergence between the mussels called *Aulacomya atra* in both Chile and South Africa points towards their being the same species, especially in view of the high interspecific divergence of *A. atra* from known sequences of *Mytilus galloprovincialis*, as well as from *Aulacomya maoriana*, which has been regarded by Dell (1964) as a subspecies of *Aulacomya atra*. However, the within-population divergences for the South African (0.4%)

and Chilean (0.7%) populations of *A. atra* are low when compared to the between-population divergence, which borders on the levels of divergence suggested by some authors as distinguishing species (Hung et al. 1999, Hebert et al. 2004).

No other sequences could be found on Genbank for *A. atra* or any of its synonyms (*Mytilus decussatus*, *M. diluculum*, *M. crenatus*, *M. americanus*, *M. ater*, *Aulacomya ater*, *A. ater ater*, *A. magellanica*, *M. magellanicus curvatus*, *M. pyriformis*, *M. oblongus*, *M. orbignyanus* and *M. magellanicus*). There seems to be a lot of confusion surrounding this species. A thorough investigation of the taxonomy of *A. atra*, which combines both molecular and morphological studies and larger sample sizes, is therefore needed.

On the basis of my evidence, two points emerge. First, the genetic difference of 2.9% between Chilean and South African material is quite high when compared to the within-population divergence. However, as pointed out above, this needs to be viewed in the context of the relative levels of intra- and interspecific variability. Moreover, my analysis was based on COI alone and the sequences contained only 315bp. Thus, further analyses are needed to test the validity of the 2.9% difference I recorded. Second, based on Genbank sequences for *A. maoriana*, there are substantial differences between it and *A. atra* populations in both Chile and South Africa (17.9 and 18.7% respectively). This supports views that the two are distinct species. Rather than according subspecific status, the name *A. maoriana* should be upheld at the level of species, supporting the morphological differences described by Beu (2004).

Identification of the unknown mussel

From my results, it is not possible to say with certainty that the Lüderitz samples are either *M. edulis* or *M. galloprovincialis*. Neither the neighbor-joining tree (Fig. 2.4), nor the maximum parsimony analysis shed any light on the phylogeny of the Lüderitz samples, as it shows one main cluster containing the Lüderitz samples and both *M. edulis* and *M. galloprovincialis* (94% and 99% bootstrap support). The nesting of *M. edulis* and *M. galloprovincialis* in one cluster in my data is well known in literature. Hilbish et al. (2000) reported a maximum parsimony tree for *Mytilus* spp 16S rRNA, which reflects this pattern. *Mytilus edulis* and *M. galloprovincialis* also grouped closely with the *Mytilus trossulus* sequences for material from North America. A *Mytilus californianus* F-type sequence was used to root the tree. Biparental inheritance of mitochondrial DNA is common in some mytilid species: females inherit mtDNA only from their mother, but they transmit it to both male and female offspring, called F-type mtDNA. Males receive mtDNA from both parents, and transfer the mtDNA they inherited from their father, to sons only (M-type mtDNA) (Zouros et al. 1994; Saavedra et al. 1997).

Mytilus edulis and *M. galloprovincialis* sequences also clustered together in a Bayesian consensus tree of the COI gene reported by Wood et al. (2007). It is, however, clear from both the cladogram and the sequence divergence in my study that the unknown Lüderitz mussels are not *Semimytilus algosus*.

A possible explanation for the ambiguity surrounding the identity of the Lüderitz samples may be found in two studies on *M. edulis* and *M. galloprovincialis* phylogeny. Hilbish et al. (2000) reported that the mtDNA of the Australasian *Mytilus* spp were closely related to North-Atlantic *M. galloprovincialis*, although a few specimens contained haplotypes found in both M. *galloprovincialis* and *M. edulis*. Daguin & Borsa (2000) found a similar pattern in the nuclear-DNA markers and came to the conclusion that this was due to the fact that some Australasian *Mytilus* spp derived from a proto-*Mytilus galloprovincialis* introgressed by *M. edulis*-like genes. *Mytilus galloprovincialis* and *M. edulis* are known to hybridise in areas where they co-occur (Daguin and Borsa 2000, Wood et al. 2003). Further analyses using additional genes might help to resolve the status of the unidentified mussels from Lüderitz.

The *Mytilus edulis* complex has a worldwide distribution (Wood et al. 2003) and was at first thought to be one species, but later found to be a complex of morphologically similar but genetically distinct species. The complex consists of *M. edulis*, *M. galloprovincialis* and *M. trossulus* (Sarver & Foltz 1993). *Mytilus galloprovincialis* is a well-known invader in many parts of the world (McDonald & Koehn 1988; Gosling 1992; Robinson et al. 2007b; Mead et al. 2011b), and has occupied much of Namibia and the southwestern coast of South Africa since the 1970s (Branch & Steffani 2004). *Mytilus edulis* though, is known to have invaded the Ukraine only (Zaitsev et al. 2004), and is unknown in Namibia. The parsimonious conclusion is that the unidentified mussels from Lüderitz are *M. galloprovincialis.*

The question arises of how *S. algosus* was introduced to South Africa. It could have been due to introductions via either the ballast water of ships or amongst oyster spat imported from Chile or it could be a natural range expansion southwards from Namibia. These possible avenues will be explored in Chapter 6. *Semimytilus algosus* is not known to have successfully invaded any other coasts globally. However, if account is taken of its rate of spread along the West Coast of South Africa, and the fact that it dominates the low intertidal on rocky shores on this coast (De Greef et al 2013), it unquestionably has the capacity to be a successful invader. Chapter 3 describes its present abundance and zonation patterns in South Africa, and Chapters 4 and 5 discuss possible reasons for its success there.

Chapter 3

Field surveys of *Semimytilus algosus* in South Africa: abundance, zonation and tolerance to wave exposure

3.1 Introduction

Several mussel species have spread from their native regions to become invasive elsewhere, in part because their pelagic larval stages are readily dispersed through various means, including wind-driven water movements, shipping and mariculture (Seed 1969; Seed 1976; Paine & Levin 1981; McQuaid & Phillips 2000; Steffani & Branch 2003; Reaugh-Flower et al. 2010; Haupt et al. 2012; Mead et al. 2013; Peters et al. 2014). Although adult mussels are also known to spread via shipping and aquaculture (Mead et al. 2013). For example, *Mytilus galloprovincialis* is cited as one of the 100 worst invasive species by the World Conservation Union (IUCN) (Lowe et al. 2000).

Invasive species have far-reaching effects on rocky shores worldwide (Braby & Somero 2005), and change both species composition and zonation patterns (Ruiz et al. 1997; Ruiz et al. 1999). Invasive mussels in particular often dominate rocky shores and affect zonation and physical conditions and may therefore have extensive effects on community composition. (Suchanek 1992; Tokeshi & Romero 1995). As foundational species they provide refuge and habitat for many other organisms and thus increase biodiversity (Suchanek 1992; Bertness et al. 2006). Sadchatheeswaran et al. (2015) recorded how the arrival of the Mediterranean mussel *M. galloprovincialis* on the West Coast of South Africa has increased habitat complexity and biodiversity, and that in zones where it has established dominance, it alters and homogenises community composition.

Zonation patterns on intertidal rocky shores

Zonation on intertidal rocky shores refers to the pattern of distribution and abundance of organisms observed between the low and the high shore (Menge & Branch 2001). Many studies have examined these patterns (Lewis 1964, Stephenson and Stephenson 1972, Underwood 1978), as well as their ecological determinants, which include both biotic and abiotic factors (Bustamante et al. 1997). Several papers have reported that the lower limits of species' distributions are often set by predators (Paine 1971; Paine 1974; Lubchenco & Menge 1978), and the upper limits by physical stress such as desiccation and high temperatures (Connell 1972; Suchanek 1978).

Competition can also play a key role in structuring intertidal communities and setting zonation limits (Connell 1961). Suchanek (1978) found that the mussel *Mytilus edulis* extends higher on the shore in areas where it co-exists with *M. californianus*. Similarly, *M. galloprovincialis* occurs higher on the shore than *Perna perna* for reasons related to their relative tolerances of wave action and desiccation, coupled with both competitive and facilitatory interactions (Zardi et al. 2006). In central Peru, Tokeshi and Romero (1995) showed that *Semimytilus algosus* outcompetes *Perumytilus purpuratus* and dominates the low to mid intertidal zone. In contrast, in central Chile its interaction with *P. purpuratus* is reversed, with *P. purpuratus* being the dominant competitor (Alvarado & Castilla 1996).

On the West Coast of South Africa *M. galloprovincialis* has until recently dominated the low to mid intertidal zones (Branch & Steffani 2004; Robinson et al. 2005). However, De Greef et al. (2013) have reported that since its recent arrival, *S. algosus* has become the dominant low-shore mussel, whereas *M. galloprovincialis* tends to prevail in the mid-high shore across its geographic range in southern Africa.

Wave exposure

Wave exposure also plays an important part in structuring intertidal populations, and specifically mussel populations (Jones & Demetropoulos 1968; Harger 1970). At wave-exposed sites there exists a trade-off between the danger of dislodgement and higher food supply. For example, wave action moderates the effects of the invasive mussel *M. galloprovincialis* on the West Coast of South Africa, due to lower growth and recruitment at sites with either very low or very high wave exposure (Steffani and Branch 2003). On the South Coast, wave action also mitigates competition between *M. galloprovincialis* and *P. perna*, because *M. galloprovincialis* has a higher mortality at wave exposed sites. This is due to the fact that *M. galloprovincialis* is more active and less strongly attached than *P. perna* (Nicastro et al. 2008).

West Coast rocky shore invasions

On the West Coast of South Africa three major invasions have altered community composition on intertidal rocky shores. The barnacle *Balanus glandula* now dominates the high shore and the Mediterranean mussel *Mytilus galloprovincialis* the mid-intertidal zone. *Mytilus galloprovincialis* has outcompeted many other organisms in the low to mid intertidal zones since its arrival in the 1970s (Branch & Steffani 2004; Robinson et al. 2007a; Branch et al. 2010). It has had far-reaching effects on the structure of South African rocky shores, including partial displacement of indigenous mussels (Hockey & Van Erkom Schurink 1992), an upwards shift of mussel beds on the shore, and an increase in overall species richness (Ruiz et al. 2002; Griffiths et al. 2005; Robinson et al. 2007a; Mead et al. 2013). However, the latest invader on South African shores, the bisexual mussel *Semimytilus algosus*, now appears to be displacing *M. galloprovincialis* on the low intertidal zone.

Semimytilus algosus

The bisexual mussel is indigenous to the Pacific coast of South America, and its distribution ranges from the coasts of Ecuador to Chiloé island in southern Chile (around 42° S) (Caro & Castilla 2004; Carranza et al. 2009a; Carranza et al. 2009b and Fig. 1.1). The species was first described in Namibia by Lamy (1931), as *Modiola pseudocapensis*, but was later identified as *S. algosus* (Kensley and Penrith 1970). Prior to my research, its distribution in Namibia was known to extend from the Kunene River to at least Walvis Bay (Kensley and Penrith 1970, Currie unpublished)

In South Africa De Greef et al. (2013) established that *S. algosus* arrived abruptly about 2 years before their 2010 survey, by which time it occupied a 500-km range along the West Coast, stretching from Brand se Baai in the north to Bloubergstrand in the south. It was absent from South Africa when Bustamante et al. (1997) and Wieters (2006) conducted extensive surveys on the West Coast during 1993-1995 and 2001-2003 respectively. It is not known to have invaded any other areas elsewhere in the world, but viable larvae were found in ballast water of ships on the Argentinian coast (Bigatti et al. 2014).

My study was designed to assess: (a) the current geographic distribution of *Semimytilus algosus* on the West Coast of South Africa, (b) its zonation patterns on rocky shores, and (c) whether it preferentially occupies sheltered or exposed areas.

Surveys were thus undertaken at a range of wave-exposed sites on the West Coast of South Africa, and covering both sheltered and exposed conditions at one site, spanning the full range of tidal heights between low spring and high spring tide. Because many of these sites included those surveyed in 2010 by De Greef et al (2013), I could also explore the extent to which the geographic distribution, zonation and responses to wave action have remained stable.

On the basis of previous research cited above on both *S. algosus* and *M. galloprovincialis*, I hypothesised that (a) the range of distribution of *S. algosus* would have expanded, (b) *S. algosus* would predominantly occupy the low shore whereas *M. galloprovincialis* would prevail in the mid-shore, and (c) *S. algosus* would be more abundant on wave-exposed than sheltered shores.

This aspect of my thesis was purely observational and correlative, but lays a foundation for probing more specific hypotheses about life-history characteristics of *S. algosus* and other mussels (Chapter 4), the causes of zonation of *S. algosus* and its possible interactions with *M. galloprovincialis* (Chapter 5), and the genetic diversity, origins and possible mode of distribution to South Africa (Chapter 6).

3.2 Methods and materials

Fieldwork

Field surveys of abundance and zonation of *S. algosus* and *M. galloprovincialis* were undertaken along the West Coast of South Africa, at roughly 100-km intervals, at Bloubergstrand, Yzerfontein, Elands Bay and Brand se Baai (Fig. 3.1). These sites were selected to allow comparison with the 2010 surveys done there by De Greef et al. (2013). In addition, searches were made to assess the occurrence (absent; scarce, with isolated individuals; or abundant, with established beds) of *S. algosus* at Hout Bay, Groenriviermond, Hondeklipbaai, Oranjemund, and Mining Licence Area 1 to the north of Oranjemund (Table 3.1, Fig. 3.1). I also drew on unpublished information provided by TB Robinson (Stellenbosch University) about the presence of *S. algosus* in False Bay.

Surveys were done at springtide during 2011 and 2012, using a 100x50cm quadrat and estimating the percentage cover of *M. galloprovincialis* and *S. algosus* in each quadrat. This was performed at vertical distances of 50cm intervals, from the low shore to the high shore, covering the full spring-tidal range of 185cm. To allow comparisons with the biomass data recorded by De Greef et al (2013), percentage cover was converted to total wet biomass using measured mean weights in 400cm^2 subsamples from areas with 100% cover. (On average, *S. algosus* 100% cover=660g; *Mytilus galloprovincialis* 100% cover=2721g). Distance along the shore was converted to shore height.

To compare the abundance of *S. algosus* at exposed and sheltered sites, surveys were undertaken at three of the sites surveyed in 2010 by De Greef et al (2013) at Elands Bay: an exposed site (EB 8), and two sheltered sites (EB 6 and EB 7). Relative wave exposure was estimated subjectively from wave height.

Data analyses

Data were analysed using Statistica v.12 (Statsoft) and were tested for normality through normality plots and homoscedacity by Levene's tests. Two separate sets of two-way ANOVA (analysis of variance) were conducted for the percentage cover data. First, sites and species were compared. Second, individual two-way ANOVAs were performed for each site, to determine: 1) whether there were significant differences between *S. algosus* and *M. galloprovincialis* abundance at different sites; 2) whether there were significant differences between *S. algosus* and *M. galloprovincialis* abundance at different shore heights. Where appropriate, ANOVAs were followed by Tukey's post-hoc tests. It was not possible to run three-way ANOVAs with sites, species and shore heights combined, as differences existed in the number of shore heights examined at each site, and in any case significant differences among sites made it more meaningful to compare zonation within sites.

3.3 Results

Geographic distribution

Semimytilus algosus ranged from Groenriviermond to Hout Bay in South Africa, but was scarce at Groenriviermond and absent at Hondeklipbaai and Oranjemund, re-appearing again at Mining License Area 1 in southern Namibia, where it was also scarce (Fig. 3.1). Together, *Mytilus galloprovincialis* and *S. algosus* covered an average of 63% of the intertidal zone from Brand se Baai to Bloubergstrand, with *S. algosus* contributing 32.4% and *M. galloprovincialis* 29.9% (Fig. 3.2A). Values for percentage cover were significantly different among sites, but not between species. There was a significant interaction between site and species because the two species differed in their relative abundance at different sites (Table 3.2, Fig. 3.2A). Separate analyses of each site showed species differed in percentage cover in all cases except Yzerfontein, with *M. galloprovincialis* being more abundant than *S. algosus* at Bloubergstrand, and the reverse at Elands Bay and Brand se Baai (Table 3.3A).

Intertidal zonation

At Bloubergstrand the two species shared similar zones, so there was no significant interaction between species and shore height (Fig. 3.2A, Table 3.3A). At Yzerfontein, Elands Bay and Brand se Baai, there were significant interaction effects (Table 3.3A) and Tukey's post-hoc tests revealed that this was because in all cases *S. algosus* dominated the low shore whereas *M. galloprovincialis* was prevalent in the mid to high shore (Fig. 3.3).

Analyses of biomass (Table 3.3B) revealed similar outcomes, except for the fact that at Yzerfontein, the species differed in overall biomass, whereas at Elands Bay they did not, thus reversing the trend that emerged from percentage cover data. Both reversals simply reflect the fact that the biomass of *S. algosus* was on average substantially less than that of *M. galloprovincialis*. Tukey's post-hoc tests confirmed that the central conclusion derived from percentage cover – that *S. algosus* was most abundant low on the shore, and *M. galloprovincialis* in the mid shore – remained unchanged for the biomass data.

In summary, *S. algosus* dominated the low shore at Yzerfontein, Elands Bay and Brand se Baai, whereas *M. galloprovincialis* dominated the mid-high shore at these sites (Fig. 3.2). However, Bloubergstrand possessed mixed mussel beds with a peak in both biomass and percentage cover on the mid-high shore (Fig. 3.2).

A comparison between my results and those of De Greef et al. (2013) at all four exposed sites showed similar patterns in biomass and zonation, at all sites (Fig. 3.2B, C).

Wave exposure

While *S. algosus* was abundant at the exposed site at Elands Bay (EB8), none were found at the sheltered sites during my survey in 2012. Comparison with the survey of De Greef et al. (2013) two years earlier (Fig. 3.2) revealed several patterns. First, at the time of that survey both *M. galloprovincialis* and *S. algosus* were present at both exposed and sheltered sites, although biomass of both species was greater at the exposed site than the sheltered site. By 2012, however, *S. algosus* had disappeared from the sheltered sites although *M. galloprovincialis* maintained a presence, again at a lower biomass than on the exposed shore. Second, in 2010 both species occurred lower on the shore at the sheltered sites than the exposed site. Third, the two species were intermixed in all zones when they co-occurred at the sheltered sites, but were differentially distributed at the exposed sites, with *S. algosus* occupying the low shore and *M. galloprovincialis* the mid shore (Fig. 3.2C). It was not possible to compare the data between the two periods statistically because of the absence of *S. algosus* from sheltered sites during my survey, but the differences were stark.

3.4 Discussion

Several factors have been cited as contributing to invasive success such as the physiological performance and life cycle traits of invasive species (Van Erkom Schurink & Griffiths 1993; Rensel et al. 2005), the nature of the recipient community and environmental conditions (Branch and Steffani 2004), and the genetic diversity of the invader (Stepien et al. 2005; Roman 2006; Rius et al. 2008).

The South African West Coast is a highly productive system due to upwelling, and supports a high filter-feeder biomass, especially on wave-beaten shores (Bustamante & Branch 1996a). The existence of a prevalently northwards current and southwards counter currents (Shannon 1985) provides means of longshore larval dispersal in both directions. Conditions on the West Coast are therefore likely to facilitate the survival, spread and proliferation of any arriving alien mussels, as is testified by the history of the *Mytilus galloprovincialis* invasion (Branch & Steffani 2004).

Geographical distribution and spread

De Greef et al. (2013) recorded that by 2010, soon after its arrival in South Africa, *Semimytilus algosus* occupied a range of 500km on the West Coast of South Africa, from Brand se Baai to Bloubergstrand. My findings show that by 2012 its range had expanded northwards to Groenriviermond, although it was still scarce there, and southwards to Hout Bay. T Robinson (pers. comm.) has since found that it has spread even further south into False Bay. The question arises whether this species will spread even further south, around the biogeographic barrier at Cape Agulhas and along the South and East Coasts. It was well established in Namibia by 1968 (Kensley & Penrith 1970; Reaugh-Flower et al. 2011), but my record of it at Mining License Area 1 constitutes a southwards range expansion in

Namibia. It is absent from Angola (GM Branch, unpublished data), suggesting that the subtropical waters of the southward-flowing Angola Current may stall or halt further northwards expansion.

Semimytilus algosus is not known to have invaded any other areas globally, but the earlier *M. galloprovincialis* invasion of South Africa and its spread to cover 2050km of the coastline may provide indications of the likely behavior of mussel invaders on this coastline. The southern African region is divided into seven major biogeographic provinces: (a) the subtropical Angola Province; (b) the cool-temperate Namib Province extending southwards to Lüderitz; (c) the cool-temperate Namaqua province stretching from Lüderitz to Cape Point; (d) the warm-temperate Agulhas Province, from Cape Point to the central Wild Coast; (e) the area between Cape Point and Cape Agulhas is considered a transition zone called the South-western Cape bioregion; (f) the subtropical East Coast Province that spans the coast from there to northern Kwazulu Natal, and a transitional region from there to (g) the tropical Indo-West Pacific (Bustamante & Branch 1996a; Sink et al. 2005; Griffiths et al. 2010; Porter et al. 2013). Lüderitz, Cape Point, Cape Agulhas and central Wild Coast form transition points between provinces, and are known to constitute barriers that slow the expansion of species ranges (Bustamante and Branch 1996). These barriers are, however, not impermeable. *Mytilus galloprovincialis* has spread through natural range expansion, from Saldanha Bay on the West Coast, past the massive upwelling cell at Lüderitz (Shannon 1985) and both the Cape Point and Cape Agulhas barriers along the South Coast of South Africa. Its eastwards range halts abruptly 20km south of East London, probably due to physiological intolerance of the warmer waters found further north (Robinson et al. 2005; Assis et al. 2015), although it could also be the result of net southward current flow, as demonstrated for *Perna perna* (Zardi et al. 2011). The same is likely true at its northern limits in Namibia, where it encounters the subtropical Angola Current. Physiological thermo-tolerance is known to affect mussel distribution patterns, as temperature influences their growth, reproduction and other physiological processes (Laudien et al. 2001; Heilmayer et al. 2004).

It seems probable that *S. algosus* will follow a similar pattern of expansion to *M. galloprovincialis*. Firstly, the new invader rapidly occupied a large portion of the West Coast of South Africa. Secondly, it has already circumnavigated the barriers at Lüderitz and Cape Point. There remains a gap in its distribution in northern South Africa, but my records of the respective northwards and southwards expansion of its range to Groenriviermond and Mining License Area 1 suggest this gap is closing.

Studies on the physiological thermo-tolerance of this species will, however, be necessary to make more informed predictions as to the likely extent of its range expansion along our coastline. In its native range, *S. algosus* occurs in temperate waters and decreases in its abundance have been reported during warm-water periods caused by El Niño-Southern Oscillation (ENSO) years, indicative of intolerance to high sea surface temperatures (Tokeshi & Romero 1995; Carstensen et al. 2010). Like *M. galloprovincialis*, its failure to penetrate from the cool temperate conditions of Namibia into the warmer waters of subtropical Angola may be a further manifestation of this.

Zonation patterns

On intertidal rocky shores, competition for space plays a major role in structuring communities (Connell 1961; Menge & Branch 2001). In its native range, *Semimytilus algosus* is found on the low-mid intertidal, where it competes for space with *Perumytilus purpuratus*. In Peru there is a distinct zonation between the two species, with *S. algosus* dominating the low intertidal and *P. purpuratus* found in smaller beds higher on the shore

(Tokeshi et al. 1989). However, the abundance of *S. algosus* is variable along its native geographic range, and on the West Coast of South America, it is more abundant in the northern portions of the coast than in Chile in the south, where it is outcompeted by *P. purpuratus* (Tokeshi & Romero 1995; Caro et al. unpublished data). These different competitive outcomes can be explained by abiotic factors, such as currents that facilitate dispersal and settlement of mussel larvae (Teske et al. 2015). Navarrete et al. (2008) concluded that the outcome of competition between these two species depended on the efficacy of interference by adult mussels, as well as the rate of recruitment of new individuals. At sites where oceanographic processes favour the dispersal and recruitment of *S. algosus*, it can co-exist with *P. purpuratus* even though the latter is competitively superior. On the South Coast of South Africa, Bownes & McQuaid (2006; 2010) found a similar pattern of co-existence between *Perna perna* on the low shore and *M. galloprovincialis* on the mid shore. They attributed this to two factors (a) *P. perna* recruitment and survival are limited on the high shore and (b) *M. galloprovincialis* is excluded from the low shore because of a high mortality rate, associated with its lower attachment strength and intolerance of the greater wave action experienced there.

On the West Coast of South Africa, *M. galloprovincialis* initially dominated the low-mid intertidal zone after its arrival (Robinson et al. 2005), but appears now to have been partially displaced by *S. algosus* on the low-intertidal zone. I found that *S. algosus* dominated the low shore at most sites I surveyed, with *M. galloprovincialis* prevailing in the mid-high intertidal zone except at Bloubergstrand, where *S. algosus* was found within predominantly *M. galloprovincialis* beds, with a peak in abundance of both species on the mid shore. Comparisons of my biomass data with those of De Greef et al. (2013) showed that patterns of zonation had not changed between the time they undertook their surveys, and my repetition of surveys at the same sites.

There are several reasons why *S. algosus* may be able to outcompete *M. galloprovincialis* on the low shore. One may be found in the enemy release hypothesis (ERH), which states that invasive species are successful because they have few or no natural enemies there, though this concept has received mixed support in the literature (Colautti et al. 2004). On the West Coast of South America, *S. algosus* is heavily preyed upon by the starfish *Heliaster helianthus*, the gastropods *Concholepas concholepas* and *Nucella crassilabrum*, and the crabs *Acanthocyclus gayi* and *A. hassleri* (Castilla 1981). In South Africa De Greef et al. (2013) reported that *S. algosus* is preyed upon by the whelk *Trochia cingulata* and that it is often found in regurgitates of the kelp gull *Larus dominicanus*. However, Branch and Steffani (2004) found that the high recruitment rate of *M. galloprovincialis*, and the comparatively low density of *T. cingulata* on the West Coast, meant that this predator is unable to control the *M. galloprovincialis* invasion.

In Chapter 4 I explore various life history characteristics of *S. algosus* relative to other mussels in the region. Amongst other things, I found that *S. algosus* has an even higher recruitment rate than *M. galloprovincialis*, and thus predation will be unlikely to control its numbers in South Africa. Parasites also have an effect on competitive abilities. Calvo-Ugarteburu & McQuaid (1998a, 1998b) came to the conclusion that the absence of trematode parasites in *M. galloprovincialis* may be one of the reasons why it is competitively superior to *Perna perna* in South Africa, but no data exist to assess the parasite load of *S. algosus*.

Secondly, competitive ability will influence the success of invaders. Both interference and overgrowth are among the strategies used by competitors to dominate space. In Chile *P. purpuratus* has been observed crushing individuals of *S. algosus* (Caro et al. unpublished).

Conversely, Tokeshi and Romero (1995) reported that increased siltation in *S. algosus* beds interferes with filtration by the sessile polychaete *Phragmatopoma moerchi*, allowing it to outcompete the polychaete. *Mytilus galloprovincialis* is also known to interfere with competitors by overgrowing them or binding them with its byssus threads, restricting movement and filter-feeding activity (Shinen & Morgan 2009).

Studies also cite life-history strategies such as high growth, reproduction and recruitment rates, as reasons for competitive success of alien species (Barkai and Branch 1989, Bownes and McQuaid 2009), and this is a topic I expand upon in Chapter 4.

Community effects of *Semimytilus algosus*

The dominance of *S. algosus* on the low intertidal zone may have either negative or positive consequences for other elements of the community. On the negative side, *S. algosus* has smaller interstitial spaces than *M. galloprovincialis* and thus is likely to support smaller infaunal assemblages (De Greef et al. 2013). However, Tokeshi et al. (1989) reported that beds of *S. algosus* support a higher biomass of the polychaetes *Pseudonereis gallapagensis* and *Halosydna johnsoni* than *Perumytilus purpuratus* beds, due to greater desiccation and lower food supply in the mid intertidal zone where *P. purpuratus* occurs, as well as larger interstitial spaces in *S. algosus* beds. They recorded that *S. algosus* is loosely attached to rock in a relatively thick (7-10mm) layer, whereas *P. purpuratus* is more tightly attached in thinner (4mm) layers. *Semimytilus algosus* is also small in size relative to other mussels on the West Coast of South Africa, and consequently rarely supports limpets on its shells. By contrast, *M. galloprovincialis* elevates the densities of recruits of the limpet *Scutellastra granularis*, boosting the densities of this limpet while reducing its maximum size and per capita reproductive output (Hockey & Van Erkom Schurink 1992; Branch & Steffani 2004;

Robinson et al. 2007a). These factors may lead to a decrease in biodiversity on the shore in zones dominated by *S. algosus*.

On the positive side, *S. algosus* falls within the optimal size class (5-60mm) for predation by a number of species. Griffiths & Hockey (1987) found that most predators prefer mussel prey within this size class. Thus *S. algosus* may have a positive effect on the abundance of predators such as the kelp gull *Larus dominicanus* and the black oystercatcher *Haematopus moquini*. Previously, invasion of the coast by *M. galloprovincialis* increased food supply and thus improved the reproductive success of *H. moquini* on the West Coast (Hockey & Van Erkom Schurink 1992), and also increased the abundance of the whelk *Trochia cingulata* (Branch and Steffani 2004). It is possible that *S. algosus* will have a similar positive effect on predators that benefit from its abundance and – in view of the thinness and fragility of its shell (see Chapter 4) – the ease with which it can be consumed. Long-term studies will be necessary to determine what the effects this species will have on biodiversity on South African rocky shores and on the abundance of individual species.

Wave exposure

De Greef et al. (2013) recorded *S. algosus* at sheltered sites they surveyed at Elands Bay, although its abundance there was about half that at nearby wave-exposed shores. However, when I resurveyed those sites, I did not find any *S. algosus*. It would seem that it can recruit into sheltered areas but cannot survive there.

At wave-exposed sites there is a trade-off between higher food supply for filter feeders, and the costs of attachment and lower survival due to high wave action (Paine & Levin 1981; Steffani & Branch 2003; Pollard & Hodgson 2016). For this reason *M. galloprovincialis* is most abundant at moderately exposed sites in southern Africa, where there is sufficient food in suspension but its condition, growth rate and survival are not compromised (Steffani & Branch 2003; Nicastro et al. 2008). It is scarce in sheltered bays, and Bustamante and Branch (1996b) have shown by field observations and modelling that supplies of particulate food are inadequate to sustain dense populations there. The success of *M. galloprovincialis* on the West Coast of South Africa and its effects on other species is therefore strongly moderated by wave action.

On the South Coast of South Africa, *M. galloprovincialis* interacts with the warm-water brown mussel *Perna perna.* While *P. perna* tends to occupy the low-shore, *M. galloprovincialis* is most abundant in the mid-shore. Reasons for this include greater attachment strength by *P. perna*, allowing it to occupy the lowest part of the shore where wave action is strongest, whereas *M. galloprovincialis* tends to be excluded from this zone, and greater tolerance of water loss by *M. galloprovincialis*, permitting it to occupy the upper zones (Zardi et al. 2006; Bownes & McQuaid 2006; 2010; Nicastro et al. 2010). In this instance, too, wave action moderates the interaction so that the two species coexist but occupy different portions of the shore, as seems the case for *S. algosus* and *M. galloprovincialis* on the West Coast.

On the Chilean coast, *S. algosus* occurs mainly in wave-protected areas (Fernandez et al. 2000). Why then is it less abundant or even absent from sheltered sites in South Africa? There are several potential reasons, one being sand inundation. The sheltered sites at Elands Bay were sand inundated (personal observations). However, in Namibia, as well as Chile, *S. algosus* is found in sand inundated areas (personal observations; Fernández et al. 2000). Sand is, therefore, unlikely to be the reason for its absence at these sites. Other reasons why *S. algosus* may be unable to maintain a presence in sheltered areas may include greater susceptibility to predators, lower recruitment rates, and diminished food supply. It is beyond the scope of this thesis to explore all these factors, but in Chapter 4 I do examine relative growth rates, reproductive outputs and differential investments in shell strength and attachment, casting light on which of these potential factors is most plausibly responsible for the invasion success of *S. algosus*. In addition, in Chapter 5 I describe experiments that explore interactions between *S. algosus* and *M. galloprovincialis* and their relative success at different heights on the shore; and in Chapter 6 the genetic diversity and likely origins and mode of distribution of *S. algosus* to South Africa are examined.

Conclusions

Semimytilus algosus is a successful invader on the West Coast of South Africa, comparable to the *M. galloprovincialis* invasion in recent history. It is likely that its geographic range would expand along the entire South African coast until halted by biogeographic barriers related to thermal limits. It consistently dominates the low intertidal zone at wave exposed sites. However, it does not seem able to maintain populations at sites with low wave exposure.

Reasons for the invasive success of this species and its differential zonation with respect to *M. galloprovincialis* on the West Coast will be developed in the following two chapters.

Chapter 4

Comparisons of life-history strategies of *S. algosus* and three other mussel species on the West Coast of South Africa

4.1 Introduction

In the intertidal zone, competition for space plays a substantial role in determining zonation patterns, community structure and diversity (Connell 1961, Dayton 1971, Menge and Branch 2001). The relative competitive abilities of species are influenced by life-history traits, such as reproductive output and recruitment rate, growth rate (Barkai and Branch 1989), shell strength (Caro et al. 2011) and byssus strength (Bell and Gosline 1996, Denny and Helmuth 2009). For example, Erlandsson et al. (2006) found that in South Africa, although the brown mussel *Perna perna* has a greater attachment strength and is relatively tolerant of storm events, under certain circumstances, it is still outcompeted by the blue mussel *Mytilus galloprovincialis* . They attributed this to the greater recruitment rate of *M. galloprovincialis*, which allows it recolonise space faster after displacement by storms. Recruitment includes settlement rate and takes into account survival of settled larvae (Bownes and McQuaid 2009). Recruitment rates are in turn affected by physical processes, pelagic larval survival and larval behaviour (Rodriguez et al.1993). For instance, Caro et al. (2011) found that on the central coast of Chile, *Semimytilus algosus* has a greater larval production rate than *Perumytilus purpuratus*. Despite this, it is outcompeted by *P. purpuratus* because the rate of arrival of *S. algosus* larvae on the shore is lower than that of *P. purpuratus*, and it settles predominantly on conspecific adults.

Growth rate can also determine whether an organism will dominate space on the rocky shore. For instance, the mussel *Mytilus edulis* can experience 60% mortality without any reduction in percent cover because it can double its size in one year (Petraitis 1995). Growth rate can be influenced by genetic differences among species and environmental factors, such as food availability and wave exposure (Steffani and Branch 2003, Van Erkom Schurink and Griffiths 1993).

Shell and byssal strength determine an organism's vulnerability to predation and wave action (Griffiths and Seiderer 1980). In Chile, *Perumytilus purpuratus* has a stronger shell and attachment strength than *S. algosus* and is known to crush individuals of *S. algosus* (Caro et al. 2011). Mussels often dominate wave-exposed shores, largely due to their ability to attach to substratum and to withstand wave action (Bell and Gosline 1996, Denny and Helmuth 2009). Byssal threads are common to all mytilid bivalves and are used for post-larval attachment to the substratum (Denny and Helmuth 2009, Pearce and LaBarbera 2009). Byssal strength is variable, as individual threads continually decay and new threads have to be produced. Therefore attachment strength depends not only on the numbers, dimensions and chemical composition of individual threads, but also on the rate of thread production (Denny and Helmuth 2009).

There often exists a trade-off between life-history traits: for example Caro and Castilla (2004) reported a decrease in growth of *S. algosus* associated with an increase in shell thickness. Mussels may also expend more energy on reproduction than on production of byssal threads, thereby reducing attachment strength (Zardi et al. 2007b, Denny and Helmuth 2009).

Native populations of *S. algosus* are found along the coast of South America from Ecuador to Chiloé Island in Chile, and recruit year round with peaks in austral winter and summer (Navarrete et al. 2008). In its natural environment, *S. algosus* is outcompeted by the thicker-shelled *Perumytilus purpuratus*, which has a negative effect on the growth and survival of *S. algosus* (Caro 2009). However, *S. algosus* can co-exist with *P. purpuratus* at local scales because of its high recruitment rate. One of the attributes that gives *P. purpuratus* an advantage is that its shell is thicker – a feature that is consistent even although it varies in response to predators (Caro and Castilla 2004).

In South Africa, studies have shown that *Mytilus galloprovincialis* , which exhibits high recruitment and growth rates (Branch and Steffani 2004), outcompetes several other mussel species. *Aulacomya atra*, on the other hand, is slow-growing (Griffiths and King 1979). Van Erkom Schurink and Griffiths (1993) compared its growth rate with that of *Choromytilus meridionalis*, *Perna perna* and *M. galloprovincialis* and found it has the slowest growth of the four species. Barkai and Branch (1989) found that *A. atra* is competitively inferior to *C. meridionalis* because the latter grows faster and is more tolerant of silting. In studies where *M. galloprovincialis* was compared to *A. atra* it was found that although *M. galloprovincialis* has a lower annual reproductive output (Van Erkom Schurink and Griffiths 1991), it still dominates exposed shores where its growth rate is highest (Steffani and Branch 2003). Furthermore, despite the fact that *A. atra* spawns three times per year and *M. galloprovincialis* only once or twice a year (Van Erkom Schurink and Griffiths 1991), settlement of *A. atra* larvae is intermittent (Griffiths and King 1979, Pollock 1979). In a comparison between *M. galloprovincialis* and three indigenous mussels, *A. atra, C. meridionalis, P. perna*, Branch and Steffani (2004) found that survivorship for *M. galloprovincialis* is much higher than for the other species.

To determine the likelihood that *Semimytilus algosus* has the ability to become be a dominant competitor on intertidal shores in South Africa, I examined several of its life-history traits – growth rate, reproductive output, recruitment, survivorship, shell and byssal strength – relative to those of another invasive mussel, *Mytilus galloprovincialis*, and two indigenous mussels, *Choromytilus meridionalis* and *Aulacomya atra*, all of which co-occur on the West Coast of South Africa.

Given (1) the rapidity with which *S. algosus* has invaded the West Coast of South Africa, (2) its domination of the low shore at many localities (De Greef et al. 2013) and (3) the fact that on its native Chilean shores and in Namibia it has high recruitment levels (Caro 2009, Reaugh-Flower 2011), I hypothesised that its life-history strategies will contribute to the competitive success of this mussel. Specifically, I tested the hypotheses that:

H1. *S. algosus* is fast-growing and will grow faster than the indigenous mussels *Choromytilus meridionalis* and *Aulacomya atra*, but that *M. galloprovincialis* will have the fastest growth rate.

H2. *S. algosus* has a higher reproductive output and recruitment rate than the other three mussel species.

H3. *S. algosus* expends much of its energy in growth and reproduction and therefore its shell and byssus strength will be low.

4.2 Methods and materials

To shed light on the relative competitive ability of *S. algosus* on the West Coast of South Africa, various life-history parameters were determined and compared with those of *Aulacomya atra*, *Choromytilus meridionalis* and *Mytilus galloprovincialis*.

Life-history strategies of mussels may differ considerably depending on wave exposure and shore heights (Van Erkom Schurink and Griffiths 1993, Steffani and Branch 2003). To standardise these effects, I therefore conducted all sampling on the low shore at Bloubergstrand (33°48'22"S, 18°27'50") (Fig. 3.1), so that I could compare the four mussel species under equivalent circumstances.

Growth rate

There are three methods for measuring the growth rate of molluscs: (1) analysing size-frequency distributions; (2) counting growth rings, and (3) measuring marked animals at regular intervals (Seed & Brown 1978). Analyses of size-frequency distributions could not be undertaken, as cohorts are known to merge rapidly in mussel populations (Seed 1969, Griffiths and King 1979). Shell ring accretion is affected by seasonal environmental changes and is not an accurate way of determining growth in mussels (Seed 1976). As a result, I employed measurements of marked individuals to determine absolute growth rates. Thirty specimens of each of the four species were labelled using Dymo tape glued onto mussels with Pratley's clear glue (Fig. 4.1). The total length of each labelled mussel, from umbo to the posterior margin, was measured monthly, for 12 months. Measurements were made to 0.1mm accuracy, using Vernier callipers. Every three months, additional mussels were labelled and measured to replace any lost to mortality.

For each species, Ford-Walford plots were drawn using the regression equation:

$$Lt+3= mLt+i$$

Lt is the length at the start and $Lt+3$ is the length after three months, m is the slope of the regression line and i is the y-intercept. Constants derived from the Ford-Walford plots were thus based on three-monthly intervals and used to draw a von Bertalanffy growth curve using the equation:

$$Lt=L\infty[1-e^{K(t-t0)}]$$

Lt is the length at time t, $L\infty=i(1-m)$ and is the asymptotic length, $K=-\log_e m$ and is the growth coefficient, and $t0$ is the theoretical age at the start of growth of settled larvae (assumed to be 0, as per Blankley & Branch 1985).

To determine whether growth rates differed significantly among species the slopes and elevations of the Ford-Walford plots were compared through analyses of covariance (ANCOVA), with initial shell length (Lt) as a covariate.

Shell strength

Shell length, height, thickness, and width of valve pairs (see Fig. 4.2; n = 30 per species) were measured using digital Vernier callipers, to 0.01mm accuracy. Failure loads of mussel shells were then measured using a Zwick 1484 universal tensile tester, with pressure being applied at a rate of 2 mm s^{-1}. Left valves of mussels were placed horizontally in the centre of the loading plate. Compressive force was then applied by placing the steel plate against the mussel so that pressure was applied to the highest part of the mussel shell. Load displacement curves up to shell breaking point were obtained.

