Ecology and Ecophysiology of *Zostera capensis*: *Responses and Acclimation to Temperature*

Cloverley Mercia Lawrence

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Dr Deena Pillay, Professor Astrid Jarre, Emer. Professor John Bolton

This thesis is dedicated to my parents.

Declaration

I, hereby declare that all the work presented in this thesis is my original work in both concept and execution (except where otherwise indicated), and that this thesis has not been submitted in whole or in part for another degree in this or any other university.

Signed by candidate

Cloverley Mercia Lawrence

Table of Contents

Chapter One

Chapter Two

Chapter Three

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Summary

As environmental norms deviate further from long-established patterns, understanding strategies that allow habitats to persist under sub-optimal conditions is critical for effective mitigation and management. It has therefore become profoundly important to understand the capacity of ecosystems to respond to anthropogenically-driven change, and maintain their function and provision of services to humankind. Species and populations that lack the ability to persist in a changing environment run the risk of declining or going extinct. Two key evolutionary processes available to organisms to cope with shifting environments are adaptation and acclimation. Adaptation is a genetically-based response, while acclimation or phenotypic plasticity is a prompt, non-genetic strategy usually triggered by a change in environmental conditions. Both adaptive and acclimative mechanisms can co-occur producing varied effects at the population level.

Seagrasses are ecosystem engineers responsible for providing an important habitat in many estuaries and on coastlines worldwide. They are, however on a global decline due to threats from human and natural influences. Thermal stress from diurnal and seasonal changes as well as climatic factors, affects ambient temperatures in seagrass habitats, which influence their productivity and distribution. Photosynthesizing plants that occur in dynamic temperature environments need to adapt or acclimate in order to avoid damage when environmental conditions are sub-optimal for growth and photosynthesis.

In South Africa, the eelgrass *Zostera capensis* provides habitats that support a diversity of invertebrates and algae which in turn supply nourishment to fish and birds in many estuarine systems. *Zostera* populations show high variability while in some estuaries, they have been completely lost, implying an associated loss in productivity and biodiversity. This study aimed to first determine the key environmental factors responsible for spatial and temporal variability in *Zostera* habitats and its associated macro-epifauna in Langebaan Lagoon, a sheltered habitat and marine protected area that forms part of the West Coast national park in South Africa. The second aim was to explore the effects of warming (above average ambient temperatures), and extreme high temperature conditions on two

morphotypes of *Zostera* using morphometry, fluorometry, chromatography and biochemistry in order to understand this species response and ability to maintain function under thermal stress. Warming can potentially benefit especially temperate species living in below optimum temperatures, while the intensely negative effects of extreme high (supra-optimal) temperatures for example from heat waves, influences their distribution. Lastly, the role of grazing was investigated to assess the influence of this trophic interaction in maintaining seagrass performance under thermal stress. The overall aim was therefore to understand the physiological strategies that enable *Zostera capensis* to adapt and acclimate to extreme temperature conditions.

Field surveys identified significant distinctions in shoot densities, leaf size and biomass of *Zostera* populations at sites close to the lagoon mouth compared to those at the southern end of the lagoon, with characteristic seasonal responses. Summer temperatures led to considerable seagrass diebacks across sites with recovery observed when conditions were more favourable. The main factors that determined spatial and temporal variability in seagrass beds were found to be exposure, temperature and turbidity. *Zostera* populations exposed for a longer time during low tide produced a small-leaved morphotype (±8.6 cm in length) that occurred in high densities (760 shoots m^{-2}). Conversely, deeper intertidal beds supported a large-leaved morphotype (±20.7 cm in length) in sparser beds (346 shoot m^{-2}). These morphological traits reflect a phenotypic response that enables a population to acclimate to particular environmental conditions that also changes its characteristics and interactions. For example, large-leaved populations are able to support a higher epiphyte load than small-leaved populations. Acclimation is a fast, non-genetic mechanism that results in structural or physiological changes in response to the prevailing environment. Gene flow between populations in the lagoon is assumed to be high and morphotypic differences between high and low shore populations are likely attributed to phenotypic rather than genetically influenced responses.

Macro-epifaunal communities associated with *Zostera* in Langebaan Lagoon were dominated by grazing invertebrates that feed largely on epiphytes. Species diversity

and richness were significantly different across sites and higher in large-leaved beds closer to the lagoon mouth as opposed to small-leaved beds. In contrast, small-leaved populations supported greater species abundances compared to large-leaved, which was dominated by two desiccation-resistant gastropods, *Assiminea* sp. and *Siphonaria compressa*. Seasonal differences in macro-epifauna were also significant due mainly to variation between warmer and cooler months. In general, abundances were highest in spring and summer while diversity and richness were highest during autumn and winter. Shoot densities and leaf width produced positive direct effects on macro-epifaunal abundances along with turbidity and oxygen. Indirectly, temperature, pH and exposure negatively affected macro-epifaunal abundance, and despite the lack of direct observations of temperature effects on macro-epifauna, temperature had a direct effect on seagrass structure and was a significant predictor of variation in five of the six seagrass metrics measured. Temperature is therefore a key factor influencing variation in macro-epifauna and seagrass structure in the lagoon.

An indoor closed mesocosm system was developed to investigate the effects of warming on both large and small-leaved morphotypes of *Zostera* to determine optimum temperatures for growth, and test the hypothesis that small-leaved morphotypes show better performance (aboveground biomass, leaf morphometrics and density) under heat stress than large-leaved morphotypes, and thereby predict the potential for expansion of seagrass stands in Langebaan Lagoon in scenarios of increasing temperature. The experiment revealed similar performance in small and large-leaved morphotypes under prolonged (six weeks) warming, with a general increase in growth metrics at cool (22^{o}C) temperatures. Significant declines were observed in supra-optimal (26^{o}C and 30^{o}C) temperatures in the two morphotypes compared to pre-treatment measurements. In addition, epiphyte loads were significantly greater on large- compared to small-leaved morphotypes. The hypothesis that small-leaved morphotypes perform better under heat stress relative to large-leaved morphotypes was refuted. In Langebaan, small-leaved populations grow in the high intertidal and are exposed to distinctive stresses from extreme high temperatures which alternate between submergence (in water) and emergence (exposed to air). Simulation of these environmental conditions were not provided in

this experiment, and these results therefore explain differences in morphotypes under submerged temperature scenarios only. These results further elucidate the ability of *Zostera* to adapt to local environmental conditions. Despite its broad thermal range from temperate to tropical waters, tolerance to thermal stress is limited, potentially confining populations to specific habitat and environmental contexts.

In a second mesocosm experiment that simulated a period of air exposure, photosynthetic rate (i.e. effective quantum yield (Y)), carbon (C), nitrogen (N) and secondary metabolites (i.e. gallic acid (GA)) were measured in each morphotype in order to determine intrinsic variability in fitness. While declines in shoot densities under heat stress were observed in the two morphotypes similar to the first experiment, small-leaved morphotypes (SLM) had higher levels of C, N and GA compared to large-leaved morphotypes (LLM). In addition, SLM had generally higher photosynthetic rates compared to LLM implying that small-leaved populations are better able to maintain productivity under heat stress. Greater photosynthetic output, above that needed for growth and metabolism, results in carbohydrates being stored as secondary metabolites, which would explain the higher phenolic content in (SLM) under warming. Photosynthetic rate was not significantly reduced in (LLM) under warming, and here photosynthetic output appears to have been channelled into maintaining above and belowground biomass (which was significantly greater in LLM compared to SLM), rather than being stored as secondary metabolites. Therefore, SLM displayed greater fitness compared to LLM based on its higher photosynthetic rates and ability to store resources (C and N) under warming.

The second experiment also investigated the interactive effects of supra-optimal temperatures and grazing on each morphotype of *Zostera* to test the hypothesis that grazing of epiphytes mitigates the effects of heat stress. This would be achieved by reducing stress from fouling thereby allowing for seagrasses to perform better under warming when grazing was present compared to non-grazed treatments. The positive effects of grazing on seagrass performance have been reported for several seagrass species particularly in mitigating the effects of temperature and these effects were marginally supported in this experiment. The direct effect of grazing was significant and positive on seagrass biomass, while the interaction between grazing and temperature had significant effects on leaf area and photosynthetic rates. The extent to which grazing influenced responses in *Zostera* morphotypes was largely a consequence of variation in biomass and leaf size. Grazing however, did not significantly influence epiphyte biomass and epiphytes generally proliferated under warming conditions for the two morphotypes despite the presence of grazers. This could potentially be the consequence of factors such as experimental grazer densities being ineffective in controlling the unpredicted extent of algal growth in heated treatments. Even so, there is a lack of direct evidence that *Siphonaria compressa*, the grazer used in this experiment, feeds exclusively on epiphyton, so these results should be interpreted with caution.

Trends in *Zostera* cover in Langebaan Lagoon show greatest declines closer to the mouth, which implies a decline in associated macro-epifaunal communities that large-leaved populations support. Low concentrations of phenolic compounds in large-leaved populations as well as their propensity to algal fouling suggests lower resilience and thus greater susceptibility to other stressors such as disease and pathogens compared to small-leaved populations.

This study has provided a clearer understanding of the key spatial and temporal factors explaining variability of *Zostera capensis* and associated macro-epifauna in Langebaan Lagoon. It is likely that climate-change induced warming will promote greater declines in *Zostera* cover with greater loss of large-leaved populations. This is expected to influence associated macro-epifaunal communities with negative effects on other trophic levels in the system. Insights into the influence of trophic interactions particularly when seagrasses are under stress were important to understanding resilience in *Zostera* populations, with particular interest in adaptation and acclimation under predicted warming scenarios.

The findings from this study have established a foundation on which to further research and monitoring of *Zostera* in Langebaan Lagoon. Efforts to maintain and restore seagrass habitats include reducing direct disturbances from e.g. trampling and bait collecting, and managing indirect human impacts. Raising awareness of the value of seagrasses at many levels of environmental governance is also critical to their protection. Effective monitoring programmes that track perpetual variability in seagrass ecosystems is essential to inform research and management initiatives. The findings from this research can be used to explore ecophysiological responses to warming and effects of grazing in marine plants in other systems.

CHAPTER ONE

General Overview and Background

Understanding the capacity of ecosystems to respond to anthropogenically-induced pressures and maintain their function and provision of services to humankind, has generated substantial interest (Bazzaz 1990, Jump and Peñuelas 2005, Pörtner et al. 2014, Reusch 2014, Short et al. 2016). Key findings have highlighted the importance of understanding stress responses to changing environments particularly to maintain fitness and performance, at species and population levels (Pörtner and Knust 2007, Pörtner 2008), while effects on biodiversity (Harley 2011, Wernberg et al. 2011, Eklöf et al. 2012, Hooper et al. 2012) and trophic interactions (Morelissen and Harley 2007, Hoekman 2010, Werner et al. 2016, Pagès et al. 2017) have also gained significance. Species and populations that lack appropriate means to contend with a changing environment run the risk of declining or going extinct. Two key evolutionary processes available to organisms and distinct strategies by which they can cope with shifting environments at both small and large-scales, are adaptation and/or acclimation.

Adaptation is a genetically based process, and while it enhances performance and fitness, the response rate is not rapid enough to keep pace with anthropogenic-driven environmental change (Sultan 2000). Acclimation or phenotypic plasticity on the other hand, is a faster, non-genetic mechanism which includes the epigenetic alteration of the genome (Duarte et al. 2018), and can be expressed in structural or physiological change in response to environmental stress. Both adaptive and acclimative mechanisms can co-occur producing varied effects at the population level (Merilä and Hendry 2014).

Rising carbon dioxide and temperatures are altering seawater chemistry with consequences on physiological performance and productivity that in turn modify community structure and behaviour of marine species at all trophic levels (Doney et al. 2012). Water depth is significant in partitioning diversity since light is a key factor determining vertical distribution particularly of autotrophs (Ferguson et al. 2016, Olesen et al. 2017). The exponential diminishing of light quality along a depth gradient due mainly to scattering and absorption, stimulates photo-acclimation plasticity displayed through several morphological and physiological adaptations to maintain photosynthesis and a positive carbon balance in a varying spectral habitat (Dattolo et al. 2017).

Photosynthesizing marine macrophytes (e.g. seagrasses and macroalgae) form the foundation of marine food webs, and fulfil important roles as both primary producers and ecosystem engineers, since they influence the structure and function of ecosystems, thus supporting high biodiversity along many coastlines worldwide (Brothers et al. 2013, Bourque and Fourqurean 2014, Teagle et al. 2017). It is therefore important to identify the ability of species and populations to adapt and acclimate to environmental variation in order to understand and model the response in natural systems to rapid anthropogenically-driven change.

1.1. Seagrass Ecosystems

1.1.1. Global Importance and Distribution of Seagrass Ecosystems

Seagrass ecosystems provide key ecological services to people and have received interest as a result of their complex biological interactions, their immense productivity, and their associations with species of value to global fisheries (Nordlund et al. 2018). Found on many tropical and temperate coastlines around the world, they form highly diverse ecosystems. Much research has focused on the distribution and extent of seagrasses (Kendrick et al. 2002, Marbà et al. 2002, , Spalding et al. 2003, Yang and Yang 2009), the factors influencing their distributional patterns (Ruiz et al. 2001, Teske and Wooldridge 2003, Bornman et al. 2008, Chefaoui et al. 2016), ecological interactions and structuring influences of associated biodiversity (Jernakoff et al. 1996, Heck et al. 2008, Rasheed et al. 2008, Vonk et al. 2015), including the influence of grazing (Heck and Valentine 1995, Duffy et al. 2003, Steele and Valentine 2015, Mutchler and Hoffman 2017), and the effects of climate change (Short and Neckles 1999, Taylor et al. 2011, Pergent et al. 2014, Short et al. 2016), particularly temperature (Leegood 1995, Olsen et al. 2012, Collier and Waycott 2014, Valle et al. 2014, Kaldy et al. 2015, Repolho et al. 2017), on seagrasses.

In many parts of the world, coastlines with seagrasses are protected by the reduction in impacts from waves and currents (Fonseca and Cahalan 1992, Ondiviela et al. 2014). The canopies of seagrass meadows have been shown to dissipate wave energy and intercept harmful pollutants (Barbier et al. 2011). Seagrasses form dense meadows that trap organic material in their root-rhizomes (Larkum et al. 2006) which stabilize marine sediments (Wabnitz et al. 2008). Seagrass habitats aid in cycling nutrients by exporting carbon, nitrogen and phosphorous into surrounding coastal and oceanic food webs (Beck et al. 2001, Duarte et al. 2006, Larkum et al. 2006). They are natural carbon sinks, and can store relatively more carbon per unit area (~17 tons C ha^{-1} year^{-1}, Duarte et al. 2005) than the Amazonian rainforest (1.02 tons C ha^{-1} year^{-1}, Grace et al. 1995). Even though they occupy an area of 0.2% of the global ocean (Duarte and Cebrián 1996), they are estimated to absorb nearly 10% of the yearly estimate of carbon buried in the ocean (Duarte et al. 2004), thus contributing to reductions in greenhouse gases in the atmosphere (Fourqurean et al. 2012, Mazarassa et al. 2015).

Globally, seagrasses are present in many intertidal and subtidal systems to depths of 50 m, and are found in tropical and cool temperate environments including the Arctic (Green and Short 2004). Despite their global spread the number of seagrass species currently stands at ~60 belonging to 15 genera derived from four main families: Posidoniaceae, Zosteraceae, Hydrocharitaceae and Cymodoceaceae, with the latter three independent lineages arising from within a single monocot order Alismatales between 70-100 million years ago (Les and Tippery 2011, Reveal and Chase 2011). Seagrass species count is taxonomically low when compared to flowering plants in the terrestrial environment (~250 000 species). The seagrass lineage is also distinct from other marine plants such as mangroves, seaweeds and salt marsh species, which have evolved from several diverse lines. Despite their modest species richness, seagrasses extend biogeographically to all but the extreme Polar Regions (Short et al. 2007), while the geographic and latitudinal ranges of other marine plants i.e. salt marsh species and mangroves, remain restricted to mainly temperate and tropical regions.

Short et al. (2007) separated seagrass habitats into six bioregions based on the main ocean they occurred in, their distributional range and species assemblage, and the influence of tropical or temperate climates. Seagrasses are represented in two tropical and four temperate bioregions, ranging in diversity from five species in the Temperate North Atlantic (Portugal to North Carolina, USA) to 24 species in the Tropical Indo-Pacific (South Asia, eastern Pacific to tropical Australia and East Africa) (Short et al. 2007). In this classification, South Africa is grouped within the Temperate Southern Oceans along with South America, temperate Australia and New Zealand, despite the presence of a subtropical zone on the north east South African coast. A total of 18 species was documented in this bioregion, with only four species recorded for South Africa: the dominant temperate species *Zostera capensis* Setchell and *Ruppia maritima* Linnaeus, and the more sparsely distributed tropical/temperate species *Halophila ovalis* and tropical *Thalassodendron ciliatum* (Short et al. 2007) due to the limited extent of tropical coastline in South Africa. *T. ciliatum* has since been separated from its rockyshore counterpart, described by Duarte et al. (2012), it is now called *T. leptocaule*. The number of seagrass species in South Africa remains at four since *T. ciliatum* has not been recorded in South Africa (Browne et al. 2013).

The eelgrass, *Zostera capensis,* is sparsely distributed in mixed seagrass meadows as far as northern Kenya but forms large monospecific stands in southern Mozambique and South Africa (Bandeira and Gell 2003), creating a critical habitat of great ecological and economic importance (Whitfield et al. 1989, Ter Morshuizen and Whitfield 1994, Barnes 2010). A revision of the Zosteraceae family by Coyer et al. (2013) grouped *Z. capensis* into the more recently described genus, *Nanozostera* (Clade III), however Jacobs and Les (2009) argued although each clade could be acknowledged as a discrete genus, robust morphological and molecular differentiation between genera was lacking. A phylogenetic assessment by Tanaka et al. (2003) suggested combining the genus *Heterozostera* with the subgenus *Zosterella* under the genus *Nanozostera.* This created further discrepancies in nomenclature since an older genus name (*Heterozostera*; Hartog 1970) takes precedence over a newer one (*Nanozostera*; Tomlinson and Posluszny 2001). Jacobs and Les (2009) proposed the clear phylogenetically differentiated lineages of *Zostera* and *Phyllospadix* be the two accepted genera within Zosteraceae. It is perhaps because of these inconsistencies that the World Register of Marine Species (WoRMS) and AlgaeBase (www.algaebase.org) retain the original genus for *Zostera capensis* which is also retained in this thesis.

Seagrass ecosystems have been under-appreciated, and distributional information is still lacking for many countries, despite the high cultural and economic value of these systems to coastal communities (Green and Short 2004, Barbier et al. 2011, Coles et al. 2011,). Distributional information aids in our understanding of the contribution of seagrass systems to regional and global productivity and biodiversity. Furthermore, these ecosystems are dynamic and subject to considerable change (Duarte et al. 2006), and quantifying their distribution helps establish a baseline on which to document long-term change (Larkum and West 1990, Hall et al. 1999, Kim et al. 2015,). A synthesis of the status and distribution of seagrasses and their associated ecosystems is critical in steering conservation and management efforts, as well as defining priorities for biodiversity and ecosystem services at national and international levels.

1.1.2. Biodiversity in Seagrass Ecosystems

Primary productivity generated from seagrasses as well as their associated benthic and epiphytic algae, creates a highly productive ecosystem. Seagrasses provide a wide spectrum of permanent and transient niches and microhabitats to a diversity of benthic and pelagic organisms (Edgar 1990, Edgar and Robertson 1992, Robbins and Bell 1994). Their 3-dimensional structure in the form of roots, stems and leaves creates a holdfast on which algae and filter-feeders attach. They in turn provide nourishment to a variety of grazing invertebrates and predators (Orth and van Montfrans 1984, Orth et al. 1984). Elevated levels of biomass and greater abundance of invertebrates have been recorded from seagrass beds compared to unvegetated sandflats (Orth and van Montfrans 1984, Edgar 1990, Edgar et al. 1994). In this way, seagrasses support a diversity of invertebrates, fish and birds and sustain a vast assemblage of epiphytic and surface-dwelling fauna uniquely associated with seagrass habitats (Heck et al. 2008, Vonk et al. 2008).

Several studies have demonstrated the use of seagrass beds as shelter by fish and invertebrate fauna as well as sites for foraging and functioning as a nursery (Perkins-Visser et al. 1996, Nagelkerken et al. 2000, Horinouchi 2007, MacArthur and Hyndes 2007, Barbier et al. 2011). A number of species including rare and/or threatened ones use seagrass habitats for part of their life cycle either as juveniles or during breeding, e.g. temperate species of seahorses (Spalding et al. 2003, Teske et al. 2007). In tropical systems, seagrasses are a primary food source for the world's largest marine herbivores i.e. manatees (Genus: *Trichechus*) and dugongs (*Dugong dugon*), as well as green seaturtles (*Chelonia mydas*) (Beck et al. 2001), all perceived to be of great aesthetic, cultural and intrinsic value. The provision of food and refugia for commercially important species highlights the economic services seagrass habitats provide (Costanza et al. 1997). Loss of seagrass habitats has been related to a decline in fish production (Jackson et al. 2001, Nordlund et al. 2018, Unsworth et al. 2019) and invertebrate biomass (Webster et al. 1998, McCloskey and Unsworth 2015), and implies a loss of associated species diversity many of high commercial and economic value (Blandon and Zu Ermgassen 2014, Tuya et al. 2014). Combatting the loss of globally productive and valued seagrass habitats is therefore becoming a priority on many conservation agendas.

Seagrasses have uniquely high light requirements for growth, ranging from 5 – 35% of surface irradiance (Dennison et al. 1993, Duarte 2007) and favour shallow coastal environments (Orth 2006). This is explained by their propensity to inhabit anoxic, sulphide-rich substrates. Photosynthesis and subsequent productivity is reduced as a result of sulphide toxicity (Holmer and Bondgaard 2001, Holmer et al. 2005). Under such reducing conditions metabolic processes in the rhizosphere must nonetheless be sustained, and the greater requisite for light is mainly to support photosynthetic activity to maintain the respiratory needs of a large non-photosynthesizing biomass (Orth et al. 2006). Seagrasses grow as a patchwork, as well as in varying shoot sizes, patterns attributed to environmental influences (Kirkman and Kuo 1990, Niu et al. 2012, Tolieng et al. 2017). The availability of space for colonization by epiphytic algae and fauna as well as their propagules is therefore influenced by seagrass morphology, surface area, growth, life-span and leaf structural properties (Jernakoff et al. 1996). The modification of seagrass shoot structure due to biological (e.g. grazing) or environmental factors, influences the space available for settlement and colonization by epibionts, which in turn affects overall biodiversity and productivity of a seagrass meadow (Orth and van Montfrans 1984, Jernakoff et al. 1996).

1.2. Threats to Seagrass Ecosystems

Seagrass meadows have declined globally by as much as 29% over a 140 year period, with the remaining meadows reported to be continuing to decline (Waycott et al. 2009). The rate of loss of seagrasses has been estimated to be up to 7% per year surpassing that of tropical forests and on par with other key service-providing ecosystems such as mangroves and coral reefs (Orth et al. 2006, Waycott et al. 2009, Short et al. 2014). Nearly a quarter of the ~60 seagrass species assessed in the IUCN's (International Union for Conservation of Nature) categorization of threatened species (Red List) was found to be at risk of extinction and listed as Endangered, Vulnerable or Near Threatened, although many species require a more thorough assessment of their status (Short et al. 2011).

The loss of seagrasses from coastal habitats globally has been attributed mainly to human impacts from pollution, eutrophication, coastal development, habitat destruction (e.g. dredging, anchoring, trampling) and harvesting (Duarte 2002, Orth et al. 2006). More specifically, seagrass loss has been linked to eutrophication of coastal waters due to excess nutrient input from effluent discharges and land-use (Ruiz and Romero 2003, Cardoso et al. 2004, Burkholder et al. 2007). Excess nutrients encourage growth of planktonic and benthic algae which compete with seagrasses for nutrients and reduce light penetration in the water column thus inhibiting seagrass photosynthesis (Short et al. 1995). Algal growth can further smother seagrasses (Walker and McComb 1992, Nelson and Lee 2001) as well as alter the balance of dissolved carbon dioxide and oxygen (Raun and Borum 2013), while toxic effluent constituents can be lethal to seagrasses (Koch and Erskine 2001, Macinnis-Ng and Ralph 2004, Govers et al. 2014, Negri et al. 2015,).

Natural stressors such as storms and floods have also contributed to seagrass loss (Preen et al. 1995, Orth et al. 2006), however these are further heightened as a result of climate change (Short and Neckles 1999, Koch et al. 2013). In general, climate change raises sea and air temperatures, alters tidal patterns, increases sea levels, and escalates flood, storm and cyclone events (Harley et al. 2006, Bijma et al. 2013, Tolieng et al. 2017). Observations since 2001 by the global seagrass monitoring network, SeagrassNet (www.seagrassnet.org), have reported seagrass declines in several regions in North and South America. Among other reasons they attributed loss of seagrasses to rising temperatures linked to global climate change (Short et al. 2006). Historically, seagrasses have been subjected to a changing climate and adapted to shifts in regime, however, this has occurred over millions of years. The rapid shift in climate especially in coastal habitats (Orth et al. 2006) may surpass a seagrass's ability to adapt (Duarte 2002). These stressors occurring across local, regional and global scales can result in extensive seagrass loss independently and synergistically, with related impacts on productivity and biodiversity, while future climate change scenarios predict further declines in seagrasses (Rasheed and Unsworth 2011, Jordà et al. 2012).

1.2.1. The Influence of Temperature on Seagrasses

In a global assessment of patterns and predictors of marine biodiversity, Tittensor et al. (2010) identified sea surface temperature as the only environmental predictor of species richness that was statistically significant across 13 taxa, including seagrasses. Temperature consistently played a key role in structuring large-scale diversity patterns in the marine environment (Tittensor et al. 2010). Warmer temperatures increase metabolic rates which can encourage greater rates of speciation resulting in a higher diversity of species (Allen et al. 2002). In addition, thermal tolerance defines a species range limit which is evident in the high species richness in warm tropical compared to cooler regions (Currie et al. 2004).

