Biogeographic patterns of endolithic cyanobacteria and their negative impacts on mussels along the South African coast

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENT FOR THE DEGREE

OF

DOCTOR OF PHILOSOPHY

OF RHODES UNIVERSITY

(MARINE BIOLOGY)

BY

ALDWIN NDHLOVU

JUNE 2019

Abstract

Endolithic cyanobacterial species occur in a wide range of environments including cold and hot deserts as well as marine systems where they attack biological material such as corals and the shells of molluscs including limpets, mussels and abalone. Endoliths live as parasites in mussel shells, where they erode and extract calcium carbonate leading to shell weakening, creating fracture holes that lead to shell collapse and death, but they also have positive effects when they lead to discolouration of mussel shells hence giving them the ability to reduce stressful heat gain during periods of extreme heat stress. Mussels are ecological engineers on which the abundance and diversity of associated species assemblages depend. Understanding how endolithic cyanobacteria affect mussels will not only help in predicting future patterns of mussel abundances, but also future patterns of the infauna that depend on them.

Firstly, I identified endolithic species infesting mussels and assessed the prevalence of endolithic parasitism in two intertidal mussel species in South Africa, the native *Perna perna* and the invasive *Mytilus galloprovincialis*. Large-scale surveys of endolithic infestation of mussels were conducted along 2500 km of the South African coast, covering three biogeographic regions: the subtropical east coast, dominated by *P. perna*, the warm temperate south coast where the indigenous species coexists with *M. galloprovincialis*, and the cool temperate west coast which is dominated by *M. galloprovincialis*. The prevalence of endolithic infestation was higher in the cool temperate bioregion than in the warm temperate and subtropical bioregions which did not differ and for *P. perna* endolithic species assemblages revealed clear groupings by bioregion. Results for endolithic induced mortality followed the same trend, with no significant difference between the two mussel species where they coexist and these results attribute biogeography of endoliths to environmental factors rather than host identity.

Secondly, I assessed energy budgets of infested and clean mussels, to evaluate the energetic cost of infestation. This involved measuring energy acquisition, expenditure, calculating scope for growth and lethal temperatures (LT_{50}s). The results revealed that endolithic cyanobacteria have a negative effect on scope for growth due to increased metabolic rates for infested mussels, with no effect of endoliths on the rates or efficiency of energy acquisition through filtration and no effect on lethal temperatures. The effects of infestation were then examined in more detail through a qualitative and quantitative analysis of mussel gonads and byssal attachment strength to the substratum. Endolithic infestation was found to affect reproduction by affecting the size (mass) of gonads, but not the density of eggs within them. Attachment strength was affected by endolithic infestation with very infested mussels requiring much less force to detach them from the substratum compared to mussels with low or no infestation. These results show that endolithic infestation affects mussel fitness by directly affecting attachment strength and by reducing their reproductive output.

Thirdly, endolithic succession within mussel shells was examined by assessing endolithic species composition in different regions of the shell and as a function of time. The results on the spatial distribution of endolith species within a shell supported those for temporal succession in shells deployed in the field. Endolithic species that were early colonists of clean shells were similar to those that were found in the distal edge, the new and growing region of the shell and species that arrived late in succession were similar to endolithic species found near the umbo, the oldest region of the shell. Overall, the study shows that endolithic cyanobacteria show the effects of biogeography on species distribution and clear patterns of succession within mussel shells. Cyanobacteria affect mussels negatively; they lead to low scope for growth and hence low growth rates, low reproductive output and reduced attachment strength for infested mussels. This, in turn is expected to have indirect consequences for other species that rely on mussels as ecological engineers for their survival.

Table of Contents

Acknowledgements

I would like to express my deep gratitude to Professor Christopher McQuaid and Dr Gerardo Zardi, my research supervisors, for their patient guidance, enthusiastic encouragement and useful critiques of this research work. Without them this thesis would not have been possible, Professor McQuaid, I must thank you for the patience demonstrated towards me and my writing skills, your guidance and support went beyond a standard student-supervisor relationship and for this reason and your professional attitude and honesty, you will always be an inspiration to my future career.

I would also like to thank Dr Cristian Monaco for your scientific advice in those long fieldtrips we embarked on along the coast, you were not just a supervisor but a friend. I would also like to thank Dr Katy Nicastro for her scientific advice and assistance in keeping my progress on schedule.

I would also like to thank Dr Nathalie Marquet (Supermarket) for giving me scientific advice and technical support especially with slide preparation for endolithic identification and I am grateful to Dr Marcos Gektidis for his scientific expertise in endolithic identification.

I would also like to extend my thanks to Jacquiline Trassiera, for technical support, Zolile Maseko and Evans Mauda my partners in "PhD-ing" with all those long nights we spent in the department doing science. I would also like to thank Ms. Nelisa Mpama for her immense help in the field even when it was cold and raining, she will be there to help, I will always be grateful for that. Another big thank you to Dr. Chari for proofreading my thesis and all the input you made, thank you.

A gratitude goes to Ticia Swanepoel, thanks for being amazing and offering your help whenever needed and a great thank you to members of the Coastal Research Group.

I would like to acknowledge the Light Microscopy Unit of CBMR-UAlg. The Microscopy Unit which was partially supported by national Portuguese funding PPBI-POCI-01-0145-FEDER-22122.

I would like to thank Rhodes University and National Research Foundation of South Africa for funding my projects.

Finally, I would like to express my deepest gratitude for the constant support, understanding and love that I received from my family.

Chapter 1 : General introduction

What are cyanobacteria?

Cyanobacteria are wide-spread prokaryotes found in freshwater lakes, streams, oceans, damp soil, moistened rocks and arid deserts (Herrero et al. 2001). They occur as single-celled, filamentous or colonial organisms, that reproduce asexually. When growing in colonies, they can be large enough to be visible to the naked eye and they can grow into toxic or non-toxic blooms in the water column. Cyanobacterial fossil structures in the form of stromatolites have been dated back to approximately 3 500 myr and living examples of stromatolites can be seen to this day (Awramik et al. 1983). Cyanobacteria, which are also known as Cyanophyta, owe their name to the blue photosynthetic pigment phycocyanin, which gives them a characteristic blue-green colouration.

Cyanobacteria fix energy through photosynthesis; making them the only prokaryotes that are photosynthetic and able to produce oxygen (Shestakov & Karbysheva 2017). Cyanobacteria are important for the earth's biosphere because their development in the early. Proterozoic was likely responsible for the production of atmospheric oxygen through their photosynthetic activities (Tomitani et al. 2006, Cockell & Herrera 2008, Shestakov & Karbysheva 2017). The atmospheric oxygen that was produced was close to modern day levels and provided the conditions for the evolution of aerobic microorganisms and thus life as we know it (Shestakov & Karbysheva 2017). Cyanobacteria inhabit a wide range of habitats. They are more common in alkaline aquatic environments, but can be found in the air, in soil and on rocks and are often the primary colonisers of surfaces. When they colonise surfaces they are responsible for adding organic matter, thus developing conditions for later colonisers to settle (Herrero et al. 2001, Adams et al. 2008). Some cyanobacteria, diazotrophic cyanobacteria, have the ability to fix

gaseous nitrogen and are important in the nitrogen cycle (Tomitani et al. 2006, Adams et al. 2008)

Phylogeny of cyanobacteria

There is evidence of the importance of cyanobacteria during the course of evolution and ecological change throughout the earth's history. Cyanobacteria have contributed to the origins of plants; the chloroplast of plants is a cyanobacterium which evolved to live within plant cells in the late Proterozoic or in the early Cambrian (Adams et al. 2008, Moreira et al. 2013). The cyanobacteria as chloroplasts took refuge in plant cells which provided them with protection making them their home. Plants benefited by gaining access to food produced by the chloroplast, this relationship is described as endosymbiosis and resulted in the creation of the eukaryotic mitochondrion (Adams et al. 2008).

Cyanobacteria display a range of different morphologies. Traditional taxonomic classifications recognised five major groups based on morphology: 1. Chrococcales which consist of unicellular or solitary cyanobacteria; 2. Pleurocapsales which consist of unicellular to pseudo filamentous, thallus-forming cyanobacteria with cells capable of multiple and binary fission; 3. Oscillotiarales comprising filamentous cyanobacteria with no cell division; 4. Nostocales which are filamentous, but marked with cell differentiation forming akinetes, enveloped, thick-walled, non-motile, dormant cells resistant to cold and desiccation, and heterocysts which are nitrogen-fixing cells; 5. Stigonematales which are cell differentiating cyanobacteria forming complex multicellular organisations (Tomitani et al. 2006, Adams et al. 2008, Knoll 2008, Moreira et al. 2013, Shestakov & Karbysheva 2017). Recent studies that use molecular data and phylogenetic analyses of cyanobacteria support some of the traditional taxonomic groupings, including those that share features such as cellular differentiation and multiple

fission, whereas groupings that are unicellular and filamentous are not supported (Cockell & Herrera 2008, Moreira et al. 2013, Shestakov & Karbysheva 2017).

Cyanobacteria have evolved to occupy a range of environments, including extreme habitats, making them very successful as a group (Li et al. 2013, Wierzchos et al. 2015). Different types of cyanobacteria have evolved to thrive in various temperature ranges, extreme pH values, different salt solutions, varying UV radiations, various levels of illumination, and hydrostatic pressure (Seckbach 2007). Consequently, they can be grouped according to their preferred habitats such as phototrophic thermophiles (heat loving), psychrophiles (cold loving), halophiles (adapted to high salt levels), acidophiles (thriving at low pH), and radiation resistant phototrophs. Most of these types of cyanobacteria are extremophiles that can exist at the edge of biological limits where they tolerate extreme physiological conditions (Hoffmann 1989, Seckbach 2007). These types are adapted to habitats that include hypersaline pools, hot-springs, deserts, alkali lakes and polar regions (Garcia-Pichel 1998).

The diversity of cyanobacteria is influenced by temperature (Hoffman 1999), which acts as a major factor limiting the geographic distribution of different cyanobacterial species. In the ocean, cyanobacterial are more abundant in the littoral zones where they form intertidal and infralittoral mats or live as endoliths in carbonate substrata (Glynn 1997, Hoffman 1999). They are often the first to colonise bare areas of rock, water and soil since they possess adaptations such as ultraviolet absorbing sheath pigments, which increase their fitness levels in relatively harsh environments. Many species of cyanobacteria are capable of living in harsh marine and other terrestrial habitats, where they provide important ecosystem functions such as nitrogen fixing and acting as food for other organisms (Seckbach 2007, Nweze 2009). They also have the ability to parasitize live organisms such as the shells of mussels (Kaehler & McQuaid 1999, Marquet et al. 2013). Some genera of cyanobacteria (e.g., *Hyella, Plectonema, Mastigocoleus*) have been found in calcareous surfaces that include reef and skeletal surfaces (Glynn 1997,

Hoffman 1999). With this invasive nature they tend to live as parasites in many species, although their activities can also be beneficial to other organisms in the community.

Parasitism

Parasitism is the relationship between two species, the host and the parasite, whereby the parasite benefits from the host by exploiting resources such as food, water, or habitat, and in doing so reduces the fitness of the host. The effects of parasitism often impair growth, reproduction, competitive ability, stress tolerance and survival of the host species (Ward et al. 2004, Wood & Johnson 2015). Parasites have a strong influence on population dynamics and ecosystems as a whole because they can influence species populations. Additionally they influence the way host species interact with other species, inter and intraspecific competition and prey-predator relationships (Dobson & Hudson 1986, Mouritsen & Poulin 2002, Lafferty 2013, Garcia-Longoria et al. 2019).

Parasites play important ecological roles in marine communities and are involved in numerous species interactions. They can facilitate interactions in all kinds of food webs, including temperate reefs and coral reefs (Lafferty 2013). Parasites have direct consequences for their hosts such as affecting their energy use and in some instances they affect host behaviours or even influence native-invasive species interactions, including how invasive species compete with native species, which can lead to changes in the ecosystem as a whole (Dobson & Hudson 1986, Torchin & Mitchell 2004, Lafferty 2013). Parasites can directly affect the host by reducing its biological fitness by draining its energy, resulting in the host having stunted growth, reduced reproductive output and at times they can have an indirect effect on their host by rendering the host vulnerable and unable to defend itself against predators. For example gastrointestinal parasites in red grouse make the red grouse individuals more susceptible to predation (Hudson & Dobson 1992, Isomursu et al. 2008). Parasites also alter the behaviour of

their host for their own benefit. Trematodes (*Microphallus*) alter the behaviour of host snails, *Potamopyrgus antipodar* such that there is reduced probability that an infected snail will be eaten by an unsuitable host of fish (*Gobiomorphus cotidia*) by making the snails hide under rocks during times when the fish is feeding (Levri 1998). Parasites will access resources such as water, heat and food from their hosts to increase their own survival but there is a trade-off between the parasite and the host as the parasite does not kill the host as this would also reduce its own survival (Torchin & Mitchell 2004).

Competitive exclusion of one parasite by another has not been widely studied especially in marine communities, but it has been noted that some parasites enhance the presence of not only other parasites, but also other non-parasite species (Mouritsen & Poulin 2002). Trematode parasites *Microphallus claviformis* enhance the coexistence of two amphipods, *Corophium volutator* and *Corophium arenarium*. *C. volutator* is competitively superior to *C. arenarium* but it incurs higher mortality due to the trematode parasite, *M. claviformis*, hence the trematode parasite is more harmful to the competitively superior amphipod and cancelling out its superiority allowing the two amphipods to coexist (Poulin 1998). With many studies revealing that parasites negatively affect host species by reducing their fitness, there is evidence that shows that parasites can be beneficiary to other species in the community. For instance, rendering host species weak is of benefit to predators relying on that host species for food (Mouritsen & Poulin 2002).

Like many other organisms, parasites are influenced by their thermal environment and environmental degradation (Lafferty 2013, Barber et al. 2016), hence with the looming effects of climate change some parasites may be affected (Cizauskas et al. 2017). Warming of the oceans is expected to result in parasites shifting to higher latitudes, occupying new environments which they did not occupy before, and at the same time some areas previously occupied by parasites are expected to lose them (Lafferty 2013). This means some communities

of parasites might collapse while others will thrive with increasing temperatures. Extreme temperatures usually stress organisms, causing them to divert energy meant for immune defence, growth and reproduction to cope with heat stress. In doing so, they can become more susceptible to parasites hence the organisms will be affected by the stress and the parasite. This will lead to a reduction in growth, reproduction, defence and potentially death to the organism. Therefore, thermal and environmental conditions are pivotal in determining the abundance and distribution of both organisms and their parasites (Barber et al. 2016).

Parasites are known to stress organisms especially in their native range, leading to organisms using more energy to defend themselves from their natural enemies (Keane et al. 2002, Torchin et al. 2009). Invasive species have been shown to suffer less from enemies such as pathogens and parasites when translocated to the invaded region as they have fewer natural enemies in introduced territories (Colautti et al. 2004). Species in their native range are subjected to parasites that coexist with them and have evolved with them. When organisms invade new territories, they can escape their natural enemies and this allows them to use energy that could have been used for defence to grow and reproduce (Colautti et al. 2004, Torchin et al. 2009). Invasive species are often successful because new habitats can offer such enemy free space in terms of predators or parasites. This study focuses on two competing ecological engineers, an indigenous mussel, *Perna perna*, and an invasive mussel, *Mytilus galloprovincialis*, that are hosts to endolithic cyanobacterial parasites. Differences in the prevalence of endolithic infestation for *M. galloprovincialis* in its native range in Portugal and in South Africa where it is invasive and competing with the indigenous *P. perna* have been observed (Marquet et al. 2013), with higher infestation in the invaded range, but different endolithic species were identified between the two regions. Even though prevalence rates of endolithic infestation are higher for *M. galloprovincialis* in South Africa than in Portugal, they might still be lower when compared to prevalence rates for *P. perna* leading to the invasive species being less affected

by endolithic parasites. Although prevalence of infestation of *M. galloprovincialis* in South Africa is greater than in Portugal, there is the possibility that prevalence of infestation for *M. galloprovincialis* is less than that for *P. perna*, in that case South Africa would effectively offer a form of enemy free space for *M. galloprovincialis*.

Importance of parasites in communities

Parasites are found almost everywhere on the planet and are associated with important processes in communities (Torchin & Mitchell 2004). Parasitism is always associated with negative effects although at times they are positive effects, parasites are known for deriving crucial resources from host species, potentially affecting their survival. Looking at communities, parasites are an important part of food web interactions because they regulate species densities in communities (Dobson & Hudson 1986, Lafferty 2013) so that, in a sense, some communities are incomplete without parasites. Parasites have both positive and negative effects on non-host species with implications that can cascade throughout the whole ecosystem (Dobson & Hudson 1986, Torchin & Mitchell 2004, Hatcher et al. 2012). They facilitate interactions between hosts, modifying competitive and consumer-resource interactions which might be beneficial to the broader community.

Some parasites act as food sources for other organisms. For example, trematode parasites are prey to fish when they swim in the water column in search of new hosts (Lafferty 2013). In other cases, parasites have been known to affect prey-predator interactions leading to increased flow of energy in communities. For example, fish that are infected with trematode parasites are 10-30 times more likely to be eaten by predatory birds, hence increasing the energy transferred from fish to birds (Lafferty 2013). In New Zealand, trematode parasites reduce the burrowing activity of invertebrates such as cockles, making them easy prey for oystercatchers, with the

shells of dead cockles supporting an epibiont community of amphipods, anemones, chitons and barnacles that use them for habitat (Thomas & Poulin 1998, Lafferty 2013).

In some communities, parasites can be used as a measure of how healthy a community is, with healthy communities said to be diverse in parasites (Dobson & Hudson 1986, Marcogliese & Cone 1997, Marcogliese 2005, Hatcher et al. 2012). It has been suggested that the presence of a parasite in a community indicates the presence of each host species that hosts each stage of that parasite's life cycle, which implies a diverse community especially if there are many hosts for different life stages (Marcogliese 2005). The loss of a host species in a community can be indicated by the absence of a parasite in that community if it uses the host to complete its life cycle, hence healthy communities are rich in parasites and environmental stressors that affect host species can be monitored by observing host-specific parasites (Marcogliese & Cone 1997, Marcogliese 2005).

Communities can be subjected to degradation by environmental stressors and this can lead to loss of host species within the community. The loss of host species will result in host-specific parasites failing as they are not able to find alternative hosts, leaving the community with only more generalist parasites. On some occasions, parasites can alter host behaviour, a phenomenon known as parasite induced host behaviour (Ponton et al. 2011). For example *Gammarus duebeni celticus*, a freshwater amphipod, tends to be more active when infected by the acanthocephalan *Echinorhynchus truttae* when it will move towards light, rather than seeking shade, making it an easier target for trout predators, the primary host for this parasite (Hatcher et al. 2012). Likewise, it has been shown that hairworms (*Paragordius tricuspidatus*) increase the water-seeking behaviour of their orthopteran hosts (*Nemobius sylvestris*) where they fall into water releasing the parasite which is favourable for their reproduction (Ponton et al. 2011).

The term parasite can be used to describe both macroparasites, such as mistletoe (parasitic plants) or organisms such as fleas and helminths, and microparasites such as protists, viruses, bacteria, and fungi. In this study, the main focus is on the endolithic cyanobacteria which act as parasites, infesting the shells of many intertidal organisms which include molluscs (Bower et al. 1994, Gehman & Harley 2019). It is thought that endolithic cyanobacteria, which are microborers, infest 50 to 80% of mussels (*Perna perna*) on the coast of South Africa, causing lethal and sub-lethal detrimental effects (Kaehler & McQuaid 1999). Endolithic cyanobacteria in mussel shells lead to a decrease in shell strength which can account for 50% of total mortality by causing shell collapse (Marquet et al. 2013) and they negatively affect the condition index, attachment strength and reduce reproduction in mussels (Kaehler & McQuaid 1999, Zardi et al. 2009).

Bioerosion

Shell degradation by cyanobacteria occurs through bioerosion, the process by which there is destruction and removal of hard surfaces by biological agents (Vogel & Frankfurt 1993, Glynn 1997). In the ocean, such substrata include minerals or lithic substances whilst the biological agents of erosion include animals (molluscs, polychaetes, and crustaceans), plants (lichens, algae) and bacteria (cyanobacteria). Mechanisms of bioerosion involve scraping, rasping, and biotic boring of hard surfaces which can be through chemical processes when bioerosion agents excrete acidic compounds, dissolving the calcium components of their hosts (Glynn 1997).

In marine communities, bioerosion is seen in corals or in the degradation of molluscan shells, whilst on land it is usually facilitated by plant roots growing into rock cracks or by lichens releasing chemical compounds that dissolve rock minerals. There are three main functional groups of bioeroders on both land and sea: macroborers, microborers and grazers (Glynn 1997). Macroborers are organisms that erode the internal structures of organisms, for example, mussel

shells and coral skeletons creating holes, hence increasing the porosity of these skeletons, making them weak, or, in terrestrial communities, wood boring insects that are destructive pests (Potter & Potter 2009) causing structural weakness, dieback and at times the death of trees and shrubs.

Microborers are tiny organisms that include algae, bacteria and fungi and these are associated with microscopic holes in the surfaces of the substrata they attack. Grazers include organisms such as fish and urchins, which normally consume algae growing on hard surfaces and as they graze on these surfaces, they incidentally scrape them, thus changing them, with implications for the process of water exchange. Limpets are also known to erode the shells of conspecifics by grazing (Day et al. 2000). Endolithic cyanobacteria are microborers of importance in this study as they play a significant role in the shells of molluscs in intertidal communities. Microborers are organisms which, through their various activities, erode and weaken calcareous reef building species and penetrate calcareous surfaces of rocks, skeletons, dead or live animals and plants (Golubic et al. 2005). Boring activities of microborers are not yet fully understood, and they are believed to differ among taxa. In some studies it is believed that they occur through the excretion of an acid which is capable of dissolving rocks thus dissolving microborers will dissolve their substrates and absorb nutrients for their benefit (Cockell & Herrera 2008) while other studies they detail microbes such as endoliths actively boring shells of bivalves. The actively boring endoliths were shown to be excavating calcium carbonate ions at the front end which promotes a spontaneous dissolution starting from the front end, transporting calcium ions along the multicellular cyanobacterial trichomes and excreting them at the distal end opening into the external medium (Garcia-Pichel et al. 2010). When microborers erode surfaces they leave behind patterns that can be analysed to identify which specific organism was involved in the process of boring (Golubic et al. 2005). Microborers weaken host species by weakening their shells, affecting their growth and reproduction

(Kaehler & McQuaid 1999, Golubic et al. 2005), and they also set up conditions for later invasions of the same host or by other organisms such sponges (Mao Che et al. 1996).

Endolithic organisms

Microboring endolithic communities are known to occupy a wide range of communities, from inhabiting a variety of rock types, mainly porous rocks such as sandstone, basalt rocks (Li et al. 2013), biological material such as coral reefs (Shashar & Stambler 1992, Gleason et al. 2017) or molluscan shells such as mussel shells (Porter & Lingle 1992, Kaehler & McQuaid 1999, Zardi et al. 2007, 2009) to occurring in the driest parts of the world such as the Atacama desert in South America (Wierzchos et al. 2006).

The ability of organisms to occupy a certain environment, is thought to be contingent on their physical requirements, such as the need for light, a tolerable temperature and nutritional requirements (Gektidis et al. 2007). Microborers can be divided into two, phototropic and heterotrophic borers. Phototrophic borers are dependent on light and their diversity is greatly influenced by light availability (Golubic et al. 2005, Gektidis et al. 2007). These are endolithic cyanobacteria which are dependent on light for their survival and they tend to be found on the upper most surfaces of substrates where they have access to light (Mao Che et al. 1996). The other group consists of heterotrophic microborers which take in organic substances as their energy sources. For example, Mao Che et al. (1996) found out that in black pearl oyster, *Pinctada margaritifera*, phototrophic endoliths dominate the outer shell surfaces whereas the inner nacreous layer was dominated by heterotrophic endoliths. The settlement success of endoliths depends on their ability to erode their hosts. Settlement normally begins with light-dependent organisms occupying the outer surfaces (e.g. cyanobacteria), which pave the way for organotrophic species that penetrate deeper, such as fungi and filter feeders (e.g. sponges) that follow later (Mao Che et al. 1996, Ćurin et al. 2014).

Endolithic organisms are contributors to primary productivity in coral communities (Shashar & Stambler 1992, Tribollet et al. 2006) and other marine communities not only because they may be photosynthetic, but because they can release nutrients by their weathering activities which release nutrients from rocks They can also improve the water holding capacity of rocks and re-cycling of important elements such as calcium by their boring activities (Li et al. 2013). Endolithic microbial activities also enhance the production of fine sediment grains through bioerosion, thus helping to shape some geological formations (Golubic et al. 2005).

Why do microorganisms bore?

1. Obtaining nutrients

Endolithic microborers require nutrients for growth and metabolic processes, and these nutrients are acquired using different methods. Endolithic microborers obtain nutrients by photosynthesising such as in the case of autotrophs, fixing gaseous nitrogen from the atmosphere (Tomitani et al. 2006, Adams et al. 2008) and they can break down chemical compounds such as in the case of endolithic heterotrophs that actively erode hard minerals (Golubic et al. 1981, Garcia-Pichel et al. 2010, Couradeau et al. 2017). The way of attaining nutrients by microorganisms influences their distribution within substrates, with some microborers found on surfaces whilst others are found within rocks (Cockell & Herrera 2008).

2. Competition for resources

Competition for resources such as living space or food is common among many organisms. Boring microorganisms compete for these resources in their environments (Hibbing et al. 2010). Some microbes excrete chemicals that are toxic or inhibitory to their competitors and if a species cannot eliminate its competitor they end up coexisting (Friedmann 1982). Phototrophs will compete for light by using light of different ranges of frequencies as they use light to make

their energy (Fredrickson & Stephanopoulos 1981), chasimoliths and heterotrophs will compete for chemicals such as carbon and nitrogen.

3. Protection from physical extremes

Environmental conditions can change rapidly from freezing conditions to very hot temperatures especially in intertidal communities. Microboring organisms living in molluscan shells can avoid desiccation and extremely high temperatures outside the shell. Molluscan shells offer improved micro-conditions to the endolithic microborers and enhance their survival (Cockell & Herrera 2008). It is not only molluscan shells that act as environmental shields in intertidal systems; calcareous rocks in arctic regions protect microborers from the freeze and thaw temperature fluctuations experienced by the surfaces of these rocks by providing microborers a habitat in the pore spaces underneath the rock surfaces (Cockell & Herrera 2008, Wierzchos et al. 2012, 2015). Microborers have evolved to finding hospitable conditions within rocks or molluscan shells where environmental conditions are extreme outside the shells or outside the rock pore spaces.

Many organisms, especially in intertidal systems, are exposed to strong wave action and wave action is important in structuring intertidal communities (Bustamante et al. 1996, Nicastro et al. 2008). Microborers that inhabit lithic environments are protected from wave action by hiding in rock pores or in the holes they form in molluscan shells (Cockell et al. 2002). If these organisms are less exposed to waves, they have the advantage of not expending energy trying to remain attached compared to those living on exposed surfaces.

4. Avoiding predation

Microborers growing inside rocks or molluscan shells can avoid being grazed by macroorganisms such as fish and snails, which cannot penetrate into these rocks or shells. However growing inside rocks does not insulate these microborers from other predators such

as microscopic fungi that can prey on microscopic eu-endoliths (boring microflora or algae living inside rocks) and this is visible as these eu-endolithic alga change their colour from green to black when they are affected by the fungi (Cockell & Herrera 2008).

Purpose of this study

The study investigated the distribution of endolithic cyanobacteria along the South African coast and how they influence mussels in intertidal communities. Two model host intertidal mussel species *Mytilus galloprovincialis* and *Perna perna* were chosen to investigate these effects. *P. perna* occurs from the lower balanoid zone to below the infralittoral fringe and is found in the south and east coasts of South Africa (Lasiak & Dye 1989, Tomalin 1995). *M. galloprovincialis* occurs in the mid- to high- intertidal zone on the south coast while on the west coast it extends to the low shore (Steffani & Branch 2003). It is native to the Mediterranean coastline but occurs as an invasive mussel on the coast of South Africa and can be found in the west and south coasts of South Africa, where it co-exists with native *P. perna* (Bownes & McQuaid 2006, Zardi et al. 2008). *Mytilus galloprovincialis* is the most successful marine invasive species in southern Africa (Robinson et al. 2005). It is believed that this invasion began in the late 1970s, probably via ballast water, on the south-west coast of South Africa (Grant & Cherry 1985). Then the species quickly expanded its distributional range along roughly 2800 of kilometres of the southern African shores (Robinson et al. 2005). Its northern limit is between the northern border of Namibia and southern Angola, while its eastern limit lies on the south-east coast of South Africa. Both range boundaries coincide with transitions between temperate and subtropical bioregions, where high water and air temperatures prevent further expansion (Assis et al. 2015). *Perna perna* dominates the east coast and coexists and competes with *M. galloprovincialis* on the southern warm-temperate coast (Bownes & McQuaid 2009). It is absent from more than 1000 km of the cold-temperate west coast due to the cold, upwelled waters of the Benguela system. North of there, it colonises rocky shores in

central Namibia and extends along the west coast of Africa to the southern Iberian Peninsula and into the Mediterranean Sea as far as the Gulf of Tunis (McQuaid et al. 2015).

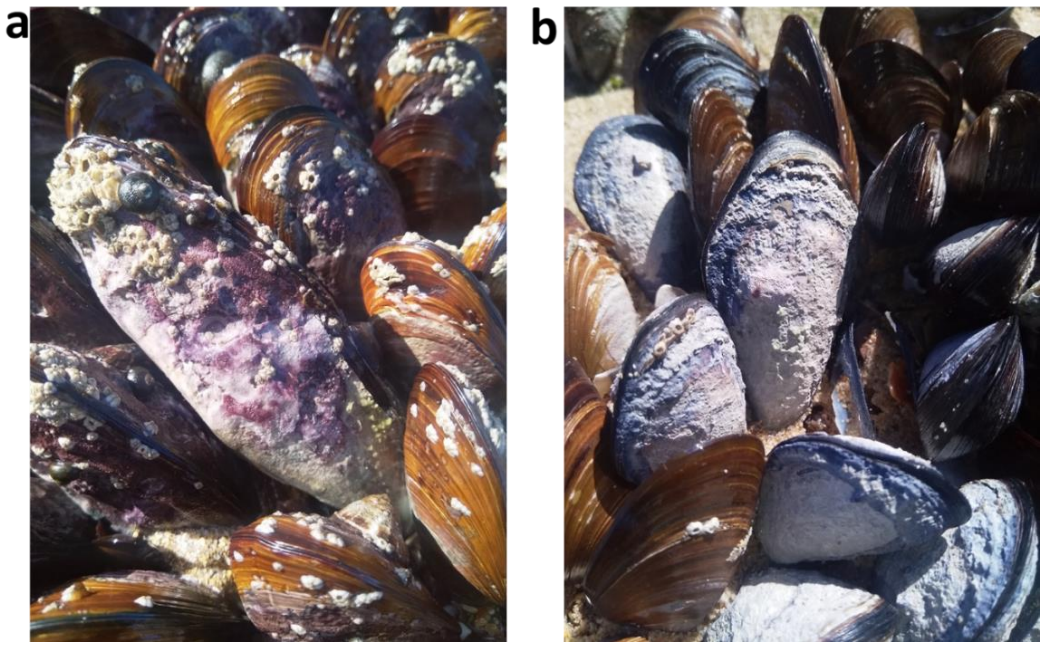

Figure 1.1: Study species: (a) *Perna perna* and (b) *Mytilus galloprovincialis*

These two-mussel species are ecological engineers and they are abundant in intertidal communities making them good candidates for this study. The thesis is sub-divided into six chapters.

Chapter 1 forms the general introduction of the thesis which deals with the history, phylogeny and characteristics of cyanobacteria. This chapter highlights the distribution of cyanobacteria in different environments, the positive and negative effects of cyanobacteria and how some species of cyanobacteria act as parasites by boring the shells of their hosts. This chapter also details the activities of cyanobacteria as endolithic microborers and how they are adapted to a wide range of environments.

Chapter 2 focuses on how biogeography influences the distribution of endolithic cyanobacteria and their infestation of mussels. This chapter investigates whether endolithic cyanobacteria

species composition differ among host mussel species and identifies the species composition of endolithic cyanobacterial communities infesting mussel shells across the three bioregions of South Africa. This chapter also investigated the lethal effects of endoliths.

Chapter 3 focuses on the energetic cost of being infested by endolithic cyanobacteria in mussels. Endoliths are known for their microboring activity, which results in the degradation of the substratum causing damage to molluscan shells. Damage to shells means mussels need to use energy to repair the damaged shells. This chapter used field and laboratory experiments to assess how endolithic cyanobacterial infestation in mussels affects growth, and its underlying physiological processes, (e.g., respiration, calcification) and whether infestation affects the temperature tolerance of mussels.

Chapter 4 sought to assess the indirect effects of endoliths by providing insight into how cyanobacterial infestation affects the reproductive output of mussels, and possible a trade-off with their attachment to the substratum.

Chapter 5 focuses on succession in the communities of endolithic cyanobacteria infesting mussel shells. This chapter deals with the temporal dynamics of endolith species composition and relates this to the distribution of endolithic species in different regions of mussel shells.

The last chapter, chapter 6, presents final conclusions in the form of a synthesis.

Chapter 2 : Biogeography influences endolithic parasitism of coexisting invasive and indigenous mussel species.

Introduction

Predicting how species composition of parasite assemblages and their pressure on their hosts may change in the future relies on understanding current patterns. With the ever changing global climatic patterns, there is an urgent need for marine ecologists to study and better understand host-parasite relationships that lead to future patterns (Lafferty 2013). Identifying current patterns of parasite richness, parasite assemblages and improving existing knowledge of parasite-induced host mortality across different bioregions with strong environmental heterogeneity is important for the understanding of distributions of parasites. This is particularly relevant when the hosts are ecological engineers on which abundance and diversity of associated assemblages are highly dependent (Badano & Marquet 2008).

Mussels are dominant intertidal ecological engineers that affect coastal species richness by increasing habitat spatial complexity, buffering against environmental extremes and providing protection from predators (Gutiérrez et al. 2003). Thus, any biotic or abiotic factors affecting intertidal mussel species may decrease ecosystem complexity with potential repercussions for the abundance and diversity of associated organisms, potentially reaching all trophic levels (Borthagaray & Carranza 2007).

Endolithic parasite infestation is one of the main biotic factors affecting intertidal mussel populations (Kaehler 1999, Kaehler & McQuaid 1999). Endoliths have a parasitic relationship with mussels (Marquet et al. 2013), they access resources from their host. The carbonate ions released during chemical dissolution of the shell are converted into carbon dioxide which is

eventually used by the cyanobacteria for photosynthesis (Garcia-Pichel et al. 2010). In addition, endoliths occupying pore spaces in coral reefs are protected from grazers such as echinoderms and scarid fish (Charpy et al. 2012).

Microbial endoliths, including phototrophs (cyanobacteria and algae) and heterotrophs (fungi) contribute to several ecologically and geologically relevant dynamics. Through biochemical dissolution, they actively bore into hard minerals, thus playing a crucial role in bioerosion and sedimentation (Glynn 1997).

Photosynthetic shell-degrading endoliths (mainly cyanobacteria) can inflict substantial damage on molluscan shells. Endolithic cyanobacteria can further negatively affect individuals' physiology, this can affect the energy budget of the host organism (Flye-Sainte-Marie et al. 2009) with negative effect on the condition, growth and reproduction efficiency of mussels (Zardi et al. 2007, 2009). Studies on mussels and oysters on the west coast of South Africa have shown that phototrophic endoliths can cause severe extensive damage to bivalves (Kaehler 1999). Other studies have shown that phototrophic endolithic cyanobacteria affect the condition index, shell growth, strength, and the byssal attachment strength of intertidal mussels (Kaehler & McQuaid 1999, Zardi et al. 2009). Phototrophic endoliths create holes in shells, weakening them and this leads to shell fracture and in some cases, this can lead to mortality. For example, 50% or more of adult mussel mortality on the south coast of South Africa is attributed to endoliths (Kaehler & McQuaid 1999, Zardi et al. 2009, Marquet et al. 2013).

The ecophysiological characteristics of endoliths vary among different cyanobacterial species and are influenced by environmental factors and on how the species is adapted to the environment it inhabits. For photoautrophic endoliths, the main limiting factor is light; this is demonstrated by the

depth related shift in species richness of photoautrophic endoliths which exhibit a decrease in species richness as the depth of water increases (Gektidis et al. 2007).

In communities where nutrients are not limiting, and temperatures are optimal, endolithic cyanobacteria will grow successfully but in temperate regions, even with high abundances of nutrients, cyanobacteria blooms are prevalent only in summer when temperatures rise (Berg & Sutula 2015, Wejnerowski et al. 2018). With varying ecophysiological properties of cyanobacteria and them being influenced by different environmental factors, different cyanobacteria species will occur in different communities. In marine communities, cyanobacteria are most diverse in intertidal communities, where they can form intertidal and infralittoral mats and live in the carbonate substrata as endoliths or form symbiotic relationships with other organisms (Hoffman 1999). Understanding the conditions that suit different cyanobacteria species in intertidal communities will help in predicting which species are likely to be found under known environmental conditions.

Two major currents dominate the South African oceanographic regimes: the cold, nutrient-enriched Benguela Current that flows northwards along the west coast of southern Africa and the warm Agulhas Current which flows on the east coast south from the tropics (Bustamante et al. 1996, Lutjeharms et al. 2000, Branch et al. 2010). Because of these two currents, the South African coastline is very different on each side of the continent. The west coast is characterised by intense upwelling phenomena that enhance coastal primary productivity, providing food for fish, marine mammals, and birds (Bustamante et al. 1996). The east coast has warm, subtropical, water that is poor in nutrients and is dominated by a sub-tropical and tropical biota. The south coast is a warm temperate region. On the south coast, the warm Agulhas Current moves further offshore driving,

in some areas, cooler upwelled waters close to shore (Bustamante et al. 1996). This region is characterised by a mix of subtropical and temperate species. Three major temperature-defined coastal marine biogeographical regions are thus described for the South African coastal biota: the cool-temperate Namaqua bioregion in the west, the warm-temperate Agulhas bioregion in the south, and the subtropical Natal bioregion in the east (Emanuel et al. 1992, Bustamante et al. 1996).

Aims

The aims of this chapter were to examine whether endolithic parasite communities conform to the biogeographic zonation identified for the fauna and flora of the South African coastal environment. Using large-scale quantitative and qualitative assessment of endolithic communities, three main questions are addressed:

1. Does endolithic species richness differ among bioregions?
2. Does the identity of the host drive parasite diversity in areas where the host species co-occur?
3. Do the rates of cyanobacteria infestation and mortality due to infestation differ across bioregions?