Shell strength was taken as the maximum force required to fracture shells. The different species spanned different ranges in shell length, and to account for this potential confounding factor, analyses of covariance (ANCOVA) were performed to test for differences in breaking force, shell thickness and the ratio between shell width and shell length for the four species, with length as a covariate. The width:length ratio was measured because of the possibility that more ‘domed’ shells with a high ratio might be stronger than ‘flatter’ shells with a low ratio. Multiple regressions were conducted to establish the relative contributions of these factors to shell strength. The assumptions of linearity, homoscedasticity, normality and independence of errors were tested using residual plots. Collinearity was assessed using Pearson’s correlation. Best models were selected using P-values ($P<0.05$). Length/breaking force and length/thickness regressions were calculated for each species and used to estimate the breaking force and thickness of standard mussels 40 mm in length. This length was chosen as it fell within the range of specimens of all four species used to measure shell strength.

Byssus strength

Overall byssal strength was determined through tensile tests, which measured the vertical pull required to break the byssus of specimens in the field. Fifty specimens per species were selected randomly on the low shore at Bloubergstrand. A retort clamp with a spring balance hooked to it was attached to each mussel and pulled steadily, perpendicular to the rock surface. The spring balance measured the pulling force (in kg) required to detach the mussel. For purposes of data analyses measurements were converted to Newton. Tenacity was calculated as detachment/A_{pl}, where A_{pl} is the valve planar area, calculated as an ellipse, using the following equation:

$$A_{pl} = \pi*(height/2)*(width/2))$$

The strength of individual byssal threads (n=3 per mussel) was measured for 15 individuals per species, following the methods of Bell and Gosline (1996). Tensile strength was measured for the distal region of all threads, as different regions of a byssal thread behave differently under tension (Bell and Gosline 1996). At the start, mussels were wet-weighed and the byssus dissected out and wet-weighed. Byssal thread thickness (n=3 per mussel) was measured to an accuracy of 0.01µm using a digital micrometer, and shell length was measured using digital Vernier callipers, to 0.1mm accuracy. Breaking strengths of individual strands were measured using an Instron 5544 tensile tester with extensiometer, applied at a rate of 10mm min^{-1}.

Stress was calculated using the formula:

$$Stress = breaking\ force/A_{pl}$$

Strain was calculated as:

$$\text{Strain}=\text{extension}/\text{initial length}.$$

A stress-strain curve was drawn and the modulus calculated (Denny 1988). The ratio of stress to strain (modulus) is a measure of the stiffness of a thread. The stiffer a material is, the greater the force needed to break it. Modulus therefore offers an explanation for the strength of a byssal thread (Denny 1988). Whilst extension of the distal region may not be directly linked to the strength of individual threads, it may enhance overall attachment strength (Moeser and Carrington 2006).

One-way ANOVA analyses were performed on natural logarithm values, due to heteroscedasticity of the untransformed data, to test for differences among species in tenacity, single-thread tensile strength, modulus, number of byssal threads and the diameter of single byssal threads. Multiple regressions were performed for each species, to establish the relative contributions of these factors to byssal tenacity.

Survival rate

Survival rates of the four species over a 12-month period were measured directly from labelled mussels in the field (see *Growth rate* above).

Reproductive output

Reproductive outputs of the four species were derived from dry flesh weight of mussels spanning a range of sizes above that at which sexual maturity is reached: 20-40 mm for *Semimytilus algosus* and *Aulacomya atra*, 30-50mm for *Mytilus galloprovincialis* and 35-70 mm for *Choromytilus meridionalis* (Steffani and Branch 2003, and personal observations).

Thirty specimens of each species were collected randomly at monthly intervals for 14 months, from the low shore at Bloubergstrand.

In the laboratory the byssus was removed, shell length, wet weight and sex were recorded, and the flesh extracted and transferred to a numbered aluminium dish and dried at 55°C for 48 hours to achieve constant weight, then weighed.

Dry weight/Length regressions were calculated for each species for each month and used to estimate the weights of standard 40-mm mussels. The estimated standard dry weight was plotted against time to create an index showing cycles of weight gain and loss reflecting gonad accumulation and gamete release. To estimate annual reproductive output, differences between peaks in weight and ensuing troughs were taken as a measure of gonadal output, and summed over the period of observation to account for the fact that more than one spawning took place. Relative reproductive output was calculated as the percentage output divided by maximum body mass of each species.

Interpretation of spawning periods was supported by histological analyses noting the change in gonad structure, by subjectively grading a gonadal smear from every female into four categories: (a) mature oocytes, (b) spawning with continuous oogenesis, (c) spent oocytes and (d) resorption, following Branch (1974) and Griffiths (1977).

Recruitment rate

Recruitment rates of the four species of mussels were determined by taking monthly scrapings from mussel beds on the low shore at Bloubergstrand, using a 10x10cm quadrat. Five replicate scrapings were taken from areas with 100% cover of each species. To compare the recruitment among shore heights at a time when all species were recruiting (March), I also collected five replicate scrapings within 10x10cm quadrats at three shore heights: low, mid and high.

Samples were sieved through a 1-mm sieve to remove sediment, and individual recruits were identified and counted under a dissecting microscope. Recruits were defined as individuals of 1-5 mm shell length (Reaugh-Flower et al. 2010).

Data were plotted against time to provide a monthly rate of recruitment. Factorial ANOVAs were used to determine differences between species and months, and any interaction between species and month, and separately to test for differences between species and shore heights, and any interaction between species and shore height.

Statistical analyses

All analyses were performed using Statistica 12 (Statsoft 2014). Assumptions of normality were tested by plotting a normality plot and then assessed visually. Homogeneity of variances were tested through a Levene's test. Where necessary the data were log-transformed to meet assumptions, and when this failed to meet assumptions, non-parametric tests were performed, as specified in the text. To determine where differences lay, post-hoc Tukey's HSD tests were performed. The significance level was set at 0.05 for all analyses.

4.3 Results

Growth

Growth rates as determined from Ford-Walford plots (Fig. 4.3) and von Bertalanffy growth curves derived from them (Fig. 4.4) showed that *Choromytilus meridionalis* had the highest growth rate (achieving 45.62 mm yr^{-1} in the first year), followed by *Mytilus galloprovincialis*

(30.72 mm yr^{-1}), while the growth rates of *Aulacomya atra* and *Semimytilus algosus* were slower (25.69 and 11.98 mm yr^{-1} respectively). Constants of the Ford-Walford plots and von Bertalanffy growth curves are recorded in Table 4.1.

One-way ANCOVA revealed significant differences among species in both the slopes and intercepts of the Ford-Walford plots. Initial length (Lt) did not significantly affect slopes among species but did have a significant effect on the y-intercepts (Table 4.2). Tukey's HSD tests established that the slopes were significantly different between *C. meridionalis* and *M. galloprovincialis*, as well as between *C. meridionalis* and *A. atra*. The intercepts were significantly different among all species except between *S. algosus* and *A. atra*.

Shell strength

Choromytilus meridionalis possessed the strongest shells, requiring a mean force of 256N to break their shells, with *M. galloprovincialis*, *A. atra*, and *S. algosus* having mean breaking forces of 217, 160 and 54N respectively (Fig. 4.5). ANCOVA detected significant differences in shell strength, thickness and the width:length ratio among species. Length had a significant effect on breaking force and shell thickness, but not on width:length ratio (Table 4.3). Tukey's HSD tests reported significant differences in breaking force among all species, except between *C. meridionalis* and *M. galloprovincialis* (Fig. 4.5). *Mytilus galloprovincialis* had the greatest mean shell thickness. Thickness was not significantly different between *A. atra* and *C. meridionalis*, but was significantly different among all other pairs of species. The ratio of width:length was greatest for *A. atra*, and all four species were significantly different from each other (Fig. 4.5).

Multiple regressions (Table 4.4) showed that thickness, length and the width:length ratio accounted for only 4% of the shell strength of *S. algosus*, none of the factors contributing significantly ($F_{3,46}=0.33$, $p=0.80$):

$y=5.41+0.10x_{thick}-0.10x_{length}+0.05x_{W:L}$ ($R^2=0.04$, $p=0.80$, SE=0.60, df 3,46).

The multiple regression for *A. atra* accounted for 38% of the variance, with both length and the ratio of width:length being significant predictors of shell strength ($F_{3,46}=11.05$, $p<0.001$):

$y=2.48+0.16x_{thick}+0.44x_{length}+0.33x_{W:L}$ ($R^2=0.38$, $p<0.001$, SE=0.48, df 3,46).

For *M. galloprovincialis*, the multiple regression explained 42% of the variance, with shell thickness significantly affecting shell strength ($F_{3,46}=12.76$, $p<0.001$):

$y=4.42+0.63x_{thick}+0.07x_{length}-0.01x_{W:L}$ ($R^2=0.42$, $p<0.001$, SE=0.40, df 3,46)

The multiple regression had the highest explanatory power for *C. meridionalis* (56% of variance), with thickness and length having significant effects on shell strength ($F_{3,46}=21.88$, $p<0.001$):

$y=1.69+0.32x_{thick}+0.53x_{length}+0.10x_{W:L}$ ($R^2=0.56$, $p<0.001$, SE=0.36, df 3,46).

Since length had a significant effect on breaking force in most instances, regressions were calculated relating shell strength to length (Table 4.5, Fig 4.6), from which the breaking forces for standard-sized 40-mm mussels were calculated for each species. Standard sized *Aulacomya atra* had the strongest shell, followed by *M. galloprovincialis* and *C. meridionalis*, and *S. algosus* had the weakest shell (Fig 4.7). To establish whether shell thickness followed the same pattern as shell strength, the thicknesses of mussels 40 mm in length were also estimated from regressions (Table 4.5). *Mytilus galloprovincialis* had a marginally greater shell thickness than *A. atra*. *Semimytilus algosus* and *C. meridionalis* had comparable thinner shells (Fig 4.7). Standard errors and statistical significance could not be calculated as the values for standard sized mussels constituted single values.

Byssus strength

Aulacomya atra exhibited the greatest tenacity, with a mean of 16.38 Nx10^3 mm^{-2} required to break the byssus. The mean tenacities for *M. galloprovincialis*, *C. meridionalis* and *S. algosus* were 9.74, 5.45 and 6.49 Nx10^3 mm^{-2} respectively (Fig 4.8a). Kruskal-Wallis tests yielded significant differences in tenacity among all species (H=90.07, $P<0.001$), except between *S. algosus* and *C. meridionalis*.

Single-thread tensile strength was marginally greater for *M. galloprovincialis* (1.41 N), than for *A. atra* (1.40 N). Mean breaking force of single threads recorded for *S. algosus* and *C. meridionalis* were 0.99 N and 0.82 N respectively (Fig. 4.8b). Means were significantly different among all species except between *C. meridionalis* and *S. algosus* and between *M. galloprovincialis* and *A. atra*. Mean modulus was not significantly different among species (Fig. 4.8c). The number of threads per byssus was however, significantly different among all four species, resembling the patterns for tenacity (Fig. 4.8d). Mean thread diameter was significantly different between *S. algosus* and both *M. galloprovincialis* and *A. atra*, and between *C. meridionalis* and *M. galloprovincialis* (Fig. 4.8e).

Multiple regressions (Table 4.6) showed that for *S. algosus*, 67% of byssal strength was explained by the factors diameter and modulus. There was a particularly strong relationship between tensile strength and modulus:

$y=-3.32+0.36x_{\text{dia}}+0.72x_{\text{mod}}$ ($R^2=0.67$, $p=0.001$, SE=0.32, df 2,11).

Both diameter of byssus threads and modulus was significant for *C. meridionalis*:

$y=-2.55+0.56x_{dia}+0.39x_{mod}$ ($R^2=0.37$, $p<0.001$, SE=0.26, df 2,37).

For *M. galloprovincialis*, modulus was the greatest and only significant predictor of byssus strength:

$y=-0.75+0.14x_{dia}+0.85x_{mod}$ ($R^2=0.69$, $p<0.001$, SE=0.26, df 2,32).

For *A. atra*, 92% of byssus strength was explained by the two factors, with modulus reported as the greatest predictor of tenacity:

$y=-0.70+0.11x_{dia}+0.98x_{mod}$ ($R^2=0.92$, $p<0.001$, SE=0.13, df 2,10).

Survival rate

Mytilus galloprovincialis exhibited the greatest survival rate, with 51% of marked mussels alive after one year, and *S. algosus* the lowest, with only 6% survivors. *C. meridionalis* and *A. atra* showed intermediate survival rates of 43% and 12% respectively (Fig. 4.9).

Reproductive output

Aulacomya atra had the greatest fluctuations in dry weight of the four species. All four species had two spawning events during the year, mostly in the austral late winter to early summer, with a smaller event during late autumn, although not during the exact same months (Fig. 4.10).

Aulacomya atra also had the highest absolute reproductive output over 14 months, at 0.72g, with *C. meridionalis* 0.66g, *M. galloprovincialis* 0.57g and *S. algosus* 0.47g (Fig. 4.11A). Relative reproductive output showed that *S. algosus* achieved 118% output compared to its maximum body mass, *C. meridionalis* 132%, *M. galloprovincialis* 84% and *A. atra* 96% (Fig. 4.11B).

Overall male-to-female sex ratios were 49:51 for *C. meridionalis*; 59:41 for *A. atra* and 64:36 for *M. galloprovincialis* (Fig. 4.12). *Semimytilus algosus* is hermaphroditic and thus sex ratio could not be calculated for it.

Recruitment rate

The recruitment rate of *Semimytilus algosus* was substantially greater than that for any of the other species. The proportions of *S. algosus* recruits in patches formed by different mussel species were 98% in conspecific patches, 79% among *C. meridionalis*, 70% in *M. galloprovincialis*, and 76% in *A. atra* patches (Fig. 4.13). Mean recruitment within conspecific patches was significantly different among species, and all four species recruited in greatest numbers to patches of their own species. Tukey's post-hoc tests showed that within patches, *S. algosus* significantly exceeded values for all the other species, but none of the other species differed from each other. Mean recruitment within conspecific patches was also significantly different among months, with a significant interaction between month and species because different species peaked in different months (Table 4.7, Fig. 4.14). Peaks in recruitment lagged just behind times when monthly dry weight attained low values that reflected spawning periods (compare Figs 4.10 & 4.14).

Mean numbers of recruits were also significantly different among shore heights, with a significant interaction between shore height and species (Table 4.8). *Semimytilus algosus* had significantly greater values than all other species on the low shore. On the mid shore its recruitment was significantly different from all species except *M. galloprovincialis*, and on the high shore there were no significant differences among species (Fig. 4.15). Recruitment of *S. algosus* declined significantly up the shore, with a three-fold reduction between the low shore and the mid shore, and a further three-fold reduction from there to the high shore, while recruitment of *M. galloprovincialis* was relatively uniformly spread over the shore, with a slight peak mid shore.

4.4 Discussion

Alien species that are successful invaders are often associated with (a) a capacity for rapid colonization and (b) predation resistance (Ehrlich 1986, Lodge 1993, Branch and Steffani 2004). The first strategy is often achieved through high reproduction and recruitment, as well as a high growth rate (Van Erkom Schurink and Griffiths 1991, 1993, Wootton 1993). In mussels, the second strategy may involve expenditure of energy on shell and/or byssus production to increase shell and attachment strength (Caro and Castilla 2004), although attainment of sufficient body size may itself provide protection (Griffiths and Seiderer 1980). For example, Griffiths and Seiderer (1980) found that West Coast rock lobsters (*Jasus lalandii*) prefer smaller rather than larger mussels as prey, but can take larger *Choromytilus meridionalis* than *Aulacomya atra*, due to the weaker shell and attachment strengths of *C. meridionalis*.

There is a trade-off between the different life-history strategies. For instance, Seed and Brown (1978) compared the growth strategies of two cockle species, *Cerastoderma edule* and *Modiolus modiolus*, and found that *C. edule* grows fast during the first year, after which growth slows when reproduction commences. *M. modiolus* reproduction is, however, delayed, allowing it to spend more of its energy on growth. The difference in strategies is due to the fact that *C. edule* is heavily predated upon in all size classes. Thus, it needs to reproduce early. *Modiolus modiolus* however, can outgrow mortality due to predation. It is therefore advantageous to grow as quickly as possible to attain this refuge in size. For *M.*

galloprovincialis, high reproductive output comes at the cost of attachment strength (Zardi et al. 2006). Bishop and Petersen (2006) reported that although the invasive Suminoe oyster *Crassostrea ariakensis* has a high growth rate and reaches maturity sooner than the indigenous Eastern oyster, *C. virginica*, it is more vulnerable to predation by the blue crab *Callinectes sapidus*, due to its weaker shell strength. They concluded that the trade-off between rapid growth and predator defences may promote invasion but may not sustain large adult populations.

The life-history strategies of *M. galloprovincialis* in South Africa are well studied and serve as an example of the characteristics that are needed to be a successful invader. It grows faster than native mussels (Hockey and Van Erkom Schurink 1992, Van Erkom Schurink and Griffiths 1993), has a relatively high annual reproductive output (Van Erkom Schurink and Griffiths 1991, Zardi et al. 2007), which converts into a high recruitment rate of up to 20 000 recruits per 100cm^2 (Harris et al. 1998). Furthermore, Hockey and Van Erkom Schurink (1992) have reported that at 50% air exposure, *M. galloprovincialis* has a survival rate double that of any native mussels. Conversely, its attachment strength is lower than that of the indigenous mussel *Perna perna* (Bownes and McQuaid 2006, Zardi et al. 2006b), so that it is at a disadvantage low on the shore where *Perna perna* dominates because of its stronger attachment and, hence, greater tolerance of wave action.

Growth rate

High growth rate allows an organism to maintain spatial dominance by occupying space more quickly than slower-growing competitors (Wootton 1993). For example, Rensel et al. (2005) found that *M. galloprovincialis* outcompetes *M. trossulus* in Puget Sound because of its higher survival and growth rate. *Mytilus galloprovincialis* also outcompetes *M. californianus* and *M. trossulus* in the Pacific Northwest, through high growth and recruitment (Shinen and

Morgan 2009). In South Africa, Barkai and Branch (1989) reported that rapid growth and the ability to withstand smothering by the other species allows *C. meridionalis* to outcompete *A. atra* at two sites on the West Coast of South Africa.

In its native range in Chile, *S. algosus* grows faster than *P. purpuratus*. However, no studies have been done on its growth rate in either Namibia or on the West Coast of South Africa since its recent arrival there. Several studies have compared the growth rates of the other three mussel species that occur there, and the rates compare favourably with mine. I found that *C. meridionalis* was the fastest-growing mussel, followed by *M. galloprovincialis* and *A. atra*. Barkai and Branch (1989) reported that in sublittoral populations *C. meridionalis* grows faster than *A. atra*. Van Erkom Schurink and Griffiths (1993) found that *A. atra* was the slowest growing species, with either *M. galloprovincialis* or *C. meridionalis* the fastest growers, depending on site. The annual growth rates they reported are also comparable to those I found, with 38mm in the first year of growth for *C. meridionalis* and 33mm for *M. galloprovincialis* in Saldanha Bay.

The spatial dominance of *Semimytilus algosus* on the low shore, as well as its high growth rate compared to its closest competitor in Chile, led me to believe that it would be fast growing in South Africa. However, I found that it had the slowest growth of the four mussel species examined, thus disproving my first hypothesis. This species must therefore use other means to achieve spatial dominance.

Shell strength

Increased shell thickness and strength can increase the survival of mussels by protecting them from wave action and predators (Raubenheimer and Cook 1990, Steffani and Branch 2003). However, this is energetically costly since as much as 26% of energy intake can be expended on shell production (Griffiths and King 1979). There also exists a trade-off between linear shell growth and shell thickness (Smith and Jennings 2000, Caro and Castilla 2004).

Semimytilus algosus had the thinnest, smallest and weakest shells overall, and *C. meridionalis* exhibited the greatest shell strength in my study. Shell strength was, however, influenced by shell length. Consequently, values of breaking force for standard-sized 40-mm mussels constituted a more valid comparison of relative strength. In those terms, *A. atra* had the strongest shell of the four species and *S. algosus* the weakest. Van Erkom Schurink and Griffiths (1993) compared the shell morphology of *A. atra*, *C. meridionalis* and *M. galloprovincialis*, and found that *A. atra* had the thickest (1.5mm) and heaviest (8.5g) shells and *C. meridionalis* the thinnest (1.1mm) and lightest (5.2g) shell. Griffiths and Seiderer (1980) also reported that *A. atra* had a much stronger shell than *C. meridionalis*. Emmanuel (2014) documented that *S. algosus* had the thinnest shell, followed by *M. galloprovincialis*, *C. meridionalis* and *A. atra*.

Shell strength is influenced by shell morphology, such as shell length and thickness (Griffiths and Seiderer 1983). Comparison of the shell morphology among the four species showed that length showed exactly the same pattern as mean shell strength (Fig. 4.5). Shell thickness and breaking force for standard sized mussels also showed a similar pattern (Fig. 4.7). Emanuel (2013) concluded that shell thickness was the best predictor of shell strength for these four mussel species. There may, however, be other factors that influence shell strength, including shell microtopographic texture and internal microstructure (Hiebenthal et al. 2013), as well as shell proportions. However, only in one instance (*A. atra*) did I find that the ratio of shell height to length influenced strength.

Attachment strength

Mussels are tethered to the rock surface by a byssus consisting of several threads, which is secreted by the foot (Bell and Gosline 1996, 1997). Securer attachment allows a competitive advantage as it reduces vulnerability to disturbance and predation and thus permits more effective occupation of space (Bell and Gosline 1996). For example, on the South Coast of South Africa, *Perna perna* has greater attachment strength than *M. galloprovincialis*, allowing it to outcompete the latter on the low shore and at sites with high wave action (Erlandsson et al. 2006, Zardi et al. 2006).

Both the number of threads, as well as thread yield and extensibility are known to increase attachment strength (Bell and Gosline 1996). Increasing the number of threads reduces the stress on each individual thread, by distributing the applied tension over a greater area. In addition, thread flexibility contributes to attachment strength by re-orientating threads in the direction of the applied tension (Bell and Gosline 1996). The modulus of a material – its stiffness or resistance to deformation – also predicts the force needed to break it. The stiffer a material the more force is needed to deform it (Denny 1988). Zardi et al. (2006) reported that the reasons for the greater attachment strength of *P. perna* are its larger number of threads as well as thicker individual threads. In Chile, *Perumytilus purpuratus* has greater attachment strength than *S. algosus* due to thicker individual byssus threads (Caro et al. 2008).

Among the species we examined, overall attachment strength (tenacity), and individual thread strength followed similar patterns, with *A. atra* and *M. galloprovincialis* having high values compared to *S. algosus* and *C. meridionalis*. The same pattern emerged among species for the number of threads per byssus and the diameter of the threads. Only measures of modulus departed from this grouping of species, with *S. algosus* having a high value. Variance was, however, high for this variable, and no significant differences emerged among species. Despite this, multiple regressions showed that modulus was the best predictor of attachment strength for three out of four of the mussel species.

Survival rate

Van Erkom Schurink and Griffiths (1993) found that *A. atra* had the lowest survival rate when compared to *M. galloprovincialis* and *C. meridionalis*, and *M. galloprovincialis* the highest survival. I found the same pattern of survival among these three species, but *S. algosus* had the lowest survival rate of the four species. This is not surprising as it had the lowest values for both shell and attachment strength, leaving it potentially vulnerable to predators, interference competition, and the effects of wave action, which have been shown to influence the survivorship of many intertidal species (Connell 1961, Dayton 1971, Paine & Levin 1981).

Further increasing its vulnerability is the fact that *S. algosus* is more prevalent on the low shore (Chapter 3), where marine predation is most intense (Bustamante et al. 1997). In addition, it is the smallest mussel on our shores, which makes it an easy prey for birds such as the kelp gull *Larus dominicanus vetula*, the black oystercatcher *Haematopus moquini* and the West Coast rock lobster *Jasus lalandii* (De Greef et al. 2013). Coleman and Hockey (2008) reported that *H. moquini* prefers mussels sized 32-36 mm long, and Griffiths and Seiderer (1980) found that *J. lalandii* preferentially selects mussels of 5-40 mm in length. *Semimytilus algosus* reaches a maximum size of 50 mm on intertidal rocks on the West Coast of South Africa (De Greef et al. 2013), which is within the critical ‘window of vulnerability’ described by Griffiths and Hockey (1987). Finally, *S. algosus* competes with *M. galloprovincialis* for space on the mid-low shore, and *M. galloprovincialis* is known to interfere with competitors by overgrowing and thus suffocating them (Shinen and Morgan 2009).

To attain the high biomass and spatial dominance observed for this species, *S. algosus* must therefore have a high reproduction rate and/or an associated high recruitment rate.

Reproduction rate

The invasive success of an organism is related to its abundance and its ability to colonise new areas (Zardi et al. 2007). One of the most important, though energetically costly, ways to achieve this is through reproductive output. Mussels can spend up to 90% of their total energy in reproduction (Seed and Suchanek 1992).

I found that *A. atra* had the greatest absolute reproductive output per individual, followed by *C. meridionalis*, *M. galloprovincialis* and *S. algosus*. Van Erkom Schurink and Griffiths (1991) found that *A. atra* had the greatest annual reproductive output of the three species they examined, with *M. galloprovincialis* in second place and *C. meridionalis* placed lowest.

Annual reproductive output is a function of the weight loss per spawning as well as the frequency of spawnings (Van Erkom Schurink and Griffiths 1991). Van Erkom Schurink and Griffiths (1991) reported two spawning events for *C. meridionalis*, *M. galloprovincialis* and *A. atra*, although they also concluded that spawning frequency and intensity is variable among years. My results show the same spawning frequency. Griffiths (1977) reported that both *C. meridionalis* and *A. atra* had three spawning events per year, and my data for *M. galloprovincialis* reflect three troughs in mass that could be interpreted as three spawning events.

Even though *Semimytilus algosus* did not have the highest absolute annual reproductive output of the four species, there are other ways of viewing its reproductive output. First, it has the advantage of being simultaneous hermaphrodite. Consequently every individual is capable of producing eggs and thus larvae, in contrast to the other species, for which females constituted 36–51% of their populations. Second, reproductive output can be expressed in relative terms as the proportional output compared with the maximum flesh mass. Standardised for 40-mm mussels, *M. galloprovincialis* had the lowest relative output (84.4% of body mass per annum), and *C. meridionalis* the highest value (132.0%); *S. algosus* also had a high relative output (118.5%). Third, reproductive output can be evaluated for the population as a whole. De Greef et al (2013; and see Chapter 3) showed that *S. algosus* and *M. galloprovincialis* now dominate wave-exposed shores on the West Coast of South Africa, contributing respectively 30-52% and 48-54% of the mussel biomass. *Aulacomya atra* and *C. meridionalis* by contrast add paltry amounts of 0-5% and 0-9%. Accurate calculation of population reproductive output is not possible without a better knowledge of subtidal populations, but it is clear that overall reproductive outputs of *S. algosus* and *M. galloprovincialis* will far exceed those of the two indigenous species.

Recruitment rate

Variation in recruitment is one of the factors that govern the distribution and abundance of mussels on rocky shores (Gaines and Roughgarden 1985, Navarette et al. 2008, Bownes and McQuaid 2009). Studies have shown that spatial variations in invertebrate recruitment are driven by several possible factors, including substratum and tidal height (Petersen 1984, Caro et al. unpublished), adult densities (Underwood et al. 1983, Harris et al. 1998, Robinson et al. 2007b, Reaugh-Flower et al. 2011; and see Chapter 5), and physical processes that affect larval dispersal and retention (McQuaid and Phillips 2000, 2006, Navarette et al. 2008).

Semimytilus algosus had by far the highest overall recruitment rate of the four mussel species. Although its reproductive output was not the highest of the four species, it translated into an exceptionally high recruitment rate. The reason for this may be that oceanically-driven dispersal favours settlement of *S. algosus* larvae at the site I examined. Several studies have found that spatial variation in recruitment may be due to physical processes that affect larval dispersal and retention at certain sites (Harris et al. 1998, Navarette et al. 2008).

Robinson et al. (2007) found a strong small-scale correlation between *M. galloprovincialis* adult density and its recruitment. For all four species, I also found that recruitment was highest in conspecific beds. However, even though the percentage of *S. algosus* recruits was highest in conspecific beds (constituting 91.72% of the recruits of all species combined), it still had significantly greater proportions of recruits than any other species within patches of the other three species. The relatively low percentage recruitment of *S. algosus* into beds of other species may reflect interspecific competition between species or preferential settlement among conspecifics – a distinction that cannot be resolved from my data. *Mytilus galloprovincialis* is known to overgrow and smother competitors (Shinen and Morgan 2009), and it is well known that interspecific competition may influence settlement behaviour (Grosberg 1981). In its native range *S. algosus* preferentially settles in conspecific beds, avoiding the beds of the dominant competitor, *Perumytilus purpuratus* (Caro et al. unpublished). Petersen (1984) found that the competitively superior *Mytilus californianus* settles on many different substrata, while *M. edulis* preferentially settles in conspecific beds, because competition is high and survival low in *M. californianus* beds.

Reaugh-Flower et al. (2011) found that recruitment of mussels is most intense on the West Coast of southern Africa, diminishes on the South Coast, and is lowest on the East Coast. This pattern follows a gradient of nutrient levels and pelagic primary production around the coast, presumably translating into greater food supplies for mussel larvae. This may contribute to the intense recruitment of *S. algosus* on the West Coast, but would not explain the differences among mussel species. Reaugh-Flower (2011) also found that mussel recruitment not only varied among sites, but also among seasons and years. My data showed that the four mussel species all had two main recruitment peaks, in early summer and autumn-early winter, although not in the same months. Nevertheless, recruitment was still consistently higher for *S. algosus* than for the other mussel species in all months. Reaugh-Flower et al. (2011) reported a similar seasonal pattern for *S. algosus* in Namibia, and for *A. atra* and *M. galloprovincialis* on the West Coast at Groenriviermond. They also found that in Namibia, *S. algosus* had the highest recruitment of the three mussel species throughout the year.

Tidal height also affects recruitment rate. For example, Bownes and McQuaid (2009) reported that *Perna perna* is excluded from the high shore due to recruitment failure, but that *M. galloprovincialis* is able to recruit to the high shore due to its higher tolerance to desiccation. I found that recruitment of *S. algosus* was substantially greater than that of the other three species low on the shore, but diminished up the shore to levels that were comparable those of the other species at the top of the shore. This is to be expected as my surveys, and those conducted by De Greef et al. (2013), showed that *S. algosus* adults occur largely on the low shore (Chapter 3). *Mytilus galloprovincialis* showed the greatest recruitment on the mid shore, again conforming to the pattern of tidal distribution found in Chapter 3 and by De Greef et al. (2013). Although small numbers of *S. algosus* did recruit to the high shore, surveys showed that adults are absent from the high shore, probably due to high recruit mortality there. Possible reasons for the respective tidal distributions of *S. algosus* and *M. galloprovincialis* will be explored and discussed in Chapter 5.

Petraitis (1995) argues that the balance between growth and mortality rules spatial dominance, but where growth is limited, recruitment must offset mortality to maintain spatial dominance. *Semimytilus algosus* clearly dominates the low shore despite its high mortality and a low growth rate, and relies on its high recruitment rate to maintain that dominance.

Predation resistance is often costly and there exists a trade-off between defence strategies and colonisation strategies (Tokeshi and Romero 1995). Thus, it would seem that *S. algosus* does not follow a defensive strategy of investing in shell and attachment strength, but rather expends energy on reproduction, leading to a very high recruitment and rapid colonisation. This was as I expected and in accordance with hypotheses 2 and 3.

Conclusions

Mytilus galloprovincialis is known as one of the 100 worst invaders globally, and to date is the most successful invader on South African rocky shores (Robinson et al. 2005). Reasons include its high growth rate (Griffiths et al. 1992, Hockey & Van Erkom Schurink 1992), relative lack of parasites (Calvo-Ugarteburu & McQuaid 1998), and tolerance of sand stress (Zardi et al. 2006) and desiccation (Hockey & Van Erkom Schurink 1992). It also has a high reproductive output (Van Erkom Schurink & Griffiths 1991) associated with high recruitment rates (Harris et al. 1998).

However, the latest invader, *S. algosus* has remarkably rapidly colonised an extensive portion of the West Coast of South Africa, where it now dominates space on the low shore at many localities (De Greef et al 2013; and see Chapter 3). Its low growth rate and high mortality appear to be more than offset by its exceptionally high recruitment rate.

In Chile, *S. algosus* is outcompeted by *Perumytilus purpuratus*, even though it has a higher growth and recruitment rate. Caro et al. (2008) propose that due to its weaker shell and byssal attachment, *S. algosus* is vulnerable to interference overgrowth by *P. purpuratus*. In South Africa *S. algosus* co-exists with dense beds of *M. galloprovincialis*, dominating the low shore but being replaced by *M. galloprovincialis* in the mid- to high shore. Bownes and McQuaid (2006) have reported that *Perna perna* and *M. galloprovincialis* co-exist on the

South Coast of South Africa due to a combination of factors. *Mytilus galloprovincialis* is more tolerant of desiccation, and can survive on the mid-high shore, whereas *P. perna* experiences weak recruitment there. On the other hand *M. galloprovincialis* experiences high mortality on the low shore due to weaker byssal attachment and thus *P. perna* dominates the low shore. Equivalent factors could also explain the co-existence of *S. algosus* and *M. galloprovincialis* on the West Coast, and their prevalence in different zones. This could also explain why *S. algosus* does not form extensive beds on the mid-high shore, despite higher recruitment than *M. galloprovincialis*. I will further expand on this idea in Chapter 5.

My study shows that the four mussel species that occupy West Coast rocky shores have adopted different life strategies. The native mussel *A. atra* expends most of its energy on predation resistance, such as shell and byssus strength. *Choromytilus meridionalis* uses its high growth rate and great size to escape predation. The two invasive mussels seem to allocate more energy to features associated with rapid colonisation and domination of space. *Mytilus galloprovincialis* employs a strategy that allows it to occupy space by growing quickly, as well as a high reproductive output, and *S. algosus* benefits from its high recruitment rate to overcome its high mortality and form dense beds on the low shore.

Life-history strategies are affected by abiotic factors such as sea temperature, wave exposure and nutrient availability and may vary at different sites and in different seasons (Hickman 1979, Bayne et al. 1993, McQuaid and Lindsay 2000). I compared the life history patterns of the four mussel species at a single site and under circumstances that ensured comparability of data. However, it is possible that results might have differed at other sites and shore heights. For example, Van Erkom Schurink and Griffiths (1993) found that *M. galloprovincialis* was the fastest-growing mussel in Algoa Bay, whereas *C. meridionalis* grew fastest in the cooler, upwelled waters of Saldanha Bay.

Several publications have also shown that on the open coast *M. galloprovincialis* settles most abundantly and is most successful on shores that experience strong but not excessive wave action (Bustamante and Branch 1996a, Steffani and Branch 2003, Branch and Steffani 2004, Branch et al. 2008, Pfaff et al. 2011), and my surveys (Chapter 3) showed that while *S. algosus* may recruit to sheltered shores, it fails to survive there. Differences in food supply and predation pressure between exposed and sheltered shores are likely explanations that will add to differences in performance of mussels among sites. It would therefore be beneficial to repeat this study at multiple sites to explore the potential roles of upwelling, food supply, local hydrography and wave action. Even without this, however, it is clear that *S. algosus* owes much of its success as an invader to its exceptionally high rates of recruitment, especially low on the shore.

The following chapter describes experiments and observations designed to tease apart factors influencing the relative success of *S. algosus* and *M. galloprovincialis* at different shore heights.

Chapter 5

Field experiments assessing survival, growth, condition, recruitment and interactions between *Semimytilus algosus* and *Mytilus galloprovincialis* at different shore heights

5.1 Introduction

Community effects:

Invasive species cannot be studied in isolation, as community interactions may determine their success, and invaders can alter community properties such as species dominance and diversity, as well as physical features of an ecosystem (Bertness 1984; Suchanek 1992; Lodge 1993). This is especially true if they act as ecosystem engineers that physically alter the environment, thereby affecting the availability of resources (Gutiérrez et al. 2003; Sousa et al. 2009; Green & Crowe 2013). Given the fact that *Semimytilus algosus* forms dense beds (De Greef et al. 2013), it may have significant engineering effects. Including increased habitat for settlement, and increased refuges for infauna and epibionts (Sousa et al. 2009). Because mussels increase habitat complexity, they often also increase biomass, diversity and species richness (Crooks & Khim 2002; Rilov et al. 2012; Sadchatheeswaran et al. 2015). For example, Robinson and Griffiths (2002) reported that mussels support a high infaunal biomass by providing additional habitat and refuge from predation. Multi-layered mussel beds are known to support higher infaunal species richness than mono-layered beds (Robinson et al. 2007a; Sadchatheeswaran et al. 2015). Mussels also constitute a food source for predators, thereby increasing predator biomass (Branch and Steffani 2004).

To some extent, all invasive species affect the invaded community through biological interactions (Ruiz et al. 1999), but not all effects are negative. Facilitation between organisms benefits at least one of the participating species by, for example, reducing biotic and physical stress and creating new habitat (Stachowicz 2001). However, these interactions can only be understood within the context of the environment in which these species occur (Bruno & Bertness 2001). In this vein, Bertness & Leonard (1997) found that the positive effects of thermal buffering by intraspecific aggregations of mussels are most pronounced in stressful environments such as the high shore. However, in less stressful zones such as the mid shore, aggregation is not beneficial and may lead to competitive effects that decrease growth rate. Kawai & Tokeshi (2006) reported that the facilitative effects of goose barnacles on mussels increase with increasing physical stress. In South Africa, several studies investigating interactions between the invasive mussel *Mytilus galloprovincialis* and the indigenous species *Perna perna* also found that positive effects of physical amelioration differ between shore heights as physical stresses change (Zardi et al. 2006; Rius & McQuaid 2006; Rius & McQuaid 2009) and facilitation is overruled by extreme physical stress on the high shore.

Interactions with indigenous species may inhibit or slow down the spread of aliens. For example, *M. galloprovincialis* is scarce on the low shore on the South Coast of South Africa, partly because it is outcompeted by *P. perna* (Rius and McQuaid 2006). However, the reverse may also be true, when species interactions benefit newcomers through facilitation (McQuaid & Arenas 2009). For instance, the abundance of the Pacific oyster *Crassostrea gigas* in northeast Canada is facilitated by the native oyster *Ostreola conchaphila* (Ruesink 2007).

Competition and facilitation go hand in hand and may even be bidirectional (Kawai and Tokeshi 2006, Rius and McQuaid 2009). To exemplify, on the low shore *P. perna* initially increases survival of *M. galloprovincialis*, but later eliminates it through competition. On the mid shore, however, *M. galloprovincialis* facilitates the survival of *P. perna*, while *P. perna* has negatively impacts on *M. galloprovincialis* (Zardi et al. 2006, Rius and McQuaid 2006).

Tolerance to physical stress

Physical stress affects the abundance of species, but also moderates interactions between them (Menge 1976; Wieters 2005; Branch et al. 2010; Scrosati et al. 2011). Physical stresses in intertidal habitats typically comprise wave action, which decreases up the shore, and exposure to air and desiccation, which are most intense on the high shore (Paine 1974;

Bustamante et al. 1997). Tolerances of organisms to these physical stresses influence zonation (Menge & Branch 2001).

In its native range, *Semimytilus algosus* is intolerant of desiccation and occurs mainly on the low shore. *Mytilus galloprovincialis*, on the other hand, has a greater tolerance to desiccation than other mussel species in South Africa and extends relatively high on the shore (Hockey & Van Erkom Schurink 1992; Nicastro et al. 2008). Due to its weaker byssus attachment however, it is more vulnerable to dislodgement by wave action than *P. perna*, limiting its abundance on the low shore (Bownes & McQuaid 2009).

To date, no studies have experimentally quantified the impact of *Semimytilus algosus* on rocky shore communities on the West Coast of South Africa. In this chapter, I employed two separate approaches to examining its role at different shore heights. First, I cleared patches of mussels and observed community responses relative to undisturbed control patches. Second, I undertook manipulative experiments in which I caged *S. algosus* and *M. galloprovincialis* at various density combinations to test their interactions.

In relation to the ecological roles and zonation patterns of *S. algosus* and *M. galloprovincialis*, I hypothesised that:

H1. Community composition will differ in the presence or absence of mussels, and among shore heights. Biomass and diversity will be greater in the presence of mussels and lower on the shore.

H2. Mussels will facilitate some species and functional groups, by providing shelter, food and substratum, but will exclude others by outcompeting them.

H3. *Mytilus galloprovincialis* will support higher biodiversity than *S. algosus* because it is larger and forms multi-layered versus mono-layered beds.

H4. *Semimytilus algosus* will predominate on the low shore and *M. galloprovincialis* on the mid shore because of an interplay between their relative tolerances to physical stress, and competition and facilitation at different heights on the shore. Specifically *S. algosus* will perform best in the low shore in terms of growth, condition, recruitment and survival, whereas recruitment of *M. galloprovincialis* will peak in the mid shore and its survival will be lowest in the low shore although its growth and condition will be highest there.

H5. Growth and survival will be greater for *M. galloprovincialis* than for *S. algosus*.

H6. Recruitment of *S. algosus* will be greater than that of *M. galloprovincialis*, and recruitment of both will be correlated with adult density.

5.2 Methods and materials

To assess (1) the zonation patterns of *S. algosus* and *M. galloprovincialis*, (2) their effects on community composition in the intertidal zone, and (3) interactions between *S. algosus* and *M. galloprovincialis*, two types of manipulative experiments were conducted: patch clearance and caging.

5.2.1 Clearance experiment

The clearance experiment consisted of three uncaged treatments. In the first treatment (hereafter called the 'settlement treatment'), plots of 0.5 x 0.5 m were scraped to remove all biota, but mussels were allowed to resettle. For the second treatment (the 'removal treatment'), plots were similarly scraped, and any mussels that subsequently settled were removed by hand, on each sampling occasion. Collectively, these two treatments are referred to as 'disturbance treatments'. Thirdly, equivalent control areas (called the 'natural treatment') were left unmanipulated. An area of approximately 10 cm was cleared around all plots to minimise reintroduction of mussels by lateral migration from adjacent areas.

Treatments were set up at three shore heights *i.e.* low, mid and high shore, with four replicates of each treatment at each shore height, and was undertaken at two comparable open-coast, wave-exposed sites: Springfontein (S33°37'16.79", E18°23'04.17"), and Schaap Island, Yzerfontein, (S33°22'33.81", E18°08'07.58") on the West Coast of South Africa.