Several factors dictate the occurrence of seagrass meadows along a coastline. Abiotic (e.g. temperature, salinity, light, and nutrients) and biotic (i.e. seed availability, disease, epiphytes and grazing) variables regulate seagrass physiology and morphology (Roca et al. 2016). Combined with anthropogenic impacts, these factors prevent or support the colonization and proliferation of seagrass meadows.

Seagrass distribution has therefore been attributed to temporal and spatial variations in biotic and abiotic environments (Hall et al. 1999, Yang and Yang 2009), however in order to unravel the main causes of seagrass change, we need to understand the extent of environmental influences, and uncouple natural versus anthropogenic effects.

The seagrass group has adapted to live in varying temperature conditions which is apparent in their global spread (Den Hartog 1970). They occur in the tropics thriving in temperatures of up to 35.5^{o}C (e.g. *Thalassia testudinum*, Biscayne Bay, USA; Zieman 1975) or in climates where temperatures reach as low as -1.6^{o}C (e.g. *Zostera marina*, Akkeshi Bay, Japan; Watanabe et al. 2005). However, within these temperature limits, individual seagrass species possess minimum and maximum ranges beyond which severe declines occur (Lee et al. 2005, Thomson et al. 2015), as well as optimal temperatures at which growth and photosynthesis peak (Niu et al. 2012). Seagrass species occurring in the same meadow have shown varying degrees of tolerance to temperatures with some more tolerant than others. This provides insight into their resilience and abilities to acclimate to temperature stress, and needs to be explored in individual species. McMillan (1984) hypothesized that tropical seagrasses from shallow intertidal sites had a greater tolerance to supra-optimal temperatures (35-37^{o}C) than those from deeper sites at lower temperature (26-32^{o}C). In experimental cultures *T. testudinum* and *Syringodium filiforme* survived after four weeks at 35°C, while some replicates of *T. testudinum* survived after four weeks at 36°C (McMillan 1984). *Halodule wrightii* displayed the greatest tolerance to sustained high temperature surviving for two weeks at 37°C.

Temperate seagrasses may be limited in their tolerance to supra-optimal temperatures since they are usually exposed to cooler water temperatures and would consequently have lower maximal growth temperatures relative to tropical/subtropical seagrasses (Lee et al. 2007). In addition, temperate seagrasses (e.g. *Zostera marina*) display seasonal variations in growth that differ from that of tropical species (Dennison 1987, Lee et al. 2005) – patterns often attributed largely to thermal stress (Lee et al. 2005).

Seagrass growth is dependent on nutrient uptake, within plant partitioning, leaf senescence and respiration all largely influenced by temperature (Marsh et al. 1986, Pérez and Romero 1992, Leegood 1995). Particular life cycle events like germination and flowering are dependent on temperature cues, while cellular level metabolic processes are altered during extreme high and low temperatures (McMillan 1980, Larkum et al. 2006). Changes, therefore, in global ocean temperatures are predicted to have severe implications on seagrass growth (Congdon et al. 2011, Rasheed and Unsworth 2011, Thom et al. 2014) and distribution of marine biodiversity in general (Currie et al. 2004, Tittensor et al. 2010, Eklöf et al. 2012, Travis et al. 2013).

Despite their thermal range, temperature fluctuations as well as periods of extreme temperatures (e.g. during heatwaves) pose a threat to seagrass acclimation and survival (Congdon et al. 2011, García et al. 2012, Valle et al. 2014, Thomson et al. 2015). Photosynthesis and respiration increase with rising temperatures, but with continued temperature elevation that exceeds maximal levels, respiration surpasses photosynthesis resulting in its rapid decline (Marsh et al. 1986, Leegood 1995). Photosynthesis and productivity of seagrasses especially in shallow areas are therefore significantly impacted by supra-optimal temperatures (Leegood 1995, Ralph 1998a, Seddon and Cheshire 2001).

Since incoming solar radiation largely influences water temperature, separating the effects of each on seagrasses has been problematic, and has given rise to conflicting assessments on the relative importance of light and temperature, summarized by Larkum et al. (1989). However, the discharge of thermal effluent from anthropogenic activities provided examples of direct influences of supra-optimal temperatures which had unpredictable and extensive impacts on seagrass populations (Thorhaug et al. 1985, Robinson 1987). Beds of *Thalassia testudinum* in Florida were eradicated from areas where thermal effluent from a power plant was sustained above 36^{o}C (Zieman 1975). An area of about 9 ha containing *T. testudinum* was completely eliminated when effluent temperatures were ~5^{o}C above ambient (~30^{o}C) (Thorhaug et al. 1973). Similarly, mixed beds of *T. testudinum* and *Syringodium filiforme* were completely eradicated in an area where effluent 7-8°C above ambient (~28.5°C) was discharged from a power plant in Kingston, Jamaica (Thorhaug 1984). In this case however, *Halodule wrightii* from the same area showed greater tolerance to heat stress and was able to survive when planted in barren areas subjected to the heated effluent, and thus able to replace functionality of the seagrass ecosystem (Thorhaug 1984). Deeper understanding of the effects of temperature on seagrass ecosystems, their tolerance limits and ability to adapt has become increasingly important.

1.2.2. Epiphytes and Fouling in Seagrass Ecosystems

Seagrass beds provide a physical substrate on which micro- and macroalgae, bacteria and invertebrates settle. Excess algae can smother seagrass leaves and reduce the absorption of nutrients and attenuation of light by seagrasses (van Montfrans et al. 1984, Mazzella and Alberte 1986, Silberstein et al. 1986, Williams and Ruckelshaus 1993, Short et al. 1995, Jernakoff et al. 1996, Hughes et al. 2004 ,). This results in reduced rates of photosynthesis and overall fitness of seagrass plants (Bologna and Heck 1999, Fong et al. 2000, Michael et al. 2008). In addition, long-lived epiphytes can weigh leaves down leading to breakage (van Montfrans et al. 1984). Warming along with increases in nutrients, through for example eutrophication, can favour algal growth causing additional stress on seagrass ecosystems.

1.3. Responses of Seagrasses to Threats

1.3.1. Acclimation in Seagrasses

The rapidly changing climate poses serious challenges by imposing firmer selection criteria forcing biological populations to respond by adapting, acclimating, migrating, or becoming extinct. Populations are thus driven away from environments to which they are adapted, and these changes are expected to hamper gene flow by upsetting the migration-adaptation interchange, likely influence productivity, and eventually alter the structure and composition of ecosystems (Davis and Shaw 2001, Townsend et al. 2003). Populations living in dynamic environments such as along coastlines, must contend with high degrees of fluctuation in environments and develop appropriate adaptive responses. These responses can be genetically driven or the result of a phenotypic response within a genotype that allows for flexibility or plasticity.

Phenotypic plasticity is the ability of a genotype to modify morphological or physiological traits in order to function in response to external disturbance (Reusch 2014). This is also referred to as acclimation, and enables populations to maintain performance across a range of environmental contexts, during the course of a lifetime. In contrast, adaptation is an evolutionary expression, mainly derived genetically via natural or sexual selection, gene flow or genetic drift, and occurs over many generations ultimately giving rise to a different ecotype (Beer et al. 2014). The processes of acclimation and adaptation may co-occur particularly when evolution is accelerated (Hairston et al. 2005).

Adaptive responses to stress is contingent on the level of genetic variation existing within a population (Reynolds et al. 2016), while intraspecific variation in genetic diversity can provide greater resilience to stress (Reusch 2006, Hughes et al. 2009), and even increase productivity in diverse populations (Rugiu et al. 2018). Plastic divergence in populations, though not genetic-based, is considered an important form of response to environmental change and creates greater variation at individual (biochemical), community and population levels of organization (Sultan 1995, Pigliucci 2005, Ruesink et al. 2012). Further evidence on the influence of environmental contexts on plasticity and facilitating adaptation to modern pressures and states, is supported in the young field of ecological epigenetics (Duarte et al. 2018).

Identifying the genetic structure driving adaptive responses has been challenging, and difficult to determine using direct observations, and requires genetic level analyses to distinguish a genotypic from a phenotypic response (Reusch 2014). However, despite the prominence of genetic and genomic techniques to assess climatically-driven evolutionary change (Oetjen and Reusch 2007, Bergmann et al. 2010), few studies have been able to link adaptation as a direct consequence of genetic changes on a phenotype (reviewed by Merilä and Hendry 2014). This lack of evidence to support genetically-driven adaptation has led to a greater focus on phenotypic plasticity, which is assumed to shape responses to present-day environmental stressors in the absence of genetically-based evidence (Merilä and Hendry 2014, Marín-Guirao et al. 2016).

Seagrasses are sensitive to environmental changes especially ones that reduce irradiance (Dennison et al. 1993, Collier et al. 2012a, 2012b), which further renders them vulnerable to other stressors i.e. temperature fluctuations (Koch et al. 2007a, Kaldy et al. 2015) and desiccation (Boese et al. 2005, Shafer et al. 2007). They have developed distinct physiological, morphological and ecological characteristics to cope with low light levels (Mazzuca et al. 2009, La Nafie et al. 2013), oxygen fluctuations (Binzer et al. 2005), ultraviolet radiation (Trocine et al. 1981, Dawson and Dennison 1996), desiccation and heat stress usually for short periods of time (Adams and Bate 1994, Björk et al. 1999, Seddon and Cheshire 2001).

A phenotypic expression in some seagrass species is the altering of morphology or downsizing i.e. reducing overall plant size and narrowing leaves (Tanaka and Nakaoka 2004, Peralta et al. 2005, Zhang et al. 2015). This trait has been associated with tolerance to exposure and desiccation, and hypothesized to explain upper limits in seagrass zonation (Tanaka and Nakaoka 2004, Boese et al. 2005, Cabaço et al. 2009, Kaewsrikhaw et al. 2016). Lower limits i.e. depth of seagrass distribution are related to interspecific competition for light (Kim et al. 2016), producing populations with sparser canopies in order to avoid self-shading, while long leaves are better able to reach surface light than shorter ones (Collier and Waycott 2014). With a changing climate, fluctuations in and increased periods of exposure to rising air and water temperatures are predicted, along with reduced oxygen and irradiance from eutrophication (Tolieng et al. 2017, Duarte et al. 2018), and the timely acclimation of many species to these new extremes is important to their survival.

A phenotypic response to temperature and nitrate was observed in *Zostera marina* revealing that increases in temperature can significantly reduce shoot size and modify internal carbon and nitrogen levels (Touchette et al. 2003). In that experiment, a physiological mechanism for the observed acclimation to heat stress was suggested (Touchette et al. 2003). Similarly, intertidal subtropical seagrasses (*Cymodocea rotundata*, *C. serrulata* and *Thalassia hemprichii*) in southwest Japan were reported to use a combination of growth strategies and morphological traits at the shoot level to cope with desiccation, such as reducing structural rigidity and downsizing (Tanaka and Nakaoka 2004).

In Padilla Bay, Washing State, USA, an increase in leaf turnover and abscission was observed in intertidal *Z. japonica* and *Z. marina* populations that experience high levels of desiccation (Shafer et al. 2007), while high vegetative reproduction followed by high shoot densities was suggested as a means to cope with desiccation in *Z. japonica* populations in Swan Lake, east China (Zhang et al. 2015). There, the dense accumulation of shoots with numerous overlapping leaves provided shade and protection from evaporation (Zhang et al. 2015). In the Baltic Sea, intra-clonal plasticity in the endemic seaweed, *Fucus radicans*, was found to demonstrate a potential for adaptation and therefore persistence in response to climate change (warming and reduced salinity) (Rugiu et al. 2018).

1.3.2. Chemical Defence in Seagrasses

Seagrasses have developed a series of biochemical strategies to cope with ecological and environmental stress. They produce a range of secondary metabolites that can provide defence against biotic stressors such as fouling, herbivory, pathogens and parasites (van Alstyne 1988, Dai and Mumper 2010), as well as abiotic factors like increases in carbon dioxide, ozone (Peñuelas and Llusià 2003, Arnold et al. 2012), light intensity (Vergeer et al. 1995, Lavola et al. 2000) and temperature (Dement et al. 1975, Tingey et al. 1980). The presence of seagrass beds have been shown to reduce bacterial pathogens in adjacent coral reefs, as well as reduce the relative abundance of pathogens that could potentially cause disease in humans (Lamb et al. 2017). The allocation of plant resources to secondary metabolite production implies a divergence of resources from primary functions such as reproduction and growth, and serves a key function in response to stress from adverse environmental conditions (Strauss et al. 2002, Neilson et al. 2013). Phenolic acids or phenolics appear to be the most abundant group of secondary metabolites and are also most frequently investigated (Waterman and Mole 1994, Robards 2003).

Secondary metabolite production can increase under warming conditions attributed to the higher rates of biosynthesis in elevated temperatures (Holopainen and Gershenzon 2010). In some cases, increases in secondary metabolites are a response to thermal stress, likely due to their anti-oxidative function, and ability to scavenge reactive oxygen species (Koch et al. 2013). Elevated levels of secondary metabolites have been recorded in land plants exposed to heat stress (Wahid et al. 2007), however, responses in seagrasses have been varied. In culture experiments, concentrations of phenolic compounds in *Zostera marina* from Lake Grevelingen, Netherlands, were reduced with increasing temperature (Vergeer et al. 1995). Similarly, Ravn et al. (1994) reported lower phenolic concentrations in *Z. marina* from Øresund, Denmark in summer as compared to spring. Conversely, concentrations of phenolics in *Z. marina* from British Columbia, Canada were high at the end of the summer active growing season and low in late winter/early spring (Harrison and Durance 1989). Reasons for these patterns are obscure, but are likely the result of the influence of temperature on photosynthesis and respiration which further influences the balance of carbon and nitrogen in plants (Waterman and Mole 1994). In addition, temperature directly affects the enzymatic biosynthesis of phenolics, while each enzyme in turn potentially possesses different temperature optima (Waterman and Mole 1994).

Studies on the bioactivity of seagrass extracts and metabolites have mainly investigated their toxicity to herbivory such as variations in response to grazing by sea urchins and fish (Vergés et al. 2007a, 2007b), as well as microbial settlement and attack from pathogens (Steele et al. 2005, Lane and Kubanek 2008, Trevathan-Tackett et al. 2015), and their antifouling potential (Todd et al. 1993), while other research has focused on variations in seagrass biological compounds in response to season or environmental factors albeit with varying patterns (Harrison and Durance 1989, Ravn et al. 1994, Vergeer et al. 1995). The direct or indirect effects of secondary metabolites on settlement and growth of epiphytes remain largely unexplored in seagrasses, along with the implications of these effects on interactions at the community level, and under the influence of anthropogenically-influenced climate change.

1.4. Trophic Interactions in Seagrass Ecosystems

Biological relationships in seagrass habitats include complex interactions between seagrasses, their associated algae, grazers (herbivores), mesopredators and piscivores. Despite direct seagrass herbivory being modest, it is globally widespread (Duarte and Cebrián 1996, Valentine and Heck 1999), whereas macroalgae provide a vital food source to a large proportion of grazers associated with seagrasses, many of which feed heavily on algal epiphytes (Orth et al. 1984, Jernakoff et al. 1996, Smit et al. 2005, Lebreton et al. 2011). Earlier studies theorised primary productivity from seagrass ecosystems to play a greater role in supporting detritivory pathways rather than contributing to the food web via direct consumption (Duarte and Cebrián 1996, Cebrián and Duarte 2001). This view has since shifted with greater evidence provided over the last three decades to support the important role of herbivory in the removal of plant biomass in marine and freshwater systems (Valentine and Heck 1999, Valentine and Duffy 2006, Poore et al. 2012, Wood et al. 2017, Scott et al. 2018) – an estimate of up to 10 times larger than that reported for terrestrial habitats (Bakker et al. 2016a).

Herbivory can influence aquatic habitats through the supply of nutrients (Allgeier et al. 2013, Hill and Heck 2015), disturbance and grazing thereby altering the composition and abundance of plants which in turn affects productivity, and carbon and nutrient loads in the surrounding ecosystem (Bakker et al. 2016a). Primary production from seagrasses and algae are now known to be strongly regulated by herbivory in coastal habitats (Bakker et al. 2016b), where the herbivoral function for seagrasses is largely performed by macrograzers such as urchins, green turtles, dugongs, manatees and fish (Valentine and Heck 1991, Valentine and Heck 1999). Mesograzing of epiphytes is mainly carried out by amphipods, gastropods, isopods and decapods (Bologna and Heck 1999). These in turn are preyed upon by mesopredators (e.g. crabs, shrimps and small bodied fish), thereby contributing to biochemical cycling, regulation of primary production and energy transfer across trophic groups (Heck et al. 2000, Heck and Valentine 2006). Increases in nutrients from for example eutrophication, favour fast-growing macro and epiphytic algae with negative effects on seagrasses. Top-down control of algal overgrowth has emerged as a key function in the maintenance and management of seagrass habitats (Eriksson et al. 2009, Reynolds et al. 2014, Campbell et al. 2017), which requires the biomass of competing epiphytes to be stemmed by maintaining a balance of both top-down (grazing) and bottom-up (nutrients levels) processes (Duffy et al. 2015).

A meta-analysis of 35 investigations compared the relative strength of nutrient and grazer effects on performance and epiphyte biomass in field and laboratory studies, on 11 tropical and four temperate seagrass species (Hughes et al. 2004). *Cymodocea rotundata*, *C. serrulata*, *Syringodium isoetifolium*, *Thalassia hemprichii*, *Enhalus acoroides*, *Halodule uninervis*, *H. wrightii*, *Ruppia maritima*, *S. filiforme*, *Zostera japonica* and *T. testudinum* were categorized as tropical/subtropical despite *R. maritima* and *Z. japonica* occurring in temperate regions (Short et al. 2007). The four temperate species analysed were *C. nodosa*, *Z. tasmanica*, *Posidonia sinuosa* and *Z. marina* (Hughes et al. 2004). The authors reported that additions of nutrients into the water column encouraged epiphyte overgrowth in contrast to sediment nutrient additions, thus supporting the concern of the decline in seagrass habitats due to coastal eutrophication (Cardoso et al. 2004, Burkholder et al. 2007). Overall grazing was found to have a negligible effect on seagrass growth and biomass, and a positive effect on shoot densities. However, when grazers that fed exclusively on epiphytic algae were analyzed separately, the results showed a negative effect on epiphyte biomass and positive effect on seagrass response variables (Hughes et al. 2004). The negative effects of eutrophication were counteracted by positive effects of epiphyte grazing albeit with similar magnitudes, prompting the suggestion that the effects of both factors be considered concurrently in further investigations.

In a recent meta-analysis of 48 distinct studies carried out in *Zostera marina* and *Fucus* spp habitats in the northern Atlantic and Baltic Sea, top-down and bottom-up effects on trophic structure were found to generally be similar, although variations in responses within trophic groups were observed (Östman et al. 2016). Similarities were due to the combined effects of both eutrophication and mesopredator release resulting in increases of macro- and micro-epiphytic algae in both seagrass and seaweed habitats. Overfishing of top-predator species has been reported to release mesopredators which in turn control grazer abundances, thus causing ephemeral algae to proliferate especially under nutrient enrichment (Heck et al. 2000, Moksnes et al. 2008, Eriksson et al. 2009, Baden et al. 2010, 2012, Svensson et al. 2017). In the *Z. marina* and *Fucus* spp habitats, eutrophication had a larger and positive effect on green as opposed to brown or red macroalgae, particularly when nitrogen levels were increased. This is because ephemeral green algae are fast growing and outcompete red and brown algae for space especially in the absence of grazers (Eklöf et al. 2012). Similarly, grazing had a stronger effect on the more palatable green algae, while the positive effects of nutrient enrichment on brown and red algae were greater when grazers were absent in the seagrass habitat. The analysis further revealed a larger bottom–up than top-down effect in *Z. marina* habitats with the addition of nitrogen which negatively influenced seagrass performance, while the effect of grazing was positively significant though minor. These studies also reported grazer abundances to have a greater top-down influence than nutrient enrichment, with total abundance of grazers negatively controlled by mesopredators and more so for isopods and amphipods than gastropods (Östman et al. 2016).

Top-down consumer control and bottom-up effects of nutrients can be influenced by direct (predation) and indirect (presence/absence) effects of predators (Amundrud et al. 2015, Hill and Heck 2015, Beerman et al. 2018) as well as herbivore identity and feeding rates (Best and Stachowicz 2012, Svensson et al. 2012), which can differ temporally (Whalen et al. 2013) and across spatial scales (Campbell et al. 2017, Donadi et al. 2017). Mesograzers (mainly crustaceans) have been recognized to fulfill a key role in controlling ephemeral algae in several eutrophic seagrass systems (Eriksson et al. 2009, Jaschinski and Sommer 2011, Reynolds et al. 2014). Greater effort is being placed on understanding the effects of eutrophication as threats from coastal nutrient enrichment increase (Baggett et al. 2010, Short et al.

2011, 2016). While some understanding has been provided on the cascading effects of the removal of top predators on seagrass habitats (Baden et al. 2010, Eriksson et al. 2011, Sieben et al. 2011, Svensson et al. 2017), grazer, seagrass and algal interactions under a changing climate appear to be species specific (Garthwin et al. 2014), and remain to be explored in many seagrass ecosystems.

1.4.1. Temperature Effects on Trophic Interactions

Global mean surface temperature of the Earth has risen by 0.6-0.8°C since 1880 (Griggs and Noguer 2002), and by 2100 a further warming of 3-4°C of the earth's atmosphere is predicted along with increases in sea surface temperatures (Domingues et al. 2008, Cheng et al. 2017, Durack et al. 2018). This is largely attributed to human activities (Tolieng et al. 2017). Despite the extensive studies on responses to rising temperatures in terrestrial plants, predictions from these models have been problematic in extrapolating responses in macroalgae and seagrasses. Field and experimental studies have provided greater clarity on thermal thresholds and tolerance mechanisms of certain algal and seagrass species (reviewed by Koch et al. 2013 for tropical and subtropical seagrasses), while the direct influences of temperature on growth and metabolism of seagrasses is relatively well known (Campbell et al. 2006). Competitive algal epiphytes may increase under elevated temperature conditions (Werner and Matthiessen 2017), and their overgrowth is likely to hamper seagrass growth and productivity (Fong et al. 2000, Hays 2005). Top-down control of algae therefore serves a key role in reducing fouling and alleviating competitive stress thus benefiting seagrass ecosystems.

Warmer temperatures influence interactions between plants and herbivores with positive and negative, direct and indirect effects on multiple species, which in turn can distort the direct negative impacts of climate change (O'Connor 2009), and further confound single species predictions of the effects of warming (Connell et al. 2011). The indirect impacts of climate change (e.g. those facilitated by species interactions such as predation or competition), are seemingly as important as those of direct effects but require quantification and comparisons with direct effects in many systems (Wernberg et al. 2012). Growing evidence implicates species interactions to be a key determinant of a community's response to climate change, since the effect on one trophic group can trigger effects on other levels (Harley 2011, Eklöf et al. 2012, Zarnetske et al. 2012). The size of this top-down or bottom-up effect may be influenced by warming by altering primary productivity (Harley et al. 2012, Collier and Waycott 2014) and the distribution and abundance of plants (Short et al. 2016), as well as a herbivore's resistance to predation (Eklöf et al. 2012), and consumption rates (O'Connor 2009). Other characteristics affected by temperature may further influence a plant's susceptibility to herbivory such as secondary metabolite production (Gong and Zhang 2014) and internal nutrient configurations (i.e. C:N ratios) (Touchette et al. 2003).

Following a meta-analysis of 18 experiments in seagrass systems that investigated the trade-off between consumption rates and resistance to predation, Eklöf et al. (2012) hypothesized that increases in temperature and CO_2 would render predation-resistant herbivores vulnerable thus weakening their ability to control primary producers. Using experimental seagrass (*Zostera marina*) mesocosms, they tested the effect of the three dominant grazing invertebrates (*Littorina littorea*, *Gammarus locusta* and *Rissoa* sp.) in controlling macroalgal biomass under both warming and ambient scenarios, and simulated predation by removing the predator-resistant amphipod, *G. locusta*. Their results showed that algal biomass increased with warming due mainly to grazer removal of out-competing green algae (*Ulva* spp.) resulting in an increase in mainly two less-palatable red and brown algal taxa. Under warming the mainly epiphytic algal community transformed to a combination of epiphytic algae and drift algal mats which grazers could not control, thus shifting dominance of the system from seagrass to algae. In that study, predation had impacted the grazer (*G. locusta*) most effective in controlling algal overgrowth, demonstrating a direct and indirect effect of climate change in altering seagrass habitats (Eklöf et al. 2012).

In a similar *Zostera marina* mesocosm experiment, the probability that the same mesograzers (*Gammarus locusta*, *Littorina littorea* and *Rissoa* sp.) mediated the combined effects of warming and CO_2 enrichment (acidification) on micro and macroalgae were investigated (Alsterberg et al. 2013). Here, no effects of acidification were detected but when analysed in combination with warming, the direct and indirect influence of acidification on seagrass, algae and mesograzers was strong and positive. Using structural equation modelling the authors uncovered warming and acidification to have a direct positive effect on microalgae in the absence of grazers, which were indirectly and negatively influenced by shading and grazing of microalgae in sediment. This study further confirms the key role of top-down control (by mesograzers) in negating direct and indirect effects of climate change (acidification and warming), and draws attention to the direct influence of climate change being as significant as indirect effects, and the importance of analysing climate factors in combination e.g. acidification and warming (Alsterberg et al. 2013).