Methodology

The study was conducted across three major temperature-defined coastal marine biogeographical regions in South Africa: the eastern subtropical, southern warm temperate and western cool temperate regions (Fig. 2.1). Three sampling sites separated by 100s of kilometres were selected within each bioregion. Within each site, two locations (A and B) approximately 300m apart were selected. At each location, *Perna perna* and *Mytilus galloprovincialis* were collected. *P. perna* and *M. galloprovincialis* are dominant intertidal mussels occupying the mid to low shore. The two mussel species overlap in the warm temperate bioregion, while the cool temperate and subtropical bioregions are dominated by only *M. galloprovincialis* and *P. perna* respectively. All samples were collected from moderately wave exposed intertidal rocky shores and at sun-exposed sites having limited shading, exposed to solar radiation for 60 % of the day (Zardi et al. 2009). In figures, the cool temperate bioregion will be referred to as cool, warm temperate as warm and subtropical bioregion as subtropical. All Analyses of Variance were run using Statistica 13 (Statsoft).

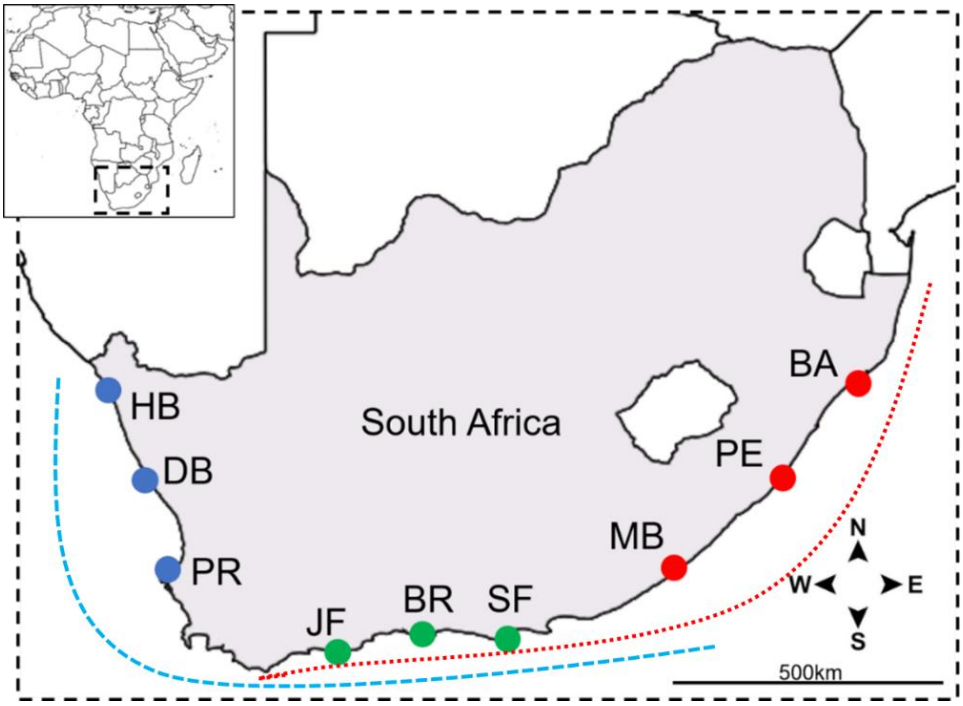

Figure 2.1: Study sites. In blue the cool temperate bioregion, in green the warm temperate bioregion and in red the subtropical bioregion, Light blue dotted line = *Mytilus galloprovincialis*, Red dotted line = *Perna perna*. Site codes refer to full names in Table 2.1.

Table 2.1: List of study sites.

Bioregion	**Site**	**Code**	**Coordinates**
Subtropical	Ballito	BA	29.53°N; 31.22°E
	Port Edward	PE	31.03°N; 30.24°E
	Morgans Bay	MB	32.71° N; 28.34°E
Warm temperate	St. Francis Bay	SF	34.21°N; 24.84°E
	Brenton-on-Sea	BR	34.08°N; 23.02°E
	Jongensfontein	JF	34.42°N; 21.38°E
Cool temperate	Paternoster	PR	32.81°N; 17.87°E
	Doring Bay	DB	31.78°N; 18.23°E
	Hondeklip Bay	HB	30.31°N; 17.27°E

Prevalence of infestation

Mussels from the following four size classes in shell length were sampled: 20-30mm, 31-40mm, 41-50mm and 51-60mm. At each location, mussels (n=5 for each size class) were haphazardly collected in the mid-mussel zone from areas of 100% mussel cover. In the laboratory, mussels were assigned to different levels of infestation following Kaehler (1999): Group A, shells with clean, intact periostracum and distinct outer periostracal striations; Group B, shells with eroding central surface portion and the outer growth lines becoming hazy; Group C, erosion of shells spreading past the central portion with grooves and pits appearing on shell surface; Group D, shells heavily pitted and becoming deformed, outer striations becoming virtually absent; Group E, shells extremely pitted, deformed and brittle, eventually developing visible holes. Prevalence is expressed as the number of individuals of a host species infected with a parasite divided by the number of hosts examined and expressed as a percentage (Margolis et al. 1982, Calvo-Ugarteburu & McQuaid 1998). To test whether biogeography had an influence on cyanobacterial infestation, for each mussel species, the overall prevalence of infested mussels (i.e., infestation categories B–E were pooled) was analysed using a three-way nested ANOVA (Analysis of Variance) with three factors; bioregion (fixed factor, three levels), site nested into bioregion (random, three levels) and size (fixed factor, four levels) as factors.

Identification of endolithic organisms

At each location, heavily infested mussels of each species present were collected (n=5 for each species). *Mytilus galloprovincialis* and *Perna perna* were collected from the cool temperate bioregion and subtropical bioregion respectively while both mussel species were sampled on the warm temperate bioregion. Once collected, mussels were immediately preserved in 4% formalin. In the laboratory, shells were cleaned with a scalpel to remove all epibiotic organisms and the soft tissue inside the valves. Cleaned shells were stored in 4% formalin.

To process the sample, each shell was broken into smaller pieces (approximately 0.5cm) and placed in 3% HCl to dissolve the calcium carbonate for 30 minutes. Under a low power stereomicroscope, the emerging endoliths were moved to a drop of immuno-mount (Shandon), fixed on a glass slide using fine forceps. After selecting all endolithic colonies until there were no more visible colonies, the immuno-mount drop was covered gently with a glass slide. All prepared slides were viewed under a Zeiss Axio Imager Z2, Apotome with Normaski lens (40x). Photographs of each endolith colony were acquired with a scale bar and used for endolith identification.

To visualise similarities and differences in endolithic communities among bioregions, non-metric multidimensional scaling (nMDS) ordinations and cluster dendrograms based on the Bray-Curtis similarity matrix were plotted (PRIMER-E v6). SIMPER (Similarity Percentage) analysis was used to identify the percentage contributions of each endolithic species to the Bray-Curtis dissimilarity metric. To examine the question of whether bioregion had an effect on cyanobacteria distribution, prevalence of endolithic infestation for each species was analysed separately using a 2-way nested ANOVA with bioregion (fixed factor with two levels) and site (random with three levels) nested into bioregion.

Lethal effects of endolithic infestation

At each location, quadrats (50cm x 50cm; n = 8) were placed in the mid-mussel zone in an area with 100% mussel cover. Within each quadrat, shells of dead mussels exhibiting extreme infestation levels (a fracture hole caused by shell collapse over the adductor muscle was visible) were counted. The assumption was that the rate of loss of empty shells in each location due to wave action or other processes was the same. Mortality was expressed as the number of dead mussels per unit area. To ensure a focus on recent mortalities, only mussel shells with a shiny

nacreous layer were counted (Kaehler and McQuaid. 1999; Marquet et al. 2013). The mortality due to infestation was analysed using a 2-way nested ANOVA with bioregion (fixed factor with three levels) and site (random with three levels) nested into bioregion.

Results

Overall, a total of 8 endolithic species were identified across all bioregions (Table 2.2). Each site had between 5 and 8 species of endolithic cyanobacteria identified (Table 2.1), with 5 of the species being common to all 9 sites but not common in all 18 locations.

Table 2.2: List of identified endolithic species in all study sites. Full site names as refer to Table 2.1.

Cyanobacterium Species	*Mytilus galloprovincialis*					
	HB	DB	PR	JF	BR	SF
Hyella balani (Lehman 1903)	X	X	X	X	X	X
Mastigocoleus testarum (Lagerheim 1886)	X	X	X	X	X	X
Solentia stratosa (Ercegovic 1932)	X	X	X	X	X	X
Plectonema terebrans (Bornet & Gomont,1889)	X	X	X	X	X	X
Hyella caespitosa (Bornet & Flahault 1889)	X	X	X	X	X	X
Hormathonema violaceo-nigrum (Ercegović 1930)		X				
Kyrthutrix dalmatica (Ercegovic 1929)	X	X	X	X	X	X
Hormathonema luteo brunneum (Ercegović 1930)	X	X	X	X		
	Perna perna					
Cyanobacterium species	JF	BR	SF	MB	PE	BA
Hyella balani (Lehman 1903)	X	X	X	X	X	X
Mastigocoleus testarum (Lagerheim 1886)	X	X	X	X	X	X
Solentia stratosa (Ercegovic 1932)	X	X	X	X	X	X
Plectonema terebrans (Bornet & Gomont,1889)	X	X	X	X	X	X
Hyella caespitosa (Bornet & Flahault 1889)	X	X	X	X	X	X
Kyrthutrix dalmatica (Ercegovic 1929)	X	X	X			
Hormathonema luteo brunneum (Ercegović 1930)	X					

Prevalence of infestation

In the cool temperate bioregion, prevalence of infestation for *M. galloprovincialis* ranged between 95% at DB and 100% at HB and PR (Fig. 2.2). In the subtropical bioregion, prevalence of infestation for *P. perna* ranged between 70% at BA and PE and 95% at MB. In the warm temperate bioregion, the two mussel species showed similar levels of infestation at all sites, with individuals from JF showing the highest levels of infestation for both species at above 95% infestation.

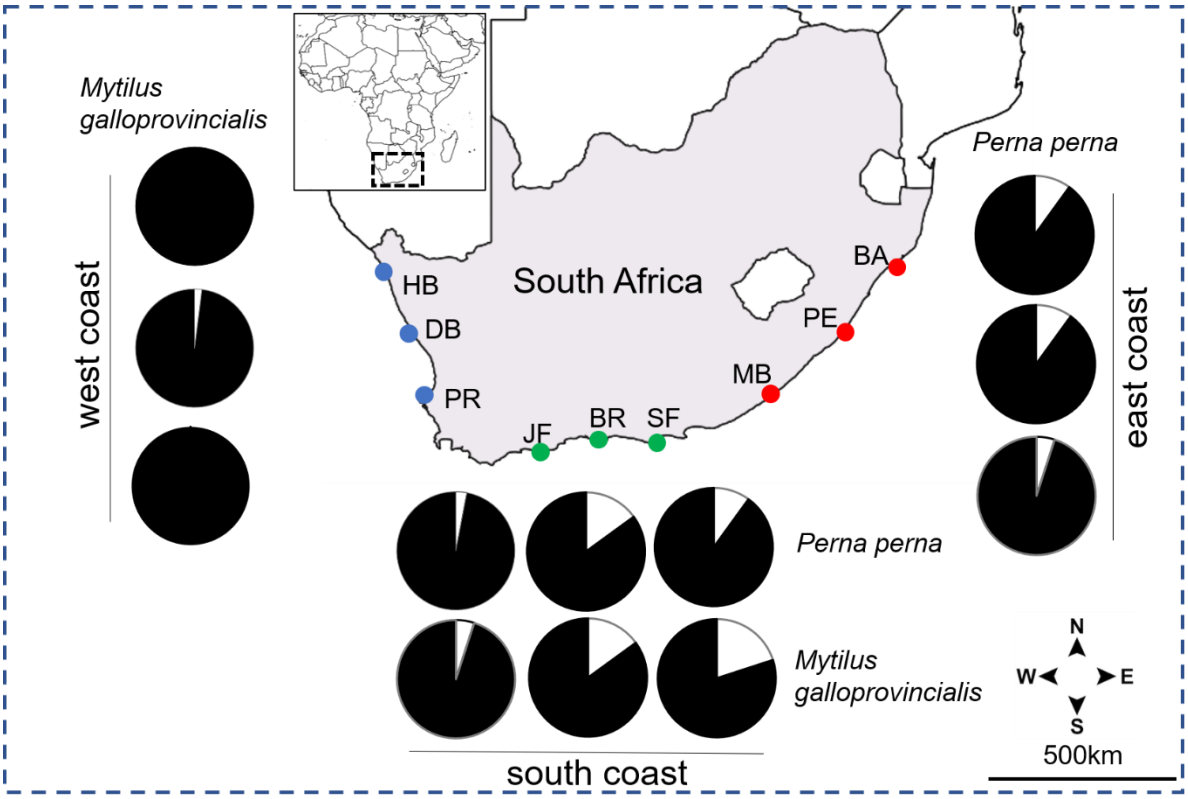

Figure 2.2: Prevalence of endolithic infestation frequencies along the west cool temperate, south warm temperate and east subtropical bioregions of South Africa. Black indicates the proportion of infested shells. Full site names refer to Table 2.1.

Infestation severity increased with size in the subtropical bioregion (sites BA, PE, MB). Some of the mussels in the size ranges 21-30cm and 31-40cm exhibited no infestation (level A). The same trend was observed in the warm temperate bioregion (SF, BR) with the exception of mussels from JF that showed no infestation only at size range 21-30cm. In the cool temperate bioregion (PR, HB), mussels exhibited infestation at levels B to E, with only hosts from DB exhibiting level A in size range 21-30cm.

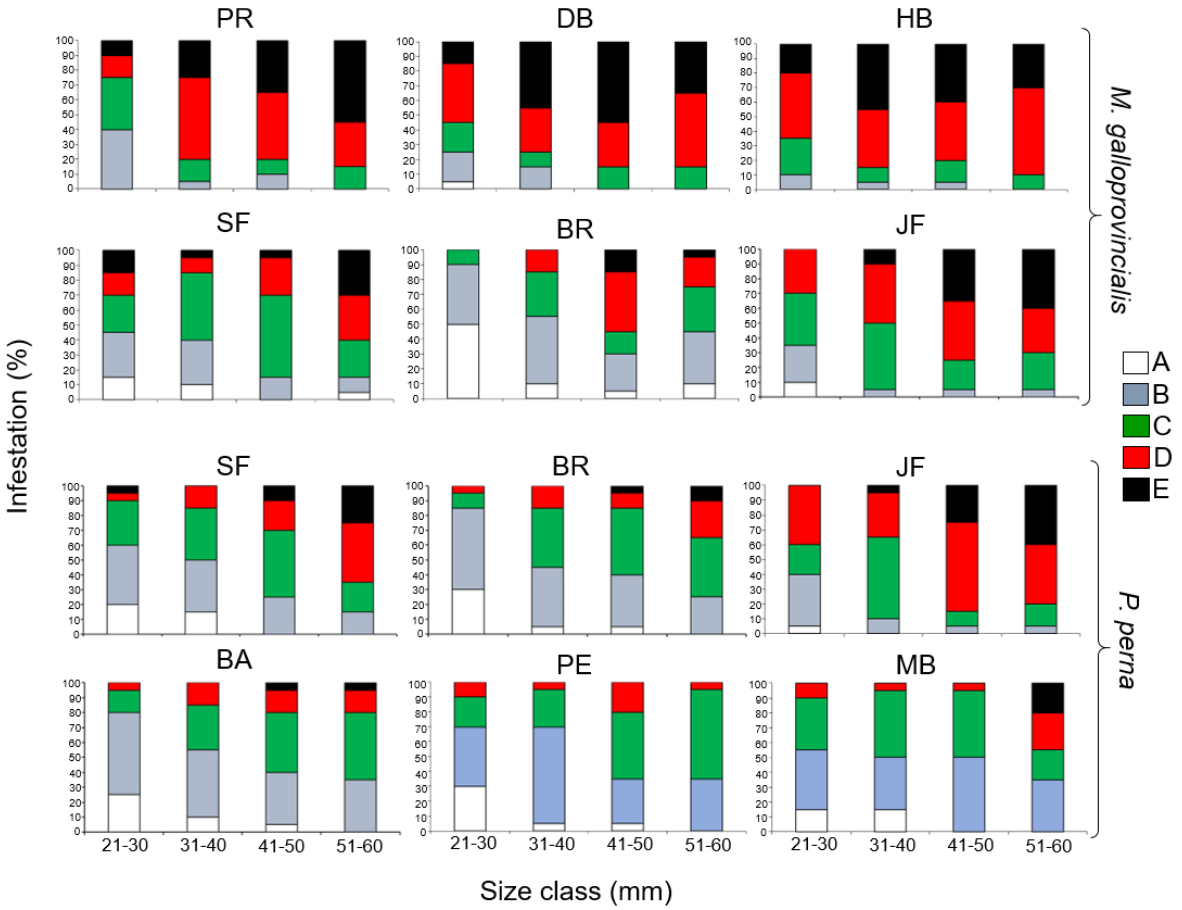

Figure 2.3: Prevalence of endoliths shown as percentages of shells exhibiting different degrees of infestation severity in each site (Groups/levels A–E), grouped into 10 mm size classes in all three bioregions of South Africa. Full site names refer to Table 2.1.

Prevalence of infestation

M. galloprovincialis (cool temperate vs warm temperate bioregion)

There was a significant difference in prevalence between the two bioregions, with high infestation in the cool temperate bioregion compared to the warm temperate bioregion, (ANOVA: $F_{1,24} = 8.467$; $p < 0.05$, Table 2.3). Site nested in bioregion (ANOVA: $F_{4,24} = 6.486$; $p < 0.05$) had a significant effect because infestation on the warm temperate bioregion in JF was significantly different from BR (Fig. 2.4). Size had a significant effect (ANOVA: $F_{3,12} = 6.743$; $p < 0.05$; Table 2.3) with the exception of sites on the cool temperate bioregion where infestation was high in all size classes (Fig. 2.5) while on the warm temperate bioregion infestation increased with size (Fig. 2.5). The cool temperate bioregion had high infestation compared to the warm temperate bioregion (Fig. 2.5), particularly for smaller size classes with infestation being similar in the larger size classes on both the cool temperate and warm temperate bioregions.

Table 2.3: Factorial ANOVA. Summary of results showing the effect of bioregion, size and site nested into region on infestation rates. Bolded p values signify a statistically significant difference between groups at $p < 0.05$.

Effect	Effect	SS	D.F	MS	F	p
Intercept	Fixed	418133.3	1	418133.3	1768.317	**0.001**
Bioregion	Fixed	2002.1	1	2002.1	8.467	**0.044**
Site (Bioregion)	Random	945.8	4	236.5	6.486	**0.005**
Size	Fixed	737.5	3	245.8	6.743	**0.006**
Site (Bioregion x Size)	Random	437.5	12	36.5	2.692	**0.019**
Bioregion x Size	Fixed	618.7	3	206.2	5.657	**0.012**
Error		325.0	24	13.5		

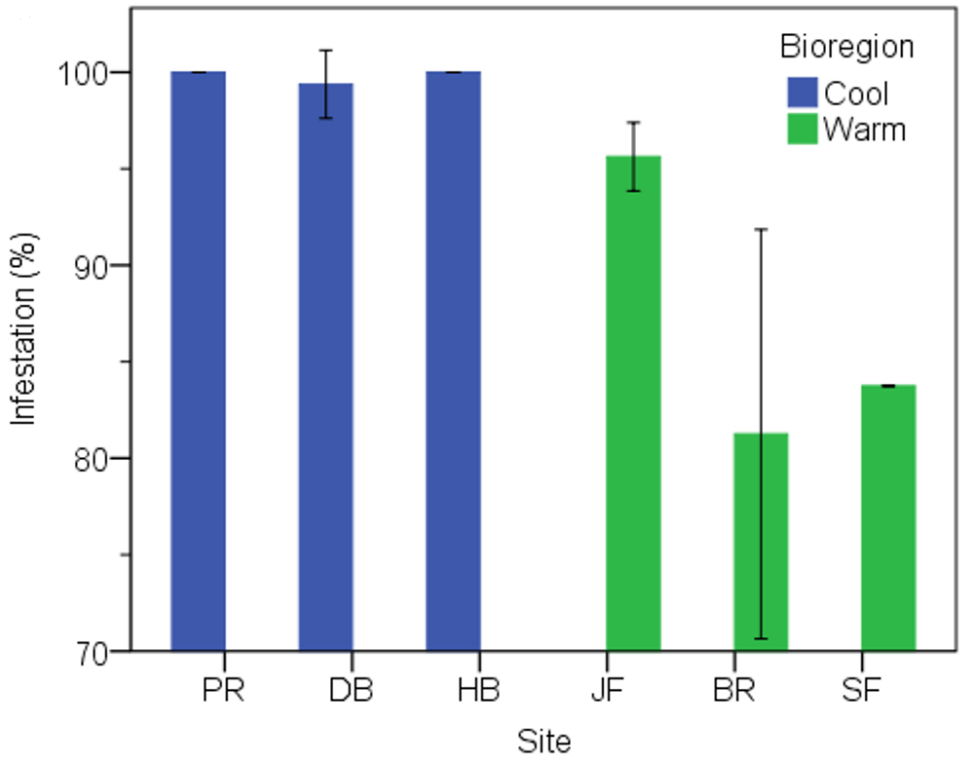

Figure 2.4: Mean infestation prevalence shown in percentages, (±SD) for *Mytilus galloprovincialis*, blue = cool temperate, green = warm temperate. Full site names of site in Table 2.1.

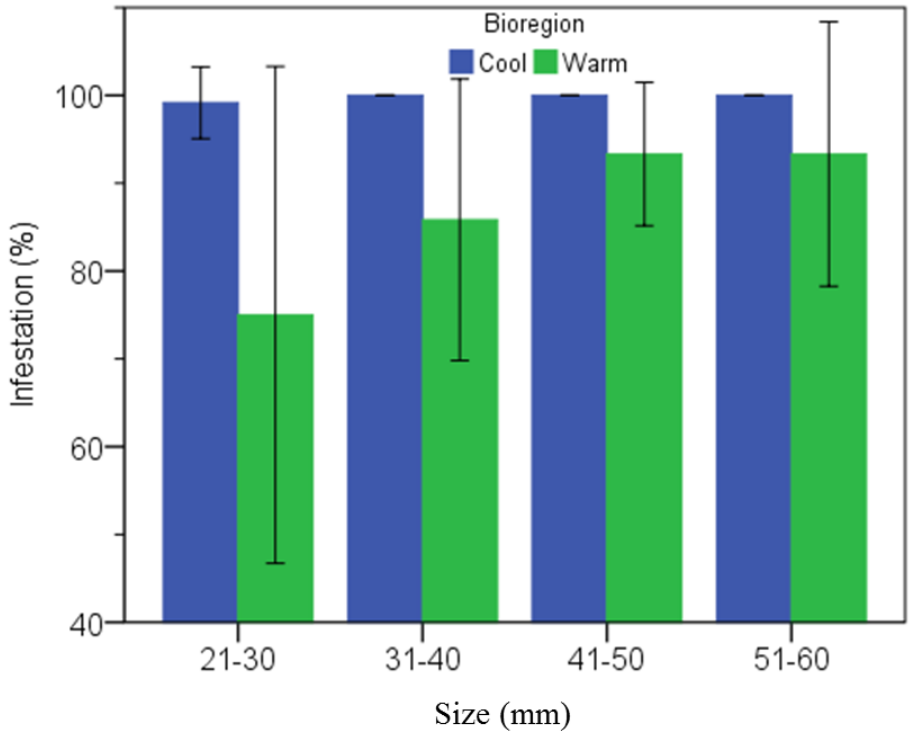

Figure 2.5: Mean prevalence infestation shown in percentages (±SD), for *Mytilus galloprovincialis* mussels for each size classes in the cool temperate and warm temperate bioregions.

***Perna perna* (subtropical vs warm temperate)**

There was no significant difference in the prevalence of infestation between the two bioregions (ANOVA: $p = 0.916$, Table 2.4). However, there was an effect of size (ANOVA: $F_{3,24} = 12.158$; $p < 0.05$) because bigger size classes (41-50mm, 51-60mm) had higher rates of infestation which were classified in categories D or E of infestation than smaller size classes (21-30mm, 31-40mm). There was a significant interaction of site nested in bioregion with size (ANOVA: $F_{12,24} = 4.222$; $p < 0.05$, Table 2.4). At all sites in both the subtropical and the warm temperate bioregion, infestation increased with size and the effect was much stronger at some sites than others (Fig. 2.7).

Table 2.4: Factorial ANOVA. Summary of results showing the effect of bioregion, size and site nested into bioregion on infestation rates in *Perna perna* mussels. Bolded p values signify a statistically significant difference between groups at $p < 0.05$.

Effect	Effect	SS	D.F	MS	F	p
Intercept	Fixed	403333.3	1	403333.3	2450.633	**0.001**
Bioregion	Fixed	2.1	1	2.1	0.013	0.916
Site (Bioregion)	Random	658.3	4	164.6	2.079	0.147
Size	Fixed	2887.5	3	962.5	12.158	**0.001**
Site (Bioregion x Size)	Random	950.0	12	79.2	4.222	**0.001**
Bioregion x Size	Fixed	18.7	3	6.2	0.079	0.970
Error		450.0	24	18.7		

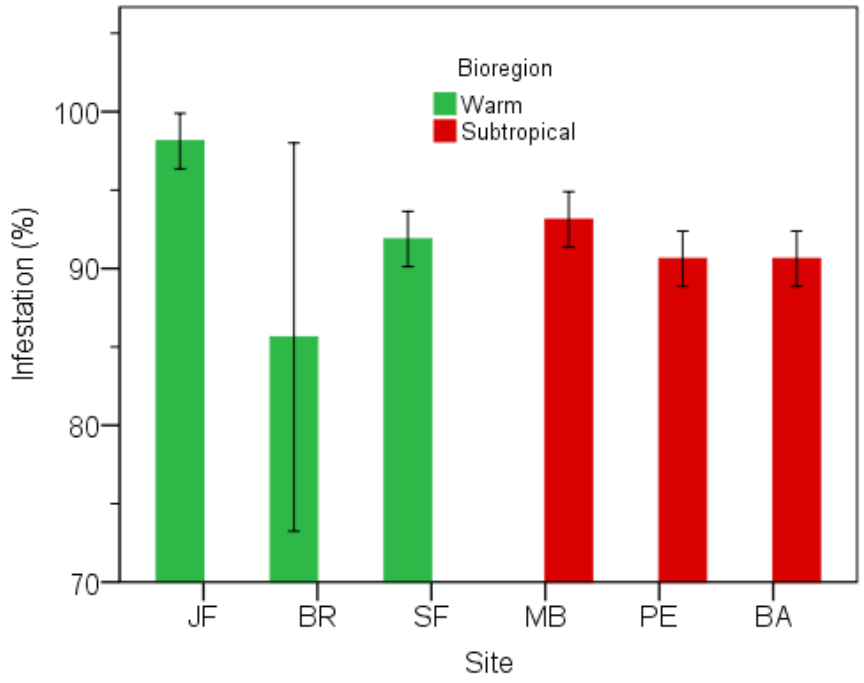

Figure 2.6: Mean prevalence infestation of *Perna perna* mussels shown in percentages, (±SD), red = subtropical, green = warm temperate. Full site names refer to Table 2.1.

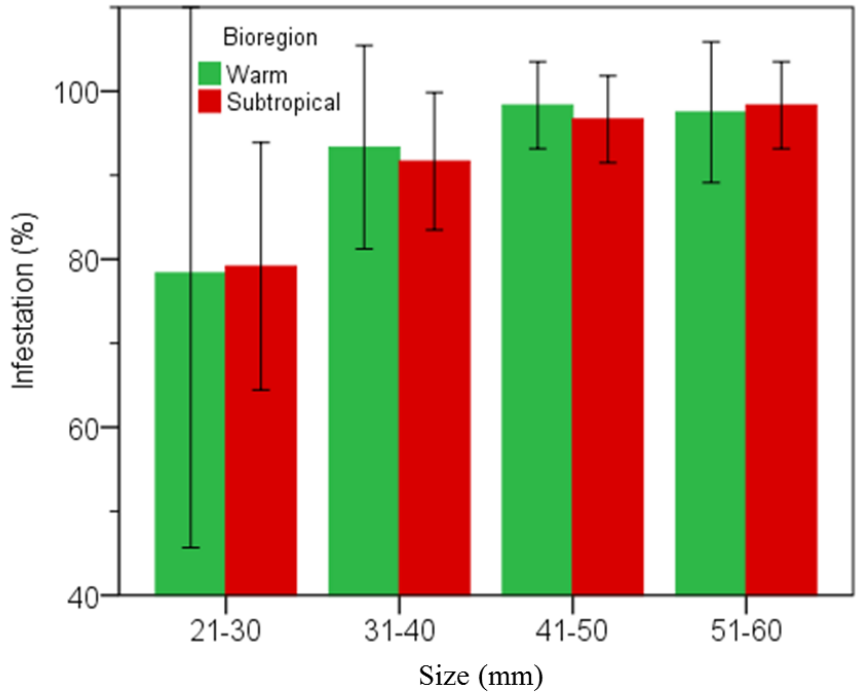

Figure 2.7: Prevalence of infestation shown in percentages (±SD), for *Perna perna* mussels for all size classes in the subtropical and warm temperate bioregions.

***M. gallopronvincialis* vs *P. perna* (warm temperate)**

There was no significant difference in the prevalence of infestation between the two species (ANOVA: $p = 0.132$; Table 2.5). There was a significant effect of size (ANOVA: $F_{3,24} = 10.47$; $p < 0.05$, Table 2.4) and a significant interaction of species x site x size (ANOVA: $F_{12,24} = 3.53$; $p < 0.05$, Table 2.5). At all sites, both species showed an increase in infestation with increasing mussel length. Smaller mussels (21-30mm, 31-40mm) were less infested than bigger mussels (41-50mm, 51-60mm) with 100% infestation rates in size classes 41-50mm and 51-60mm (post hoc, TUKEY HSD, Fig. 2.8). For both species, the prevalence of infestation in BR was 60% for hosts belonging to the smaller size classes and this level increased to more than 75% at BR for bigger size classes (Fig. 2.8) and there was a stronger influence of size in JF with prevalence of infestation being very high in all size classes.

Table 2.5: Factorial ANOVA, summary of results showing the effect of bioregion, size and site nested into bioregion on the prevalence of infestation. Bolded p values signify a statistically significant difference between groups at $p < 0.05$.

Effect	Effect	SS	D.F	MS	F	p
Intercept	Fixed	385208.3	1	385208.3	506.923	**0.002**
Species	Fixed	252.1	1	252.1	6.127	0.132
Site	Random	1519.8	2	759.9	18.468	0.051
Size	Fixed	3004.2	3	1001.4	10.468	**0.001**
Species x Site	Random	82.3	2	41.1	0.430	0.660
Species x Size	Fixed	35.4	3	11.8	0.123	0.944
Species x Site x Size	Random	1147.9	12	95.7	3.532	**0.004**
Error		650.0	24	27.1		

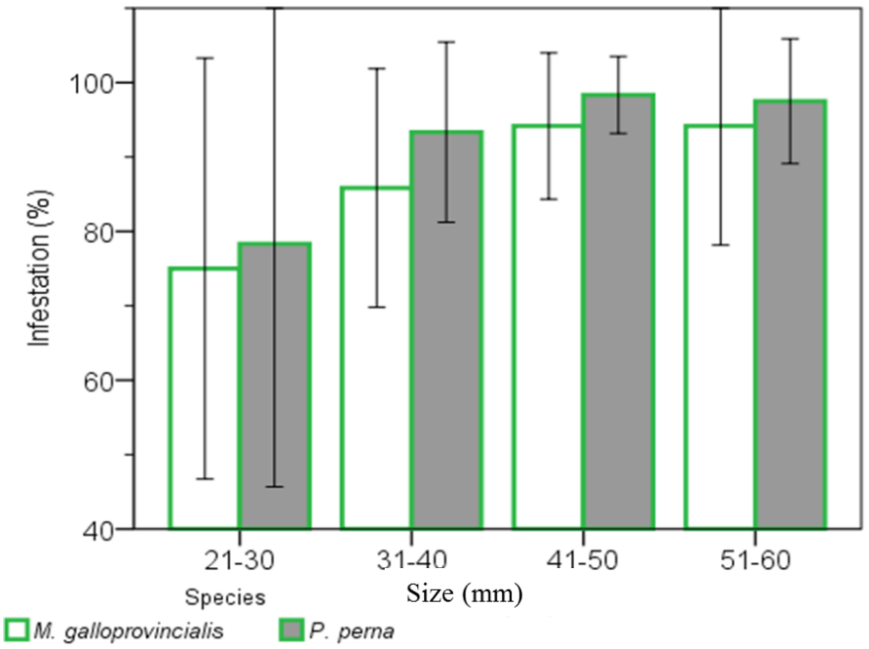

Figure 2.8: Mean prevalence infestation for *Mytilus galloprovincialis* and *Perna perna* mussels shown in percentages (±SD) for all size classes in the warm temperate bioregion.

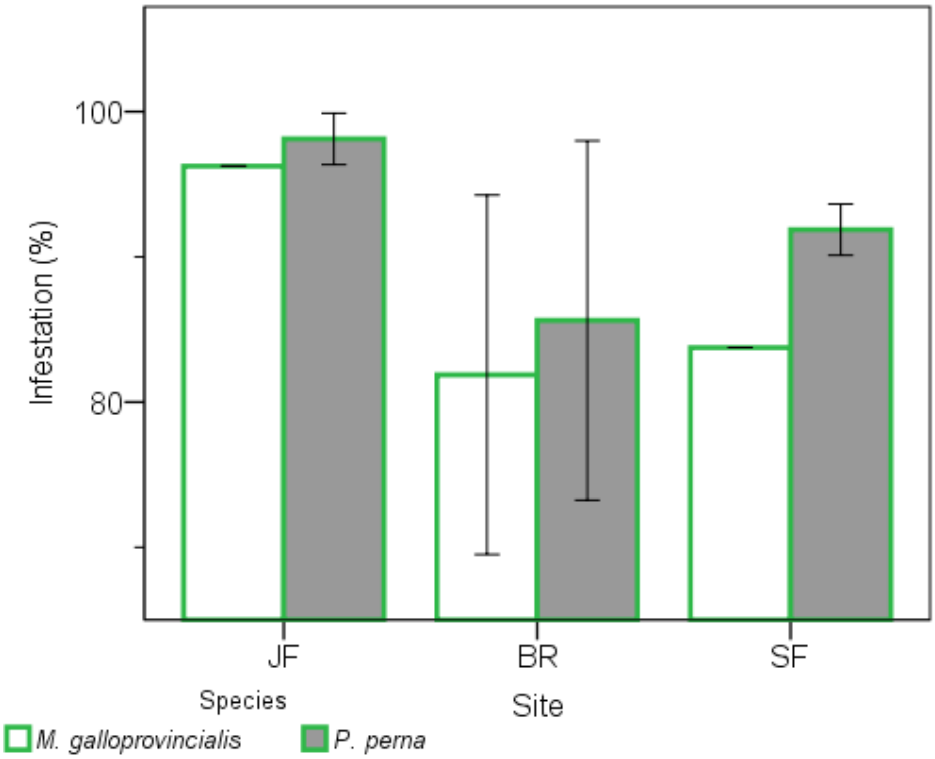

Figure 2.9: Mean prevalence infestation shown in percentages, (±SD), for *Perna perna* and *Mytilus galloprovincialis* mussels in the warm temperate bioregion. Full site names refer to Table 2.1.

Endolithic organisms:

M. galloprovincialis (cool temperate vs warm temperate)

The dendrogram and nMDS did not reveal a clear difference in the composition of the cyanobacterial community between the two bioregions (Fig. 2.10 and Fig. 2.11). In the nMDS plots, there was partial overlapping between sites from the two bioregions (Fig. 2.11).

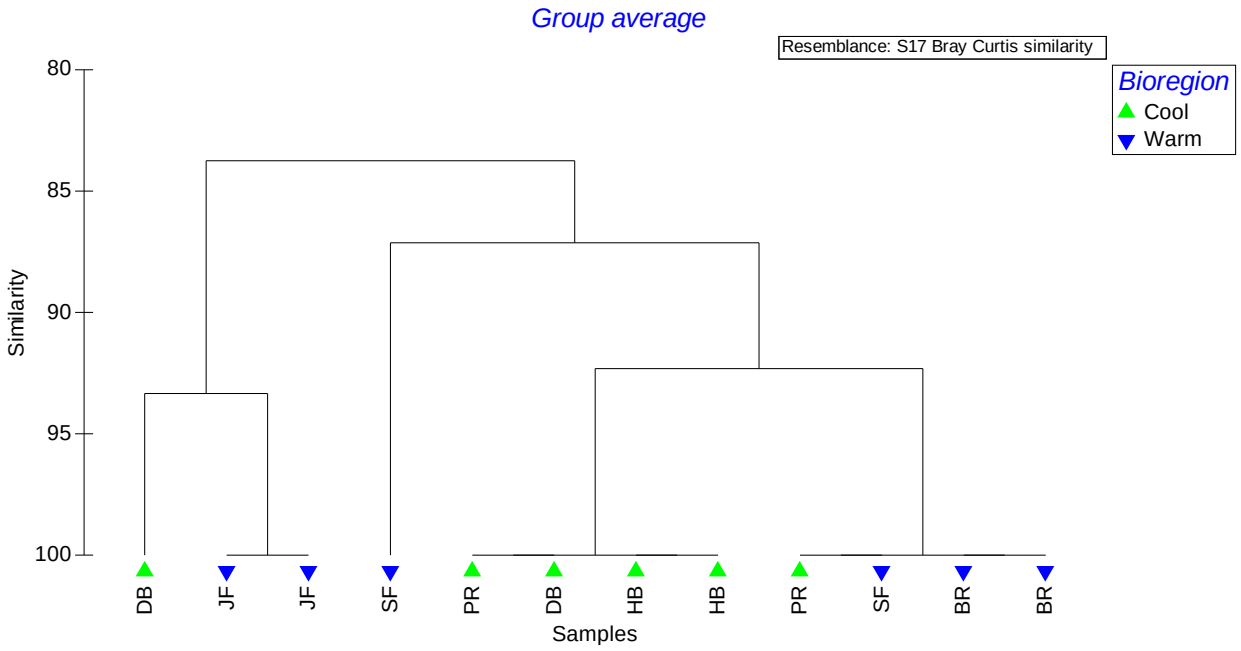

Figure 2.10: Results of classification analysis of endolithic species distribution based on Bray Curtis similarity matrix for *Mytilus galloprovincialis*, location pooled. Site codes refer to full names in Table 2.1.