Succession was monitored at one-, two-, four-, six-, eight- and ten-month sampling periods, by estimating percentage cover of all taxa in the field, supplemented by photographs. At the end of the experiment the treatments were destructively sampled. All organisms within the plots were identified, counted and wet weighed. Percentage cover and biomass data generated similar results for all taxa except encrusting algae, which were better quantified as percentage cover because they fragmented when scraped. For organisms with an upright conformation, biomass is a more appropriate measurement of abundance than percentage cover. As a result, I presented data for biomass of all groups except for encrusting algae, for which percentage cover data were used. Percentage cover data were however, used to investigate differences in community composition over time, as biomass data were available only for the final period when treatments were destructively sampled. Samples were taken both around the edge of plots (in four areas of 500 cm^2 each) and in a comparable area of 500 cm^2 in the centre of the plot, but as these generated similar results, I present only the data for the centre of the plots.

To determine the size composition of mussels found within treatments at the end of the experiment, mussels from each treatment, at each of three shore heights, were measured using Vernier callipers to 0.1mm accuracy, and size-frequency plots constructed.

Statistical analyses

Multivariate analyses were conducted in PRIMER v6. Data for community composition were fourth-root transformed to reduce the effect of extreme values, and subjected to Bray-Curtis resemblance analyses. MDS ordinations were used to assess differences in community structure among treatments and shore heights at each site. PERMANOVA analyses were performed to test whether differences in community structure were statistically significant. For these analyses, shore height was nested within site and treatment within shore height. Post-hoc pairwise tests were conducted among shore heights, and treatments. SIMPER analyses identified which species contributed most to dissimilarity among treatments. The following diversity indices were calculated using the DIVERSE function: (1) species richness (S); (2) Shannon-Wiener diversity (H'); (3) Margalef's diversity index (d) and (4) Pielou's evenness (J'). As similar patterns emerged from the first three and the last did not yield any significant differences, only data for species richness are presented.

The assumptions of normality and homogeneity of variances were tested through visually assessing a normality ordination and Levene's test. Where assumptions of normality or homoscedasticity could not be met despite transformations, non-parametric tests were performed. In particular, due to heteroscedasticity, Mann-Whitney U-tests were performed to determine whether diversity indices differed between sites, using Statistica 12.

Species and functional groups that contributed up to 80% of differences among treatments in SIMPER analyses were analysed individually. Functional groups consisted of: (1) algae (17 macroalgal species combined); (2) barnacles (*Balanus glandula*, *Chthamalus dentatus*, *Notomegabalanus algicola*); (3) infauna (the isopod *Ischyromene huttoni*, the amphipods *Hyale grandicornis* and *Paramoera capensis*, and the nereid worms *Pernereis nuntia vallata* and *Pseudonereis capensis*); (4) predatory whelks (*Burnupena lagenaria*, *Nucella dubia*, *Nucella squamosa*, *Trochia cingulata*) and anemones (*Bunodactis reynaudi* and *Anthothoe stimpsoni*). Individual attention was given to three species because of their distinctive responses: the limpet *Scutellastra granularis*; the anemone *Bunodactis reynaudi*, and the pulmonate seaslug *Onchidella maculata.* Two-way factorial ANOVAs and Tukey's post-hoc tests were performed to determine whether differences in abundance of these taxa were significant among treatment, shore height and the interaction between these factors.

Community composition was also investigated over three time periods at each site: (A) March 2013, two weeks after the start of the experiment, (B) June 2013, and (C) March 2014 at the end of the experiment. MDS ordinations were used to portray whether community structure at each site differed among treatments, shore heights, and over time. To further investigate the causes of differences in community structure over time, data for four time periods were used to compare diversity indices: March 2013, April 2013, June 2013 and March 2014, based on percentage cover as a measure of abundance, as biomass data were available only for the final period. To avoid pseudo-replication, repeated measures ANOVAs were conducted. To determine whether diversity indices were significantly different over time, among shore heights and treatments, and whether there were any interactions between factors, ANOVAs were performed.

5.2.2 Caging experiment

The caging experiment had three main aims: to explore interactions between *S. algosus* and *M. galloprovincialis*, to do so at different shore heights, and to test for intra- and inter-specific density effects. It was conducted at Springfontein (S33°37'16.79" E18°23'04.17") and consisted of eight treatments, *i.e.*, five caged, and three ‘Cage controls’. The caged treatments consisted of (1) 15 *Mytilus galloprovincialis* plus 15 *Semimytilus algosus* per cage (hereafter called the ‘combination treatment’); (2) 30 *M. galloprovincialis*, zero *S. algosus* (high density *M. galloprovincialis*); (3) zero *M. galloprovincialis*, 30 *S. algosus* (high density *S. algosus*); (4) 15 *M. galloprovincialis* and zero *S. algosus* (low density *M. galloprovincialis*); (5) zero *M. galloprovincialis* and 15 *S. algosus* (low density *S. algosus*). Treatments 6, 7 and 8 repeated the combinations of mussels in 1, 2 and 3, but instead of retaining the mussels in cages, they were installed in mesh tunnels of comparable size that were removed once the mussels had attached themselves to the rock face, thus creating a cage-less treatment that could be compared with equivalent caged treatments to assess potential cage effects.

The caged treatments were set up at three shore heights, *i.e.* low, mid and high shore and the cage controls at two shore heights (low and high shore), with four replicates of each treatment at each of these shore heights. For treatments 1-8, the substratum was scraped to remove all biota in 10x10cm quadrats. Adult mussels (20-50mm) were collected from the low shore, rinsed and/or cleaned to remove all epibionts, and installed inside the scraped areas in combinations as per the relevant treatment. A band of approximately 10cm was cleared around these installations to minimise reintroduction of epibionts from adjacent mussels. The installed mussels were then covered with cheesecloth to hold them in position temporarily, to allow them to attach to the substratum. The cheesecloth disintegrated and disappeared within 3-7 days. Wire cages of 10x10cm with a height of 6cm and a 5cm overhang, constructed out of stainless-steel 2-mm mesh, were placed over the each of the replicates. Cages were roofed with stainless steel 5-mm mesh, which was attached to the top of the cages with cable ties so that the roofs could easily be removed during monitoring and processing (Fig. 5.1). For the cage controls (treatments 6-8), mesh tunnels of the same size as the cages were constructed from plastic mesh and attached to the substratum over batches of mussels held in position by cheesecloth. The mesh tunnels were removed after one month, leaving the mussels uncaged.

Survival in all treatments was monitored by counting the remaining mussels in each cage after two weeks, one, two, four and six months after the experiment was set up. The installations were destructively sampled at the end of the six months to determine the community composition within treatments, as well as the growth, condition and recruitment of the mussels. To determine the growth of mussels within treatments, notches were filed in the growing edge of the mussel shells at the start of the experiment. Growth was measured at the end as the difference in length between the notch and the new growing edge, using

Vernier callipers accurate to 0.1mm. The body condition of surviving *M. galloprovincialis* and *S. algosus* specimens was determined at the end of the experiment. Condition index was obtained by dissecting out the flesh of each mussel and drying it in an oven at 54°C for 48h, then calculating condition index (as per Steffani and Branch 2003) as the ratio between dry flesh weight (g) and shell length (mm). Recruits (1-5mm) were identified and counted in all samples.

As a complementary procedure to expand exploration of the relationship between recruitment and adult density, randomly selected natural clumps of mussels of variable density were caged to create conditions comparable with caged treatments (hereafter called the control+cage treatment). Size-frequency plots were also constructed for this treatment at the end of the experiment to obtain an indication of size frequencies and relative abundances at different shore levels, in the natural population. Data were scaled up to represent mean numbers per square meter for 5-mm size classes.

Condition was also calculated for mussels in these controls, and compared to that of mussels in unmanipulated patches without cages (hereafter called 'uncaged controls'), to determine cage effects.

Statistical analyses

To determine whether growth, recruitment and survival were significantly affected by species, density, shore height and their interactions, three-way factorial ANOVAS were conducted, with species, shore height and treatment as fixed factors. Because condition index is related to the size of the mussels and *S. algosus* was significantly smaller than *M. galloprovincialis*, two-way factorial ANOVAS were conducted for the two species separately. Post-hoc Tukey's tests were performed to determine which of the factors explained differences and to explore any interactions.

To determine whether cages had an effect on the four variables measured (growth, survival, condition and recruitment), cages were compared to cage controls within equivalent treatments and shore heights. Three-way factorial ANOVAs were conducted with plots (e.g. cage or cage control) and treatment as fixed factors and shore height as a random factor. Differences between individual treatments within zones were accepted as significant if there was no overlap in their 95% confidence intervals.

To investigate whether communities differed among caged treatments, as well as among cage controls, PERMANOVA analyses were performed. Data were fourth-root transformed to reduce the effect of extreme values, and subjected to Bray-Curtis resemblance analyses. Treatment was nested within shore height. DIVERSE and SIMPER analyses were also conducted as for the clearance experiment. Two-way factorial ANOVAs were performed to determine whether diversity indices were significantly different among shore heights and treatments.

Data were analysed using Primer version 6+ (Anderson et al. 2008; Clarke & Gorley 2006) and Statistica 12 (Statsoft 2012).

5.3 Results

5.3.1 Clearance experiment:

Comparison of community composition between sites

PERMANOVA indicated no significant difference in community structure between sites (Pseudo-F_1=1.29, P(perm)=0.32). The MDS ordination showed that the communities at sites were closely related except for the low-shore samples, where the sites formed separate clusters (Fig. 5.2). None of the diversity indices were significantly different between sites (Table 5.1), but in reporting the data below, I have shown the two sites separately.

Comparisons among shore heights and treatments

At the end of the experiment, community composition was significantly different among shore heights and treatments (PERMANOVA: Pseudo-F_4=2.73, P(perm)=0.001; Pseudo-F_{12}=2.54, P(perm)=0.001 respectively). Treatments were significantly different, depending on site and shore height (Table 5.2).

ANOVAS revealed that all four measures of diversity (number of species, Margalef's diversity, Shannon-Wiener diversity and Pielou's evenness) differed significantly among shore heights, bar one exception: Pielou's evenness index J' at Springfontein (Table 5.3). Post-hoc comparisons indicated that these differences were attributable to the low shore having significantly greater values than the mid- and high-shore, which did not differ. Treatment rarely had a significant effect.

Community composition over time

Differences in community composition among shore heights (based on percentage cover) became more pronounced over time, whereas differences among treatments were initially clear-cut, but became less prominent over time (Fig. 5.3).

In March 2013, one month after commencement of the experiment, community structure was significantly different among treatments at both Springfontein (Pseudo-F_6=10.35, P(perm)=0.001) and Yzerfontein (Pseudo-F_6=8.27, P(perm)=0.001). However, shore heights were not at that stage significantly different (P(perm)>0.05). Pairwise tests reported that at all shore heights the natural treatment was significantly different from the removal and settlement treatments, but the latter two did not differ significantly (Fig. 5.3A).

By June 2013, PERMANOVA detected significant differences among shore heights at Springfontein, with the low shore being significantly different from the other shore heights (low:mid t=2.60, P(perm)=0.01; low:high t=2.78, P(perm)=0.02). Shore heights were not yet significantly different at Yzerfontein, despite the low shore separating from other zones in the

MDS. Community structure no longer differed significantly among treatments at either site (Fig. 5.3B).

In March 2014, both shore height (Pseudo-F_4=4.83, P(perm)=0.02) and treatments (Pseudo-F_6=2.51, P(perm)=0.001) were significantly different at Springfontein, with the MDS ordination clearly showing a separation between the low shore and the other two shore heights (Fig. 5.3C). PERMANOVA once again indicated that the natural treatment was significantly different from the other treatments, but only on the low and mid shore (P(perm)<0.03 in all cases). At Yzerfontein only shore height was significantly different (Pseudo-F_4=5.63, P(perm)=0.01), the MDS ordination showing that the low shore formed a separate cluster from the mid and high shore (Fig. 5.3C).

Repeated measures ANOVAs reported that at both sites species richness, Shannon-Wiener and Margalef's diversity were significantly different among months, shore heights and treatments. Only species richness is shown (Fig. 5.4), as the other two indices showed the same patterns. Post-hoc comparisons showed that treatments differed in March 2013 and sometimes April 2013, but not thereafter, and that treatment effects were significant only in the mid and low shore, hence significant interactions between time x shore height, and time x treatment. Two patterns emerged. First, treatments never differed significantly in the high shore. Second, while treatments did differ in the mid and low shore, the differences disappeared quite quickly, never being perpetuated beyond the second sampling period. Third, when differences did exist, values for the natural treatment were higher than in the disturbance treatments.

Algal assemblages (Fig. 5.5) showed clear succession over time in the disturbance treatments at Springfontein. Diatoms dominated the low shore in March 2013, followed by ephemeral algae in June 2013 and corticated algae in March 2014 samples. In the natural treatment corticated and ephemeral algae co-existed most of the time and their combined percentage cover was always lower that in either of the disturbance treatments.

At Yzerfontein algae did not follow the same succession. Ephemeral algae dominated the algal population in all three treatments, and in the majority of the four periods. However, the natural treatment had an even spread of corticated algae over time. This was not the case in the disturbance treatments, which only supported corticated algae in one month each (removal-April; settlement-June). Cover of all algae combined was greater in the disturbance treatments than the natural treatment (Fig.5.5), as was the case at Springfontein as well.

Biomass and species responses

At the end of the experiment, mean biomass for all species combined (Fig. 5.6) was highest on the low shore at both sites, but only significantly so at Springfontein ($F_{2,27}$=39.52, p<0.001). Treatments were also only significantly different at Springfontein, and only on the low shore ($F_{2,27}$=6.70, p=0.004), with a significant interaction between these factors ($F_{4,27}$=4.99, p=0.003), because of an unexpectedly high biomass in the removal treatment caused by prolific settlements of *S. algosus* and *A. atra* (see below).

Three mussel species were recorded within each of the three treatments at both sites: *Semimytilus algosus*, *Mytilus galloprovincialis* and *Aulacomya atra* (Fig. 5.7). A comparison at each site showed that at Springfontein *M. galloprovincialis* had significantly greater mean biomass (460g.500cm^{-2}) than *A. atra* (146g.500cm^{-2}) or *S. algosus* (71g.500cm^{-2}) ($F_{2,105}$=8.22, p<0.001) over all shore heights and treatments. The latter two species did not differ significantly from each other. At Yzerfontein *M. galloprovincialis* also had the highest mean biomass (402g.500cm^{-2}), but did not differ significantly from *S. algosus* (200g.500cm^{-2}). However, *A. atra* had significantly lower biomass (7g.500cm^{-2}), compared to the other two species ($F_{2,105}$=10.03, p<0.001), over all shore heights and treatments.

Biomass was always significantly greater on the low shore, for all three mussel species and at both sites ($p<0.0001$ in all instances), except in the case of *M. galloprovincialis* at Yzerfontein. At Springfontein there were significant interactions between shore height and treatment for *A. atra* and *S. algosus* ($F_{2,105}=4.97$, $p<0.001$; $F_{2,105}=51.1$, $p<0.001$ respectively). At Yzerfontein *A. atra* biomass was significantly higher on the low shore ($F_{2,105}=17.59$, $p<0.001$) and in the removal treatments ($F_{2,105}=4.11$, $p=0.03$), with a significant interaction between the two factors ($F_{4,105}=3.51$, $p=0.02$) as these differences were specific to the low shore. Neither of the factors was significantly different for *M. galloprovincialis* at Yzerfontein.

The size-composition data (Figs 5.8 & 5.9) showed several trends. (1) At both sites, *S. algosus* was concentrated in the low shore, to the point that it was virtually absent from the mid and high shore at Springfontein. (2) *Aulacomya atra* settled at all shore levels but rarely achieved adult sizes (and then only in natural plots). (3) *Mytilus galloprovincialis* occurred at all shore heights, generally in greater abundance in the natural plots, and attained sizes that were indicative of arrival by lateral immigration as well as recruitment. (4) At Springfontein, *A. atra* recruitment was greatest in the disturbance treatments, a possible indication of competition between it and the other two mussel species at this site. However, at Yzerfontein its recruitment was equal between the natural and removal treatment, and relatively low in the settlement treatment (Fig. 5.9).

SIMPER analyses conducted among treatments, within shore heights and sites, reported that (excluding mussels) 17 of the 40 species that contributed 80% of the dissimilarity among treatment were algae, three were barnacles, six were species that feed on mussels and five were species that find refuge within mussel beds (nereid worms, isopods and amphipods), eight were herbivores, and one was the sponge *Hymeniacidon perlevis* (see methods for other species names).

Algal species (Fig. 5.10A) were significantly more abundant on the low shore (Springfontein: $F_{2,27}$=8.41, p=0.001; Yzerfontein: $F_{2,27}$=9.69, p<0.001), but did no differ among treatments, despite its biomass being markedly higher in the disturbance treatments at Springfontein.

Barnacle assemblages consisted of three species. On the high shore, all barnacles were *Balanus glandula*. On the mid shore, 55% of barnacles were *B. glandula* and 45% *Chthamalus dentatus*. On the low shore, barnacles occurred on mussels and consisted of 65% *Notomegabalanus algicola* and 35% *B. glandula*. Barnacles were combined for the analyses (Fig. 5.10B) and its biomass was found to be significantly different among shore heights ($F_{2,27}$=22.18, p<0.001) and treatments ($F_{2,27}$=4.73, p=0.02), with a significant interaction between the two factors ($F_{4,27}$=4.62, p=0.01) at Springfontein, where barnacle biomass was greatest on the low shore, and significantly greater in the natural treatment than other treatments at that shore height. Yzerfontein showed no significant difference for barnacles, among either of the factors, although they followed the same pattern of greater biomass low on the shore that was displayed at Springfontein.

Infaunal species (Fig. 5.10C) were significantly different among shore heights at Springfontein ($F_{2,27}$=6.02, p=0.01), with biomass being highest on the low shore. Infaunal biomass was significantly different among treatments at Yzerfontein ($F_{2,27}$=6.07, p=0.01), the removal treatment having significantly lower biomass than the natural treatment in the mid and high shore.

Predators (Fig. 5.10D) had significantly less biomass on the high shore than low shore at both sites (Springfontein: $F_{2,27}$=3.76, p=0.04; Yzerfontein: $F_{2,27}$=5.99, p=0.01). Treatments did not differ significantly, although predators were, without exception, always more abundant in the natural treatment.

Three individual species that contributed to the SIMPER analyses also showed distinct responses among factors. *Scutellastra granularis* biomass (Fig. 5.11A) was significantly greater in the natural than in the removal treatment at Springfontein ($F_{2,27}$=4.80, p=0.02). At Yzerfontein the mid shore had significantly higher biomass than the low shore ($F_{2,27}$=7.10, p=0.003). There was also a significant interaction between shore height and treatment ($F_{4,27}$=4.68, p=0.005), which post-hoc tests revealed was because of its higher mid-shore abundance in the settlement treatment.

Bunodactis reynaudi (Fig. 5.11B) was severely depleted in the disturbance treatments, at both sites. At Springfontein its biomass was significantly different among shore heights and among treatments ($F_{2,27}$=178.80, p<0.001; $F_{2,27}$=157.91, p<0.001 respectively) with a significant interaction between factors ($F_{4,27}$=126.93, p<0.001) because significant treatment effects were limited to the low shore. At Yzerfontein *B. reynaudi* was significantly different only among treatments ($F_{2,27}$=3.68, p=0.04), having significantly greater biomass in the natural than the disturbance treatments in the mid and high shore.

Onchidella maculata (Fig. 5.11C) also showed a reduction in disturbance treatments. At Springfontein, its biomass was significantly different among shore heights and treatments ($F_{2,27}$=4.80, p=0.02; $F_{2,27}$=12.86, p<0.001 respectively), with a significant interaction between factors ($F_{4,27}$=4.83, p=0.004), being greater in the natural treatment than the disturbance treatments in the mid shore. At Yzerfontein, although the differences were non-significant, the biomass of *O. maculata* in the natural treatment was strikingly greater than in the disturbance plots in the mid shore, repeating the pattern at Springfontein.

5.3.2 Caging experiment:

Growth

In cages, growth rate (Fig. 5.12) was consistently higher on the low shore for both *M. galloprovincialis* and *S. algosus*, and *M. galloprovincialis* grew significantly faster than *S. algosus*. Growth was not significantly different among treatments ($F_{2,638}$=0.6, p=0.5), but was significantly greater on the low shore that at other shore heights ($F_{2,638}$=104.34, p<0.001) and between species ($F_{1,638}$=44.89, p<0.001). The interaction between species and shore height was also significant ($F_{2,638}$=8.51, p<0.001), but no other interactions were significant. Tukey's HSD tests revealed that growth of *M. galloprovincialis* was significantly greater than that of *S. algosus* in: (1) single-species treatments of 15 mussels per cage on the low shore (p<0.001), (2) single-species treatments with 30 mussels per cage on the low (p<0.001) and mid shore (p<0.001), and close to significant on the high shore (p=0.05); (3) in combination treatments on the mid shore only (p=0.001). On the low shore, the growth of *S. algosus* was greatest in the combination treatment, compared to the other two treatments, but not significantly so (high density: mean=4.59mm, SE=0.81; low density: mean=4.61mm, SE=0.73; combination: mean=9.32mm, SE=4.20).

Growth in cage controls could only be determined for *M. galloprovincialis*, due to high mortality for *S. algosus*, which reduced numbers to the point where its growth could not be evaluated. *Mytilus galloprovincialis* growth was not significantly different between cages and cage controls ($F_{1,337}$=0.005, p=0.94), showing that cages had no significant effect on the growth of this species (Fig. 5.13A). Patterns of growth for *M. galloprovincialis* in cage controls followed those in cages, being significantly higher on the low than the high shore ($F_{1,337}$= 59.32, p<0.001), but not differing significantly among treatments ($F_{1,337}$=2.89, p=0.09). There was no interaction between shore height and treatment ($F_{1,337}$=0.43, p=0.51).

Condition

The condition index (Fig. 5.12) was not significantly different for *S. algosus* for either shore height ($F_{2,164}$=2.33, p=0.1) or treatment ($F_{2,164}$=0.97, p=0.38). Both factors were, however, significant for *M. galloprovincialis*, with a significant interaction between them (Shore height: $F_{2,476}$=27.73, p<0.001; Treatment: $F_{2,476}$=7.83, p<0.001; shore height x treatment: $F_{4,476}$=4.17, p=0.002). For high-density treatments, *M. galloprovincialis* was in better condition on the low shore (mean=9.89, SE=0.31) and mid shore (mean=9.96, SE=0.38), compared to the high shore (mean=5.49, SE=0.48, p<0.001 in both cases). In the combination treatments differences were significant between the high (mean=7.36, SE=0.62) and low shore (mean=11.31, SE=0.80, p<0.001), but neither of these differed significantly from the mid shore (mean=9.63, SE=0.65). The condition index of *M. galloprovincialis* was not significantly different among the various density treatments in the low and high shore (p>0.05), except between the combination treatment (mean=11.31, SE=0.80) and the low density treatment (mean=7.76, SE=0.50), on the low shore (p=0.02). on the mid shore, condition was significantly less in the low density treatment (mean=8, SE=0.62) than in the high density treatment (mean=9.96, SE=0.38, p=0.03). *Semimytilus algosus* also had a higher condition index in the high density (mean=7.39, SE=1.6) and combination (mean=7.59, SE=2.6) treatments, compared to the low density treatment (mean=3.48, SE=0.8) on the mid shore, though not significantly so (p>0.05). However, on the high shore *S. algosus* had the lowest condition in the combination treatment (combination: mean=1.38, SE=0.01; high density: mean=9.93, SE=2.8; low density: mean=11.41, SE=4.1; although the differences were again not significant: p>0.05).

In cage controls, due to high mortality of *S. algosus*, condition could only be determined for *M. galloprovincialis* Fig. 5.13B). Cages negatively affected condition of *M. galloprovincialis*, with cage controls having significantly higher condition than mussels in cages, in both the combination and high density treatments ($F_{1,312}$=64.55, P<0.001). *Mytilus galloprovincialis* condition was also compared among controls+cage and uncaged controls. Controls without cages had significantly higher condition than controls with cages ($F_{1,312}$=58.12, p<0.001). Both outcomes indicate cages had a negative effect on condition. Within cage controls, *M. galloprovincialis* condition was not significantly different between shore heights ($F_{1,78}$=1.3, p=0.26), or between density treatments ($F_{1,78}$=1.22, p=0.27), nor was there any interaction between these factors (F_1=0.001, p=0.97).

Recruitment

In cages, *M. galloprovincialis* had significantly higher recruitment than *S. algosus* (Fig. 5.12; $F_{1,54}$=14.95, p<0.001). There was an interaction between species and shore height ($F_{2,54}$=3.28, p=0.04), as recruitment of the two species were not significantly different on the high shore (p>0.05).

Recruitment of *M. galloprovincialis* was significantly less in the high than the mid or low shore (p=0.02), but low and mid shore recruitment was not significantly different (p>0.05). Treatments did not differ significantly ($F_{2,54}$=1.84, p=0.17). Recruitment of *S. algosus* could not be statistically examined because of zero variance at two shore heights, but its recruitment was limited to the low shore (Fig. 5.12). Several patterns emerged (1) Recruitment for *M. galloprovincialis* was highest on the low and mid shore. (2) *Semimytilus algosus* recruitment was limited to the low shore. (3) *Mytilus galloprovincialis* recruitment was higher in cages containing 30 *M. galloprovincialis* (mean=357, SE=164) than those with 15 *M. galloprovincialis* (mean=119, SE=78) but not significantly so. (4) Recruitment of *M. galloprovincialis* was always greater than that of *S. algosus* at all three shore levels.

Comparisons showed that *M. galloprovincialis* recruitment within the cages was significantly greater than that within cage controls ($F_{1,24}$=19.41, p<0.001; Fig. 5.13C). Shore height also had a significant effect within cage controls ($F_{1,24}$=11.51, p=0.002), being 20-fold greater on the low shore than on the high shore. A significant interaction existed between the cage effect and shore height ($F_{2,24}$=9.71, p<0.001), as the cage effect was stronger on the low shore than on the high shore (Fig.5.13C).

There was a clear relationship between adult density and recruitment for *M. galloprovincialis* when data for all treatments and control plots were combined (Fig. 5.14), and recruitment declined up the shore. This relationship could not be established for *S. algosus* due to its relatively low recruitment and zero values for recruits in the mid and high shore samples.

Survival

Mytilus galloprovincialis had a significantly higher survival rate than *S. algosus* at all shore heights and across all treatments ($F_{1,54}$=48.46, p<0.001). Survival of both species was not significantly different among shore heights ($F_{2,54}$=2.02, p=0.14), but post-hoc comparisons revealed that survival of *M. galloprovincialis* was significantly less in the low shore than either the mid or high shores (Fig. 5.12). Survival was also not significantly different among treatments ($F_{2,54}$=1.10, p=0.34). There were no interactions between the factors.

Within cage controls survival was again significantly greater for *M. galloprovincialis* than for *S. algosus* ($F_{1,24}$=14.36, p=0.001). Shore height had no significant effect ($F_{1,24}$=1.60, p=0.22). The effects of treatment were difficult to test because *Semimytilus algosus* survived only in the combination treatments, and even there, survival was very low (Low shore: mean=0.75, SE=0.48; High shore: mean=0.25, SE=0.25). *Mytilus galloprovincialis* had significantly greater survival in the high density treatment on the high shore, compared to the other treatments ($F_{1,48}$=0.83, p<0.05). None of the other treatments differed significantly (Fig 5.13D).

Mytilus galloprovincialis survival was significantly different between cages and cage controls ($p<0.001$), being consistently higher within cages (Fig. 5.13D), at both the low and high shore, and in both the high density and combination treatments.

Size-frequency plots for control+cage treatments (Fig. 5.15) showed that recruits of *S. algosus* (<5mm) were confined to the low shore, and while isolated juveniles (5-20mm) were located in the mid to high shore, adults (>20mm) were also recorded only on the low shore. *Mytilus galloprovincialis* recruits occurred at all shore levels but at decreasing frequencies up the shore. Adults of *M. galloprovincialis* (>30mm) displayed the same pattern (Fig. 5.15), and the size distribution was truncated in the high shore, where adults were practically absent.

Community structure within caged and cage control treatments

Community structure in cages at the end of the experiment was significantly different among shore heights (Pseudo-$F_2=5.39$, P(perm)=0.001), and among treatments (Pseudo-$F_{15}=1.97$, P(perm)=0.001). Pairwise PERMANOVA reported that all three shore heights differed significantly from each other ($p<0.002$ in all cases). Treatments were all significantly different from each other on the low shore, apart from (1) the low density treatments of the two species, and (2) the low density treatment for both species and the combination treatment. In the mid shore, none of the treatments differed significantly. On the high shore the low density *S. algosus* treatment was significantly different from its high density counterpart and from the low density *M. galloprovincialis* treatment (Table 5.4).

Factorial ANOVAs reported that both species richness (S) and Shannon-Wiener diversity (H′) were significantly different among all shore heights (S: $F_{2,41}=24.62$, $p<0.001$; H': $F_{2,41}=22.15$, $p<0.001$) and among treatments (S: $F_{5,41}=4.52$, $p=0.004$; H': $F_{5,41}=3.67$, $p=0.01$). Only species richness is shown (Fig. 5.16), as the Shannon-Wiener diversity index displayed the same outcomes. Post-hoc tests showed that richness differed significantly among all three shore heights ($p<0.05$ in all comparisons). On the low shore, both indices were significantly less in the low density *S. algosus* treatment, relative to all the high density treatments, with the low-density *M. galloprovincialis* treatment being intermediate. The high density treatments had the highest species richness and diversity, similar to those of combination treatments. On the mid and high shores, species richness and Shannon-Wiener diversity were not significantly different among treatments, and the same was true in the high shore, although the low-density *S. algosus* treatment consistently had the lowest values. Neither Pielou's evenness, nor Margalef's diversity differed among shore heights (J': $F_{2,41}=2.59$, $p=0.09$; d: $F_{2,41}=1.28$, $p=0.29$) or treatments (J': $F_{4,41}=0.41$, $p=0.80$; d: $F_{4,41}=0.55$, $p=0.69$). Comparing equivalent densities, there were no significant differences in diversity between the two mussel species.

SIMPER analyses were conducted and the species that contributed to 80% of the dissimilarity were compared among treatments, pooled across heights (Fig. 5.17). ANOVAs reported that biomasses of only four of the 28 species were significantly different among treatments. The barnacle *Notomegabalanus algicola*, which occurs on mussel shells, was more abundant in the high density *M. galloprovincialis* cages than in other treatments, and notably absent or rare in cages housing both high density and low density *S. algosus*. *Burnupena lagenaria*, a whelk that feeds on mussels, was also more abundant in high-density *M. galloprovincialis* cages. The alga *Tayloriella tenebrosa* was not found in the *M. galloprovincialis* treatments but its biomass values were low overall, so no great importance should be attached to this difference. The amphipod *Paramoera capensis* was absent from the low density *M. galloprovincialis* treatment, but its biomass was likewise consistently low. *Polysiphonia* sp. occurred only in the combination treatment, as did two other algae – *Endorachne binghamiae* and *Laminaria pallida*. The overall conclusion is that individual species differed relatively little among the treatments

Community structure of cage controls was not significantly different between shore heights (Pseudo-F_1=2.09, P(perm)=0. 12) or among treatments (Pseudo-F_3=1.25, P(perm)=0.33). None of the diversity indices for cage controls were significantly different among shore heights or treatments (p>0.05) either.

Community structure was not significantly different between cages and cage controls (Pseudo-F_1=0.68, P(perm)=0. 62). When diversity indices were compared between cages and cage controls, Shannon-Wiener diversity (U=2, p<0.001), Margalef's diversity (U=23.85, p=0.007) and Pielou's evenness (U=29, p=0.01) were significantly greater in cages than cage controls.

5.4 Discussion

The hypotheses that I advanced in the Introduction were addressed through the twin avenues of the clearance experiment (which concentrated on community responses to removal of mussels) and the caging experiment (with a focus on the effects of shore height and interactions of *M. galloprovincialis* and *S. algosus* and their influences on life history characteristics of the two species). Hypothesis 1 is attended to by both approaches, Hypothesis 2 by the clearance experiment, and Hypotheses 3-7 by the caging experiment. Tables 5.5 and 5.6 summarise the clearance and the caging experiments respectively. Both showed that mussels strongly affected community composition.

5.4.1 Clearance experiment:

I hypothesised that *M. galloprovincialis* and *S. algosus*, as ecosystem engineers, would increase diversity relative to plots from which they were removed, but would exclude some species through competition for space. Mussels provide complex habitat structure that offers refuge for small invertebrates from predation and physical stress (Bertness et al. 2006; Borthagaray & Carranza 2007). They further increase biodiversity by providing substratum for settlement by other organisms such as barnacles and limpets (Griffiths et al. 1992; Hockey & Van Erkom Schurink 1992; Firstater et al. 2010). *Mytilus galloprovincialis* exemplifies the effects of ecosystem engineers on rocky shore communities in South Africa. Robinson et al. (2007a) found that it increases species richness and invertebrate density on the mid shore for three reasons. Firstly, physical stress is alleviated by the presence of *M. galloprovincialis*. Secondly, habitat complexity is increased by the mussels. Lastly, habitat becomes less patchy due to mussel beds dominating a large proportion of the rock face. In Peru colonisation by *S. algosus* has also been shown to increase species richness (Tokeshi & Romero 1995).

The importance of shelled molluscs as ecosystem engineers depends on specific shell traits, as well as the spatial arrangement of the shells (Gutiérrez et al. 2003; Firstater et al. 2010). In Peru, Tokeshi et al. (1989) reported that, relative to beds of *Perumytilus purpuratus*, *S. algosus* beds support higher densities and larger individuals of the polychaete *Pseudonereis gallapagensis*. They attributed this to the facts that (a) *S. algosus* occurs on the low shore where desiccation stress is reduced, and (b) *S. algosus* provides more and larger interstitial spaces for the polychaete to inhabitat, because it forms a multi-layered, loosely attached mussel bed. In South Africa, *M. galloprovincialis* beds have been shown to support a greater infaunal abundance than *Aulacomya atra* because they form deeper beds (Griffiths et al. 1992). The larger, smoother shells of *M. galloprovincialis* also provide better space for colonisation by the limpet *Scutellastra granularis* (Griffiths et al. 1992). In general *S. algosus* beds did not support *S. granularis* communities, due to its smaller shell. *Semimytilus algosus* is also likely to exclude larger limpets, such as, *Scutellastra argenvillei* and *S. cochlear* through competition for space. However, in the removal experiment this was not unambiguously demonstrated as mussel beds contained a mix of *M. galloprovincialis* and *S. algosus* and removal involved removal of both species. However at Springfontein *S. granularis* biomass was significantly reduced in the absence of mussels.

In my clearance experiments, community composition was significantly different among shore heights and treatments at the end of the experiment. A comparison of community composition over one year showed clearly that as the disturbance plots recovered, nearing diversity in the natural treatment, shore height effects became more pronounced and treatment effects diminished. These results were expected, as studies have shown that abiotic factors such as desiccation cause a gradient in species richness and abundance up the shore (Connell 1972; Bustamante et al. 1997), with distinct communities being established at different shore levels (Boaventura et al. 2002). Disturbance is also known to increase diversity on rocky shores by removing dominant species and creating space for recolonization by other species (Dayton 1971; Lubchenco & Menge 1978). However, it may also decrease diversity (Addessi 1994). With regards to the treatment effects, I found that removal of mussels reduced diversity initially but relatively rapid encroachment by new mussels and/or colonisation by algae allowed a recovery of diversity within two to four months. Algae are also known to be engineering species (Hull 1997; Chapman et al. 2005), and they may have supported a similar species richness and diversity to mussel beds. Robinson et al. (2007a) reported that although community structure on the low shore changed when *M. galloprovincialis* beds replaced *A. atra* beds, total species richness did not change, because *M. galloprovincialis* did not change the nature of the habitat available. Ecosystem engineers increase species richness at landscape level, when: (a) there are species that are limited to the newly engineered habitat for at least part of their life cycle (Wright et al. 2002) or (b) the engineer can provide an environment different from other available substrata (Borthagaray &

Carranza 2007). Algae are also known to support unique epifaunal assemblages (Dean & Connell 1987). Specifically, corticated algae have a complex structure that provides refugia for epifuanal species such as amphipods (Gibbons 1988; Parker et al. 2001).

In my clearance experiments, community composition, diversity and biomass did not differ between sites, but the successional sequence and the responses of individual species did differ. Thus, while broad patterns were consistent at both sites, details of individual species' responses were not. Community composition was significantly different among shore heights and treatments at the end of the experiment. A comparison of community composition over one year showed clearly that as the disturbance plots recovered, nearing diversity in the natural treatment, shore height effects became more pronounced and treatment effects diminished. These results were expected, as studies have shown that abiotic factors such as desiccation cause a gradient in species richness and abundance up the shore (Connell 1972; Bustamante et al. 1997). Disturbance is also known to increase diversity on rocky shores by removing dominant species and creating space for recolonization by other species (Dayton 1971; Lubchenco & Menge 1978). However, it may also decrease diversity (Addessi 1994). With regards to the treatment effects, I found that removal of mussels reduced diversity initially but relatively rapid encroachment by new mussels and/or colonisation by algae allowed a recovery of diversity within two to four months. Algae are also known to be engineering species (Hawkins & Hartnoll 1983; Hull 1997; Chapman et al. 2005; Crowe et al. 2011), and they may have supported a similar species richness and diversity to mussel beds. Robinson et al. (2007a) reported that although community structure on the low shore changed when *M. galloprovincialis* beds replaced *A. atra* beds, total species richness did not change, because *M. galloprovincialis* did not change the nature of the habitat available. Ecosystem engineers increase species richness at landscape level, when: (a) there are species that are limited to the newly engineered habitat for at least part of their life cycle (Wright et al. 2002) or (b) the engineer can provide an environment different from other available substrata (Borthagaray & Carranza 2007). Algae are also known to support unique epifaunal assemblages (Dean & Connell 1987). Specifically, corticated algae have a complex structure that provide refugia for epifaunal species such as amphipods (Gibbons 1988; Parker et al. 2001).

Worldwide, rocky-shore algal succession after disturbance often follows a regular pattern. Diatoms are frequent early successional species, but quickly disappear as ephemeral algae appear, followed by corticated algae (McCook & Chapman 1997; Kim 1997; Robinson et al. 2008). Removal or thinning of grazers such as limpets (which was associated with removal of mussels in my experiments) often leads to a burgeoning of ephemeral algae, which decline as grazers return, and are replaced by corticated algae (Jenkins et al. 2005; Hawkins et al. 2008). Branch et al. (2013a) found that ephemeral algae appear early in succession after disturbances, followed by perennial corticated algae, which contributed to a decline in the former, aided by the effects of grazers when they become established. I found that in both disturbance treatments at Springfontein, algae showed a clear successional sequence that followed this pattern. Diatoms dominated at first, but were replaced by ephemeral algae, which were supplanted by corticated algae after approximately four months. In natural treatments, ephemeral and corticated algae co-existed, with the former being most abundant, and neither achieved the cover they attained in the disturbance plots. The overall biomass of ephemeral algae was also higher in the absence of mussels than in the natural treatments, at both sites. Yzerfontein did not show the same clear successional pattern, but had high ephemeral algal biomass, co-occurring with corticated algae. Though the sites showed different successional responses to the removal of mussels, both sites displayed an increase in algal biomass in the disturbance treatments from which mussels were removed. Thus, (a) mussels appeared to competitively diminish algae, and (b) it is possible that the increased abundance of ephemeral and corticated algae provided habitat for a sufficient number of species to increase diversity to levels equal to those found in mussel beds.

Although diversity was not significantly different among treatments at the end of the experiment, individual species showed significant differences among treatments.

Biomass for all species combined was generally greatest in the natural treatment, but the only statistically significant outcomes were that (a) biomass declined up shore at Springfontein, and (b) biomass was significantly higher in the removal treatment, but only on the low shore at Springfontein. The high value for biomass for this particular treatment consisted mostly of *S. algosus*, and was caused by two processes of colonisation: (a) a large settlement event during the last month of the experiment; (b) immigration of adult mussels into the cleared space. Tokeshi and Romero (1995) reported that *S. algosus* utilises three methods for filling gaps: colonisation by recruits; colonisation by adult mussels drifting in the water column, and lateral movement by attached adults. They found that *S. algosus* colonisation through recruitment was much slower than through adult migration, which colonised cleared space quite quickly. They attributed this to the fact that recruitment is dependent upon season and oceanographical dispersal, whereas adult migration is continual. Size composition data indicated that at Springfontein *S. algosus* recruitment was greater in the removal treatment than that in the other treatments. At Yzerfontein it was equal to recruitment in the other treatments. The size composition data also indicated that at both sites adults of both *S. algosus* and *M. galloprovincialis* immigrated into the disturbance treatments. I have no means of determining whether their arrival was by drift or lateral movements of adults.

SIMPER analyses provided greater insight into the impacts of *M. galloprovincialis* and *S. algosus* on community structure. I found three types of interactions potentially occurring between these mussels and other organisms.

(1) *Competition for space*:

I found larger numbers of *A. atra* in the disturbance treatments on the low shore at Springfontein, relative to the very few individuals within the natural treatment. The size-composition data suggested that at Springfontein, recruitment of *A. atra* was greatest in the disturbance treatments – a possible indication of competition between it and the other two mussel species at this site. However, on the low shore at Yzerfontein, its recruitment was similar between the natural and removal treatments, and relatively low in the settlement treatment, so support for the argument that it may be competitively excluded is not strong. *Aulacomya atra* is known to have a slower growth rate and a lower reproductive output than *M. galloprovincialis*, and is also less tolerant to desiccation (Van Erkom Schurink and Griffiths 1991, 1993; Branch and Steffani 2004). It is thus not surprising that it can be outcompeted by *M. galloprovincialis* (Robinson et al. 2007a, Branch et al. 2010). Most *A. atra* individuals fell within the 5-10mm size class. This suggests that *A. atra* colonised the disturbance treatments through recruitment, but that survival through to an adult stage was minimal. Sadchatheeswaran et al. 2015 found that *A. atra* density increased after the arrival of *S. algosus* at Marcus Island. The population of *A. ater* there also consisted of mainly small individuals. It would seem that *A. atra* can recruit and settle within intertidal mussel patches, but does not grow to adulthood. The limited evidence for competition restricting *A. ater* was a contrast with the clear effects of shore height, for at both sites it was significantly more abundant low on the shore, particularly strikingly so at Springfontein.