Most mesocosm experiments show warming to generate stronger top-down control of primary production due mostly to consumer respiration rates being faster than photosynthetic rates (O' Connor 2009, Kordas et al. 2011, Gilbert et al. 2016). This strong top-down control was also supported in an *in situ* warming experiment in the northern Baltic Sea that used cages to exclude predatory fish (Svensson et al. 2017). In that experiment, the negative effect of excluding fish on algal biomass was strengthened by warming, however algal growth was favoured via a different mechanism. Heated temperatures in the test site were beyond the physiological tolerance of smaller invertivorous fish, driving larger fish to target grazing invertebrates (i.e. gammarids) instead. In that study, warming appeared to have restructured a coastal food web by reducing it from four trophic levels to three, due to a predator-driven alteration of herbivore behaviour and not direct changes in herbivore abundance (Svensson et al. 2017), and demonstrates a climate-driven effect on trophic interactions that can alter trophic structures in seagrass systems.

1.4.2. Top Down Control and Effects of Grazing

The cascading effect of depleting top-down control by predators has been shown to be as important as nutrient enrichment in controlling large-scale abundance of macroalgae (Moksnes et al. 2008, Eriksson et al. 2009, Baden et al. 2010, Östman et al. 2016). This is achieved through the increase in mesopredators (small predatory fish) as well as the alteration of size structure, abundance and distribution of macro- and micro-grazers, contributing to an increase in production of ephemeral algae in several seagrass systems (Eriksson et al. 2009). The control of algal fouling by grazing invertebrates is a key regulatory function which can have a positive effect on the physiological performance and resilience of seagrasses (Baden et al. 2010, Lewis and Anderson 2012).

Top-down control is important in regulating abundance and composition of primary production and consumption in both tropical and temperate seagrass ecosystems. For example, Fong et al. (2000) demonstrated the significant interactive role of grazers and epiphytic algae in influencing growth and survival of *Zostera japonica* in Lai Chi Wo, Hong Kong. In a caging experiment, they found the dominant grazers, *Clithon* spp. to play a critical role in controlling epiphytic algal biomass, when control cages without grazing significantly reduced the percentage cover and increased sloughing of seagrass leaves. Similarly, experimental nutrient enrichment and predator exclusion in a *Thalassia testudinum* habitat in St. Joseph Bay, Gulf of Mexico reported significant effects of nutrients to be limited, while greater effects of fish exclusion were detected (Heck et al. 2000). As predicted, grazer abundances were significantly reduced in fish enclosures, however these enclosures also produced an unexpected reduction in algal biomass due to fish consuming algal epiphytes along with grazers, resulting in an indirect positive effect on the seagrass (Heck et al. 2000). In another *T. testudinum* mesocosm experiment in the northern Gulf of Mexico, a significant negative effect of grazing on algal epiphytes and a positive effect on seagrass growth was recorded (Hays 2005), and highlighted the importance of dense seagrass stands in preventing the reduction of mesograzers, as well as the role of even small grazers in controlling epiphyte biomass.

Stronger top-down than bottom-up processes were found to regulate ephemeral algae and grazers in temperate *Zostera marina* beds in western Sweden (Moksnes et al. 2008). Here, a large mesograzer (*Gammarus locusta*) was highly effective in lowering epiphytic algal biomass on seagrass leaves when predators were excluded in a caging experiment. Predation by fish and other predators were reported to significantly reduce (by 90-95%) mesograzer abundances resulting in an increase in meiofauna and macroalgal biomass up to six fold. The effects of nutrient additions were not significant on macroalgal biomass in this system, except for a temporary bloom of mainly green algae and an increase in mesograzer biomass in predator-exclusion treatments (Moksnes et al. 2008). In this study, a trophic cascade at the community-scale driven by strong top-down predator control, seemed to reduce functionality of mesograzers, resulting in a significant increase in macroalgae on seagrass leaves. This was mainly due to the strength of the trophic interaction being largely based on two functionally dominant species (*Ulva* spp. and *G. locusta*) that were also the most susceptible to consumption, causing these effects to permeate a trophically complex system (Moksnes et al. 2008).

Top-down control of mesograzers as a result of predation pressure can greatly influence the effects of eutrophication further demonstrated in a predator-controlled *in situ* cage experiment at three regions in the Baltic–Skagerrak (Baden et al. 2010). In this experiment, *Zostera marina* growth was reduced significantly following strong responses in ephemeral algae to nutrient additions along the Swedish west coast where mesograzer abundances were found to be generally low. The opposite effect was observed in Baltic areas where high densities of mesograzers occur, and algal biomass response to enrichment was not significant. On the Swedish coast, intermediate predator (small fish and crabs) control significantly reduced mesograzer abundances (>98%) while this effect was absent at sites in the Baltic (Baden et al. 2010). These observations were attributed to the combined effects of overfishing and nutrient enrichment on trophic interactions that differed across regions. Overfishing of top predators on the Swedish west coast since the 1980s appeared to have caused a significant increase in mesopredator biomass resulting in the virtual absence of mesograzers (Baden et al. 2012). Coupled with enhanced eutrophication in the region for over eight decades, seagrasses were reported to have declined by 60% (Baden et al. 2003) due mainly to the overgrowth of filamentous macroalgae (Baden et al. 2012).

Species identity and richness can influence the main interactive effects influencing epiphytes and grazing and even regulate top-down control in seagrass habitats (Duffy 2001). A global study spanning 15 sites across the Atlantic and Pacific ranges of *Zostera marina*, tested the influence of top-down and bottom-up forces on naturally occurring environmental and biodiversity gradients (Duffy et al. 2015). Grazers were reduced and nutrients added factorially in 40 plots at each site, in a four week field experiment. Generally, no effects of nutrient additions were detected while top-down control of algal biomass was negligible, however differences in seagrass and grazer diversity across sites were more influential in predicting algal and grazer biomass than environmental contexts. Algal biomass was demonstrated to be influenced more strongly by grazers (top-down) than by nutrients (bottom-up). Findings from this large-scale experiment corresponded with previous local-scale studies (Hughes et al. 2004, 2010, Reynolds et al. 2014), and provided global and local support for the importance of biodiversity and top-down control in influencing threatened seagrass habitats (Duffy et al. 2015).

Understanding factors that allude to the resilience of seagrass habitats are important to aid our understanding of the effects of stressors, particularly eutrophication and competition with algae. Ecological resilience is defined by Holling (1973) as "*the capacity of an ecosystem to absorb repeated disturbances or shocks, and adapt to change without fundamentally switching to an alternative stable state*", and relates to the ability of an ecosystem to resist stress and recover from degradation or loss (Unsworth et al. 2015). Reducing stress from fouling may allow a seagrass meadow to withstand further stress since photosynthesis, respiration and overall plant fitness can still be maintained (Baden et al. 2012). Understanding the resilience of seagrass species is of critical importance if we are to understand how climate change will affect productivity and ecosystem services.

1.5. Seagrasses in South Africa

Of the four seagrass species recorded for South Africa, the temperate dwarf eelgrass, *Zostera capensis* is dominant. *Z. capensis,* forms dense meadows from cool temperate to subtropical waters off southern and south eastern Africa, but is patchy and interspersed with other seagrass species as it extends into tropical waters of east Africa (Bandeira and Gell 2003). Growing in limited stretches of soft substrates in coastal lagoons and estuaries, *Z. capensis* is vulnerable to coastal development, and despite being fast-growing, colonization is slow and growth is hampered by pollution, sedimentation and human disturbance (Bandeira and Gell 2003). The harvesting of bivalve molluscs from seagrass meadows in Mozambique has posed a serious threat to *Z. capensis* cover which has declined considerably in harvesting areas (Green and Short 2004).

The distribution and extent of *Z. capensis* in Tanzania, Kenya and Madagascar is largely unknown, however its total extent including in South Africa is estimated to be less than 2 000 km^2 (Short et al. 2012). It grows mainly in soft substrates of estuaries, lagoons and intertidal mudflats and can survive partially or fully submerged (Edgecumbe 1980, Adams and Bate 1994). Smaller plants produced on intertidal mudflats have shorter reproductive shoots and short leaves (20 – 30 cm), while those growing in lagoons are described as having long reproductive stalks and leaves of up to 115 cm (Phillips et al. 1988).

Previously, Begg (1978) and Heydorn and Tinnley (1980) reported the presence of *Zostera capensis* collectively in 26 estuaries in South Africa including the harbours of Richards Bay and Durban on the east coast, while Bandeira and Gell (2003) described *Z. capensis* from 17 estuaries along the southeast coast of South Africa with a total aerial cover of 7.07 km^2. Allanson and Baird (1999) noted that generally *Z. capensis* was found in estuaries that were permanently open to the sea ranging from the Olifants River on the west coast to St Lucia on the east coast of South Africa (Fig. 1.1). The National Biodiversity Assessment estimated 25% (approximately 75) of the nearly 300 functional estuaries in South Africa to be permanently connected to the sea, and noted the presence of *Z. capensis* in most permanently open estuaries (van Niekerk et al. 2012). The exact presence of *Z. capensis* in each estuary was not determined. It was grouped instead with two submerged macrophytes: *Ruppia maritima* (synonym: *cirrhosa;* spiral ditchgrass), and *Stuckenia pectinata* (pondweed), to collectively cover an area of 13.27 km^2 in 68 estuaries (van Niekerk et al. 2012). However, a recent review reports the presence of *Z. capensis* in 62 estuaries in South Africa from Kosi Bay (east coast) to the Olifants Estuary (west coast) and an estimated cover of 10.4 km^2(Fig 1.1) (Adams

2016). These studies highlight the variations in assessments of, as well as variability in, *Z. capensis* cover in South African estuaries.

Fewer estuaries in South Africa now support *Zostera capensis* populations, which have been reduced to a few hectares containing small beds (Bandeira and Gell 2003). The loss of *Z. capensis* from estuaries in South Africa has been attributed to flooding (Talbot and Bate 1987, Hanekom and Baird 1988), silt deposition (Hanekom and Baird 1988, Cyrus et al. 2008), and sediment smothering from bioturbation (Siebert and Branch 2006). Barnes (2010) observed substantial diebacks of seagrass beds in the Knysna Estuary and attributed this to increasing summer temperatures and considerable modification of the shoreline as a result of development. Extreme environmental events predicted with climate change may see an increase in temperature and floods leading to further loss of *Z. capensis*.

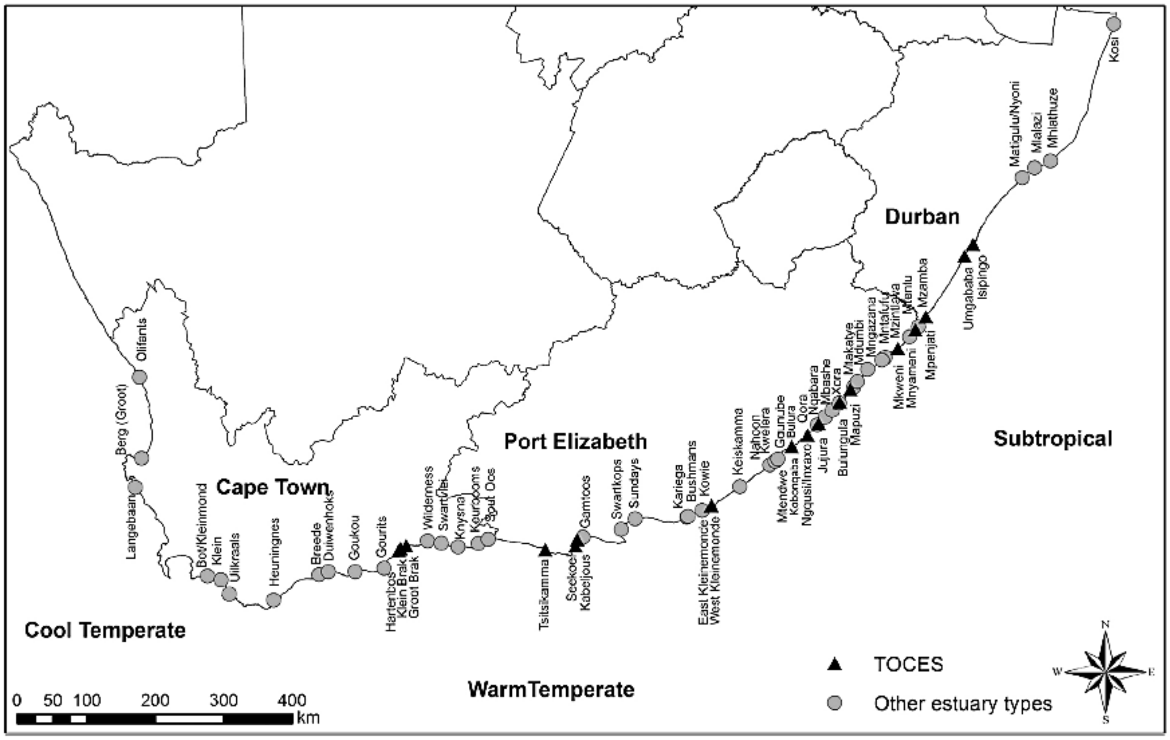


Figure 1.1. Positions of estuaries in South Africa that contain *Zostera capensis* including temporarily open/closed estuaries (TOCES) (Adams 2016).

1.5.1. **Zostera capensis** ***in Langebaan Lagoon***

In South Africa, a sizeable population of *Zostera capensis* is found in Langebaan Lagoon, on the west of South Africa. Langebaan Lagoon (18°03'E, 33°08'S) is a marine lagoon proclaimed as a marine reserve that forms part of the West Coast National Park (Fig. 1.2). It has also been declared a RAMSAR site for its significance as a wetland and layover for migratory birds, and is of ecological and conservation significance (Day 1959, Christie 1981, Compton 2001). The lagoon is ~15 km long with a maximum width of 4 km. It is connected to a large bay (Saldanha Bay) and is entirely marine with a mouth to the sea in the north and no riverine inflow – freshwater input is from groundwater seepage during the rainy season (Whitfield 2005). The lagoon is largely intertidal and experiences a spring tidal range between 1.8 m at the mouth and 1.5 m at the head (Day 1959b). The benthic substrate comprises mainly sandy sediment while various algal species, salt marsh grass (*Spartina* sp.) and seagrass (*Z. capensis*) make up the vegetation within and around the lagoon edge (Schils et al. 2001).

The lagoon and adjacent bay have been subject to numerous impacts from human activities. Saldanha Bay is an industrial port that handles bulk cargo. The economy of the region is centered on industries created by harbour-related activities, fishing and fish-processing plants, as well as a steel manufacturing industry (Welman and Ferreira 2016). Following the construction of an iron-ore jetty, a multi-purpose cargo terminal was added during the 1980s to facilitate the export of lead, copper and iron-ore and the import of oil (Welman and Ferreira 2016). These developments have altered hydrodynamics of the bay and significantly affected abundances in some species such as the seaweed *Gracilaria* spp (Rothman et al. 2009).

Red algae, *Gracilaria gracilis* and *Gracilariopsis longissima* occur in the bay and lagoon. In the 1950s and -60s, *Gracilaria* beach-cast supported a thriving industry in Saldanha Bay supplying as much as 20 tons of agar (Anderson et al. 1989). Collecting of algae was prohibited in Langebaan Lagoon due to its RAMSAR status, however, dredging activities and the building of a loading jetty in Saldanha Bay in the 70s led to a collapse in *Gracilaria* production that has not recovered (Rothman et al. 2009; pers. observation). *Gracilaria* can loosely attach to mud and sand substrates (Simons 1977) which makes their detachment easy. Their production in the lagoon has been variable and they have often been observed entangled in *Zostera* beds or washed up on the shore.

Saldanha Bay is regarded as a suitable site for mariculture in South Africa, due to the sheltered nature of the bay, and as a result 430 ha of sea space has been earmarked for farming of mainly oysters, mussels and finfish (Clark et al. 2016). In addition, further developments and expansions are planned for the port under the auspices of Operation Phakisa, a presidential directive aimed at strengthening the national economy and food security by accelerating economically-driven initiatives that exploit the marine environment (van Wyk 2015, Welman and Ferreira 2016). This includes expansion of the aquaculture sector in Saldanha Bay, a feat that is likely to enhance contamination, organic enrichment, and the influx of alien species in the bay and lagoon (Griffiths et al. 2009). Due to rising concerns for the overall health of this ecosystem, studies were commissioned in the 70s to act as a benchmark against which further monitoring of change could be assessed.

An analysis of satellite images (Landsat time series archive: 1985-2012) revealed dynamic interannual and/or seasonal variability in seagrass cover in the lagoon, while Pillay et al. (2010) used aerial photography to demonstrate that approximately 38% of seagrass cover had been lost from 1960 to 2007, and reported a total remaining area of 22 ha. Exact reasons for the decline were unclear, although dredging operations in the adjacent bay, and human disturbance associated with trampling and bait collecting were hypothesized as possible causes. Two other significant crashes in seagrass cover were identified in 1976 and 2003 (Angel et al. 2006), with the 1976 crash also linked to harbour dredging activities. A more recent review of the distributional status of *Zostera capensis* in South African estuaries reported seagrass cover in Langebaan Lagoon to be 85.8 ha (Adams 2016). Black and white aerial images as was used in the 2010 study, are problematic to interpret since shading and shadows can make it difficult to accurately quantify and distinguish seagrass cover from that of other submerged macrophytes e.g. *Gracilaria* spp. Therefore, the estimate of 22 ha should be used with caution. These studies however, highlight the need to understand variability of *Z. capensis* in the lagoon, and the importance of using technologies such as remote sensing and GIS to provide reliable assessments of coverage, and as a result clearer understanding of temporal and spatial patterns (Bax et al. 2019, Duffy et al. 2019).

Figure 1.2: Map of Saldanha Bay and the adjacent Langebaan Lagoon, west coast of South Africa. *Source: NASA ISS ISS030-E-275092*

1.6. Mesocosm Experiments in Seagrass Research

A mesocosm is an artificial experimental system that is a compromise between the macrocosm provided by nature and the microcosm created in a laboratory (Odum 1984). While experiments at the ecosystem scale may be realistic, they are often limited by low resolutions, and reduced experimental control (Quinn and Keough 2002). Mesocosms on the other hand can be replicated and still retain a degree of authenticity not completely achievable in micro laboratory experiments (Odum 1984, Crossland and La Point 1992).

Mesocosms are fully or partially enclosed outdoor or indoor systems designed for controlled experiments that provide an alternate to *in situ* sampling approaches

(Grantham et al. 2012). They usually comprise of tanks supplied with continuous sea water, where flow rate, current velocities, as well as water depth and sediment ratios can be artificially constructed and manipulated. Environmental variables such as light/shade, temperature, pH and salinity can also be controlled. This allows for a single environmental factor to be manipulated between control and treatment tanks, and in this way, the influence of a particular factor on a plant's characteristics can be determined (Short 1987). Mesocosms can therefore be used to assess the impact or impacts of single or multiple parameters on seagrass performance.

Mesocosms have been considered a useful experimental approach in seagrass studies and widely used to determine responses and interactions in seagrass habitats (Short 1987, Short et al. 1995, Sandoval-Gil et al. 2012, Marín-Guirao et al. 2013, Dierssen et al. 2015, ,). However, extrapolations from mesocosm experiments to the natural environment cannot easily be achieved, and is largely determined by the scale of experiment (Ahn and Mitsch 2002). In addition, mesocosm experiments can be expensive since they often require much equipment and technical expertise in their construction (Graney et al. 1989). Moreover, without meticulous design, close monitoring and control of all variables, the influence of response parameters on experimental outcomes can be difficult to extract and interpret (Graney et al. 1989).

Against the backdrop presented above, this thesis comprises two parts: the first deals with documenting the temporal and spatial changes in seagrass habitats and associated invertebrate communities at five locations in Langebaan Lagoon. The second part uses mesocosm experiments to describe the response in divergent *Zostera capensis* morphotypes to temperature and grazing, in order to further understand ecosystem processes in seagrass communities in Langebaan Lagoon.

1.7. Aims and Overview of the Thesis

This study aimed firstly to determine key environmental factors that best explain spatial and temporal variability in *Zostera capensis* habitats and their associated macro-epifauna in Langebaan Lagoon. Secondly, the effects of warming on two morphotypes of *Zostera* was explored in order to understand the ability of this species to maintain function under thermal stress. Lastly, the role of grazing in maintaining seagrass performance under warming was investigated. The overall aim was therefore to understand the physiological strategies that enable *Zostera* to adapt and acclimate to extreme temperature conditions.

The initial study design had included a component to investigate the trophic effects of mesopredatory fish with potential impacts on seagrasses however, an adequate background literature on fish related to seagrass habitats in Langebaan was limited at the time. Additional investigations needed to support this question expanded this study beyond its scope, and this component was omitted. Similarly, disturbance by the bioturbating sandprawn, *Callichirus kraussi* was not considered since this question was resolved in previous research (Siebert and Branch 2005, 2007). Field experiments revealed *Zostera* cover to expand in the absence of *C. kraussi*, although when introduced into treatments with *C. kraussi* present, sandprawn densities declined only briefly, before outcompeting the seagrasses, implying sandprawns had a greater impact on seagrass establishment than the other way around. Because of the threat of climate change and rising global temperatures, and the direct/indirect effects on shifting environmental factors, the focus of this study remained on the effects of abiotic variables and seagrass responses to particularly heat stress.

In Chapter Two**,** I conducted field surveys over four seasons to characterize the spatial and temporal variability of *Zostera capensis* distribution in Langebaan Lagoon, and determine the key environmental factors explaining the observed variability. I then describe seagrass-associated macro-epifaunal assemblages as well as investigate the influence of environmental variables and seagrass structure in determining macro-epifaunal community structure (Chapter Three). Two morphotypes of *Zostera* emerged and remaining chapters consider them separately. Chapter Four describes the results of a mesocosm experiment conducted to investigate the effects of temperature which was found to be a key factor influencing *Zostera* performance parameters. The response in density, biomass, morphometrics and algal fouling in the two morphotypes were measured under four thermal conditions (18, 22, 26 and 30^{o}C). Lastly, a second mesocosm experiment was undertaken to gain a mechanistic understanding of the top-down effects of grazing on the ecophysiology of each morphotype of *Zostera* under warming (Chapter Five). A general discussion and conclusions are provided in Chapter Six.

CHAPTER TWO

Spatial and Temporal Variability of *Zostera capensis* in Langebaan Lagoon

2.1. Introduction

Atmospheric carbon dioxide (CO_2) levels increased by 41% from eighteenth century preindustrial levels of ~280 ppm (parts per million) to an annual average of 402 ppm by 2017 (Dlugokencky and Tans 2018) attributed mainly to anthropogenic activities. This rate of increase is an order of magnitude faster than has been historically documented, and concentrations are higher than that recorded for the last few million years (Monastersky 2013). The ocean acts as a sink, taking up nearly a third of atmospheric carbon (Sabine et al. 2004, Doney et al. 2009), thus curbing the consequences of even greater CO_2 levels on the Earth's climate. This oceanic absorption of CO_2 leads to chemical changes in seawater that include acidification (reduced pH), and increases in total dissolved inorganic carbon (DIC), while reducing saturation levels of calcium carbonate and carbonate ion (Doney et al. 2009). Surface ocean pH is 0.1 units lower than preindustrial levels (pH 8.2) (Orr et al. 2005), and is predicted to drop by a further 0.14 to 0.4 by 2100 under current anthropogenic CO_2 emission rates (Gattuso et al. 2015). Elevated CO_2 has been shown to have a positive effect on growth and photosynthesis of several seagrass species, since seagrasses are carbon-limited under prevailing DIC levels in the ocean (Koch et al. 2013, Borum et al. 2016, Olsen 2018), and thus overall increases in productivity and biomass are expected under reduced water column pH (Duarte et al. 2018). However, the response of seagrasses to acidification appears to be species-specific and dependent on a seagrass's ability to exploit different species of DIC, further compounded by the interactive effects of other factors such as the availability of nutrients, irradiance quality and competition (Koch et al. 2013, Cox et al. 2015, Olsen 2018).

Another effect of anthropogenic activities is increasing eutrophication in shallow coastal and estuarine environments which impedes light availability thus promoting anoxic (no oxygen) or hypoxic (low oxygen) conditions (Howarth et al. 2011). This is amplified in warm water when oxygen solubility is lower (Shaffer et al. 2009), while respiration of organic matter is enhanced (Quiñones-Rivera et al. 2010, Rabalais et al. 2010). Prolonged anoxia increases sulfide levels in sediment, whereas water column hypoxia influences internal oxygen conditions, both negatively affecting photosynthetic rates, metabolism and growth in seagrasses (Koch et al. 2007b, Povidisa et al. 2009, Raun and Borum 2013). Die-offs have been reported for seagrass meadows under hypoxic conditions (Holmer and Bondgaard 2001, Sheilds et al. 2018) and the suggested reasons for this include a combination of intrusion of sediment sulfide and anoxic plant tissue, exacerbated by a greater demand for oxygen in warmer water to meet respiratory needs (Koch et al. 2001, Koch et al. 2007b). Since nearshore environments receive a higher output of organic matter from fluvial and estuarine sources than the open ocean, local scale variability in dissolved oxygen is higher in coastal compared to oceanic waters (Soetaert et al. 2009, Gilbert et al. 2010). Seagrasses are adapted anatomically and physiologically to contend with submersion as gases are efficiently diffused by well-formed aerenchyma tissue in the leaves, and oxygen loss through the root system is well buffered (Papenbrock 2012, Brodersen et al. 2018). However, prolonged hypoxic and anoxic conditions retard growth (Holmer and Bondgaard 2001), resulting in the eventual loss of seagrass habitats (Hall et al. 2016). Restoration of these important systems depends on interventions that reduce eutrophication and improve water quality.

Seagrass ecosystems regularly face fluctuations in the environment over time and space. Confined to nearshore subtidal and intertidal zones, they are influenced by air and water temperatures and have evolved to live in environments with varying temperatures evident by their global spread (Den Hartog 1970). Seagrasses persist in tropical and temperate waters, and variations within these environments can cause changes in net seagrass biomass and densities, as well as alter reproductive output (Wabnitz et al. 2008). However, both tropical and temperate seagrasses have optimal conditions in which growth and photosynthesis are at their peak (Niu et al. 2012). Even within these geographical temperature limits, fluctuations as well as extreme temperature shifts pose a threat to seagrass acclimation and survival.