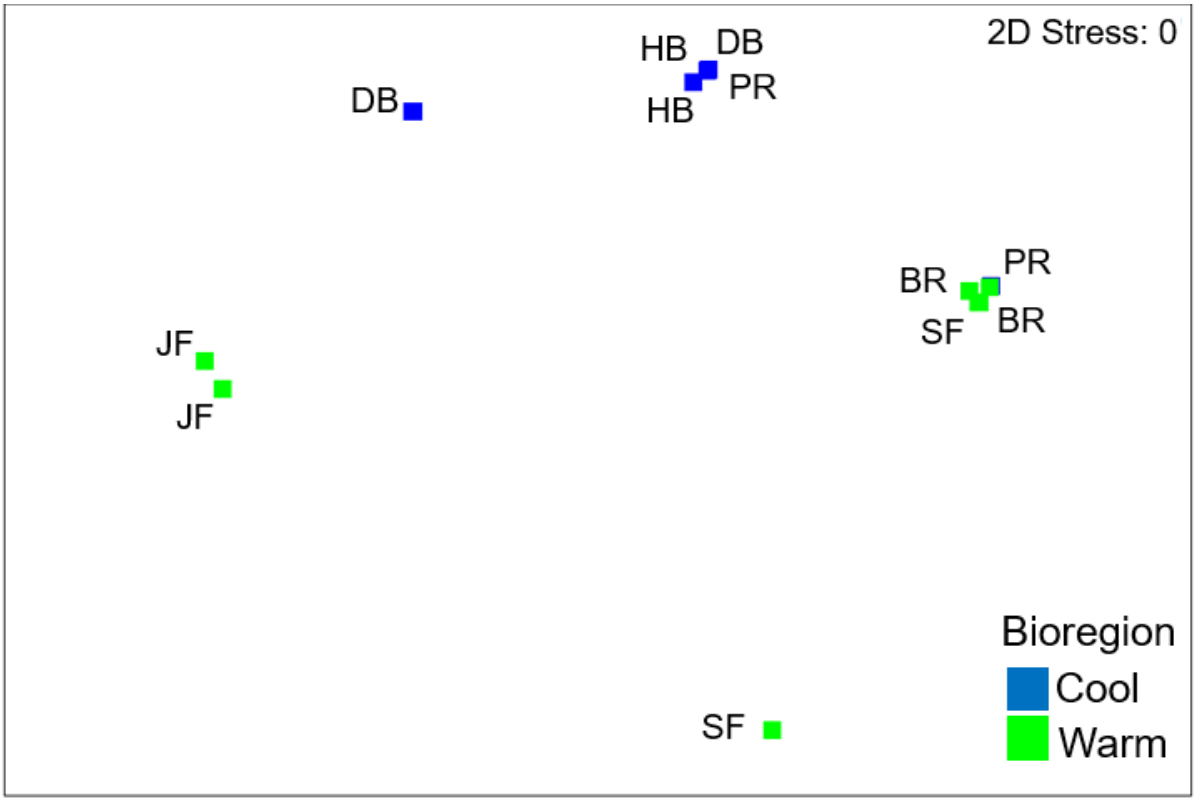

Figure 2.11: Non-metric multidimensional scaling (NMDs), based on presence-absence of cyanobacterial species using Bray–Curtis distance for *Mytilus galloprovincialis*, location pooled. Site codes refer to full names refer to Table 2.1.

Similarities between bioregions based on endolith species contributions were assessed using SIMPER (PRIMER-E v6). The species that contributed most to regional differences were *Hormathonema violaceonigrum* (27.78%), *Hormathonema luteo brunneum* (26.85%), *Kyrthutrix dalmatica* (23.15%) and *Hyella balani* (19.44%). Eight endolith species were identified in mussel shells collected on the cool temperate coast, while seven species were identified from warm temperate mussels with *Hormathonema luteo nigrum* being unique to the cool temperate bioregion.

For *Mytilus galloprovincialis* (cool temperate and warm temperate bioregions), shells from the cool temperate bioregion eight species of endoliths were identified while the warm temperate

bioregion seven species (Fig. 2.12) were identified with *Hormathonema luteo nigrum* as the species unique to the cool temperate bioregion. Moreover, bioregion had no effect on species richness within shells (ANOVA: p = 0.192; Table 2.6).

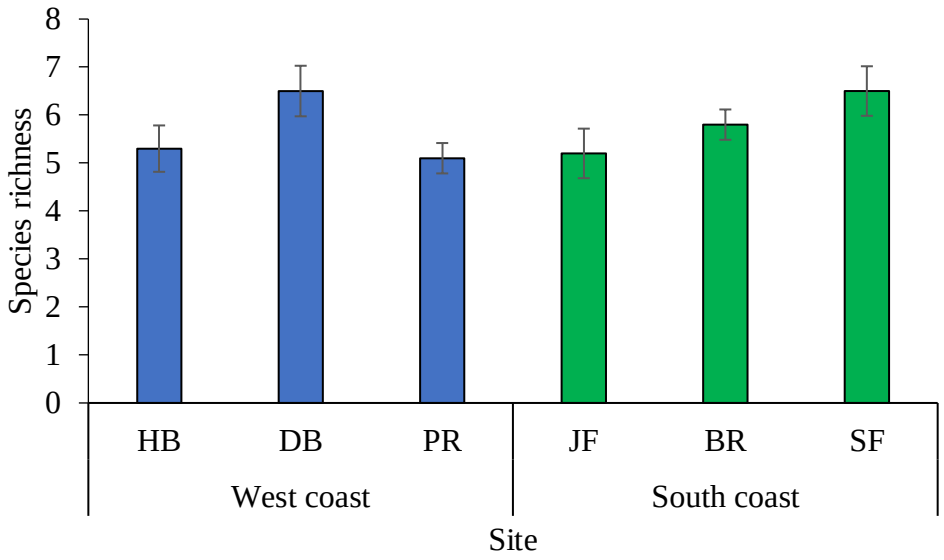

Figure 2.12: Mean endolithic species richness (+SD) identified in *Mytilus galloprovincialis*, location pooled in the cool temperate and warm temperate bioregions of South Africa. Full site names refer to Table 2.1.

Table 2.6: Factorial ANOVA, summary of results showing the effect of bioregion and site on endolithic species richness. Values of p in bold signify a statistically significant difference between groups at $p < 0.05$.

Effect	Effect	SS	D.F	MS	F	p
Intercept	Fixed	9.242	1	9.242	3201.297	**0.001**
Bioregion	Fixed	0.007	1	0.007	2.462	0.192
Site (Bioregion)	Random	0.012	4	0.003	3.312	0.093
Error		0.005	6	0.001		

Endolithic organisms:

Perna perna (subtropical vs warm temperate)

A dendrogram based on the presence or absence of endolithic species suggested a clear-cut grouping in terms of bioregion (Fig. 2.13), with the exception of one location at SF. This is explained by the absence of *Hormathonema luteo brunneum* in some of the shells from SF while this species was present in all other shells from the warm temperate bioregion sites.

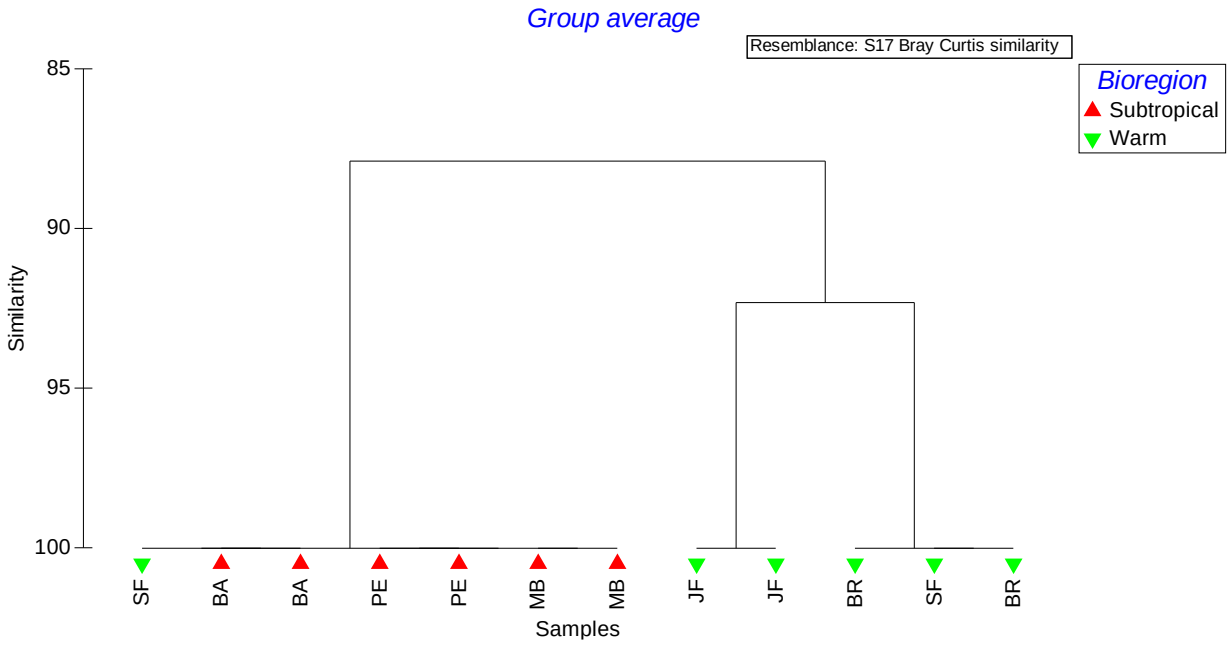

Figure 2.13: Results of classification analysis of endolithic species distribution based on Bray Curtis similarity matrix for *Perna perna*, location pooled. Site codes refer to full names in Table 2.1.

Non-metric Multi-Dimensional Scaling plot showed similarity in endolithic community composition between bioregions (Fig. 2.14) with clear-cut grouping of the subtropical and warm temperate bioregions except for SF.

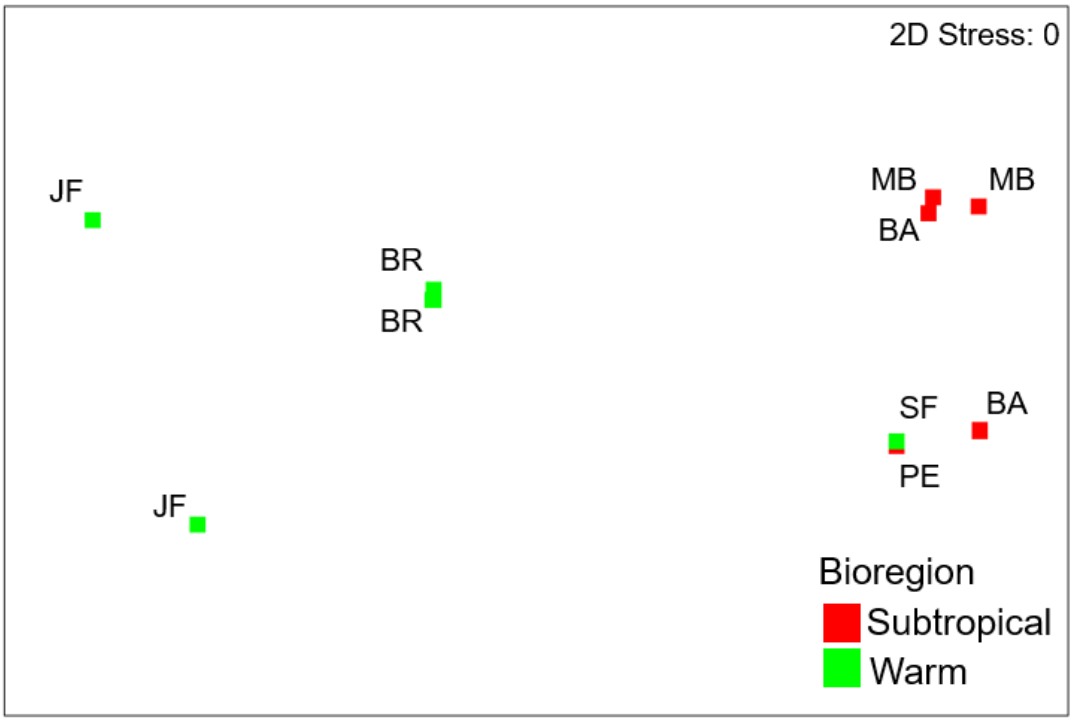

Figure 2.14: Non-metric multidimensional scaling (NMDs), based on presence-absence of cyanobacterial species using Bray–Curtis distance for *Perna perna*, location pooled. Site codes refer to full names refer to Table 2.1.

Species contributions to similarity (SIMPER; PRIMER-E v6) were used to assess similarities between bioregions. The three species that contributed most to geographical differences were *Hormathonema luteo brunneum* (67.57%), *Mastigocoleus testarum* (21.62%) and *Kyrthutrix dalmatic*a (10.81%). Five and seven species were identified on the subtropical and warm temperate bioregions respectively, with *Hormathonema luteobrunneum* and *Kyrthutrix dalmatica* being unique to warm temperate shores.

For *Perna perna* on the east coast, the maximum number of species identified was five, while in the warm temperate bioregion seven species were identified, with *Hormathonema luteo brunneum* and *Kyrthutrix dalmatica* being unique to the warm temperate bioregion (Fig. 2.15).

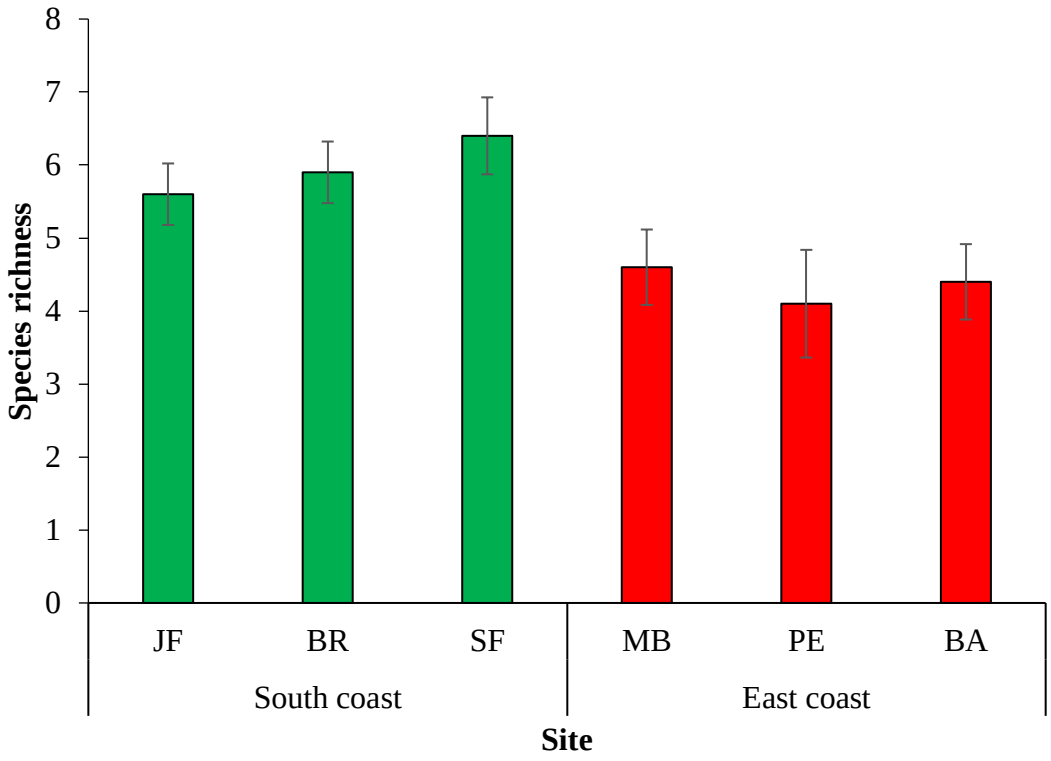

Figure 2.15: Mean endolithic species richness (+SD) identified in *Perna perna*, location pooled in the south and east coast of South Africa. Full site names refer to Table 2.1.

Species richness showed a similar pattern. Bioregion had an effect on cyanobacteria richness (ANOVA: $F_{1,6} = 11.604$; $p < 0.05$; Table 2.7).

Table 2.7: Factorial ANOVA, summary of results showing the effect of bioregion and site on endolithic species richness. Bolded p values signify a statistically significant difference between groups at $p < 0.05$.

Effect	Effect	SS	D.F	MS	F	p
Intercept	Fixed	7.728	1	7.728	2900.981	**0.001**
Bioregion	Fixed	0.031	1	0.031	11.604	**0.027**
Site (Bioregion)	Random	0.011	4	0.003	1.878	0.234
Error		0.009	6	0.001		

Endolithic organisms:

M. galloprovincialis vs *P. perna* (warm temperate)

The dendrogram and nMDS did not reveal a clear difference in the cyanobacterial community composition between the two host species, *M. galloprovincialis* and *P. perna* (Fig. 2.16 and Fig. 2.17). In the nMDS plots, there is partial overlapping between the two species (Fig. 2.17).

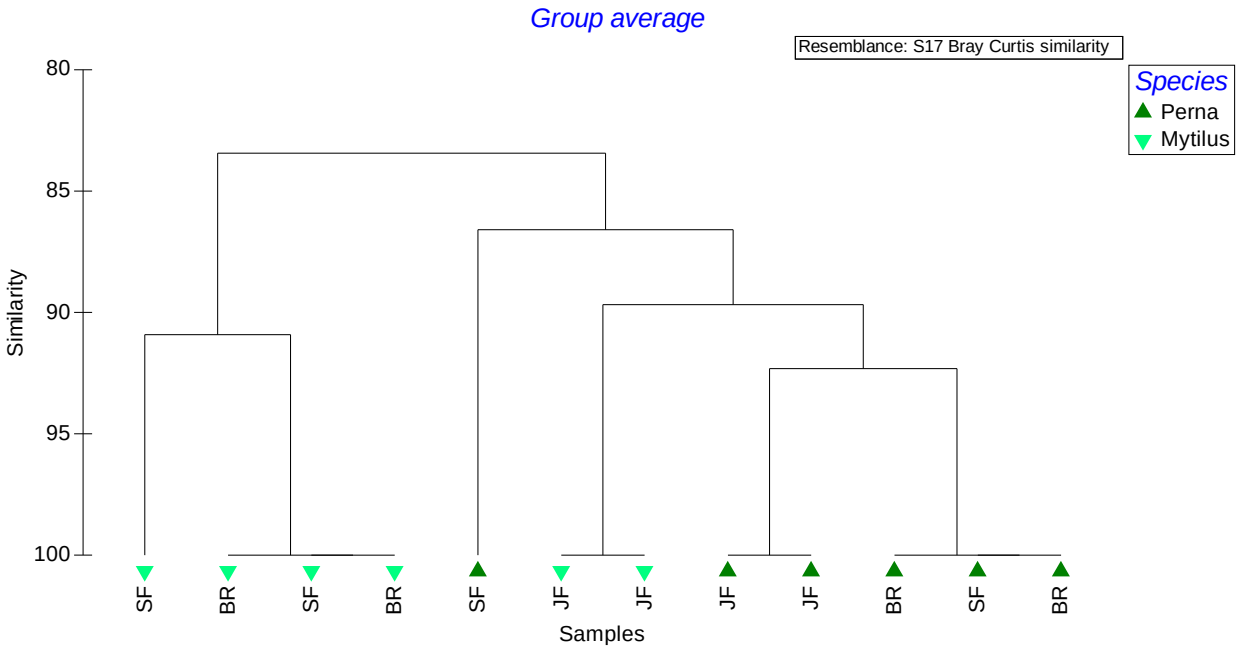

Figure 2.16: Results of classification analysis of endolithic species distribution based on Bray Curtis similarity matrix for *Perna perna* and *Mytilus galloprovincialis* mussels, location pooled. Site codes refer to full names in Table 2.1.

The lack of species' effect is also clear in the nMDS plot (Fig. 2.17), which had an acceptable stress level (stress = 0.01) and displayed partial overlapping between *P. perna* and *M. galloprovincialis* samples.

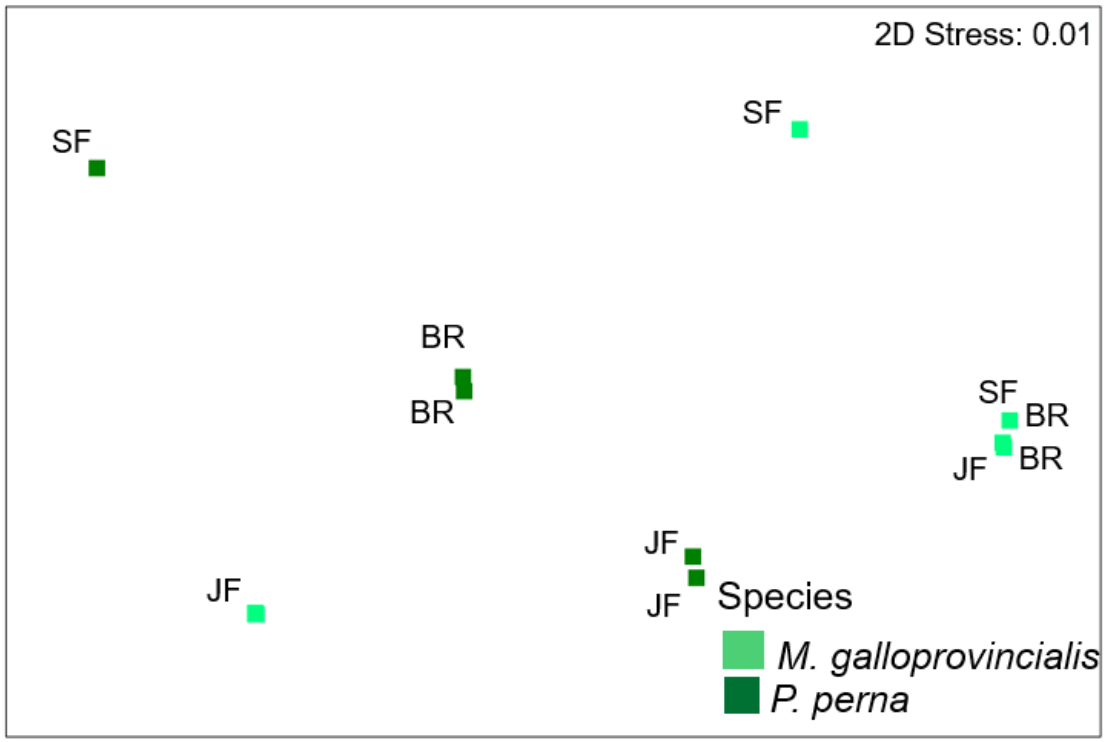

Figure 2.17: Non-metric multidimensional scaling (nMDS) plot of endolithic communities in *P. perna* and *M. galloprovincialis* on the warm temperate bioregion. Scaling was based on Bray–Curtis similarity distances. Site codes refer to full names in Table 2.1

Similarities between *M. galloprovincialis* and *P. perna* based on endolith species contributions were assessed using SIMPER (PRIMER-E v6). *Hormathonema luteo brunneum* and *Kyrthutrix dalmatica* accounted for the highest contributions, 54.59% and 45.41% respectively. In both mussel species, seven endolith species were identified.

In the warm temperate bioregion, eight species of endoliths were identified from *M. galloprovincialis* shells, while the seven species were identified for *P. perna* (Fig. 2.18) with *Hormathonema luteo brunneum* as the species unique to *M. galloprovincialis*. Species richness showed a similar pattern, site had a significant effect on cyanobacterial richness (ANOVA: $F_{2,6} = 29.875$; $p < 0.05$, Table 2.8) with Tukey HSD post-hoc tests indicating that the only significant difference among sites or species was *Mytilus galloprovincialis* SF vs JF.

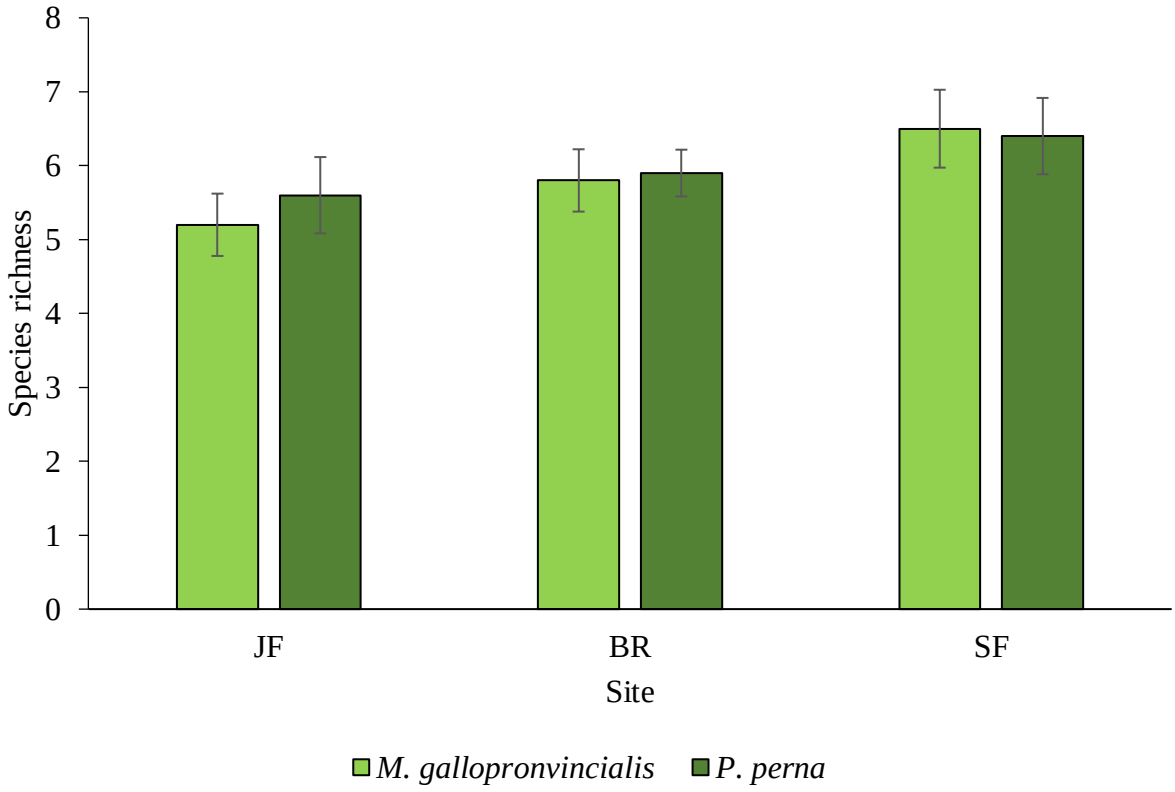

Figure 2.18: Mean endolithic species richness (+SD) identified in *Perna perna* and *Mytilus galloprovincialis* mussels in the warm temperate bioregion of South Africa. Full site names refer to Table 2.1.

Table 2.8: Factorial ANOVA, summary of results showing the influence of bioregion and site on endolithic cyanobacteria distribution based on species richness for each site. Bolded p values signify a statistically significant difference between groups at $p < 0.05$.

Effect	Effect	SS	D.F	MS	F	p
Intercept	Fixed	8.836	1	8.836	1055.360	**0.001**
Species	Fixed	0.000	1	0.000	1.000	0.423
Site	Random	0.017	2	0.008	29.875	**0.032**
Species x Site	Random	0.001	2	0.000	0.273	0.770
Error		0.006	6	0.001		

Lethal effects of endolithic infestation

For *M. galloprovincialis*, all sites in the cool temperate bioregion had high mortality rates with an average of five dead mussels/m², with the warm temperate bioregion having an average of one dead mussel/m². *Perna perna* on the subtropical bioregion had an average mortality of 1.5 dead mussels/m² compared to the warm temperate bioregion with a mortality rate of 2.1 dead mussels/m² on average (Fig. 2.19 and 2.20). For *P. perna*, endolith induced mortality rates did not differ between the two bioregions (ANOVA: $F_{1,42} = 6.612$; $p = 0.062$; Table 2.9). For *M. galloprovincialis*, mussel mortality differed significantly between the two bioregions; the cool temperate bioregion had significantly higher mortality rates than the warm temperate bioregion (ANOVA: $F_{1,42} = 136.5$; $p < 0.05$; Table 2.10). There was a significant effect of the nested factor site (ANOVA: $F_{4,42} = 3.758$; $p < 0.05$) because, in the warm temperate bioregion, mortality rates in JF were significantly lower than those in SF ($p < 0.019$, TUKEY HSD).

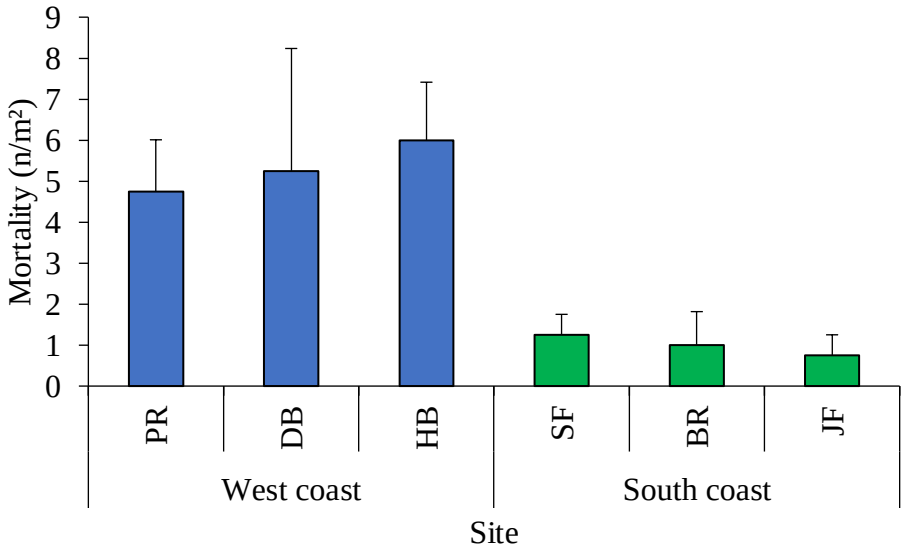

Figure 2.19: Mean endolithic induced mortality (+SD) for *Mytilus galloprovincialis* in the warm temperate and cool temperate bioregions of South Africa. Full site names refer to Table 2.1.

Table 2.10: Factorial ANOVA, summary of results for mortality rates showing the effects of bioregion and site for *Mytilus galloprovincialis* mussels. Bolded p values signify a statistically significant difference between groups at $p < 0.05$.

Effect	DF	MS	F	P
Bioregion	1	176.3	136.5	**0.001**
Site (Bioregion)	4	1.3	1.2	0.331

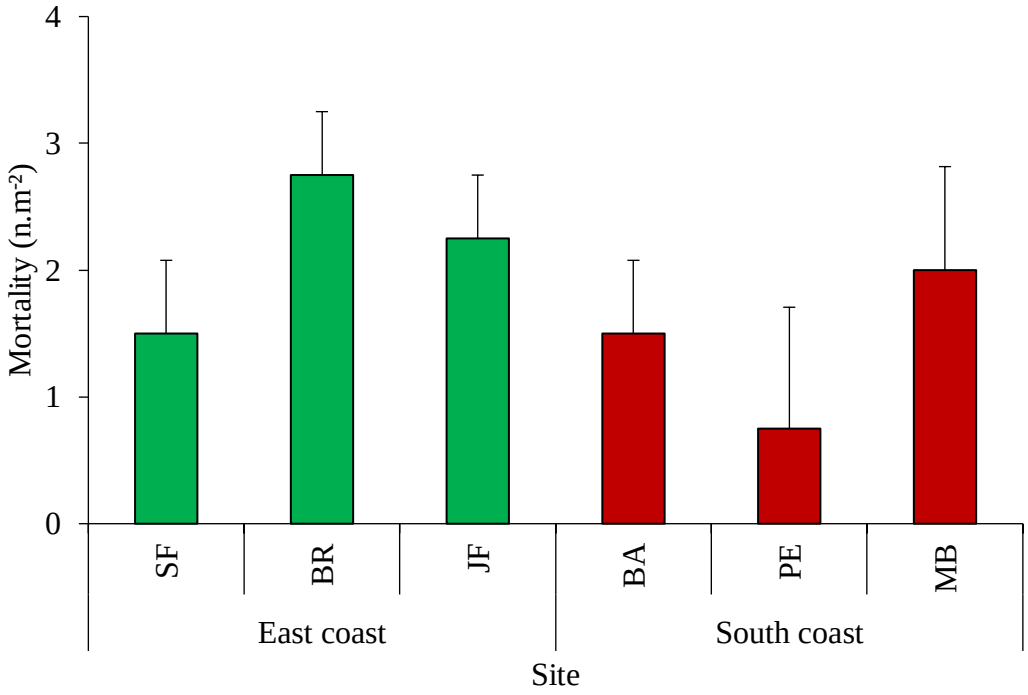

Figure 2.20: Mean endolithic induced mortality (+SD) for *Perna perna* mussels in the warm temperate and subtropical bioregions of South Africa. Full site names refer to Table 2.1.

Table 2.9: Factorial ANOVA, summary of results for mortality rates showing the effects of bioregion and site for *Perna perna* mussels. Bolded p values signify a statistically significant difference between groups at $p < 0.05$.

Effect	DF	MS	F	P
Bioregion	1	11.02	6.612	0.062
Site (Bioregion)	4	1.67	3.758	**0.011**

Discussion

This study provides a quantitative and qualitative assessment of endolithic communities, and their lethal effects on mussels across distinct bioregions. Results show that endolithic induced erosion is intense and ubiquitous in intertidal mussel populations across three distinct bioregions of South Africa. Along approximately 2000 km of the South African coastline, the prevalence of infestation was recorded to be more than 75% in all populations, with infested individuals hosting between three and seven species of endolithic cyanobacteria each. Previous work has shown that marine calcifying organisms are affected by endolithic parasitism globally (Porter & Lingle 1992, Gleason et al. 2017) and the findings of this study support this idea. The results show that biogeography has an influence on the prevalence and species richness of phototrophic shell-degrading endoliths and on endolith-induced mortality in mussels. There was high prevalence of endolithic infestation and high mortality due to endolithic cyanobacteria along the cool temperate west coast compared to the other two bioregions, the warm temperate south and the subtropical east coasts.

In the case of *M. galloprovincialis* there was a significant difference in the prevalence of infestation, with high infestation on the cool temperate bioregion and low infestation on the warm temperate bioregion. For *P. perna* mussels, there was no significant difference in the prevalence of infestation between the warm temperate and subtropical bioregions. When comparing the two-mussel species, *M. gallopronvincialis* and *P. perna* in the warm temperate bioregion where they co-occur, there was no significant difference in the prevalence of infestation. However, size had an influence in the prevalence of infestation with infestation increasing with mussel size. There were clear-cut groupings in terms of bioregion shown by the dendrogram based on the presence or absence of endolithic species in the subtropical vs warm temperate bioregions where *P. perna* occurs. This indicates that, while bioregion had no effect on infestation rates for *P. perna*, it did

influence the species richness and species distribution of the endolithic community. The two bioregions, warm temperate and subtropical bioregions where *P. perna* occurs showed no significant difference in prevalence of infestation, nor was there a significant difference in terms of endolithic induced mortality between the two-mussel species in the warm temperate bioregion. *M. galloprovincialis* occurs in two bioregions and there was significantly greater mortality in the cool temperate bioregion than the warm temperate bioregion. This suggests that the differences in induced mortality due to endoliths was mainly due to bioregion. The only difference in prevalence of cyanobacteria infestation was between the cool temperate and the warm temperate bioregions, with the cool temperate bioregion having higher infestation levels which in turn was correlated with higher mortality compared to the other two bioregions. Higher infestation and mortality may reflect the positive effects on cyanobacteria of greater nutrient availability on the west coast.

Endolithic cyanobacteria occupy a wide range of environmental conditions, hence their abundance and distribution in marine living organisms such as molluscs is influenced by numerous factors, these include temperature, water, light and nutrient availability (Friedmann 1982), though in this case the only significant effect of bioregion on cyanobacteria was cool temperate vs warm temperate bioregion.

Prevalence of Infestation

The approach was to investigate if the prevalence of endolithic infestation was different among bioregions. Overall, all sites showed high prevalence levels of infestation, with more that 75% of mussels infested at each site for all size classes. Other studies have shown relatively low infestation. For example, Marquet et al. (2013) showed relatively low infestation levels for *Mytilus galloprovincialis* in Portugal. Results showed that the prevalence of endolithic cyanobacteria in *Mytilus galloprovincialis* was higher in the cool temperate bioregion compared to the warm

temperate bioregion (Fig. 2.2). For *Perna perna* (subtropical vs warm temperate) results show that the prevalence of endolithic infestation was high in the cool temperate than warm temperate (Fig. 2.2). Where the two-mussel species coexisted in the warm temperate bioregion there was no difference between the two-mussel species in terms of the prevalence of infestation, with infestation increasing with shell size. The findings of this study show that infestation increasing with mussel size supported the results of Marquet et al. (2013) for *M. galloprovincialis*. There was a clear correlation of size and percentage infestation with prevalence of endolithic infestation increasing with size for both species in this study.

Invasive species are thought to be vulnerable to parasites in areas where they colonise as they are not adapted to the new environment (Keane et al. 2002, Li et al. 2015), but comparing the two mussel species in the warm temperate bioregion where they coexist, there was no difference in the prevalence of endolithic infestation. This suggests that endolithic cyanobacteria affect both the invasive species and the native species in a similar way and to a similar degree. Even though *M. galloprovincialis* is invasive in South Africa, there is a possibility that it acquired new enemies in the form of endolithic parasites which are also affecting the native species *P. perna*. Results from Marquet et al. 2013 showed that *M. galloprovincialis* which is invasive in South Africa suffers more from endolithic infestation than in the native range in Portugal. This finding is in contrast with previous studies which have shown that *M. galloprovincialis* has weak shells compared to *P. perna* hence *M. galloprovincialis* suffer more prevalence of infestation compared to *P. perna* (Zardi et al. 2009).

Endolithic organisms

The aim was to investigate if endolithic species richness changes with bioregion and whether the distribution of endolithic organisms was different among bioregions. In the subtropical and warm temperate bioregions where *Perna perna* mussels were compared, there was a clear-cut grouping with samples collected from the subtropical bioregion being the same to each other and this trend was observed in the warm temperate bioregion, with warm temperate bioregion sites grouping together except JF (Fig. 2.10). This grouping by bioregion may be attributed to the fact that endolithic organisms are influenced by their location, this suggests that different climatic conditions experienced in these two bioregions determine which organisms can exist there. Populations are known to develop characteristics that make them suitable or be adaptive to particular conditions that they might experience over a prolonged period of time in a particular area following the principle of adaptation (Pigliucci 1996). Endolithic organisms in these two bioregions might have developed certain characteristics that allow them to adapt and evolve to suit the environments of these bioregions.