Algal species also increased in abundance in disturbed patches, at both sites. Algae are known to compete with mussels for space (Dayton 1971; Crowe et al. 2011) and their abundance is known to increase after disturbance (Sousa 1984; Robinson et al. 2008). However, studies have also linked increases in algal abundance to the removal of grazers during disturbance (Kim 1997; Jenkins et al., 2005; Coleman et al. 2006; Branch et al. 2013a). In my study, abundance of the limpet *Scutellastra granularis* was reduced in the absence of mussels, and this may have contributed to the increase in algal abundance in disturbance treatments.

(2) *Facilitation:*

Barnacles on the low shore were associated with mussels, and were most abundant in the natural treatment, but only at Springfontein, where low-shore barnacles occurred predominantly on mussel shells. Mussel shells are known to provide substrata for settlement by other organisms, such as algae (Albrecht 1998) and limpets (Griffiths et al. 1992). However, at Yzerfontein overall barnacle biomass was much lower than at Springfontein, and their abundance was not significantly different among treatments.

My study showed a direct link between abundance of the limpet *S. granularis* and the presence of mussels. It is well established that *S. granularis* recruits and small adults survive better on *M. galloprovincialis* shells than on bare rock (Hockey & Van Erkom Schurink 1992; Branch et al. 2010; Sadchatheeswaran et al. 2015), and Branch et al. (2010) reported that although large adults of *S. granularis* are displaced by *M. galloprovincialis*, its overall abundance increases in the presence of this mussel.

The naked mollusc *Onchidella maculata* was also more abundant in the mid-shore natural treatment at both sites, supporting the conclusion of Sadchatheeswaran et al. (2015) that *O. maculata* secures protection from desiccation within *M. galloprovincialis* beds.

(3) *Predator/prey interactions*:

The whelk *Trochia cingulata* is an important predator of *M. galloprovincialis*. Its abundance is highest within mussel beds, and has increased following the arrival of *M. galloprovincialis* (Branch & Steffani 2004). Several mollusc species that prey on mussels, such as *Burnupena lagenaria*, *Nucella squamosa*, *N. dubia* and *Trochia cingulata*, tended to be less abundant in removal and settlement treatments than in natural patches, possibly because of a reduction of their food source in the disturbance treatments. These trends were, however, non-significant, and shore height was the overreaching factor influencing their abundance.

The anemone *Bunodactis reynaudi* was in many cases significantly more abundant in the natural treatments at both sites. It is known to feed on dislodged mussels, and whelks, and to secure shelter in mussel beds (Branch et al. 2013b).

In summary, mussels did support unique assemblages, and favoured some species while disfavouring others, but their removal resulted in only short-term reductions in total diversity and species richness. The influence of mussels at both sites was strongly related to shore height. Sadchatheeswaran et al. (2015) similarly reported that changes in community structure following the arrival of alien species could not be explained by changes in habitat complexity alone, but was also influenced by shore height. Vertical zonation affects community structure due to variations in physical stress at different shore heights (Connell 1972; Bustamante et al. 1997). Biotic interactions and physiological traits of species inhabiting different zones also influence community structure (Sadchatheeswaran et al. 2015).

The outcomes that I recorded in the clearance experiment provided strong correlative evidence of the manner in which mussels influence communities. To examine this in a more rigorous manner, I complemented this study with a caging experiment to determine the mechanisms of co-existence between the two mussels.

5.4.2 Caging experiment:

The caging experiment was designed to explore the performances of, and interactions between, *M. galloprovincialis* and *S. algosus* at various shore heights. It was spurred by earlier reports that *M. galloprovincialis* and *S. algosus* co-exist on rocky shores, but that a clear vertical zonation pattern is evident, with *S. algosus* occurring on the low shore and *M. galloprovincialis* on the mid shore, and mixed beds in the transition area between these zones (De Greef et al. 2013; Chapter 3). Comparable patterns have been recorded on the South Coast between the indigenous mussel *Perna perna* and the alien invasive *M. galloprovincialis*, which co-exist because of (a) differences in tolerance to wave action and desiccation, and (b) a balance between competition and facilitation (Rius & McQuaid 2006; Zardi et al. 2006; Bownes & McQuaid 2009; Bownes & McQuaid 2010).

Physical stresses in the intertidal zone are related to shore height, as wave action decreases and desiccation and thermal stress increase with shore height (Rius & McQuaid 2009). Increased wave action on the low shore negatively affects the survival of *M. galloprovincialis* (Rius & McQuaid 2009), whereas higher on the shore, desiccation stress sets upper limits for this species. *Perna perna* is less affected by wave action than *M. galloprovincialis* because of its greater powers of attachment, and therefore dominates the low shore, but is less tolerant to desiccation, so it does not extend as high up the shore as *M. galloprovincialis* (Rius & McQuaid 2006; Zardi et al. 2006; Bownes & McQuaid 2009; Bownes & McQuaid 2010).

In its native range, *S. algosus* is found on the low to mid shore (Tokeshi & Romero 1995) because it is intolerant to desiccation higher on the shore. On South African rocky shores it also predominates low on the shore, although its upper limit is extended into the mid shore in mixed beds with *M. galloprovincialis* (De Greef et al. 2013; Chapter 3). It has weak byssal strength and experiences high mortality rates, but offsets this with high rates of recruitment (Chapter 4).

Biotic interactions such as facilitation and competition also affects co-existence on the shore (Erlandsson et al. 2011). Facilitation between species is thought to be most significant in situations where physical stress is high (Stephens & Bertness 1991) but less important under conditions that are benevolent, and under extreme conditions it is overridden by physical stress (Rius & McQuaid 2009). There is also a switch from competition to facilitation, with increased physical stress, although at high stress levels competition may again become significant due to a reduction in resources (Kawai & Tokeshi 2007).

Growth

A clear species-effect on growth emerged, as *M. galloprovincialis* grew significantly faster than *S. algosus* within cages at all three shore heights. *Mytilus galloprovincialis* is known for its high growth rate compared to other mussel species in South Africa (Hockey & Van Erkom Schurink 1992; Van Erkom Schurink & Griffiths 1993; Branch & Steffani 2004), a fact that has contributed to its competitive success in South Africa (Branch & Steffani 2004). When I compared this mussel to other mussels in Chapter 4, I also found that it had a higher growth rate.

Shore height also affected growth, across all treatments and in both cages and cage controls. Both *M. galloprovincialis* and *S. algosus* had significantly higher growth on the low shore, and growth decreased with increasing shore height. Van Erkom Schurink & Griffiths (1993) showed the same for *M. galloprovincialis*, *A. atra* and *Choromytilus meridionalis* in South Africa. Eckersley & Scrosati (2012) reported that desiccation stress on the high shore reduces the growth rate of the mussel *Mytilus edulis*. Bertness & Grosholz (1985) also found that the ribbed mussel *Geukensia demissa* experiences lower growth and settlement rates with increased tidal height, but greater survival higher on the shore. They attributed these respective patterns to lower food supply on the high shore due to decreased water flow, and release from predation higher on the shore.

Treatment effects involving different densities and combinations of species had no significant effect on growth for either species. There was, however, a pattern related to shore height that might hint at facilitation between species. On the low shore where wave stress is highest, *S. algosus* grew faster in the combination treatment where it was held with *M. galloprovincialis* than in monospecific plots of its own species. No pattern was distinguishable on the mid shore. Wave stress is known to influence the performance of mussels as they have to expend more energy on byssus production to prevent dislodgement. Buffering by *M. galloprovincialis* from wave action may allow *S. algosus* to expend more energy on growth rather than attachment. No pattern was apparent for *M. galloprovincialis*. Wave action does, however, promote food supply, and *M. galloprovincialis* prevails on wave-beaten rather than sheltered shores (Bustamante & Branch 1996b), Similarly, McQuaid & Mostert (2010) demonstrated that a reduction of water flow by baffles placed around *Perna perna* diminished its growth rate. Wave action is thus a two-edged sword, increasing food supply but also demanding greater attachment strength for survival.

Condition

Condition index is an important measure of the health of mussels (Seed & Suchanek 1992) and thus a good indicator of stress. Condition index is also linked to gonadal mass and thus reproductive output and the competitive ability of mussels (Branch & Steffani 2004).

Both shore height and density had a significant effect on condition of *M. galloprovincialis*, which was highest on the low and mid shores. *Semimytilus algosus* condition was highest on the low shore though not significantly so. These trends mirror the zonation patterns of these mussels and implies that the lower portions of the shore provide more favourable conditions for these species.

High-density aggregation is known to ameliorate heat stress and desiccation (Eckersley & Scrosati 2012). Indeed, at least in the mid shore, *M. galloprovincialis* had a lower condition index in low-density plots than either high-density or combination plots, and *S. algosus* showed the same (albeit non-significant) trend. Both instances point towards facilitation by high-density and mixed-species treatments compared with single-species low-density treatments. Given the fact that *S. algosus* has a low tolerance to desiccation, dense aggregation should particularly benefit it on the high shore. However, in that zone its condition was negatively affected by the presence of *M. galloprovincialis*, implying a competitive interaction. Food supply is only available to filter feeders during periods of submergence. Thus, the high shore only has a short period when food is available (Bertness & Grosholz 1985). It is probable that *S. algosus* condition is thus affected by competition for food in this zone. Efficient resource utilisation also influences the outcome of competition between mussels. Alexander et al. (2015) reported that M. galloprovincialis takes up food more efficiently than S. algosus and Aulacomya atra, and A. atra was the least efficient of the three species. They linked per capita resource use by mussels to their biomass on the shore and concluded that efficient resource use could explain why M. galloprovincialis dominates the shore.

Recruitment

Recruitment into cages as Springfontein again showed a strong species-specific effect. *Semimytilus algosus* had significantly lower recruitment than *M. galloprovincialis*. This contrasts with what I recorded in Chapter 4 for another site, Bloubergstrand, where *S. algosus* had higher recruitment than *M. galloprovincialis*, particularly on the low and mid shore. Although the data were gathered in different years, it is likely that differences between the sites were real, as Reaugh-Flower et al. (2011) also found that although *S. algosus* had a higher recruitment than *M. galloprovincialis* in Namibia, recruitment varied substantially among sites, as well as temporally between months and years. Spatial variations in invertebrate recruitment are caused by several possible factors, including substratum and tidal height (Petersen 1984, Caro et al. pers. comm.), adult densities (Underwood et al. 1983; Harris et al. 1998; Robinson et al. 2007b; Reaugh-Flower et al. 2011), and physical processes that affect larval dispersal and retention (McQuaid & Phillips 2000; McQuaid & Lawrie 2005; McQuaid & Phillips 2006; Navarrete et al. 2008).

There was a clear relationship between the density of adults of *M. galloprovincialis* and its recruitment in the caging experiment, both in comparisons of 30 versus 15 mussels per cage, and in regressions incorporating data covering a wider spectrum of densities from cages and controls. As *S. algosus* recruitment on the mid and high shore was very low it was not possible to examine whether a comparable relationship existed for it. However, size-frequency analyses of controls+cage showed that no *S. algosus* adults were found on the mid and high shore, reflecting the virtual absence of recruits into cages in those zones.

Recruitment showed a clear shore-height effect in both cages and cage controls. *Semimytilus algosus* recruited almost exclusively to the low shore, while *M. galloprovincialis* recruited to all zones but prevalently to the low and mid shore. This again echoes zonation patterns for these mussels, as well as adult abundance (Chapter 3; De Greef et al. 2013).

Recruitment was not significantly different among treatments. *Mytilus galloprovincialis* recruitment was, however, strongly related to conspecific density, at all shore heights. Its recruitment is known to be sensitive to variations in adult density (Harris et al. 1998; Robinson et al. 2007b), leading to a positive feedback in which high adult densities promote recruitment, boosting adult populations. Conversely, removal or thinning of adult mussels diminishes recruitment, as has been demonstrated for *M. galloprovincialis* on the West Coast (Robinson et al. 2007b) and for *Perna perna* on the Southeast Coast (Erlandsson & McQuaid 2004).

Survival

Survival of *M. galloprovincialis* was consistently higher than for *S. algosus*. *Semimytilus algosus* is especially vulnerable to predators, for two reasons: (a) it has a weak shell (Chapter 4), and (b) it is a relatively small mussel, and for its entire adult life falls within the critical 'window of vulnerability' described by Griffiths and Hockey (1987). It is also susceptible to dislodgement due to high wave action as it has weak byssal attachment (Chapter 4), and predominates in the low shore where wave action is most intense.

Survival of *S. algosus* among shore heights was not significantly different. However, survival of *M. galloprovincialis* within cages was significantly less on the low shore than on the mid and high shore. This pattern was surprising given the higher growth and condition found on the low shore, for both these species. As mussels were largely protected from predators by cages, the high mortality on the low shore is probably due to higher wave action there. *Mytilus galloprovincialis* is known to be vulnerable to the higher wave action on the low shore (Bownes & McQuaid 2010), and *S. algosus* has even weaker byssal strength than *M. galloprovincialis* (Chapter 4).

Survival was significantly greater in cages than cage controls for *M. galloprovincialis*, indicating that cages protected it from various sources of mortality including wave stress and predation. However, *S. algosus* survival was not significantly different between cages and cage controls, probably because its survival was very low regardless of cage protection. Nevertheless, cage controls showed similar patterns of survivorship to those in cages, for both species. Menge & Branch (2001) found that predation pressure is highest on the low shore and is often responsible for setting the lower limits of species zonation. On the other hand, predation is unlikely to affect *M. galloprovincialis* as strongly as it does *S. algosus*, as it is bigger than *S. algosus* and grows faster, thus allowing it to escape the critical 'window of vulnerability' (Griffiths & Hockey 1987). Greatest differences in survival between the two species were evident high on the shore, reflecting the greater vulnerability of *S. algosus* to desiccation. Treatments had no significant effect on survival.

Community structure

Sadchatheeswaran et al. (2015) found that the arrival of the alien mussel *M. galloprovincialis* at Marcus Island on the west coast of South Africa increased habitat complexity, with a resultant increase in species richness and diversity. However, Robinson et al. (2007a) found that diversity and species richness did not increase significantly when *Aulacomya atra* beds were replaced by *M. galloprovincialis* beds on the low shore, and they suggested the replacement of one mussel species by another would not have changed habitat complexity significantly. I attempted to establish whether community structure and diversity were significantly different between *S. algosus* treatments and *M. galloprovincialis* treatments. Sadchatheeswaran et al. (2015) speculated that relative to *M. galloprovincialis*, *S. algosus* will reduce habitat complexity and species richness because of its smaller shell and habit of forming monolayered beds. However, Tokeshi et al. (1989) reported that polychaetes were more abundant in *S. algosus* beds than in *Perumytilus purpuratus* beds, due to greater habitat complexity of the former species. I found that *S. algosus* treatments supported similar species richness and diversity to those of *M. galloprovincialis* treatments.

SIMPER analyses showed that there were certain species that were associated with one or the other of the mussel species. The barnacle *Notomegabalanus algicola* occurs on mussel shells and was most abundant in the high density *M. galloprovincialis* treatment low on the shore. This was true even when this treatment was compared to control+cage treatments. The algal species *Tayloriella tenebrosa* was more abundant in the high density *S. algosus* treatment, and was absent from *M. galloprovincialis* treatments. Due to its smaller size, *S. algosus* supports fewer limpets, and therefore grazing among *S. algosus* is reduced and algae can proliferate. Subtle differences in the species mix therefore emerged between the two mussels, even although overall diversity did not differ.

Cage effects

It is not unusual in field experiments to fail to assess caging effects because of the practical difficulties of instituting cage controls (see, for example Jackson et al. 2009; Caro et al. 2011; Caro et al. unpublished). I did employ experimental (cage) controls, although they were imperfect in that they, like the cages themselves, involved disturbance. Nevertheless, they did allow assessment of caging effects, and revealed that the cages affected some of the variables. Condition was negatively affected by cages whereas survival increased in cages. Recruitment was greater in cages, but only on the low shore. Growth was not affected by cages. Community structure within comparable treatments were not significantly different between cages and cage controls, neither was species richness. However, the differences between cages and cage controls do not invalidate my conclusions because, firstly, I compared the variables examined under consistent conditions of caging and with known densities and combinations. Secondly, in most cases the trends that emerged from the cage controls supported the trends displayed in the cages, even if the levels of response differed.

Conclusion

The clearance experiment revealed that mussels had an initial effect on diversity and species richness at both study sites, but that the system recovered within two to four months, as other habitat-forming species replaced the role of mussels, and the mussels themselves recovered relatively fast. Shore height prevalently affected biodiversity in the long run, with biomass and diversity declining with increasing shore height in both sets of experiments. As predicted, mussels facilitated some species, and competed for space with others, reducing their numbers.

I also examined the mechanisms for the co-existence of *S. algosus* and *M. galloprovincialis* on the shore, through the caging experiment. Space is limited on rocky shores and competition for space thus plays an important role in structuring communities (Connell 1961, Dayton 1971, Menge and Branch 2001). *Mytilus galloprovincialis* seems to be competitively superior to *S. algosus*, given its life-history strategies (Chapter 4 and this chapter). How then is it possible that these species co-exist? The answer lies in tolerance to physical stress. Given the high mortality rate and low recruitment and growth rate of *S. algosus* on the high shore, it clearly cannot tolerate the level of desiccation found there. Tokeshi et al. (1989) also attributed the zonation pattern in Peru between *S. algosus* (which occurs only on the low to mid shore), and *Perumytilus purpuratus* (which occurs above it), to differences in tolerance to desiccation between the two species. Even though the recruitment and growth rates for *M. galloprovincialis* were much lower on the high shore than on the mid shore, it is still able to recruit and survive in the high shore, because it has a high tolerance to desiccation (Hockey and van Erkom-Schurink 1992). Bownes and McQuaid (2009) likewise reported that *M. galloprovincialis* survives better on the high shore than *P. perna* and attributed this to its higher tolerance to desiccation. On the other hand, *M. galloprovincialis* is not abundant on the low shore, as it is intolerant of high wave action, despite its higher growth and condition there (Bownes & McQuaid 2009). Even though *S. algosus* has weak byssal strength and high mortality on the low shore, it is able to offset these effects through high recruitment.

There also exists a trade-off in competitive ability with tidal height (Griffiths & Hockey 1987; Bertness & Leonard 1997; Rius & McQuaid 2009). For example, mussel growth decreases with increasing shore height (Bertness & Grosholz 1985; Eckersley & Scrosati 2012). Both *M. galloprovincialis* and *S. algosus* had better growth and body condition on the low shore, but *M. galloprovincialis* achieved its peak recruitment on the low-mid shore, with significantly less recruitment in the high shore than either the mid or low shores. Both growth and condition are related to food supply, which is greater on the low shore due to longer periods of immersion and higher water flow (Bustamante & Branch 1996b). The high recruitment of *M. galloprovincialis* on the low and mid shore is most likely due to a combination of (a) higher adult density there, as discussed above, and (b) longer periods of submergence. The absence of recruitment of *S. algosus* from the mid and high shores is the likely cause of the rarity of its adults in these zones.

Facilitation between these two species may also contribute to co-existence, although my results yielded only weak support for this. *Semimytilus algosus* achieves highest abundance on the low intertidal zone and does not survive on the high shore. Its vertical range is extended when it occurs in mixed beds with *M. galloprovincialis* (Chapter 3). Possible modes of facilitation are through the amelioration of abiotic stress or the reduction of harmful biological interactions such as predation (Bruno et al. 2003; Kawai & Tokeshi 2004). If we return to the example of the co-existence of *M. galloprovincialis* and *P. perna* on the South

Coast, on the mid shore *P. perna* is protected from desiccation by the *M. galloprovincialis* matrix (Rius and McQuaid 2009). On the low shore *M. galloprovincialis* survival initially increases in mixed beds as it is protected from physical stress by the *P. perna* matrix (Rius and McQuaid 2006). Facilitation is strongly related to the stress gradient, as explained above and in Rius & McQuaid (2009). I did find trends that pointed towards facilitation in my study, though none were significant. For example, within cages on the mid shore *S. algosus* condition was greater when combined with *M. galloprovincialis*, or in high density cages, relative to its condition in low-density cages. *Mytilus galloprovincialis* also appears to benefit from the presence of *S. algosus*, as its condition on the low shore was greater in combination treatments, indicating that there is mutual facilitation through amelioration of physical stress, in the form of wave action on the low shore, and desiccation further up the shore.

Overall, shore height emerged as the most important factor dictating the differences in zonation between *M. galloprovincialis* and *S. algosus*, with *M. galloprovincialis* being more tolerant of desiccation and therefore able to survive higher up the shore. Growth and condition were strongly negatively related to shore height, and although survival of adults was significantly related to shore height only in the case of *M. galloprovincialis*, recruitment differed for both species, being virtually non-existent in all but the low shore for *S. algosus*, and very limited in the high shore for *M. galloprovincialis*. The restriction of *S. algosus* recruitment to the low shore is likely the main determinant of its low-shore zonation, while *M. galloprovincialis* recruited in almost equal numbers in the low and mid shore, but at very low levels high on the high shore.

Chapter 6

Phylogeography of the invasive mussel *Semimytilus algosus* in South Africa relative to natural populations in Chile and Namibia

6.1 Introduction

Worldwide, the spread of alien species in marine ecosystems has increased (Robinson et al. 2005; Hoegh-Guldberg & Bruno 2010, Ricciardi 2015) with mussels frequently mentioned (Savini et al. 2010; Mead et al. 2011a; Crocetta 2012). In South Africa, 86 alien and 39 cryptic marine and estuarine species, from 17 taxonomic groups, are known (Mead et al. 2011a). Of the 86 species, three have been associated with major invasions on the West Coast. Firstly, the Mediterranean mussel *Mytilus galloprovincialis* appeared in the early 1970s and became the prevalent rocky-shore organism (Mead et al. 2011b). Secondly, the barnacle *Balanus glandula* established itself in the 1990s and now occupies much of the high shore in exposed areas (Laird and Griffiths 2008). Thirdly, and most recently, the bisexual mussel *Semimytilus algosus* was discovered (Mead et al. 2011b) and currently dominates the low shore (De Greef et al. 2013).

Many studies have attempted to determine which characteristics enable introduced species to become invasive in a new environment (Lodge 1993; Johnson & Carlton 1996). For example, certain life-history strategies increase ability to compete for space (Van Erkom Schurink & Griffiths 1993; Rensel et al. 2005), and in Chapter 4 I described the life-history strategies that contribute to the invasive success of *S. algosus*. Release from natural predators or parasites in the invaded area has also been cited as a reason for invasive success (Torchin et al. 2001; Colautti et al. 2004), but Lodge (1993) came to the conclusion that the best predictor of an invasive species success, is simply its history of invasion elsewhere.

Relevant to this chapter is the fact that high genetic diversity is known to increase the fitness of non-indigenous species by improving the chance they will be able to adapt to their new circumstances, survive and become invasive (Lee 2002; Stepien et al. 2005; Roman 2006; Rius et al. 2008).

Genetic diversity in introduced species

One would expect an invasive population to exhibit reduced genetic diversity, when compared to source populations, due to founder effects (Darling et al. 2008). For example, Darling et al. (2008) found marked reductions of genetic diversity between native and invasive populations of *Carcinus maenas*, and ascribed this to a combination of founder effects and genetic drift due to isolation from the native population. However, Rius et al. (2014b), in their review of marine invasion genetics in Europe, concluded that colonizing populations usually were not genetically less diverse than native populations.

Propagule pressure, which is the number of individuals introduced as well as the number of introduction events in a system, can increase the genetic variation in alien populations and thus add to their invasive potential (Kolar & Lodge 2001; Von Holle & Simberloff 2005; Roman & Darling 2007; Facon et al. 2008; Darling et al. 2008; Briski et al. 2012). For example, Rius et al. (2014a) found that the populations of invasive ascidians in South Africa were genetically relatively diverse and attributed this to high propagule pressure. Dlugosch and Parker (2008) also reported that multiple introductions moderate founder effects, by increasing the effective population size and thus genetic diversity. They also noted that invasive populations of St John’s wort, *Hypericum canariense*, evolved quickly after arrival in new habitats, despite initially having a low genetic diversity, and concluded that the importance of founder effects are overestimated. Another example of invasive success despite a population bottleneck, is that of the bluespotted cornetfish, *Fistularia commersonii*. This species underwent a severe bottleneck which reduced its genetic diversity to only two mitochondrial haplotypes, yet it still successfully invaded a large area of the Mediterranean Sea (Golani et al. 2007).

Another factor that influences genetic diversity in invasive populations, and thus their success, is genetic diversity in the source population. Invasive populations that come from a genetically diverse source population, will share its high diversity (Holland 2000) and it has been shown that low population-genetic structuring in the native range can also explain invasion success (Gaither et al. 2013; Jackson et al. 2015). Linked to this is the effective population size of the introduced population. Effective population size (Ne) measures the number of individuals that contribute genetically to the next generation (Wright 1969). Thus, a large local effective population size is likely to be associated with high genetic diversity (Dupont et al. 2007), which could be transferred to non-native ranges.

The effect of life-history strategies on genetic diversity

Phylogeographic conclusions cannot be drawn in isolation, but need to be augmented by an understanding of the biology and ecology of the study subject (Bowen et al. 2014). Life history traits, such as long-lived pelagic larval stages can influence gene flow (Weersing and Toonen 2009, Selkoe and Toonen 2011, Selkoe et al. 2014, Wright et al. 2015) and it is commonly accepted that organisms with long-lived pelagic larval stages will show high levels of gene flow and little or no genetic structure, as the larvae can disperse over great distances (Apte & Gardner 2001; Neethling et al. 2008; Palumbi 1994). However several studies have reported that dispersal potential is not always a good predictor of gene flow (Barber et al. 2002; Ayre et al. 2009; Riginos et al. 2011). Sivasundar & Palumbi (2010) noted that larval dispersal of Pacific rockfish species cannot be considered in isolation, because there is an interaction between dispersal, settlement behaviour and environmental factors such as upwelling. In Florida, Bingham & Young (1991) compared swimming versus non-swimming larvae of the ascidian *Ecteinascidia turbinata*, and found that swimming larvae prefer to settle in conspecific colonies and, thus, the dispersal rate and distance of dispersal were less for swimming larvae than non-swimming larvae. Gaither et al. (2013) used population structure (F_{ST}) as a proxy for realized dispersal. F_{ST} takes into account species-level features that aid invader success – for example, life history, behaviour, habitat requirements, population size, colonisation history and ecology. They concluded that it is useful in predicting the extent of spread of invasive species once established in the invaded area.

Oceanographic systems influence gene flow

Oceanographic systems, such as upwelling (Lessios et al. 2003; Teske et al. 2008; Henriques et al. 2014), coastal currents or eddies (Teske et al. 2007; Zardi et al. 2011) and deep-water troughs (Shaw et al. 2004) are also known to influence gene flow. On the West Coast of South Africa the northward-flowing Benguela Current facilitates unidirectional gene flow patterns (Von der Heyden 2009, Teske et al. 2011), although nearshore southward-flowing countercurrents may counteract this to some extent. On the South Coast the westward flowing Agulhas Current impacts gene flow, resulting in mainly east to west gene-flow patterns (Neethling et al. 2008). However, some species employ inshore counter-currents to facilitate eastward gene flow (Von der Heyden et al. 2008). Sea urchins (*Parechinus angulosus*) also show a measure of bidirectional gene flow on the South Coast (Muller et al. 2012). Mussel larvae are passively dispersed and are therefore dependent on environmental conditions such as wind and current direction (Narváez et al. 2006; Teske et al. 2007). For instance, on the South Coast of South Africa McQuaid and Phillips (2000) found that prevailing dispersal of *Mytilus galloprovincialis* occurs in the direction of the major wind-driven currents.

Furthermore, the wind-driven Lüderitz upwelling cell separates the Northern Benguela from the Southern Benguela and is believed to be a semi-permanent barrier to the transport of planktotrophic larvae (Zardi et al. 2007b).

Freshwater influxes from large rivers such as the Amazon are also believed to serve as barriers to gene flow (Muss et al. 2001; Rocha et al. 2002). In southern Africa, Von der Heyden et al. (2007) hypothesised that the freshwater influx of the Orange River may form a barrier between South Africa and Namibia, thereby affecting the larval dispersal of Cape hakes *Merluccius capensis* and *Merluccius paradoxus* between the two countries. However, they concluded that there was little genetic divergence between Namibian and South African stocks of *Merluccius capensis*, indicating that gene flow does occur between South African and Namibian hake despite the Orange River barrier. The deep-water hake *Merluccius paradoxus*, however, showed population structuring for mature fish. Branch and Steffani (2004) also reported that the abovementioned barriers did not prevent the northward spread of *M. galloprovincialis* from Saldanha Bay to as far as northern Namibia, although it did retard its northward passage. The mussel *Perna perna* also showed very little genetic divergence between populations in Namibia and South Africa, despite a large gap in the distribution of the species on the West Coast of South Africa (Grant et al. 1992; Zardi et al. 2007a).

Oceanography of the West Coast of southern Africa

The West Coast of southern Africa is a cool-temperate region that comprises two biogeographic provinces called the Namaqua and Namib Provinces, divided just north of Lüderitz (Emanuel et al. 1992). The entire area has several strong upwelling cells and is dominated by the cold (~12 °C) northwards-flowing Benguela Current (Walker et al. 1984; Shannon 1985). Examination of the genetic structure of the clinid *Clinus cottoides* has revealed that it exhibits a northward gene-flow pattern on the West Coast, as would be expected from the influence of the prevalently northward flowing Benguela current (Von der Heyden et al. 2008). However, some studies have shown no genetic divergence between South African and Namibian populations, for example the Cape hakes *Merluccius capensis* and *M. paradoxus* (Von der Heyden et al. 2007), and the mussel *M. galloprovincialis* (Zardi et al. 2007b).

Laudien et al. (2003) found two genetically distinct populations of the surfclam *Donax serra* at Meob Bay and Langstrand in Namibia and attributed this to the influence of the Lüderitz upwelling cell, which is shown to cause a biological discontinuity near Meob Bay (Agenbag & Shannon 1988).

Phylogeography

Phylogeography is a useful tool to elucidate the processes that govern geographical distribution of genealogical lineages within a species (Avise 2000). For example, comparisons of genetic composition between introduced and source populations can be used to identify the most probable source population (Gaither et al. 2013).

Davies et al. (1999) recommended that neutral nuclear markers such as microsatellites, introns, randomly amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) be used in phylogeographic studies of invasive species. The reasons for this are: (a) bottleneck effects are amplified in mtDNA due to its smaller effective population size (Hartl & Clark 1997), and (b) although allozymes preserve more genetic diversity than mtDNA during bottleneck events, genetic variation may be underestimated as only part of the existent genetic variation can be detected using protein markers (Davies et al. 1999). Nuclear genes have been successfully used in several phylogeographic studies (Hare

2001). However, mitochondrial DNA is most commonly used in these studies due to the fact that it is inexpensive and easy to sequence. MtDNA also has a higher mutation rate and smaller effective population size than nuclear loci, which allows for high nucleotide sequence variation (Avise 2009). Avise (2009) states further that nuclear markers are not ideal for this kind of study, due to the slow evolution of nuclear sequences and the fact that intragenic recombination hides genealogical patterns in nuclear loci. Zink & Barrowclough (2008) compared mtDNA and nuclear loci and found that mtDNA does reliably predict phylogeographical patterns and population structure. Specifically, the mtDNA gene cytochrome oxidase I (COI) is a widely employed tool in phylogeography as its mutation rate is conservative enough to give information on the origins and range expansion of invasive species (Avise 2000).

Aims

In this chapter, I consider the phylogeography of the invasive species *S. algosus* in southern Africa, to determine its origin. To achieve this goal I employed a molecular approach to assess the population genetic structure between populations from Chile, Namibia and South Africa using the mtDNA COI gene.

Because (a) *S. algosus* originated in Chile, and (b) founder effects are likely to have been influenced by differences in distance between regions and duration of time since its respective invasions of Namibia and South Africa, I hypothesised that:

H1: *Semimytilus algosus* will exhibit genetic differentiation among Chilean, Namibian and South African populations, the magnitude of which will be greater between Chile and South Africa than between Namibia and South Africa.

H2: The genetic diversity of *S. algosus* populations will be less in Namibia than Chile, and further reduced between Namibia and South Africa.

6.2 Methods and materials

To assess genetic differences between populations from Chile, Namibia and South Africa and variations among sites within these populations, thirty individuals were collected at each of five sites on the West Coast of South Africa: Hout Bay (S34°02′5333″ E18°21′389″), Melkbosstrand (S33°43′2015″ E18°26′3347″), Lamberts Bay (S32°06′507″ E18°18′217″), Baboon Point at Elands Bay (S32°19′1880″ E18°18′4777″) and Groenriviermond (S30°50′50″ E17°34′27″); at three sites in Namibia: Langstrand near Walvis Bay (S22°48′31.73″ E14°32′35.17″), Cape Cross (S21°45′23.48″ E13°57′55.48″) and at Terrace Bay in northern Namibia (S19°59′53.31″ E13° 1′57.93″) (Fig. 6.1), and at three sites in Chile: Pichilemu (S34°25′37.24″ W72°02′57.11″) in the south, ECIM sur (S33°30′07.27″ W71°02′57.11″) and Temblador (S29°54′20.17″ W71°21′32.70″) (Fig. 6.2). The material was fixed in 98% alcohol on collection.

DNA extraction, PCR and sequencing

All DNA was extracted using the Nucleospin extraction kit (Macherey-Nagel), following the manufacturer’s protocol. PCR amplification of the 5' end of the mitochondrial DNA cytochrome oxidase I gene (COI) used a species-specific forward primer (SemMytF1; GGA ATA TGA AGA GGT TTG GTT GGA G) and the universal reverse primer, HCO2198 (Folmer et al. 1994). PCR amplification were performed in a 25µl solution containing 2µl DNA, 13.4µl distilled water, 2.5 µl Qiagen PCR buffer, 2.5µl dNTPS, 2 µl Magnesium, 1.25

µl of each primer and 0.1 µl Taq DNA polymerase (Qiagen). The PCR cycle comprised one initial denaturation step at 93°C for 3 minutes, 35 cycles of denaturation at 93°C for 30 seconds each, annealing at 52°C for 30 seconds, extension at 72°C for one minute and final extension at 72°C for 5 minutes. PCR products were visually checked on 1% agarose gels stained with ethidium bromide. Clean bands were then gel purified using a Gel Extraction kit (Qiagen) and sent for sequencing. Sequences were generated using BigDye terminators (Applied Biosystems) and run on an ABI3100 automated sequencer. Sequences were aligned by eye in BioEdit (Hall, 1999) and were translated to ensure no stop-codons were present (this ensures that no pseudogenes are used in the analyses), using EMBOSS Transeq (http://www.ebi.ac.uk/Tools/st/emboss_transeq).

Genetic and statistical analyses

Sequences were aligned by eye using BioEdit (Hall 1999) and collapsed into haplotypes using Collapse 1.2 (http://darwin.uvigo.es). Haplotype diversity (*h*) and nucleotide diversity (π) were calculated using Arlequin v.3.5.1.3 (Excoffier et al. 2005). Samples were divided into three populations according to the country of origin and used for analyses using an analysis of molecular variance (AMOVA) (Excoffier et al. 1992). Traditional bifurcating tree-building algorithms often lack the power to solve intraspecies relationships (Posada & Crandall 2001). Thus, parsimony networks were constructed instead, both for each of the three populations, as well as for the three populations combined (Only the combined network is shown). TCS v.1.21 was used for all networks (Clement et al. 2000). SAMOVA was conducted to partition the populations into genetically homogenous groups (Dupanloup et al. 2002). SAMOVA define groups of populations that are geographically homogeneous and have the highest possible differentiation from each other. Thus it also identifies genetic barriers between these groups, and it does not require pre-definition of populations, as is the case for AMOVA.

6.3 Results

The COI gene for 226 individuals from 11 localities in the three countries was sequenced, and 515 base pairs amplified. In total, 133 different haplotypes from the entire dataset were identified. The Chilean population had 72 polymorphic sites (65 transitions, 11 transversions), yielding 51 haplotypes of which 41 were unique. Namibia had 47 polymorphic sites (43 transitions, 4 transversions), yielding 35 haplotypes with 21 of those being unique. Finally, South Africa had 73 polymorphic sites (69 transitions, 12 transversions), yielding 66 haplotypes of which 54 were unique. The most common haplotype was shared by 19% of individuals amongst all three populations.

For Chile, Namibia and South Africa, haplotype diversity (h) was very similar, respectively being 0.95 (±0.022); 0.96 (±0.018) and 0.96 (±0.013), and nucleotide diversity (π) was also quite similar, with respective values of 0.0065 (±0.003); 0.0069 (±0.0039) and 0.0065 (±0.0037) (Table 6.1).

AMOVA (Table 6.2) did, however, recover shallow genetic structuring between Chile and South Africa (F_{ST}=0.01, P=0.003) but not between Chile and Namibia (F_{ST}=0.004, P=0.14) or between South Africa and Namibia (F_{ST}=-0.003, P=0.74). F_{ST} values between sites sampled within South Africa, Namibia and Chile, respectively, were not significant (P>0.05) (Table 6.3).

The connection limit for the statistical parsimony network showed that topologies that connected haplotypes by 8 or fewer steps had a 95% probability of being correct. The network demonstrated a star shape with a high number of unique haplotypes that were closely related to a single, common haplotype (Fig. 6.3). Five haplotypes were shared by all three countries. An additional five haplotypes were shared by South Africa and Namibia, three by Chile and Namibia and two by Chile and South Africa. The most divergent haplotype, with three mutational steps, was from Lamberts Bay, South Africa.

SAMOVA reported the greatest Φ_{ct} when seven groups were selected ($\Phi_{ct} = 0.0189$, $P<0.0001$). However none of the groups included were geographically meaningful (Table 6.4).

6.4 Discussion

I tested the hypotheses that (a) *S. algosus* would exhibit genetic differentiation among Chilean, Namibian and South African populations, with greater differences between Chile and South Africa than that between Namibia and South Africa; (b) The genetic diversity of *S. algosus* populations would decrease from Chile to Namibia and, to a lesser extent, from Namibia to South Africa. However these hypotheses were refuted by the data. There was shallow genetic structure between Chile and South Africa, but no indication of population structure was found between the South African and Namibian populations of *S. algosus*, as shown by both the AMOVA and SAMOVA analyses. A lack of population genetic structure is generally taken to indicate high levels of gene flow among populations (Williams & Benzie 1997; Wei et al. 2013). The diversity indices also yielded similar values for heterozygosity, haplotype and nucleotide diversity for all three populations, with high h and low π.

In a newly invasive population one might expect to find low levels of genetic variation if it was established by a founder event (Darling et al. 2008). However, many studies have reported no evidence of founder events in invasive species (Rius et al. 2014b). I found no indication of a founder event in the South African population of *S. algosus*, even though it is clearly the youngest population of the three countries. This implies that *S. algosus* is unlikely to have arrived in South Africa via a single introduction of a limited number of founder individuals via, for example, ballast water or amongst imported oyster spat. High genetic diversity in an introduced population is possible if propagule pressure was high, if effective population size of the invading population was great (Gaither et al. 2012), or if the source population showed a lack of population structure (Gaither et al. 2013). Thus, the high haplotype diversity in the South African population can be explained by a natural range expansion with high propagule pressure and from a population with high genetic diversity and a lack of genetic structure. The Namibian population, although not endemic, is at least 83 years old, and has a haplotype diversity reflective of the native Chilean sites, and no population structure. Of particular interest is the fact that the haplotype network combining the three populations showed a high number of unique haplotypes. This pattern in a haplotype network is indicative of a large effective population size (Ovenden et al. 2007; Leffler et al. 2012). The effective population size is the number of individuals that contribute genetically to subsequent generations (Hellberg et al. 2002). To exemplify, Mkare et al. (2014) found that panaeid prawns in Ungwana Bay, Kenya had panmictic populations with almost no shared haplotypes in the parsimony network. They attributed this to the large effective population sizes of these prawns. Silva et al. (2010) reported the same genetic pattern for the mangrove crab *Perisesarma guttatum*, describing many rare haplotypes within each population, due to the high maternal effective population size.

Larval dispersal is one of the ways alien mussels spread in introduced areas (Johnson & Carlton 1996; Branch & Steffani 2004). *Semimytilus algosus* has a pelagic larval stage lasting between 17 and 30 days (Campos & Ramorino 1979), which could easily have been dispersed by currents. McQuaid & Phillips (2000) reported that mytilid larvae are dispersed as passive particles in the water column, conveyed by wind-driven currents. Taking this fact into account there are two stumbling blocks to a natural range expansion from Namibia, southwards to South Africa.

Firstly, the Benguela Current facilitates predominantly northward dispersal, as has been demonstrated for the *Mytilus galloprovincialis* invasion mentioned in the introduction (Branch & Steffani 2004). However, variations do occur in this current, for example during times of the Benguela El Niño a slackening or even a reversal of the usual northerly flow of the Benguela occurs, together with an increase in sea temperatures (Shannon 1985). Inshore and deep countercurrents also exist. Changes in ocean currents would affect the dispersal of larvae and together with changes in environmental cues such as water temperature, may determine where and when larvae settle (Blamey 2010).