Of the many factors influencing seagrass growth, productivity is mainly controlled by temperature, light, and nutrient availability (Dennison et al. 1993, Dunton 1994, Herbert and Fourqurean 2009). These key elements along with hydrodynamics of the system affect the spatial distribution of seagrasses (Schanz and Asmus 2003) as well as biochemical processes that regulate growth and reproduction (Dunton 1994, Lee et al. 2007). Several studies have been done to determine the effects of fluctuating temperatures (Campbell et al. 2006, Koch et al. 2007a), light limitation (Koch and Erskine 2001, Mvungi et al. 2012), extreme tidal exposure (Adams and Bate 1994, Unsworth et al. 2012, Petrou et al. 2013b), excess nutrients (Frankovich et al. 2009, Baggett et al. 2010) as well as their interactions with biodiversity (Blake and Duffy 2012, Eklöf et al. 2012) in a range of seagrass ecosystems with the aim of understanding seagrass-environment dynamics.

Several factors, often acting collectively, are deemed responsible for the decline of seagrasses worldwide (Orth et al. 2006, Short et al. 2006). These factors include eutrophication and coastal development, trophic interactions, and storm events linked to climate change (Short et al. 2006). Both natural and anthropogenic activities therefore, influence various processes responsible for seagrass meadow dynamics (Orth et al. 2012, Pollard and Greenway 2013). These activities can alter the stability of an ecosystem, which is sometimes easily detected in catastrophic events such as storms, cyclones and floods (Pollard and Greenway 2013), or are so subtle that immediate detection is evaded and change is gradual (Marbà et al. 2004, Pillay et al. 2010). Investigating the causes of seagrass decline is often only initiated once substantial habitat loss has occurred and even then the source of the decline is difficult to isolate. Changes that lead to lower productivity and even habitat loss may have severe implications on associated species and important ecosystem services (Duarte 2002, Orth et al. 2006, Barbier et al. 2011). Understanding the influence of the environment is therefore important to identifying and detecting key influences of seagrass growth, distribution and decline, and to particularly detect subtle change.

In South Africa, there are a few places remaining with monospecific stands of *Zostera capensis* which forms an important habitat in estuaries and coastal environments. One such place is Langebaan Lagoon supporting several large meadows of *Z. capensis*, an eelgrass listed by the IUCN (The World Conservation Union) as a “Vulnerable” species of conservation concern because of its restricted range and declining population trend (Short et al. 2011). Seagrass cover in the tidal lagoon was estimated at ~58 ha in 1960 and reported to have declined to 22 ha by

2009 (Pillay et al. 2010), however, by 2014 seagrass cover was assessed to be 85.8 ha (Adams 2016). Understanding the natural variability and identifying the environmental factors that shape *Z. capensis* populations will broaden our knowledge of how this temperate seagrass responds to fluctuating environments and predicted extremes. Variability including acclimation strategies to environmental conditions can be identified by examining seasonal environmental influences on morphometric parameters.

In this chapter, I document the spatial and temporal patterns of distribution in *Zostera capensis* in Langebaan Lagoon, and identify particular environmental factors influencing variability in density, biomass and morphometrics of *Zostera*. This is a critical first step towards understanding variability, and essential when establishing long-term initiatives to monitor change in seagrass habitats. Monitoring seagrass abundance and distribution, as well as tracking environmental parameters, are a prerequisite to understanding natural dynamism, and allows for detection of trends and anomalies. The prolonged effects of anomalies can cause shifts in ecosystem stable states, alter ecosystem functions and lead to loss of productivity and biodiversity (Duarte 2002, Dennison 2009, Winters et al. 2011, Fraser et al. 2014). Knowledge of the influence of environmental factors on ecosystem states aids in identifying effects of natural and human-induced stress as well as the effectiveness of conservation efforts. Furthermore, the results can be used to inform predictions of environmental effects under future climate change scenarios, and contribute to the wider knowledge of seagrass ecosystems (Orth et al. 2006, Short et al. 2007).

The following key questions are addressed:

1. How do *Zostera capensis* morphometrics and epiphyte algal biomass vary spatially and temporally in Langebaan Lagoon?
2. To what extent do the selected environmental factors explain variability in seagrass metrics?
3. To what extent do localized environmental differences vary between sites?
4. Which environmental variables support the highest outputs of seagrass morphological metrics and epiphyte algal biomass?

5. Does *Z. capensis* display acclimation strategies and can these strategies be explained by environmental or site-specific contexts? And if so, what are the key factors that produce these acclimations?

2.2. Methods

2.2.1. Site Selection and Sampling Period

Intertidal seagrass populations at five sites in Langebaan Lagoon - Centre Banks, Oesterval, Klein Oesterval, Bottelary, and Geelbek – covering an area of 10 542 m^2 were studied (Fig. 2.1). These sites were selected based on previous spatial descriptions of *Zostera* in Langebaan Lagoon (Day 1959), further substantiated my analysing satellite (Landsat time series archive - 1985-2012) and aerial imagery (1960-2009, Pillay et al. 2010) that confirmed the consistent seasonal and/or interannual colonisation of these areas by seagrasses albeit with a high degree of variability. Centre Banks, Klein Oesterval and Oesterval are located closer to the lagoon mouth, while Bottelary and Geelbek occur closer to the head of the lagoon (Fig. 2.1). Sampling was carried out during four austral seasons: autumn (April), winter (July), spring (October) and summer (February). The number of beds or patches sampled at each site was selected according to the total number of beds or overall seagrass cover each site contained. For example, Oesterval had ~20 beds and 10 were sampled while beds at Klein Oesterval and Bottelary ranged from three to rarely more than five. At these sites only three beds were sampled each season. Centre Banks and Geelbek each had essentially one continuous seagrass stand >200 m long, and here samples were taken every 50 m.

2.2.2. Seagrass Metrics Measured

Five core samples (10 cm diameter x 15 cm depth; 0.0079 m^2) of seagrass shoots were collected arbitrarily in each bed or bed section during spring low tide when beds were completely exposed. Shoot densities varied within beds and since core samples were taken randomly, areas with low and high seagrass cover were sampled. Seagrasses from each core were gently rinsed in the field to remove sediment and faunal epiphytes then placed in a bag and transported to the University of Cape Town for analyses. In the laboratory, the principal seagrass biological and foliar parameters (shoot densities, leaf densities, leaf length, leaf width and aboveground biomass) were calculated for each core sample. Short shoots (hereafter referred to as "shoots") and leaves were counted and the average width and length of 20% of the longest leaves in the core were recorded. Seagrass shoots were separated from their root/rhizomes and leaves were dried at 60°C to a constant dry weight (~12 hours). This short drying period was due to the small shoots/leaves and low biomass characteristic of this species. Shoots were then weighed to obtain aboveground dry weight (g m^{-2}). Epiphytic algae were gently scraped off all leaves per core into a pre-weighed petri dish using the back of a scalpel blade. Epiphytic algal biomass dry weight m^{-2} was obtained after drying at 60°C till constant weight was reached (~11 hours).

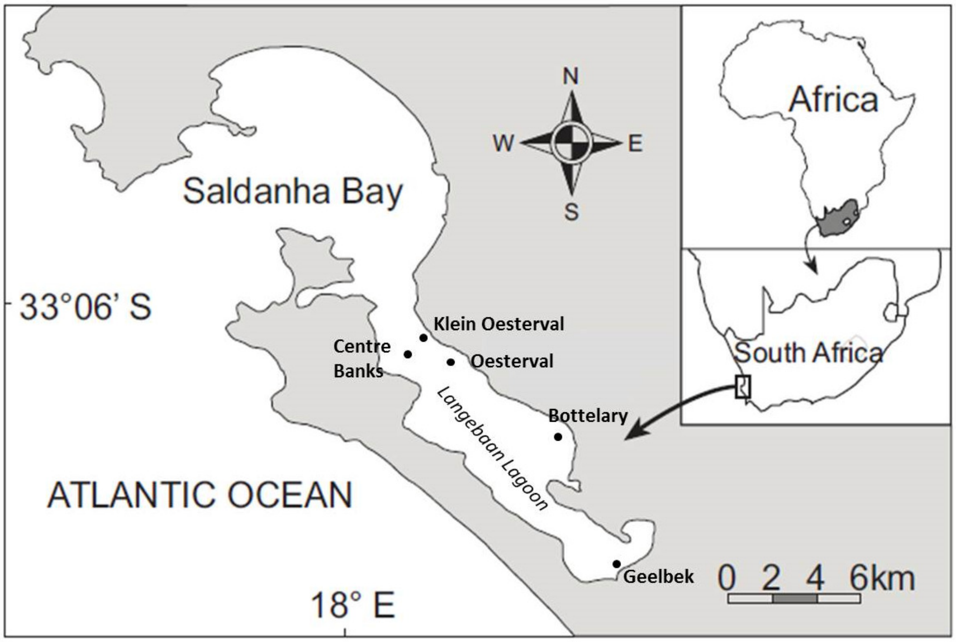


Figure 2.1: Map of Langebaan Lagoon on the west coast of South Africa indicating the five major *Zostera capensis* populations sampled in this study.

2.2.3. Environmental Variables

Measurements of temperature, pH, turbidity, salinity, dissolved oxygen and chlorophyll *a* (chl *a*) were taken monthly during a similar time of day, over a 12 month period, *in situ* at each site during spring high tide using a handheld multiparameter probe (Conductivity Temperature Depth profiler, Model: YSI 6820 V2-2V). According to a bathymetry model of Langebaan Lagoon developed by Flemming (1977), average water depth at high tide is estimated at 2 m at Centre Banks, 1.5 m at Klein Oesterval and Oesterval, and 1 m at Bottelary and Geelbek. Langebaan Lagoon experiences a semi-diurnal tidal regime with an average spring tidal range of 1.4 m and a maximum astronomical tide range of 2 m (Day 1981). Maximal tidal range in South Africa is 2 m and as a result estuarine systems have been described as micro-tidal (Whitfield 1992). Centre Banks is a sand bank between the eastern and western shores and is closest to the lagoon mouth (Fig 2.1). It experiences shorter tidal exposure times compared to the other sites where seagrass beds grow mostly along the shoreline edge and are exposed for longer. Seagrass meadows at Geelbek for example, form a continuous narrow strip fringing the southern edge of the lagoon and experienced a maximum exposure time of up to three hours during spring low tide.

During low tide, it is assumed that all sites are subjected to the same solar irradiance intensity, however since sites occurred at varying depths, the duration for which they are exposed is expected to be different. Direct measurements of light were not recorded instead turbidity was measured as a proxy for water column irradiance and to assess variability of light reaching seagrass plants during high tide, since suspended particulate matter has been found to influence light levels reaching seagrass plants (Livingston et al. 1998). Beds were assigned an exposure level based on the average amount of time they remained out of the water during spring low tide as well as distance (m) from the high water mark. They were classified as ‘high shore’ if beds were within 10 m of the spring high water mark, ‘mid shore’ between 10 -50 m and ‘low shore’ if greater than 50 m.

2.2.4. Statistical Analyses

Data were explored using draftsman plots, histograms and scatterplots. Multi-panel scatterplots and correlation analyses indicated strong collinearity between temperature and pH (Spearman $R_s = 0.87$, $p = 0.001$), leaf and shoot densities (Spearman $R_s = 0.92$, $p = 0.001$) as well as leaf length and leaf width (Spearman $R_s =$

0.85, *p* = 0.001). Therefore, leaf density, leaf width and pH were excluded from analyses because their collinearity would confound observed variation (Lipovetsky and Conklin 2001).

In order to statistically relate the effects of environmental conditions to seagrass morphological metrics and epiphyte biomass, 2-tailed Pearson correlations were carried out between seagrass metrics and environmental variables averaged across three months, and with a one month lag preceding the seagrass sampling event. One month was assumed a reasonable response time for a small-leaved seagrass such as *Zostera capensis* since a similar species (e.g. *Z. noltei*) was observed to delay its response to seasonal fluctuations in this period relative to a large-leaved seagrass such as *Z. marina* (Marba et al. 1996). Correlations were higher between seagrass metrics and seasonal averages of environmental variables compared to measurements recorded one month before (refer to Appendix I for correlation results), meaning that seasonal environmental factors were better predictors of growth responses of *Z. capensis* in Langebaan Lagoon. This was the case for all environmental variables with the exception of oxygen which showed a slightly stronger and significant correlation to leaf length and width in the preceding month (-0.58 and -0.61 respectively) than over the season (-0.47 and -0.53 respectively). Given that this difference was marginal, seasonal averages of all environmental variables were therefore used to predict responses in seagrass morphological metrics in subsequent analyses.

2.2.4.1. Multivariate Analyses

Multivariate analyses were used to explore the effects of Seasons (fixed factor: four levels) and Sites (random factor nested within season: five levels) on variations in three seagrass morphological metrics (leaf length, shoot density and aboveground biomass (referred to as seagrass biomass) and epiphytic algal biomass per core ($0.0079m^2$) using PERMANOVA+ for PRIMER (Plymouth Routines in Multivariate Ecological Research, version 6; Clarke and Gorley, 2006). Visual assessment of site differences in seagrass metrics was done using ordination techniques (non-metric multi-dimensional scaling (MDS) and principal coordinates (PCO)). Vectors correlating environmental variables were overlain on PCO plots. Seagrass variables were 4th root transformed to down weight the influence of large variances, and differences were calculated as Euclidean distances. 'Bed' was used as the sampling unit which represented the mean of five replicate core samples.

Permutational multivariate analysis of variance (PERMANOVA, Anderson et al. 2008) was performed using sequential Type I Sums of Squares to test the null hypothesis of no differences among groups, on 9999 permutations generating a pseudo-F test statistic and corresponding P (perm) value (Anderson 2005). For acceptable contrasts for site and season factors, α was set at 0.05 for the analyses. PERMANOVA is robust to differences in multivariate dispersion among group centroids in balanced designs (Anderson 2005). To test for homogeneity in relative group spread by comparing average distance values of each observation to their group centroid, PERMDISP (9999 permutations) including post hoc pair-wise comparisons, were run (Anderson 2006, 2017). Thereafter, a similarity percentage breakdown analysis (SIMPER) on Euclidean distances was done to calculate the contribution of each seagrass variable to the observed dissimilarity between sites.

To determine the extent to which selected environmental factors explain variability in seagrass metrics, environmental predictor covariates that most strongly influenced the multivariate assemblage of seagrass and epiphyte variables were assessed using a distance-based linear model in PERMANOVA (DistLM, with selection procedure 'best' and criteria 'AIC'). DistLM calculates the proportion of variability contributed by each environmental variable and partitions variation in seagrass metrics according to univariate multiple regression modelling based on selected predictor variables (Anderson et al. 2008). In this assessment, all possible combinations of predictor variables are explored and the best models are selected based on Akaike's information criterion (AIC) (Akaike 1974) and adjusted R^2 values. Small AIC values indicate a better model fit, therefore the lowest AIC value signals the most efficient model to explain variation (Anderson et al. 2008).

2.2.4.2. Univariate Analyses

Environmental variables (water temperature, salinity, pH, turbidity, chlorophyll *a* and oxygen) were "normalised" before analysis by subtracting the mean from each value for each variable and then dividing by their standard deviation. This is usually necessary for bringing environmental data which have different scales and units into proportion with each other in order to derive meaningful outputs. Thereafter, two-factor ANOVAs of each environmental variable with sites (random) and seasons (fixed) were performed to test for localized differences and variations in the measured environmental parameters, followed by Tukey multiple comparisons post hoc testing.

To test the effect of site (random factor) and season (fixed factor) on each seagrass metric including algal epiphyte biomass, two-factor analyses of variance (ANOVA) were performed followed by post hoc tests (Tukey multiple comparisons). If assumptions for normality were not met following a Shapiro-Wilk's test, seagrass variables were transformed (logged or logged +1).

Following the initial analyses of the multivariate seagrass metric dataset, one categorical (exposure level/shore height) and five continuous environmental variables (water temperature, salinity, turbidity, dissolved oxygen and chlorophyll *a*) and combinations thereof were tested as likely predictors of variation in seagrass metrics using generalized additive mixed models (GAMMs) (Wood and Augustin 2002). GAMMs allow for flexibility in modelling non-linear relationships and contain model functions constructed on a penalized regression-spline approach that incorporates smoothness selections in the estimation process (Wood 2017). The degree of smoothing provided by each smooth term is treated as a random effect and expressed as "effective degrees of freedom" (edf) estimated based on its variance in a mixed modelling structure (Wood 2017). Smoothers with high edf values (>8) indicate a highly non-linear curve, while a straight line has an edf of 1 (Zuur et al. 2009, Wood 2017).

GAMMs were evaluated for each seagrass metric and included smoother functions for continuous environmental variables only and a random effect for 'site'. Seagrass metrics were transformed (logged or logged +1) if assumptions for normality were not met following a Shapiro-Wilk's test. Knots were selected incrementally to produce relationships that were biologically meaningful, and checked for over-specification (Wood 2017). Model assumptions were also assessed by inspecting diagnostic plots of random effects and residuals. Most parsimonious models were derived after evaluating the significance of the random effect in the full model. This was followed by determining optimal combinations of predictor variables by opting for low AIC values and high R^2 values, while selecting for only significant predictor variables in the final model. Models were configured on a Gaussian distribution with a maximum likelihood function (Zuur et al. 2009), and tested using the R package 'mgcv' (Wood and Scheipl 2014) within the "nlme" library (Pinheiro et al. 2016) using the R statistical software 3.6.0 (The R Development Core Team, 2015; www.r-project.org).

Significance for all tests was assessed at $\alpha < 0.05$, and variance is expressed as standard error (SE) of the mean.

2.3. Results

2.3.1. S*eagrass morphometrics and epiphyte biomass*

2.3.1.1. Multivariate analyses

PERMANOVA indicated significant variation among sites and the interaction between sites and seasons (Table 2.1). Contrasts in morphological variables and epiphytic algal biomass were significant across sites, and the interaction of site and season contributed slightly more to the explained variation than site only (Table 2.1). Despite sites being >90% similar in the multivariate space a spatial pattern with distance from the lagoon mouth was visually evident (Fig. 2.2A). Seasonal differences were not significant and accounted for the least variation (17%) which was confirmed visually except for a slight separation in variability of morphological metrics at Centre Banks in summer (Fig. 2.2B). A large percentage (51%) of multivariate variation in morphology was unexplained.

Multivariate dispersion of centroids for ‘site’ was further assessed in a PCO due to its significance and the nested ranking of this factor. Site differences in multivariate dispersion was found to be significant (PERMDISP: $p < 0.001$) most notable by significant differences between two sites (Oesterval and Klein Oesterval) and the other three sites. The evidence of a morphological distinction along environmental gradients was supported visually along PCO1 although differences between Bottelary and Geelbek are evident along PCO2 (Fig 2.3).

The relative contribution of each morphological metric to site distinctions was determined in a SIMPER analyses. Shoot density contributed the highest proportion (>60%) of variation to within and between site differences for all sites with a few exceptions. At Bottelary, epiphytic algal biomass contributed more (41.85%) to the explained variation than number of shoots (35.78%). Variation between seagrass metrics at Centre Banks vs Geelbek and Bottelary was explained more by leaf length than by shoot densities, which are evident in their spatial and temporal contrasts (Fig 2.4).

Table 2.1. PERMANOVA results based on Euclidean distances comparing seagrass variables (leaf length, shoot density, seagrass and epiphyte biomass) sampled across five sites (random) and four seasons (fixed). Effect size was calculated from the estimated contribution of components of variation for each factor. *P* values are significant at α < 0.05 and in bold.

Source of variation	df	MS	Pseudo-F	*p*	Unique permutations	Effect Size
Season	3	8.18	2.06	0.092	9943	17%
Site	4	10.46	20.66	**<0.001**	9929	53%
Season x Site	12	3.03	5.99	**<0.001**	9902	54%
Residual	79	0.51				51%

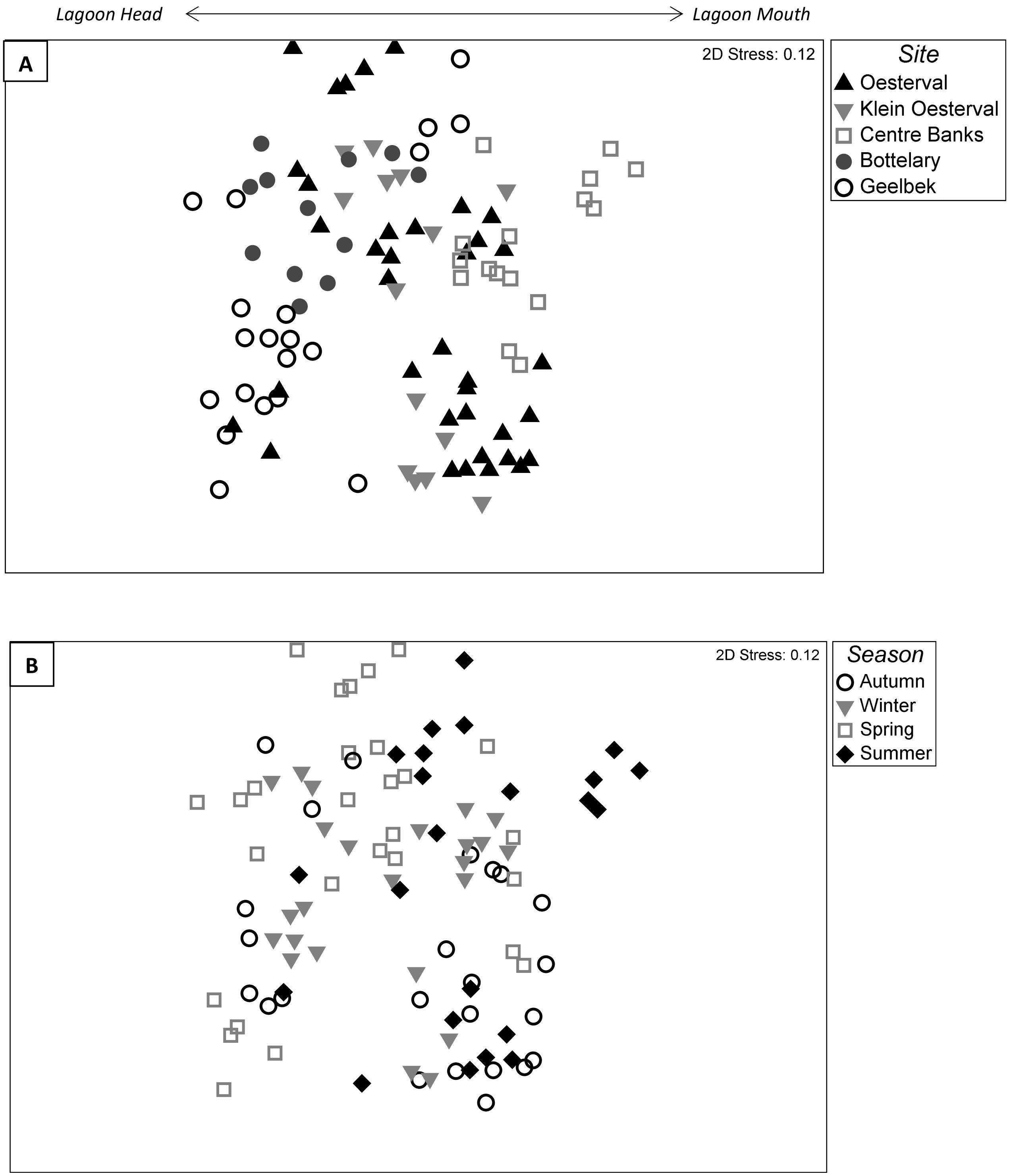


Figure 2.2: Multi-dimensional scaling ordination of distances among centroids based on Euclidean distances for site (A) nested within season (B) distributions of morphometrics and associated epiphyte biomass of *Zostera capensis* in Langebaan Lagoon.

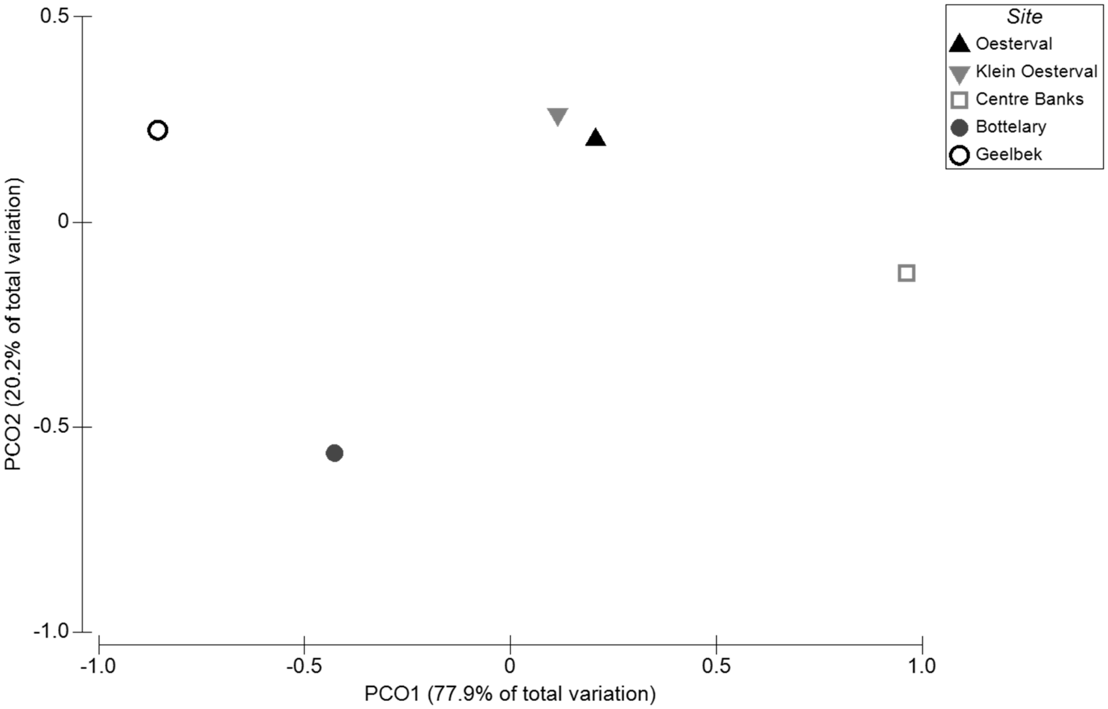


Figure 2.3. Metric ordination (PCO) of site centroids based on Euclidean distances among variable groups representing seagrass metrics and algal epiphyte biomass from five sites in Langebaan Lagoon.