Endolithic organisms in these two bioregions are not different even though the cool temperate bioregion is colder and experiences higher nutrient levels through upwelling than the warm temperate bioregion (Bustamante et al. 1996, Branch et al. 2010). The warm temperate bioregion is not different when compared to the cool temperate bioregion with endolithic species that are found in the two bioregions being the same. Similarly, in the warm temperate bioregion, where endolithic organisms were compared between the two-mussel species, there was no separation of cyanobacterial communities between the two. This suggests that endolithic organisms which are parasitic on mussels do not have a preference between hosts, they colonise mussel shells in the same way regardless of what mussel species they belong to. Phototrophic parasites have been

described before as extremely generalist parasites, affecting a wide range of hosts (Torchin et al. 2002, Torchin & Mitchell 2004, Marquet et al. 2013) and this is reflected in the similar levels of infestation in the two mussel species, despite differences in their periostracum structure (Bers et al. 2010).

Species richness

Species richness is the simplest way to describe community or regional diversity (Gotelli & Colwell 2001). The species pool hypothesis described by Zobel (1997) states that the number of species occupying a particular habitat should be determined by the commonness (similar quality) of that particular region. In this study, I focused on three South African bioregions, identifying 8 and 7 endolithic species on *Mytilus galloprovincialis* in the cool temperate and warm temperate bioregions respectively. In the warm temperate bioregion and subtropical bioregion where *Perna perna* occurs, there were seven and five species of endolithic cyanobacteria, respectively. In the warm temperate bioregion, where the two-mussel species coexist, seven species of endoliths where identified for both mussel species. This study supports the hypothesis that species occur because of commonness in habitat (Zobel 1997) as in the warm temperate bioregion there was the same number of endolithic species for both mussels. This supports the idea that the habitat, rather than host mussel species, influences endolithic species. There is a clear gradient of increasing temperatures and decreasing nutrient availability as you move from cool temperate west coast to the subtropical east coast highlighting the differences in the three bioregions of South Africa. A comparison of the cool temperate vs the warm temperate bioregions, showed no difference in species richness as these two bioregions experience very similar climatic conditions with the warm temperate bioregion having upwelling regimes that are common in the cool temperate bioregion. The warm temperate bioregion supported two more endolithic species than the subtropical

bioregion, perhaps due to the fact that the subtropical bioregion is much more oligotrophic, leading to low species richness despite higher temperatures. These results indicate that bioregions with high nutrient availability have a strong positive influence on endolithic species prevalence and species richness.

Lethal effects of endolithic infestation

I investigated whether mortality due to infestation varies in different geographic regions. Results show that, for *Mytilus galloprovincialis*, there was higher endolithic induced mortality in the cool temperate bioregion compared to the warm temperate bioregion. In contrast, endolithic induced mortality in *Perna perna* showed no significant difference between the warm temperate and subtropical bioregions. This trend follows what was observed with the results of prevalence of infestation of endolithic cyanobacteria where there was high prevalence in the cool temperate bioregion compared to the other bioregions. The high mortality in the cool temperate bioregion where there is the invasive *M. galloprovincialis* cannot be attributed to the mussel species being an invasive species thus having new enemies of cyanobacteria that attack the species. This is because in the warm temperate bioregion where the two-mussel species coexist there is no significant difference between the two-mussel species seen which is due to endolithic induced mortality. High endolithic induced mortality on the west coast compared to the other two bioregions might be a result of high prevalence of endoliths in the cool temperate bioregion which in turn leads to high bioerosion of mussel shells resulting in their collapse; thus, leading to death. Invasive species in the invaded range are known to have new enemies that they have never encountered, which might cause severe damage to their hosts. Alternatively, invasive species might have no enemies in the new environment. When species invade, they tend to filter out their native parasites thus being able to survive with less or no parasites in their new environment

(Torchin et al. 2002, 2003). In this study there is no evidence to suggest that endolithic cyanobacteria selectively infested mussel species differently as the native, *P. perna* and the invasive *M. galloprovincialis* had similar endolithic induced mortality where they coexisted. The only known physical defence system against attacks in shells of bivalves is the periostracum (Bers et al. 2010) which is different between the two mussel species with *P. perna* having a thick layer compared to the invasive *M. galloprovincialis* (Zardi et al. 2009). This suggests that endolithic cyanobacteria are generalist parasites, generalist parasites affect a wide range of hosts (Marquet et al. 2013), they are not specific to certain individual species.

Overall, *M. galloprovincialis* and *P. perna* did not differ on the warm temperate bioregion where they coexist, the main differences in terms of cyanobacterial species richness, prevalence of infestation and endolithic induced mortality were between the cool temperate bioregion and the other two bioregions, warm temperate and subtropical bioregion which possibly reflects nutrient availability. The results from this chapter show that endolithic infestation is intense and ubiquitous in intertidal mussels and that biogeography is a strong driver of endolithic species distribution, while host identity is not. Where the two mussel hosts co-occur, there were no significant differences in levels of endolith prevalence or intensity of infestation, suggesting that endolithic parasites are not host specific. This in turn implies that there is no enemy release for the invasive species as it suffers from endolithic cyanobacteria the same way as the indigenous species. Mortality results show that endoliths parasites can be lethal to organisms thus they have the ability to modulate populations in communities.

Chapter 3 : Energetic costs of being infested: how endolithic cyanobacteria affect the energy budgets of mussels.

Introduction

Parasites are common across natural systems, in terrestrial and aquatic communities where they have major impacts on the development, performance and growth of host species (Mouritsen & Poulin 2002). They have important effects on population dynamics and community structure (Torchin et al. 2002, 2015, Hatcher et al. 2012, Lafferty 2013), and therefore there is much interest in the mechanisms by which they affect host organisms.

Infestation of organisms by parasites comes with a cost. Parasites affect their host's energy use by draining resources such as water and food, which can lead to reduced growth, reduced reproductive output and failure of the host species to defend itself from other harmful factors such as predators or increases in environmental temperatures (Mouritsen & Poulin 2002, Flye-Sainte-Marie et al. 2009). Most parasites have strong negative effects on their hosts; individuals that are infested by parasites often show signs of stress and they are likely to have limited growth as they must channel resources to fighting the parasite or repairing damage caused by the parasite (Dobson & Hudson 1986, Mouritsen & Poulin 2002).

While the direct effects of parasites on hosts are always negative, they can indirectly benefit non-host species in some cases (Mouritsen & Poulin 2002, Hatcher et al. 2012). For example, snails infected by trematodes were more likely to be found in open spaces such as surfaces of boulders were they can be easily preyed upon by the predatory birds, which are the trematodes' primary host, compared to uninfected snails (Granovitch 1992). Similarly, the metacercarial stage of a trematode found on the surface of a killifish brain affects the neuromodulators such that the

response to stress by killifish is lowered hence making it more susceptible to predation than non-affected fish (Lafferty, 2013), resulting in more food for predatory birds.

Endolithic cyanobacteria can be found in mollusc shells (e.g., bivalves) where they derive minerals at a cost to the host and thus act as parasites (Marquet et al. 2013). Endoliths bore into bivalve shells where they mine carbonates and contribute immensely to the erosion of bivalve shells (Garcia-Pichel et al. 2010). The resultant degradation increases the need for shell maintenance, which can affect the energy budget of the organism. Higher cost of maintenance in the host may impact reproduction by affecting gametogenesis and can also lead to reduced growth (Flye-Sainte-Marie et al. 2009). Previous studies have described the effects of other parasites on the energy budgets of bivalves. These studies have looked at the mechanical effects caused by trematodes and pinnotherid crabs that live between the valves, thus reducing the physical space for the gonads to grow and interfere with the filtration of food particles, thus reducing ingestion(Lafferty & Kuris 2009, Leung et al. 2009).

The rate at which organisms grow is a major determinant of their population dynamics (Alfaro et al. 2008). Growth rate in an organism is a good indicator of stress (Widdows & Staff 2006) because it integrates major physiological responses in organisms. These responses include the balance between processes of energy acquisition (feeding and digestion) and energy expenditure (metabolism and excretion). Each of these physiological responses can be measured and easily converted to the common denominator of energy. Directly measuring energy allocation to growth is difficult, but scope for growth (SFG) provides a simple measure of the energy status of an organism. SFG is the surplus energy available for growth beyond what is required for maintenance, and it is measured from physiological responses derived from the differences between energy acquisition (rate of feeding and digestion) and energy expenditure (metabolic rate) (Naylor et al.

1989, Widdows & Staff 2006). The SFG has been successfully used to assess the influence of contaminants, temperature, ocean acidification and food limitation in many marine species (Brett 1976, Naylor et al. 1989, Widdows & Staff 2006, Resgalla et al. 2007, Sarà & Pusceddu 2008). Similarly, SFG can be used to assess the effects of cyanobacterial infestation on mussels.

Several studies have examined the physical damage caused by cyanobacteria on shellfish species such as mussels (Golubic et al. 2005, Ćurin et al. 2014), including differences in shell growth and reproduction between clean and infested mussels (Kaehler & McQuaid 1999). This chapter focusses on how endolithic infestation affects mussel SFG. Intertidal communities experience extreme environmental conditions, where organisms barely have enough energy for maintenance, growth and reproduction due to tidal exposure, heat stress, wave action, and food limitation (Helmuth & Hofmann 2001). Parasites add to the challenge because they increase maintenance costs (Mouritsen & Poulin 2002). Endolithic cyanobacteria can inflict severe shell damage to mussels by dissolving the calcium carbonate in mussel shells, hence weakening the shell and causing fracture holes (Kaehler 1999, Kaehler & McQuaid 1999, Garcia-Pichel et al. 2010) and this damage may eventually lead to death through shell collapse (Marquet et al. 2013). Mussels are exposed during low tide and during the low tide period they cannot feed as they are filter feeders, so any additional energy cost could be critical to their ability to maintain themselves. With parasitic endolithic cyanobacteria eroding mussel shells, energy which could be used for growth must be channelled into shell repair (Zardi et al. 2007, 2009). This is necessary to ensure survivorship of individual mussels but presumably reduces the energy remaining for growth and reproduction.

The increased energy costs of infection may also influence the ability of intertidal mussels to cope with thermal stress. The maintenance costs are expected to increase to support the extra cost of

repair due to damage in shells. This extra cost may lead to a drop in the ability to tolerate high temperatures and a reduction in lethal temperatures for infested mussels as intertidal mussels rely on an energetically expensive heat shock response to cope with thermal stress (Buckley et al. 2001, Hochachka & Somero 2002, Somero 2002). Energy limitation and environmentally induced stress have the ability to affect thermal tolerance (Sokolova et al. 2012) as higher maintenance costs can lead to a reduction in lethal temperatures between infested and clean mussels. For organisms such as mussels, balancing the energy budget is important for adaptation and tolerance to environmental stressors. Oxygen supply and temperatures become lethal when organisms cannot maintain their basic energy requirements (Sokolova & Portner 2001, Sokolova et al. 2012). For sedentary intertidal organisms such as mussels, a reduction in lethal temperatures could be critical because they often experience temperatures close to their thermal limits during low tide (Connell 1972, Tagliarolo & McQuaid 2016). If endolithic cyanobacteria reduce lethal temperatures, this has implications for individual mussel survival and population dynamics under climate change, as this would pose a serious threat to infested mussels, with the risk of death in some cases.

Mussels are important ecological engineers (Gutiérrez et al. 2003, Borthagaray & Carranza 2007) and research on the effects of endolithic cyanobacteria on the individual energetics of mussels can offer a means to better understanding and predict the effects of endolithic parasites on not only their hosts but also the many other organisms that are dependent on the ecological engineer for survival. By aggregating into dense beds, mussels modify the nature and complexity of the substratum for other benthic organisms (Borthagaray & Carranza 2007). Mussels provide refuge from predators, wave action and thermal stress (Jurgens & Gaylord 2018), they affect the flow of particles in benthic communities and also act as substratum for some benthic organisms (Gutiérrez et al. 2003). Thus, endolithic cyanobacteria can have an indirect effect on the benthic community

composition by affecting mussels and this study will offer some understanding of how endoliths can affect their mussel hosts and thus, indirectly, the communities that depend on them.

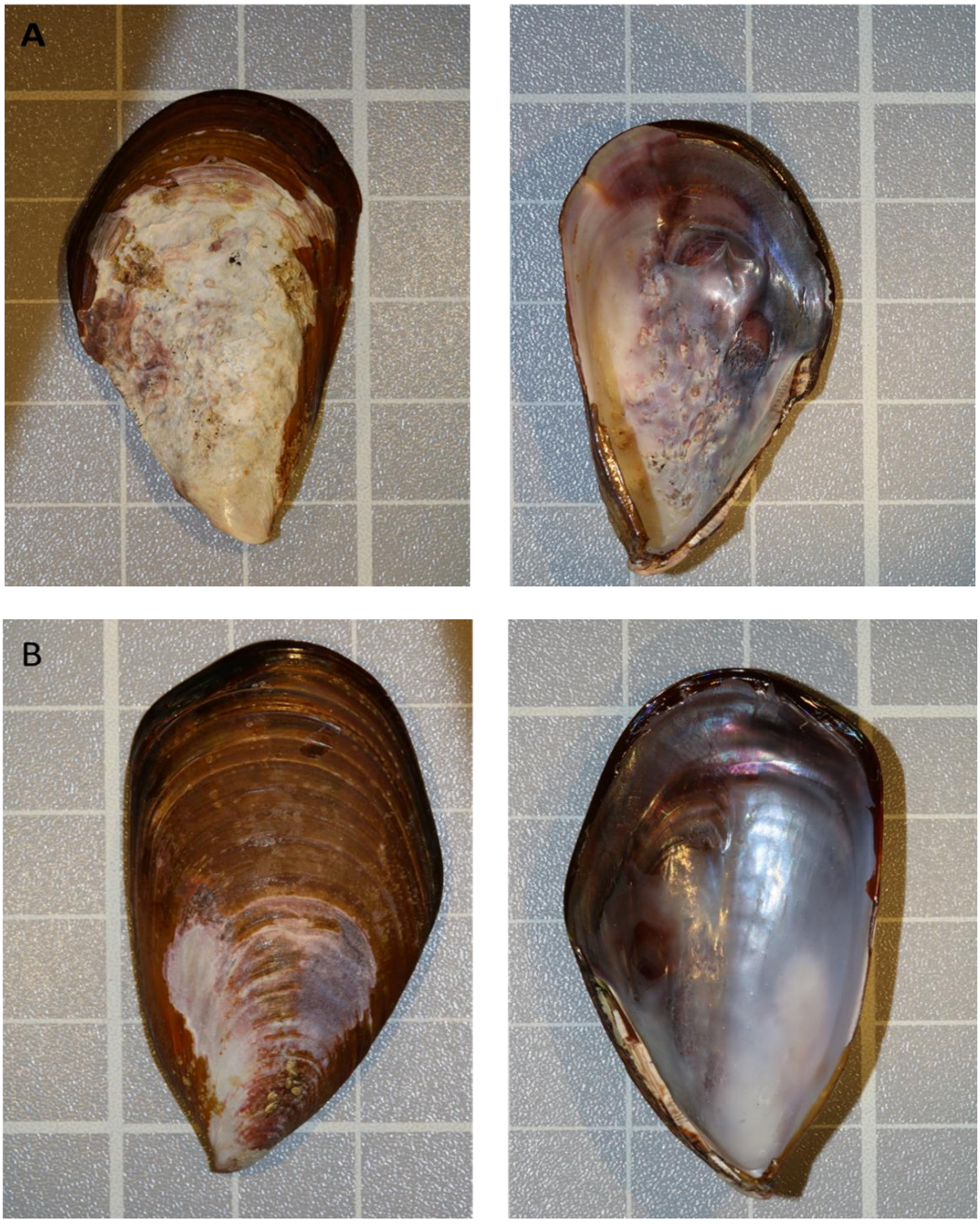

Figure 3.1: A = infested *Perna perna* shell with a rough outside corresponding with the damage inside with a rough light-coloured nacreous layer. B = clean *P. perna* shell with no sign of infestation and the inside showing a shine nacreous layer which is intact.

Aims

In this study, a field experiment was conducted to compare growth and reproduction between clean and infested mussels. In addition, laboratory experiments were used to assess the energy budgets of infested and clean mussels by measuring the components of SFG (standard metabolic rates, clearance rates, ammonium excretion, absorption efficiency), calcification rates and lethal temperatures. SFG was calculated to estimate the energy that is available after assimilation for reproduction and growth. Calcification rates were measured to quantify the amount of calcium carbonate used by mussels to build and maintain their shells. Lethal temperatures were measured to ascertain if infestation has an influence on the ability of mussels to tolerate rising temperatures. The hypotheses were that endolithic cyanobacteria will have an influence on the components of scope for growth, reproduction, growth, shell building and lethal temperatures. Specifically, I addressed the following questions:

1. What are the effects of endolithic cyanobacterial infestation on mussel growth rate and reproductive output?
2. What are the effects of endolithic cyanobacterial infestation on calcium uptake, shell structure (buoyant weight) and scope for growth in mussels?
3. What is the effect of endolithic cyanobacterial infestation on the thermal tolerance of mussels?

Methodology

Balancing energy acquisition and energy expenditure allows the quantification of the remaining energy, i.e. the SFG, or the energy available for reproduction and growth. To achieve this, laboratory experiments were carried out to measure all the components for scope for growth. These laboratory experiments were coupled with field experiments involving the measurement of individual growth, reproduction and buoyant weight for infested and clean mussels. *Perna perna* exists as two genetic lineages that are largely allopatric, but co-exist along approximately 200km of coastline in the study area (Zardi et al. 2015). As these two lineages have been shown to have different physiological adaptations to temperature (Tagliarolo & McQuaid 2015), the individuals used for measuring the components for scope for growth were collected from outside the overlap area of the two *P. perna* lineages on the western side of the overlap region.

Field growth and reproduction

Growth

Growth was measured as the change in shell length of individual mussels over time. The growth experiment was conducted in summer (November 2017 – February 2018) at Kidd's Beach (32º 55' 14.2'' S, 27º 29' 18.0''). For each group (infested and clean) of mussels, 100 individuals of ~35 mm (mean = 36mm, SD = 0.13mm) in shell length were marked in the field using paper tags which were glued (Loctite super glue) on the posterior growing edge of the mussel (McQuaid & Mostert 2010). After a period of 100 days, marked mussels were collected and taken to the laboratory where antero-posterior growth was measured using a pair of callipers with a resolution of 0.01mm. At the end of the experiment, growth was calculated as the difference between the new growing edge of the mussel shell (final length) and where the tag was located on the shell (initial length) and standardised to growth per month.

Calcification measurements

Clean and infested *Perna perna* of ~4cm in length were collected from the mid-mussel zone at Brenton-on-Sea and transported to the laboratory in an insulated cooler box (in air) and the transportation time was less than 4 hours. In the laboratory, mussels were cleaned of epibionts and placed in aerated sea water with those that showed signs of spawning being removed immediately. Mussels were allowed to recover from sampling stress for a period of 5 days under standardised conditions at 20˚C temperature in aerated sea water before they were used for the experiment. Sea water was changed every day to maintain water quality. Mussels were kept in sea water and the conditions were standardised so that conditions such as food availability, salinity, and dissolved oxygen were held constant and measurements could reflect the unconfounded physiological responses underlying the effects of endolithic infestation.

To assess the effect of endolithic infestation on mussel shell production in the field, the buoyant weight of infested and clean mussels after a growth period was measured. Buoyant weight of mussel shells was considered as the weight of a mussel weighed under water. As the specific density of soft tissues is assumed to be equal to that of seawater. This method can effectively measure changes in shell mass alone (Molina et al. 2005). I used a calibrated balance (SARTORIUS, Sanas calibration laboratory) with a resolution of ±0.001 mg fitted with a hook from which mussels were hung while submerged in natural seawater. Buoyant weight was measured, and individuals were marked (n = 88) differently for identification and taken back to the rocky shore for a period of 100 days. Metal quadrats (20cm x 20cm) attached to the rocks using rawl plugs and screws with a canvas mesh (5mm x 5 mm) covering mussels. This setup was used to hold the mussels in place until they attached themselves naturally to the rocks. Consequently, mussels were monitored every two weeks. After 4 weeks, mussels had attached to the rocks and

the metal quadrats and the mesh were removed. After a further period of 100 days (total of 121 days), the mussels were returned to the laboratory and the buoyant weight was re-measured.

Additionally, a laboratory experiment using the alkalinity anomaly technique (Smith & Key 1975) was carried out to measure rates of calcification to see if any differences in shell weight change in the field were due to differences in instantaneous calcification rate. Although this was a lab experiment, it was included in this section so that it can link with the field measurement of calcification. The alkalinity anomaly technique is based on the fact that total alkalinity decreases by 2 equivalents for each mole of $CaCO_3$ precipitated. A total alkalinity mini-titrator and pH meter (Hanas HI 84531 model) were used to measure total alkalinity for a duration of 1 hour (n = 12 for each group). Net calcification rates (G) were calculated using the following equation (Smith & Key 1975, Tagliarolo et al. 2012):

$$G = - (\Delta TA \times v)/ 2 \times (t1-t0)$$

Where ΔTA is the variation of total alkalinity during incubation ($\mu mol\ l^{-1}$), v is net bottle volume (l), and t1-t0 is incubation time (h).

Reproduction

To examine differences in reproductive output between clean and infested mussels, I used empirical relationships between gonad dry weight and shell length (Berry 1978). A once-off collection of *Perna perna* mussels ranging between ≥ 10 mm shell length and the largest individual I could find (65 mm) were collected in the mid-shore region from Brenton-on-Sea (34˚ 04' 31.7" S, 23˚ 01' 29.5" E). I collected 32 individuals of each group (infested and clean) which were preserved in 70% ethanol for later analyses. The reproductive condition f females and males is difficult to distinguish for different size classes, therefore mussels where not sexed. Once in the

laboratory, shell length was measured, each individual was dissected, and the somatic tissues were separated from gonad tissue. The gonad tissue of each individual mussel was dried at 60°C in the oven for 48 hours and weighed using a calibrated balance (Mettler Toledo), with a resolution 0.1mg.

Laboratory experiments

Scope for Growth

Collection of organisms and preparation

All mussels used to quantify energy budget components, infested (heavily infested mussels) and non-infested (clean), were collected in April 2016 from the mid-shore at Brenton-on-Sea, and immediately transported to the laboratory (~ 4 hours) in an insulated cooler box fitted with ice packs and wet paper-towels to maintain low temperature and high humidity. On arrival in the laboratory, mussels were cleaned of any epibionts and placed in 20-L tanks containing aerated, unfiltered natural seawater collected from Port Alfred. Water was replaced daily to maintain quality. Tanks were kept in a controlled environment room maintained at 20˚C and programmed with a light:dark cycle of 12:12 hours. Very few individuals (less than 2%) showed signs of stress-induced spawning and these were removed immediately. Animals were acclimated under these conditions for 7 days and then starved in filtered seawater for 24 hours before the experiments. The same individuals were used for all energy budget component measurements following this sequence: clearance rate, absorption efficiency and standard metabolic rate.

Clearance rate measurements

Prior to the experiments, 10 infested and 10 clean mussels were placed in 0.47µm filtered sea water for 1 hour to allow flushing out of faeces and pseudofaeces. The shell of each mussel was marked with a permanent marker for individual identification. Mussel clearance rates were measured using glass beakers (4500ml, one mussel per beaker) containing filtered seawater (0.47µm), gently stirred by a magnetic stirrer to ensure particles remained in suspension. Mussels were allowed to acclimate for 15 minutes until the valves were open, suggesting that pumping had resumed. As soon as they started pumping, a commercial alga paste (Brightwell Aquatics, Phytogreen-M) was added at an initial concentration of 0.43mg/ml (Widdows and Staff. 2006). Preliminary trials indicated that there was no pseudofaecal formation at this concentration. From each beaker, two 500-ml samples were collected soon (15 minutes) after the algal concentration was added and then at the end of the experiment (after 1.5 hours). Two mussel-free beakers were used as controls. Pre-weighed dry and ashed glass fibre filters (GFC, Merck Millipore, 0.47µm) were used to filter all samples. Dry weight of the filtrate was obtained by drying samples for 24 hours at 60˚C, and ash-free dry weight was determined after burning samples for 4 hours at 450˚C. A calibrated balance (SARTORIUS, Sanas calibration laboratory) with a resolution of ±0.001 mg was used to weigh the samples. Clearance rate (l/h) was calculated by subtracting the initial weight from final weight of the filters and recorded as weight of filtered material in mg (Petersen et al. 2004).

Absorption efficiency

Absorption efficiency (AE) was calculated based on the ratio of organic matter absorbed to organic matter ingested (Conover 1966):

$$AE = (F - E)/[(1 - E) \times F]$$

Where F = ash free dry weight:dry weight ratio of the food, and E = ash free dry weight:dry weight ratio of the faeces.

Faeces were collected 6 hours after the ingestion measurements, using the same individuals. Faeces were drawn from the beaker floor using a 10-ml pipette and concentrated in a 50 ml centrifuge tube and allowed to settle before the overlying water was removed using a pipette. Ammonium formate (0.5 ml) was added to the centrifuge tube and again any overlying liquid was removed using a pipette. This process was repeated twice to remove seawater salts from the faecal samples. To gather enough material from faeces for analysis, measurements from 4 mussels were pooled together, with two groups of 4 mussels used from each treatment. The faecal samples were placed on pre-muffled GFC (glass micro-fibre) filters and dried to constant weight in the oven (60˚C) for 24 hours. After drying in the oven, the samples were weighed and then burnt in the furnace (450˚C) for 4 hours and then weighed again giving one estimate of absorption efficiency (the average of the two duplicates) for each infestation treatment used.

Standard metabolic rate measurements

Prior to measuring standard metabolic rates, mussels were placed in 0.47µm filtered sea water for 24 hours to recover and to ensure that mussels were in post absorptive state. Standard metabolic rate measurements were based on closed system respirometry, which was done in a controlled

environment. Using closed glass chambers (220 ml) with optical oxygen sensors attached to the chamber on the inside, oxygen consumption by individual mussels was measured. This method is non-invasive and allows measurement of oxygen with minimal disturbance to the mussel. In a controlled environment room set to 20˚C, oxygen-saturated filtered seawater was added to each chamber. An individual mussel was then placed inside each chamber, and the chamber was then sealed. A magnetic stirrer was used to mix the water inside the chamber. Mussels were allowed to acclimate for 15 minutes before measurements began. The rate of oxygen concentration in the chamber was recorded every 5 minutes for 70 minutes using an optical sensor (PreSens, Fibox 3) calibrated according to the manufacturer's guidelines. Oxygen consumption measurements were done in the dark to prevent photosynthesis by endolithic cyanobacteria, and two chambers which did not contain mussels were used as controls. Oxygen consumption rate was then calculated following Widdows and Staff (2006) using the equation:

$$\text{Rate of } O_2 \text{ uptake (mg } O_2\ h^{-1}) = [C(t_0) - (C(t_1)] \times (Vr) \times 60(t_1 - t_0)^{-1}$$

Where t_0 and t_1 = start and finish times (min) of the measurement period, C(t) = concentration of oxygen in the water (mg O_2 L^{-1}) at time t, Vr = volume of chamber minus the animal.

At the end of the experiment, the volume of water in the chambers was measured, shell length of each mussel was measured and tissues from the shells were dissected and placed in a pre-weighed aluminium foil container. The tissue was dried at 60°C for 48h, weighed and then burned in a muffle furnace at 450°C for 4h and re-weighed to determine the ash-free dry weight. All oxygen consumption measurements were corrected to standard body size measurements (soft-tissue dry weight) for each mussel using allometric scaling and then converted to energy equivalents (J g^{-1} h^{-1}) where 1mg of O_2 = 14.100 J (Brey 2001).

Ammonium excretion

Ammonium is the major waste product in aquatic molluscs (Bishop et al. 1983, Tagliarolo et al. 2012), and ammonium excreted is energy lost and therefore unavailable as scope for growth (SFG). Clean and infested *Perna perna* mussels (n = 12, size ~4cm) collected from Brenton-on-Sea and kept in a controlled environment for 7 days as above, were used to calculate ammonium excretion. After the 7-day period of acclimation, each individual mussel was placed in a 220 ml chamber filled with filtered sea water and incubated for 70 minutes. Water samples (1000 µl) were collected at the beginning and at the end of the experiment. The production of ammonium was determined using the phenol-hypochlorite method (Rosas et al. 2003). Sample colouration was measured at 630 nm using A Genesys 20 spectrophotometer and milli-Q water was used as a blank with ammonium excretion expressed in µmol NH_4 g $AFDW^{-1}$ h^{-1}.

Calculation of scope for growth

After measuring the energy budget components, the measurements were corrected to a standard body size (soft-tissue dry weight, mg) and then converted to energy equivalents (J g^{-1} h^{-1}) and used to calculate the energy available for growth and reproduction following Widdows and Staff (2006) equation:

$$SFG = A - R$$

Where SFG = Scope for growth, A = Energy absorbed (Energy absorbed = energy consumed; Clearance rate (mg l^{-1}) x food absorption efficiency x energy content of algae, product used to feed mussels = 20.043J g^{-1} based on product specifications. R = Respiration (J g^{-1} h^{-1}) and excretion in terms of ammonium excretion (J g^{-1} h^{-1}).

Lethal Temperature LT_{50} experiments

Mussels for LT_{50} experiments were collected from Brenton-on-Sea and transported to the laboratory as mentioned above. In the laboratory, mussels were cleaned of any epibionts and placed in 20-L tanks containing aerated, unfiltered natural seawater collected from Port Alfred which was replaced daily to maintain quality. Tanks were kept in a controlled environment room maintained at 20˚C. Mussels were acclimated under these conditions for 7 days. Mortality at a range of air temperatures experienced by mussels on the rocky shores (23°C, 26°C, 28.8°C, 31.7°C, 34.8°C, 37.5°C, 38.7°C, 39.8°C and 40.5°C) was examined by simulating a 2-hour low tide period in a chamber which was placed inside a controlled water bath (Grant TXF200-R4). Groups of 10 infested and 10 clean mussels were subjected to each temperature treatment at the same time. For the first hour, temperatures were raised gradually from an initial temperature of 20°C to the treatment temperature and maintained at that temperature for another hour. Mussels were then removed from the controlled environmental chamber and placed in aerated sea water for 24 hours to recover in a controlled environment at 20˚C. At the end of the 24-hour period, dead mussels were counted with dead mussels gapping even when water was removed. Data were expressed as the number of mussels that were dead at each temperature and the temperature required to kill 50% of the mussels was estimated based on logistic regression models using generalized linear models in R (R Core Team 2017). LT_{50} values and 95% confidence intervals for each treatment were estimated and the difference between treatments was then determined based on the overlap between the confidence intervals.

Data analysis

For growth, a One-way Analysis of variance (ANOVA) with infestation as a fixed factor (two levels) was used to compare infested and clean mussels. The effects of infestation on the individual components of SFG were tested separately from overall SFG. All these tests were run using a One-

way ANOVA to compare infested and clean mussels. SFG for each individual was calculated and a One-way ANOVA was used to compare clean and infested. Reproductive output data were analysed using log10-transformed data to meet the assumptions of a parametric test. A One-way Analysis of Covariance (ANCOVA) was conducted on the reproduction data with infestation as a fixed factor (two levels) and shell length as a co-variate. All statistical tests were done using Statistica 13 (Statsoft).

Results

Field growth and reproduction

Growth

Clean mussels exhibited significantly higher growth rates than mussels that were infested by endolithic cyanobacteria (One-way ANOVA: $F_{(1, 88)} = 15.003$, $p = 0.001$, Fig. 3.2). This was evident, with clean mussels growing faster than infested mussels.

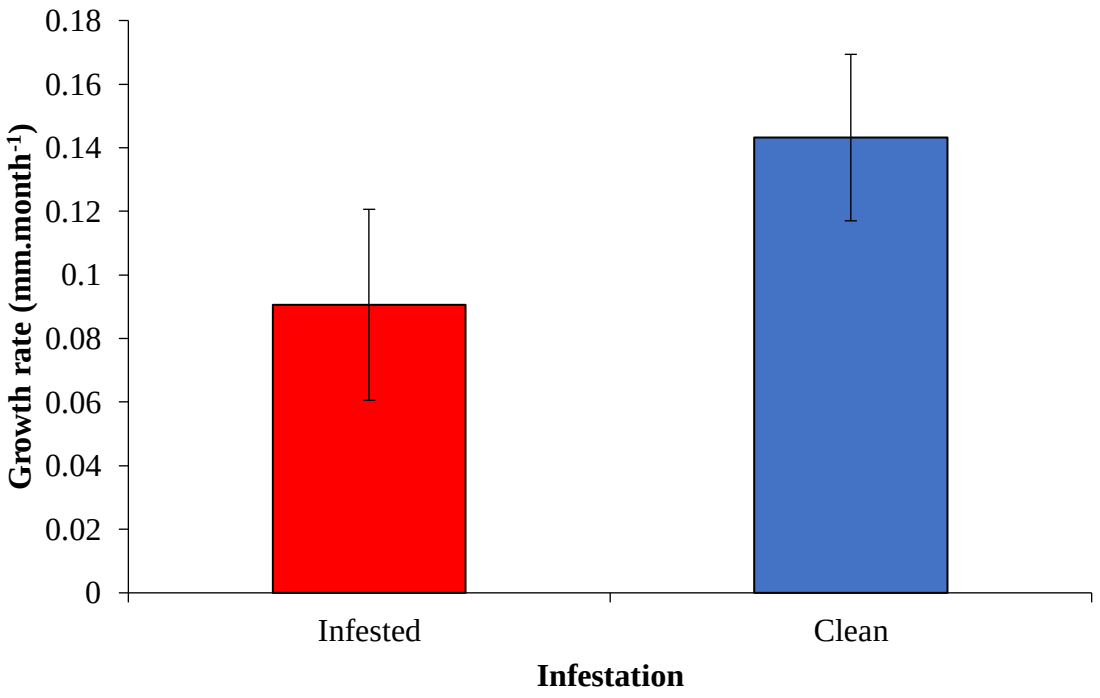

Figure 3.2: Mean (±SD) growth rate (mm/month) of infested and clean *Perna perna* mussels measured in situ (n = 45 for each group).

Calcification: Field experiment

There was a significant difference in buoyant weight change between clean and infested mussels (One-way ANOVA: $F_{(1, 86)} = 44.881$, $p < 0.001$; Fig. 3.3). Clean mussels showed more rapid increases in shell weight than infested mussels.

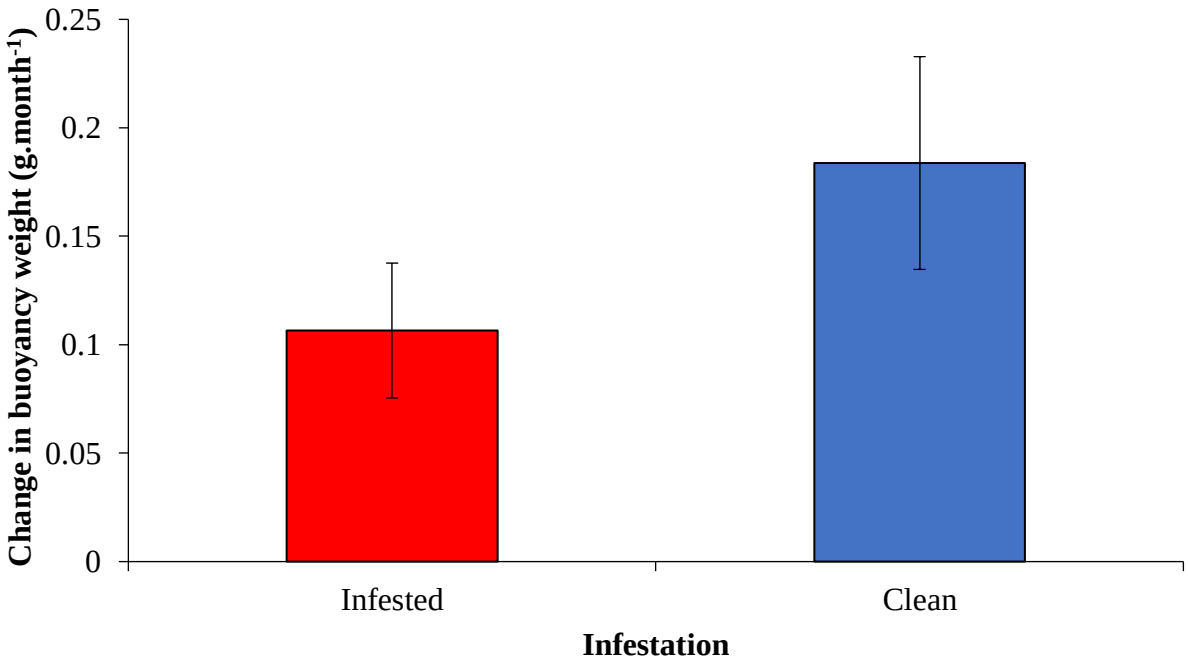

Figure 3.3: Mean buoyant weight for infested and clean mussels (n = 44 for each group). Values are means ±SD.

Calcification ($CaCO_3$ absorption): Laboratory experiment

The rate at which $CaCO_3$ was absorbed by mussels did not differ significantly between the two groups (One-way ANOVA: $F_{(1, 22)} = 1.483$, $p > 0.05$, Fig. 3.4).

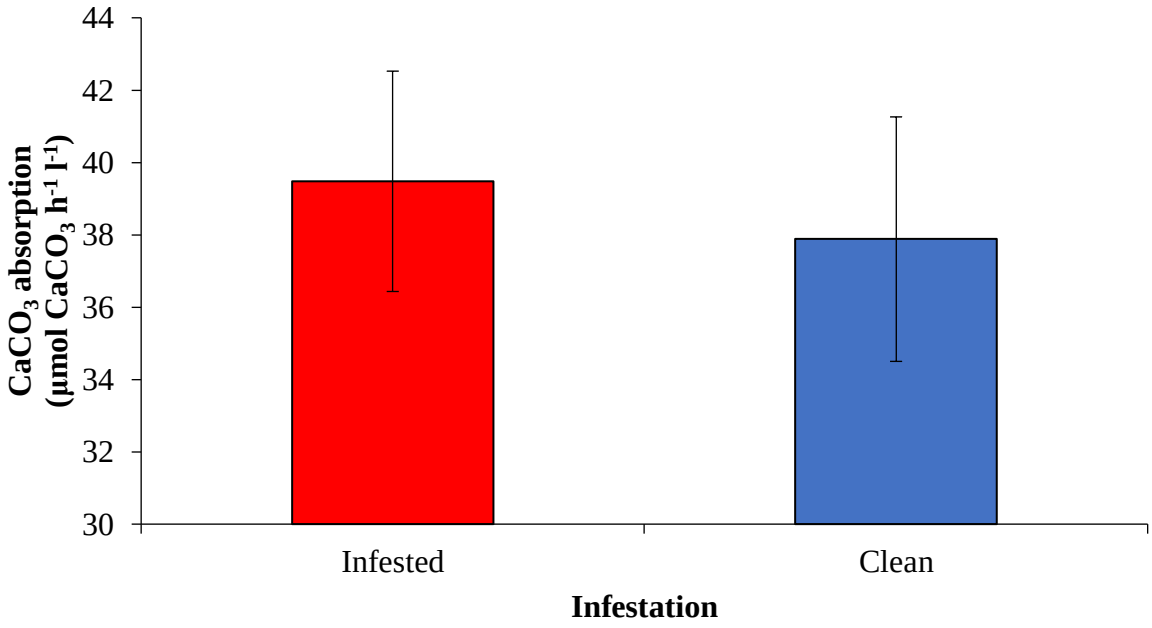

Figure 3.4: Mean calcification rates for infested and clean mussels (n = 12 for each group). Values are means ±SD.