Secondly, the Lüderitz upwelling cell as well as the outflow of the Orange River may serve as barriers to gene flow (Von der Heyden et al. 2007; Zardi et al. 2007a). However these barriers are not impermeable and research has shown that gene flow does occur between South Africa and Namibia (Grant et al. 1992; Branch and Steffani 2004, Von der Heyden et al. 2007), though most studies show a northward trend (Hockey & Van Erkom Schurink 1992; Von der Heyden et al. 2008) as discussed above. Following a survey in 1968-1969 Kensley & Penrith (1970) reported that *S. algosus* occurred only north of Swakopmund. Later surveys in 2007 by the Namibian Ministry of Fisheries and Marine Resources did however reveal the presence of *S. algosus* in Lüderitz (B. Currie unpublished) and in 2014 the species was collected in Mining Area M170-175, just north of Oranjemund (S28°16' 46" E16°02'45") (G.M. Branch, personal communication), clearly indicating that *S. algosus* has spread southwards within Namibia, as well as extending past the Lüderitz upwelling cell to South Africa.

Furthermore, the AMOVA results indicates that F_{ST} for the South African population does not differ significantly from that of the Namibian population, suggesting high propagule pressure and a large effective population size in the Namibian population. The evidence suggests that *S. algosus* was first introduced to Namibia, from Chile, most likely through human-mediated transport in view of the great distance between the two regions and the absence of direct currents that would provide a connection, and then to South Africa through a secondary (and probably natural) expansion from Namibia. The lack of genetic structure across the introduced range, together with the fact that values for genetic diversity are very similar between the three populations, contradicts a stepping-stone colonisation. Expansion must have been simultaneous over the entire range, with high levels of gene flow between distant areas.

Semimytilus algosus has gradually spread across the Namibian coastline (Kensley and Penrith 1970, B. Currie unpublished), but it was not until 2009 that this species was found in South Africa, and it rapidly colonising almost the entire West Coast. There is often an extended time lag between the introduction and expansion of invasive species (Crooks & Soule 2001). The lag time may be due to (a) low population density in the initial introduction, or (b) environmental or ecological barriers (Crooks 2005). As discussed above, *S. algosus* has a high genetic diversity in Namibia, equal to that in its native region in Chile. Thus, the lag time in its spread to South Africa is unlikely to have been due to a low population density at introduction in Namibia, but rather due to an environmental barrier such as the Lüderitz upwelling cell or the Orange River outflow. Rapid colonisation of introduced species after a lag period may be due to the fact that conditions have become favourable for the invader due, for example, to changes in environmental conditions or habitat quality creating a niche opportunity (Crooks and Soulè 1999; Shea & Chesson 2002; Golani et al. 2007). Climate change causes changes in sea surface temperatures, sea-atmosphere exchange, and ocean current speed and direction (Rouault et al. 2010; Brochier et al. 2013). This in turn, may alter dispersal routes of larvae (Andrello et al. 2015). *Semimytilus algosus* may have benefited from a relaxation of the Lüderitz upwelling cell, combined with a cooling trend in sea surface temperature (SST) on the West Coast, caused by increased upwelling (Rouault et al. 2010).

F_{ST} values for all sites within the three populations were not significantly different, reflecting panmixia in all three populations. The lack of population structure could again be explained by a large effective population size (Hellberg et al. 2002). Large effective population size increases genetic diversity (Dupont et al. 2003) and this, in combination with the exceptionally high settlement rates exhibited by *S. algosus* (Reaugh-Flower et al. 2011), could explain the high haplotype variation in the invasive population, and may have contributed to the invasive success of this species along the entire West Coast of South Africa. A single flood of larvae sufficiently large to have been representative of the genetic diversity of Namibia, carried past the Lüderitz barrier and distributed southwards by a reversal of currents or by inshore counter-currents, may account for the rapid arrival, spread and genetic diversity of the South African population. It is possible that alternatively (or additionally), ongoing gene flow may have contributed to the high genetic diversity in South Africa. However, (1) the semi-permanent nature of the Lüderitz barrier, (2) the long delay between arrival of *S. algosus* in Namibia and its appearance in South Africa, (3) the rapidity and extent of its colonisation of the West Coast of South Africa, and (4) the emergence of unique haplotypes there all argue against ongoing gene flow. The presence of unique haplotypes in such a young population may however, be an artefact of the relatively small sample size. Further study may be necessary to fully investigate the phylogeny of this species in South Africa.

The haplotype network confirms panmixia and features a central common haplotype with a high number of single haplotypes that are closely related to it. Studies have shown that a star-shaped haplotype network such as this is indicative of a population expansion with one ancestral haplotype (Hewitt 1996; Hewitt 2000; Avise 2000; Nordborg & Innan 2002; Teske et al. 2014). Further evidence of a past population expansion in *S. algosus* is the fact that analyses showed high h but low π in all three populations. Grant & Bowen (1998) came to the conclusion that for marine fish, high haplotype diversity together with low nucleotide diversity signifies a population expansion after a period of low effective population size. This would suggest that the source population of *S. algosus* underwent a population expansion. This expansion could not have been recent as mitochondrial DNA is only sensitive to historical population expansions (Avise 2000; De Jong et al. 2011).

Population expansions of *S. algosus* in Chile could be explained by the fact that Chile has been subject to fluctuations in climate due to several glaciation events (Ruzzante et al. 2008). These fluctuations influence species distributions, but little is known about the effects of glaciation on the phylogeography of marine organisms in Chile. Most studies there have been conducted on terrestrial (Rodríguez-serrano et al. 2006; Victoriano et al. 2008) or freshwater organisms (Ruzzante et al. 2008; Unmack et al. 2009). The only marine organisms studied in this regard are several kelp species (Fraser et al. 2010) and the mollusk *Concholepas concholepas* (Cárdenas et al. 2009). During the last glacial maximum (LGM) during the Pleistocene, an extensive ice sheet covered southern Chile from 39°S, extending westward to the edge of the continental shelf, and probably eradicated several rocky shore species. Central and northern Chile, however were not affected by this glaciation (Fraser et al. 2010). Fraser et al. (2010) found that mitochondrial (COI) and chloroplast (rbcL) DNA analyses showed that the kelp *Durvillaea antarctica* recently recolonized southern Chile. Cárdenas et al. (2009)

reported that *C. concholepas* showed a slight decrease in genetic variation from north to south, though not significantly so, and (more importantly) the haplotype network featured a large number of unique haplotypes related to a common central haplotype, indicating a rapid population expansion. They calculated the date of onset of the expansion at 400 000 years ago during a warmer interglacial period in the Pleistocene. It is therefore possible, given the similarities in the haplotype network of *S. algosus* to that of *C. concholepas*, that the geographic range of *S. algosus* was contracted during glaciation and that the population then expanded during warmer interglacial periods.

Rates of mitochondrial evolution do, however, differ between species (Shearer et al. 2002) and mytilids are known to have an exceptionally high rate of mtDNA divergence (Hoeh et al. 1996). Further study to calculate the mutation rate of the mtCOI for *S. algosus* would allow estimation of the date of commencement of the population expansion for this species. However, this falls outside the scope of my study.

Conclusions

The results discussed above answer two important questions. Firstly the origin of the *S. algosus* invasion in South Africa was proven. The high genetic diversity of the South African population would not have been possible if it had arrived in South Africa via a single, small introduction in ballast water or amongst oyster spat. The genetic diversity of this population is probably due to high propagule pressure at introduction, a potentially large effective population size, and possibly (though less likely), ongoing gene flow. Thus, introduction to South Africa was most probably due to a natural range expansion from Namibia via larval dispersal. Furthermore, a stepping stone mode of expansion would have shown an increase in differentiation between sites in South Africa with increased geographic distance from Namibia. However, there was no indication of a geographic cline from north to south.

Secondly, *Semimytilus algosus* was extremely successful in invading rocky shores on the West Coast of South Africa, spreading rapidly and outcompeting other organisms. This success is due to the characteristics described in previous chapters, particularly its high recruitment rate, but is likely also related to its high genetic diversity.

Chapter 7

Synthesis and conclusions

An unidentified mussel was detected at Elands Bay in 2009. Based on its morphology it was identified as *Semimytilus algosus* (De Greef et al. 2013). However, mussels are known to have high phenotypic plasticity (Seed 1968), and identification based on morphology alone is not always accurate. Comparisons of DNA sequences can however, be used to verify the identify mussel species (Toro 1998; Rego et al. 2002; Wood et al. 2003; Blair et al. 2006; Santaclara et al. 2006). In Chapter 2, I sought to confirm the identity of this mussel by comparing its mitochondrial gene cytochrome oxidase I (COI), to that of known specimens of *S. algosus* from Chile and Namibia. I found that the invader is indeed *Semimytilus algosus* (Gould, 1850) (Chapter 2; De Greef et al. 2013).

As a complimentary procedure I compared the mitochondrial DNA (COI) sequences for mussels known as *Aulacomya atra*, from both Chile and South Africa, because *A. atra* is considered native to both regions (Branch et al. 2010; Bouchet & Gofas 2011). No prior studies have been conducted to establish whether they are indeed the same species, but my genetic comparisons verify that this is the case.

De Greef et al. (2013) also conducted surveys to establish the range of *S. algosus* along the West Coast in South Africa. They found that it covered 500km from Brand se Baai in the north to Bloubergstrand in the south. In Chapter 3 I describe how I resurveyed De Greef's sites and additional sites to determine: (a) whether the range of this mussel is expanding the coast, and (b) to confirm its zonation on the rocks. I found that it occurs from Groenriviersmond in the Northern Cape to Hout Bay in the south, and seem to be spreading southward. It has also spread southwards in Namibia and now extends almost as far as Oranjemund. Like De Greef et al., I also found that at most localities it is most abundant on the low shore, with *M. galloprovincialis* on the mid shore. Mixed beds are found in the transition between the low and mid shore (Chapter 3). I did however, find that while De

Greef et al. recorded *S. algosus* at both exposed and sheltered sites in Elands Bay, it failed to maintain a presence at the sheltered site.

I then investigated possible reasons for the rapid spread of *S. algosus* across the West Coast, as well as its concentration on the low shore, by (a) comparing life histories among mussel species and (b) the genetic variation of *S. algosus* in South Africa relative to that found in Namibian and Chilean specimens.

Competitive ability is strongly related to rapid colonisation, through high reproductive output, recruitment and growth rate (Branch & Steffani 2004). Thus, in Chapter 4, I compared the life-history strategies of *S. algosus* to those of the three other mussel species that occur abundantly on the West Coast. I found that it had a relatively slow growth rate, low byssal and shell strength and resultant high mortality. Its reproductive output was also comparatively low, but translated into an exceptionally high rate of recruitment, which appears to be the main factor driving its invasive success.

I also wanted to determine what the effect of *S. algosus* will be on South African rocky shores, given its recent arrival and rapid spread on the West Coast. I explored its effects on biodiversity, as well as its interactions with its closest competitor, *Mytilus galloprovincialis*, through field experiments described in Chapter 5. I discovered that its influence on the ecosystem, as well as, its interactions with *M. galloprovincialis*, were strongly related to shore height. Both species grow fastest and have highest body condition low on the shore, but due to its intolerance of abiotic stresses, *S. algosus* rarely recruits to the mid and high shores, or if it does recruit there, its survival is low.

In one set of experiments, at two sites, I removed all mussels from patches on the shore, and showed that this initially diminished biodiversity. However, this effect disappeared after two to four months as the system recovered, or mussels were replaced by other foundation species. For example algae served to boost diversity to levels comparable to those prior to removal of the mussels. Though, differences in diversity among shore heights persisted throughout the experiment, with biodiversity consistently lower with increased shore height. Removal of *M. galloprovincialis* and *S. algosus* did affect particular species. Abundance of the limpet *Scutellastra granularis* and the slug *Onchidium maculata* declined, but recruitment of the ribbed mussel *Aulacomya atra* rose, although it failed to survive to adulthood in the intertidal zone when in competition with these mussels.

In its native range *S. algosus* is known to only occur on the low shore, due to intolerance to desiccation (Tokeshi & Romero 1995). In a second set of experiments, also described in Chapter 5, I caged different densities of *S. algosus* and *M. galloprovincialis* separately and together. In this study I found that *S. algosus* was intolerant of the high levels of desiccation on the high shore and thrived only on the low shore. There were some pointers that it may be facilitated by *M. galloprovincialis*, and extends its range toward the mid shore among beds of *M. galloprovincialis*. *Mytilus galloprovincialis* thrives on the mid shore, but due to its greater tolerance to desiccation than other mussel species, it can survive on the high shore as well (Hockey and van Erkom-Schurink 1992; Bownes and McQuaid 2009). It is also found on the low shore, but only in low numbers due to intolerance to high wave action (Bownes & McQuaid 2009).

Sadchatheeswaran et al. (2015) suggested that *S. algosus* beds would reduce habitat complexity and have lower species richness and biodiversity than *M. galloprovincialis* beds, because *S. algosus* forms mono-layered beds (De Greef et al. 2013) and has much smaller shells than *M. galloprovincialis*. However, I found that it supported similar species richness and biodiversity to that within *M. galloprovincialis* beds. Some species, such as certain algal species, were more abundant within *S. algosus* beds, possibly because *S. algosus* supports fewer grazers, because of its smaller shell.

Genetic diversity is known to increase the fitness of invasive species, and to improve their chances of survival and becoming invasive. (Stepien et al. 2005; Roman & Darling 2007; Rius et al. 2008). In Chapter 6 I demonstrated that *S. algosus* exhibits high genetic diversity in all three populations examined: Chile, Namibia and South Africa. Population structure in the native range is also a good predictor of the extent of spread and thus invasive success in marine invaders (Gaither et al. 2013; Jackson et al. 2015). There was little indication of population structure for this species among these populations, but the high genetic diversity and rapid development of unique haplotypes may contribute to its invasive success in South Africa.

In summary, central features of *S. algosus* that emerged were its intolerance of high-shore conditions that largely restricted it to the low shore. Its slow growth and vulnerability to predators because of its weak shell and weak powers of attachment and its exceptional rate of recruitment to the shore. Also its high genetic diversity, which may increase its adaptability to new circumstances and contribute to its invasive capability.

Semimytilus algosus is not known to have invaded any other areas in the world to date, and no studies have been conducted on the invasive potential of this species. However, its invasion history in southern Africa, along with the findings in this study, points towards its being a highly invasive species warranting close observation worldwide. Bigatti et al. (2014) have already found viable *S. algosus* spat in the ballast water of a ship in the Nuevo Gulf, Argentina, and recommended close monitoring of the rocky shore in that area to determine whether this species will become established.

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Figure 1.1: Map of South America showing the distribution of *Semimytilus algosus* along the Pacific coast. Sampling sites are indicated with ○. Species are indicated with initials: Sa- *Semimytilus algosus* and Aa- *Aulacomya atra*. Map produced by Saachi Sadchatheeswaran.

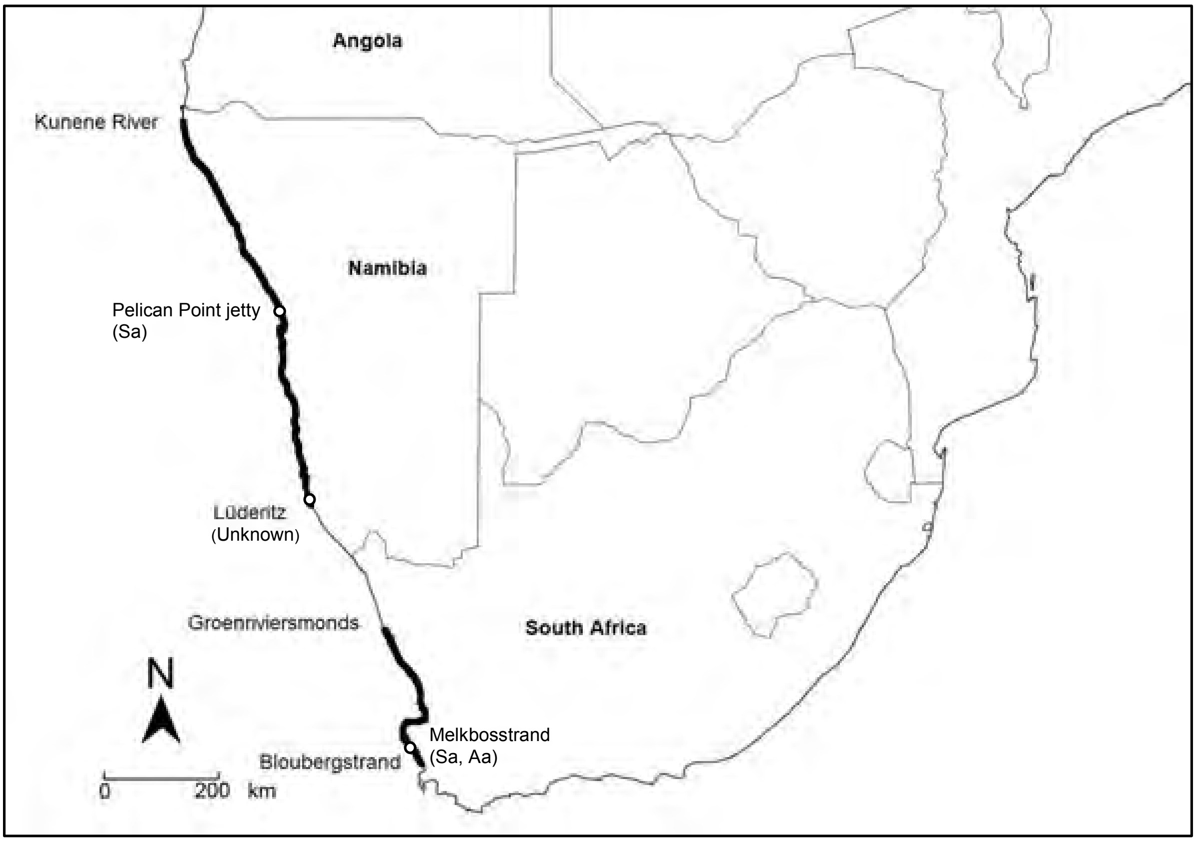

Figure 1.2: Map of Southern Africa showing the distribution of *Semimytilus algosus* in Namibia and South Africa. Sampling sites are indicated with ○. Species are indicated with initials: Sa- *Semimytilus algosus*; Aa- *Aulacomya atra*; unknown- the unknown mussel. Map produced by Saachi Sadchatheeswaran.

Figure 2.1: Photograph of *Semimytilus algosus* shells on a bed of *S. algosus* mussels, showing both the inside and the outside of the shell. Photograph courtesy of Prof. George Branch.

Figure 2.2: Photograph of *Aulacomya atra* shells, top row from South Africa and bottom row from Chile. Courtesy of Prof. George Branch.

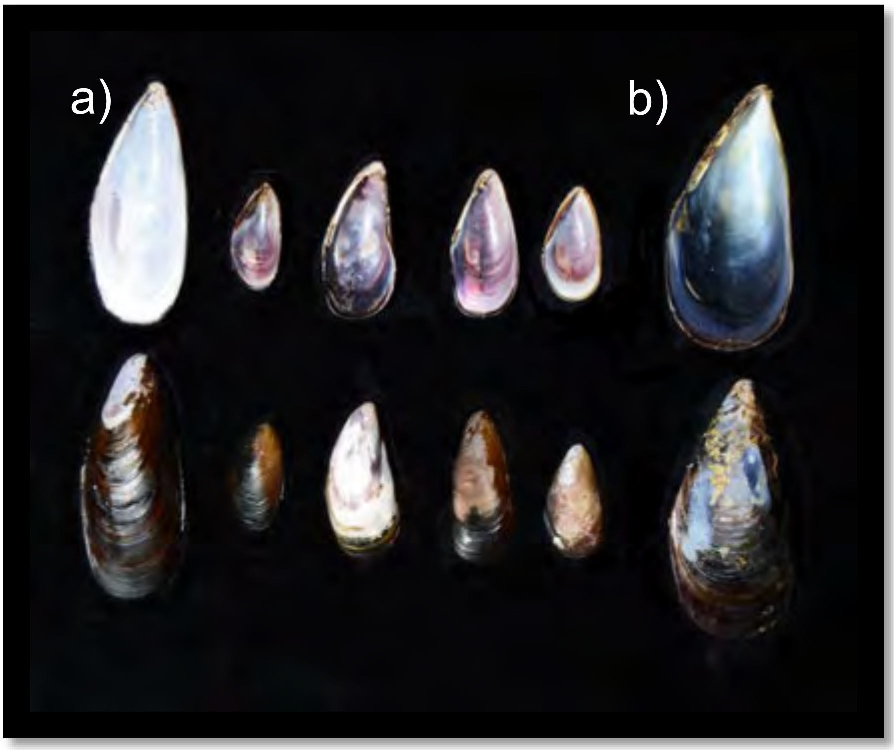

Figure 2.3: Photograph showing the differences in morphology between a) *Semimytilus algosus* (five shells on the left) and b) *Mytilus galloprovincialis* (right). Photograph courtesy of Prof. George Branch.

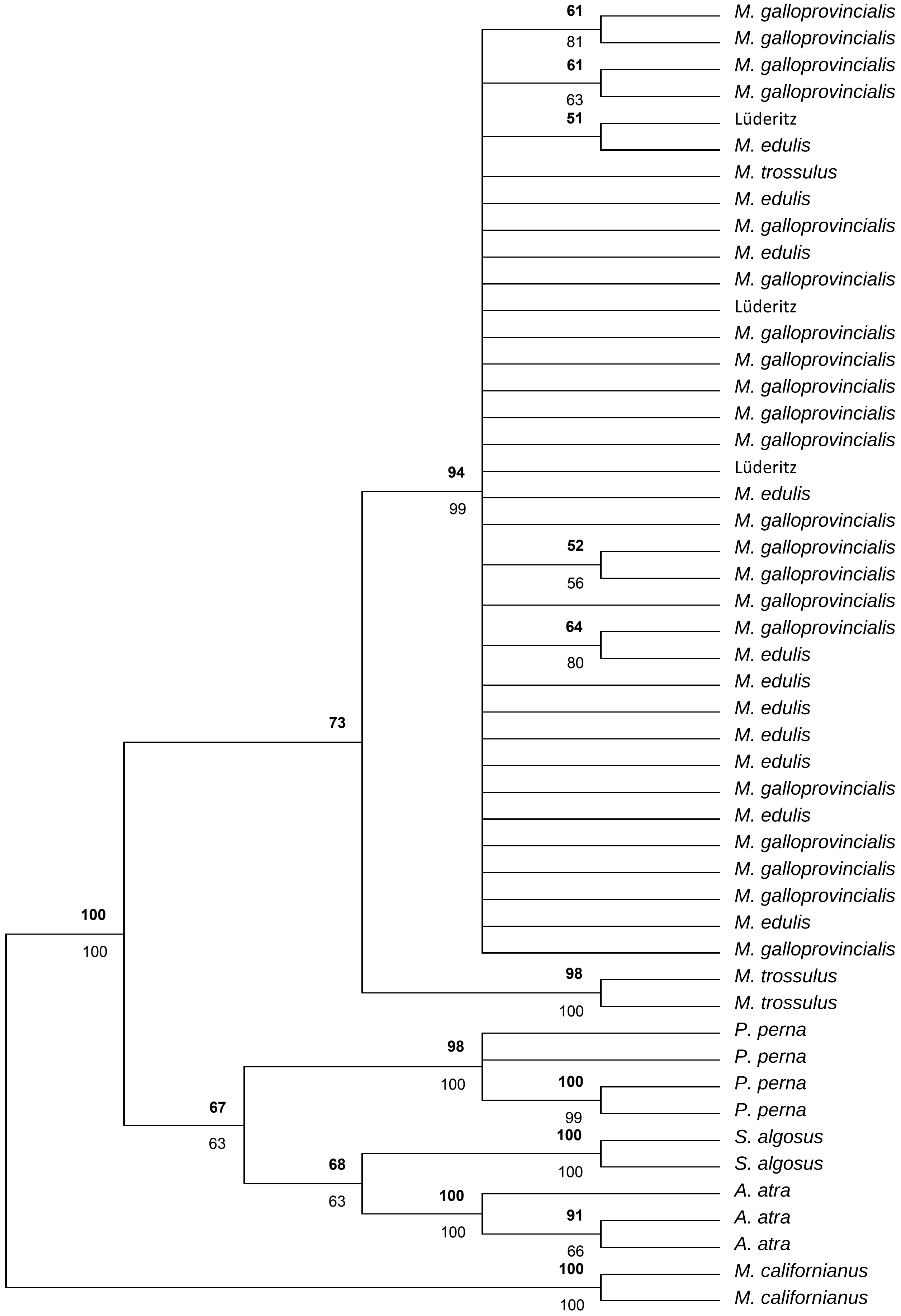

Figure 2.4: Neighbor-Joining tree employing the Tajima-Nei method. Bootstrap support values are given above the nodes. Bootstrap support for the maximum parsimony tree is given below the nodes.

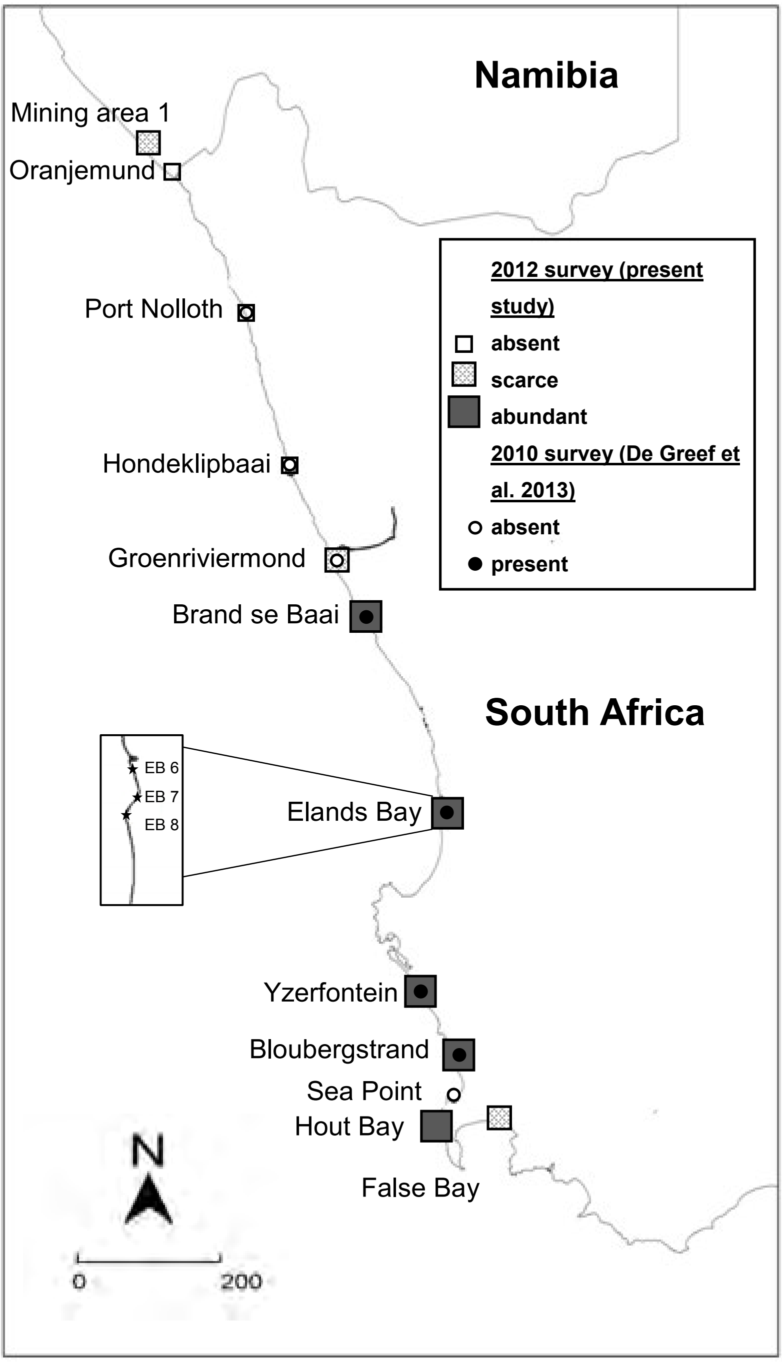

Fig. 3.1: Map of the West Coast of South Africa showing all sites surveyed during this study, and those conducted by De Greef in 2010. Solid squares indicate the presence of *Semimytilus algosus* and open squares the absence thereof, shaded squares signifies low abundance. A circle denotes sites surveyed by De Greef et al. (2013), with open circles denoting sites where no *S. algosus* were found, and closed circles where *S. algosus* were present.

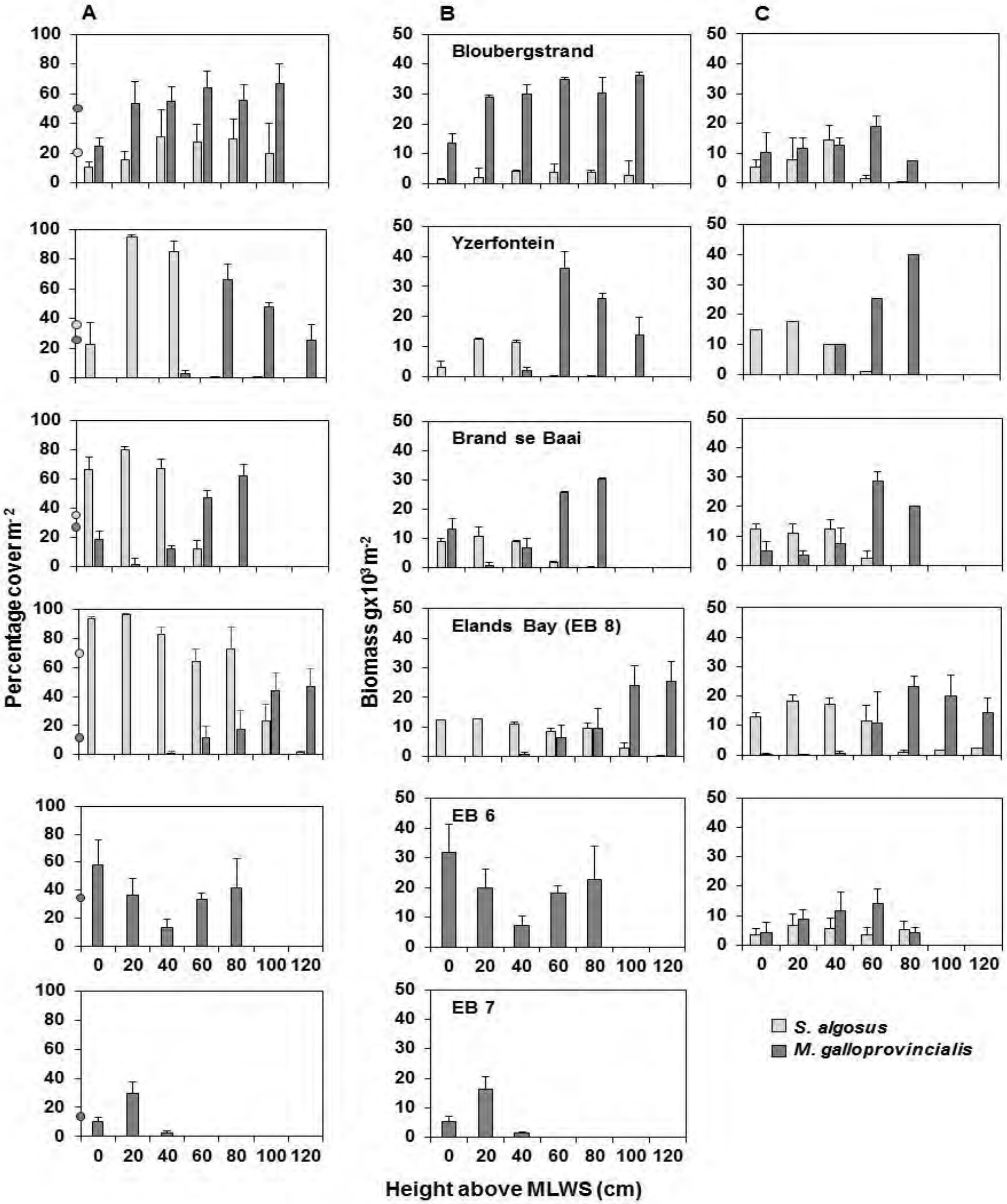

Figure 3.2: Bar graphs comparing: A. percentage cover; B. biomass in surveys carried out for this study in 2012; C. biomass (gx10^3 m^{-2}) for surveys conducted by De Greef in 2010, for all sites. All sites were exposed except the sheltered sites EB6 and EB7. The mean % cover for each species at the different sites are indicated by circles. Error bars denote +1 SE.

Figure 3.3: Photograph showing zonation on the shore. *Semimytilus algosus* occurred on the low shore and *Mytilus galloprovincialis* on the mid-high shore. Photograph courtesy of Professor George Branch.

Figure 4.1: Photograph of a labelled *M. galloprovincialis* mussel.

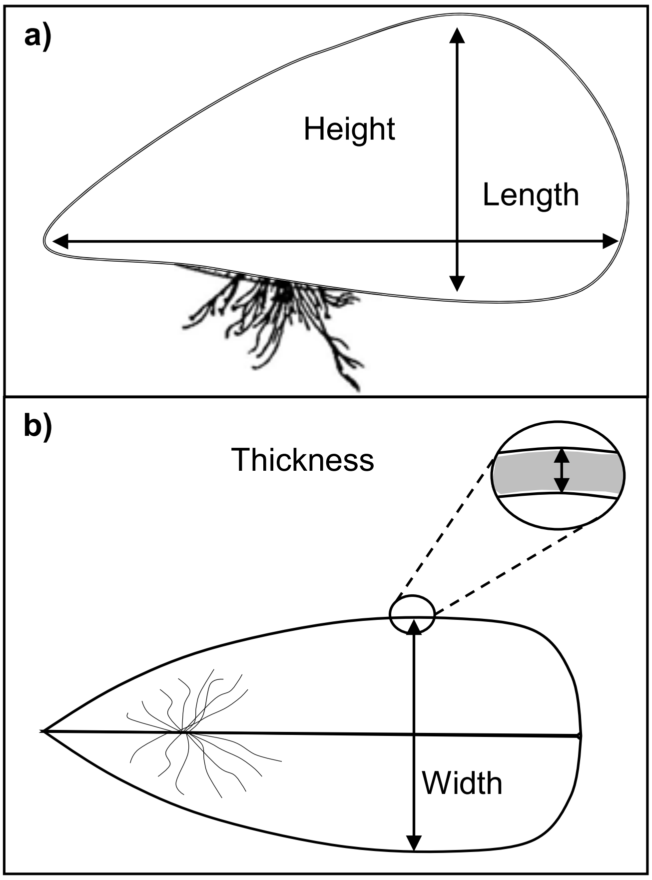

Figure 4.2: Measurements taken of shell morphology: a) Lateral view: maximum shell length, and shell height (mm) taken at tallest part of each shell; b) Ventral view: shell width (mm) taken at the widest part of single shell, and shell thickness (mm) taken at the widest part of the shell.

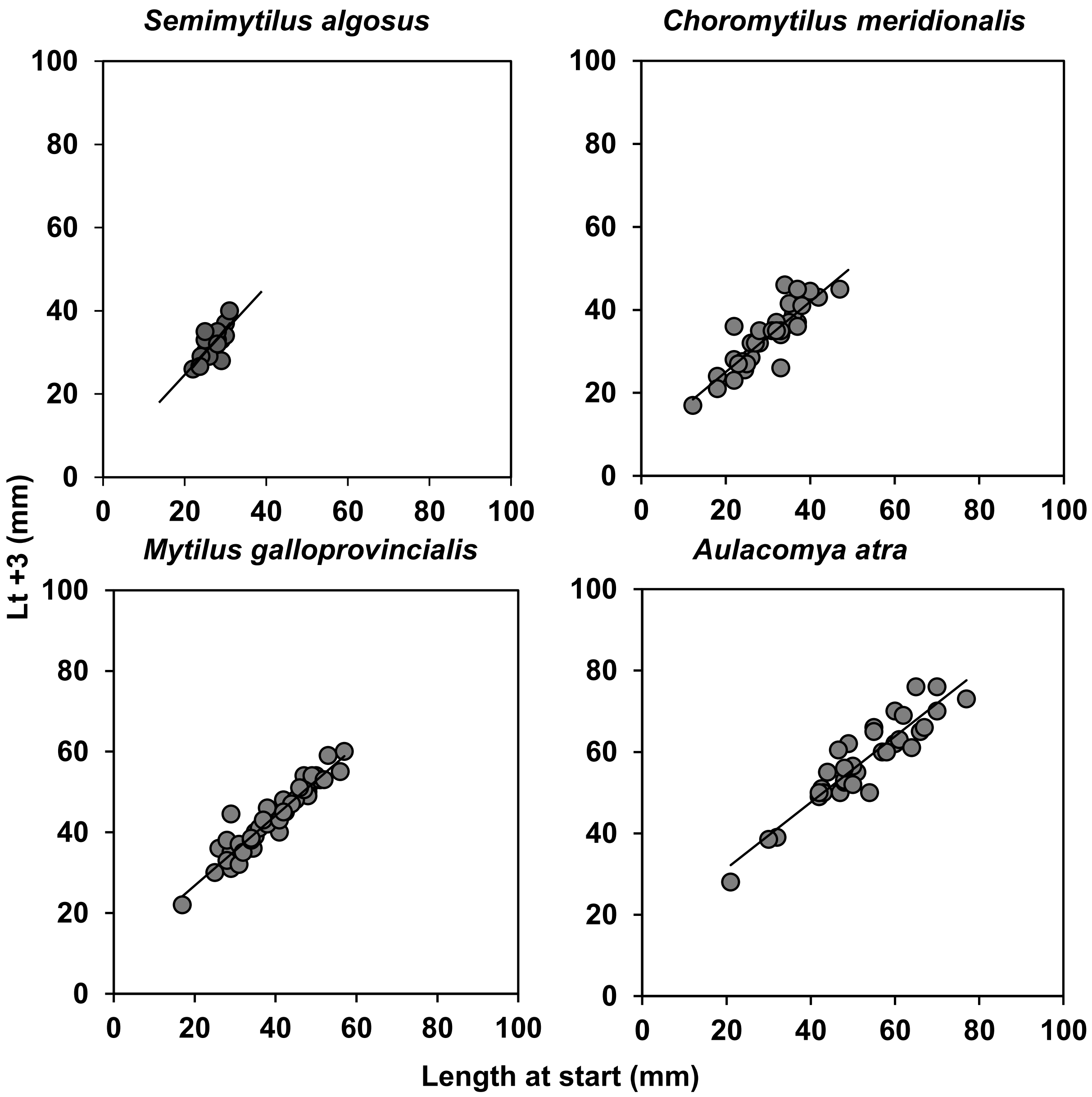

Figure 4.3: Ford-Walford plots for the four species of mussels found on the West Coast of South Africa: *Semimytilus algosus*, *Mytilus galloprovincialis*, *Choromytilus meridionalis* and *Aulacomya atra*. Lt+3 is the length after three months.

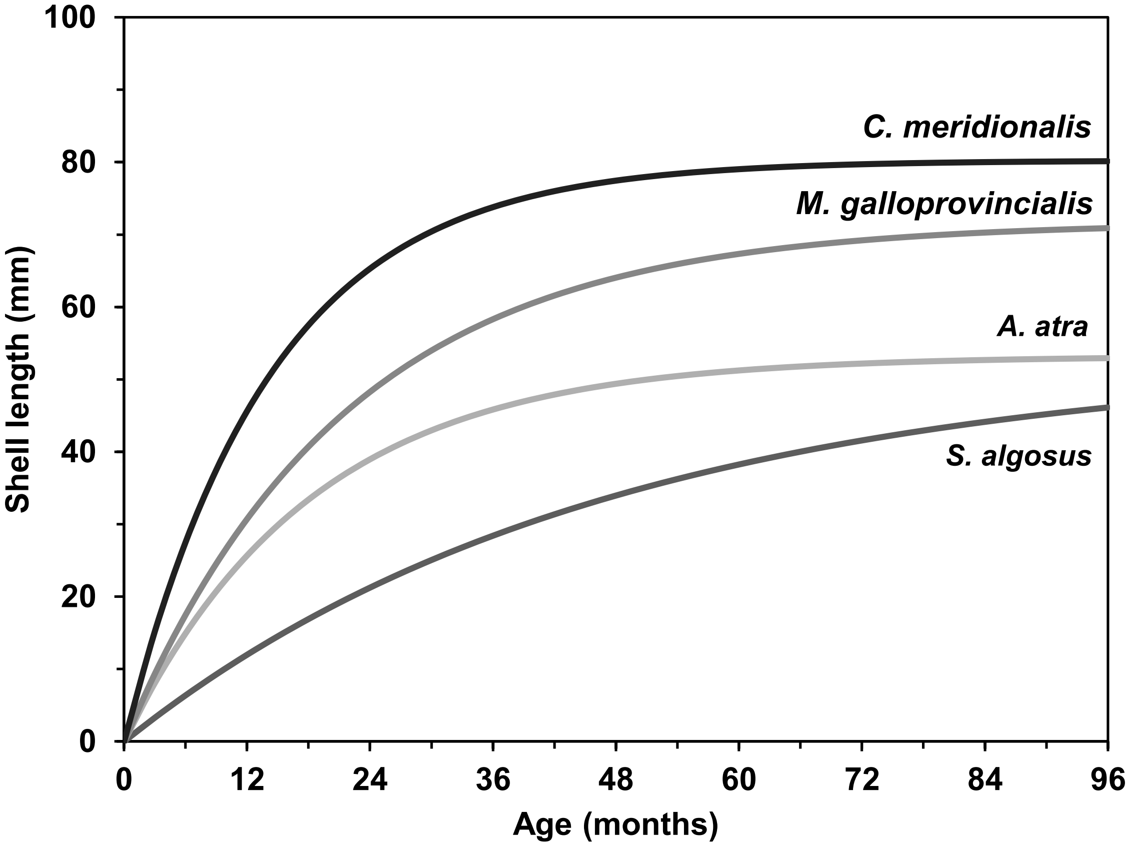

Figure 4.4: Von Bertalanffy growth curves for *Semimytilus algosus*, *Aulacomya atra*, *Mytilus galloprovincialis* and *Choromytilus meridionalis*.