2.3.1.2. Univariate analyses

Shoot densities differed significantly across sites ($F_{4,\ 494} = 117.22$, $p < 0.001$) and seasons ($F_{3,\ 494} = 75.5$, $p < 0.001$). Bottelary and Geelbek had similar means and highest average densities of shoots (744 and 769 m^{-2} respectively) (Fig. 2.4A). Average shoot densities were also similar at Oesterval and Klein Oesterval (412 and 344 m^{-2} respectively), while seagrass beds at Centre Banks were the least dense (195 m^{-2}) (Fig. 2.4A). The average lengths of seagrass leaves were also shorter at Geelbek and Bottelary compared to Centre Banks, Klein Oesterval and Oesterval (Fig. 2.4B). Summer produced the lowest shoot densities for all sites except Oesterval which had lowest densities in autumn (Fig. 2.4A). All sites produced longer leaves in autumn and winter, with the exception of Centre Banks where leaves were shortest in winter and longest in summer and autumn (Fig. 2.4B). Average length of leaves differed significantly across sites ($F_{4,494} = 179.49$, $p < 0.001$) and seasons ($F_{3,494} = 29.29$, $p < 0.001$) due to variability in mean leaf lengths at all sites (Tukey HSD, $p < 0.05$).

Seagrass beds at Oesterval produced the highest average aboveground biomass (9.72 g dry wt m^{-2}) across all seasons, followed by Centre Banks (9.45 g dry wt m^{-2}) and Klein Oesterval (8.04 g dry wt m^{-2}). Seagrass biomass at Bottelary was comparable to sites closer to the mouth while biomass at Geelbek was consistently low for all seasons (Fig. 2.4C). Seagrass biomass differed significantly between sites ($F_{4,\ 494}$ = 29.71, $p < 0.001$) and seasons ($F_{3,\ 494}$ = 57.23, $p < 0.001$) due mainly to high variability between Geelbek and the other four sites (Tukey HSD, $p < 0.05$). On average seagrass biomass was lowest in summer in the lagoon and higher in autumn and winter (Fig. 2.4C).

In general, epiphytic algal biomass did not appear to show a correlation with leaf length or density. Algae were found on seagrass leaves at Klein Oesterval in all four seasons, while no algae were recorded at Oesterval, Centre Banks and Bottelary in winter, or from Geelbek during the entire sampling period (Fig. 2.4D). On average highest algal biomass was recorded on seagrass leaves at Centre Banks, followed by Bottelary, Oesterval and Klein Oesterval (Fig. 2.3D). These differences were significant across sites ($F_{4,494}$ = 7.12, $p < 0.001$), seasons ($F_{3,494}$ = 22.03, $p < 0.001$) and their interaction ($F_{12,494}$ = 8.22, $p < 0.001$) due to variation in mean algal biomass between Geelbek and the other four sites (Tukey HSD, $p < 0.05$).

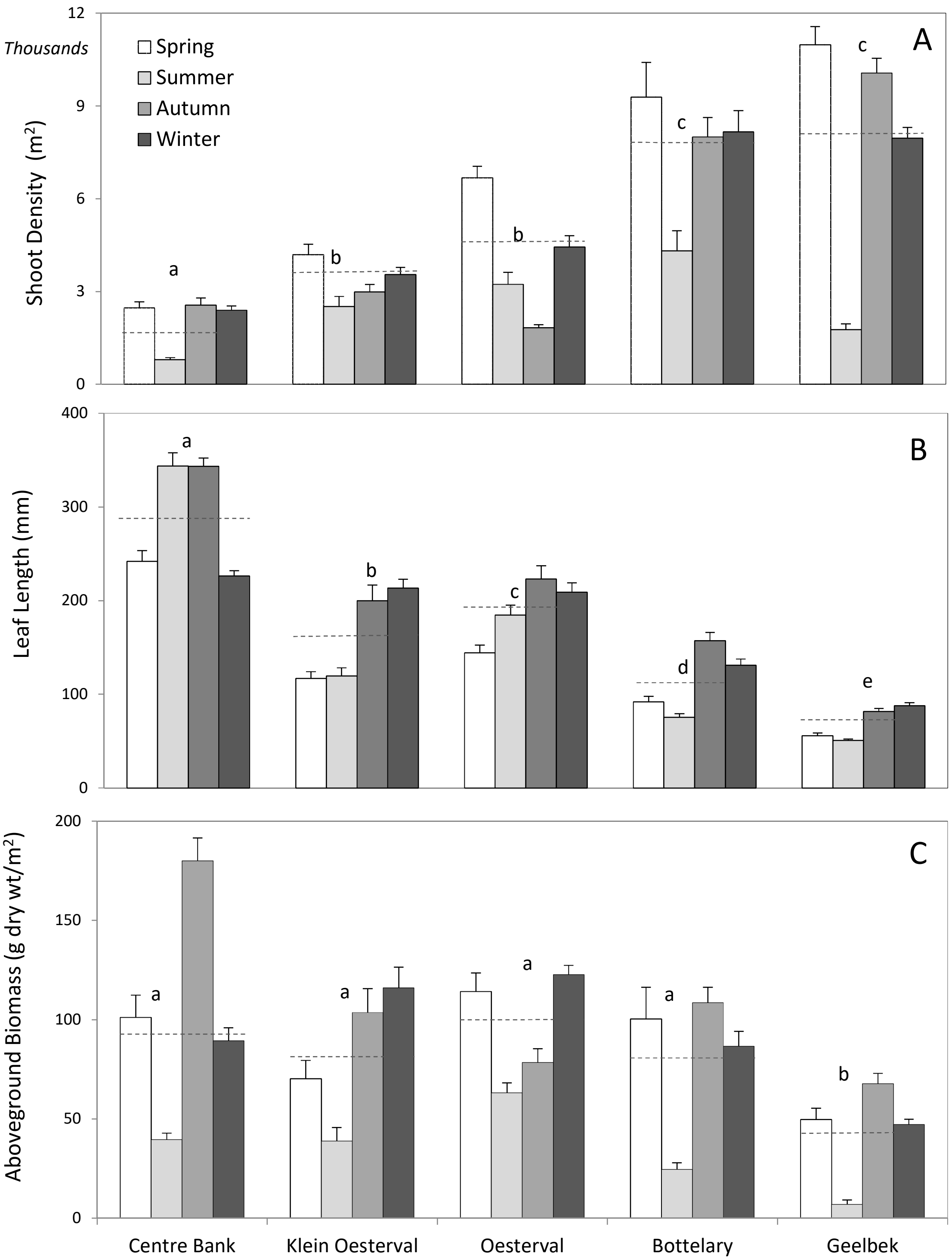


Figure 2.4. Spatial variation in mean shoot density (A), leaf length (B), aboveground biomass (C), and epiphytic algal biomass (D) of *Zostera capensis* across four seasons. No epiphytic algae were recorded from Geelbek in all seasons sampled. Small letters indicate similar means, indicated by dotted lines, not significantly different when compared using an *a posteriori* Tukey HSD test. Error bars represent +1SE (refer to Appendix II for site and seasonal means per seagrass metric).

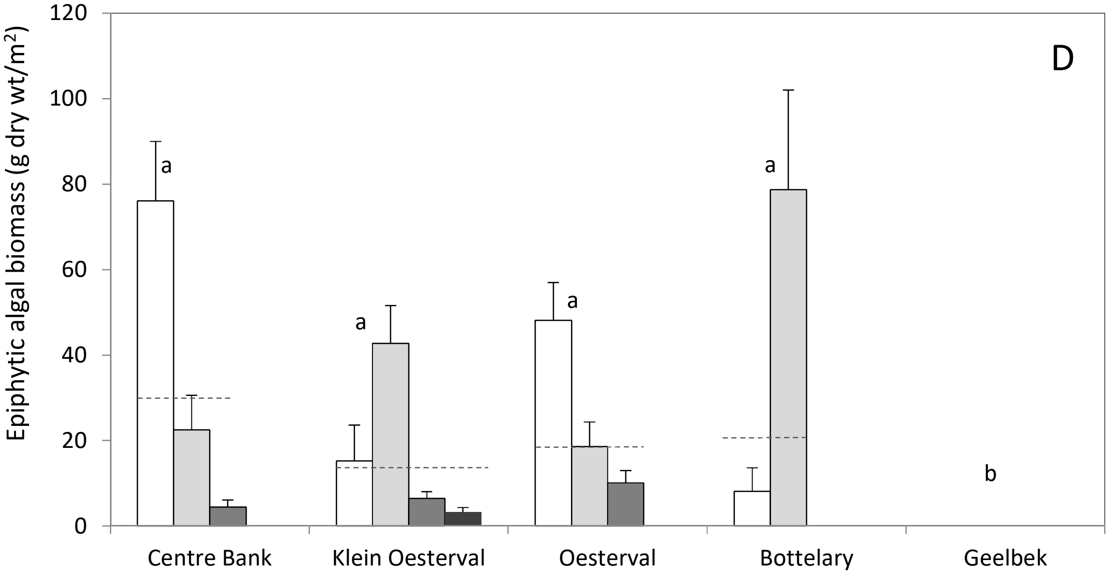


Figure 2.3. Continued.

2.3.2. *Spatial and Temporal Patterns in Environmental Variables*

Langebaan lagoon exhibits an environmental gradient from the mouth to the upper reaches of the lagoon. Average water temperature increased with distance from the mouth, with temperatures at Bottelary and Geelbek being consistently warmer compared to Klein Oesterval and Oesterval (Table 2.2). Water temperature at Centre Banks was the lowest on average throughout the study. Highest temperatures in the lagoon were recorded at Geelbek followed by Bottelary. Maximum temperature values were generally similar and lower near the mouth at Klein Oesterval, Oesterval and Centre Banks (Table 2.2). Water temperature differences were significant across sites ($F_{4,141} = 28.23$, $p < 0.001$), seasons ($F_{3,\ 141} = 93.51$, $p < 0.001$) and their interaction ($F_{12,141} = 2.22$, $p = 0.015$) explained mainly by variation in means between Centre Banks, Bottelary and Geelbek (Tukey HSD, $p < 0.05$, Table 2.2). Mean temperatures differed across all seasons with the exception of spring and autumn (Tukey HSD, $p < 0.05$).

Salinity levels between sites ($F_{4,\ 141} = 13.39$, $p < 0.001$), seasons ($F_{3,\ 141} = 8.74$, $p < 0.001$) and their interaction ($F_{12,\ 141} = 9.75$, $p < 0.001$) differed significantly. Geelbek and Bottelary experienced highest salinity levels while Centre Banks, Klein Oesterval and Oesterval had similar average salinities (Table 2.2). Variation at the site level was explained by the significantly higher salinity levels at Geelbek compared to the other four sites (Tukey HSD, $p < 0.05$), while salinities across the remaining four sites remained homogenous. Seasonal variation was explained by the significant differences between salinities in summer and autumn, and summer and winter (Tukey HSD, $p < 0.05$, Table 2.2).

Water column pH levels differed significantly between sites ($F_{4,\,141} = 2.98$, $p = 0.022$), seasons ($F_{3,\,141} = 93.44$, $p < 0.001$) and their interaction ($F_{12,\,141} = 2.30$, $p = 0.011$). This was mainly due to pH at Oesterval being significantly different to levels at Centre Banks, Geelbek and Bottelary (Tukey HSD, $p < 0.05$). On average pH at Geelbek were 0.33 levels higher i.e. more alkaline (8.21) compared to the other four sites, however Centre Banks experienced the greatest range in pH with a minimum of 6.88 in winter and a maximum of 9.67 in summer (Table 2.2). All sites experienced alkaline conditions in summer compared to winter with the exception of Klein Oesterval where alkalinity was higher in spring.

Average turbidity in the water column was highest at Geelbek with maximum levels reaching almost five times higher than other sites (Table 2.2). This implies lower light levels in the water column at Geelbek. Average turbidity across the other four sites ranged from 5.73 – 8.57 NTUs (Table 2.2). Turbidity differed significantly across sites ($F_{4,\,141} = 22.37$, $p < 0.001$) but not across seasons ($F_{3,\,141} = 2.48$, NS). The interaction between site and season was also significant ($F_{12,\,141} = 2.55$, $p = 0.005$) driven mainly by the high differences in turbidity between Geelbek and the other sites (Tukey HSD, $p < 0.05$, Table 2.2).

A generally high level of dissolved oxygen (DO) was recorded at Geelbek across all seasons with the highest value of 26.44 mg/L recorded in autumn (Table 2.2). Centre Banks experienced lowest DO levels, while DO at Klein Oesterval, Oesterval and Bottelary were similar (Table 2.2). DO between sites differed significantly ($F_{4,141} = 6.75$, $p = 0.001$) due to differences in means between sites closer to the mouth (Centre Banks, Oesterval and Klein Oesterval) and those closer to the end (Geelbek and Bottelary) of the lagoon (Tukey HSD, $p < 0.05$, Table 2.2). Seasonal differences were not significant ($F_{3,141} = 1.8$, NS), while the interaction between sites and seasons were ($F_{12,140} = 4.32$, $p = <0.001$).

Dissolved chlorophyll *a* (chl *a*) was significantly different at the site level ($F_{4,141} = 20.760$, $p < 0.001$) due to a higher mean recorded at Geelbek compared to the other sites (Tukey HSD, $p < 0.05$, Table 2.2). Average chl *a* was relatively stable at the other sites and ranged from 1.46 mg/L at Klein Oesterval, 1.71 mg/L at Oesterval, 2.01 mg/L at Bottelary and 2.10 mg/L at Centre Banks. No differences in chl *a* levels were found between seasons ($F_{3,\ 141} = 1.089$, NS) or the interaction between site and season ($F_{12,\ 141} = 1.013$, NS).

Table 2.2: Abiotic variables recorded over 12 months at five sites in Langebaan Lagoon. Means ± 1 standard error, minimum and maximum values are presented. Small letters represent homogenous means between sites that are not significantly different when compared using Tukey HSD testing.

		Centre Banks	**Klein Oesterval**	**Oesterval**	**Bottelary**	**Geelbek**
Water temperature (^{o}C)		15.86^{a} ±0.46	18.64^{abc} ±0.75	17.89^{ab} ±0.65	20.74^{bc} ±0.93	21.42^{c} ±0.92
	Min	12.68	13.62	13.07	14.68	15.01
	Max	20.15	25.53	23.78	27.68	28.49
Salinity		34.26^{a} ±0.21	34.40^{a} ±0.21	34.71^{a} ±0.20	34.86^{a} ±0.29	35.76^{b} ±0.55
	Min	32.38	32.40	32.80	32.30	31.87
	Max	35.80	36.30	36.13	37.32	40.65
pH		7.78^{a} ±0.19	7.88^{bc} ±0.13	7.86^{c} ±0.14	7.98^{ab} ±0.13	8.21^{a} ±0.13
	Min	6.88	7.09	7.06	7.08	7.31
	Max	9.67	9.48	9.48	9.32	9.28
Turbidity (Nephelometric turbidity units)		6.99^{a} ±0.58	5.73^{a} ±0.39	6.66^{a} ±0.45	8.57^{a} ±0.69	23.79^{b} ±3.07
	Min	4.40	3.60	4.30	5.10	7.70
	Max	16.10	12.90	14.00	18.00	73.30
Oxygen (mg/litre)		7.52^{a} ±0.16	8.66^{ab} ±0.26	8.26^{ab} ±0.70	8.83^{b} ±0.27	11.76^{c} ±1.92
	Min	6.17	6.84	6.24	7.32	7.32
	Max	8.40	16.40	9.60	10.65	26.44
Chlorophyll *a* (mg/litre)		2.10^{a} ±0.21	1.46^{a} ±0.11	1.71^{a} ±0.10	2.01^{a} ±0.16	5.63^{b} ±0.69
	Min	1.00	0.70	1.00	0.90	1.70
	Max	7.30	3.40	3.60	4.10	15.50
Exposure/shore height (m)		Low shore	Low/mid/high shore	Mid/low shore	High shore	High shore

According to the marginal test from the distance-based linear model, exposure contributed the greatest proportion of variation in seagrass morphological metrics and epiphyte biomass between sites (Table 2.3). This was followed by turbidity, oxygen, salinity and chlorophyll *a* (Table 2.3). Variability in seagrass metrics provided by temperature was 4.11%. The PCO ordination confirmed these responses visually, as the longest trajectories indicating the strength of the Spearman correlations were provided by exposure, turbidity and oxygen (Fig 2.4). The best model solution based on lowest AIC and highest R^2 values suggested all six environmental variables contributed to the observed patterns in seagrass morphometrics and algal epiphyte biomass (Table 2.3).

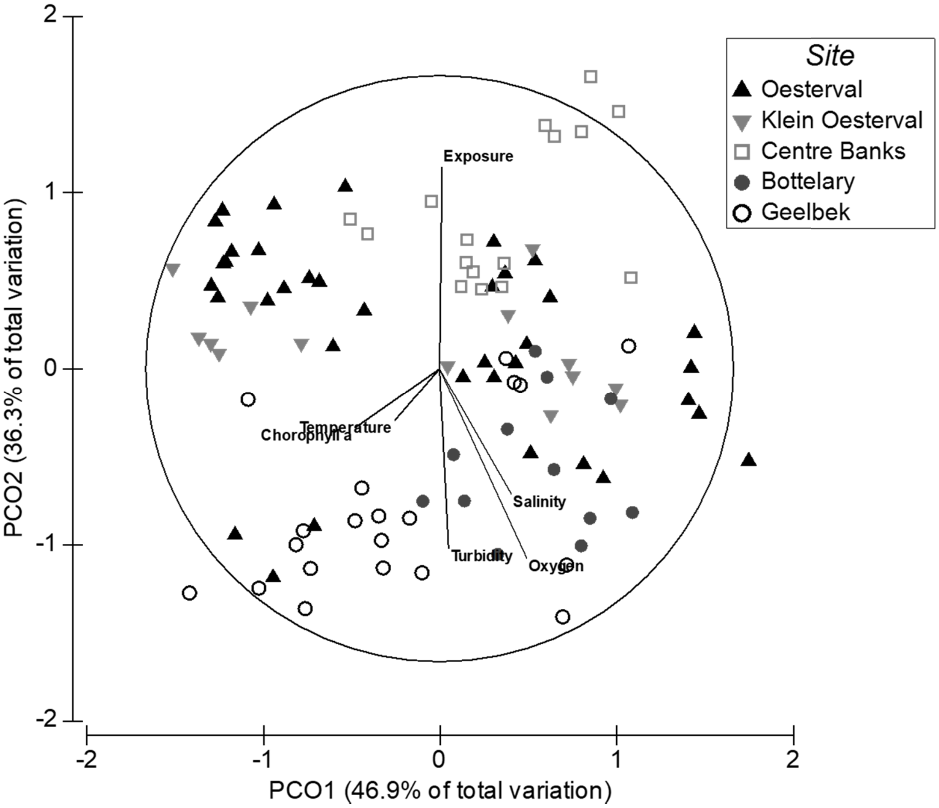


Figure 2.4. PCO of seagrass metrics and algal epiphyte biomass from five sites in Langebaan Lagoon partitioned based on Euclidean distances between group centroids. Six environmental vectors are overlain, and lengths and direction of trajectories indicate the correlative strength of the relationship between environmental variables and the PCO axes.

Table 2.3: Results of distance-based multiple regression modelling (DistLM) used to estimate the proportion of variation explained by continuous and categorical environmental variables on seagrass morphometrics and algal epiphyte biomass. Overall five best model solutions are presented ranked by fit based on AIC values. *P* values are significant at < 0.05 and in bold.

		Marginal Tests	
Variable	**Pseudo-F**	***p***	**Proportion of variation**
Temperature	4.16	**0.009**	4.11%
Salinity	8.78	**<0.001**	8.30%
Turbidity	13.86	**<0.001**	12.51%
Oxygen	13.72	**<0.001**	12.39%
Chlorophyll *a*	6.29	**<0.001**	6.09%
Exposure	19.21	**<0.001**	16.53%

Overall best solutions			
AIC	**R^2**	**Number of variables**	**Selections**
6.26	0.36	6	Temperature, Salinity, Turbidity, O_2, Chl *a*, Exposure
7.76	0.34	5	Temperature, Salinity, Turbidity, Chl *a*, Exposure
9.31	0.32	5	Temperature, Turbidity, O_2, Chl *a*, Exposure
9.59	0.32	5	Temperature, Salinity, Turbidity, O_2, Exposure
10.16	0.31	4	Temperature, Salinity, Turbidity, Exposure

Results from generalized additive mixed modelling showed patterns in seagrass densities in Langebaan Lagoon to be largely (>80%) predicted by water temperature, turbidity, oxygen, salinity and exposure (Table 2.4). High seagrass densities were not supported in low and midshore positions, while densities were predicted to decline in water temperatures above 22°C (Fig. 2.5).

Warmer water temperature and higher salinity is predicted to have a significant negative effect on leaf length, a response similarly observed for turbidity (Table 2.4). This is supported by observations of longer leaves at cooler temperatures closer to the mouth, and shorter leaves in more turbid conditions at the end of the lagoon. Leaf width on the other hand, is predicted to decrease with increasing levels of chlorophyll *a* but increase with higher levels of dissolved oxygen. Increasing salinity is also predicted to produce narrower leaves (Fig. 2.5), while low and mid shore levels are predicted to support longer/wider leaves (Table 2.4).

Water temperature, oxygen and salinity were the principle factors influencing aboveground biomass accounting for 69% of variation (Table 2.4). Exposure was not a significant predictor of aboveground biomass, whereas warmer temperature and higher oxygen is expected to negatively influence seagrass biomass (Fig. 2.5).

Four environmental factors were significant in predicting patterns in epiphytic algal biomass (Table 2.4). Warmer temperature is predicted to favour algal epiphyte growth along with increasing levels of chl *a* (Fig. 2.5), which will be supported in low shore stands. In contrast, decreasing water clarity and salinity are expected to negatively influence algal epiphyte growth. These variables only explained 29% of variation in algal biomass implying that other factors, possibly biotic and abiotic, are better predictors of epiphytic algal growth.

Table 2.4: Summaries of generalized additive mixed models for top performing models predicting the responses of five seagrass metrics and algal epiphyte biomass to one categorical and five continuous environmental predictors. Estimates (Est) and standard errors (SE) of parametric coefficients are presented, along with approximate significance of thin plate regression spline smoother (s) terms and estimated degrees of freedom (Edf) for predictor variables. All variables are significant at $p < 0.05$.