Reproduction

There was a strong positive correlation between gonad mass and shell length for both infested (r = 0.733, n = 32, p = 0.001) and clean mussels (r = 0.868, n = 32, p 0.001). Gonad dry mass was lower in infested than clean mussels (Fig. 3.5).

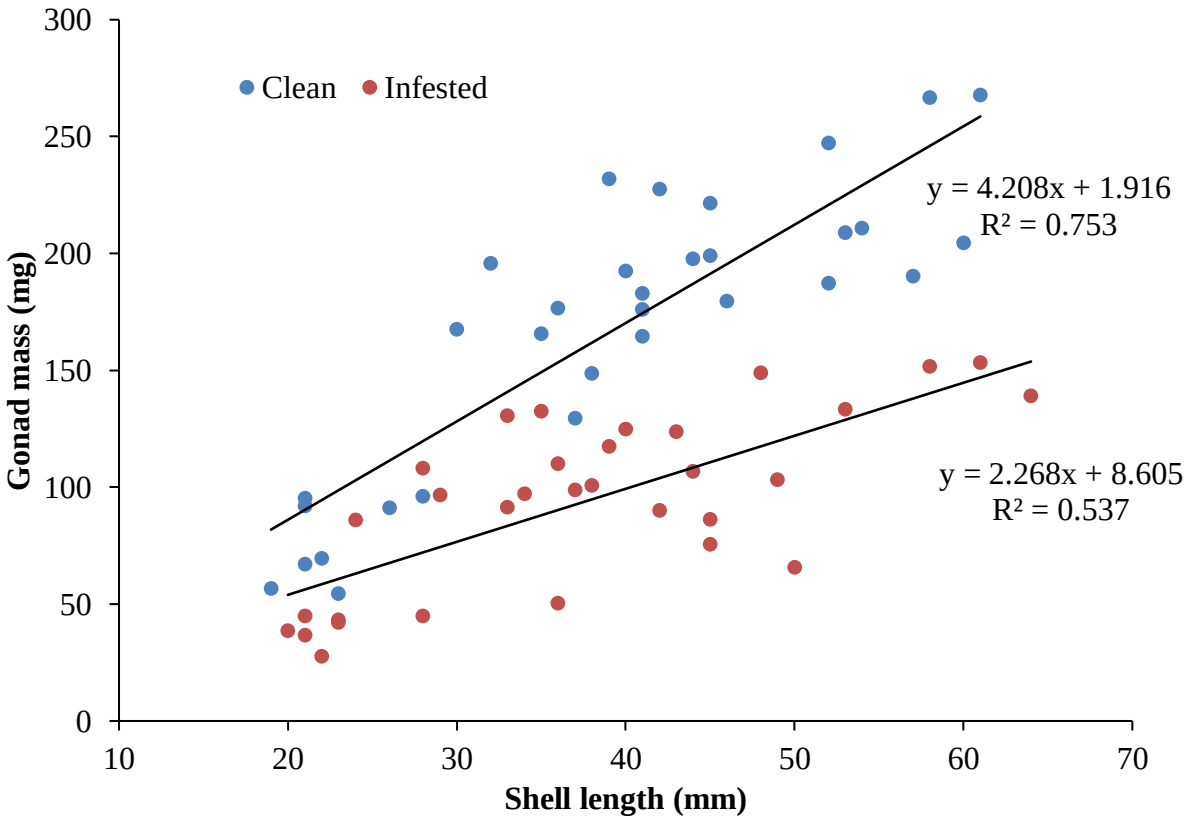

Figure 3.5: Linear regression between shell length and gonad dry mass of *P. perna*. Red = infested and Blue = clean mussels.

Data were tested for normality (Shapiro Wilk) and failed, then data were log10-transformed after which the normality test was passed ($p > 0.05$). One-way ANCOVA of transformed data showed that both infestation ($p < 0.05$, Table 3.1) and shell length ($F_{(1,61)} = 138.862$, $p < 0.05$) had significant effects on gonad mass, but there was no interaction between infestation and shell length ($p > 0.05$).

Table 3.1: Summary statistics of One-way ANCOVA to assess the influence of infestation and shell length on gonad mass. Bolded p values signify a statistically significant difference between groups at $p < 0.05$.

Effect	SS	D.F	MS	F	p
Intercept	241.029	1	241.029	141.874	**0.001**
Shell length	235.911	1	235.911	138.862	**0.001**
Infestation	140.165	1	140.165	82.504	**0.001**
Error	103.633	61	1.699		

Laboratory measurements

Scope for Growth

Energy absorbed

There was no significant difference in clearance rates between infested and clean mussels (One-way ANOVA: $F_{(1, 18)} = 1.832$, $p > 0.05$, Fig. 3.6), suggesting that mussels from both treatments cleared the same amount of water during the experiment. There was only a minor, non-significant (ANOVA: $P > 0.05$, $n = 10$) difference in absorption efficiency between the two groups: infested = 0.54 and clean = 0.52.

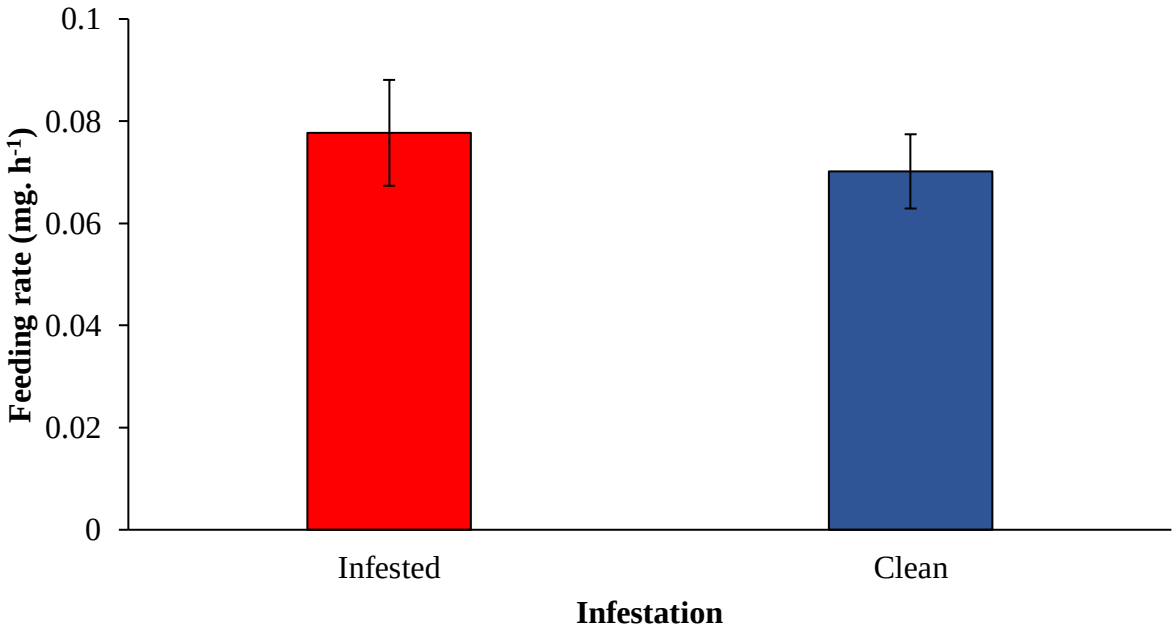

Figure 3.6: Mean clearance rates for infested and clean mussels, vertical bars represent ±SD, n = 10 for each group.

Standard metabolic rate

Standard metabolic rate was higher for infested than clean mussels (One-way ANOVA: $F_{(1, 18)}$ = 218.154, p = 0.001, Fig. 3.7) with higher respiratory rates for infested than clean mussels. Infested mussels exhibited average maintenance costs of 6 J g^{-1} h^{-1} compared to an average of 4.1 J g^{-1} h^{-1} for clean mussels.

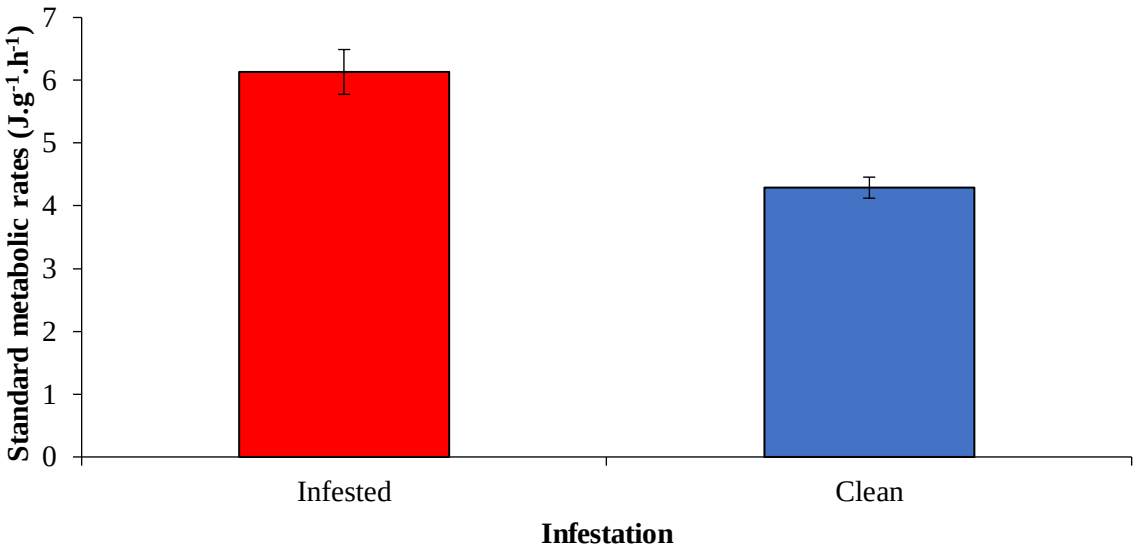

Figure 3.7: Mean (±SD) metabolic rates for infested and clean mussels, n = 10 for each group.

Ammonium excretion

The amount of ammonium excreted by infested and clean mussels was not significantly different (One-way ANOVA: $F_{(1, 22)} = 0.191$, $p > 0.05$, Fig. 3.8). Clean mussels and infested mussels excreted similar amounts of ammonium.

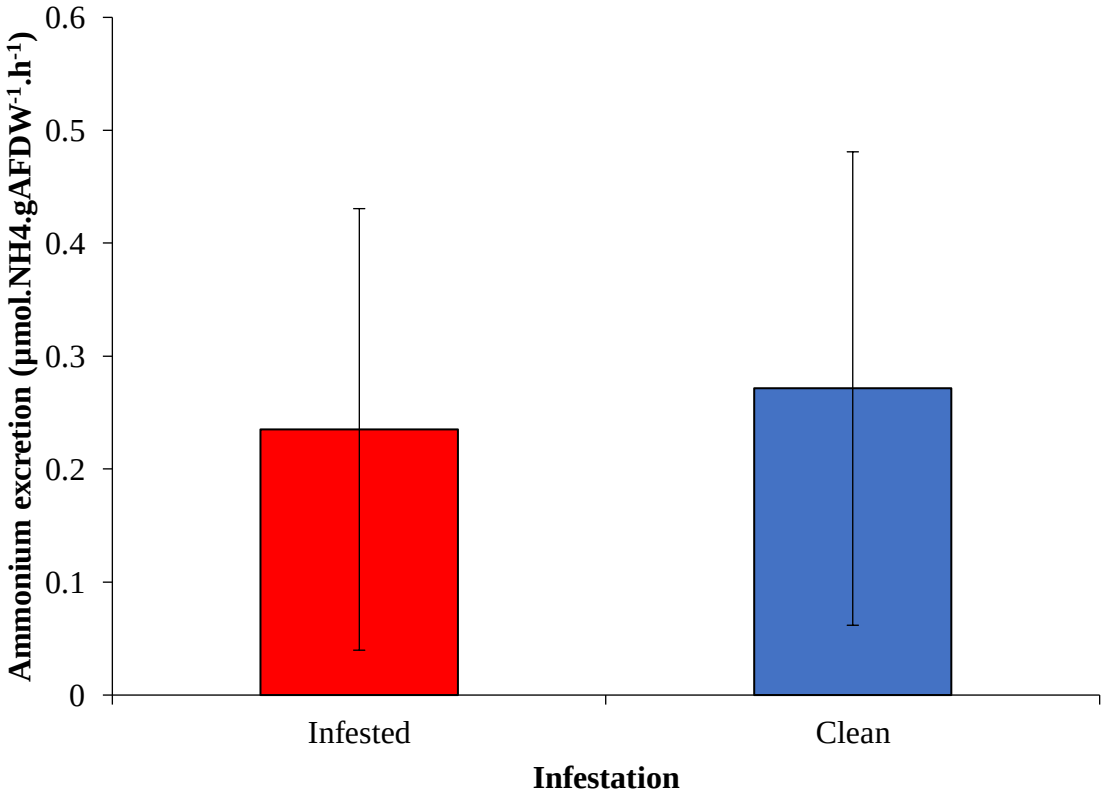

Figure 3.8: Mean ammonium excretion for infested and clean mussels, vertical bars represent ±SD (N = 12 for each treatment).

Scope for Growth

There was a significant difference in scope for growth between infested vs clean mussels (One-way ANOVA: $F_{(1, 18)} = 19.055$, $p = 0.001$, Fig. 3.9), with more energy available for growth and/or reproduction in clean mussels.

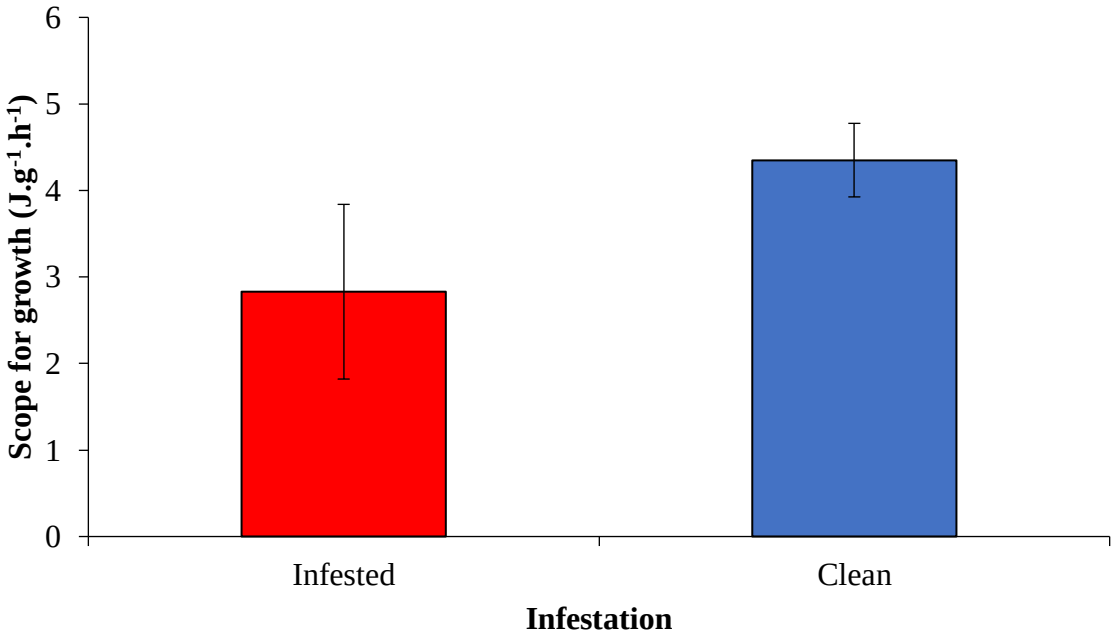

Figure 3.9: Mean scope for growth of infested and clean mussels. Values are means ± SD, n = 10 for each group.

Lethal temperatures

The LT_{50} estimates for clean and infested mussels were similar, at 38.23˚C and 38.08˚C respectively (Fig. 3.10). An overlap of the 95% confidence intervals between the two groups' LT_{50} values indicates no significant difference.

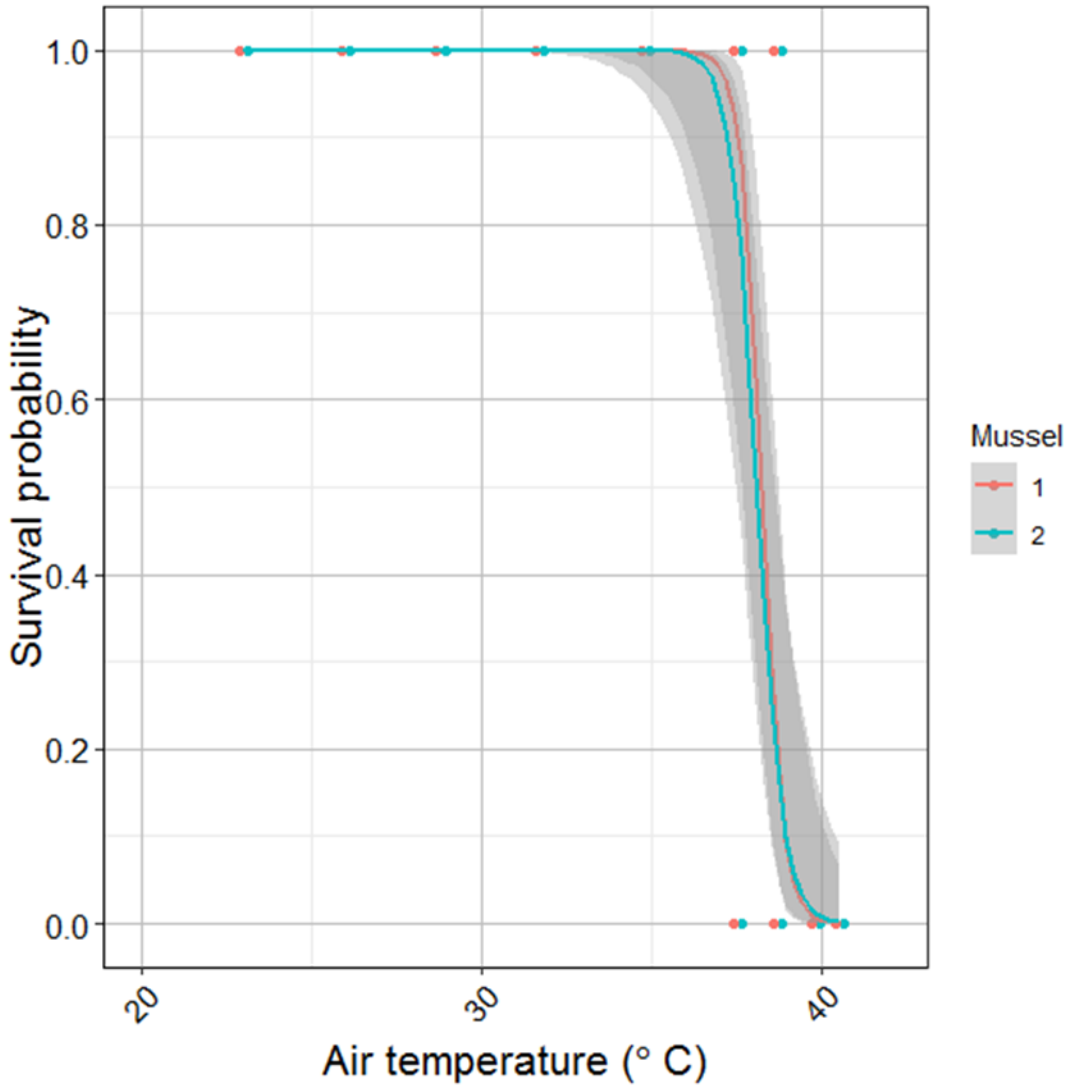

Figure 3.10: Survival probability as a function of air temperature for *Perna perna*. Logistic regression fits are provided for clean (blue) and infested (red) mussels, along with 95% confidence intervals (grey shaded areas), n = 10 for each treatment in each temperature range. Points are displaced slightly on the x-axis.

Discussion

This chapter investigated whether endolithic cyanobacteria have a negative influence on scope for growth in intertidal mussels and hence reduce the energy remaining for reproduction and growth. This chapter also looked at how mussels that have reduced energy due to endolithic cyanobacteria are able to cope with high temperatures by measuring lethal temperatures. The hypothesis of this study was that endolithic cyanobacteria have a negative influence on the components of scope for growth (Clearance rates, Absorption efficiency, Standard metabolic rates) that would be detectable through an influence on reproduction, growth and lethal temperatures (LT_{50}) in mussels because they divert energy for shell repair. The results show that endolithic cyanobacteria have a negative effect on scope for growth, growth rate, shell building and reproduction. Similarly, many studies have looked at how other factors such as temperature can affect metabolic rates in intertidal mussels (Sokolova et al. 2012) and how endolithic cyanobacteria negatively affect growth rates in mussels (Kaehler & McQuaid 1999). Some studies have also used mussels to measure scope for growth (Widdows et al. 1995, Widdows & Staff 2006, Fly & Hilbish 2013), calcification (Gazeau et al. 2007, Stumpp et al. 2011), reproduction and lethal temperatures (Helmuth & Hofmann 2001). In the current study, effects of endolithic cyanobacteria were assessed. In line with my results, earlier studies (Kaehler & McQuaid 1999, Marquet et al. 2013) have similarly demonstrated that endolithic cyanobacteria have negative effects on mussel shells by reducing SFG.

Scope for growth

Infestation in organisms affects the host by increasing the maintenance cost. For example the brown ring disease in Manila clam (*Venerupis philippinarum*) doubles maintenance costs during an infection compared to clean individuals (Flye-Sainte-Marie et al. 2009). Previous studies have measured scope for growth in mussels (Widdows et al. 1995, Widdows & Staff 2006, Fly & Hilbish 2013) and the boring activity of endolithic cyanobacteria has been shown to take up

calcium carbonate from mussel shells possibly leading to reduced SFG in mussels (Garcia-Pichel et al. 2010). In the current study, infested mussels had low SFG compared to clean mussels indicating less energy available for growth and reproduction (Fly & Hilbish 2013). Even though there was no negative SFG in this study, infested mussels had less energy left as SFG compared to clean mussels and having reduced energy available for growth and reproduction is risky for intertidal organisms as they inhabit a stressful habitat.

Marked differences were observed in standard metabolic rate measurements, a component of scope for growth, explaining the significant differences in scope for growth (SFG) between infested and clean mussels. The results suggest that endolithic cyanobacteria do influence metabolism in mussels with infested mussels losing more energy compared to clean mussels. The components that resulted in the differences in scope for growth involved processes that have to do with energy expenditure or energy loss, standard metabolic rate measurements. This shows that infested mussels lose more energy compared to clean mussels. There was no difference in clearance rates, assimilation efficiency or ammonium excretion, with the only significant difference observed in standard metabolic rates. Ammonium excretion which is also a process involving losing energy showed no significant difference between infested and clean mussels. This shows that not all components of scope for growth that involve energy loss contributed to low scope for growth in infested mussels. However, clearance rate measurements which is a component of scope for growth that involves energy acquisition showed no differences between infested and clean mussels. This shows that the amount of food taken in by mussels, energy consumed by mussels is not influenced by the presence of endolithic cyanobacteria. The amount of food consumed does not depend on the status of the mussel in terms of being infested or clean.

Some environmental stressors have been shown to lead to a compensatory response where feeding rates increase to maintain basal energy needs, this was observed when temperature increases resulted in increases in respiration, clearance rates and ammonium excretion in the ribbed mussel *Geukensia demissa* (Wilbur & Hilbish 1989). In the current study, endolithic cyanobacteria infestation did not lead to an increase in the amount of food consumed but there were significant differences in standard metabolic rates. The difference in scope for growth was due to more energy lost through increased metabolic rates in infested mussels and this might be due to the fact that mussels are prioritizing energy allocation to meet basal energy demands such as repair and maintenance of shells over growth and reproduction.

Growth and reproduction

In line with the results for SFG, when measured empirically, both growth rates and reproduction were significantly reduced in infested mussels. Infested mussels had lower growth rates than clean mussels (Fig. 3.1). The results also showed that endolithic cyanobacterial infestation has a strong influence on gonad mass, with infested mussels having less gonad mass than uninfected mussels of the same size (Fig. 3.2). The observed reduction in actual measured growth and reproduction corroborates the results of SFG which indicated there was less energy left as SFG for infested mussels compared to clean mussels. This is supported by the field growth and reproduction measurements which showed that infested mussels had lower growth rates and lower gonad mass compared to clean mussels. These results support those of Kaehler & McQuaid (1999), who also showed that gonad dry mass of infested mussels was substantially lower than that of clean *Perna perna* mussels and this difference was entirely due to endolithic cyanobacteria infestation. The results from the current study suggest that endolithic cyanobacteria affect reproduction by reducing the mass of gonad tissue produced in mussels hence reducing their fitness directly.

However, the results from this study which focussed on growth rate, contradicted results by (Kaehler & McQuaid 1999) who used *in situ* shell markings of *P. perna* and found that infested and clean mussels showed similar growth rates and differed only in shell thickness and rates of shell repair which were faster in infested shells than in clean shells (Kaehler & McQuaid 1999). The different results from these two studies might be due to the differences in sample sizes, with the current study having a larger sample size and a longer experimental period compared to n = 36 for each group and 30 days experimental period in the experiment by Kaehler & McQuaid (1999). Since growth rates of organisms are good indicators of stress (Widdows & Staff 2006), the low growth rates of infested mussels is a sign that endolithic cyanobacteria in mussel shells are stressors to their host. Increased maintenance costs due to endolithic cyanobacteria eroding mussel shells could also lead to energy trade-offs in individual mussels where mussels try to maintain basal energy needs and reduce energy channelled towards growth and reproduction. Mussels have been shown to have energy trade- offs whereby they invest more energy into survival than to reproduction (Petes et al. 2008). The current study results show that infested mussels have low growth rates and low gonad mass compared to clean mussels, this presumably represents such a trade-off, with more energy channelled to meet basal energy demands for survival and probably for shell repair than for growth and reproduction.

Calcification

Field experiments of calcification showed that infested mussels are significantly different to clean mussels. Infested mussels had a lower buoyant weight change compared to clean mussels, which might have been due to the fact that the same amount of energy is channelled to shell building, but endolithic cyanobacteria erode shells of infested mussels thus leading to low buoyant weight. This is likely because infested mussels channel less energy to shell building as they use more energy in

other physiological processes (e.g., standard metabolic rates), hence there is less energy for shell building.

Laboratory experiments of calcification when measuring the rate of $CaCO_3$ absorption showed that there was no difference between the two groups; infested and clean mussels. This shows that mussels, either infested or not, absorb $CaCO_3$ at a same rate but if they are infested, they are faced with the risk of losing calcium which is continuously being mined by endoliths; hence resulting in reduced buoyant weight. Field experiments suggest that buoyant weight is lower for infested mussels and there is no compensation for this through increased $CaCO_3$ uptake supported by the laboratory calcification results which show no significant differences in $CaCO_3$ uptake between infested and clean mussels.

Previous research has focussed on how changes in pH and carbon dioxide had affected calcifying marine organisms (Gazeau et al. 2007, Melzner et al. 2011, Rodolfo-Metalpa et al. 2011, Stumpp et al. 2011). It has been demonstrated that factors that lead to changes in pH such as ocean acidification will alter calcification in calcifying organisms such as in the case of *Mytilus edulis* (Gazeau et al. 2007). With increasing atmospheric carbon dioxide concentrations expected, ocean surface pH levels are expected to lower which is further expected to decrease calcification in marine organisms leading to reduction in skeletal growth in corals and molluscs (Rodolfo-Metalpa et al. 2011). With increase in atmospheric carbon dioxide and ocean acidification affecting molluscs, the effect of endolithic cyanobacteria together with the effects caused by ocean acidification, they will act together to affect shell building in mussels. This study has shown that endolithic infested mussels experience low calcification compared to clean mussels in field experiments, despite similar rates of calcium uptake, which will be another big factor negatively

affecting mussel shell building at the same time as other factors brought in by the effects of climate change.

Lethal temperatures

Lethal temperatures (LT_{50}) did not differ between infested and clean mussels, suggesting that despite increasing maintenance costs, endolithic cyanobacteria do not limit the ability of mussels to cope with thermal stress. The prediction was that infested mussels would have lower lethal temperatures, and this might be a big concern for mussels as they occur in intertidal communities where temperatures change rapidly and can even reach very critical limits (Fields et al. 1993). As sea and air temperatures continue rising due to climate change (Fields et al. 1993, Lathlean et al. 2011), it is important to know if endolithic cyanobacteria are not indirectly reducing the ability of mussels to cope with heat stress. Endolithic cyanobacteria stress mussels and increase their maintenance costs which would leave less energy available to build an energetically expensive heat shock response and such mussels might be expected to exhibit a lower lethal temperature.

Several studies have focused on physiological stress posed by heat that affects mussels in intertidal communities (Helmuth & Hofmann 2001, Tomanek 2002, Schneider 2014, Nishizaki et al. 2015), and thermal stress has been considered as one of the factors that determine the distribution of organisms in intertidal communities (Helmuth & Hofmann 2001). This study showed that there were no significant differences in lethal temperatures as a result of endolithic infestation. This might be due to the fact that both infested and clean mussels have the ability to activate heat shock proteins in their bodies, which triggers a process of heat-shock response to combat the effect of increasing temperatures as mussels are known to possess heat-shock proteins (Buckley et al. 2001, Hochachka & Somero 2002, Somero 2002, Schneider 2014). It has also been shown that endoliths reduce heat stress in mussels, the boring endolithic activities in mussel shells reveal the light grey

colouring which reflects heat hence reducing heat absorbed by mussels thus endolithic infestation is a benefit in combating heat stress (Zardi et al. 2016).

This chapter shows that endolithic cyanobacterial species have direct sublethal effects on mussels. Endolithic infestation reduces scope for growth hence reduced growth rates and reproduction in mussels. These results show that endolithic infestation reduces fitness in mussels, and this can lead to not only negative effects on mussels, but potential impacts on other organisms, especially benthic fauna that are dependent on the ecological engineering activities of mussels. Although these results are generally applicable, they are based on two mussel species collected from the coast of South Africa. Similar studies in other parts of the world may yield different results. Therefore, there may be a need to investigate the effects of endoliths on mussels across a variety of climatic conditions around the world.

Chapter 4 : Effects of endolithic cyanobacteria on the reproductive output and attachment strength of *Mytilus galloprovincialis* and *Perna perna*.

Introduction

Ecological engineers are important to communities and studying factors that influence them helps provide insight into how other species might be affected in the community at large. Intertidal mussels are important ecological engineers, they cover rock surfaces thus influencing biodiversity in these communities (Seed & Suchanek 1992; Zardi et al. 2007). Mussels modify habitat, making it more suitable for themselves and for other organisms(Jones et al. 1994, Karatayev et al. 2002, Borthagaray & Carranza 2007, Arribas et al. 2014). They aggregate to form mussel beds which contribute positively to other fauna by moderating temperature, providing refuge and habitat to associated fauna (Suchanek 1985, Seed & Suchanek 1992). As filter feeders, mussels are responsible for recycling nutrients in the water column, they are responsible for increasing sedimentation and they are also important biological indicators for environmental stress (Karatayev et al. 2002; Borthagaray and Carranza 2006).

Any direct or indirect process that affect mussels will have an indirect effect on the epifauna that is dependent on mussels and their engineering ability. The current study focuses on the influence of endolithic cyanobacteria on mussels as ecological engineers in intertidal communities. The study assesses how endolithic cyanobacteria affect mussels by looking at how infestation affects mussel attachment strength to the substratum and how infestation can influence the reproductive output of mussels by looking at gonad density and gonadosomatic index.

Mussels in intertidal communities require energy to produce byssal threads to attach themselves to the substratum and still have energy to grow and reproduce. Wave action in intertidal communities is one of the main factors affecting mussel survival (Seed & Suchanek 1992; Zardi et al. 2007). Because they inhabit wave-swept environments, intertidal mussels need to be able to withstand the forces exerted by waves and achieve this by producing byssal threads for attachment to the substratum. Byssal threads are silky fibres made of proteins that are produced by mussels to use as holdfasts to the substratum (Carrington & Gosline 2004). Mussels need to attach firmly to the substratum or risk being swept away by waves which are constantly washing these communities.

Byssus production in mussels is costly, it is estimated that mussels use about 8–15% of their energy expenditure in byssus production (Zardi et al. 2007). Mussels produce varying numbers of byssal threads of different thickness to help attach to the substratum depending on the season, salinity, size of mussel and the hydrodynamic stress experienced at that particular time (Young 1985, Seed & Suchanek 1992, Blattenbauer et al. 2012). Endolithic cyanobacteria living as parasites in mussel shells alter energy use and could reduce the energy left for mussels to produce adequate byssal threads to survive in the intertidal communities or to reproduce. Mussels not only need to have strong byssal threads, they need constantly to replace individual byssal threads as these have a relatively short life span.

Reproductive investment is important for adult mussels. Stressed individual mussels might show low ecological fitness levels and have constrained survival and reproduction capabilities (Petes et al. 2008). Individual adult mussels need to have high levels of good health so that they can be able to produce viable gametes. Reproduction is a costly process that requires energy investment, and it has been shown that when organisms are stressed, channelling of energy to reproduction might

be altered with more energy being channelled to physiological stress responses leading to reduced reproduction (Helmuth & Hofmann 2001, Petes et al. 2008).

A number of studies have shown that environmental stressors such as temperature and food quality negatively affect reproduction in mussels (Wacker & Von Elert 2003, Petes et al. 2007, 2008). Other factors such as wave action in intertidal communities have been shown to affect reproductive output in mussels (e.g. Zardi et al. 2007). Endolithic cyanobacterial infestation has been shown to reduce scope for growth which is energy available for growth (chapter 3). This study focussed on assessing if infestation of mussels by endoliths will ultimately lead to low reproductive output. Eggs produced by mussels can be assessed in two ways, a qualitative assessment which considers the size and maturity of eggs produced or a quantitative assessment which considers egg composition and the number of eggs produced (Wacker & von Elert 2003).

Mussels are exposed during low tide and during this time they do not eat, so any additional energy cost could be critical to their ability to maintain themselves. With parasitic endolithic cyanobacteria eroding mussel shells, there will be a trade-off with energy which can be used for growth and reproduction being channelled to shell repair and attachment to substratum to ensure survivorship of individual mussels (Zardi et al. 2007, 2009) and this may result in mussels having little energy for reproduction or byssal threads for attachment.

Purpose of this study

Many studies in the past have focused on tissue growth and reproduction in bivalves (Widdows et al. 1995, Lauzon-Guay et al. 2005, Widdows & Staff 2006, Petes et al. 2008, Fearman et al. 2009). In this study the focus was on how endolithic cyanobacterial infestation affects the reproductive output and attachment strength of molluscs. Gametogenesis, which is the process of gamete formation, can be affected by many factors such as food availability and temperature but for this study, the focus was on how endolithic cyanobacteria affects reproductive output in mussels. The purpose of this study was to investigate if endolithic cyanobacteria have an effect on the reproductive output (gonad production) and attachment to the substratum of mussels. Reproductive output was assessed using histological techniques to measure the density of eggs per unit area and the gonadosomatic index of female mussels. This was done to test the hypotheses that endolithic cyanobacteria will have a negative influence on reproduction and attachment strength in mussels. This was addressed by answering the following questions:

1. Does infestation have a negative effect on the reproductive output of individual mussels?
2. Does endolithic infestation have a negative effect on the strength of mussel attachment to the substratum?

Methods

Reproductive output

Reproduction was measured using two different methods: histology method which involved calculating density of fully developed female mussel eggs (eggs per unit area) and calculating the gonadosomatic index of female mussel eggs as described below. At the size range (3.5 – 4.5 cm) used for this experiment, it was possible differentiate between males and females using the colour of the gonads, therefore female mussels were used.

Histology

Heavily infested female mussels of 3.5 – 4.5 cm in shell length (Level E as described in chapter 2, n = 10) and clean (Level A as described in chapter 2, n = 10) mussels were collected from Brenton-on-Sea. They were collected every two months for the first 6 months of the experimental period in 2017 (August, October and December) and for every month of the last part of the experimental period in 2018 (January-June) to ensure that the peak period of reproduction was sampled as observed in previous studies) with the peak periods observed in April and January for *Mytilus galloprovincialis* and *Perna perna* respectively (Zardi et al. 2007). Mussels were collected in the mid-mussel zone for both species and stored in 70% ethanol and transported to the laboratory. In the laboratory, the reproductive status of mussels was determined by taking a small portion of gonad (~0.1 g) tissue cut from the middle region of the mantle lobe and fixed in Bouin's solution for histological examination. The remaining gonad tissue and the rest of the body were dried at 60°C for 2 days. The fixed gonad tissue was dehydrated in ascending concentrations of alcohol, washed in xylene and then embedded in paraplast. Since the reproductive tissues of both mussel species (*P. perna* and *M. galloprovincialis*) are homogeneously distributed around the mantle (Lowe et al. 1982, Ndzipa 2002), only a section (6 μm) was cut from each sample and stained with haematoxylin and eosin. A representative section through the gonad tissue was used to determine

the reproductive status of each individual using a light microscope fitted with a camera. Photos of the reproductive tissues (female gonad) were taken using a light microscope (Olympus BX50) with a camera (Olympus SC30) installed in the microscope, with a scale bar inserted for each photograph. The density of eggs was calculated by counting the number of eggs per unit area in each photograph and it is referred to as gonad density in this chapter.

Gonadosomatic index (GSI)

Mussels were collected at Brenton-on-Sea and brought to the laboratory following the same methods used for histology. In the laboratory, each individual female mussel was dissected, and the gonads were separated from the body tissue. Gonads and the body tissues were dried in an oven at 60̊ C for 48 hours. To measure the reproductive condition of mussels, the gonadosomatic index (GSI) was estimated. The GSI was calculated by multiplying the mantle gamete fraction by the dry weight of gonads and then dividing that total by the dry body weight. The mantle gamete fraction was calculated by assigning a grid of 64 points on a photo of gonads taken following the histology methods described above. The gamete fraction was calculated as the number of points that fell on developing or ripe eggs divided by the total number of points. The GSI was then calculated by multiplying the mantle gamete fraction by the dry weight of gonads, tissue that was reproductive, and then dividing that total by the dry body weight, including the mantle (Roff 1993, Zardi et al. 2007).