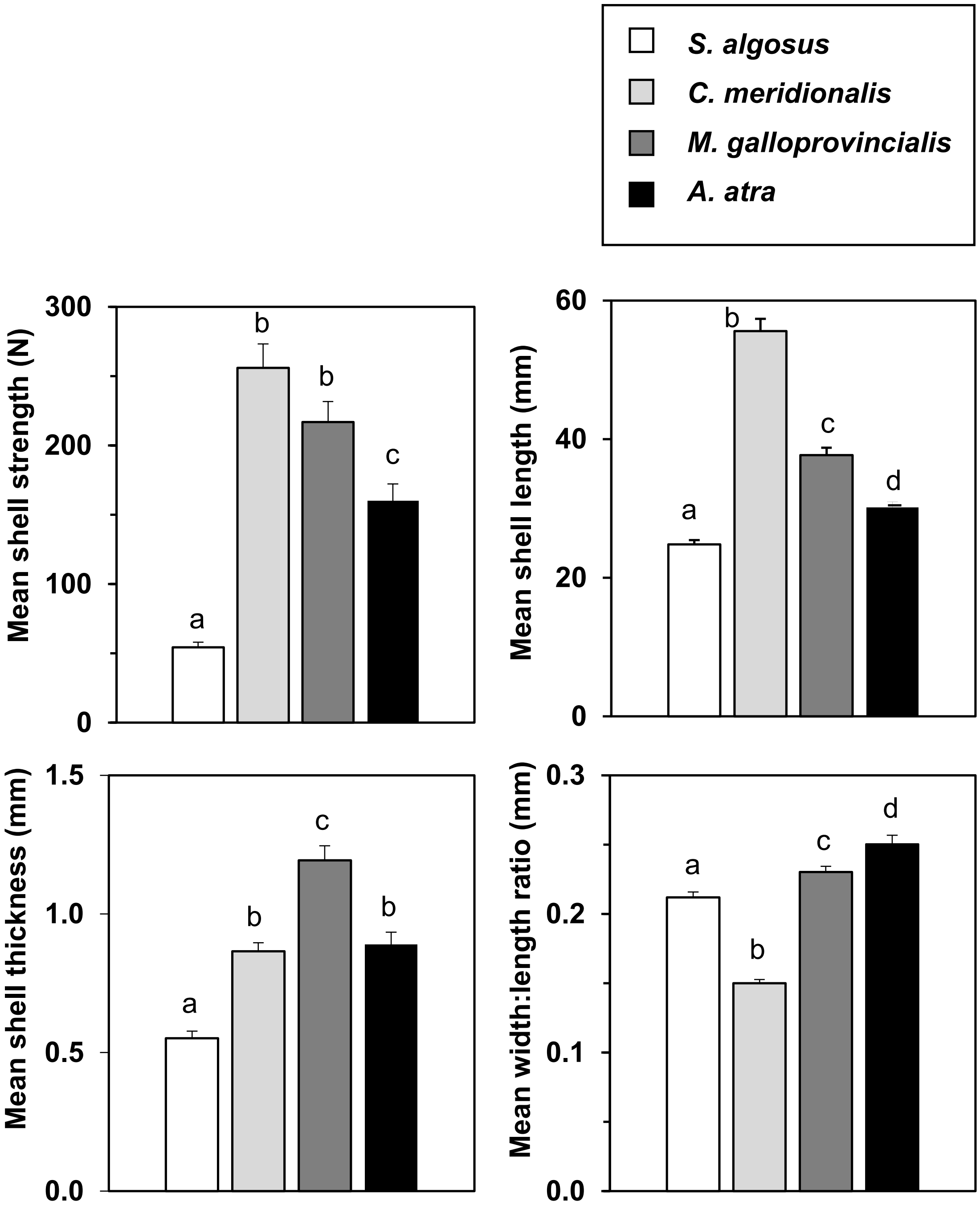

Figure 4.5: Comparison of mean breaking force, shell length, thickness and width:length ratio. Error bars +SE. Letters indicate significant differences. Statistical analyses were performed on the log-transformed data, due to heteroscedasticity.

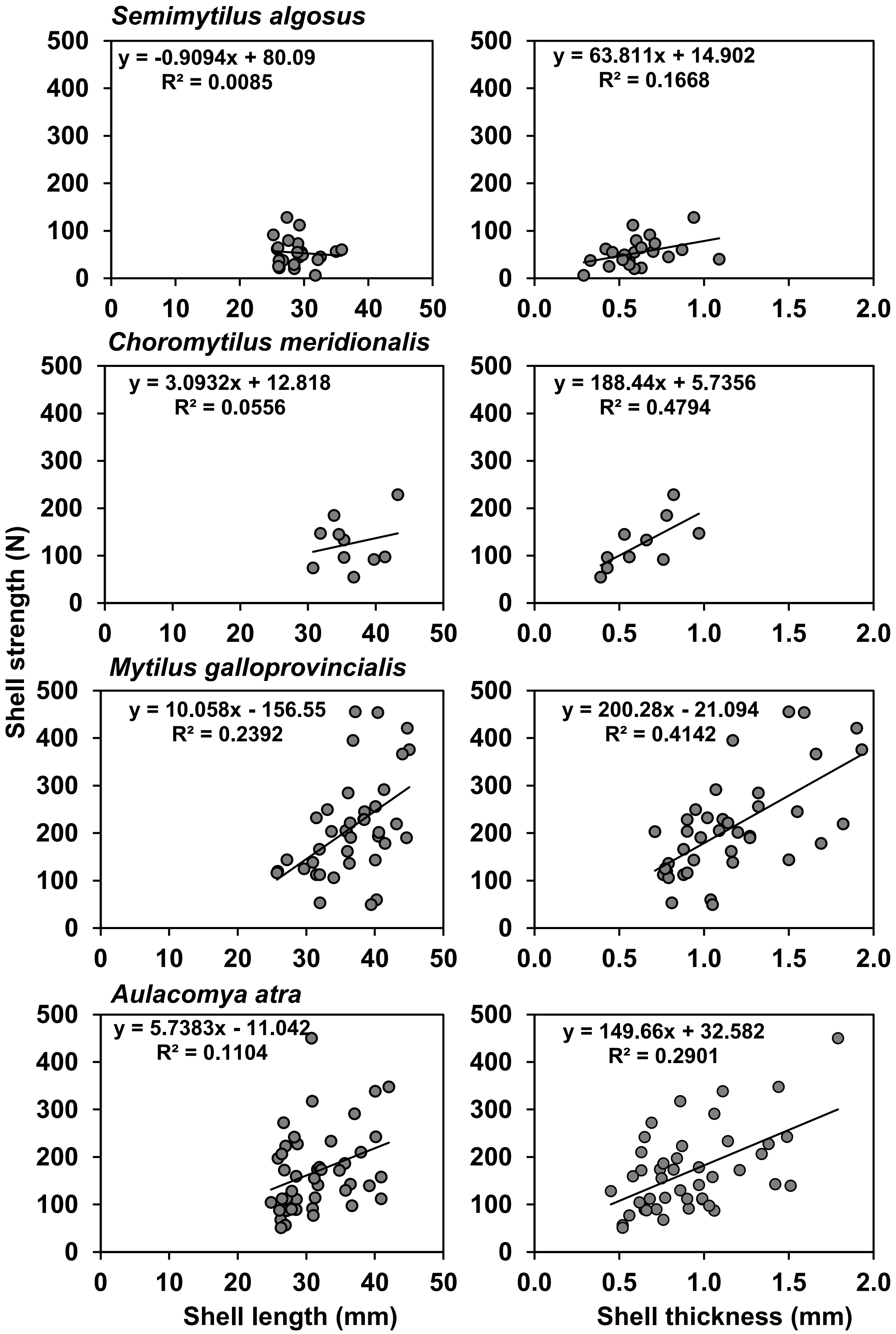

Figure 4.6: Linear regressions between shell length and shell strength (left) and shell thickness and shell strength (right), for mussels in the size range 25-45mm.

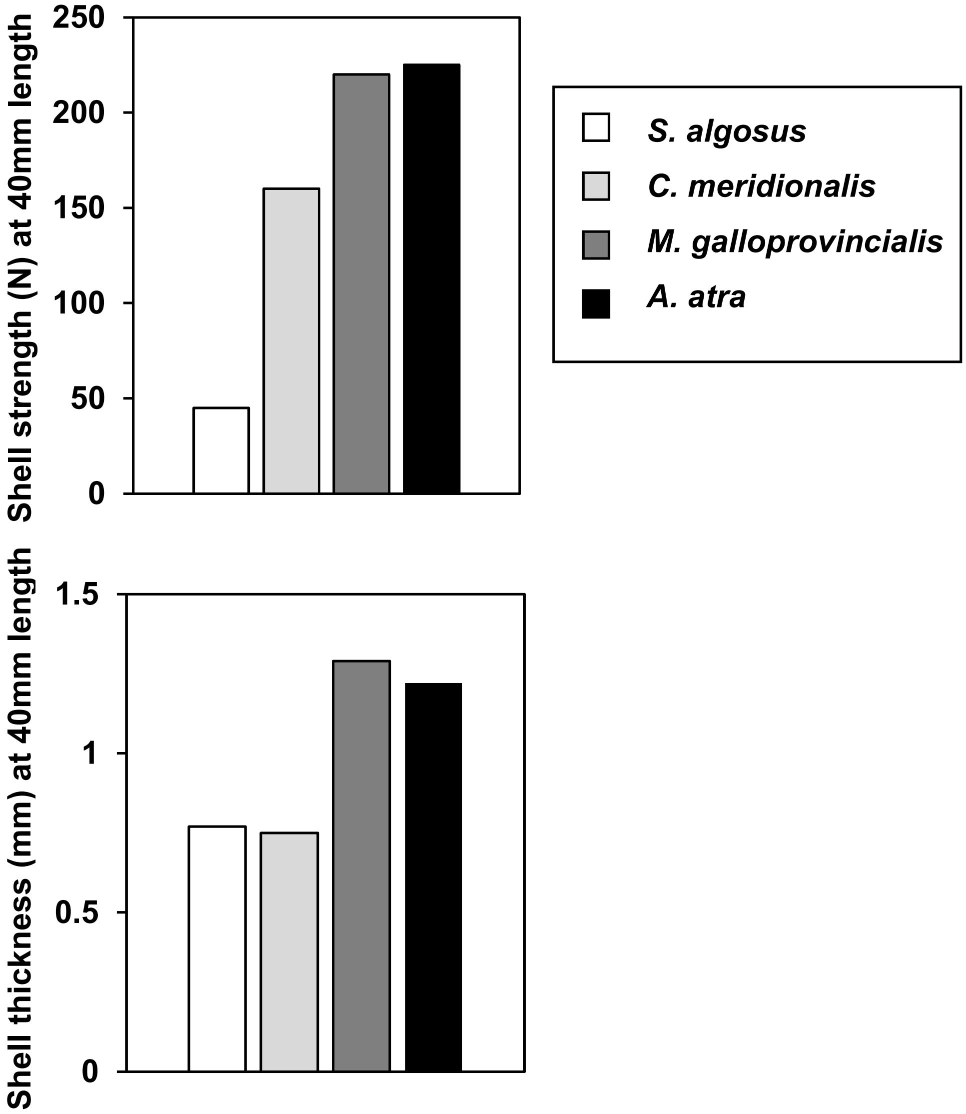

Figure 4.7: Comparison of shell strength and shell thickness for standard-sized, 40-mm mussels.

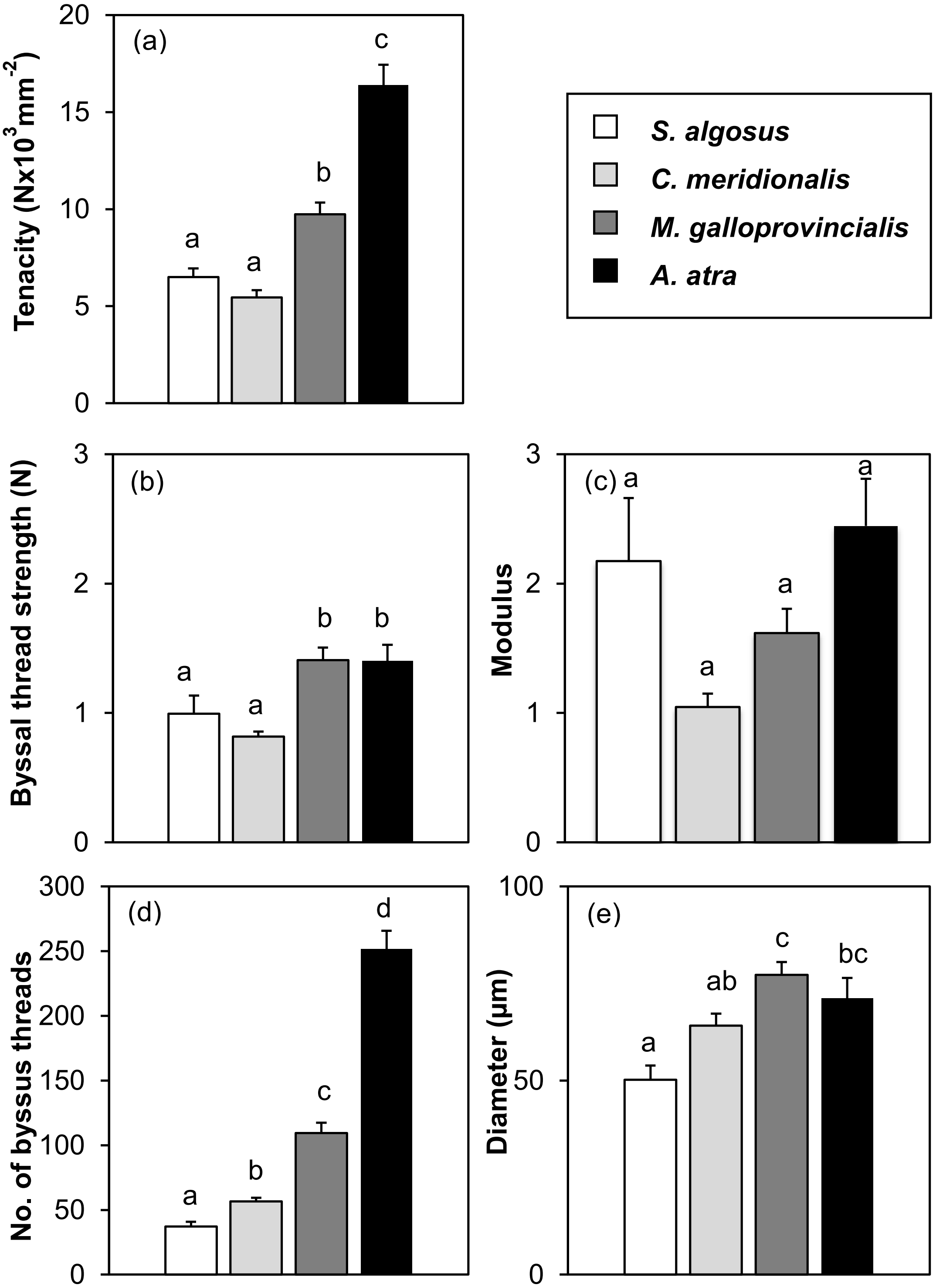

Figure 4.8: Bargraphs comparing (a) overall tenacity; (b) single byssal thread strength; (c) mean modulus; (d) number of byssus threads; (e) mean diameter of byssus threads, between mussel species. Error bars +SE. Letters indicate significant differences among species. Statistical analyses were performed on the log-transformed data, due to heteroscedasticity.

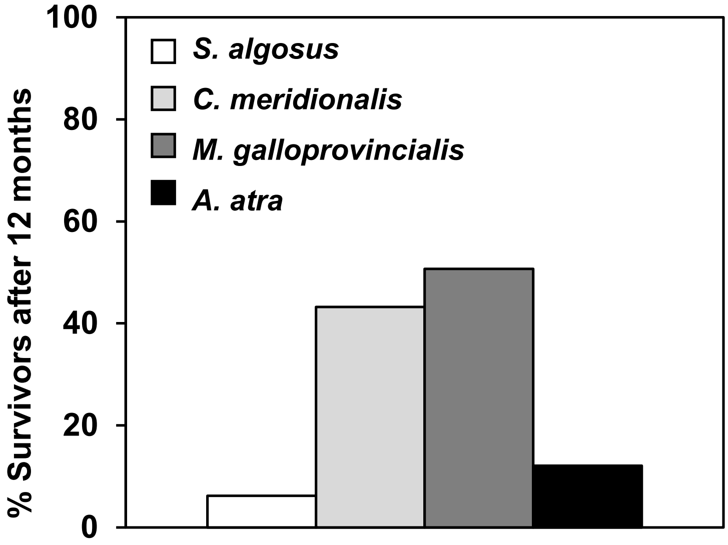

Figure 4.9: Comparison of % survival after 12 months, among the mussels *S. algosus*, *C. meridionalis*, *M. galloprovincialis* and *A. atra*.

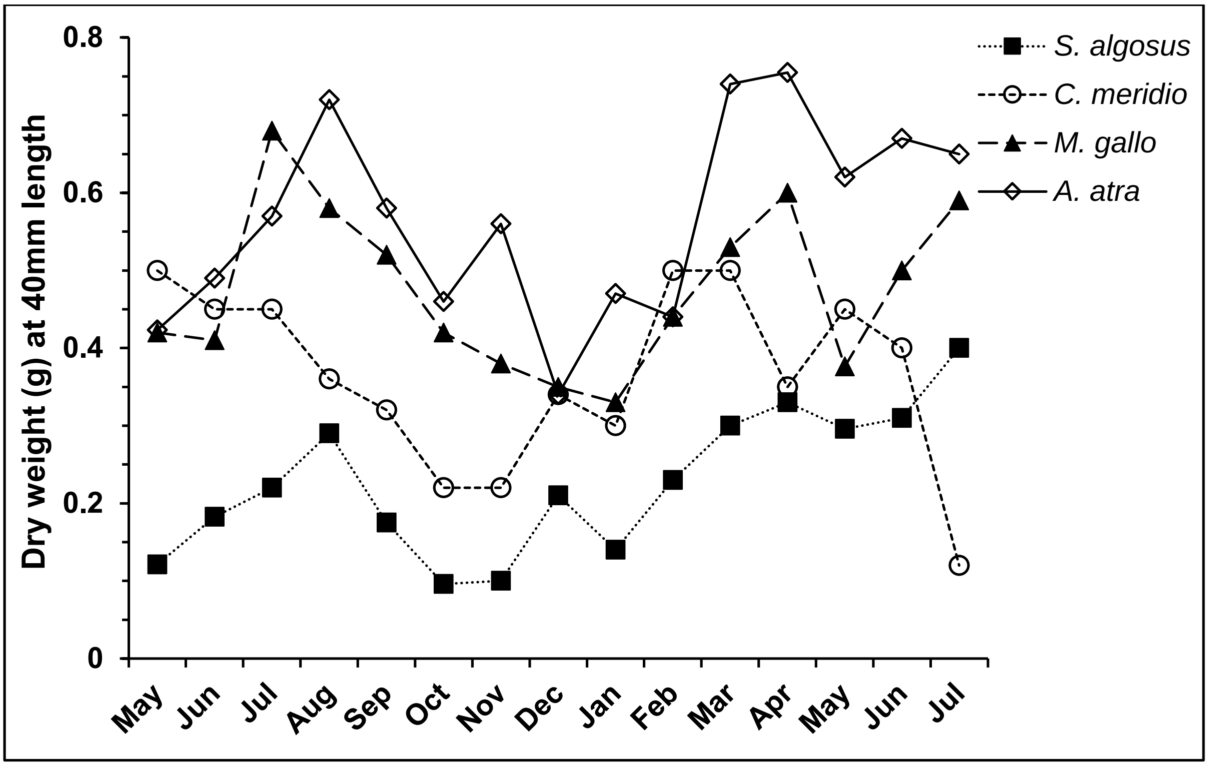

Figure 4.10: Monthly dry flesh weight at 40mm length, for four mussel species, *Semimytilus algosus*, *Aulacomya atra*, *Mytilus galloprovincialis* and *Choromytilus meridionalis*.

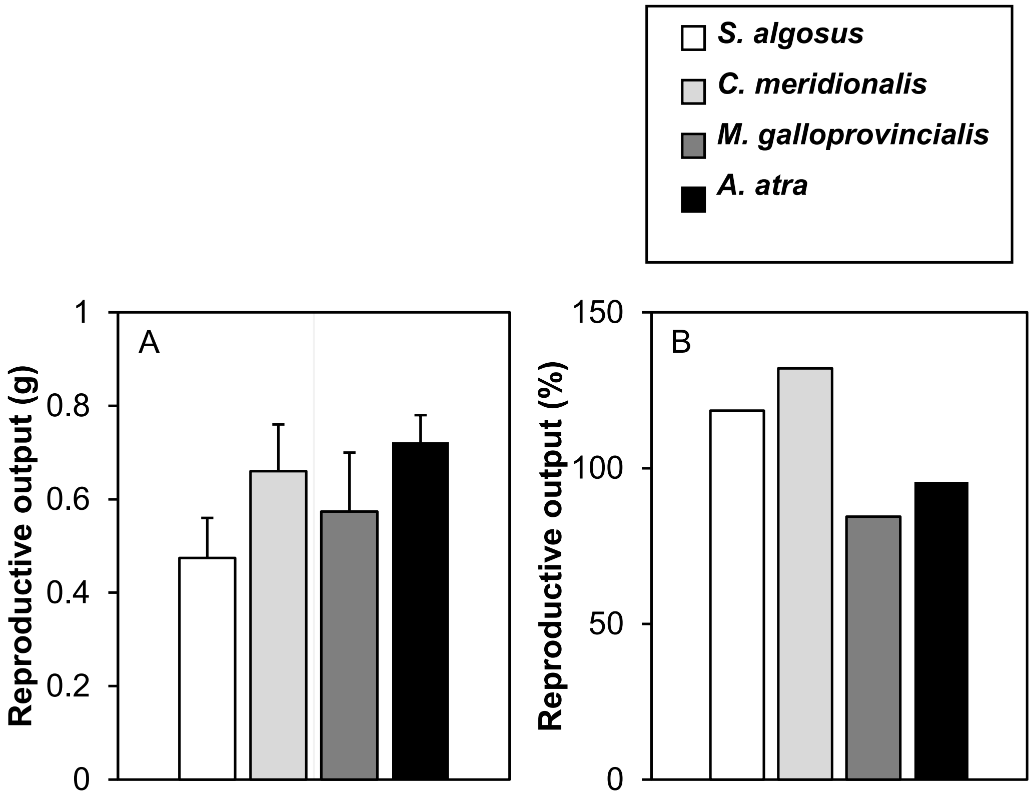

Figure 4.11: Comparison of (A) absolute reproductive output and (B) reproductive output as a percentage of body mass, for 40-mm sized mussels, over 14 months. Error bars +SE.

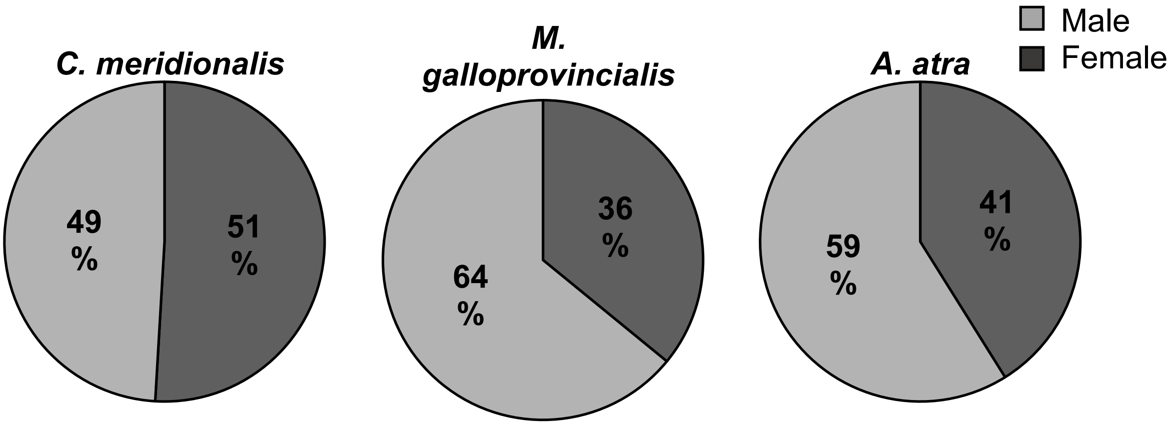

Figure 4.12: Sex ratios for *Mytilus galloprovincialis*, *Choromytilus meridionalis* and *Aulacomya atra*. (*S. algosus* is omitted as it is bisexual.)

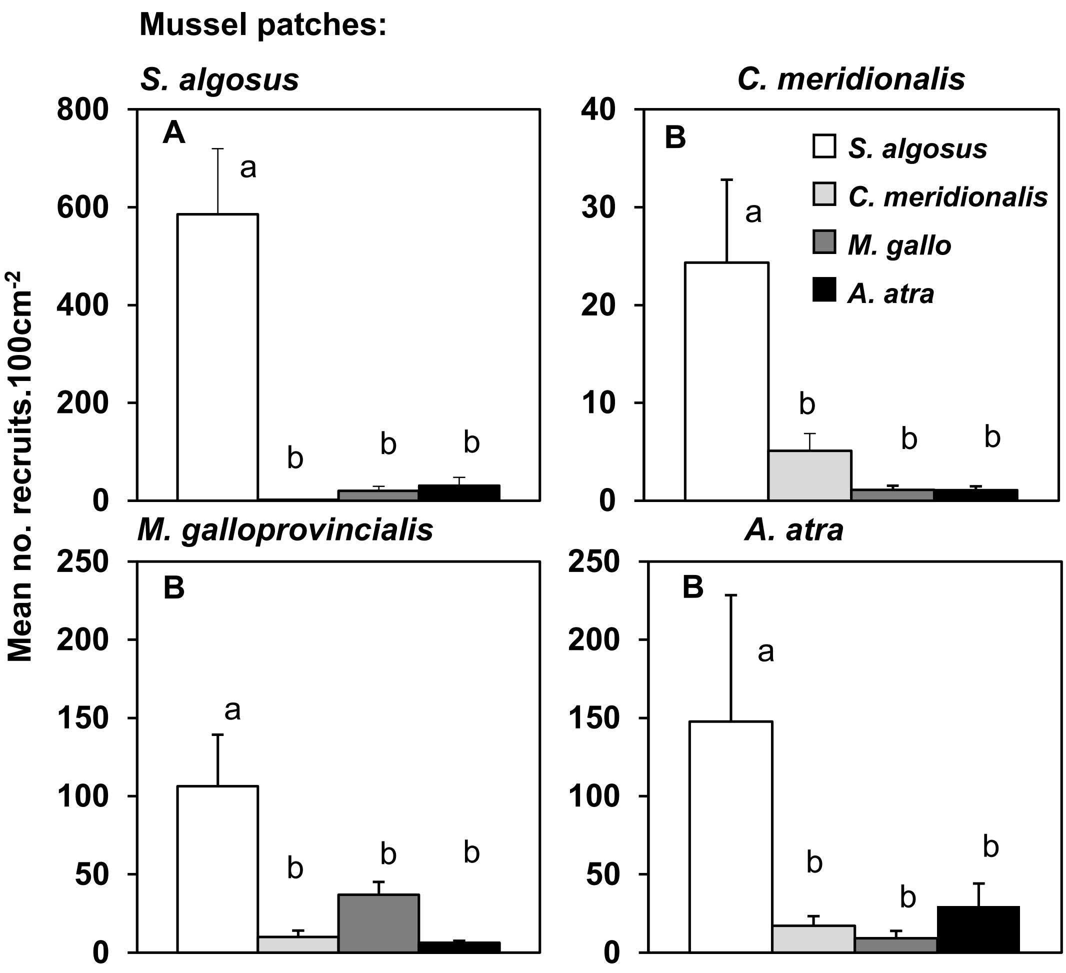

Figure 4.13: Mean number of recruits, sampled within patches with 100% cover of each of the four different mussel species. Error bars +SE. Capital letters show significant differences between patches, and small letters indicate differences among species within patches. Note differences in scale among patches of the different species.

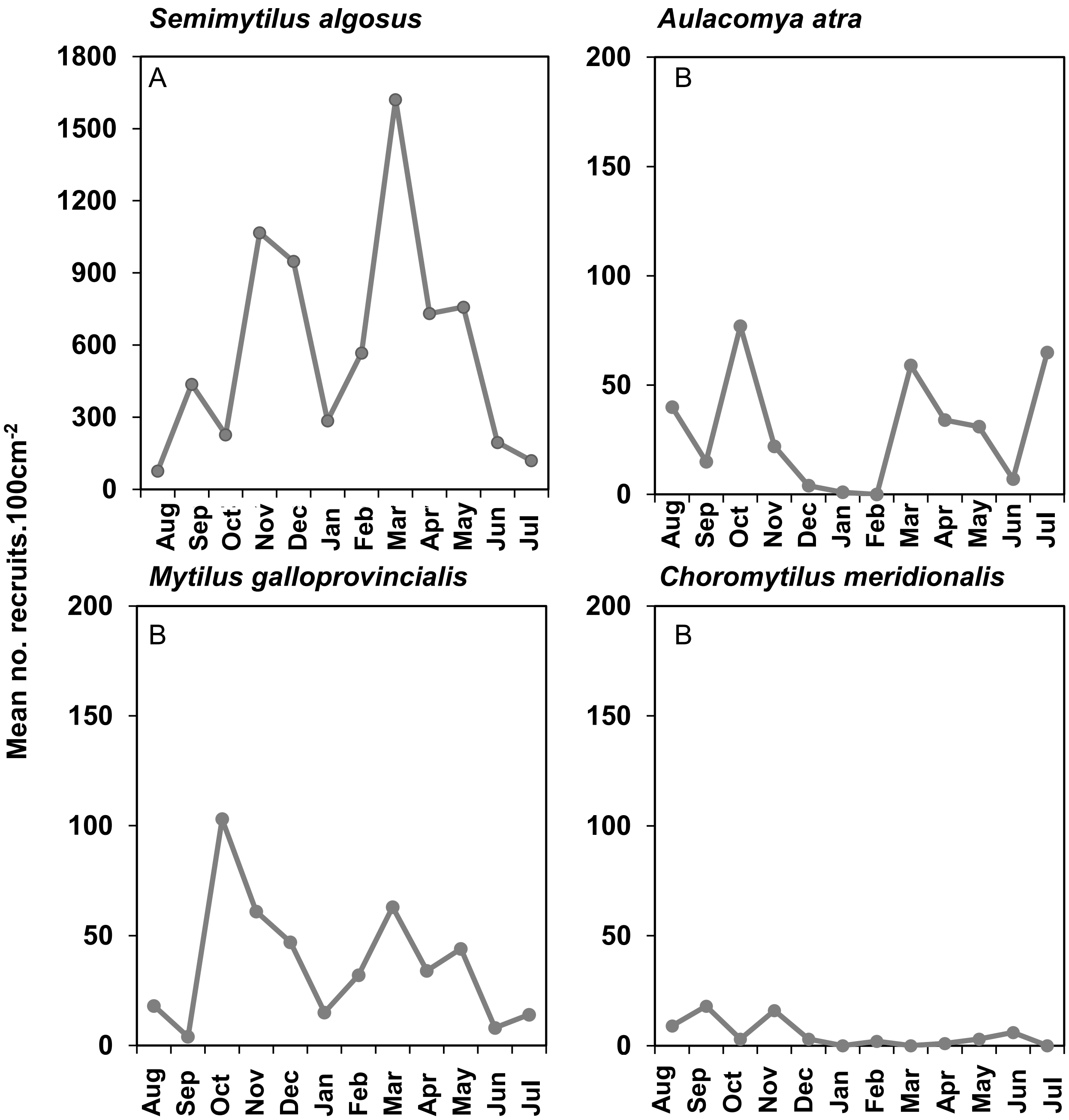

Figure 4.14: Mean monthly recruitment within conspecific patches from August 2012-July 2013. Note the different scale used for *S. algosus*. Letters indicate significant differences between species.

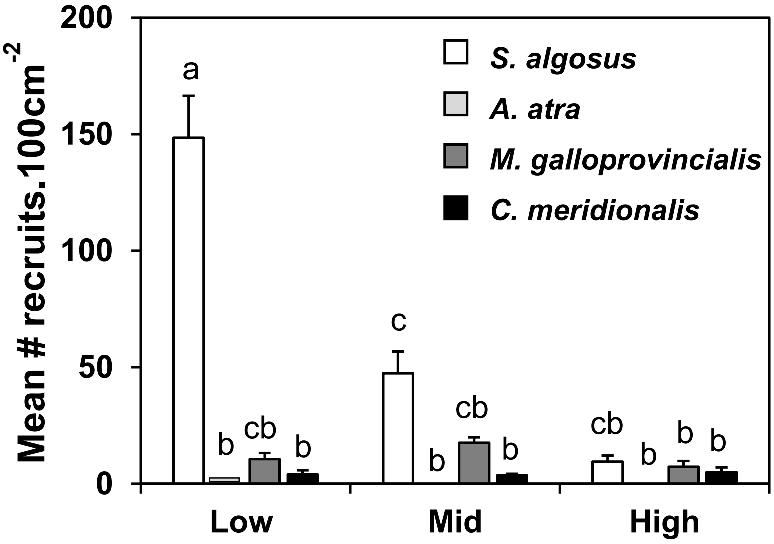

Figure 4.15: Mean number of recruits for *Semimytilus algosus*, *Aulacomya atra*, *Mytilus galloprovincialis* and *Choromytilus meridionalis* at different shore heights. Errors bars +SE. Letters indicate significant differences among species and shore levels.

Figure 5.1: Photograph showing the cages installed for the caging experiment.

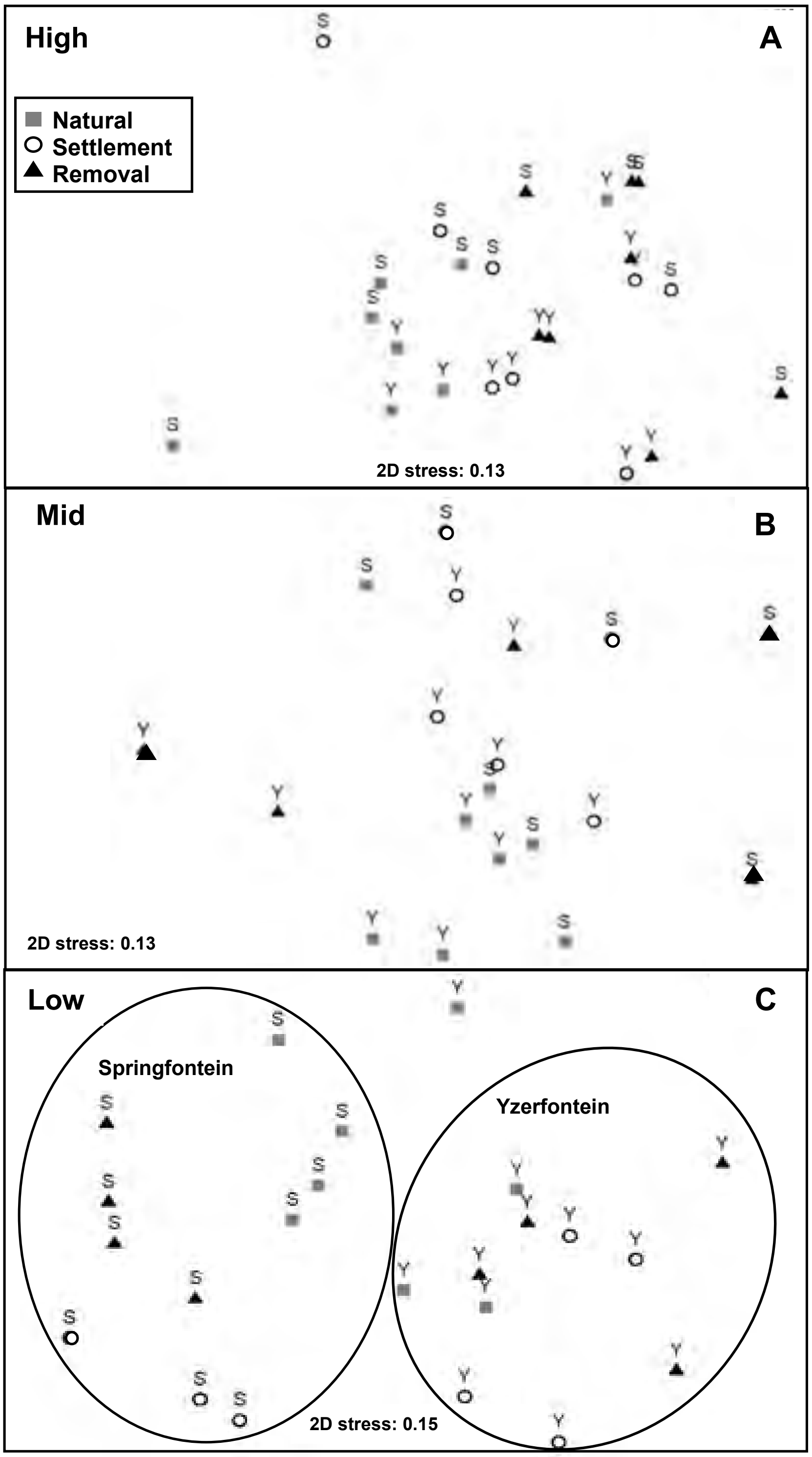

Figure 5.2: MDS ordination plots (based on biomass) comparing community structure between two sites (Yzerfontein – Y, Springfontein – S), among three treatments (see key), at three shore heights (high; mid and low shore) at the end of the experiment. Each data point represents one sample; n=4 per shore height per treatment, at both sites N=72. On the low shore the two sites formed separate clusters.

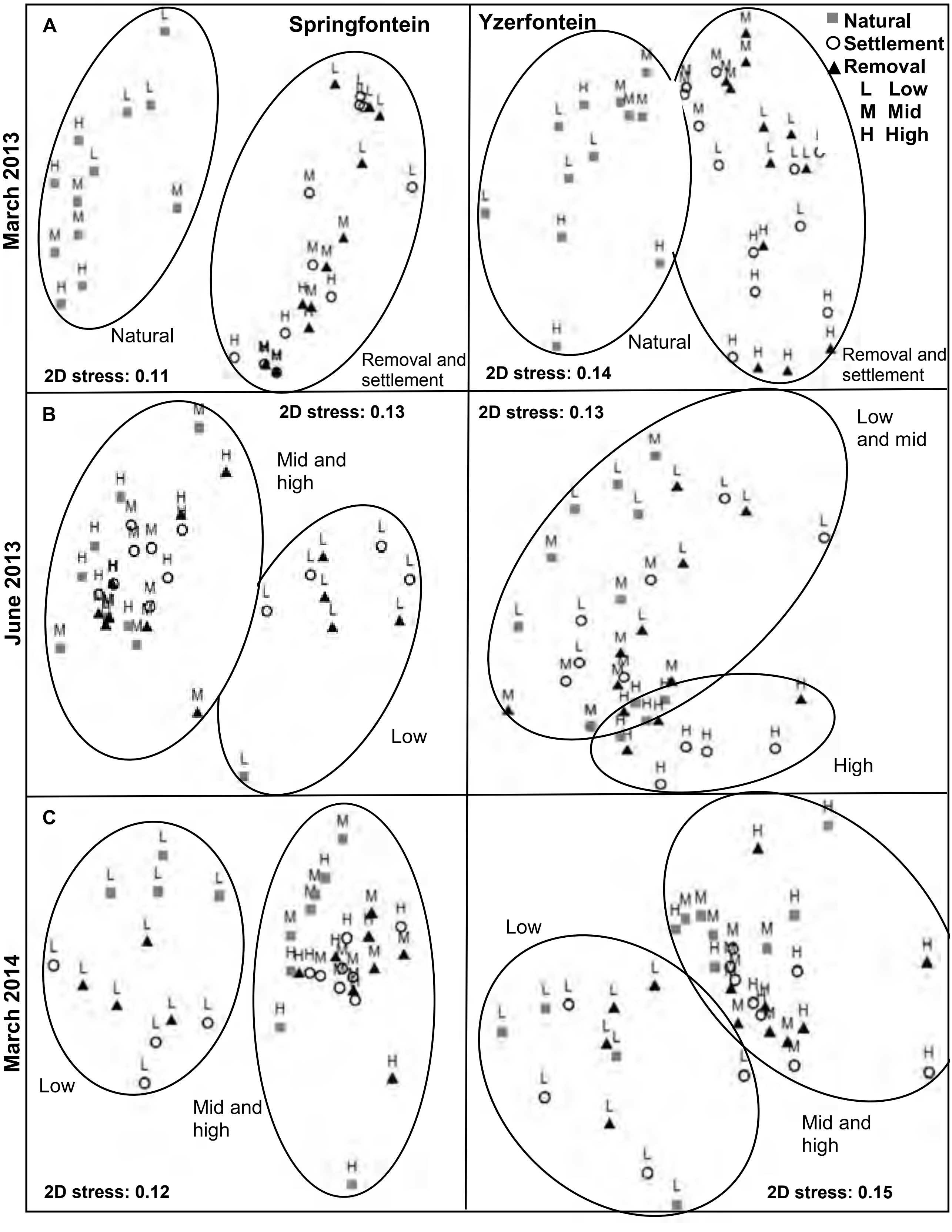

Figure 5.3: MDS ordination comparing community structure at two sites after 2 weeks (March 2013), 4 months (June 2013) and 1 year (March 2014), among three treatments, within three shore heights, based on percentage cover data. Circles indicate significant differences. Each data point represents one sample; N=36.