	Parametric coefficients				**Non-parametric smooth terms**			
	Est	**SE**	***t***	***p***	**Predictor**	**Edf**	***F***	***p***
Shoot density (R^2 = 83%)								
Intercept	6.28	0.06	107.19	0.00	s(Temperature)	3.59	6.75	0.00
Exposure Low	-0.66	0.10	-6.95	0.00	s(Turbidity)	3.60	13.41	0.00
Exposure Mid	-0.64	0.10	-6.17	0.00	s(Oxygen)	1.88	8.41	0.00
					s(Salinity)	2.90	17.97	0.00
Leaf density (R^2 = 83%)								
Intercept	5.13	0.06	87.44	0.00	s(Temperature)	3.48	6.72	0.00
Exposure Low	-0.64	0.09	-6.93	0.00	s(Turbidity)	3.62	12.98	0.00
Exposure Mid	-0.62	0.08	-6.13	0.00	s(Oxygen)	1.64	8.36	0.00
					s(Salinity)	2.78	18.01	0.00
Leaf length (R^2 = 76%)								
Intercept	4.82	0.08	61.92	0.00	s(Temperature)	1.00	11.69	0.00
Exposure Low	0.32	0.08	3.92	0.00	s(Turbidity)	2.55	5.85	0.00
Exposure Mid	0.21	0.08	2.60	0.01	s(Salinity)	1.00	11.07	0.00
Leaf width (R^2 = 71%)								
Intercept	0.27	0.04	6.32	0.00	s(Chl *a*)	1.00	9.73	0.00
Exposure Low	0.27	0.04	6.13	0.00	s(Oxygen)	1.00	5.62	0.02
Exposure Mid	0.20	0.04	4.69	0.00	s(Salinity)	1.00	12.05	0.00
Aboveground biomass (R^2 = 69%)								
Intercept	-0.72	0.07	-9.72	0.00	s(Temperature)	1.00	22.78	0.00
					s(Oxygen)	1.80	6.92	0.00
					s(Salinity)	2.18	5.29	0.00
Algal epiphyte biomass (R^2 = 29%)								
Intercept	0.07	0.03	2.49	0.02	s(Temperature)	1.00	7.32	0.01
Exposure Low	0.11	0.05	2.42	0.02	s(Chl *a*)	1.80	4.14	0.01
					s(Turbidity)	1.00	11.74	0.01
					s(Salinity)	2.90	3.55	0.02

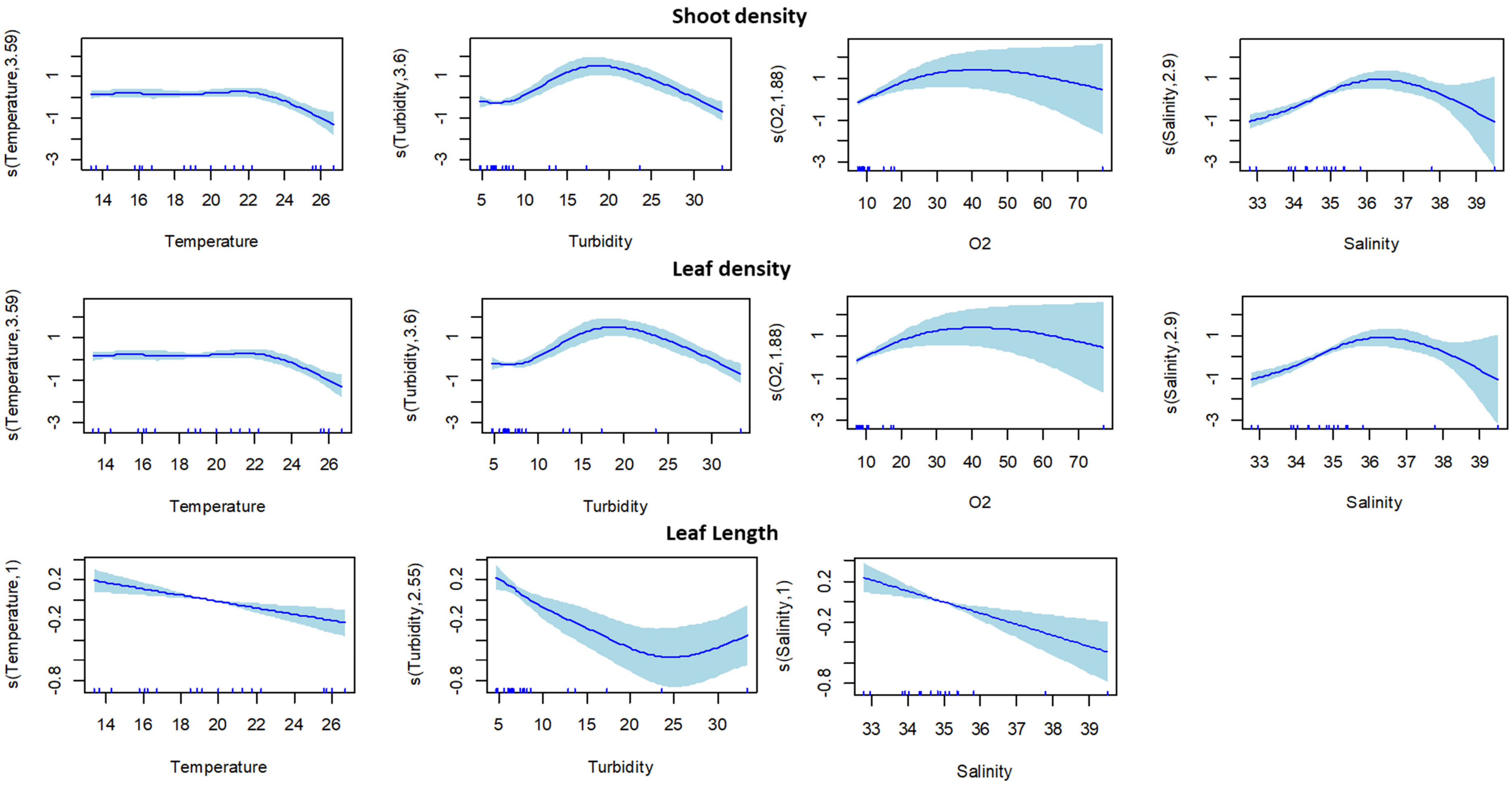


Figure 2.5: Plots of significant environmental predictors with fitted smooth terms showing linear and non-linear relationships with six seagrass metrics from five sites in Langebaan Lagoon. Smoothed curves are represented by solid lines shaded by 95% confidence intervals. Short vertical lines (rug) on the x-axis indicate actual observations for each variable forming the basis of the model's response. Y-axis indicates the "component smooth" centred on zero representing partial residuals from the model fit.

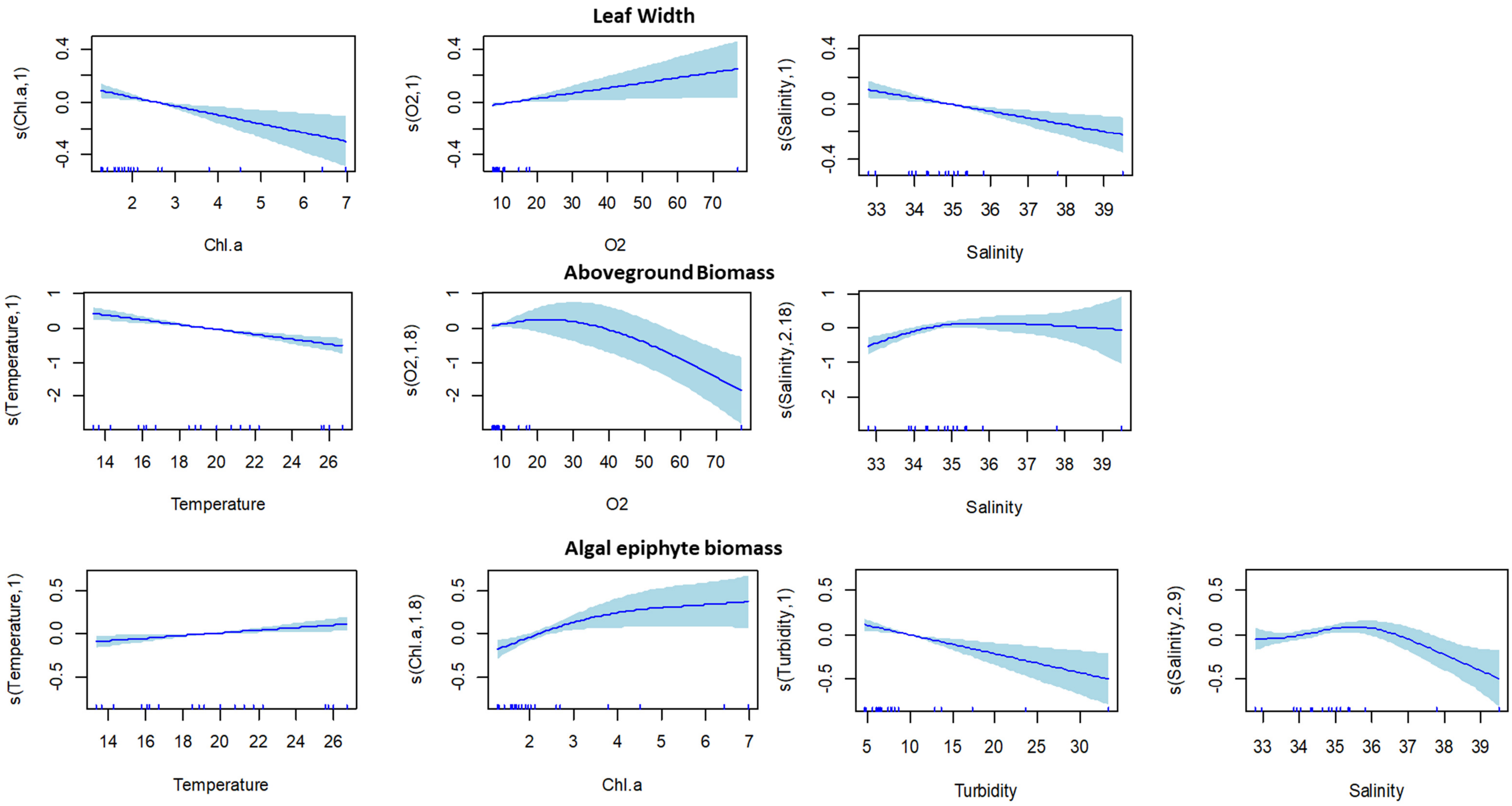


Figure 2.5. Continued.

2.4. Discussion

Seagrass growth parameters were significantly different across sites and seasons in Langebaan Lagoon largely due to differences between stands closer to the mouth (Centre Banks, Klein Oesterval and Oesterval) and those at the end of the lagoon (Bottelary and Geelbek). Environmental conditions between these sites were significantly different but not always across seasons indicating a greater influence of the local environment on seagrass patterns compared to seasonal forcing. These results are correlative and do not infer causation, which can only be determined through experimental manipulation. These findings imply small scale influences on seagrasses are as important if not more important than large scale global and climatic factors. Therefore, greater effort in research and management at smaller scales may effect positive change.

2.4.1. Temporal Patterns in Seagrass Metrics

The densest stands of *Zostera capensis* were measured in early summer (October), which declined to lowest densities by the end of summer, indicating the effect of supra-optimal temperature and light conditions on seagrass growth. These observations align with the general cycle of growth in temperate seagrasses that display high seasonal fluctuations (Duarte 1989), and is related to broad temperature and light variations that occur throughout the year (Duarte 1989, Pérez-Lloréns and Niell 1993, Marbà et al. 1996). Seasonal environmental fluctuations influence seagrass productivity and growth, especially in temperate regions when shoot formation rates during winter are slower as growth is restricted by cold and low light levels, while growth increases in early summer concurrent with increasing temperature and irradiance optima (Bigley and Harrison 1986, Marbà et al. 1996).

Other studies on temperate seagrass populations have shown spring and early summer to be the time when densities and biomass are at their peak (Duarte 1989, Olesen and Sand-Jensen 1994). High densities are then followed by a rapid reduction in shoot cover the likely cause of which is heat stress resulting in the loss of shoots and leaves (Bergmann et al. 2010). In this study, densities of *Z. capensis* were greatly reduced by the end of summer particularly at Centre Banks and

Geelbek, and generally became denser again along with an increase in biomass during cooler temperatures in late autumn and winter. Rapid leaf growth in *Z. capensis* was documented experimentally following subjection to heat stress and desiccation (Adams and Bate 1994), thus demonstrating the ability of *Z. capensis* to recover when temperatures are favorable. This flexibility in seagrasses may be a key feature of their capacity to acclimate under a range of environmental conditions (Duarte et al. 2006, Lee et al. 2007).

Longest leaves, high biomass particularly in autumn, and lowest shoot density were recorded from the seagrass population at Centre Banks. Being approximately in the middle of the lagoon, the sand bank is the first to be submerged by the incoming tide allowing for seagrass plants to experience a shorter emergence period during spring tides and remain submerged during neap tides (Day 1959, pers. observation). In addition, this site does not appear to completely drain during spring low tide but retains a layer of water at the base of the seagrass plants and across much of the bed. Water temperatures at Centre Banks were cooler on average throughout the year compared to other sites and ranged from 13-20^{o}C. Temperature ranges at the other sites were higher (13-29°C), significantly so at Bottelary and Geelbek. Edgcumbe (1980) measured optimal temperatures for *Zostera capensis* reproductive and vegetative growth which ranged between 15-20°C in the laboratory, and noted decreased growth rates when plants were continuously exposed to <10^{o}C and >26^{o}C. This is consistent with the optimum temperature range proposed for *Z. marina* in Korea where limitations in growth were observed above 20^{o}C (Lee et al. 2005). Water temperatures that exceed optimum levels lead to an increase in leaf respiration and reduction of photosynthesis in seagrasses. This is a limiting growth factor during summer in some seagrasses (Penhale and Wetzel 1983, Marsh et al. 1986, Lee et al. 2005, Kim et al. 2012), and also plays a key role in influencing seagrass growth in Langebaan Lagoon.

All sites experienced on average low water column pH in winter and higher pH conditions in summer, a generally common trend in estuaries and nearshore environments (Daniel et al. 2013, Fietzke et al. 2015) since reduced pH benefits seagrass photosynthesis which is heightened under greater light and temperature conditions (Buapet et al. 2013, Koch et al. 2013, Cox et al. 2015, Borum et al. 2016). Given the strong negative correlation between plant biomass and water column pH in this study, reduced pH is also assumed to have a positive influence on seagrass performance in Langebaan. Ecosystem engineers such as seagrasses, corals and macroalgae are able to biologically influence water column pH at local scales (meters to kilometers) (Hofmann et al. 2011, Duarte et al. 2013), through the exchange of CO_2 during photosynthesis, respiration (autotrophs), and shell formation (calcifying organisms) (Hurd et al. 2009). Consequently, both range and frequency of pH may fluctuate considerably across temporal and spatial scales (Hofmann et al. 2011, Saderne et al. 2013), thereby masking local and global effects of acidification on coastal ecosystems (Duarte et al. 2013). A key requirement to elucidate these effects are *in situ* long-term, high-resolution observations of pH which the *ex situ* experiments in this study could not accommodate.

Similar to pH, oxygen levels can show daily variation at small spatial scales (Felisberto et al. 2015) and while seagrass size and biomass were lower at Geelbek, dissolved oxygen (DO) levels were generally higher compared to sites closer to the mouth. A combination of both biological and abiotic factors likely played a greater role in influencing seagrass performance at Geelbek than oxygen. While it was the third highest contributor to seagrass variation (after exposure and turbidity), DO was selected as a significant predictor of seagrass density and biomass in the GAMM models. DO levels were possibly influenced by photosynthetic rates (Silva et al. 2009) and land-based runoff enriched by organic matter from adjacent salt marshes at Geelbek and Bottelary (Whitfield 2005). In addition, the higher concentration of particulate matter in the water column at Geelbek is likely to be phytoplankton which can affect respiratorily- and photosynthetically-driven DO levels (Clavier et al. 2011).

Highest temperature, salinity, turbidity, oxygen and chlorophyll *a* levels across all seasons were recorded at Geelbek, and coupled with the long period of emergence created distinct local conditions that fostered unique seagrass growth patterns (i.e. shorter leaves and lower biomass). It was also the shallowest site during high tide (Flemming 1977) where seagrasses were concentrated at the upper intertidal zone and rarely spread into mid or lower zones. Located at the southern end of the lagoon, tidal flow patterns resulted in increased suspended particles (highest average turbidity of 23.79 NTU) which reduced light in the water column, while light reaching seagrass beds closer to the mouth was likely similar given the comparable depths at which these populations occur (Flemming 1977). The lower irradiance during submergence is a possible reason for the narrow fringing bed formation, since the limited favourable light confines plants to this restricted band. Light variation and exposure were key factors influencing the observed differences in seagrass growth patterns between sites, supported by statistical models that attributed highest variation to exposure and turbidity (DistLM) which were also significant predictors of all seagrass metrics (GAMMs). Suspended sediment has been found to have a direct impact on seagrasses since declining light levels lead to loss and changes in seagrass community structure (Livingston et al. 1998). The migration of *Zostera capensis* and many other seagrass species to upper shoreline positions where they are exposed to supra-optimal air temperatures for extended periods, suggests these plants are better able to cope with high UV and desiccation but not low irradiance (Bjork et al. 1999).

2.4.2. Environmental Influences on Seagrass Metrics

Environmental variables contributed to 66% of the observed variation in seagrass parameters, and of these variables, exposure level as a consequence of position on the shore contributed the most (19.21%) spatial variability. A morphological acclimation in the form of downsizing in upper intertidal populations is a feature of many seagrass species including *Zostera capensis*. Populations with smaller shoots and narrow leaves like those found at Geelbek and Bottelary, are a characteristic feature associated with tolerance to desiccation in intertidal seagrasses since a general reduction in size reduces rates of water loss (Pérez-Lloréns and Niell 1993, Tanaka and Nakaoka 2004, Cabaço et al. 2009,). Here phenotypic plasticity is demonstrated through small and narrow leaves since damage to photosynthetic tissue are reduced in shorter leaves (Tanaka and Nakaoka 2004, Shafer et al. 2007). Björk et al. (1999) observed that *Halophila ovalis* from Zanzibar, East Africa, coped with desiccation in the upper intertidal by flexing down their thin, malleable leaves to flatten out on the sediment surface during low tide, and remain moistened by sediment pore-water and inflow from upper levels of the shore. In the same tidal zone, *Halodule wrightii* formed dense stands that allowed for leaves to overlap during emersion, and thereby avoid drying out when not in direct contact with the moist sediment (Björk et al. 1999). Both these strategies were observed for *Z. capensis* in upper intertidal sites in Langebaan.

Phenotypic plasticity has been observed in seagrass populations around the world, demonstrating an immediate response to environmental conditions in a single generation. This is a key strategy to acclimatise, and displays resilience and an ability to survive in sub-optimal conditions. Intertidal populations of *Zostera japonica*, *Z. noltei* and *Z. marina* produce short, narrow leaves compared to subtidal populations (Bigley and Harrison 1986, Pérez-Lloréns and Niell 1993, Peralta et al. 2000). In other instances, reductions in shoot size from subtidal to intertidal zones have been observed in *Z. capricorni, Halodule uninervis* and *Halophila ovalis* in Australia (Dawson and Dennison 1996), *H. wrightii* in Florida (Phillips 1967), *Cymodocea rotundata* and *Thalassia hemprichii* in Japan (Tanaka and Nakaoka 2004) and *H. ovalis* in Thailand (Kaewsrikhaw et al. 2016). Den Hartog (1970) documented similar acclimations in *H. wrightii* and *H. ovalis*. Large, upright leaves of low intertidal, and subtidal populations are difficult to flex downwards onto the sand, and therefore more vulnerable to desiccation, as was described in *C. rotundata* and *T. hemprichii* populations in East Africa, which died when exposed during extremely low tides (Bjork et al. 1999).

A recent study determined genomic outliers in populations of *Zostera capensis* from 12 sites along the west and south coasts of South Africa, Mozambique and southern Kenya (Phair et al. 2019). The assessment included populations separately from Geelbek (small-leaved morphotype) and Oesterval (large-leaved morphotype) in Langebaan Lagoon. A pooled genomic approach was used to identify differences across population genomes by detecting neutral genomic variation and potential outlier loci. The findings showed that sites did not emerge as significantly different nor was there significant population structuring, however a general east and west clustering of populations was evident based on variation in frequency of outlier loci (Phair et al. 2019). West and south coast (temperate) sites were found to be similar and differed from east coast (subtropical/tropical) populations in nucleotide diversity, except for Oesterval which showed a closer relation to east than west coast populations.

Within a site, seagrasses appear to be highly clonal with low genomic diversity suggesting high connectivity and gene flow (Phair et al. 2019). Gene flow (mostly vegetatively and possibly through seed dispersal) within seagrass populations in Langebaan Lagoon are assumed to be high and the observed differences between high (Oesterval) and low (Geelbek) shore populations are likely attributed to phenotypic responses to environmental conditions rather than genetic differences. In addition, the two morphotypes were not observed to co-occur within the same site i.e. average leaf length/widths were consistently larger at sites closer to the mouth and smaller in high shore sites further away (Chapter Two). There was therefore no indication that the morphological expressions were not solely as a result of the prevailing environmental conditions.

In a similar assessment, Oetgen and Reusch (2007) investigated the genetic basis for environmental acclimation in three paired populations of *Zostera marina* from the Wadden Sea, Germany using genome scans of marker loci. Their results also suggested a relatively high gene flow and low genetic differentiation among all submerged and exposed populations. However, a possible functional link with environmental characteristics that explained divergences for some populations, was a locus responsible for channelling water across cell membranes (Oetjen and Reusch 2007). Similar genetic expressions that suppress or stimulate loci to adapt to local environmental conditions, might be responsible for differences in east and west coast populations of *Z. capensis*.

While specialised acclimations to reduce rates of water loss during air exposure are not apparent in leaf tissue of upper intertidal seagrasses (Björk et al. 1999, Tanaka and Nakaoka 2004), the ability to tolerate desiccation is certainly key to their survival. *Zostera capensis* has a thin leaf cuticle which is perforated and fragile in places and seems to lack a physiological barrier to desiccation (Barnabas et al. 1977). Adams and Bate (1994) measured the response of *Z. capensis* to desiccation stress and noted that recovery was largely due to new leaf growth and not rehydration of desiccated leaves. Faster leaf turnover rates have been observed in smaller compared to larger seagrass plants (Marbà et al. 2006), further evidenced in the average lifespan of individual leaves of the small *Z. japonica* which is >10 days less than that of the larger leaves of *Z. marina* (Lee et al. 2006). High leaf turnover rates appear to be a strategy of small-leaved populations to offset the energy costs of leaf production compared to investing in repairing desiccation damage (Shafer et al. 2007). High leaf production also leads to denser meadows with overlapping leaves, thus providing protection from water loss and further compensating for the absence of a robust cuticle layer (Barnabas et al. 1977, Adams and Bate 1994).

Environmental conditions at the scale of site could elucidate observed differences in leaf morphology and biomass across Langebaan Lagoon. Other environmental factors not accounted for in this study such as flow rate, wave energy, water retention time, as well as nutrient levels in the sediment and water column can differ at small (tens of meters) spatial scales (Cardoso et al. 2004, Borg et al. 2005, Delgard et al. 2016) and are likely to influence seagrass growth in addition to seasonal variability (Alcoverro et al. 1995, Duarte et al. 2006). For example, investigations of *Zostera marina* beds in San Diego Bay, USA found sites within the bay to differ from those closer to the mouth specifically in density and biomass (Moore and Hovel 2010) as was also observed in Langebaan. This is most likely the effect of gradients from the mouth to the head of the lagoon that influence factors such as salinity, the transport of nutrients as well as water retention time (Largier et al. 1997). In addition, the hydrodynamics of the system such as current patterns and wave energy can affect the exchange of gases and uptake of nutrients in seagrass plants (Schanz and Asmus 2003).

In shallow, sandy areas along the coast of Puget Sound, USA, wave energy was the main factor that determined the distribution of *Zostera marina* and accounted for 86% of variability in seagrass percent cover (Stevens and Lacy 2012). Similarly, meadows of *Posidonia oceanica* in Malta had low overall cover, more complex patch shapes and lower within-patch structural complexity along a low to high gradient of wave exposure (Pace et al. 2017). Barr et al. (2008) demonstrated that wave surge increased wet mass and that bulk flow rates increased access to nutrients in the macroalga *Ulva australis,* particularly at saturating light levels. Mainstream velocity of seawater influences rates of nutrient uptake in macroalgae, and is a key factor in determining macroalgae productivity, community structure and physiology (Hurd 2000, Barr et al. 2008). This feature could well extend to seagrasses and provide further reasoning for the occurrence of larger plants closer to the mouth of Langebaan Lagoon, where water velocity and flow rates are likely to be higher, than at the end of the lagoon.

Environmental forcing alone may not be responsible for seagrass growth but may include species-specific traits that influence plant physiological responses to exposure, temperature and light limitations (Tanaka and Nakaoka 2004, Staehr and Borum 2011). Intrinsic factors such as the ability to store and use resources as well as partition resources for growth (i.e. rhizome internodes, leaf growth or reproduction) (Duarte 1991, Alcoverro et al. 1999) may also contribute to patterns in seagrass morphological responses to environmental conditions, and further explain the temporal and spatial patterns in *Zostera capensis* in Langebaan Lagoon. Intraspecific acclimation to environmental contexts is evident in *Z. capensis* through the expression of specific morphological traits. This concept is explored further in Chapters Four and Five.

Increasing global temperatures are predicted to have major consequences for growth of intertidal seagrasses (Rasheed and Unsworth, 2011, Hammer et al. 2018) particularly at sites that are exposed for longer periods (Valle et al. 2014) and where ambient water temperatures are likely to exceed thermal optima for growth and photosynthesis such as at Geelbek. There are numerous effects on plant growth and metabolism as a result of temperature. These include reductions in metabolic rates due to limitations on enzyme activity, light capture and nutrient uptake at low temperatures (Staehr and Borum 2011). With increasing temperatures these limitations gradually disappear and metabolic rates rise along with the heightened demand for nutrients creating a state of nutrient limitation (Staehr and Borum 2011). Metabolic rates are reduced due to the restraint in enzyme capacity beyond optimum temperatures while supra-optimal temperatures eventually exhaust metabolic functioning through incapacitation or the denaturing of proteins (Atkin and Tjoelker 2003) thereby limiting productivity. Abe et al. (2008) experimentally tested the effect of increased water temperature on *Zostera marina* seedlings and found that plants are able to adapt to changing temperatures and display a range in tolerance levels but possess optimum conditions for growth. Optimal temperatures for net productivity and maximum photosynthesis investigated in three tropical seagrasses species (*Z. muelleri*, *Halodule uninervis* and *Cymodocea serrulata*) over a latitudinal range of >1,500 km, found the capacity for acclimation to be limited, but concluded that thermal optima can be useful in clarifying a species vulnerability to ocean warming in both present and future scenarios (Collier et al. 2017).

In Langebaan Lagoon, the high variation in seagrass morphological metrics between sites, and observed specifically between Centre Banks and Geelbek provide insight into intraspecific acclimation strategies to cope with effects of temperature and exposure in *Zostera capensis*. The implications of an increase in temperature at Langebaan could result in further population changes where the small-leaved morphotype replaces the large-leaved with subsequent lower biomass thereby reducing structural and habitat complexity. Warming could further drive small-leaved populations to their environmental limits leading to population decline and overall loss of seagrasses in this system.

2.5. Conclusions

Zostera capensis displays significant spatial and temporal variation in Langebaan Lagoon. Biomass and morphometric responses in *Z. capensis* were largely explained by variation in exposure and temperature. Denser seagrass beds with low biomass were found to experience higher temperatures while cooler temperatures favoured greater biomass in sparser beds. Seagrass stands growing closer to the lagoon mouth and experiencing shorter emergence times had larger shoots than populations exposed for longer and growing further away from the mouth. All environmental variables measured were essential in explaining patterns in seagrass response variables with the exception of chlorophyll *a*, however exposure and temperature contributed the highest variation in explaining these patterns. The threat of increasing air temperature as a result of global warming, will likely have a major effect on *Z. capensis* growth by way of large-leaved plants with high biomass giving way to short, dense beds with low biomass, possibly driving this species to the edge of its thermal tolerance range in Langebaan Lagoon.