GSI = (mantle gamete fraction x weight of gonads)/dry body weight

To compare clean and infested mussels for both species, I compared GSI for infested and clean mussels for the whole duration of the study period. I also compared GSI when mussels had their

peak reproduction periods, which was January 2018 for both species, in order to compare maximum reproductive output rather than fluctuations of gonad tissue development.

Attachment strength

Five levels of infestation as described in chapter 2 (n = 20 for each level of infestation) in both mussel species *Perna perna* and *Mytilus galloprovincialis* were tested *in situ* for attachment strength. A hand used drill was used to produce a 2mm hole near the posterior margin of the shell and a fishhook was inserted into the hole. The fishhook was connected with a fishing line to a spring scale (Chatillon-N.Y.-U.S.A.-MODELIN- 25). The scale was gradually pulled normal to the surface of the rock until dislodgement occurred. The spring scale recorded kilograms, to convert kilograms to Force (N), a conversion of 1kg = 9.81 N was used. Tested individuals were separated by at least 20cm to avoid any dislodgement effects from adjacent mussels (Nicastro et al. 2010).

Statistical Analysis

All data fulfilled the pre-requisites for parametric analysis (Shapiro Wilk's test, $p > 0.05$). To compare density of mussel eggs and GSI between clean and infested mussels, data were analysed using a Two-way ANOVA with two factors, status (2 levels infested or clean) as a fixed factor and month (9 levels) as a random factor. To compare attachment strength for different levels of infestation, a Two-way ANOVA with species (2 levels, *Mytilus galloprovincialis* and *Perna perna*), infestation (5 levels A-E) and the interaction of species and infestation as fixed factors was used to analyse the data.

Results

Reproductive output

Histology (*Perna perna*)

There was an increase in gonad density starting from the month of August through to January when the highest densities were recorded (Fig 4.1). From the month of February gonad density fluctuated through to June. A density of more than 30 eggs per unit was recorded in all months throughout the study period.

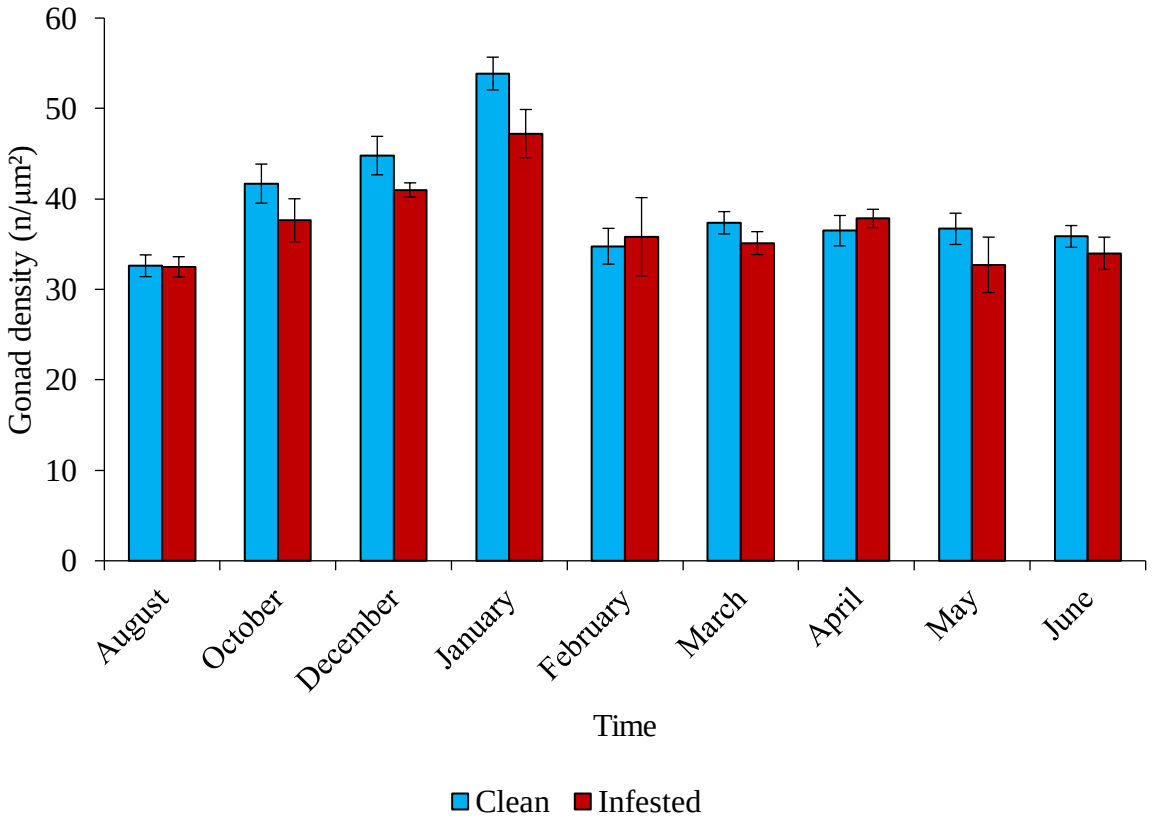

Figure 4.1: Gonad density for *Perna perna* mussels for clean and infested mussels (values are mean ±SD) from August 2017 – June 2018.

Gonad density varied among months (ANOVA: $F_{8,162}$ = 18.44; $p < 0.05$; Table 4.1), and a significant effect of status (ANOVA: $F_{1,162}$ = 6.972; $p < 0.05$; Table 4.1) with clean mussels having higher densities compared to infested mussels. There was a significant interaction of month x status (ANOVA: $F_{8,162}$ = 8.616; $p < 0.05$; Table 4.1) with January and December having significantly higher densities for clean mussels compared to infested mussels (Post hoc, TUKEY HSD; $p < 0.05$), these were the only months with significant differences, and these were the peak reproductive months.

Table 4.1: Factorial ANOVA, summary of results of gonad density for *Perna perna* mussels showing the effect of infestation. Bolded p values signify a statistically significant difference between groups at $p < 0.05$.

Effect	Effect	SS	D.F	MS	F	p
Intercept	Fixed	454.702	1	454.702	7055.325	**0.001**
Month	Random	0.516	8	0.064	18.444	**0.001**
Status	Fixed	0.024	1	0.024	6.971	**0.030**
Month x Status	Random	0.028	8	0.003	8.616	**0.001**
Error		0.066	162	0.001		

Histology (*Mytilus galloprovincialis*)

M. galloprovincialis showed less seasonality in reproduction, with very little variation among months in gonad density. This was particularly true for infested individuals (Fig. 4.2).

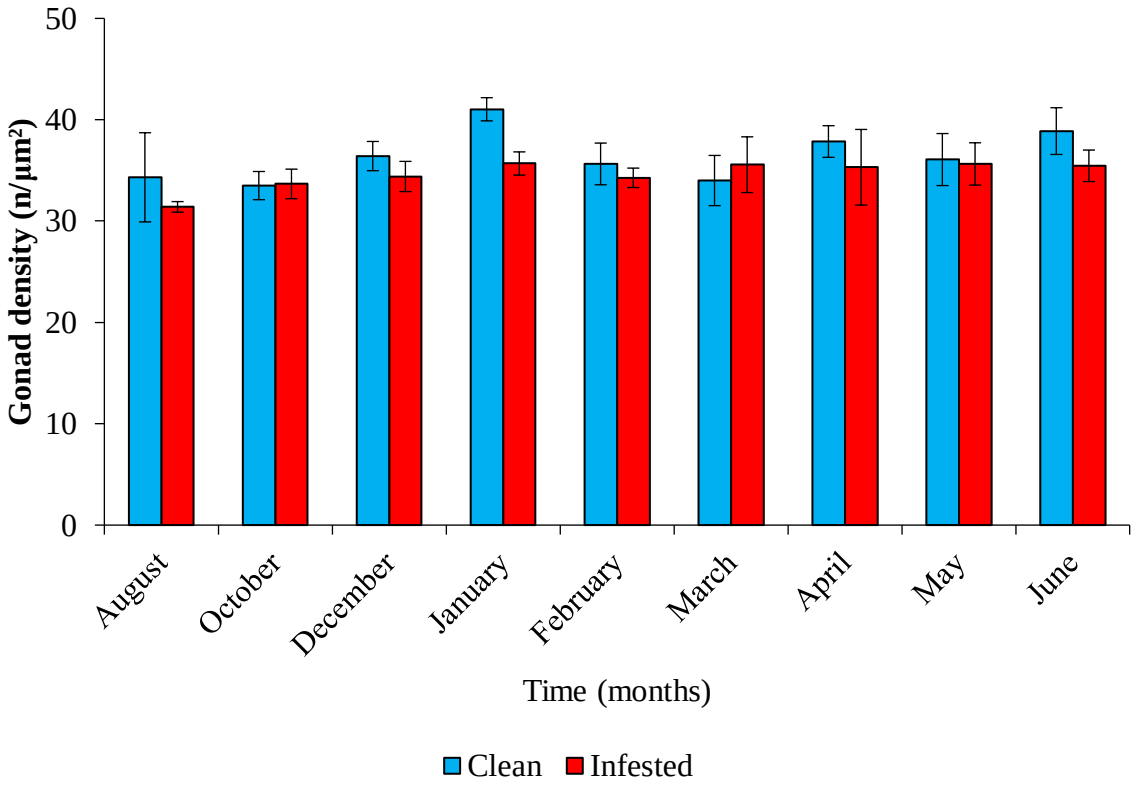

Figure 4.2: Gonad density for *Mytilus galloprovincialis* mussels for clean and infested mussels (values are mean ±SD) from August 2017 – June 2018.

There was no significant effect of month on gonad density (ANOVA: $F_{8,162} = 2.591$; $p = 0.1$; Table 4.2), but a significant effect of status on gonad density (ANOVA: $F_{1,162} = 7.05$; $p < 0.05$; Table 4.2) with clean mussels having higher densities compared to infested mussels. There was also a significant month x status interaction on gonad density (ANOVA: $F_{8,162} = 6.42$; $p < 0.05$), with August and January being the only two months having significantly higher densities for clean mussels compared to infested mussels (Posthoc, TUKEY HSD).

Table 4.2: Factorial ANOVA, summary of results of gonad density for *Mytilus galloprovincialis* mussels showing the effect of infestation. Bolded p values signify a statistically significant difference between groups at $p < 0.05$.

Effect	Effect	SS	D.F	MS	F	p
Intercept	Fixed	439.733	1	439.733	45278.537	**0.001**
Month	Random	0.078	8	0.010	2.591	0.100
Status	Fixed	0.026	1	0.026	7.050	**0.029**
Month x Status	Random	0.030	8	0.004	6.420	**0.001**
Error		0.095	162	0.001		

Gonadosomatic index

Perna perna

The gonadosomatic index (GSI) fluctuated throughout the study period. There were two peaks for GSI with the highest peak period reached in the month of January and another peak in April. Clean mussels recorded an average peak of 0.25 and infested mussels recorded an average peak of 0.15 in January (Fig. 4.3). Soon after the January peak there was a sharp decline in GSI, presumably indicating spawning, and then GSI started to increase from March till it reached another peak in April.

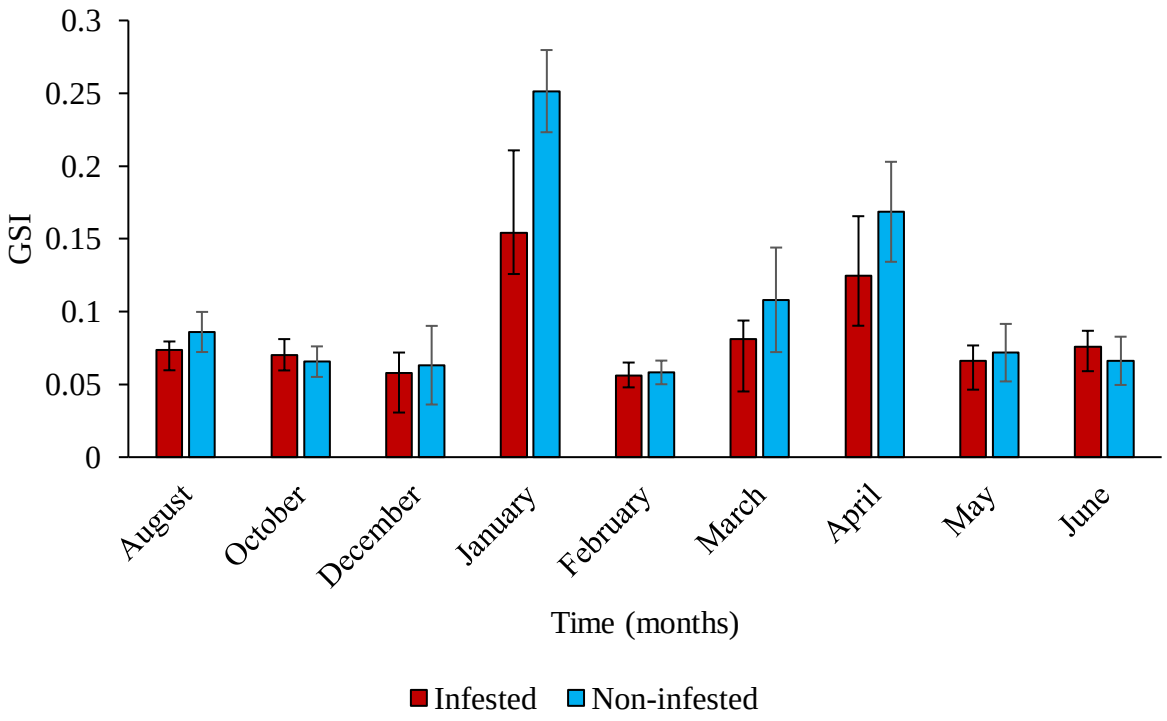

Figure 4.3: Gonadosomatic Index for *Perna perna* mussels for clean and infested mussels (values are mean ±SD) from from August 2017 – June 2018.

There was a significant difference in GSI in terms of month (ANOVA: $F_{8,162} = 9.264$; $p < 0.05$; Table 4.3) and status (ANOVA: $F_{1,162} = 13.217$; $p < 0.05$; Table 4.3). There were significant effects of month and status (ANOVA: $F_{8,162} = 4.079$; $p < 0.05$; Table 4.3), with clean mussels having a higher GSI than infested mussels only in the months of January and April when there were peaks in GSI, showing the effects of infestation were only discernible during peak periods (Posthoc, TUKEY HSD; $p < 0.05$).

Table 4.3: Factorial ANOVA, summary of results of GSI for *Perna perna* mussels showing the effects of month and infestation. Bolded p values signify a statistically significant difference between groups at $p < 0.05$.

Effect	Effect	SS	D.F	MS	F	p
Intercept	Fixed	2739.601	1	2739.601	112.731	**0.001**
Month	Random	194.417	8	24.302	9.264	**0.002**
Status	Fixed	34.673	1	34.673	13.217	**0.007**
Month x Status	Random	20.986	8	2.623	4.079	**0.001**
Error		104.186	162	0.643		

Mytilus galloprovincialis

The gonadosomatic index (GSI) fluctuated throughout the study period, the peak period was reached in the month of August with clean mussels showing a mean peak of 0.29 and infested mussels recording 0.24 (Fig 4.4). Another peak in GSI was observed in the month of April with both infested and clean mussels recording just below 0.25 (Fig 4.4).

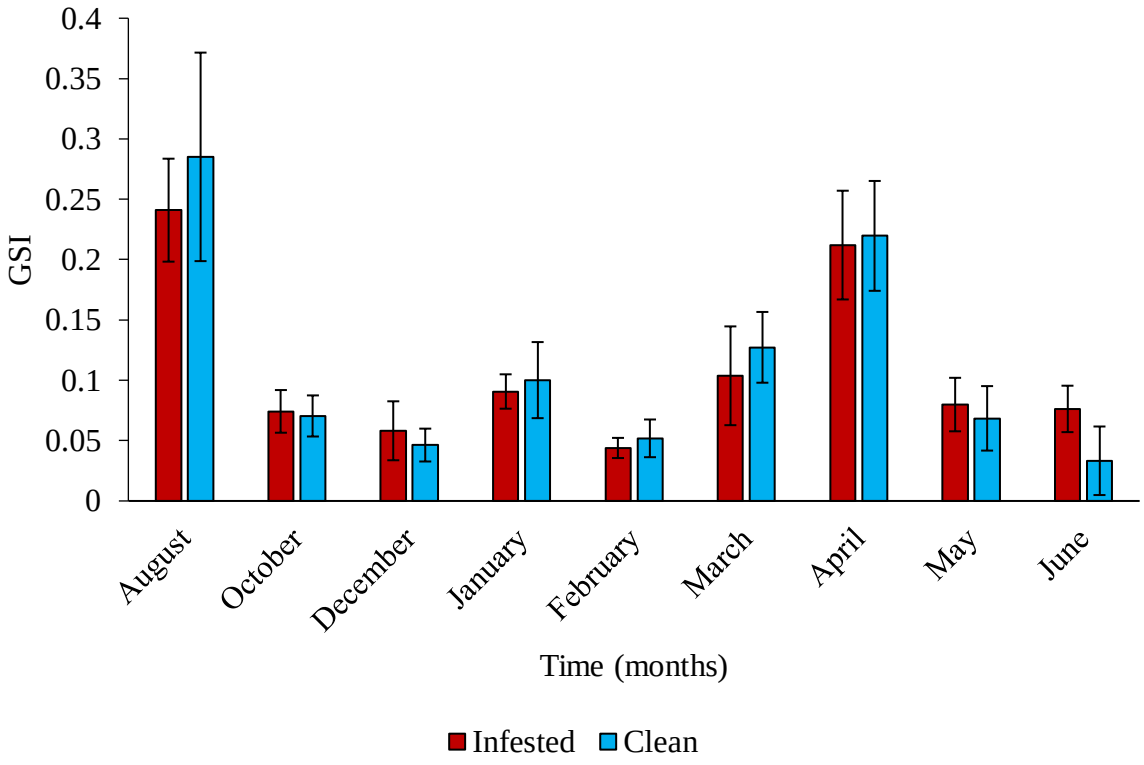

Figure 4.4: Gonadosomatic Index for *Mytilus galloprovincialis* mussels for clean and infested mussels (values are mean ±SD) from August 2017 – June 2018.

There was a significant effect of month on GSI (ANOVA: $F_{8,162} = 5.236$; $p < 0.05$; Table 4.5). Although peak values for clean mussels exceeded those of infested mussels, the differences were not significant, probably because of the large SD values (ANOVA: $F_{1,162} = 1.76$; $p = 0.221$; Table 4.5). There was, however, a significant interaction between month and status (ANOVA: $F_{8,162}$ =

5.83; $p < 0.05$; Table 4.5), with differences in GSI levels being stronger in some of the months (Posthoc, TUKEY HSD).

Table 4.5: Factorial ANOVA, summary of results of GSI for *Mytilus galloprovincialis* mussels showing the effect of infestation. Bolded p values signify a statistically significant difference between groups at $p < 0.05$.

Effect	Effect	SS	D.F	MS	F	p
Intercept	Fixed	2163.507	1	2163.507	83.884	**0.001**
Month	Random	206.332	8	25.792	5.236	**0.015**
Status	Fixed	8.667	1	8.667	1.760	0.221
Month x Status	Random	39.405	8	4.926	5.830	**0.001**
Error		136.859	162	0.845		

Attachment strength

There was no significant difference between the two mussel species, *Perna perna* and *Mytilus galloprovincialis*, in attachment strength (i.e. no significant effect of species; $p = 0.69$; Table 4.7). Infestation had a significant effect on attachment strength (ANOVA: $F_{4,190} = 87.149$; $p < 0.05$; Table 4.7) and there was a significant interaction of species and infestation (ANOVA: $F_{4,190} = 3.887$; $p < 0.05$; Table 4.7). For *M. galloprovincialis* levels A-B were not significantly different from each other but were both different from levels D and E (ANOVA: TUKEY HSD; $p < 0.05$; Fig 4.5) with level C being significantly different from neither of these two groups. The pattern for *P. perna* differed only in detail; levels A, B, C were significantly different from levels D and E (ANOVA: TUKEY HSD; $p < 0.05$; Fig 4.5). This shows that the more infested a mussel becomes, the less force is required to be detach it from the substratum.

Table 4.7: Two-way ANOVA, summary of results for attachment strength to substratum showing the effect of species, different levels of infestation and their interaction for *Perna perna* and *Mytilus galloprovincialis*. Bolded p values signify a statistically significant difference between groups at $p < 0.05$.

Effect	SS	D.F	MS	F	p
Intercept	295007.2	1	295007.2	8519.829	**0.001**
Species	5.6	1	5.6	0.160	0.690
Infestation	12070.5	4	3017.6	87.149	**0.001**
Species x Infestation	538.4	4	134.6	3.887	**0.005**
Error	6578.9	190	34.6		

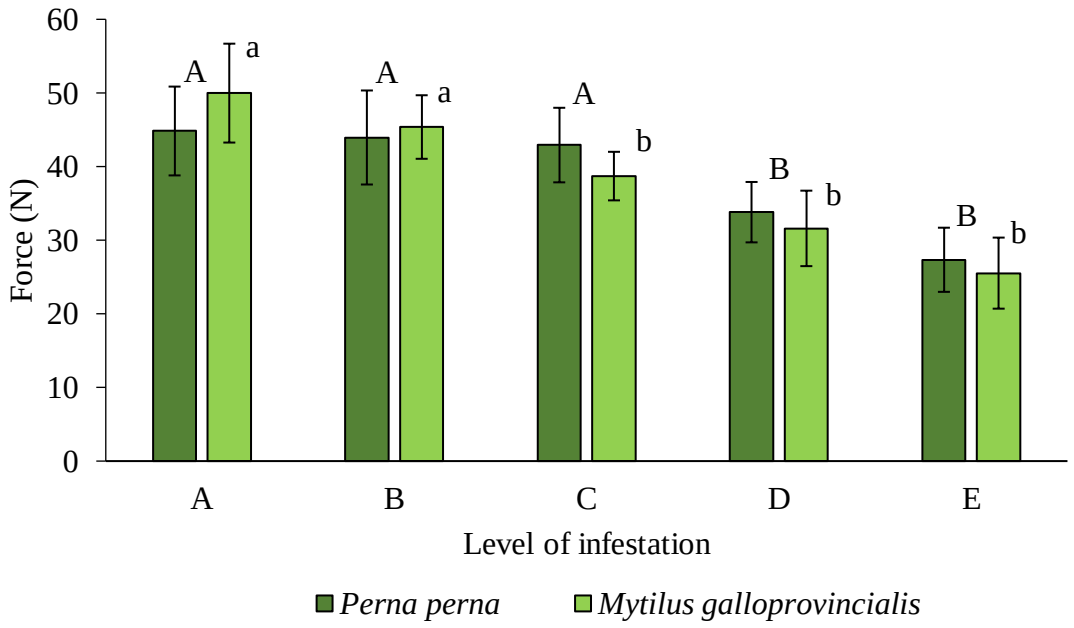

Figure 4.5: Attachment strength in Newtons for different levels of infestation in 4cm *P. perna* and *M. galloprovincialis* mussels (values are mean ±SD). Homogenous groups are indicated by uppercase letters for *Perna perna* and lower-case letters for *Mytilus galloprovincialis*.

Discussion

Endolithic cyanobacteria act as parasites to mussels by continuously eroding their shells. Results show that endolithic cyanobacteria infestation has a negative effect on mussel reproduction, measured as GSI and the density of eggs within the ovary, and on attachment to the substratum. Infested mussels had low gonad density and low GSI compared to clean mussels and attachment strength to the substratum decreased with the level of infestation.

Reproductive output

In the case of *Perna perna*, the results showed that there was a significant interaction of month and status. Reproduction in mussels is known to follow seasonal cycles (Fearman et al. 2009), and this was confirmed here, though seasonality differed between mussel species, as shown by Zardi et al. (2007). The influence of infestation was only significant in months when there was high density of eggs, just before spawning, when infested mussels had lower densities than clean mussels. In the case of *Mytilus galloprovincialis*, the results followed the same trend as for *P. perna*, showing a strong influence of infestation in months when there were high gonad densities. Throughout the whole study period, neither mussel species had zero-density, showing that there were always eggs present, although their numbers fluctuated from month to month.

Likewise, the gonadosomatic index (GSI) fluctuated throughout the study period for both mussel species and both showed a major and a minor spawning event. Reproduction can be altered by reducing the size (mass) of the gonads or by reducing the density of eggs within the gonad but the size of eggs did not differ between infested and clean mussels (Unpub data). There was no correlation between GSI and the density of eggs which means that reproduction was altered by reducing the size (mass) of gonads. Samples for both mussel species were collected at the same location where they experienced the same environmental conditions, but they had different peak

periods of spawning suggesting that spawning in mussels might be driven by endogenous influences.

Different mussel species, *P. perna* and *M. galloprovincialis* have been shown to have different peak periods of reproduction (Zardi et al. 2007) and this was also observed in the current study with the two species having peak reproduction periods in different months. There was a significant difference between infested and clean mussels for both mussel species with infested mussels having a low gonadosomatic index (GSI) compared to clean mussels, but these significant differences were observed only during peak reproductive periods. Other studies have shown how factors such as temperature, food quality and quantity affect reproduction in mussels (Wacker & Von Elert 2003, Petes et al. 2007, Fearman et al. 2009), and intertidal mussels are subject to considerable environmental stress, including wave action and exposure to heat and desiccation stress during low tide.

The additional stress of parasitism by endolithic cyanobacteria seems to tip the balance in terms of reproduction, with the need to channel energy to shell repair resulting in infested mussels having low GSI. This mirrors the results for Scope for Growth (chapter 3), with infested mussels having reduced SFG, i.e., energy that can be used for growth and reproduction. Previous studies have shown that parasites reduce reproduction in marine species. Parasitic larval trematodes have been shown to reduce the population density of salt marsh snail *Cerithidea californica* by castrating the snails (Lafferty 1993, Lafferty & Kuris 2009) and trematode parasites were found to reduce the competitive ability of *P. perna* mussels (Calvo-Ugarteburu & McQuaid 1998).

The two measures of reproductive output gave different results; the GSI data showed two peaks which were clearly visible, but the density data were much less clear. With the GSI data, the

changes in reproductive output seem to be linked to increased mass of gonad (GSI) rather than density of eggs within gonads.

Attachment strength

The results show that infestation affects attachment to the substratum for both mussel species. For *P. perna*, infestation starts to have a significant effect when the level of infestation reaches levels of infestation C, D and E as described by Gektidis et al. (2007) and Marquet et al. (2013). This shows that at the first levels of infestation, level A (clean) and B when the infestation starts to be visible, there is no significant difference between clean and infested mussels in terms of attachment strength. Infestation starts to lower attachment strength as it spreads throughout the mussel shell with clear, significant differences as infestation increases to levels C, D and E. For *M. galloprovincialis* infestation at the first three levels, A, B and C shows no significant difference between but level D and E are significantly different from level A and B with C not different from all other levels of infestation. In other words, the effects of infestation on attachment strength become discernible at an earlier stage for *P. perna*. This aligns well with the fact that *P. perna* adopts a resistance approach to dealing with wave action, growing more and stronger byssal threads than *M. galloprovincialis*, which adopts a less resistant approach when dealing with wave action (Zardi et al. 2007). The difference becomes important in terms of competitive interactions between the two (Robinson et al. 2005). The implication is that *P. perna* channels a higher proportion of its energy into attachment than *M. galloprovincialis* so that the negative effects of infestation become obvious earlier in *M. galloprovincialis*.

Previous studies have looked at how other factors affect mussel attachment strength. Some studies have looked at how hydrodynamic stress affects attachment strength (Hunt & Scheibling 2001,

Zardi et al. 2007). There are other factors that are thought to affect byssus production including temperature, salinity, agitation and seasonality (Young 1985, Lachance et al. 2008), and factors that affect byssus production will have an effect on attachment strength. Mussels occur in intertidal communities where they face high hydrodynamic forces. They need to attach themselves to the substratum to avoid dislodgement. This study focused on how different levels of infestation affect mussel attachment strength in intertidal communities where they are exposed to high hydrodynamic conditions. Attachment strength has been shown to be correlated to hydrodynamic stress (Nicastro et al. 2010), in this study, results show that with an increase in infestation attachment strength is reduced. For heavily infested mussels, this means they can be dislodged easily compared to clean mussels as they have low attachment strength when there is increased wave action.

Endolithic cyanobacterial infestation affects attachment of *M. galloprovincialis* more than *P. perna*, with significant effects in *M. galloprovincialis* observed starting from level C of infestation compared to *P. perna* where significant effects were observed starting from level D of infestation. When it came to reproduction infestation seemed to affect *P. perna* more than *M. galloprovincialis* with significant differences observed in both GSI and gonad mass whereas in *M. galloprovincialis*, the observed significant difference was only in gonad density. The results show that infestation by endolithic cyanobacteria has a negative influence on mussels as also shown in chapters 2 and 3. Endolithic cyanobacterial infestation leads to reduced reproduction and attachment strength leading to mussels having a low reproductive output and potentially being easily washed off by waves in intertidal communities as there is less energy channeled to reproduction and attachment strength.

Chapter 5 : Endolithic cyanobacterial succession in mussel shells

Introduction

Ecological succession is a series of progressive changes or sequences of colonisation in a community by which the structure of a biological community evolves. It refers to observed sequences or changes in vegetation associations or animal groupings (Drury & Nisbet 1973, Walker & Leonard 1975, Fierer et al. 2010). Some of these sequences occur in time, with changes observed in different time periods and some sequences being observed in space with succession being influenced by the shape and area of the habitat (Cook et al. 2005b).

There are two major types of succession referred to in ecology, allogenic and autogenic succession. Allogenic succession is succession driven by the abiotic components of an ecosystem; of which it is physically induced by changes in the environment that alter geophysical-chemical processes (Walker & Leonard 1975, Begon et al. 1986). Allogenic succession usually takes several years, even hundreds of years to occur, and the species that occupy newly exposed landforms are called primary successors (Begon et al. 1986). These changes may be brought about by factors that provide virgin substratum such as volcanic eruptions, flooding, drought and earthquakes. Autogenic succession is succession driven by biotic components of the ecosystem. These biotic components may include plants facilitating processes such as production of detritus, water and nutrient uptake and nitrogen fixation which can lead to changes in the ecosystem (Walker & Leonard 1975).

Succession can also be considered primary or secondary. Primary succession occurs when a community develops from barren areas or surfaces that are virtually lifeless or a change in vegetation which occurs on previously unvegetated terrain (Begon et al. 1986). These places

include areas that have just experienced lava flow, newly formed sand dunes and rocks from retreating glaciers. This is succession driven by forces that involve changes in soil conditions, such as gradual accumulation of soil and accumulation of nitrogen (Begon et al. 1986, Jumpponen et al. 1998). Secondary succession occurs when a previously existing community has been removed following a severe disturbance. Disturbances that can lead to secondary succession include volcanic eruptions, actions of forest thinning, floods, fire and wind.

There are three ways in which changes can happen in a community for both primary and secondary succession; facilitation, inhibition and tolerance (Armesto & Pickett 1986). These three ways can happen separately or simultaneously at different stages in successional time. Primary successors can play an important role as facilitators, paving the way for later colonisers that are less tolerant to extreme conditions. Facilitation is the process by which early or first species colonisers alter the environment making it suitable for entry by new species (Connell & Slatyer 1977, Begon et al. 1986). The early successional or pioneer species enhance the establishment and survival of the new colonisers in the system and this enhancement brings about changes in microclimate, including changes in the physical and chemical properties of the soil which are facilitated by the early colonisers (Jumpponen et al. 1998). However, in some cases early successors can inhibit or resist the establishment of other species. This mechanism of inhibition was observed in a study of succession in algal communities where early successional alga, *Ulva*, was found to inhibit the recruitment of perennial red algae. This inhibition by the early successor alga was broken when the a crab (*Pachygrapsus crassipes*) started grazing the earlier successor opening up space for the later red alga coloniser to dominate the system (Sousa 1979). When pioneer species coexist with later succession species, succession occurs through tolerance. Under the tolerance scenario, late succession species are able to grow and reproduce even in the presence of pioneer species hence

the two are able to tolerate each other and exist in the same place at the same time period (Armesto & Pickett 1986, Passy & Larson 2011). Tolerance can be passive or active (McCormick & Stevenson 1991). Passive tolerance happens in earlier stages where there is coexistence of species with no suppression of growth of early colonisers by late colonisers. Active tolerance happens in the late stages of succession when species with better access to resources start to suppress the growth of early colonizers (McCormick & Stevenson 1991, Passy & Larson 2011). Succession allows development of a community by allowing new areas to be colonised, damaged communities to be recolonised and allows organisms to adapt to changes in the environment (McCook 1994). There is considerable documented evidence of succession in plant communities (Fierer et al. 2010), and several studies have also looked at the process of succession using microorganisms (Redford & Fierer 2009, Fierer et al. 2010, Cobaugh et al. 2015). Given that microbes are ubiquitous around the world and form diverse communities (Fierer et al. 2010), looking at succession in endolithic cyanobacteria which are microbes and occur in varying environments will help our understanding of ecological succession. Understanding endolithic succession in mussels will bring about knowledge on how endolithic communities change in time and also to assess if there are endolithic cyanobacterial species that are involved in the processes of facilitation, inhibition and tolerance of other endolithic communities.

Succession can also be degradative. Degradative succession is observed in dead organic matter over a relatively short timescale (months to years). This occurs when dead matter such as a carcass, dead plant matter or faecal deposits are exploited by microorganisms and detrivorous organisms (Cobaugh et al. 2015). Detritivores feed in sequence, each group releasing nutrients that are utilized by the next group in the sequence until the resources are exhausted (Begon et al. 1986). Endolithic cyanobacteria organisms are degradative to mussel shells, they actively erode mussel

shells (Garcia-Pichel et al. 2010) and they can be responsible for up to 50% of adult mussel mortality (Kaehler & McQuaid 1999, Zardi et al. 2009, Marquet et al. 2013). Mussels are important ecological engineers in intertidal communities thus looking at succession of endolithic cyanobacteria in mussel shells will give an insight into how these degradative microorganisms change in time and space within mussel shells. In this study, the focus was on succession in endolithic cyanobacteria which are microbes found in mussel shells (Kaehler & McQuaid 1999, Zardi et al. 2009, Marquet et al. 2013).

Aims

The aim of this chapter was to examine how endolithic succession in mussels occurs through time and across space within mussel shells taking advantage of the fact that the umbo represents the oldest region and the distal edge the newest regions of a shell. Using a large-scale quantitative and qualitative assessment of endolithic communities, four main questions are addressed:

1. Do endoliths exhibit succession in mussel shells?
2. Are different levels of infestation in mussels associated with different species of endolithic cyanobacteria?
3. Does endolithic succession differ between mussel species?
4. Does the distribution of endolithic cyanobacteria species differ in different regions (umbo, centre and the growing region/distal edge/apex) of a mussel shell?

Methodology

To assess endolithic cyanobacterial succession in mussels, an assessment of succession in time and endolithic succession in space (within a mussel shell) was conducted. Succession in time was considered as changes in endolithic species communities in mussels with time while succession in space was considered as the distribution of endolithic species in different regions of a mussel shell. All samples for the succession experiment were collected from Brenton-on-Sea (34.08°N; 23.02°E) on the south coast of South Africa.

Succession in time

To assess succession in time, 30 clean (no sign of endolithic erosion) *Perna perna* and 30 *Mytilus galloprovincialis* mussels of ~ 4cm in length were collected in the mid-mussel zone and transported to the laboratory. In the lab, mussels were cleaned of epibionts and the soft tissues were taken out so that the remaining shells could be used for the experiment. The periostracum was artificially removed using a knife to allow easy colonisation since it is the layer that protects mussels from endoliths (Zardi et al. 2009). The shells were deployed in the mid-mussel region where they were exposed to waves and sunlight, shells were deployed from April 2017- April 2018. Using A-788 Z-Spar Splash Compound (Kopper's Co., USA) each valve was deployed to hard rock surfaces at Brenton-on-Sea. After deployment of shells, 10 shells (5 for each species) were collected every month for the first 4 months of the study period and every two months for the last 8 months of the study period and taken to the laboratory. In the laboratory, collected shells were assigned to the five different levels of infestation described in chapter 2, with zero infestation being A and E being the most infested shell. Endolithic cyanobacteria species were identified using the methods described in chapter 2 for each shell. Endolithic species richness for each shell was recorded for each time period throughout the study period.

Within-shell succession in space

To examine succession within a mussel shell, heavily infested *Perna perna* and *Mytilus galloprovincialis* mussels (Level E, n = 10 for each species) were collected. Collected mussels were immediately preserved in 4% formalin and transported to the laboratory. In the laboratory, each mussel shell was cut into 3 regions, the umbo, which is the oldest part of the shell, the centre which is the middle part of the shell and the distal edge which is the growing new part of the shell. In each region endolithic cyanobacterial species were identified. Endolithic cyanobacteria species were identified using the methods described in chapter 2 for each shell. All samples were collected in Brenton-on-Sea, on a moderately wave exposed intertidal rocky shore and at sun-exposed rocks.

Data Analysis

To visualise similarities and differences in endolithic assemblages, succession in time and assemblages of endolithic species within different regions of mussel shells, non-metric multidimensional scaling (nMDS) ordinations and dendrograms based on cluster analysis of Bray-Curtis similarity matrices were plotted (PRIMER-E v6). A SIMPER analysis in PRIMER-E v6 was used to assess the endolithic species percentage contributions to differences among the three regions of a mussel shell. Endolithic species differences in different regions of a shell were analysed using a permutational analysis of variance (PERMANOVA) based on the Bray Curtis similarity measures in PRIMER-E v6 with mussel species (fixed factor with two levels) and region (fixed factor with three levels) as factors. Changes in endolithic species richness with time were analysed using a 2-way ANOVA with mussel species (fixed factor with two levels) and time (fixed factor with six levels) and endolithic species richness of each time period as the dependent factor.

Results

Succession in time

Level of infestation with time

At the end of the experiment, all shells deployed exhibited level E of infestation. A dendrogram based on the different levels of infestation shows that there was a clear-cut grouping, which occurred by month with mussels of the same age grouping together regardless of host species (Fig. 5.1). This shows that infestation increased at the same rate for both species with samples from the same month grouping together for both mussel species except for the month of February where *Mytilus galloprovincialis* grouped with *Perna perna* and *M. galloprovincialis* samples from the month of April. This shows that samples of the same month had the same level of infestation which was gradually increasing from level A till level E in all samples collected at the end of the experiment.