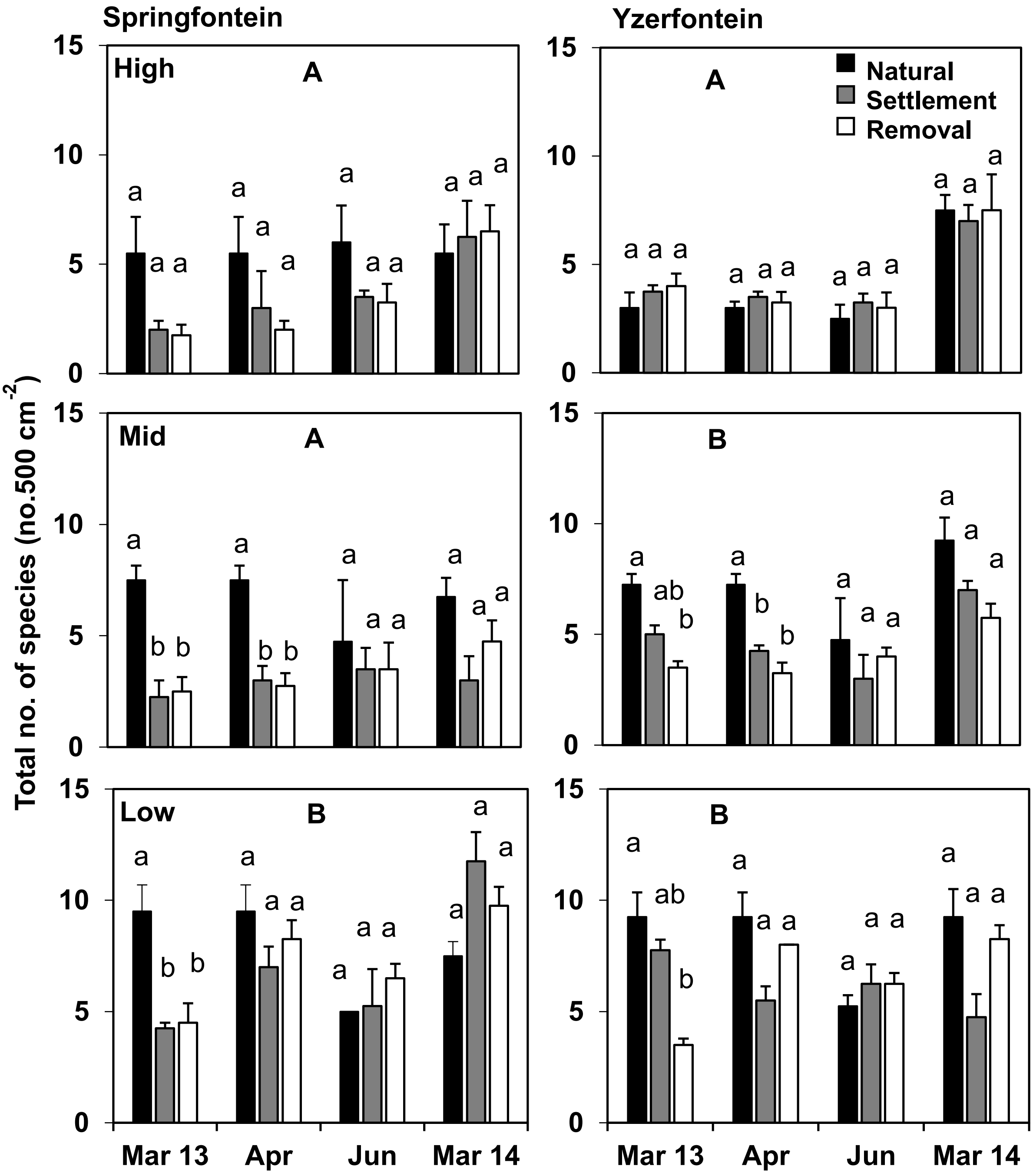

Figure 5.4: Comparison of species richness (total number of species) at two sites, among treatments, months and shore heights. Error bars +1SE. Capital letters indicate significant differences among shore heights and small letters among treatments within shore heights.

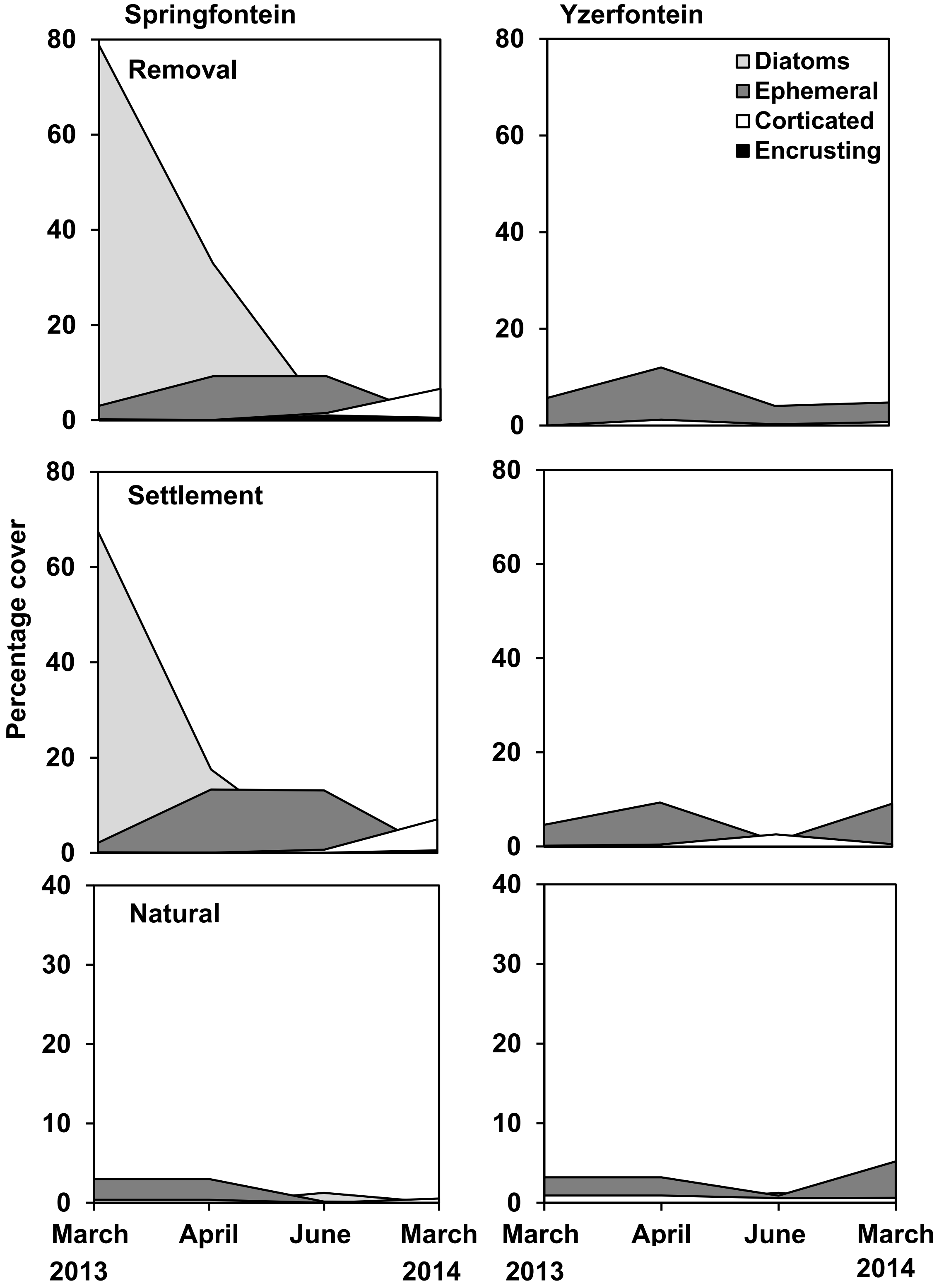

Figure 5.5: Algal responses over one year, within three treatments on the low shore at Springfontein (left), and Yzerfontein (right). Note scale differences for natural treatment.

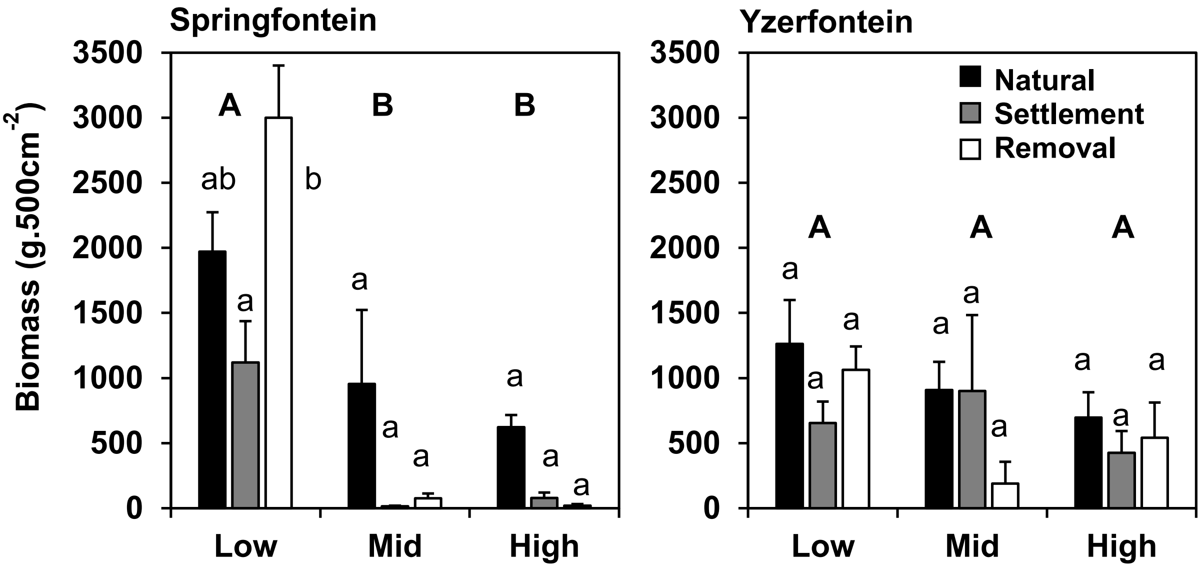

Figure 5.6: Variability in mean (+1SE) biomass at two sites among shore heights and treatments on termination of the clearance experiment. Capital letters indicate significant differences among shore heights and small letters among treatments within shore heights.

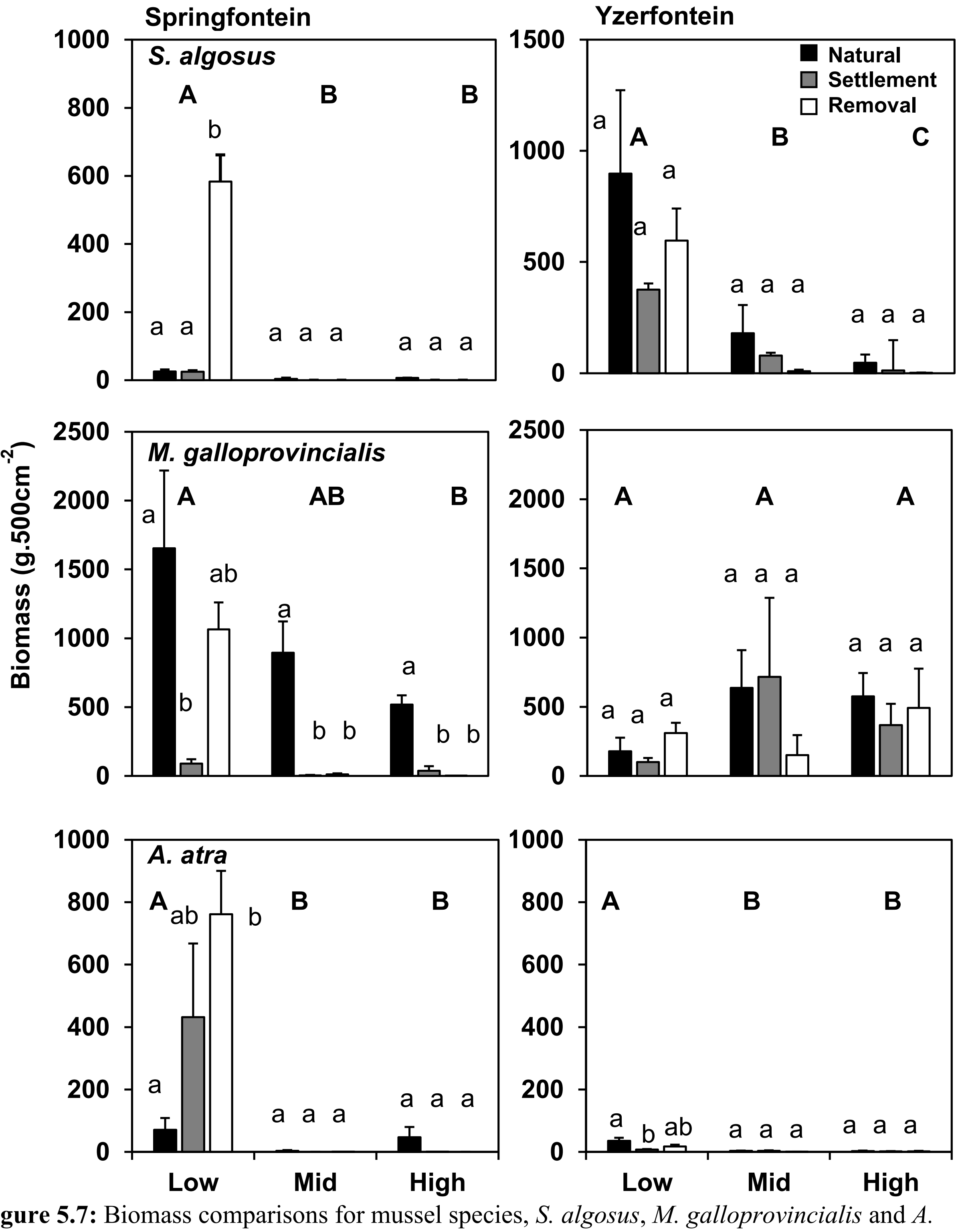

Figure 5.7: Biomass comparisons for mussel species, *S. algosus*, *M. galloprovincialis* and *A. atra* at the termination of the clearance experiment, at two sites and among shore heights and treatments. Capital letters indicate significant differences among shore heights and small letters among treatments within shore heights. Error bars +1SE. Note differences in scale among species.

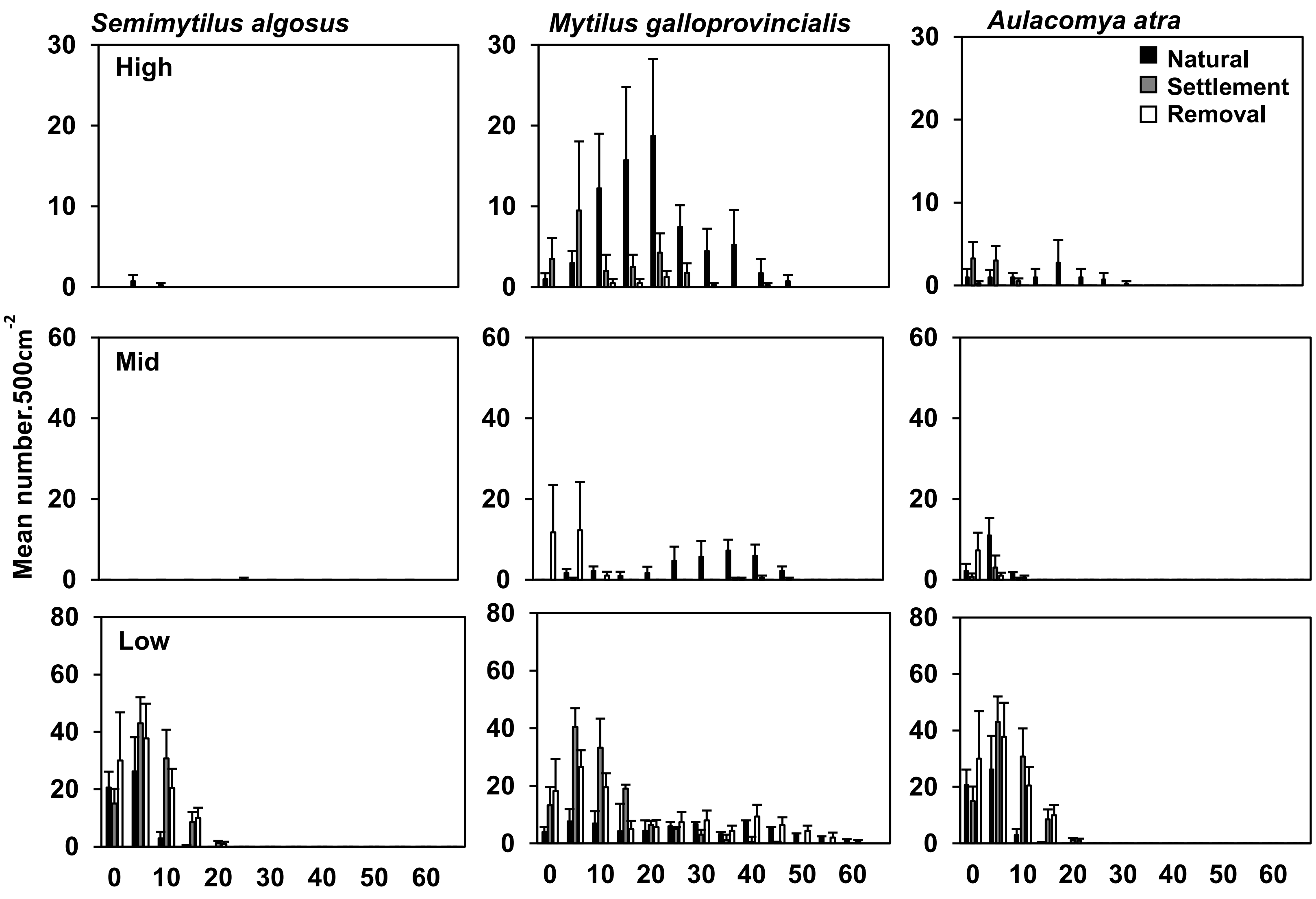

Figure 5.8: Size-composition of three mussel species at Springfontein, at three shore heights and within three treatments. Error bars +1SE. Note scale differences among shore heights.

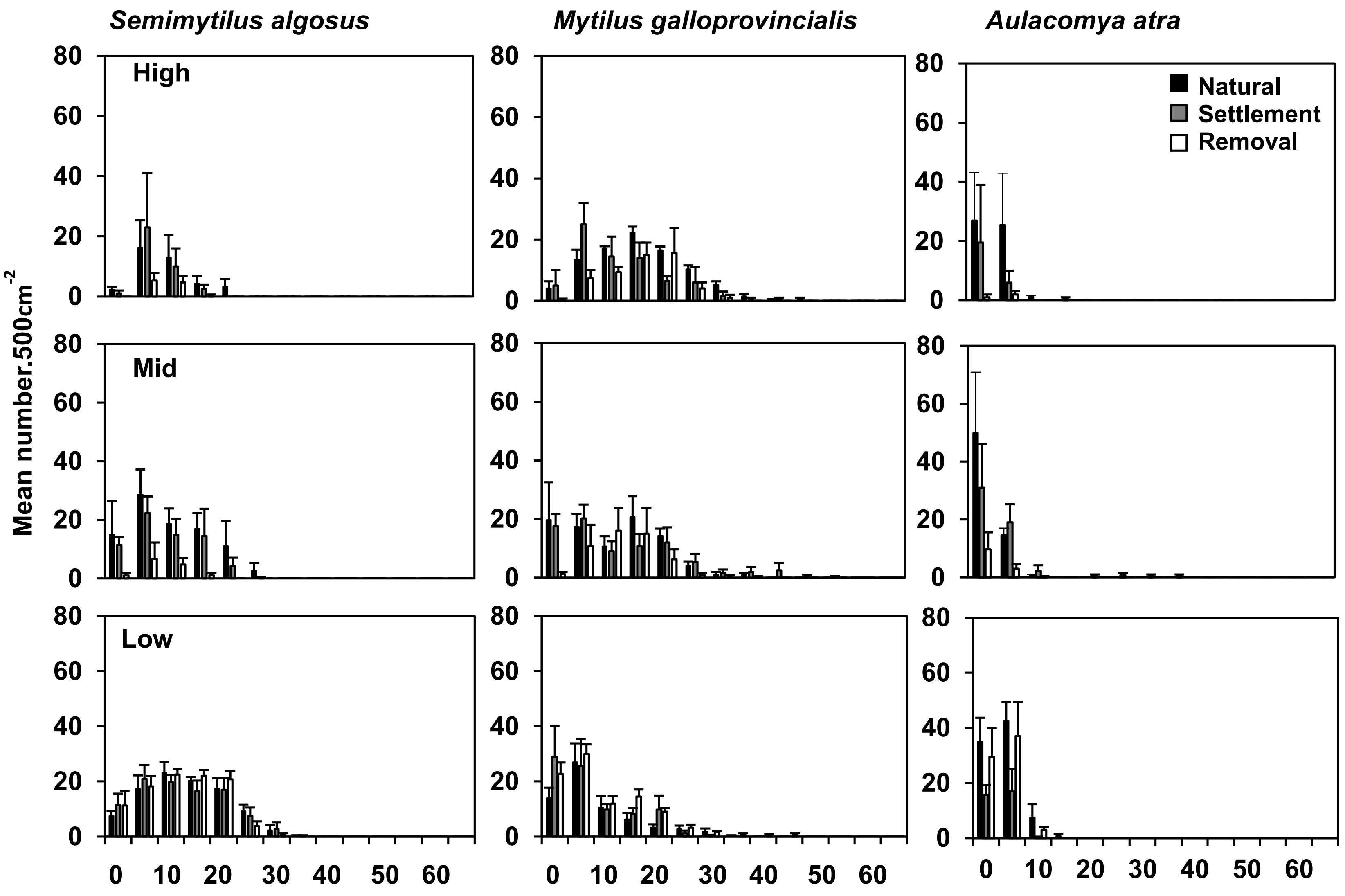

Figure 5.9: Size-composition of three mussel species at Yzerfontein, at three shore heights and within three treatments. Error bars +1SE.

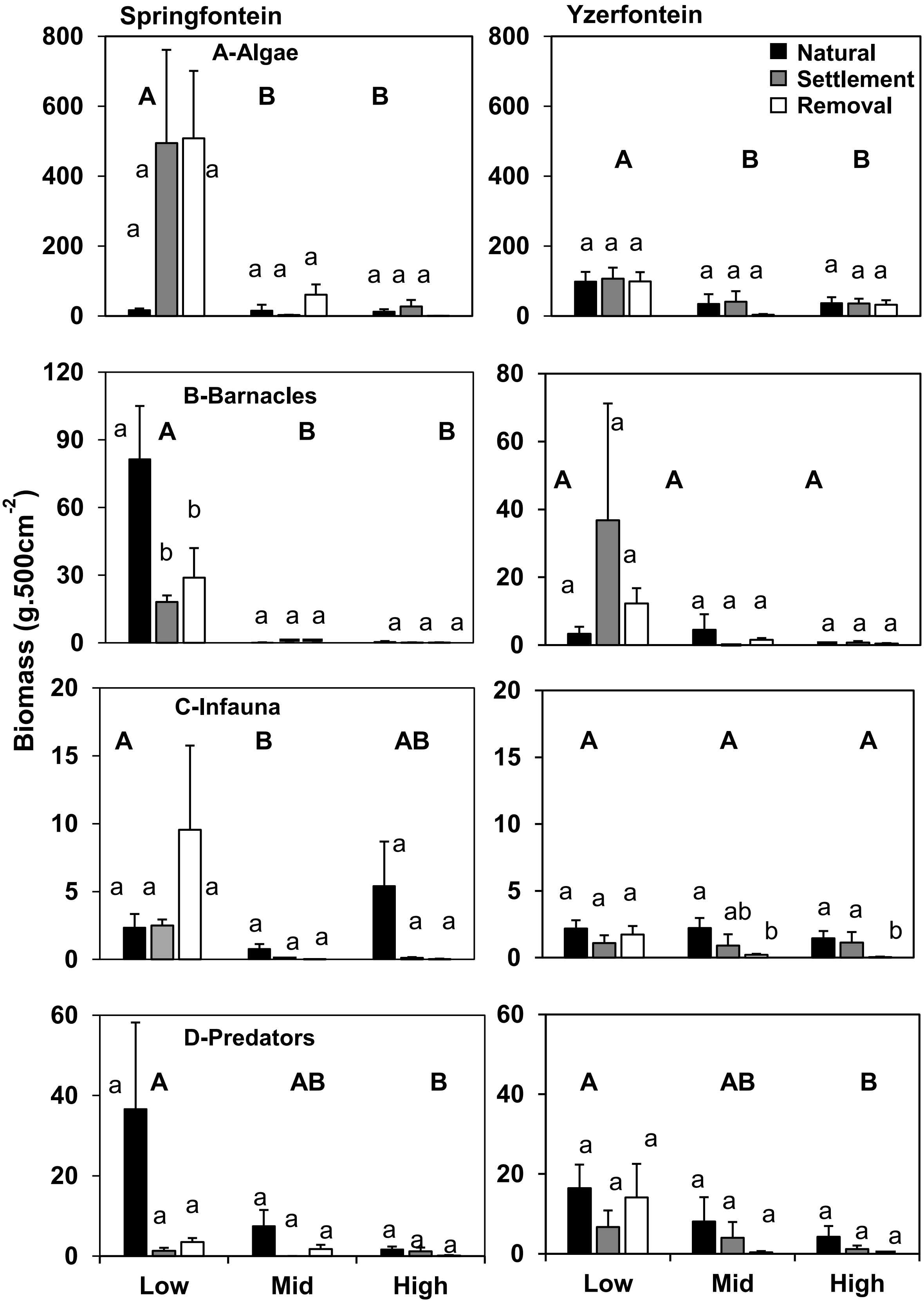

Figure 5.10: Biomass comparisons at the termination of the clearance experiment, for functional groups contributing to 80% of dissimilarity among treatments. Capital letters indicate significant differences among shore heights and small letters among treatments within shore heights. Error bars +1SE.

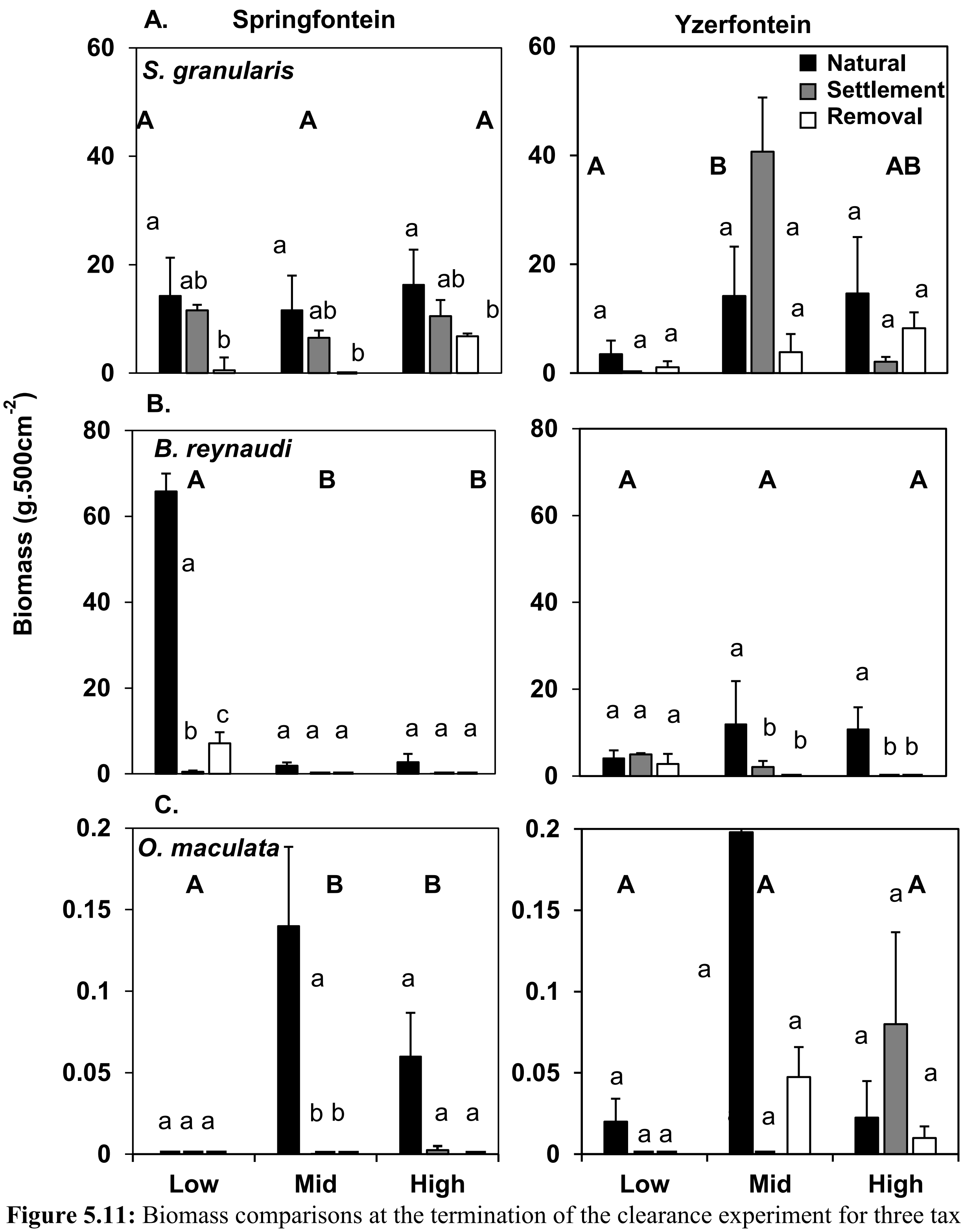

Figure 5.11: Biomass comparisons at the termination of the clearance experiment for three taxa, contributing to 80% of dissimilarity among treatments. Capital letters indicate significant differences among shore heights and small letters among treatments within shore heights. Error bars +1SE.

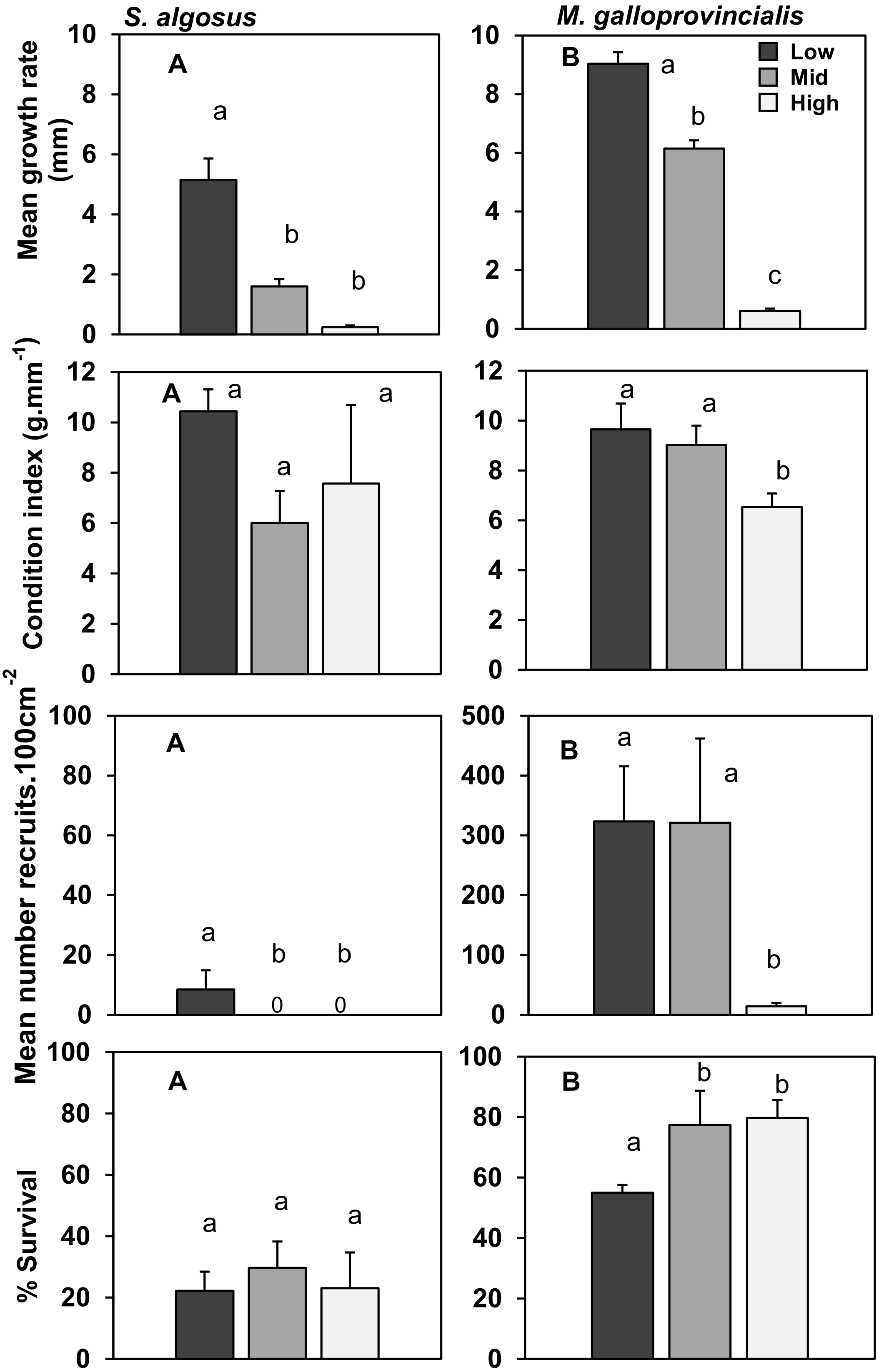

Figure 5.12: Variation in mean growth rate, condition, recruitment and survival (+SE), for mussels within cages, between species and among three shore heights, low, mid and high. Letters that differ indicate significant differences: capital letters for comparisons between species, and lower-case for comparisons among shore heights within species.

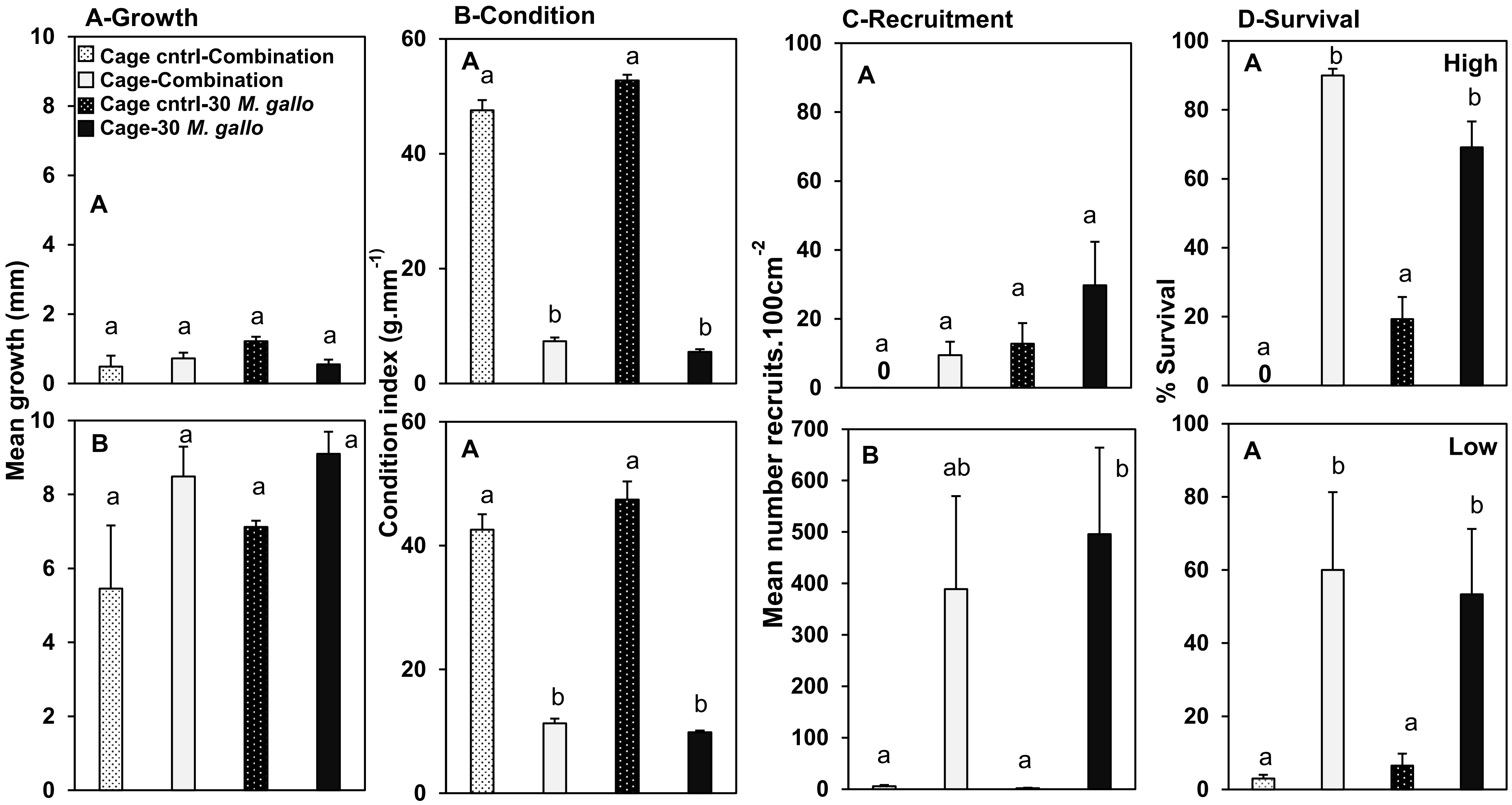

Figure 5.13: Comparison of *M. galloprovincialis* in cage controls (patterned fill) and cages (solid fill), at two shore heights (high and low) and within two treatments. Letters indicate significant differences, capital letters for comparisons between shore heights and small letters for comparisons among treatments within shore heights. Error bars +1 SE. Data for *S. algosus* not shown, as its survival was virtually zero except in the low shore

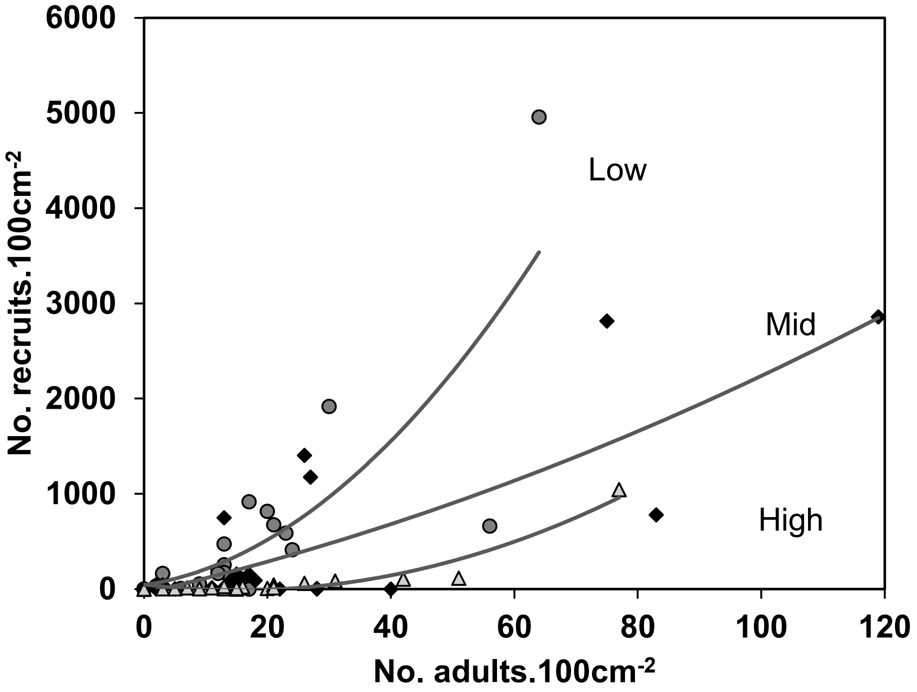

Figure 5.14: Relationship of recruitment to adult density for *M. galloprovincialis* at different shore heights, across all treatments. *S. algosus* was not included as its recruitment was too low for meaningful analysis.

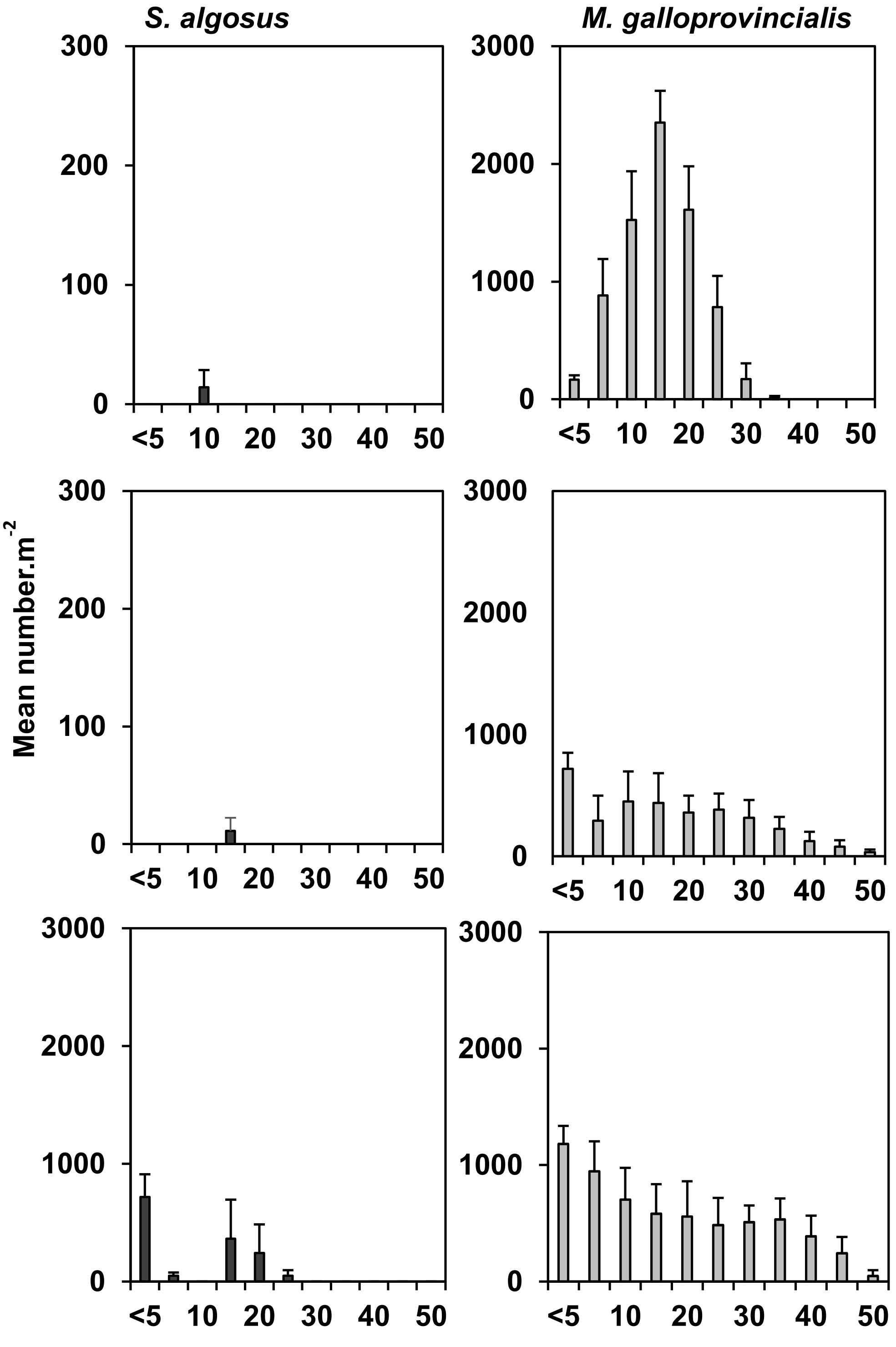

Figure 5.15: Size-frequency of mussels within control+cage treatments, at three shore heights. Note differences in scale. <5mm x100 on the mid and low shore. Error bars +1SE.