This study provides the first insight into patterns in response of *Zostera capensis* to seasonal environmental conditions in Langebaan lagoon. Although declines in density and biomass were high in summer, these parameters had increased in cooler months when environmental conditions were favourable. This indicates the capacity of *Z. capensis* populations to recover from thermal stress, and alludes to underlying mechanisms such as allocation of resources (e.g. C and N), as survival strategies (explored further in the following chapters). The considerable increase in cover based on studies carried out in 2009 to those reported in 2014 further highlights this point.

A longer sampling regime would provide annual trends in response patterns and a deeper insight into understanding long term factors influencing seagrass growth in the lagoon. The synchronised interactions of environment, seagrass nutrient requirements and sediment dynamics on seagrass distribution in Langebaan Lagoon require further investigation. The additional effects of human activities from development, dredging and trampling may also benefit our understanding of the dynamics that have led to historical declines in *Z. capensis* in Langebaan Lagoon and are further discussed in Chapter Six.

CHAPTER THREE

Spatial and Temporal Differences in Macro-epifauna associated with *Zostera capensis* in Langebaan Lagoon: *Influences of Environmental Variables and Seagrass Structure*

3.1. Introduction

The need to preserve biological diversity and ensure its perpetuation stems mainly from the concept that biodiversity loss would hamper the functioning of ecosystems and thereby compromise the variety of ecosystem services that society benefits from (Costanza et al. 1997, McCann 2000, Hooper et al. 2012). Generally, a biologically diverse ecosystem implies that more than one species can fulfill a specific function (functional redundancy), thus ensuring that functioning ecosystems are maintained, even if one species is affected by a stress e.g. from disease or over-exploitation. Species that fulfill similar functions may respond differently to stressors which enhances the resilience of an ecosystem, and maintains the stability of ecosystem states, with a greater likelihood of recovery from stress or disturbance (Elmqvist et al. 2003). Sustaining biodiversity is therefore essential for enhancing a complex system's ability to contend with change and lowers sensitivity to species loss. The more stable an ecosystem is, the greater its role in providing services such as the recycling of carbon dioxide, producing oxygen and maintaining productive fisheries (Ghilarov 2000, Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2012).

3.1.1. Macro-epifauna and Seagrass Structure

Benthic macrofauna of coastal ecosystems comprise mainly molluscs, amphipods, decapods and polychaetes which often serve as the key trophic link between primary producers and pelagic consumers (Heck et al. 2008). The main constituents therefore, in a typical seagrass food web include seagrass plants, periphyton, epiphytic algae, detritivores, invertebrate grazers, vertebrate grazers, meso-predators, piscivorous predators and humans (Duffy 2006). Here, macrofauna provide a crucial link in the food chain to intermediate and larger predators, associated directly and indirectly with seagrass habitats (Heck et al. 2008, Baden et al. 2010). Seagrass structure supports macrofaunal assemblages occurring largely as epifauna (living on seagrass blades), and infauna (dwelling in or on sediment surfaces) (Webster et al. 1998, Bologna and Heck 1999, Klumpp and Kwak 2005, Leopardas et al. 2014,).

A large diversity of fauna and algae are associated with seagrass habitats despite the plants themselves having a limited diversity. For example, Orth et al. (1984) compared densities of faunal communities from seagrass and unvegetated areas from several studies and found seagrass meadows to support a richer assemblage of fauna compared to nearby unvegetated areas in both tropical and temperate systems. These patterns have also been described in other studies attributed mainly to the structural complexity of seagrass habitats, the abundance of food found within them and the greater stability of sediment compared to bare sandflats (Orth et al. 1984, Bos et al. 2007, McCloskey and Unsworth 2015, Carr et al. 2016). There are however, exceptions to this paradigm. Disturbance by callianassids for example, influence faunal assemblages by supporting mainly burrowing infauna only to be replaced by epifauna and seagrass-specific burrowers when seagrasses are present, and can discount dissimilarities in species diversity indices between callianassid-dominated sandflats and seagrass habitats (Barnes and Barnes 2012). When localities without callianassid bioturbators were investigated in the Knysna Estuary, South Africa, seagrass habitats were found to support fewer individuals than bare sandflats, and although seagrasses supported more species due to the number of epifauna, the numerical differences were statistically negligible (Barnes and Barnes 2014). Unvegetated sandflats generally support a different suite of species (Casares and Creed 2008), and despite their often lower diversity compared to vegetated areas, they still provide a significant contribution to overall diversity in nearshore coastal environments.

Abundances of many macrofaunal species have been found to positively correlate with plant morphology; mainly plant canopy and root-rhizome structure (Orth et al. 1984). In *Zostera capensis* beds in Mozambique, macrofaunal patterns were attributed to structure provided by enhanced habitat complexity produced by seagrass standing crop and consequent biomass (Paula et al. 2001). This paradigm also holds true for smaller seagrasses that provide lower structural complexity such as *Halophila decipiens*, which was found to have significantly greater macrofaunal diversity, richness and density in the seagrass habitat compared to bare sand in Guanabara Bay, Brazil (Casares and Creed 2008).

Seagrass stems and leaves provide a structure on which epiphytes, periphyton and encrusting algae attach. Epiphytes comprise collectively of micro- and macroalgae while periphyton is described as a mucus-like layer coating seagrass blades comprising largely of diatoms, blue green, red, and filamentous algae, particulate material, bacteria and microfauna (van Montfrans et al. 1984, Klumpp et al. 1992). Algae and periphyton are highly nutritious and easily digested by grazing invertebrates which find both food and shelter in seagrass beds (Schneider and Mann 1991, Edgar 1992, McNeely et al. 2001). Furthermore, the structural substrate provided by seagrasses for photosynthesizing epiphytes allows for primary productivity from seagrass ecosystems to be substantially increased (Orth and van Montfrans 1984, van Montfrans et al. 1984, Smit et al. 2005), however an excess of algal epiphytes can also become problematic for seagrasses.

Eutrophication of coastal waters due mainly to land-use and effluent discharges have been linked to seagrass loss (Ruiz and Romero 2003, Cardoso et al. 2004, Burkholder et al. 2007). While toxic effluent can be lethal to seagrasses (Koch and Erskine 2001, Macinnis-Ng and Ralph 2004, Govers et al. 2014, Negri et al. 2015), an excess of nutrients encourages planktonic and benthic algal growth which compete with seagrasses for nutrients and reduce light levels in the water column further reducing conditions for seagrass photosynthesis (Short et al. 1995). In addition, epiphytic algal growth can smother seagrasses (Walker and McComb 1992, Nelson and Lee 2001) and alter the dissolved carbon dioxide/oxygen balance (Raun and Borum 2013). In some cases long-lived epiphytes can weigh down seagrass leaves leading to breakage (van Montfrans et al. 1984). Climate warming along with increases in coastal eutrophication, are predicted to favour algal growth posing additional stress on seagrass ecosystems.

3.1.2. Influence of Environmental Variables on Faunal Assemblages Associated with Seagrasses

Environmental effects particularly from temperature, irradiance, and salinity on seagrass growth and distribution are well documented (see review by Lee et al. 2007; Chapter Two). In general, seagrass growth displays a distinct seasonal trend where growth increases during spring and summer, and decreases in autumn and winter (Vermaat et al. 1987, Dunton 1990, Dunton 1994, Huong et al. 2003). This has been attributed mainly to the individual or interactive effects of temperature and light (Iverson and Bittaker 1986, Dennison 1987, Nejrup and Pedersen 2008).

Salinity is a key environmental factor affecting the distribution, biomass and productivity of macrofauna in many estuarine and lagoon habitats (Whitfield 1992, Al-Wedaei et al. 2011, Vuorinen et al. 2015). This is evident in the general spread of species along a salinity gradient from seawater levels at the mouth of an estuary, through brackish levels in the middle reaches, until a freshwater source is encountered in the upper reaches (Allanson and Baird 1999, Barnes and Ellwood 2012). For example, seawater-derived salinity was found to be the main environmental factor explaining distribution of taxa from freshwater and saline assemblages surveyed in 28 coastal water bodies (estuaries, shallows bays, lagoons) in Sussex, England (Joyce et al. 2005). Similarly, in the Celestun coastal lagoon, Mexico, benthic community structure sampled along a salinity gradient, was influenced spatially by salinity, while temporally sediment characteristics influenced species diversity but not abundance (Pech et al. 2007).

Altering of salinity levels through evaporation, flooding, water abstraction, pollution and flow reduction (Allanson and Baird 1999, van Niekerk et al. 2013) has consequences for macrofaunal community structure and abundance. In the Swartkops estuary, South Africa, where salinity levels are usually close to that of seawater, other factors such as sediment characteristics were the main influence on macrobenthos (McLachlan and Grindley 1974). Following a flood event in this system, salinity had dropped markedly altering macrobenthic distribution, patterns which were still observed two years after the flood (McLachlan and Grindley 1974). Teske and Wooldridge (2003) noted that endemic mud and sand fauna in estuaries in the Eastern Cape, South Africa, were limited by substrate characteristics, while salinity limited species from marine and freshwater habitats. Conditions in Langebaan Lagoon are similar to that of estuaries, except there is no direct freshwater input. Seepage from groundwater has been suggested (Allanson and Baird 1999) and supported by the presence of freshwater-associated macrophytes such as *Phragmites australis* and *Typha capensis* on the south east banks of the lagoon (Christie 1981). The enclosed nature of the lagoon provides a relatively stable tidal environment where water residence times of >30 days have been recorded in the inner basin (Largier et al. 1997). Variability in salinity levels in Langebaan were observed between the mouth and the head of the lagoon (Chapter Two), and while no effect of freshwater input was detected, evaporation as a result of diurnal heating was the likely cause of the slight elevated salinities at Bottelary and Geelbek (Chapter Two) and in the salt marsh creeks (Day 1959, Flemming 1977) at the head of the lagoon.

3.1.3. Macrofauna in Langebaan Lagoon

In non-quantitative surveys of *Zostera capensis* beds, Day (1959) observed macrofauna in Langebaan Lagoon to be distributed along a salinity gradient from marine (occurring mainly in Saldanha Bay) to estuarine (occurring mainly in the lagoon). He attributed this to shelter i.e. reduced wave action and its indirect effect on substrate, rather than temperature or salinity, and described faunal community characteristics in Langebaan to be similar to that of other estuaries in the Western Cape, South Africa (Day 1959). Specific descriptions of seagrass-related macrofauna both spatially and temporally in *Zostera* beds in Langebaan, as well as the influence of environmental variables, is lacking and is important in aiding our understanding of macrofaunal diversity within this system.

The decline of seagrass in Langebaan Lagoon has raised questions on the influence of epiphytic algae on seagrasses as well as patterns in associated grazing epifauna. In the following study, a subset of macro-epifauna were used to quantify community indices using visual counts. The technique is different to traditional methods to quantify infauna i.e. digging and sieving of sediments, which are relevant to evaluating a general suite of fauna associated with seagrass and unvegetated habitats (Raz-Guzman and Grizzle 2001). Visual counts carried out in daylight confines estimates of faunal composition to represent non-cryptic, diurnal, sessile species (Edgar et al. 2001), while sampling during low tide can further under represent potentially important ecological species (Pearman et al. 2016). However, traditional methods of coring might also fail to capture cryptic components as well as species patterns altered by succession and colonization, especially between sessile and motile epibenthic communities (Moura et al. 2008), and thus similarly categorise a subset of the faunal assemblage.

Since this study focuses on the extent to which epifaunal species composition differs between populations of seagrasses from vegetated sites only, a subset of species that mainly graze on seagrass and epiphytic algae was assessed. A visual assessment was therefore considered an appropriate method to determine the degree to which different seagrass morphologies support epibenthic diversity. Visual techniques to assess a subset of macrofauna *in situ* have been used elsewhere (Vonk et al. 2008, Källén et al. 2012, Lee et al. 2012, Poulos et al. 2013,) to address specific questions and provide meaningful patterns in diversity and species composition (Kuenen and Debrot 1995, Vellend et al. 2008). In addition, epifauna are the principal focus of studies in other habitats such as coral reefs and kelp forests, since infaunal evaluations while greatly enhancing overall diversity, requires immense destruction and is often avoided. A much larger literature therefore exists on surface fauna in these ecosystems.

In this chapter, I aim to describe the spatial and temporal variation in macro-epifaunal community structure (abundance, richness, and diversity) in *Zostera capensis* populations in Langebaan lagoon, and identify the key seagrass structural (biomass, leaf morphometrics, density and algal biomass) and environmental variables (temperature, salinity, pH, turbidity, oxygen, chlorophyll *a*) that contribute to variation in abundances. This provides a framework with which to further explore the influence of trophic interactions (i.e. grazing and epiphytic microalgae) and the effects of temperature on *Z. capensis* (Chapter Five).

3.2. Methods

3.2.1. Study Site

Macro-epifauna were assessed in intertidal seagrass habitats in Langebaan Lagoon on the west coast of South Africa (18°03'E, 33°08'S). Five sites: Centre Banks, Klein Oesterval and Oesterval (closer to the lagoon mouth), Bottelary and Geelbek (further away from the mouth) were sampled. These sites were chosen because they contained the highest coverage of *Zostera capensis* in the lagoon (refer to Chapter Two for more detailed descriptions and site map Fig. 2.1).

3.2.2. Seagrass Metrics

Seagrass morphometric and epiphyte biomass response variables are the same as those used in Chapter Two. In summary, five cores of seagrasses (Ø 10 cm x 15 cm deep, $0.0079m^2$) were taken randomly within beds of *Zostera capensis* at each of the five sites: Centre Banks, Oesterval and Klein Oesterval, Geelbek and Bottelary, across four austral seasons (spring, summer, autumn and winter) during spring low tide. Core samples were rinsed with seawater on site and stored in labelled bags for transportation to the University of Cape Town. In the laboratory, densities of seagrass shoots and leaves were recorded, as well as average lengths and widths of the longest 20% of leaves. Roots/rhizomes were then separated from leaves, and epiphytic algae gently scraped off all leaves with a scalpel blade. Aboveground dry weight of seagrasses and algal epiphytes were obtained by weighing samples that were dried at 60^oC for ~12 hours which is when constant dry weight was reached (see Chapter Two: Methods).

3.2.3. Macro-epifauna Sampling

In order to determine the relative influence of plant structure on macro-epifaunal diversity and whether morphologically different seagrass populations support a different epibenthic diversity, invertebrate fauna observed in the seagrass canopy and sediment surface was estimated. A visual census was used to quantify densities of macro-epifauna in $0.5m^2$ quadrats at a size fraction > 3mm, during spring low tide when seagrass beds were completely exposed. This method was used to quantify a subset of seagrass-associated fauna (mainly invertebrates that graze on seagrass leaves and epiphyton), and maximise the area sampled in the timeframe available during spring low tide (Edgar et al. 2001). For example, a much greater area of seagrass bed could be sampled using $0.5m^2$ quadrats than would have been achieved using cores ($0.0079m^2$). The technique employed is different to traditional methods used to quantify infauna i.e. digging and sieving of sediments, which are relevant to evaluating a general suite of fauna associated with seagrass and unvegetated habitats.

Five 0.5m^2 quadrats were placed randomly in each bed or seagrass patch sampled in each of the five sites (n = 420) and across four seasons. All visible fauna on, among and below seagrass leaves within each quadrat were identified and counted. Species were identified *in situ* using field guides while specimens of those that could not be identified were collected for later identification. Species were finally categorised into phyla and functional groups using species identification guidebooks (Branch et al. 2012) and the World Register of Marine Species (www.marinespecies.org) as well as consultation with local experts from the University of Cape Town.

3.2.4. Environmental Variables

Environmental variables are the same as described in Chapter Two. Temperature, pH, turbidity, salinity, oxygen and chlorophyll *a* were recorded monthly at each of the five sites during spring high tide using a handheld CTD (Conductivity Temperature Depth profiler (YSI 6820 V2-2V)). The average of three readings taken approximately 10m apart constituted one measurement (see Chapter Two: 2.2.3. Environmental Variables).

3.2.5. Statistical Analyses

Correlations between seagrass and environmental variables were explored using multi-panel scatterplots and rank correlations. Correlations of (±) 0.7 - 0.9 indicating strong co-linearity were identified between shoot and leaf densities, leaf length and width, and temperature and pH (refer to Chapter Two: 2.2.4). These variables are excluded from multivariate analyses since co-linear variables can confound the true degree of variability (Lipovetsky and Conklin 2001).

In this Chapter, ‘bed’ (nested within site within season) was used as the sampling unit representing the average number of macro-epifaunal species counted per five quadrats (0.5m^2 x 5).

Macro-epifaunal community structure was measured using the community indices of abundance, species richness and species diversity. The abundance of species was determined as the total number of individuals (*N*) recorded across all sites and seasons. Species richness was measured according to the Margalef index (d):

$$d = (S - 1) / \log N$$

where, S represents the total number of species and *N* the total number of individuals (Margalef 1968).

Species diversity was first determined after the Shannon-Wiener index (H') (Shannon 1963) and calculated using the following formula:

$$H' = \sum_{i-1}^{s} - (Pi * \log (Pi))$$

where, *S* is the number of species observed and P is the proportional abundance of individuals from the sample total of species *i*. Diversity indices were then converted to “effective number of species” by calculating the exponential (exp (H)) of each diversity value after Hill (1973). This transforms the non-linear Shannon-Wiener index to a linear form that permits statistical comparisons that are intuitive, while the unit “number of species” allows for diversity comparisons across metrics (Jost et al. 2006, Jost et al. 2010).

3.2.5.1. Univariate Analyses

Each community index was assessed visually using histograms and q-q plots. Variances in the data were tested for normality (Shapiro-Wilk’s test) and homogeneity (Levene’s Test) and were natural log transformed where necessary to meet assumptions for parametric analyses. Two-way factorial ANOVAs were used to test for main and interactive effects of site (random factor: five levels) and season (fixed factor: four levels) on each community index: abundance (*N*), richness (d) and diversity (H'). This was followed by Tukey’s HSD post hoc tests. Univariate analyses were performed using Sigma Stat (IBM SPSS Statistics 25).

3.2.5.2. Multivariate Analyses

Macro-epifaunal species abundances were compared across seasons (fixed) and sites (random factor nested within season) using multivariate data testing in PRIMER (Plymouth Routines in Multivariate Ecological Research; Version 6.1.11; Clarke and Gorley, 2006). Species abundance measures were considered an adequate metric on which to assess the effects of seagrass structure, epiphyte biomass and environmental factors since macro-epifauna were largely comprised of grazing invertebrates whose densities were important in controlling algal epiphytes (Hovel et al. 2002, Vonk et al. 2010). Data were fourth root transformed to down-weight the influence of large variances, before translating into an S17 Bray-Curtis similarity matrix (Bray and Curtis 1957). Unconstrained multi-dimensional scaling (MDS) and cluster dendograms provided visual representation of relationships between variables in ordination space. Multivariate analyses were also used to test the null hypothesis of no differences in macro-epifaunal species abundance across season and site groups and their interaction using Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson et al. 2008). Within group differences as well as comparisons of species composition between groups (i.e. beta diversity, Anderson et al. 2006) were tested using Permutational Analysis of Multivariate Dispersions (PERMDISP (9999 permutations)). Thereafter, a SIMPER (similarity percentage breakdown) analysis was carried out to determine the relative contribution of individual species to community structure at each site across all seasons.

The relative importance of direct and indirect effects of environmental factors and seagrass variables on macro-epifauna was first assessed individually and jointly using multivariate assessment techniques. To determine which environmental factors (temperature, salinity, turbidity, pH, oxygen, chlorophyll a and exposure) and seagrass metrics (shoot density, leaf length, aboveground biomass, algal epiphyte biomass) best predict variability in macro-epifaunal community structure, a distance-based linear model (DistLM, selection procedure 'best' and criteria 'AIC') was used (PRIMER/PERMANOVA, Version 6.1.11; Clarke and Gorley, 2006). DistLM calculates the proportion of variability contributed by each factor using multiple regression modelling to partition variation according to selected predictor variables (Anderson et al. 2008). After all possible model combinations were explored, the five best models that explain variability based on smallest AIC values and adjusted R^2 derivatives are presented.

To partition the net effects of environmental variables (exogenous) and seagrass structure (endogenous) on macro-epifaunal abundance (endogenous) into direct and indirect effects, maximum-likelihood estimated structural equation models (SEM) were created in AMOS (IBM SPSS Amos version 26 Graphics, Arbuckle 2010). Structural equation modelling is a multivariate statistical analysis framework that is based on techniques from factor and path analyses (Grace 2003). It allows for direct and indirect relationships between observed and unobserved (latent) variables to be examined through paths that indicate statistical dependency, described by parameters that specify the magnitude of effect (direct or indirect) of independent variables on dependent variables (observed or latent) (Grace 2003).

A full model was first specified based on theory and hypothesised relationships that (i) exogenous environmental variables: temperature, salinity, pH and oxygen would positively or negatively influence endogenous variables: seagrass biomass, leaf length/width, shoot density, epiphytic algal biomass, chlorophyll *a* (chl *a*) and macro-epifaunal abundance; while (ii) turbidity and exposure would negatively influence all endogenous variables; and (iii) seagrass density, leaf length/width, epiphytic algae and chl *a* would positively influence macro-epifaunal abundances. Stepwise model selection was performed in which variables with non-significant paths i.e. regression coefficients with $p > 0.05$ were removed until all remaining paths were significant ($p < 0.05$) (Grace 2003). Model fit was assessed using the chi-square value and the root mean square error of approximation (RMSEA) as measures of goodness-of-fit and evaluated by ensuring observed and predicted covariance matrices were aligned. The chi-square test is considered a reasonable measure of model fit for sample sizes of 75 to 200 (Fan et al. 1999) – sample size in this study was 99. Models were then adjusted to produce a low chi-square value with a corresponding p value > 0.05 denoting observed and fitted models were not significantly different, and an RMSEA < 0.08 indicating acceptable fit (Grace 2003). The final model output presents standardized coefficients to compare the strength of direct and indirect effects.

Factors were considered significant at $\alpha =/< 0.05$ in all analyses and variance is expressed as standard error (SE) of the mean.

3.3. Results

Grazing macro-invertebrates comprised the largest percentage (53% m^{-2}) of total macro-epifauna recorded in seagrass beds in Langebaan Lagoon (Table 3.1). This group was dominated by the generalist microgastropod *Assiminea globulus* (31.45% m^{-2}) and the pulmonate limpet *Siphonaria compressa* (16.12% m^{-2}). The keyhole limpet *Fissurella mutabilis* was found only in beds in the low shore zone comprising 3.26% m^{-2} of total macro-epifaunal abundance (Table 3.1). Seagrass beds also supported 43.45% m^{-2} of predatory species, mainly the alien anemone, *Sargartia ornata* (43.38% m^{-2}). Dominant at sites closer to the lagoon mouth, this species was generally found in spring and autumn. In its native habitat (Mediterranean and Western Europe), *S. ornata* attaches to kelps and rocky shores, however in Langebaan Lagoon it is found among *Zostera* shoots attached to loose rocks and feeds mainly on amphipods and polychaetes (Robinson and Swart 2015).

The hermit crab, *Diogenes brevirostris* had a dual function as grazer and deposit feeder and comprised 2.67% of total macro-epifaunal abundance. A small percentage (0.83%) of suspension feeding organisms was also encountered, the most abundant being a solitary ascidian, *Pyura stolonifera* (0.4%), found mainly growing in sediment between seagrass shoots (Table 3.1).

Table 3.1: Species, functional groups and number of individuals m^{-2} recorded in five populations of *Zostera capensis* at Langebaan Lagoon.

Phylum	**Family**	**Species**	**Functional Group**	**#Indiv.m^{-2}**
Annelida	Polychaeta	Fan worms	Suspension feeder	81
Annelida	Polychaeta	Polychaete	Suspension feeder	9
Annelida	Polychaeta	Tube polychaete	Suspension feeder	25
Arthropoda	Crustacea	*Cymadusa filosa*	Grazer	2
Arthropoda	Crustacea	*Hymenosoma obiculare*	Grazer	3
Arthropoda	Crustacea	*Danielella edwardsii*	Deposit/Grazer	19
Chordata	Ascidiacea	*Pyura stolonifera*	Suspension feeder	158
Cnidaria	Anthozoa	*Halianthella annularis*	Predator	20
Cnidaria	Anthozoa	*Sargartia ornata* (alien)	Predator	16 971
Crustacea	Brachyura	*Diogenes brevirostris*	Deposit/Grazer	1 046
Echinodermata	Asteroidea	*Parvulastra exigua*	Grazer	585
Mollusca	Bivalvia	*Choromytilus meridionalis*	Suspension feeder	7
Mollusca	Bivalvia	*Venerupis corrugata*	Suspension feeder	14
Mollusca	Gastropoda	*Assiminea globulus*	Grazer	12 303
Mollusca	Gastropoda	*Crepidula fornicata*	Grazer	192
Mollusca	Gastropoda	*Fissurella mutabilis*	Grazer	1 277
Mollusca	Gastropoda	*Gibbula* spp	Grazer	1
Mollusca	Gastropoda	*Gibbula zonata*	Grazer	1
Mollusca	Gastropoda	*Hydrobia* sp.	Grazer	52
Mollusca	Gastropoda	*Nassarius kraussianus*	Grazer	7
Mollusca	Gastropoda	*Nucella dubia*	Predator	5
Mollusca	Gastropoda	*Oxystele antoni*	Grazer	5
Mollusca	Gastropoda	*Turritella capensis*	Suspension feeder	30
Mollusca	Gastropoda	*Siphonaria compressa*	Grazer	6 305

3.3.1. *Macro-epifaunal Community Structure*

3.3.1.1. Abundance

Macro-epifaunal average abundances (i.e. number of individuals) were lower at the mouth compared to the head of the lagoon (Fig. 3.1A). Species abundances across all seasons was highest at Bottelary (136.68 m^{-2}) followed by Geelbek (83.78 m^{-2}). This was due to high densities of the gastropods *Assiminea globulus* and *Siphonaria compressa*. Average species abundance at Oesterval was 79.14 m^{-2}, Centre Banks 68.84 m^{-2} with the lowest abundance recorded at Klein Oesterval 23.66 m^{-2}. These sites were dominated by the anemone, *Sargartia ornata*. Centre Banks supported the greatest average abundance of *Fissurella mutabilis* (26.55 m^{-2}), while the hermit crab, *Diogenes brevirostris* was most abundant at Klein Oesterval (36.74 m^{-2}). *A. globulus* was also prevalent at high shore sites at Oesterval while *F. mutabilis* was only found at sites closer to the mouth and not at Bottelary or Geelbek. Similarly, *A. globulus* was not detected at Centre Banks and an average of 1.26 individuals was found at Klein Oesterval in winter.