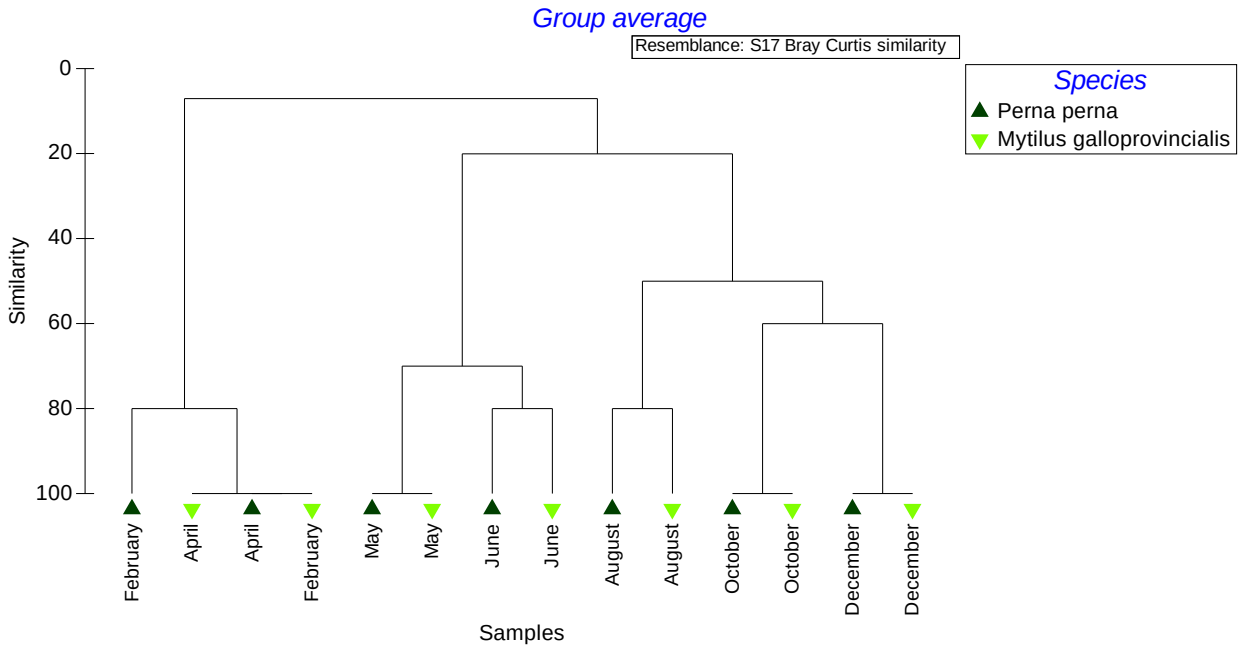

Figure 5.1: Results of levels of infestation over time between *Mytilus galloprovincialis* and *Perna perna* based on Bray Curtis similarity matrix from February 2017 – June 2018.

The Non-metric Multi-Dimensional Scaling (nMDS) plot support results shown by the dendrogram, showing similarity in infestation levels with time in both mussel species. Levels of infestation results for month were grouping together in both species which shows that infestation for both species was at the same level at a given time period for both species except for the month of February (Fig. 5.2) where *M. galloprovincialis* samples collected in April grouped with some samples for P. *perna* collected in February.

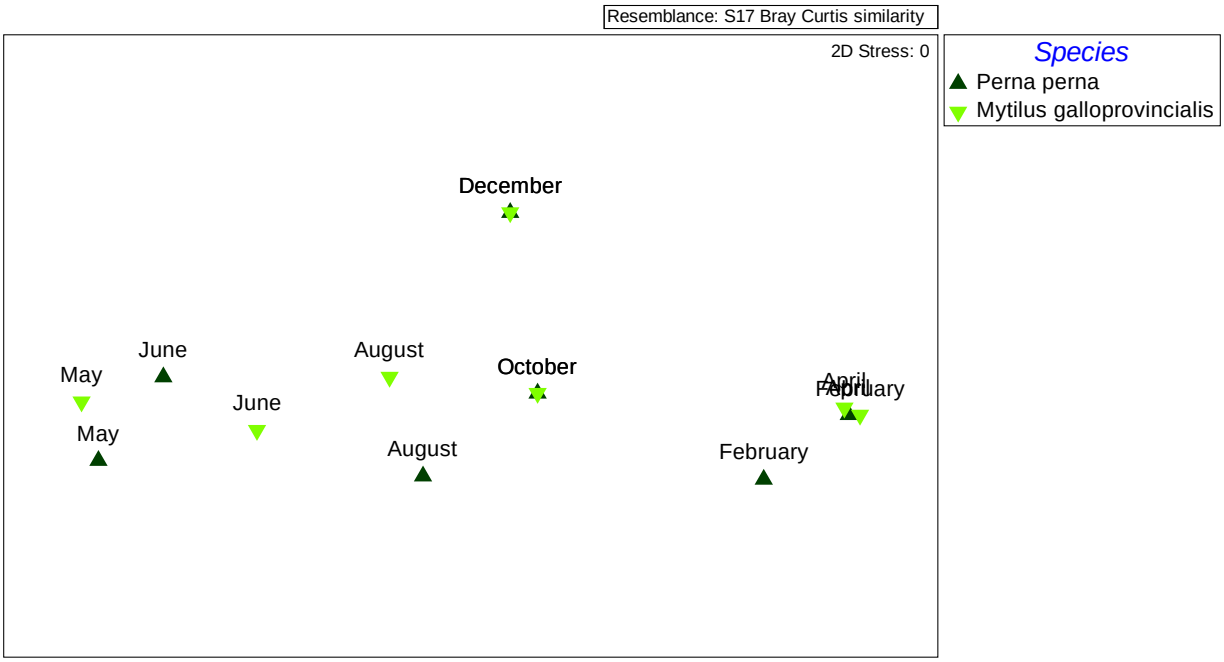

Figure 5.2: Non-metric Multi-Dimensional Scaling plot showing the dissimilarity between of levels of infestation over time between *Mytilus galloprovincialis* and *Perna perna* based on a Bray Curtis similarity matrix from February 2017 – June 2018.

Species richness with time

The mean number of endolithic cyanobacteria in shells continually increased for *Mytilus galloprovincialis* throughout the study period with endolithic species reaching 5 or more endolithic species in the month of December and this stayed the same till the end of the experimental period in April. For *Perna perna* mussels, the number of endolithic cyanobacterial species increased from the start of the experiment till February 2018 where it reached more than 5 endolithic species and the same number of endolithic species were observed at the end of the experiment in April.

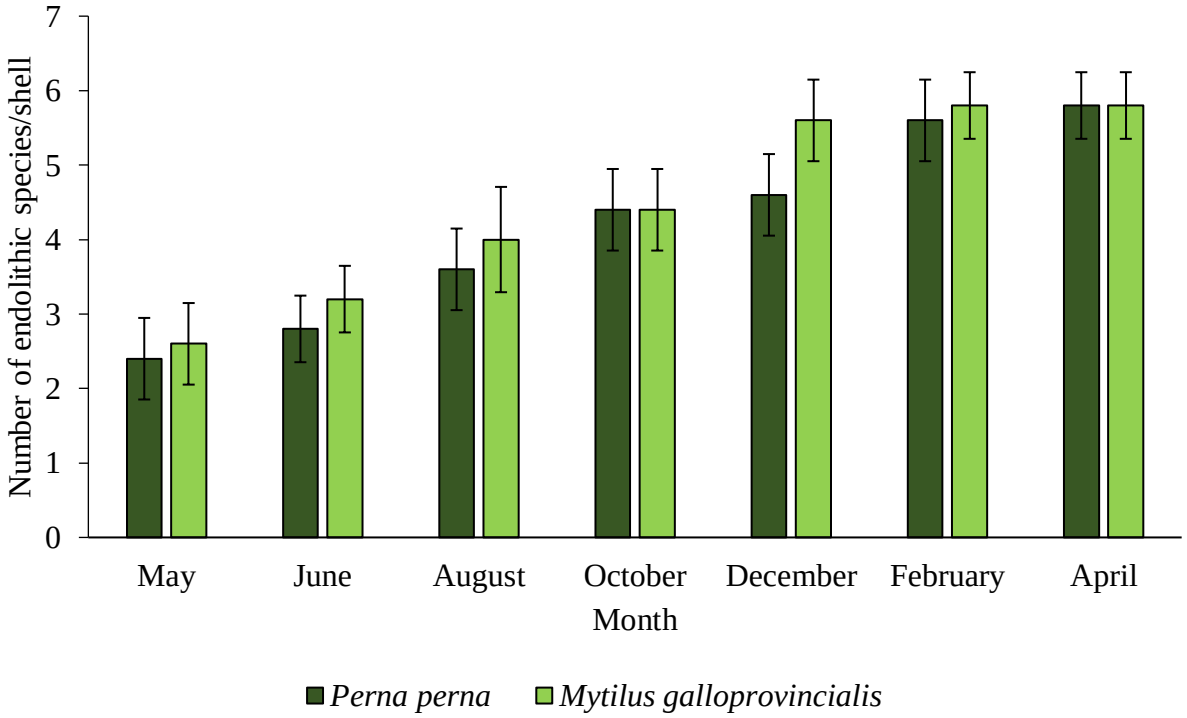

Figure 5.3: Mean endolithic species distribution (+SD) over time for *Mytilus galloprovincialis* and *Perna perna* from May 2017 – April 2018.

The mean number of cyanobacterial species per shell was significantly affected by host species (ANOVA: $F_{1,56} = 6.205$; $p < 0.05$, Table 5.1.) with *Mytilus galloprovincialis* having more endolithic species and time (ANOVA: $F_{6,56} = 60.034$; $p < 0.05$, Table 5.1.) but there was no significant effect of the interaction of time and species (ANOVA: $F_{6,56} = 1.06$; $p = 0.398$, Table 5.1).

Table 5.1: Factorial ANOVA. Summary of results showing the effect of species and time on the number of endolithic species over time. Bolded p values signify a statistically significant difference between groups at $p < 0.05$.

Effect	SS	D.F	MS	F	p
Intercept	1311.557	1	1311.557	4708.154	**0.001**
Species	1.729	1	1.729	6.205	**0.016**
Time	100.343	6	16.724	60.034	**0.001**
Species x Time	1.771	6	0.295	1.060	0.398
Error	15.600	56	0.279		

Changes in endolithic species community structure with time

The number of endolithic species increased with time for both mussel species. This number increased to 4 in the month of August for *Perna perna* and to 4 in June for *Mytilus galloprovincialis,* with endolithic species identified reaching 5 in August for *M. galloprovincialis* and 5 in October for *P. perna* (Table 5.2). There was no difference in the cyanobacterial species colonising the two host species with colonisation following the same sequence. Early colonisers, which were first to appear in a shell, included *Hyella caespitosa*, *P. terebrans* and *M. testarum*. The late successional species included *Hyella balani*, *Hormathonema luteo brunneum* and *Hormathonema luteo brunneum* and they only appeared after 10 months for *P. perna* and 8 months for *M. galloprovincialis*. *H. balani* and *H. luteo brunneum* were species that were observed in the later stages of the experiment, from October for *H. balani* and from February for *H. luteo brunneum* in *P. perna* and from August for *H. balani* and from December for *H. luteo brunneum* in *M. galloprovincialis* mussels.

Table 5.2: List of identified endolithic species for every time period sampled, species list pooled for all replicates of each time/species combination.

	Perna perna						
Endolithic species	May	June	August	October	December	February	April
Hyella caespitosa	X	X	X	X	X	X	X
Plectonema terebrans	X	X	X	X	X	X	X
Mastigocoleus testarum	X	X	X	X	X	X	X
Solentia stratosa			X	X	X	X	X
Hyella balani				X	X	X	X
Hormathonema luteo brunneum						X	X
	Mytilus galloprovincialis						
Endolithic species	May	June	August	October	December	February	April
Hyella caespitosa	X	X	X	X	X	X	X
Plectonema terebrans	X	X	X	X	X	X	X
Mastigocoleus testarum	X	X	X	X	X	X	X
Solentia stratosa		X	X	X	X	X	X
Hyella balani			X	X	X	X	X
Hormathonema luteo brunneum					X	X	X

The dendrogram and nMDS did not reveal a clear difference in the endolithic community structure between the two host species, *M. galloprovincialis* and *P. perna* over time (Figs. 5.4 and 5.5). The nMDS shows that there was some partial overlap of months between the two species (Fig. 5.5), endolithic species identified for in both mussel species showed minor similarities in terms of month with shells collected at the same time being similar regardless of the host species.

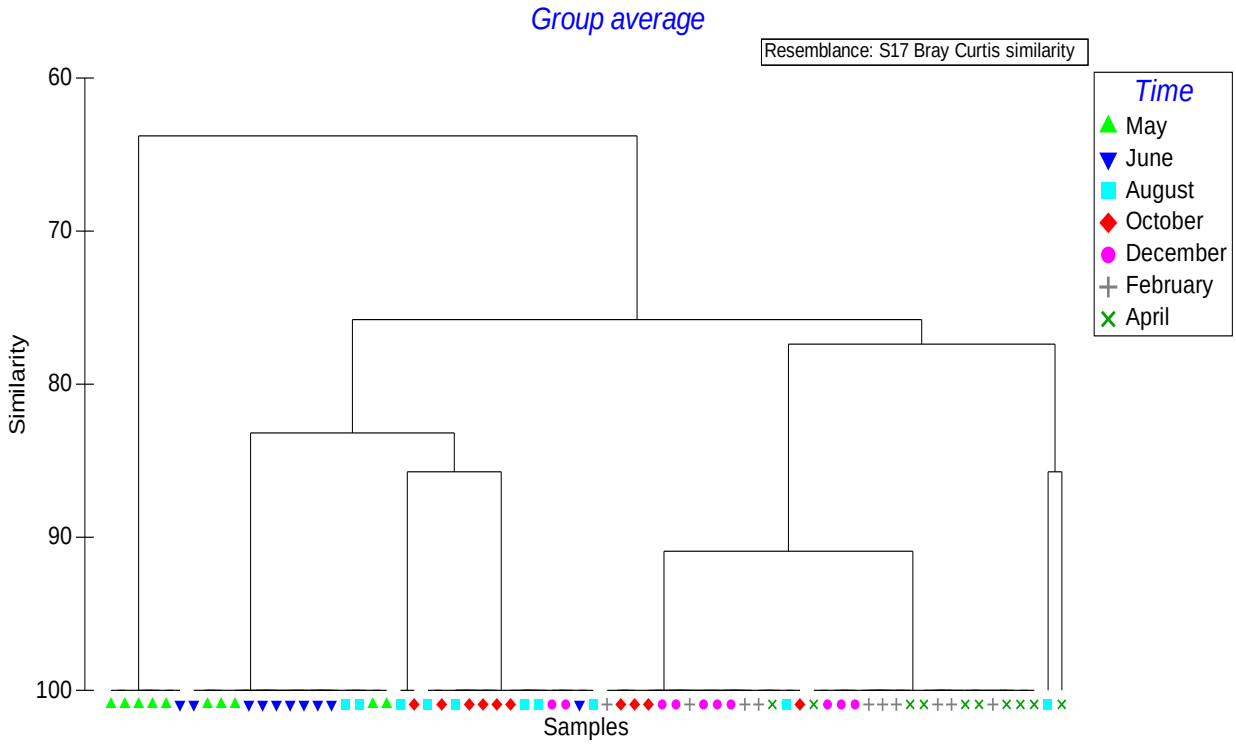

Figure 5.4: Results of endolithic species community structure over time for *Mytilus galloprovincialis* and *Perna perna* based on the Bray Curtis similarity matrix from May 2017 – April 2018.

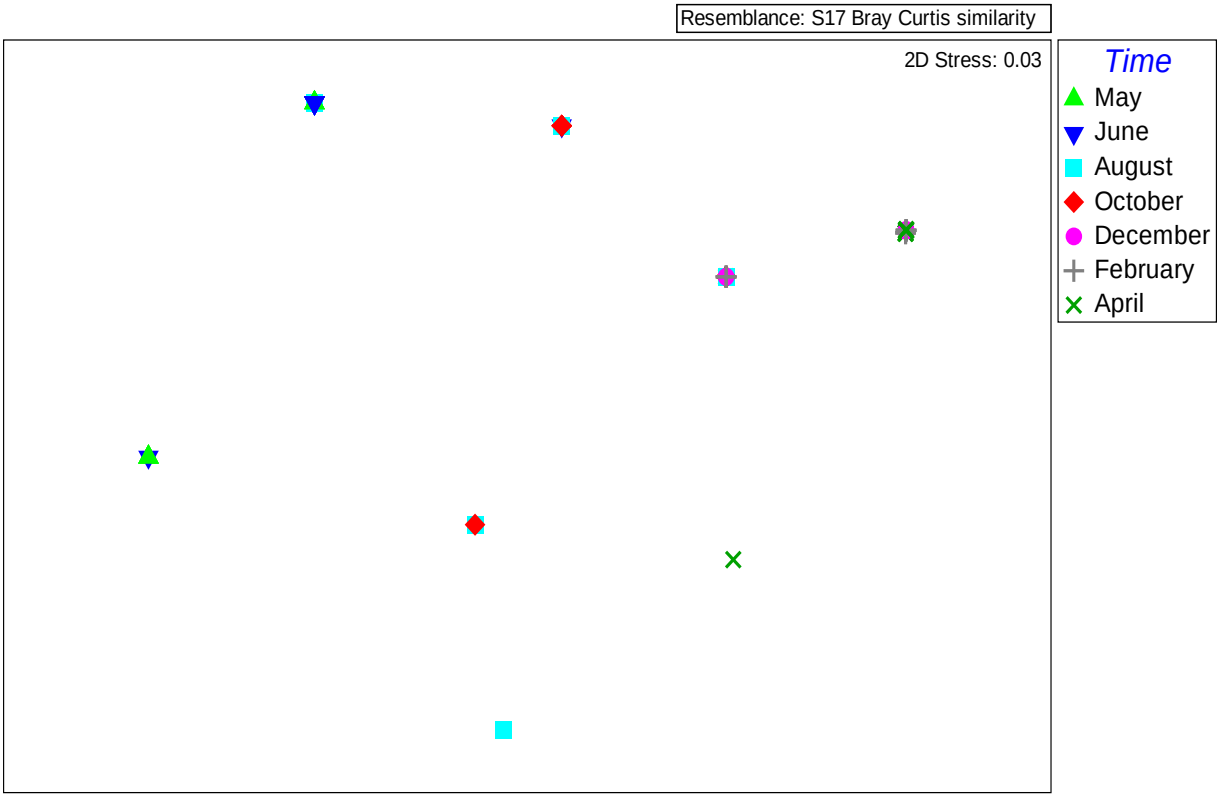

Figure 5.5: Non-metric Multi-Dimensional Scaling plot showing the dissimilarity endolithic species community structure over time for *Mytilus galloprovincialis* and *Perna perna* based on Bray Curtis similarity matrix from May 2017 – April 2018.

Succession in space

Succession in a mussel shell (umbo, centre and the growing region/distal edge)

The dendrogram and nMDS revealed partial differences in the distribution of endolithic cyanobacteria species in different regions of mussel shells (Fig. 5.6 and 5.7). The nMDS shows some partial overlap in the groupings with regards to region (Fig. 5.7). Samples from the umbo grouped together with some overlap with samples from the centre and distal edge with samples for the centre also overlapping with samples from the distal edge. There were no clear divisions among regions, but a trend along the length of the shell was observed for both mussel species.

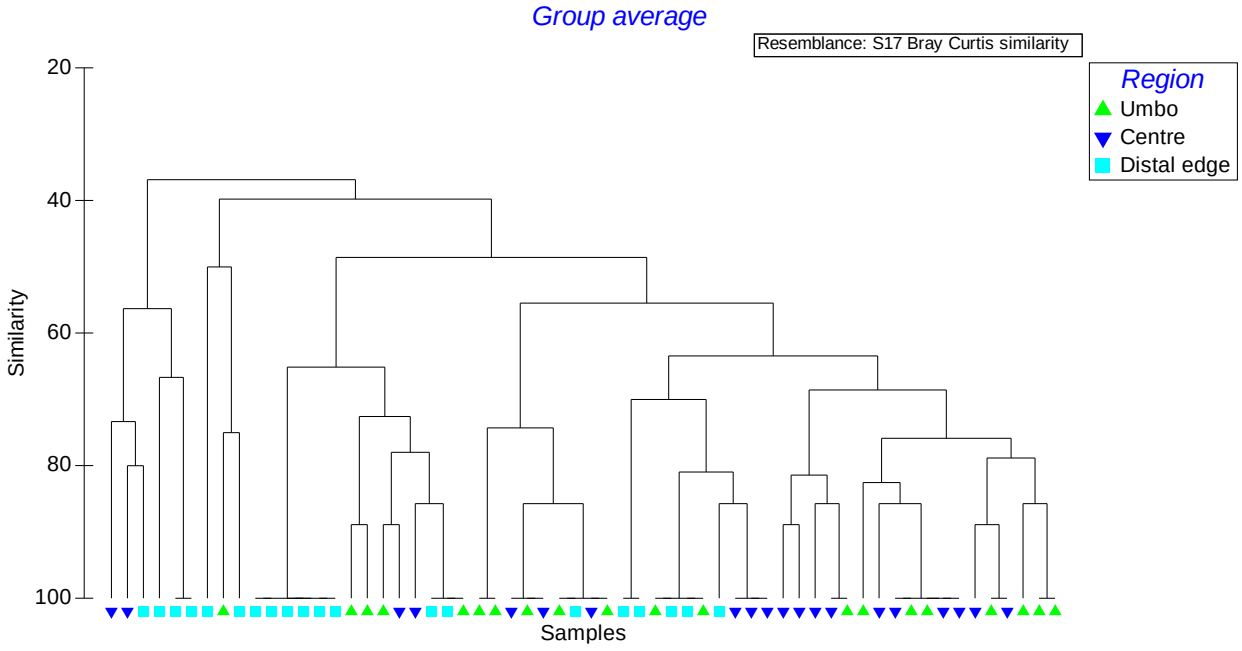

Figure 5.6: Results of the distribution of endolithic cyanobacteria species in different regions (umbo, centre and the growing region/distal edge/apex of the mussel) for *Perna perna* mussel shells based on the Bray Curtis similarity matrix from May 2017 – April 2018.

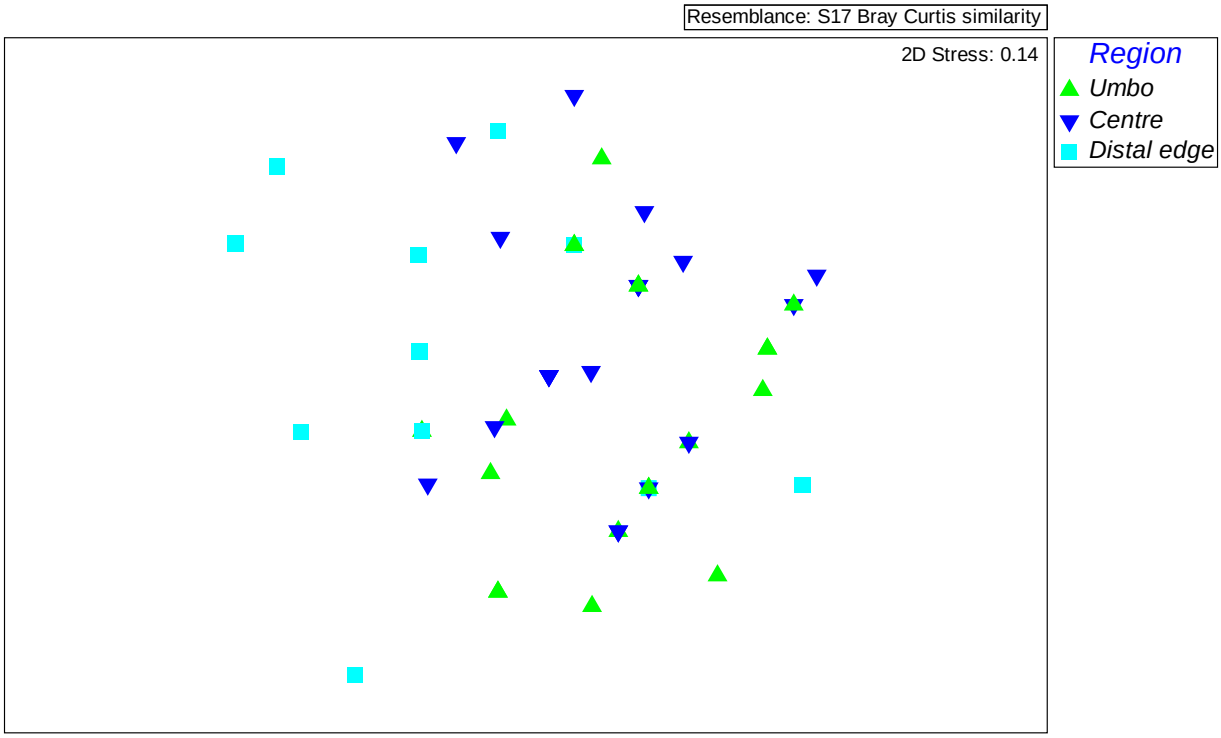

Figure 5.7: Non-metric Multi-Dimensional Scaling plot showing the dissimilarity in the distribution of endolithic cyanobacteria species in different regions (umbo, centre and the growing region/distal edge/apex of the mussel) of a mussel shell based on the Bray Curtis similarity matrix from May 2017 – April 2018.

PERMANOVA results based on the Bray Curtis matrix indicated a significant difference among regions (PERMANOVA: p = 0.001, Table 5.3) but no significant effect of mussel species (PERMANOVA: p = 0.376) and no significant interaction of species with region (PERMANOVA: p = 0.162). This showed that region had a strong influence on the community structure of endolithic species communities in a mussel shell.

Table 5.3: PERMANOVA table of results; influence of species, region and interactions on community structure of endolithic species in a mussel shell.

Source	D.F	SS	MS	Pseudo-F	P(perm)	Unique perms
Species	1	1129	1129	1.1315	0.376	999
Region	2	20832	10416	10.438	0.001	999
Species x Region	2	3566	1783	1.7869	0.162	999
Res	54	53884	997.85			
Total	59	79410				

The endolithic communities in the umbo were not significantly different from the endolithic communities in the centre (PERMANOVA: p = 0.188) but the endolithic communities in the umbo were different from endolithic communities in the distal edge (PERMANOVA: p = 0.001) and the centre was significantly different from the distal edge (PERMANOVA: p = 0.001). Table 5.4 shows percentage dissimilarities among the 3 different regions of a mussel's shell. The umbo was 62.01% similar to the centre and 48.09% similar to the distal edge while the centre was 48.04% similar to the distal edge. This shows that the umbo and the centre were more similar in terms of endolithic species community but different from the distal edge, this is shown by percentage contributions to percentage dissimilarities for all three regions when compared to each other (Table 5.5). All 3 regions shared most of the species except for *Hyella balani* and *Hormathonema luteo*

brunneum which was only found in the centre and distal edge (Table 5.6) in *Perna perna* mussels and only in *Mytilus galloprovincialis* mussels was found only in the centre and distal edge.

Table 5.4: Percentage similarities among different regions of a mussel shell in terms of endolithic species composition.

	Umbo	Centre	Distal edge
Umbo			
Centre	62.012		
Distal edge	42.089	48.036	

Table 5.5: Percentage disimilarities in different regions of a mussel shell in terms of endolithic species composition.

Umbo & Centre		Umbo & Distal edge		Centre & Distal edge	
Species	% dissimilarity	Species	% dissimilarity	Species	% dissimilarity
P. terebrans	19.84	*H. caespitosa*	19.51	*S. stratosa*	20.86
H. balani	18.52	*S. stratosa*	17.93	*H. caespitosa*	19.99
M. testarum	18.3	*P. terebrans*	17.89	*M. testarum*	15.89
S. stratosa	13.33	*H. balani*	16.74	*H. balani*	13.81

Table 5.6: List of identified endolithic species for each mussel region, species list pooled for all replicates.

Mytilus galloprovincialis			
	Umbo	Centre	Distal end
Hyella caespitosa	X	X	X
Plectonema terebrans	X	X	X
Mastigocoleus testarum	X	X	X
Solentia stratosa	X	X	X
Hyella balani	X	X	
Hormathonema luteo brunneum	X		

Perna perna			
	Umbo	Centre	Distal end
Hyella caespitosa	X	X	X
Plectonema terebrans	X	X	X
Mastigocoleus testarum	X	X	X
Solentia stratosa	X	X	
Hyella balani	X	X	
Hormathonema luteo brunneum	X		

Discussion

The results showed clear-cut groupings in infestation for both mussel species, *Mytilus galloprovincialis* and *Perna perna*. The two mussel species showed the same level of infestation with time. Infestation was shown to increase at the same rate in both mussel species and this shows that endolithic cyanobacteria infestation happens at almost the same rate for both mussel species. The periostracum was removed at the beginning of the experiment from shells for both species so that settling of endolithic cyanobacteria can be easy. *P. perna* mussels are known to have a thicker periostracum compared to *M. galloprovincialis* (Zardi et al. 2009). The removal of the periostracum meant that the protective barrier was removed, and this might have influenced rates of colonisation, masking possible differences between host species when the periostracum is intact.

At the first sampling period in May an average of just above two endolithic species were recorded for both species. The numbers of endolithic species increased with time and they reached their maximum in December and February for *M. galloprovincialis* and *P. perna* respectively. This shows that the number of endolithic cyanobacteria increased from the start of the experiment to the end of the sampling period when six endolithic cyanobacteria species where identified. This shows that early cyanobacteria colonisers were not replaced by late colonisers but coexisted with them. There was accumulation of endolithic species over time, with endoliths identified in the early stages of the experiment being present even at the end suggesting that there was little or no competition between early colonists and late colonist species. Pioneer endolithic species coexisted with late colonisers suggesting that there was tolerance where late colonists occupy the same area at the same time with pioneer species (Armesto & Pickett 1986) rather than inhibition or elimination of pioneer species by late colonists. Other studies have shown tolerance in succession, this was observed in subtidal zones where early encrusting macroalgae coexisted with later

overgrowth by turf-forming immigrants without experiencing any reduction in growth and reproduction (Passy & Larson 2011). Pioneer species might have a better colonising ability and be the first endoliths to settle on the shell and once they settle the do not prevent other endoliths from settling and colonising the shell, thus they were present at both the start and the end of the experiment.

Host species had a minor influence on the endolithic species colonising them, with similar numbers of endolithic species for most of the study period, a difference appearing only in December when there were less than 5 species identified for *P. perna* and more than 5 species identified for *M. galloprovincialis*. In the following months there were no significant differences in numbers of endolithic species identified between *P. perna* and *M. galloprovincialis* mussels till the end of the experimental period. This shows that endolithic species were not selective when settling in mussel shells, they did not have a preference, they settled in mussels' shells at the same rate for both mussel species and this complements the results from chapter 2 which showed that the prevalence rate by endolithic cyanobacteria in mussels was the same.

This study showed that succession was not different between the two mussel species, prevalence due to endolithic cyanobacteria increased with time, species richness of endolithic cyanobacterial species also increased with time. Species such as *Hyella caespitosa*, *Plectonema terebrans* and *Mastigocoleus testarum* appeared first then they were followed by species such as *Solentia stratosa* and *Hyella balani* which were then followed by *Hormathonema luteo brunneum*. The species that were early colonisers did not inhibit late colonisers, they coexisted together as early coloniser species where still found in the later stages of the experiment. This suggests that early colonisers acted as facilitators for endolithic species that were late colonisers species, or they tolerated late

coloniser species or earlier colonisers were quicker to settle and colonise the shell compared to later colonisers and thus they ended up coexisting in the same mussel shell.

There was a significant difference in the distribution of endolithic cyanobacteria within the different regions of mussel shells. Species such as *Hyella balani* and *Hormathonema luteo brunnneum* were found in the umbo and in the distal edge except for *Hyella balani* which was found in all three regions in *Mytilus galloprovincialis*. Succession in time was mirrored in spatial distribution of endolithic species along the shell. Species identified as early colonisers (e.g. *Hyella caespitosa, Plectonema terebrans* and *Mastigocoleus testarum*) also characterised the distal edge of the shell. Again, tolerance was exhibited in the form of a gradient of species presence along the shell.

There was overlapping in species distribution in different shell regions with regions close to each other being similar to each other. The umbo was more similar to the centre than it was when compared to the distal edge. Spatial influences are expected in succession as the way species are dispersed plays an important role in how adjacent areas or regions receive new species (Chang & Turner 2019). The source of species to colonise an area is important in succession (Backhaus et al. 1994, Verheyen & Hermy 2001). Succession has been known to be influenced by the distance of source of colonists (Butaye et al. 2001, Cook et al. 2005a, Dzwonko 2019) with places that are closer to the source of colonists being colonised faster than those that a further away. Areas or regions that are closer to each other are likely to be the same as species can easily be dispersed between those regions. Regions closer to each other within a mussel shell were more similar to each other. This might be due to that some regions might have acted as sources of endoliths to other adjacent regions, or simply reflect the dispersal abilities of the species involved.

The results of this chapter clearly show endolithic cyanobacteria succession in mussel shells, there was succession in time with endolithic species identified increasing with time. Succession in space was also observed with different regions of the shell having different endolithic communities when compared to each other and the differences were more pronounced in regions that were further apart from each other, the umbo and the distal edge. Although there was a relatively low level of replication in this chapter, focusing on succession in mussel shells showed that in such cases one can trade time for space because one can equate space and age. Developing an understanding of succession across varying communities will help to access whether there are universal rules guiding the process of succession.

Chapter 6 : General Discussion

Mussels play an important role as ecological engineers in rocky intertidal communities. They form beds which are home to many other species that find refuge and protection there (Borthagaray & Carranza 2007, Arribas et al. 2014). Since mussels act as ecological engineers, any disturbance to them is bound to have a detrimental effect on the many other species that are dependent on them for their survival. Endolithic cyanobacteria live as parasites in mussel shells, they erode and weaken the shells of mussels and they lead to lethal and sub-lethal effects thus endolithic cyanobacterial species modulate the engineering processes of mussels (Marquet et al. 2013, Ndhlovu et al. 2019).

In this study, I assessed the prevalence and lethal impact of endolithic parasitism by endolithic cyanobacteria on two mussel species that are important ecological engineers in intertidal communities, *Perna perna* and *Mytilus galloprovincialis*. The focus was on assessing how endolithic cyanobacteria can affect the energy budgets of mussels, how the effects on the energy budget can affect growth and reproduction, how infestation affects attachment strength to the substratum and how endolithic succession in mussels can happen in space (within mussel shells) and in time.

The thesis assessed the geographic distribution of endolithic cyanobacterial species around the South African coast including the effects of biogeography and host identity. Chapter 2 addressed the following questions: (1) does endolithic species richness differ among bioregions? (2) Does the identity of the host drive parasite diversity? (3) Do the rates of cyanobacterial infestation and mortality due to infestation differ across bioregions? The results showed that infestation increased

with mussel size with no differences between the two mussel species. I found significantly higher infestation in the cool temperate bioregion than in the warm temperate and the subtropical bioregions with the results from the warm temperate and subtropical bioregion being marginally non-significant different in terms of prevalence of infestation.

Endolith induced mortality rates through shell collapse mirrored the patterns for prevalence. For *P. perna*, endolith species assemblages revealed clear grouping by bioregions. My findings indicate that biogeography affects cyanobacterial species composition and that differences between biogeographic regions are driven by environmental conditions rather than host identity. Endolithic cyanobacteria have been shown to occupy a wide range of environmental conditions, their distribution and abundance is influenced by numerous environmental factors including temperature, water, light and nutrient availability (Friedmann 1982, Omelon 2008). Microbial community composition is believed to be influenced by physical and chemical characteristics of their hosts as well as the microenvironmental conditions that are driven by climate (Omelon 2008). In this study where two mussel species, *M. galloprovincialis* and *P. perna* were used to assess the distribution of endolithic cyanobacteria along the three biogeographic regions of the South African coastline, no influence of host characteristics was observed, with no significant differences in prevalence or endolith induced mortality between the two mussel species where they coexist in the warm temperate bioregion. This was despite the fact that the two mussel species are known to be different in terms of shell strength, with *P. perna* having a thicker shell and a thicker periostracum than the invasive *M. galloprovincialis* (Zardi et al. 2009, Bers et al. 2010).

There were more endolith species in the cool temperate bioregion than in the other two bioregions (Table 2.2). This clearly indicates that the driving force for biogeographic differences is climate rather than the physical properties of the host mussels. High prevalence of infestation, high

mortality attributed to endoliths and high endolithic species richness were correlated with environmental factors that are associated with high population densities of organisms as the cool temperate bioregion is characterised by intense upwelling that enhances coastal nutrient availability and primary productivity (Bustamante et al. 1996).

Endolithic cyanobacteria were shown to have a negative influence on scope for growth, reducing the energy available for reproduction and growth. Similarly, endoliths were shown to negatively affect shell building and reproduction in chapter 3. The hypotheses were that endolithic cyanobacteria would have a negative influence on the components of scope for growth, reproduction, growth, shell building and lethal temperatures. It is known that endolithic cyanobacteria live as parasites in mussels (Marquet et al. 2013), and parasites are usually associated with negative impacts on their hosts. The experiments described in chapter 3 showed that, although infested and clean mussels did not differ significantly in rates of food uptake, they did differ in energy expenditure through increased standard metabolic rate.

Chapter 4 further explored the effects of endolithic cyanobacteria on reproductive output (gonad production) and attachment to the substratum, as these are two major energy sinks for mussels and because these contribute to the mussel's survival and fitness. The results showed that infestation has a negative influence on gonad mass and attachment strength to the substratum. This supports the finding of chapter 3 that endolithic infestation leads to low scope for growth for infested mussels. Negative effects of infestation were observed in both mussel species and this suggests that endolithic cyanobacteria species exert the same effects to their host regardless of their identity. Factors such as reduced food availability and increased temperatures negatively influence reproduction and attachment strength (Schurink & Griffiths 1993, Carrington 2002, Lachance et al. 2008). The ability of mussels to survive in intertidal communities is tied to the production of

byssal threads, allowing strong attachment to the substratum (Carrington et al. 2008). With endoliths reducing energy available as scope for growth (chapter 3), there is little energy left for reproduction or to produce byssus making them vulnerable to strong waves.

Endolithic parasites are normally associated with having negative effects on mussels. Endolithic infestation leads to reduced gonad mass for infested mussels (Kaehler & McQuaid 1999, Chapter 4). It leads to death by weakening mussel shells which leads to shell collapse and ultimately death (Marquet et al. 2013, Chapter 2). However, endolithic infestation has also been shown to have positive impacts on the host. Endolithic cyanobacteria have been shown to alter host morphology and reduce mussel vulnerability to high environmental temperatures. Endolithic infestation in mussels is associated with the discolouration of the dark-coloured periostracum to a light grey colour and this grey colouration gives mussels the ability to reduce stressful heat gain during low tide or during periods of extreme heat stress (Zardi et al. 2016, Gehman & Harley 2019). Endolithic infestation was shown to reduce individual body temperatures and infested individuals suffered low mortality compared to non-infested individuals during high heat stress (Zardi et al. 2016). This shows that endolithic organisms not only have negative impacts on their host as shown in results for scope for growth in chapter 3, but they can also have positive impacts especially in periods of extreme heat stress. With the prospect of global warming, endoliths might be a benefit to individual mussels by having a symbiotic relationship with mussels protecting them from heat stress while benefiting from inhabiting and using resources obtained from mussel shells (Zardi et al. 2016).