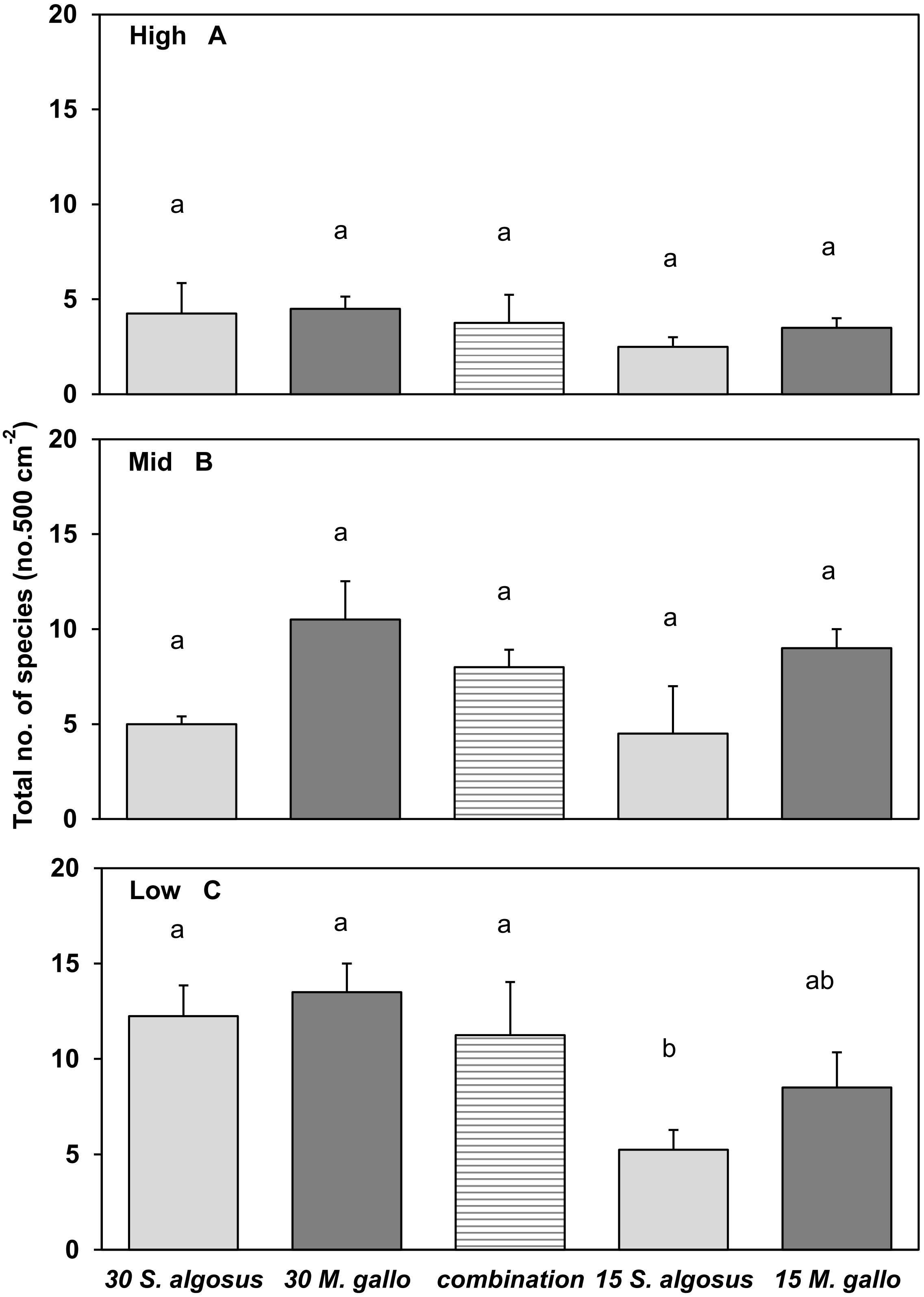

Figure 5.16: Species richness among treatments within cages, at three shore heights. Letters indicate significant differences. Capital letters for shore heights and small letters among treatments. Error bars +1SE.

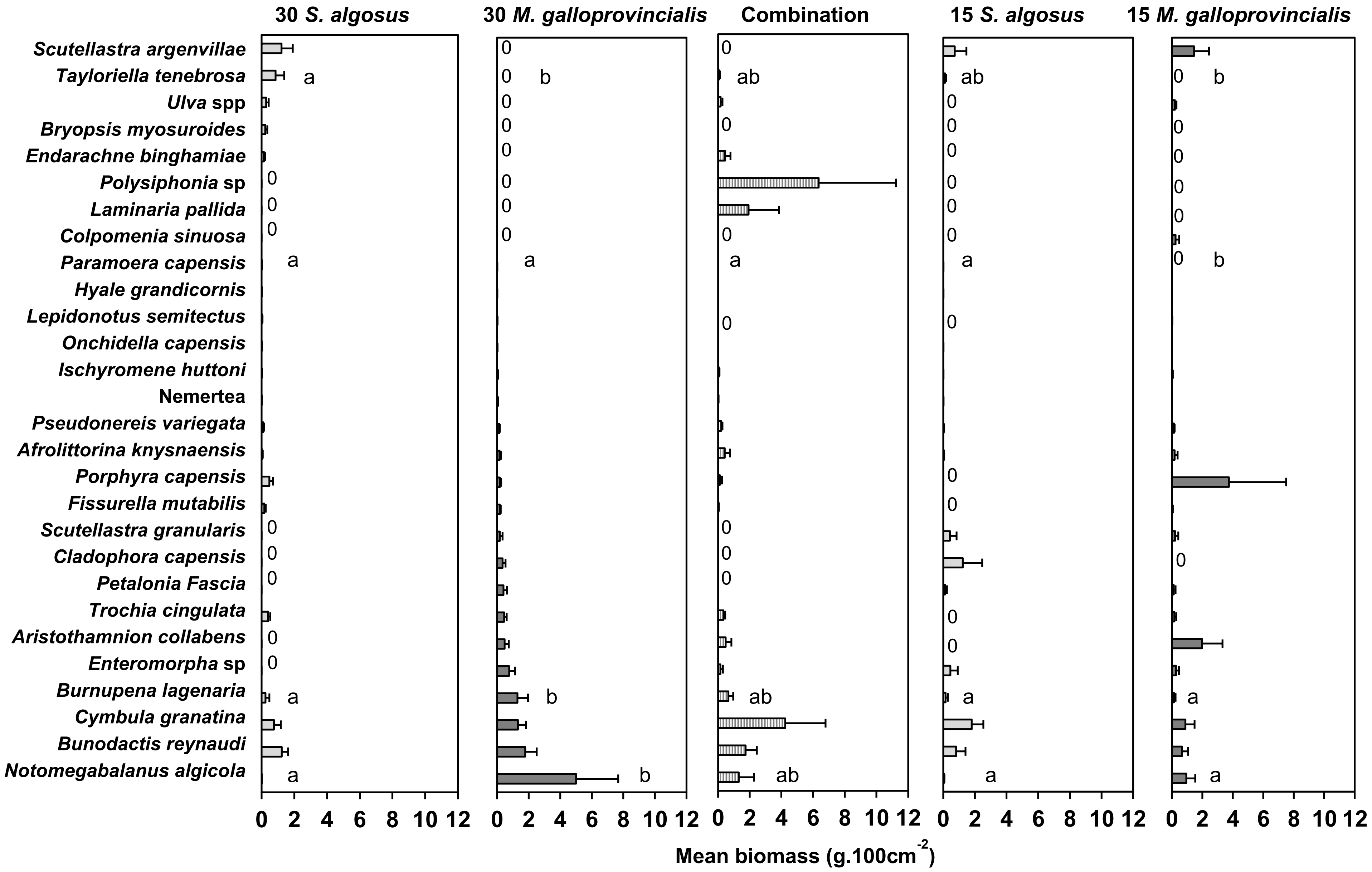

Figure 5.17: Comparison of mean biomass of species that contributed 80% to differences among caged treatments, 30 *S. algosus*, 30 *M. galloprovincialis*, 15 *S. algosus*, 15 *M. galloprovincialis*, and the combination treatment consisted of 15 of each species. Letters indicate significant differences among treatments. Error bars +1 SE.

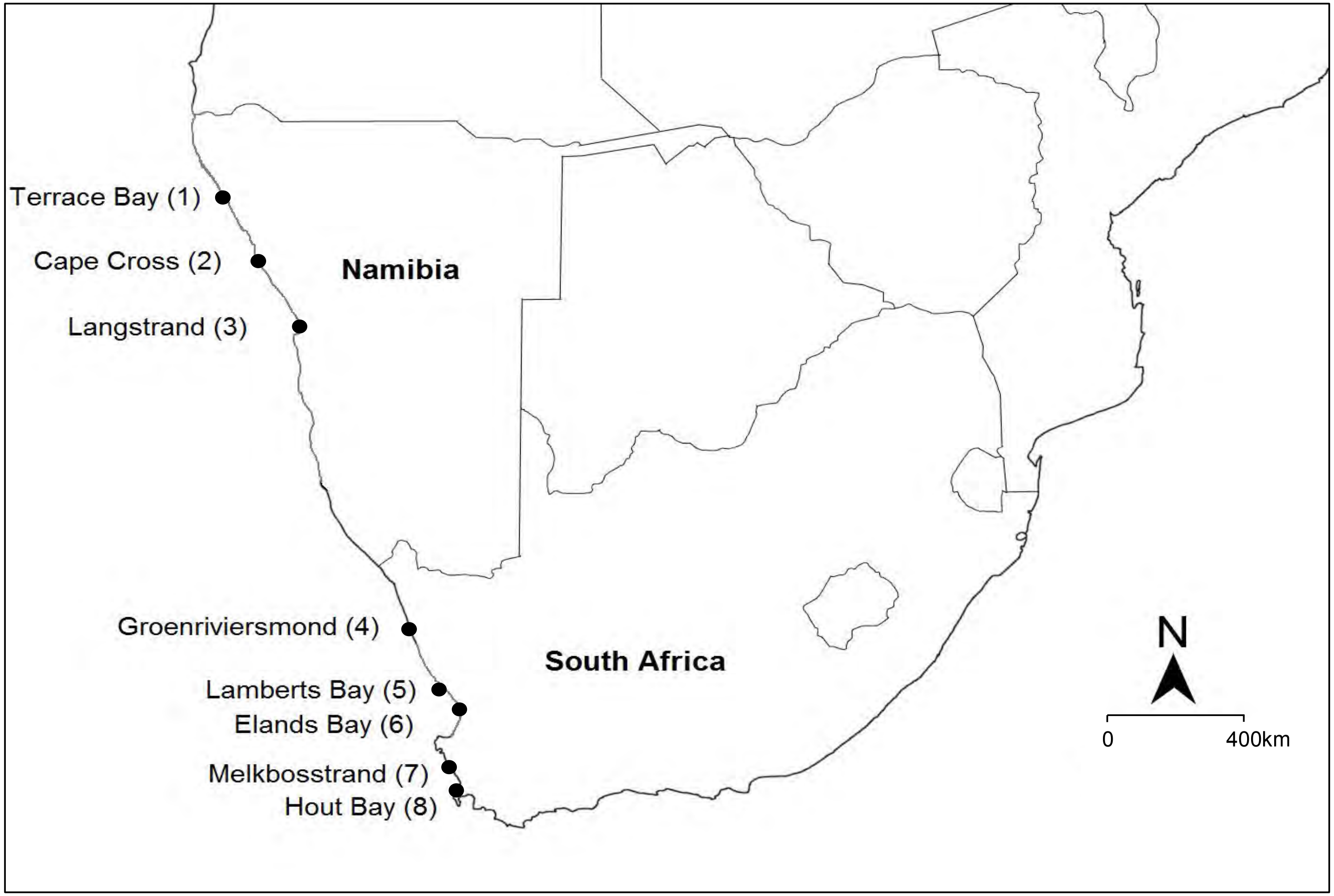

Figure 6.1: Map of southern Africa. ● Indicates the sample sites in Namibia and South Africa, numbering refers to Table 6.4.

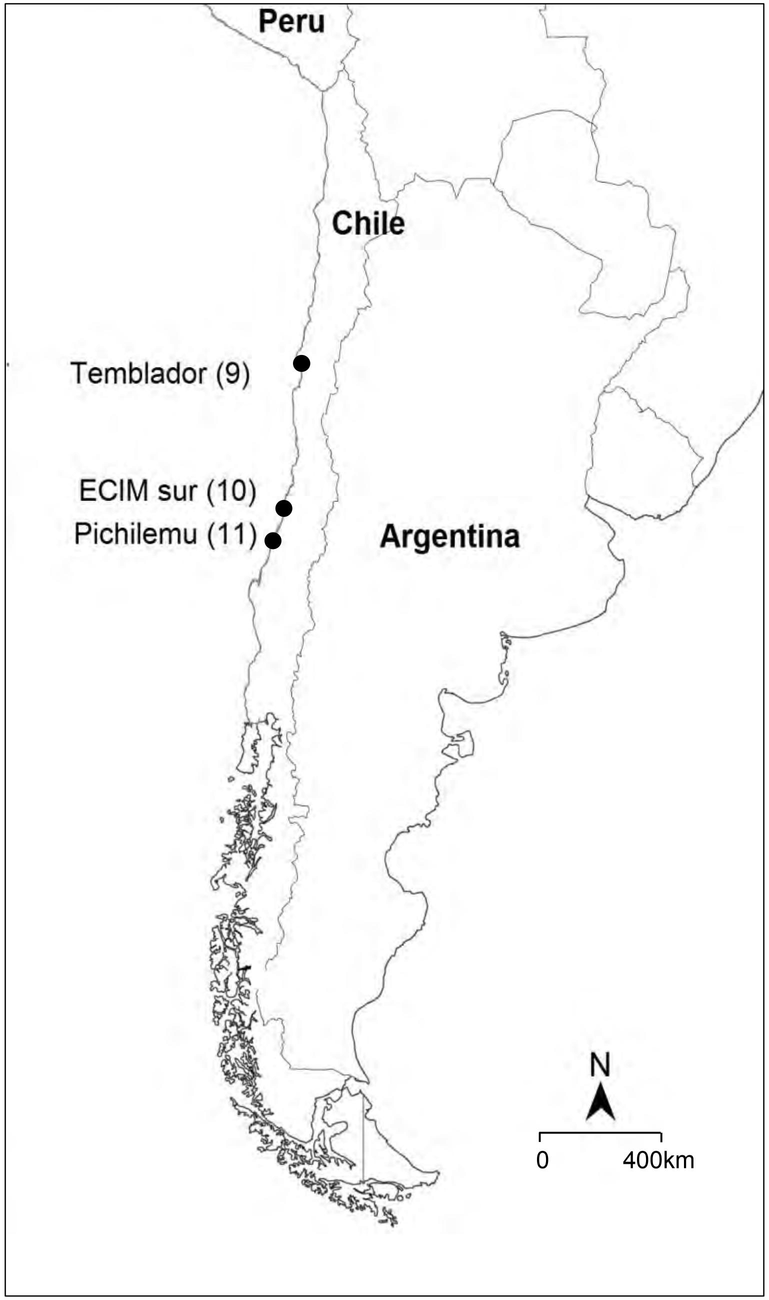

Figure 6.2: Map of Southern America.

● Indicates sample sites; numbering refers to Table 6.4.

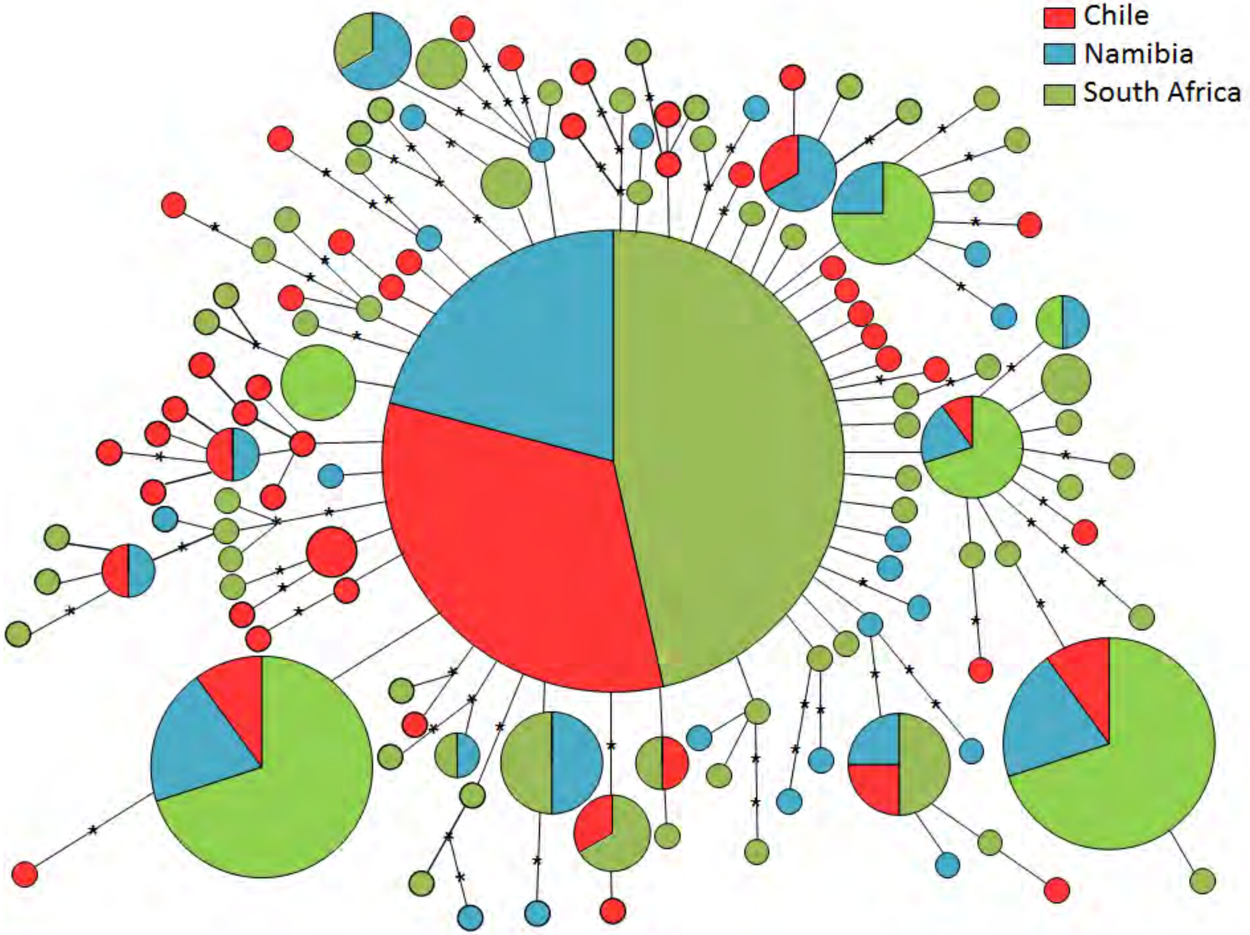

Figure 6.3: Haplotype network for *Semimytilus algosus*. Size of circles is proportional to the frequency of each haplotype. Black stars are interior node haplotypes that were not present in the samples.

Table 2.1: List of reference samples taken from GenBank to supplement genetic analysis. COI refers to the cytochrome oxidase I gene.

Species	**Country**	**GenBank no. (COI)**	**Reference**
Semimytilus algosus identification			
Mytilus galloprovincialis	South Africa	DQ351477	Zardi et al. (2007a)
		DQ351490	
		DQ917605	Wood et al. (2007)
Brachidontes rodrigeuzii	Argentina	KC844460	Trovant et al. (2013)
		KC844464	
Aulacomya atra identification			
Aulacomya atra maoriana	New Zealand	DQ917614	Wood et al. (2007)
		DQ917615	
Mytilus galloprovincialis	South Africa	DQ351477	Zardi et al. (2007a)
		DQ351490	
		DQ351478	
Identification of the unknown mussel			
Mytilus edulis	Canada	KF643859	Layton et al. (2014)
	Wales	DQ917606	Wood et al. (2007)
	Mediterranean	AF241936-44	Wares & Cunningham (2001)
	North-Atlantic	AY130034	Riginos et al. (2004)
Mytilus californianus	USA	U73812	Beagley et al. (1997)
		U73811	
Perna perna	South Africa	DQ351427	Zardi et al. (2007)
		DQ351428	
		DQ351429	
Semimytilus algosus	Chile	JX891528	De Greef et al. (2013)
		JX891530	
Mytilus trossulus	USA	AY130061	Riginos et al. (2004)
	Canada	KF644043	Layton et al. (2014)
	Canada	KF643946	Layton et al. (2014)
M. galloprovincialis	South Africa	DQ351477-97	Zardi et al. (2007a)

Table 2.2: Mean percentage sequence divergence for *Semimytilus algosus* from South Africa, Chile, Namibia and GenBank sequences for *Mytilus galloprovincialis* and *Brachidontes rodrigeuzii*.

	South Africa	**Chile**	**Namibia**	***M. gallo***	***B. rodrigeuzii***
South Africa	-				
Chile	0.5				
Namibia	0.6	0.7			
M. galloprovincialis	41.1	41.0	41.0		
B. rodriguezii	24.6	24.5	24.8	40.4	-

Table 2.3: Mean percentage sequence divergence for *Aulacomya atra* samples from South Africa and Chile, and Genbank sequences of *Aulacomya maoriana* and *Mytilus galloprovincialis*.

	A. atra **S. Africa**	***A. atra*** **Chile**	***A. maoriana***	***M. gallo***
A. atra **S. Africa**	-	-	-	-
A. atra **Chile**	2.9	-	-	-
Aulacomya maoriana	18.7	17.9	-	-
Mytilus galloprovincialis	49.9	49.5	50.7	-

Table 2.4: Mean percentage sequence divergence, within populations.

Population	***Mean % divergence***
A. atra **South Africa**	0.4
A. atra **Chile**	0.7
Aulacomya maoriana	2.9
Mytilus galloprovincialis	1.0

Table 3.1: GPS coordinates of all sites surveyed. Asterisks indicate sites where surveys of zonation were undertaken.

Site	*GPS coordinates*
Hout Bay	S34°02′11″, E18°20′40″
*Bloubergstrand**	S33°48′22″, E18°27′50″
*Yzerfontein**	S33°20′46″, E18°09′15″
*Elands Bay (6)**	S32°18′57″, E18°19′43″
*Elands Bay (7)**	S32°19′03″, E18°19′18″
*Elands Bay (8)**	S32°18′54″, E18°18′59″
*Brand se Baai**	S31°14′03″, E17°50′14″
Groenriviermond	S30°50′50″, E17°34′27″
Hondeklipbaai	S30°28′31″, E17°17′09″
Port Nolloth	S29°40′57″, E17°07′28″
Oranjemund	S28°35′07″, E16°23′28″
Mining License Area 1	S28°16´46″; E16°02´45″

Table 3.2: Results of the factorial ANOVA comparing mean % cover among sites, and between species. Significant differences are in bold.

Factor	**df**	***MS***	**P**	**F**
Site	3	2740.00	4.18	**0.01**
Species	1	363.24	0.55	0.46
Interaction	3	11705.47	17.87	**<0.001**

Table 3.3: Results of the factorial ANOVA for all sites, comparing % cover and biomass between species and between shore heights. Significant differences are in bold.

Sites	*Factor*		**A.** *Percentage cover*			**B.** *Biomass*		
		df	**MS**	**F**	**P**	**MS**	**F**	**P**
Bloubergstrand	**Shore height**	12	1554.7	2.9	**0.003**	629397	2.95	**0.003**
	Species	1	15092.6	28.5	**<0.001**	26705099	125.3	**<0.001**
	Interaction	12	873.1	1.6	0.11	506982	2.4	**0.02**
Yzerfontein	**Shore height**	5	1814.9	11.9	**<0.001**	464270	4.4	**0.005**
	Species	1	367.1	3.5	0.07	1358491	12.8	**0.001**
	Interaction	5	731.6	46.2	**<0.001**	1396780	13.2	**<0.001**
Elands Bay	**Shore height**	18	544.4	1.9	**0.03**	231935	2.2	**0.01**
(EB8)	**Species**	1	92084.2	312.3	**<0.001**	336587	3.2	0.1
	Interaction	18	74703.5	14.1	**<0.001**	879481	8.4	**<0.001**
Brand se Baai	**Shore height**	9	312.6	1.5	0.17	425057	6.1	**<0.001**
	Species	1	4898.5	23.6	**<0.001**	4747014	67.6	**<0.001**
	Interaction	9	6768.4	32.6	**<0.001**	1584426	22.6	**<0.001**

Table 4.1: Constants of the Ford-Walford and Von Bertalanffy equations for the four mussel species: m is the slope of the line and i is the y-intercept, R^2 the coefficient of determination; $L\infty=i(1-m)$ is the asymptotic length, and $K=-\log_e m$ is the growth coefficient.

	Ford-Walford			*Von Bertalanffy*	
Species	**m**	**i**	**R^2**	**$L\infty$**	**K**
S. algosus	1.10	3.51	0.53	52.89	0.06
C. meridionalis	0.81	15.21	0.85	80.22	0.21
M. galloprovincialis	0.87	9.36	0.91	71.71	0.14
A. atra	0.85	8.08	0.77	53.22	0.16

Table 4.2: Results of the ANCOVA analyses comparing the slope and intercepts of the Ford-Walford plots between species, with initial length (Lt) as a covariate. Significant differences are indicated in bold.

Variable	**df**	**MS**	**F**	**P**
Lt (covariate)	1	2.18	2.21	0.14
Slope (m)	3	4.86	4.92	**0.003**
Lt (covariate)	1	3702.46	55.22	**<0.001**
y-intercept (i)	3	375.86	5.60	**0.001**

Table 4.3: Results of the ANCOVA analyses comparing breaking force, shell thickness and the ratio of width:length among species, with shell length as a covariate. Significant differences are indicated in bold.

Variable	df	MS	F	P
Length (covariate)	1	11.68	43.56	**<0.001**
Breaking force	3	7.56	28.18	**<0.001**
Length (covariate)	1	5.96	85.26	**<0.001**
Thickness	3	3.68	52.60	**<0.001**
Length (covariate)	1	0.04	2.43	0.12
Width:length	3	1.40	77.89	**<0.001**

Table 4.4: Results of the multiple regression relating shell breaking force to: thickness, and width:length ratio. Significant differences are indicated in bold.

Species		**b**	**SE**	**t**	**p**
S. algosus	Thickness	0.10	0.18	0.58	0.57
	Length	-0.10	0.17	-0.57	0.57
	Width:length	0.05	0.17	0.33	0.74
C. meridionalis	Thickness	0.32	0.15	2.20	**0.03**
	Length	0.53	0.15	3.57	**<0.001**
	Width:length	0.10	0.12	0.80	0.43
M. galloprovincialis	Thickness	0.63	0.19	3.29	**0.002**
	Length	0.07	0.17	0.40	0.69
	Width:length	-0.01	0.13	-0.11	0.92
A. atra	Thickness	0.16	0.16	0.98	0.33
	Length	0.44	0.14	3.09	**0.003**
	Width:length	0.33	0.14	2.29	**0.03**

Table 4.5: Constants of the length/strength and length/thickness regressions for the four mussel species: m is the slope of the line and i the intercept, and R^2 the coefficient of determination.

Regression	**Species**	**m**	**i**	**R^2**
Length/strength	*S. algosus*	- 0.27	60.98	0.002
	C. meridionalis	6.35	- 96.94	0.43
	M. galloprovincialis	7.62	- 70.41	0.30
	A. atra	6.09	- 23.63	0.19
Length/thickness	*S. algosus*	0.01	0.19	0.14
	C. meridionalis	0.01	0.32	0.34
	M. galloprovincialis	0.04	- 0.17	0.53
	A. atra	0.02	0.20	0.20

Table 4.6: Results of the multiple regression relating single byssus breaking force to thread diameter and to modulus. Modulus is the stiffness or resistance to deformation of byssal threads. Significant effects are indicated in bold.

Species		**b**	**SE**	**t**	**p**
S. algosus	Diameter	0.36	0.16	2.26	**0.03**
	Modulus	0.72	0.16	4.46	**0.005**
C. meridionalis	Diameter	0.56	0.13	4.35	**<0.001**
	Modulus	0.39	0.13	2.99	**0.005**
M. galloprovincialis	Diameter	0.14	0.10	1.44	0.16
	Modulus	0.85	0.10	8.77	**<0.001**
A. atra	Diameter	0.11	0.08	1.39	0.19
	Modulus	0.98	0.08	11.86	**<0.001**

Table 4.7: Results of the factorial ANOVA, comparing mean recruitment among species and among months. Significant differences are indicated in bold.

Factor	**df**	**MS**	**F**	**p**
Month	8	12549	2.17	**0.03**
Species	3	182930	31.61	**<0.001**
Month v. species	24	11803	2.04	**0.005**

Table 4.8: Results of the factorial ANOVA, comparing mean recruitment between species and between shore heights. Significant differences are indicated in bold.

Factor	**df**	**MS**	**F**	**p**
Shore height	2	12290	16.79	**<0.001**
Species	3	25034	34.21	**<0.001**
Shore height v. sp	6	12922	17.66	**<0.001**

Table 5.1: Results of the Mann-Whitney U-tests, comparing diversity indices based on biomass at the end of the clearance experiment between sites, across shore heights. S=species richness, d=Margalef's diversity, H'=Shannon-Wiener diversity and J'=Pielou's evenness. Significant differences are indicated in bold.

Biomass	***df***	***U***	***p***
S	36	560.5	0.33
d	36	634.0	0.88
H′	36	548.0	0.26
J′	36	514.0	0.13

Table 5.2: Results of pairwise post-hoc tests, comparing community structure among treatments, at different shore heights, for (A) Springfontein and (B) Yzerfontein. Numbers in bold indicate significant differences.

(A) Springfontein

Shore height	Treatment	t	p
Low	Remove:resettle	1.65	**0.029**
	Remove:natural	2.20	**0.028**
	Resettle:natural	3.00	**0.031**
Mid	Remove:resettle	2.09	**0.033**
	Remove:natural	1.89	**0.03**
	Resettle:natural	1.77	0.08
High	Remove:resettle	0.86	0.46
	Remove:natural	1.57	**0.03**
	Resettle:natural	1.64	**0.031**

(B) Yzerfontein

Shore height	Treatment	t	p
Low	Remove:resettle	0.93	0.67
	Remove:natural	1.42	0.078
	Resettle:natural	1.45	0.08
Mid	Remove:resettle	1.83	**0.036**
	Remove:natural	1.95	**0.023**
	Resettle:natural	1.16	0.23
High	Remove:resettle	0.85	0.66
	Remove:natural	0.99	0.39
	Resettle:natural	0.70	1

Table 5.3: Results of the one-way ANOVA tests comparing mean diversity indices at the end of the clearance experiment at (A) Springfontein and (B) Yzerfontein, among shore heights and among treatments. Numbers in bold indicates significant differences. S=number of species; d=Margalef's diversity index; J'=Pielou's evenness index; H'=Shannon-Wiener diversity index. Due to heteroscedasticity Kruskal-Wallis tests were performed for d, J' and H' at both Springfontein and Yzerfontein.

(A) Springfontein		**S**		**d**		**J'**		**H'**	
Effect	**df**	**F**	**p**	**F/H**	**p**	**H**	**p**	**H**	**p**
Shore height	2,36	13.71	**<0.001**	12.00	**0.002**	4.21	0.12	16.44	**<0.001**
Treatment	2,36	2.10	0.14	0.31	0.74	6.38	**0.04**	3.73	0.15

(B) Yzerfontein		**S**		**d**		**J'**		**H'**	
Effect	**df**	**F**	**p**	**F**	**p**	**H**	**p**	**H**	**p**
Shore height	2,36	10.68	**<0.001**	8.82	**<0.001**	7.18	**0.03**	15.47	**<0.001**
Treatment	2,36	2.97	0.06	3.19	0.054	0.22	0.81	1.92	0.16

Table 5.4: Results of the pairwise tests, comparing community structure within cages among treatments. Sa= *Semimytilus algosus*, Mg= *Mytilus galloprovincialis*. Significant values are in bold.

Shore height	Treatment	t	P
Low	30Sa:30Mg	3.44	**0.04**
	30Sa:15Sa	1.90	**0.03**
	30Sa:15Mg	1.90	**0.02**
	30Sa:combination	1.86	**0.03**
	15Sa:15Mg	0.86	0.86
	15Sa:combination	1.33	0.09
	30Mg:15Sa	1.76	**0.04**
	30Mg:15Mg	1.57	**0.03**
	30Mg:combination	2.02	**0.02**
	15Mg:combination	1.58	0.13
Mid	30Sa:30Mg	1.96	0.51
	30Sa:15Sa	1.47	0.07
	30Sa:15Mg	0.73	0.80
	30Sa:combination	1.32	0.12
	15Sa:15Mg	0.92	1.00
	15Sa:combination	1.84	0.05
	30Mg:15Sa	1.27	0.14
	30Mg:15Mg	0.86	0.54
	30Mg:combination	0.91	0.69
	15Mg:combination	1.33	0.06
High	30Sa:30Mg	1.15	0.27
	30Sa:15Sa	2.1	**0.03**
	30Sa:15Mg	1.01	0.41
	30Sa:combination	1.45	0.08
	15Sa:15Mg	1.82	**0.03**
	15Sa:combination	1.32	0.19
	30Mg:15Sa	1.70	0.06
	30Mg:15Mg	0.94	0.47
	30Mg:combination	0.84	0.60
	15Mg:combination	1.48	0.07

Table 5.5: Summary of the results from the clearance experiment

MEASURES ***FACTORS:*** ***VARIABLES:***	***Sites***	***Shore height***	***Treatment***	***Conclusions***
Community composition	Not significantly different	Significant effect	Significant but only initially	Sites similar Treatment initially has effects. Shore height dominates by 2-4 months. System recovers fast
Diversity	Not significantly different	Significant effect in almost all cases. Low>mid & high	Significant depending on site and shore height	Sites similar Diversity initially reduced in disturbance treatments but recovery rapid (2-4 months) as other habitat-forming species colonise and mussels return via immigration and settlement
Time	Succession from diatom to ephemeral to corticated at Springfontein. No obvious succession at Yzerfontein	Differences become more prominent over time	Differences become less prominent over time Diversity differences disappear after 2-4 months	Succession follows typical pattern at Springfontein but not Yzerfontein
Biomass	Not significantly different	Significant decline up shore	Significant only for Springfontein low shore	Initial treatment effects disappear by end, when treatment effects are non-significant, and shore height effects dominate
Intertidal groups/taxa				
***S. granularis* & barnacles**	Response differs between sites	Low<mid & high at Yzerfontein Low>mid & high at Springfontein	Natural>Removal at Springfontein	Facilitation of *S. granularis* by mussels, at Springfontein only
B. reynaudi	Similar treatment effects at both sites	Low>mid & high at Springfontein	Natural>disturbance (but in different zones)	Clear facilitation (mussels= food) at both sites
O. maculata	Similar treatment effects at both sites	Low<mid & high at both sites. Only Springfontein was significant	Natural>disturbance treatments in mid shore (zone of greatest abundance)	Facilitation at both sites (provision of shelter), in zone of maximum abundance
***A. atra* & Algae**	Response differs between sites	Low>high at both sites	Natural<disturbance at Springfontein	Competition in low shore at Springfontein
S. algosus	Sites differ	Low>mid & high at both sites	Not clear-cut	Huge recruitment to Removal plots at Springfontein
M. galloprovincialis	Sites differ	Low>high at Springfontein But not at Yzerfontein	Natural>disturbance at Springfontein Not at Yzerfontein	Recovery fast at Yzerfontein

Table 5.6: Summary of the results for the caging experiment

COMPARED IN:	**Cages**			**Cage controls**			**Cage v. cage controls**
FACTORS: VARIABLES:	***Species***	***Shore height***	***Treatment***	***Species***	***Shore height***	***Treatment***	
Growth	*M. galloprovincialis* >*S. algosus*	Significant: Low shore>mid & high	Not significant	Not applicable	Significant: Low>high	Not significant	Not significantly different; shore height trends same.
Condition	Comparison not meaningful	*S. algosus*: not significant. *M. galloprovincialis*: high<low & mid	*S. algosus*-: not significant *M. galloprovincialis*: 30>15 on mid shore	Not applicable	Not significant	Not significant	Condition of *M. galloprovincialis* better in cage controls than cages.
Recruitment	*M. galloprovincialis* >*S. algosus*	Significant for both spp.: Low shore >mid & high	Not significant *S. algosus*: combination>others *M. galloprovincialis*: 30>15	*M. galloprovincialis* >*S. algosus*	Low>high	Not significant	Cage>cage control on the low shore only; no difference in high shore.
Survival	*M. gallo*> *S. algosus*	Not significant	Not significant	*M. galloprovincialis* >*S. algosus*	Not significant	Not significant but *S. algosus* survives only in combo	*S. algosus*: not significant. *M. galloprovincialis*: Cage>cage control. Shore height trends the same.
Community structure	Not applicable	Significant	Significant	Not applicable	Not significant	Not significant	Not significant
Diversity	Not applicable	Significant	Significant. *S. algosus*: 30>15 but=combination. *M. galloprovincialis*: Not significant but same trend	Not applicable	Not significant	Not significant	H', d and J': Cage>cage control

Table 6.1: Molecular diversity indices for all sampling locations, combined for all data sets. (N=number of sequences; S=number of polymorphic sites; N_{hap} = nr of haplotypes; h=haplotype diversity; π=nucleotide diversity).

Locality	N	S	N_{hap}	h	π
Chile	65	72	51	0.95±0.02	0.0068±0.004
Namibia	50	73	35	0.96±0.02	0.0069±0.004
South Africa	108	47	66	0.96±0.01	0.0065±0.004

Table 6.2: AMOVA F_{ST} pairwise difference among populations. Significance is indicated in bold, P<0.01; other comparisons were not significant, P>0.05.

Locality	***Chile***	***Namibia***	***South Africa***
Chile	0.000	-	-
Namibia	0.004	0.000	-
South Africa	**0.01**	-0.003	0.000

Table 6.3: AMOVA F_{ST} pairwise differences within (A) Chilean, (B) Namibian and (C) South African populations. None were significant, P>0.05.

Population (A)	***Pichilemu***	***ECIM***	***Temblador***		
Pichilemu	0.000	-	-		
ECIM	-0.012	0.000	-		
Temblador	0.010	0.011	0.000		
Population (B)	**Langstrand**	**Cape Cross**	**Terrace Bay**		
Langstrand	0.000	-	-		
Cape Cross	-0.001	0.000	-		
Terrace Bay	-0.009	-0.015	0.000		
Population (C)	**Hout Bay**	**Melkbosstrand**	**Baboon Point**	**Lamberts Bay**	**Groenriviersmond**
Hout Bay	0.000	-	-	-	-
Melkbosstrand	0.009	0.000	-	-	-
Baboon Point	0.013	-0.011	0.000	-	
Lamberts Bay	0.002	-0.001	-0.001	0.000	-
Groenriviersmond	0.007	-0.004	-0.004	0.006	0.000

Table 6.4: SAMOVA results, highest values for Φ_{ct} is in bold. Groups 1-3 are from Namibia: 1=Terrace Bay; 2=Cape Cross; 3=Langstrand. Groups 4-8 are from South Africa: 4=Groenriviermond; 5=Lamberts Bay; 6=Elands Bay; 7=Melkbosstrand; 8=Hout Bay (see Figure 6.1). Groups 9-11 are from Chile: 9=Temblador; 10=ECIM sur; 11=Pichilemu (see Figure 6.2).

# groups	*Spatial structure*	Φ_{ct}	*P*
2	(1 2 3 4 5 6 7 8 9) (3 10)	0.0139	<0.001
3	(2 9) (10 11) (1 3 4 5 6 7 8)	0.015	<0.001
4	(2 9) (4) (10 11) (1 3 5 6 7 8)	0.0164	<0.001
5	(1 3 6 7 8) (2 9) (5) (10 11) (4)	0.0181	<0.001
6	(4) (1 3 7 8) (10 11) (6) (2 9) (5)	0.0184	<0.001
7	(6) (3 7 8) (10 11) (4) (5) (1 2) (9)	**0.0189**	<0.001