Another component of the thesis was to examine endolithic succession in mussels through time and in space across the shell. The results in chapter 5 provide evidence of succession through time. This is seen by the increased effects of the same endoliths over time, starting from clean to heavily infested (level D and E) shells. It has been said that if a community of plants or animals is sampled

at different times, there is likely to be differences between the two time periods and also it is more likely that samples collected at adjacent time periods will be similar or the same (Usher 1979). This is also evident in the results from this study (chapter 5), samples collected in successive months were more similar than those collected at times that were further apart and also endolithic species identified at each time interval changed, with increasing numbers of endolith species with each time interval sampled.

Early stages of colonization are generally associated with low species diversity (Ohtonen et al. 1999) and this can be seen in the current study where in the early stages very few endolithic species where identified in shells with the number of endolithic species increasing with time. All organisms are said to affect or be affected by the environment they occupy (Hastings et al. 2007), if the organisms change the environment they occupy they can modulate conditions for other species. Species that arrive later can replace the initial occupants or they can coexist with the earlier occupants.

In my study in chapter 5, I showed that the earlier endolithic colonists coexisted with the late endolithic colonisers. Succession over time was shown in microbial communities, a study of microbes under canopies of mycorrhizal and nonmycorrhizal plants showed not only that biomass of microbes increased with time but that microbial communities shifted from bacterial-dominated to fungal-dominated with fungi species replacing bacteria species (Ohtonen et al. 1999). This was not the case in this study in chapter 5 where the initial colonisers were not replaced by later colonisers but rather coexisted with them.

There was also evidence of succession in space with some regions of the shell having different endolithic species. The distal edge was different from the other two, older regions, the umbo and

the centre, with both *P. perna* and *M. galloprovincialis* exhibiting the same trend. Although many studies tend to focus on chronosequences when studying succession (e.g. Boerner 1985, Walker et al. 2010), there is evidence that spatial differences in sites might be observed as sites tend to have different environmental characteristics (McDonald et al. 2007).

A micro diversity study of *Synechocossus* cyanobacteria showed that there was succession in time and space where the spatial distribution of *Synechocossus* was shown to be higher in summer than in winter and also higher in coastal waters than within an estuary (Mackey et al. 2017). Chapter 5 looked at very fine spatial scales, the results for succession within a shell supported results for succession in time. Endolithic species that were early colonists were similar to the endoliths found in the distal edge which is the growing region of the shell and assemblages of endoliths that were identified in later stages, or late colonists, were similar to those found in the umbo which is the oldest region.

Conclusion and perspectives

Mussels play an important role as habitat forming species (Jones et al. 1994, Gutiérrez et al. 2003). This thesis demonstrates how endolithic cyanobacterial species affect mussels. The influence of endolithic cyanobacterial species on mussels is important as it affects other species that are dependent on mussels as habitat forming species, with clean and infested mussel beds shown to support different infaunal communities (Zardi, unpublished data).

The present thesis demonstrates that the distribution and abundance of endolithic cyanobacterial species are highly dependent on bioregion, with each bioregion experiencing different climatic conditions with high prevalence of infestation and high mortality due to endoliths in the cool temperate bioregion where nutrient levels are higher. This study also revealed that endolithic cyanobacteria affect mussel species the same way regardless of host identity in terms of prevalence

of infestation, mortality and even the process of succession. The enemy release theory is not supported by these findings as the invasive mussel is affected in the same way as the indigenous mussel. This was in contrast with findings from other studies that determined that the invasive mussel *Mytilus galloprovincialis* suffers more from endoliths than the indigenous *Perna perna* (Zardi et al. 2009). Endolithic cyanobacteria were also found to negatively affect the energy budget of mussels resulting in infested mussels having low scope for growth and hence low growth rates and this was also observed with low reproductive output for infested mussels compared to clean mussels. This shows that endoliths as parasites reduce fitness of organisms by reducing their reproductive output as there is less energy channelled to growth and reproduction. This thesis shows that endolithic cyanobacteria have a strong influence on mussels, they negatively affect the energy budget of mussels and hence they potentially have indirect consequences for other species that rely on mussels as ecological engineers for their survival.

Overall, this thesis demonstrates the overarching role played by parasites in shaping communities which is often overlooked. There is considerable evidence on the ways that parasites negatively affect their hosts (Wood & Johnson 2015). This thesis provides a springboard for future studies to focus on the potential impacts posed by parasites on communities rather than their hosts, as the influence of parasites can indirectly have cascading effects on communities especially when they infest ecological engineers.

References

Adams D, DeRuyter Y, Bauwe H, Elhai J, Bergman B, Flores E, Blankenship R, Borner T, Forchhammer K, Fromme P, Kaplan A, Herrero A, Knoll A, Garcia-Pichel F, Kondo T, Hagemann M, Liberton M, Hess W, Los D, Kahlon S, Luque I, Meeks J, Ran L, Mullineaux C, Raymond J, Murata N, Sivonen K, Muro-Pastor A, Stal L, Ogawa T, Suzuki I, Pakrasi H, Swingley W, Terauchi K, Xu X, Wolk C, Zehr J, Zinchenko V (2008) The cyanobacteria: molecular biology, genomics, and evolution. Herrero A, Flores E (eds) Caister Academic Press, Norfolk, UK.

Alfaro AC, Webb SC, Barnaby C (2008) Variability of growth, health, and population turnover within mussel beds of Perna canaliculus in northern New Zealand. Mar Biol Res 4:376–383.

Armesto JJ, Pickett STA (1986) Removal experiments to test mechanisms of plant succession in oldfields. Vegetatio 66:85–93.

Arribas LP, Donnarumma L, Palomo MG, Scrosati RA (2014) Intertidal mussels as ecosystem engineers: Their associated invertebrate biodiversity under contrasting wave exposures. Mar Biodivers 44:203–211.

Assis J, Zupan M, Nicastro KR, Zardi GI, McQuaid CD, Serrão EA (2015) Oceanographic conditions limit the spread of a marine invader along southern African shores. PLoS One 10:1–17.

Awramik SM, Schopf JW, Walter MR (1983) Filamentous fossil bacteria from the Archean of western Australia. Precambrian Res 20:357–374.

Backhaus J, Haems H, Krause M, Heath H (1994) An hypothesis concerining the space-time succession of Calanus finmarchicus in northen North Sea. J Mar Sci:169–180.

Badano EI, Marquet PA (2008) Ecosystem engineering affects ecosystem functioning in high-Andean landscapes. Oecologia 155:821–829.

Barber I, Berkhout BW, Ismail Z (2016) Thermal change and the dynamics of multi-host parasite life cycles in aquatic ecosystems. Integr Comp Biol 56:561–572.

Begon M, Harper J, Townsend C (1986) Ecology. Individuals, populations and communities., Second Edi. Blackwell Scientific Publications, Oxford.

Berg M, Sutula M (2015) Factors affecting the growth of cyanobacteria with special emphasis on the Sacramento-San Joaquin Delta. Southern California Coastal Water Research Project Technical Report 869. Appl Sci.

Berry PF (1978) Reproduction, growth and production in the mussel, Perna perna (Linnaeus), on the east coast of South Africa. South African Assoc Mar Biol Res Investig Rep 48:1–28.

Bers A V., Diaz ER, da Gama BAP, Vieira-Silva F, Dobretsov S, Valdivia N, Thiel M, Scardino AJ, McQuaid CD, Sudgen HE, Thomason JC, Wahl M (2010) Relevance of mytilid shell microtopographies for fouling defence - a global comparison. Biofouling 26:367–377.

Bishop S, Ellis L, JM B (1983) Amino acid metabolism in molluscs. In: Hochachka PW (ed) The Mollusca: metabolic biochemistry and molecular biomechanics, Volume 1. Academic Press, New York.

Blattenbauer A, Knowlen M, Newcomb L (2012) Variation in byssal thread production by

Mytilus trossulus with temperature. Mar Ecol Prog Ser 1250:1–19.

Boerner REJ (1985) Alternate pathways of succession on the Lake Erie Islands. Vegetatio 63:35–44.

Borthagaray AI, Carranza A (2007) Mussels as ecosystem engineers: Their contribution to species richness in a rocky littoral community. Acta Oecologica 31:243–250.

Bower SM, McGladdery SE, Price IM (1994) Synopsis of infectious diseases and parasites of commercially exploited shellfish: Vibrio spp. (Larval and juvenile Vibriosis) of oysters. Annu Rev fish Dis 4:1–199.

Bownes SJ, McQuaid CD (2009) Mechanisms of habitat segregation between an invasive and an indigenous mussel: Settlement, post-settlement mortality and recruitment. Mar Biol 156:991–1006.

Bownes SJ, McQuaid CD (2006) Will the invasive mussel Mytilus galloprovincialis Lamarck replace the indigenous Perna perna L. on the south coast of South Africa? J Exp Mar Bio Ecol 338:140–151.

Branch G, Griffiths C, Branch M, Beckley L (2010) Two Oceans: A guide to the marine life of southern Africa. Struik Publishers, Cape Town, South Africa.

Brett JR (1976) Scope for metabolism and growth of Sockeye Salmon, Oncorhynchus nerka , and some related energetics. J Fish Res Board Canada 33:307–313.

Brey T (2001) Population dynamics in benthic invertebrates. A virtual handbook. http://www.awi-bremerhaven.de/Benthic/Ecosystem/FoodWeb/Handbook/main.html..

Alfred Wegener Institute for Polar and Marine Research, Germany.

Buckley B, Owen M, Hofmann G (2001) Adjusting the thermostat: the threshold induction temperature for the heat-shock response in intertidal mussels (genus Mytilus) changes as a function of thermal history. J Exp Biol 204:3571–3579.

Bustamante RH, Branch GM, Biogeography J, May N, Branch M (1996) Large scale patterns and trophic structure of southern African rocky shores : the roles of geographic variation and wave exposure. J Biogeogr 23:339–351.

Butaye J, Jacquemyn H, Honnay O, Hermy M (2001) The species pool concept applied to forests in a fragmented landscape: Dispersal limitation versus habitat limitation. J Veg Sci 13:27–34.

Calvo-Ugarteburu G, McQuaid CD (1998) Parasitism and introduced species: Epidemiology of trematodes in the intertidal mussels Perna perna and Mytilus galloprovincialis. J Exp Mar Bio Ecol 220:47–65.

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

Carrington E, Gosline JM (2004) Mechanical design of mussel byssus: Load cycle and strain rate dependence. Am Malacol Bull 18:135–142.

Carrington E, Moeser GM, Thompson SB, Coutts LC, Craig CA (2008) Mussel attachment on rocky shores: The effect of flow on byssus production. Integr Comp Biol 48:801–807.

Chang CC, Turner BL (2019) Ecological succession in a changing world. J Ecol 107:503–509.

Charpy L, Casareto BE, Langlade MJ, Suzuki Y (2012) Cyanobacteria in coral reef ecosystems: A review. J Mar Biol 2012:1–9.

Cizauskas CA, Phillips AJ, Carlson CJ, Dougherty ER, Harris NC, Burgio KR, Clements CF (2017) Parasite vulnerability to climate change: an evidence-based functional trait approach. R Soc Open Sci 4:160535.

Cobaugh KL, Schaeffer SM, DeBruyn JM (2015) Functional and structural succession of soil microbial communities below decomposing human cadavers. PLoS One 10:1–20.

Cockell CS, Herrera A (2008) Why are some microorganisms boring? Trends Microbiol 16:101–106.

Cockell CS, Lee P, Osinski G, Horneck G, Broady P (2002) Impact-induced microbial endolithic habitats. Meteorit Planet Sci 37:1287–1298.

Colautti RI, Ricciardi A, Grigorovich IA, Macisaac HJ (2004) Is invasion success explained by the enemy release hypothesis? Ecol Lett 7:721-733. doi: 10.1111/j.1461–0248.2004.00616.x.

Connell JH (1972) Community interactions on marine rocky intertidal shores. Annu Rev Ecol Syst 3:169–192.

Connell JH, Slatyer RO (1977) Mechanisms of succession in natural communities and their role in community stability and organization. Am Nat 111:1119–1144.

Conover R (1966) Assimilation of organic matter by zooplankton. Limnol Oceanogr 11:338–345.

Cook WM, Patrick LB, Yao J, Holt RD, Foster BL (2005a) Secondary succession in an experimentally fragmented landscape: Community patterns across space and time. Ecol Soc Am 86:1267–1279.

Cook WM, Patrick LB, Yao J, Holt RD, Foster BL (2005b) Secondary Succession in an Experimentally Fragmented Landscape: Community Patterns Across Space and Time. Ecology 86:1267–1279.

Couradeau E, Roush D, Guida BS, Garcia-Pichel F (2017) Diversity and mineral substrate preference in endolithic microbial communities from marine intertidal outcrops (Isla de Mona, Puerto Rico). Biogeosciences 14:311–324.

Ćurin M, Peharda M, Calcinai B, Golubić S (2014) Incidence of damaging endolith infestation of the edible mytilid bivalve Modiolus barbatus. Mar Biol Res 10:179–189.

Day EG, Branch GM, Viljoen C (2000) How costly is molluscan shell erosion? A comparison of two patellid limpets with contrasting shell structures. J Exp Mar Bio Ecol 243:185–208.

Dobson AP, Hudson PJ (1986) Parasites, disease and the structure of ecological communities. Tree I:1–12.

Drury WH, Nisbet ICT (1973) Lilac of Arnold Arboretum of Harvard University. J Arnold Arbor 54:331–368.

Dzwonko Z (2019) Effect of proximity to ancient deciduous woodland on restoration of the field layer vegetation in a pine plantation. Nord Soc Oikos 24:198–204.

Emanuel BP, Bustamante RH, Branch GM, Eekhout S, Odendaal FJ (1992) A zoogeographic

and functional approach to the selection of marine reserves on the west coast of South Africa. South African J Mar Sci 12:341–354.

Fearman J-A, Bolch CJS, Moltschaniwskyj NA (2009) Energy Storage and Reproduction in Mussels, Mytilus galloprovincialis : The Influence of Diet Quality . J Shellfish Res 28:305–312.

Fields P, Graham J, Rosenblatt R, Somero G (1993) Effects of expected global climate change on marine faunas. Elsevier Sci Publ 8:361–367.

Fierer N, Nemergut D, Knight R, Craine JM (2010) Changes through time: Integrating microorganisms into the study of succession. Res Microbiol 161:635–642.

Fly EK, Hilbish TJ (2013) Physiological energetics and biogeographic range limits of three congeneric mussel species. Oecologia 172:35–46.

Flye-Sainte-Marie J, Jean F, Paillard C, Kooijman S (2009) A quantitative estimation of the energetic cost of brown ring disease in the Manila clam using Dynamic Energy Budget theory. J Sea Res 62:114–123.

Fredrickson AG, Stephanopoulos G (1981) Microbial competition. Am Assoc Adv Sci Stable 213:972–979.

Friedmann EI (1982) Endolithic microorganisms in the Antarctic cold desert. Science (80-) 215:1045–1053.

Garcia-Longoria L, Marzal A, De Lope F, Garamszegi L (2019) Host-parasite interaction explains variation in the prevalence of avian haemosporidians at the community level. PLoS

One 14:1–17.

Garcia-Pichel F (1998) Solar ultraviolet and the evolutionary history of cyanobacteria. Orig Life Evol Biosph 28:321–347.

Garcia-Pichel F, Ramirez-Reinat E, Gao Q (2010) Microbial excavation of solid carbonates powered by P-type ATPase-mediated transcellular Ca2+ transport. Proc Natl Acad Sci 107:21749–21754.

Gazeau F, Quiblier C, Jansen JM, Gattuso JP, Middelburg JJ, Heip CHR (2007) Impact of elevated CO2 on shellfish calcification. Geophys Res Lett 34:1–5.

Gehman AM, Harley CDG (2019) Symbiotic endolithic microbes alter host morphology and reduce host vulnerability to high environmental temperatures. Ecosphere 10:e02683.

Gektidis M, Dubinsky Z, Goffredo S (2007) Microendoliths of the shallow euphotic zone in open and shaded habitats at 30°N - Eilat, Israel - paleoecological implications. Facies 53:43–55.

Gleason FH, Gadd GM, Pitt JI, Larkum AWD (2017) The roles of endolithic fungi in bioerosion and disease in marine ecosystems. I. General concepts. Mycology 8:205–215.

Glynn PW (1997) Bioerosion and coral reef growth: a dynamic balance. Life death coral reefs:68–95.

Golubic S, Friedmann I, Schneider J (1981) The lithobiontic ecological niche, with special reference To microorganisms. J Sedimantary Petrol 51:475–478.

Golubic S, Radtke G, Le Campion-Alsumard T (2005) Endolithic fungi in marine ecosystems. Trends Microbiol 13:229–235.

Gotelli N, Colwell R (2001) Quantifying biodiversity procedures and pitfalls in the measurement species richness_ecoLet_2001.pdf. Ecol Lett:379–391.

Granovitch A (1992) The Littorina saxatilis (Olivi) in the White Sea. In: *In Proceedings of the third International Symposium on Littorinid Biology*. p 255–263

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

Gutiérrez JL, Jones CG, Strayer DL, Iribarne OO (2003) Mollusks as ecosystem engineers: The role of shell production in aquatic habitats. Oikos 101:79–90.

Hastings A, Byers JE, Crooks JA, Cuddington K, Jones CG, Lambrinos JG, Talley TS, Wilson WG (2007) Ecosystem engineering in space and time. Ecol Lett 10:153–164.

Hatcher MJ, Dick JTA, Dunn AM (2012) Diverse effects of parasites in ecosystems: Linking interdependent processes. Front Ecol Environ 10:186–194.

Helmuth BST, Hofmann GE (2001) Microhabitats, thermal heterogeniety, and patterns of physiological stress in the rocky intertidal zone. Biol Bull 201:374–384.

Herrero A, Muro-pastor AM, Flores E (2001) Minireview nitrogen control in cyanobacteria. J Bacteriol 183:411–425.

Hibbing ME, Fuqua C, Parsek MR, Peterson SB (2010) Bacterial competition: surviving and thriving in the microbial jungle. Natl Rev Microbiol 8:15–25.

Hochachka P, Somero G (2002) Biochemical adaptation. Trends Biochem Sci 10:88–89.

Hoffman L (1999) Marine cyanobacteria in tropical regions: Diversity and ecology. Eur J Phycol

34:371–379.

Hoffmann L (1989) Algae of terrestrial habitats. Springer 55:77–105.

Hudson PJ, Dobson AP (1992) Do parasites make prey vulnerable to predation? Red grouse and parasites. J Anim Ecol 61:681–692.

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

Isomursu M, Rätti O, Helle P, Hollmén T (2008) Parasitized grouse are more vulnerable to predation as revealed by a dog-assisted hunting study. Ann Zool Fennici 45:496–502.

Jones CG, Lawton JH, Shachak M (1994) Organisms as ecosystem engineers. Oikos 69:373–386.

Jumpponen A, Mattson K, Trappe JM, Ohtonen R (1998) Effects of established willows on primary succession on Lyman glacier forefront, North Cascade Range, Washington, U.S.A.: Evidence for simultaneous canopy inhibition and soil facilitation. Arct Alp Res 30:31–39.

Jurgens LJ, Gaylord B (2018) Physical effects of habitat-forming species override latitudinal trends in temperature. Ecol Lett 21:190–196.

Kaehler S (1999) Incidence and distribution of phototrophic shell-degrading endoliths of the brown mussel Perna perna. Mar Biol 135:505–514.

Kaehler S, McQuaid CD (1999) Lethal and sub-lethal effects of phototrophic endoliths attacking the shell of the intertidal mussel Perna perna. Mar Biol 135:497–503.

Karatayev A, Burlakova L, Padilla D (2002) Invasive aquatic species of Europe. Distribution,

impacts and management. Invasive Aquat Speicies Eur:433–446.

Keane RM, Crawley MJ, Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. Trends Ecol Evol 17:164–170.

Knoll AH (2008) Cyanobacteria and earth history. In: *Herrero A,Flores E (eds) The cyanobacteria: molecular biology, genomicsand evolution. Caister Academic Press, Norfolk*. p 1–20

Lachance AA, Myrand B, Tremblay R, Koutitonsky V, Carrington E (2008) Biotic and abiotic factors influencing attachment strength of blue mussels Mytilus edulis in suspended culture. Aquat Biol 2:119–129.

Lafferty K, Kuris A (2009) Parasitic castration: the evolution and ecology of body snatchers. Trends Parasitol 25:564–572.

Lafferty KD (1993) Effects of parasitic castration on growth, reproduction and population dynamics of the marine snail Cerithidea californica. Mar Ecol Prog Ser 96:229–237.

Lafferty KD (2013) Parasites in Marine Food Webs. Bull Mar Sci 89:123–134.

Lasiak T, Dye A (1989) The ecology of the brown Mussel Perna perna in Transkei , Southern Africa : Implications for the management of a traditional food resource. Biol Conserv 47:245–257.

Lathlean JA, Ayre DJ, Minchinton TE (2011) Rocky intertidal temperature variability along the southeast coast of Australia: Comparing data from in situ loggers, satellite-derived SST and terrestrial weather stations. Mar Ecol Prog Ser 439:83–95.

Lauzon-Guay JS, Dionne M, Barbeau MA, Hamilton DJ (2005) Effects of seed size and density on growth, tissue-to-shell ratio and survival of cultivated mussels (Mytilus edulis) in Prince Edward Island, Canada. Aquaculture 250:652–665.

Leung T, Donald K, Keeney D, Koehler A, Peoples R, Poulin R (2009) New dimension in soliton studies. Bull Am Phys Soc 43:857–865.

Levri EP (1998) The influence of non-host predators on parasite-induced behavioral changes in a freshwater snail. Oikos 81:531–537.

Li J, Yang B, Yan Q, Zhang J, Yan M, Li M (2015) Effects of a native parasitic plant on an exotic invader decrease with increasing host age. AoB Plants 7:1–10.

Li S, Shi Y, Zhang Q, Liao X, Zhu L, Lou K (2013) Phylogenetic diversity of endolithic bacteria in Bole granite rock in Xinjiang. Acta Ecol Sin 33:178–184.

Lowe D, Moore M, Bayne B (1982) Aspects of gametogenesis in the marine mussel Mytilus edulis L. J Mar Biol Assoc United Kingdom 62:133–1459.

Lutjeharms JRE, Cooper J, Roberts M (2000) Upwelling at the inshore edge of the Agulhas Current. Cont Shelf Res 20:737–761.

Mackey KRM, Hunter-Cevera K, Britten GL, Murphy LG, Sogin ML, Huber JA (2017) Seasonal succession and spatial patterns of synechococcus microdiversity in a salt marsh estuary revealed through 16s rRNA gene oligotyping. Front Microbiol 8:1–11.

Mao Che L, Le Campion-Alsumard T, Boury-Esnault N, Payri C, Golubic S, Bézac C (1996) Biodegradation of shells of the black pearl oyster, Pinctada margaritifera var. cumingii, by

microborers and sponges of French Polynesia. Mar Biol 126:509–519.

Marcogliese DJ (2005) Parasites of the superorganism: Are they indicators of ecosystem health? Int J Parasitol 35:705–16.

Marcogliese DJ, Cone DK (1997) Food webs: A plea for parasites. Trends Ecol Evol 12:320–325.

Margolis L, Esch G, Holmes JC, Kuris AM, Schad GA (1982) The use of ecological terms in parasitology (Report of an Ad Hoc committee of the American society of parasitologists). J Parasitol 68:131–133.

Marquet N, Nicastro KR, Gektidis M, McQuaid CD, Pearson GA, Serrão EA, Zardi GI (2013) Comparison of phototrophic shell-degrading endoliths in invasive and native populations of the intertidal mussel Mytilus galloprovincialis. Biol Invasions 15:1253–1272.

McCook L (1994) Understanding ecological community succession: Causal models and theories , a review. Vegetatio 110:115–147.

McCormick P, Stevenson R (1991) Mechanisms of benthic algal succession in Lotic. Ecol Soc Am 72:1835–1848.

McDonald RI, Halpin PN, Urban DL (2007) Monitoring succession from space: A case study from the North Carolina Piedmont. Appl Veg Sci 10:193–203.

McQuaid CD, Mostert BP (2010) The effects of within-shore water movement on growth of the intertidal mussel Perna perna : An experimental field test of bottom-up control at centimetre scales. J Exp Mar Bio Ecol 384:119–123.

McQuaid CD, Porri F, Nicastro KR, Zardi GI (2015) Simple, scale-dependent patterns emerge from very complex effects-an example from the intertidal mussels mytilus galloprovincialis and perna perna. Oceanogr Mar Biol An Annu Rev 53:127–156.

Melzner F, Stange P, Trübenbach K, Thomsen J, Casties I, Panknin U, Gorb SN, Gutowska MA (2011) Food supply and seawater pCO_2 impact calcification and internal shell dissolution in the blue mussel Mytilus edulis. PLoS One 6:in the Blue Mussel Mytie24223. doi:10.1371/journal.

Molina R, Hanlon S, Savidge T, Bogan A, Levine J (2005) Buoyant weight technique: Application to freshwater bivalves. Am Malacol Bull 20:49–53.

Moreira C, Vasconcelos V, Antunes A (2013) Phylogeny and biogeography of cyanobacteria and their produced toxins. Mar Drugs 11:4350–4369.

Mouritsen KN, Poulin R (2002) Parasitism, community structure and biodiversity in intertidal ecosystems. Parasitology 124:101–117.

Naylor C, Malby L, Callow P (1989) Scope for growth in *Gammarus pulex*, a freshwater detritivore. Hydrobiologia 188:517–523.

Ndhlovu A, McQuaid C, Monaco C, Marquet N, Nicastro K, Gektidis M, Zardi G (2019) Biogeographical Patterns of Endolithic Infestation in an Invasive and an Indigenous Intertidal Marine Ecosystem Engineer. Diversity 11.

Ndzipa V (2002) Synchronisation of breeding in populations of the brown mussel Perna perna on the south coast of South Africa. Rhodes University

Nicastro KR, Zardi GI, McQuaid CD (2010) Differential reproductive investment, attachment strength and mortality of invasive and indigenous mussels across heterogeneous environments. Biol Invasions 12:2165–2177.

Nicastro KR, Zardi GI, McQuaid CD (2008) Movement behaviour and mortality in invasive and indigenous mussels: Resilience and resistance strategies at different spatial scales. Mar Ecol Prog Ser 372:119–126.

Nishizaki M, Barron S, Carew E (2015) Thermal stress increases fluctuating asymmetry in marine mussels: environmental variation and developmental instability. Ecosphere 6:1–15.

Nweze N (2009) Ecological implications and roles of cyanobacteria (cyanophyta) in food security – A review. Plant Prod Res J 13:8–14.

Ohtonen R, Fritze H, Pennanen T, Jumpponen A, Trappe J (1999) Ecosystem properties and microbial community changes in primary succession on a glacier forefront. Oecologia 119:239–246.

Omelon CR (2008) Endolithic microbial communities in polar desert habitats. Geomicrobiol J 25:404–414.

Passy SI, Larson CA (2011) Succession in stream biofilms is an environmentally driven gradient of stress tolerance. Microb Ecol 62:414–424.

Petes LE, Menge BA, Harris AL (2008) Intertidal mussels exhibit energetic trade-offs between reproduction and stress resistance. Ecol Monogr 78:387–402.

Petes LE, Menge BA, Murphy GD (2007) Environmental stress decreases survival, growth, and

reproduction in New Zealand mussels. J Exp Mar Bio Ecol 351:83–91.

Pigliucci M (1996) How organisms respond to environmental changes: from phenotypes to molecules (and vice versa). Am J Phys Anthropol 5347:168–173.

Ponton F, Otálora-Luna F, Lefvre T, Guerin PM, Lebarbenchon C, Duneau D, Biron DG, Thomas F (2011) Water-seeking behavior in worm-infected crickets and reversibility of parasitic manipulation. Behav Ecol 22:392–400.

Porter D, Lingle WL (1992) Endolithic thraustochytrid marine fungi from planted shell fragments. Mycol Soc Am 84:289–299.

Potter DA, Potter MF (2009) Insect borers of trees and shrubs. Coop Ext Serv Univ Kentucky Coll Agric Food Environ 43:1–7.

Poulin R (1998) The functional importance of parasites in animal communities: many roles at many levels? Int J Parasitol 29:903–914.

Redford AJ, Fierer N (2009) Bacterial succession on the leaf surface: A novel system for studying successional dynamics. Microb Ecol 58:189–198.

Resgalla C, Elisângela J, Brasil DS, Salomão LC (2007) The effect of temperature and salinity on the physiological rates of the mussel Perna perna (Linnaeus 1758). Brazilian Arch Biol Technol 50:543–556.

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

Rodolfo-Metalpa R, Houlbrèque F, Tambutté É, Boisson F, Baggini C, Patti FP, Jeffree R, Fine

M, Foggo A, Gattuso JP, Hall-Spencer JM (2011) Coral and mollusc resistance to ocean acidification adversely affected by warming. Nat Clim Chang 1:308–312.

Roff D (1993) The evolution of life histories: Theory and analysis. Rev Fish Biol Fish 3:384–385.

Rosas C, Sánchez A, Chimal ME, Brito R (2003) Manual de Métodos para la Evaluación del Balance Energético en Crustáceos. Jornadas Iberoam Nutr en Acuic 7:1–43.

Sarà G, Pusceddu A (2008) Scope for growth of Mytilus galloprovincialis (Lmk., 1819) in oligotrophic coastal waters (Southern Tyrrhenian Sea, Italy). Mar Biol 156:117–126.

Schneider KR (2014) Mussel under a variety of thermal conditions heat stress in the intertidal: Comparing survival and growth of an invasive and native mussel under a variety of thermal conditions. Biol Bull 215:253–264.

Schurink van E, Griffiths CL (1993) Factors affecting relative rates of growth in four South African mussel species. Aquaculture 109:257–273.

Seckbach J (ed) (2007) Algae and cyanobacteria in extreme environments, 11th ed. The Hebrew University of Jerusalem, Israel, Jerusalem, Israel.

Seed R, Suchanek T (1992) The mussel Mytilus: Ecology, physiology, genetics and culture. J Exp Mar Bio Ecol 174:281–283.

Shashar N, Stambler N (1992) Endolithic algae within corals - life in an extreme environment. J Exp Mar Bio Ecol 163:277–286.

Shestakov S V, Karbysheva EA (2017) The origin and evolution of cyanobacteria. Biol Bull Rev

7:259–272.

Smith AS V, Key GS (1975) Carbon dioxide and metabolism in marine environments carbon. Limnol Oceanogr 20:493–495.

Sokolova I, Frederich M, Bagwe R, Lannig G, Sukhotin A (2012) Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. Mar Environ Res 79:1–15.

Sokolova IM, Portner H (2001) Physiological adaptations to high intertidal life involve improved water conservation abilities and metabolic rate depression in. Mar Ecol Prog Ser 224:171–186.

Somero GN (2002) Thermal physiology and vertical zonation of intertidal animals: Optima, limits, and costs of living. Integr Comp Biol 42:780–789.

Sousa W (1979) Experimental investigations of disturbance and ecological succession in a rocky intertidal algal community. Ecol Soc Am 49:227–254.

Steffani CN, Branch GM (2003) Spatial comparisons of populations of an indigenous limpet scutellastra argenvillei and an alien mussel mytilus galloprovincialis along a gradient of wave energy. African J Mar Sci 25:195–212.

Stumpp M, Wren J, Melzner F, Thorndyke MC, Dupont ST (2011) CO2 induced seawater acidification impacts sea urchin larval development I: Elevated metabolic rates decrease scope for growth and induce developmental delay. Comp Biochem Physiol - A Mol Integr Physiol 160:331–340.

Suchanek TH (1985) Mussels and their role in structuring rocky shore communities. Moore P, Seed R (eds) Hodder & Stoughton Press, London, UK.

Tagliarolo M, Clavier J, Chauvaud L, Koken M, Grall J (2012) Metabolism in blue mussel: Intertidal and subtidal beds compared. Aquat Biol 17:167–180.

Tagliarolo M, McQuaid CD (2016) Field measurements indicate unexpected, serious underestimation of mussel heart rates and thermal tolerance by laboratory studies. PLoS One 11:e0146341. doi:10.1371/journal.pone.0146341.

Tagliarolo M, McQuaid CD (2015) Sub-lethal and sub-specific temperature effects are better predictors of mussel distribution than thermal tolerance. Mar Ecol Prog Ser 535:145–159.

Thomas F, Poulin R (1998) Manipulation of a mollusc by a trophically transmitted parasite: Convergent evolution or phylogenetic inheritance? Parasitology 116:431–436.

Tomalin BJ (1995) Growth and mortality rates of brown mussels Perna perna (Linnaeus) in Kwazulu-Natal : a comparison between sites and methods using non-parametric length-based analysis. South African Assoc Mar Sci 16:241–254.

Tomanek L (2002) Physiological ecology of rocky intertidal organisms: A synergy of concepts. Integr Comp Biol 42:771–775.

Tomitani A, Knoll A, Cavanaugh C, Ohno T (2006) The evolutionary diversification of cyanobacteria: Molecular-phylogenetic and paleontological perspectives. Proc Natl Acad Sci 103:442–447.

Torchin EM, Miura O, Hechinger RF (2015) Parasite species richness and intensity of

interspecific interactions increase with latitude in two wide-ranging hosts. Ecol Soc Am 2:183–190.

Torchin ME, Lafferty KD, Dobson AP, McKenzie VJ, Kuris AM (2003) Introduced species and their missing parasites. Nature 421:628–630.

Torchin ME, Lafferty KD, Kuris AM (2002) Parasites and marine invasions. Parasitology 124.

Torchin ME, Lafferty KD, Torchin ME, Lafferty KD (2009) Escape from Parasites 11.1 Introduction. Biol Invasions Mar Ecosyst:203–214.

Torchin ME, Mitchell CE (2004) Parasites, pathogens, and invasions by plants and animals. Front Ecol Environ 2:183–190.

Tribollet A, Langdon C, Golubic S, Atkinson M (2006) Endolithic microflora are major primary producers in dead carbonate substrates of Hawaiian coral reefs. J Phycol 42:292–303.

Usher BYMB (1979) Markovian approaches to ecological succession. Br Ecol Soc 48:413–426.

Verheyen K, Hermy M (2001) The relative importance of dispersal limitation in secondary forest succession. J Ecol 89:829–840.

Vogel K, Frankfurt M (1993) Bioeroders in fossil reefs. Erlangen:109–113.

Wacker A, Von Elert E (2003) Food quality controls reproduction of the zebra mussel (Dreissena polymorpha). Oecologia 135:332–338.

Walker LR, Wardle DA, Bardgett RD, Clarkson BD (2010) The use of chronosequences in studies of ecological succession and soil development. J Ecol 98:725–736.

Walker R, Leonard P (1975) Ecological succession as an aspect of sructure in fossil communities. Paleobiology 1:238–257.

Ward ME, Shields JD, Van Dover CL (2004) Parasitism in species of Bathymodiolus (Bivalvia: Mytilidae) mussels from deep-sea seep and hydrothermal vents. Dis Aquat Organ 62:1–16.

Wejnerowski Ł, Rzymski P, Kokociński M, Meriluoto J (2018) The structure and toxicity of winter cyanobacterial bloom in a eutrophic lake of the temperate zone. Ecotoxicology 27:752–760.

Widdows AJ, Donkin P, Brinsley MD, Evans S V, Salkeld PN, Franklin A, Law RJ, Waldock MJ, Widdows J, Donkin P, Brinsley MD (1995) Scope for growth and contaminant levels in North Sea mussels Mytilus edulis. 127:131–148.

Widdows J, Staff F (2006) ICES techniques in marine environmental sciences. determination of perfluoroalkyl compounds in water, sediment, and biota.

Wierzchos J, Ascaso C, McKay CP (2006) Endolithic cyanobacteria in halite rocks from the hyperarid core of the Atacama desert. Astrobiology 6:415–422.

Wierzchos J, DiRuggiero J, Vítek P, Artieda O, Souza-Egipsy V, Škaloud P, Tisza MJ, Davila AF, Vílchez C, Garbayo I, Ascaso C (2015) Adaptation strategies of endolithic chlorophototrophs to survive the hyperarid and extreme solar radiation environment of the Atacama Desert. Front Microbiol 6:1–17.

Wierzchos J, de los Ríos A, Ascaso C (2012) Microorganisms in desert rocks: The edge of life on Earth. Int Microbiol 15:173–183.

Wilbur AE, Hilbish TJ (1989) Physiological energetics of the ribbed mussel Geukensia demissa (Dillwyn) in response to increased temperature. J Exp Mar Bio Ecol 131:161–170.

Wood C, Johnson T (2015) A world without parasites: exploring the hidden ecology of infection. Front Ecol Environ 13:425–434.

Young GA (1985) Byssus-thread formation by the mussel Mytilus edulis: effects of environmental factors. Mar Ecol Prog Ser 21:261–2.

Zardi GI, McQuaid CD, Nicastro KR (2007) Balancing survival and reproduction: Seasonality of wave action, attachment strength and reproductive output in indigenous Perna perna and invasive Mytilus galloprovincialis mussels. Mar Ecol Prog Ser 334:155–163.

Zardi GI, Nicastro KR, McQuaid CD, Castilho R, Costa J, Serrão EA, Pearson GA (2015) Intraspecific genetic lineages of a marine mussel show behavioural divergence and spatial segregation over a tropical/subtropical biogeographic transition. BMC Evol Biol 15:1–11.

Zardi GI, Nicastro KR, McQuaid CD, Erlandsson J (2008) Sand and wave induced mortality in invasive (Mytilus galloprovincialis) and indigenous (Perna perna) mussels. Mar Biol 153:853–858.

Zardi GI, Nicastro KR, McQuaid CD, Gektidis M (2009) Effects of endolithic parasitism on invasive and indigenous mussels in a variable physical environment. PLoS One 4:https://doi.org/10.1371/journal.pone.0006560.

Zardi GI, Nicastro KR, Mcquaid CD, Ng TPT, Lathlean J, Seuront L (2016) Enemies with benefits: parasitic endoliths protect mussels against heat stress. Sci Rep 6:https://doi.org/10.1038/srep31413.

Zobel M (1997) The relative of species pools in determining plant species richness: an alternative explanation of species coexistence? Trends Ecol Evol 12:266–269.