Differences in macro-epifaunal species abundance were significant across sites ($F_{4,97} = 10.80$, $p < 0.001$, Fig. 3.1A). Mean abundances at Centre Banks and Klein Oesterval were similar while Oesterval, Bottelary and Geelbek displayed homogenous means (Tukey HSD, $p < 0.05$, Fig. 3.1A). Despite the high abundance (252.93), of individuals at Centre Banks in autumn there were no significant seasonal differences in abundance between sites ($F_{3,97} = 2.66$, NS), however, the interaction between site and season proved significant ($F_{12,97} = 6.68$, $p < 0.001$).

3.3.1.2. Richness

In contrast to abundances, macro-epifaunal species richness was higher closer to the lagoon mouth and lowest at the head (Fig. 3.1B). Centre Banks had the highest average species richness (1.48) across all seasons followed by Klein Oesterval (1.27) and Oesterval (1.13), while the lowest number of species was recorded at Bottelary and Geelbek (0.53 and 0.55 respectively). These differences were significant across sites ($F_{4,97} = 8{,}01$, $p < 0.001$), seasons ($F_{3,97} = 3{,}64$, $p = 0.02$) and their interaction ($F_{12,97} = 3{,}09$, $p = 0.001$). Post hoc analysis found significant differences in means between each of the sites closer to the mouth with the two sites at the head (Tukey HSD, $p < 0.05$, Fig. 3.1B). Seasonal variation was due to a significant difference in species richness in summer and autumn (Tukey HSD, $p = 0.04$).

3.3.1.3. Diversity

Species diversity had a similar pattern to richness and was highest closer to the lagoon mouth and lowest at the head (Fig. 3.1C). Highest species diversity was recorded at Klein Oesterval (2.40) followed by Centre Banks (2.28), Oesterval (2.05), and Geelbek (1.81) – diversity was lowest at Bottelary (1.47). These differences were significant across sites ($F_{4,97} = 4.20$, $p = 0.004$) attributed mainly to differences in mean diversity between Bottelary and Centre Banks, and Bottelary and Klein Oesterval (Tukey HSD, $p = 0.006$). Seasonal differences in diversity were also significant ($F_{3,97} = 6.54$, $p = 0.001$) as well as the interaction between site and season ($F_{12,97} = 2.70$, $p = 0.004$) since diversity varied between warmer and cooler months (Tukey HSD, $p < 0.05$).

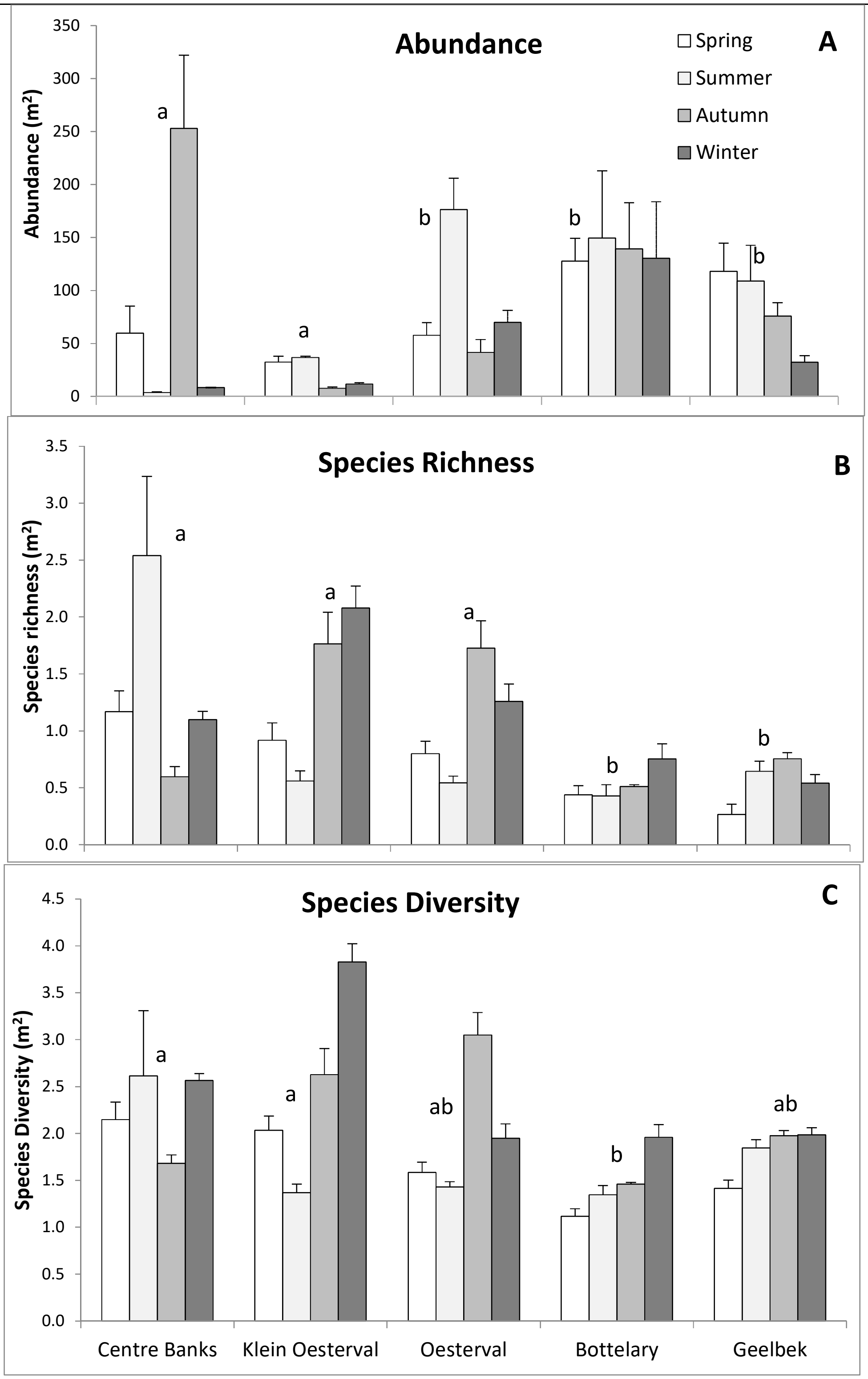


Figure 3.1: Means (+1SE) of species (A) abundance, (B) richness and (C) diversity (exp('H)) at five sites arranged from closest to the mouth (left) to the head (right) of Langebaan Lagoon across four seasons. Small letters that are shared between sites denote means that did not differ significantly (Tukey HSD, $p < 0.05$)

3.3.2. Spatial and Temporal Patterns in Macro-epifaunal Communities

Both site and season were found to significantly influence variation in macro-epifaunal abundance in *Zostera capensis* habitats in Langebaan Lagoon (PERMANOVA, $p < 0.001$; Table 3.2). Site differences contributed the most (36.83%) to the observed variation and more than twice that of seasonal differences (17.28%). A significant proportion of variation (21.33%) was explained by the interaction between site and season while 24.56% of macro-epifaunal variation was unaccounted for (Table 3.2). Site differences were evident in the clear spatial separation of macro-epifaunal abundance in the dendogram and MDS plot showing a 60% dissimilarity in two grouping, one comprising Centre Banks, Klein Oesterval and Oesterval (closer to the mouth), and the other Bottelary and Geelbek (closer to the head of the lagoon) (Fig. 3.2 A&B).

Beta-diversity differed between sites (PERMDISP: $F = 17.8$, $p < 0.01$), with pairwise comparisons confirming differences in macro-epifaunal abundances between sites at the mouth and those at the lagoon head (pairwise PERMDISP $t = 2.48$, $p < 0.04$). The species *Siphonaria compressa* and *Assiminea globulus* contributed largely to the observed dissimilarity contributing as much as 91% to the observed variation (Table 3.3). *Fissurella mutabilis*, *Parvulastra exigua*, *Diogenes brevirostris* and *Pyura stolonifera* were the main species accounting for >60% of the observed patterns at Centre Banks, Klein Oesterval and Oesterval (Table 3.3).

Table 3.2: PERMANOVA main test based on Bray Curtis similarities comparing macro-epifaunal abundances (4th root transformed) sampled across four Seasons (fixed) and five Sites (random). Effect size was calculated as a proportion of the estimated contribution of components of variation for each factor. *P* values in bold denote significance at *a* <0.05.

Source of variation	**df**	**MS**	**Pseudo-F**	***P***	**Unique Permutations**	**Effect Size**
Season	3	13 288.00	2.97	**<0.001**	9922	17.28%
Site	4	31 481.00	42.96	**<0.001**	9931	36.83%
Season x Site	12	3 291.50	4.49	**<0.001**	9876	21.33%
Residual	79	732.82				24.56%

Despite the lack of clear seasonal distinctions spatially, sites assessed individually showed seasonal groupings at degrees of similarity that ranged from 25 – 75% (Fig. 3.3). MDS plots separated species abundances in spring from summer, autumn and winter at Centre Banks and Klein Oesterval. Oesterval abundances were separated into two groups: spring and summer, and autumn and winter (Fig. 3.3). No clear seasonal grouping was observed for Bottelary, while abundance in autumn emerged as different from other seasons at Geelbek (Fig. 3.3). Generally, beta-diversity was not seasonally significant (PERMDISP: $F = 3.24$, $p = 0.09$), however pairwise comparisons produced significant differences between macro-epifaunal abundances in autumn and winter (pairwise PERMDISP $t = 3.31$, $p < 0.01$) and winter and summer (pairwise PERMDISP $t = 3.19$, $p < 0.01$).

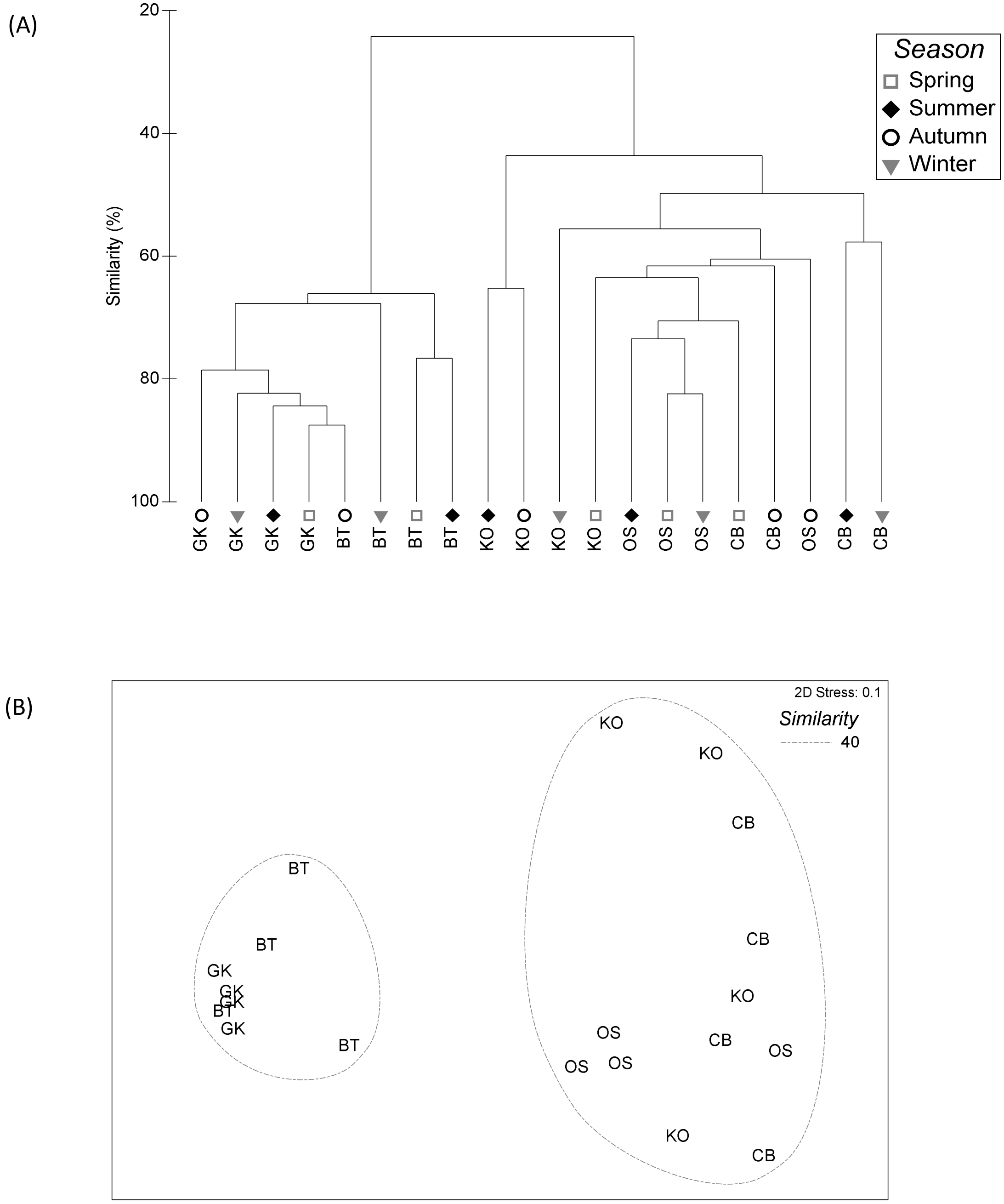


Figure 3.2: Dendogram (A) and multi-dimensional scaling plot (B) of seasonal average macro-epifaunal community structure (abundance) in *Zostera capensis* beds at five sites in Langebaan Lagoon: Centre Banks (CB), Klein Oesterval (KO), Oesterval (OS), Bottelary (BT) and Geelbek (GK). Circle delineations indicate a 40% similarity in groups.

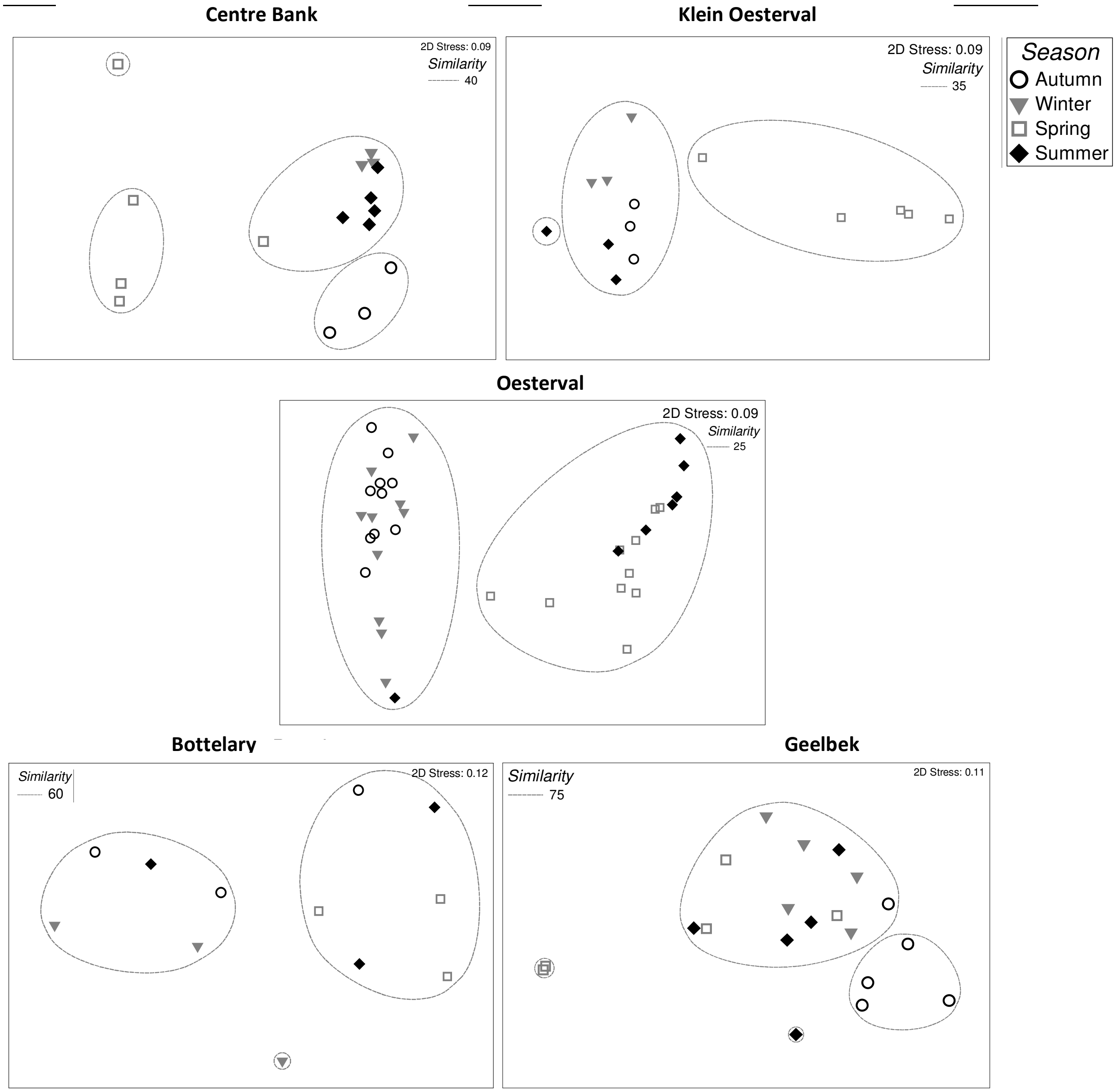


Figure 3.3: Multi-dimensional scaling plots of seasonal average macro-epifaunal species abundances in *Zostera capensis* beds at individual sites at Langebaan Lagoon. Circles represent percentage group similarities which vary per site.

Table 3.3: Results of a similarities contribution (SIMPER) analysis showing average percentage dissimilarity and the key macro-epifauna species contributing to 60% dissimilarity between sites. % Cumulative contributions are also given.

Species	**Average % cover of species**		**%Contribution**	**%Cumulative**
	Bottelary	**Centre Banks**	Average dissimilarity = 87.22	
Siphonaria compressa	2.47	0.04	33.20	33.20
Assiminea globulus	1.69	0	20.31	53.51
Fissurella mutabilis	0	0.87	11.07	64.58
	Bottelary	**Geelbek**	Average dissimilarity = 42.26	
Assiminea globulus	1.69	2.43	39.85	39.85
Siphonaria compressa	2.47	1.91	22.53	62.38
	Centre Banks	**Geelbek**	Average dissimilarity = 90.36	
Assiminea globulus	0	2.43	31.62	31.62
Siphonaria compressa	0.04	1.91	22.97	54.59
Diogenes brevirostris	0.87	0	11.3	65.9
	Bottelary	**Klein Oesterval**	Average dissimilarity = 87.64	
Siphonaria compressa	2.47	0	32.46	32.46
Assiminea globulus	1.69	0.29	18.93	51.38
Diogenes brevirostris	0.31	1.42	14.57	65.95
	Centre Banks	**Klein Oesterval**	Average dissimilarity = 57.94	
Fissurella mutabilis	0.87	1.03	20.18	20.18
Parvulastra exigua	0.73	0.16	16.78	36.96
Diogenes brevirostris	0.87	1.42	16.7	53.65
Pyura stolonifera	0.37	0.05	9.22	62.87
	Geelbek	**Klein Oesterval**	Average dissimilarity = 91.00	
Assiminea globulus	2.43	0.29	27.06	27.06
Siphonaria compressa	1.91	0	22.45	49.51
Diogenes brevirostris	0	1.42	17.33	66.84
	Bottelary	**Oesterval**	Average dissimilarity = 82.08	
Siphonaria compressa	2.47	0.09	31.01	31.01
Assiminea globulus	1.69	0.42	20.05	51.06
Fissurella mutabilis	0	0.87	10.3	61.36
	Centre Banks	**Oesterval**	Average dissimilarity = 54.43	
Fissurella mutabilis	0.87	0.87	19.57	19.57
Parvulastra exigua	0.73	0.96	13.43	33.00
Assiminea globulus	0	0.42	10.89	43.89
Diogenes brevirostris	0.87	0.97	10.84	54.73
Crepidula fornicata	0.35	0.30	10.61	65.34
	Geelbek	**Oesterval**	Average dissimilarity = 81.21	
Assiminea globulus	2.43	0.42	27.42	27.42
Siphonaria compressa	1.91	0.09	22.61	50.03
Diogenes brevirostris	0	0.97	12.47	62.50
	Klein Oesterval	**Oesterval**	Average dissimilarity = 57.97	
Parvulastra exigua	0.16	0.96	18.46	18.46
Fissurella mutabilis	1.03	0.87	16.20	34.66
Assiminea globulus	0.29	0.42	13.49	48.15
Diogenes brevirostris	1.42	0.97	13.00	61.15

3.3.3. Influence of Environmental and Seagrass Structural Variables on Macro-epifaunal Abundance

All seagrass variables including epiphytic algal biomass were significant in explaining variability in macro-epifaunal abundance (PERMANOVA: DistLM, $p < 0.01$, Table 3.4). Distance-based linear models were first run without the variable leaf width given its strong correlation with leaf length however, a better model fit was achieved when leaf width was included. Leaf width contributed the highest proportion of variation in macro-epifaunal abundance followed by leaf length, density, seagrass biomass and epiphytic algal biomass in the marginal test (Table 3.4). All seagrass variables were selected in the first model that best explained variability in macro-epifaunal abundance determined by lowest AIC and highest R^2 values. The second best model containing four variables (i.e. density, leaf width, epiphytic algae and seagrass biomass), and with an AIC value < 2 levels from the first model, would also be adequate in explaining the observed variation (Table 3.4).

The influence of environmental variables only on macro-epifaunal abundance as assessed in a DistLM marginal test, found exposure to contribute the highest proportion of variation followed by chl *a* and turbidity (Table 3.5). Smaller proportions of variation were provided by temperature, salinity and oxygen (Table 3.5). All environmental variable contributions were significant ($p < 0.001$), and the top two models determined by AIC and R^2 parameters, that best explained variation in macro-epifauna abundance selected five (all) and four (without turbidity) environmental variables (Table 3.5).

The final structural equation model (SEM) used to explore the influence of environmental variables and seagrass structural metrics on macro-epifauna showed no direct effects of temperature, salinity, pH and exposure on macro-epifaunal abundance (chi-square = 90.66, df = 45, $p = 0.35$, RMSEA = 0.083; Fig. 3.4B). Only turbidity and oxygen displayed strong negative (-0.85) and positive (1.09) direct effects respectively. However, indirectly temperature, pH, exposure and oxygen negatively affected macro-epifaunal abundance while turbidity had a positive indirect effect (Table 3.6). Although all environmental variables appeared to directly influence leaf length, this did not translate into indirect effects on macro-epifaunal abundance. This was not the case for leaf width which was negatively influenced by temperature and turbidity, and positively influenced by pH and exposure, and produced a positive direct effect explaining 65% of variation in macro-epifaunal abundance (Fig. 3.4B, Table 3.6). Similarly, shoot density had a positive direct effect on macro-epifaunal abundance accounting for 65% of variation (Fig 3.4B), while shoot densities were negatively influenced by pH, oxygen and exposure and positively affected by turbidity (Table 3.6). No other seagrass metrics emerged to directly influence macro-epifaunal abundances.

Table 3.4: Results of a distance-based linear model displaying the proportion of variability explained by seagrass structural variables on patterns in macro-epifaunal abundance. A marginal test was produced and the overall five best solutions ranked by AIC and R^2 model fit parameters are presented. *P* values are significant at < 0.05 and in bold.

	Marginal Test		
Variable	**Pseudo F**	***P***	**Prop. of variation**
Leaf density	29.28	**0.001**	23.19%
Leaf length	31.29	**0.001**	24.39%
Leaf width	41.14	**0.001**	29.78%
Epiphytic algal biomass	6.62	**0.001**	6.39%
Seagrass biomass	9.05	**0.001**	8.53%

Overall best solution			
AIC	**R^2**	**# Var.**	**Variable Selections**
737.13	0.43	5	All
738.50	0.41	4	Density, leaf width, epi-algal biomass, seagrass biomass
739.78	0.40	4	Density, leaf length, leaf width, seagrass biomass
740.89	0.40	4	Density, leaf length, leaf width, epi-algal biomass
741.12	0.39	4	Density, leaf length, epi-algal biomass, seagrass biomass

Table 3.5: Distance-based linear modelling results displaying the proportion of variation explained by environmental variables on patterns in macro-epifaunal abundance using the "best" procedure, AIC and R^2 selection criteria. Overall five best model solutions are presented ranked by fit based on AIC values. *P* values are significant at < 0.05 and in bold.

	Marginal Test		
Variable	**Pseudo F**	***P***	**Prop. of variation**
Temperature	13.02	**<0.001**	11.84%
Salinity	10.72	**<0.001**	9.96%
Turbidity	27.88	**<0.001**	22.32%
Oxygen	7.93	**<0.001**	7.56%
Chlorophyll *a*	29.22	**<0.001**	23.15%
Exposure	40.14	**<0.001**	29.27%

Overall best solutions

AIC	**R^2**	**# Var.**	**Variable Selections**
729.54	0.48	6	Temperature, salinity, turbidity, O_2, chl *a*, exposure
730.88	0.46	5	Temperature, salinity, O_2, chl *a*, exposure
731.54	0.46	5	Temperature, salinity, turbidity, chl *a*, exposure
732.01	0.46	5	Temperature, salinity, turbidity, O_2, exposure
732.21	0.45	4	Temperature, salinity, turbidity, exposure

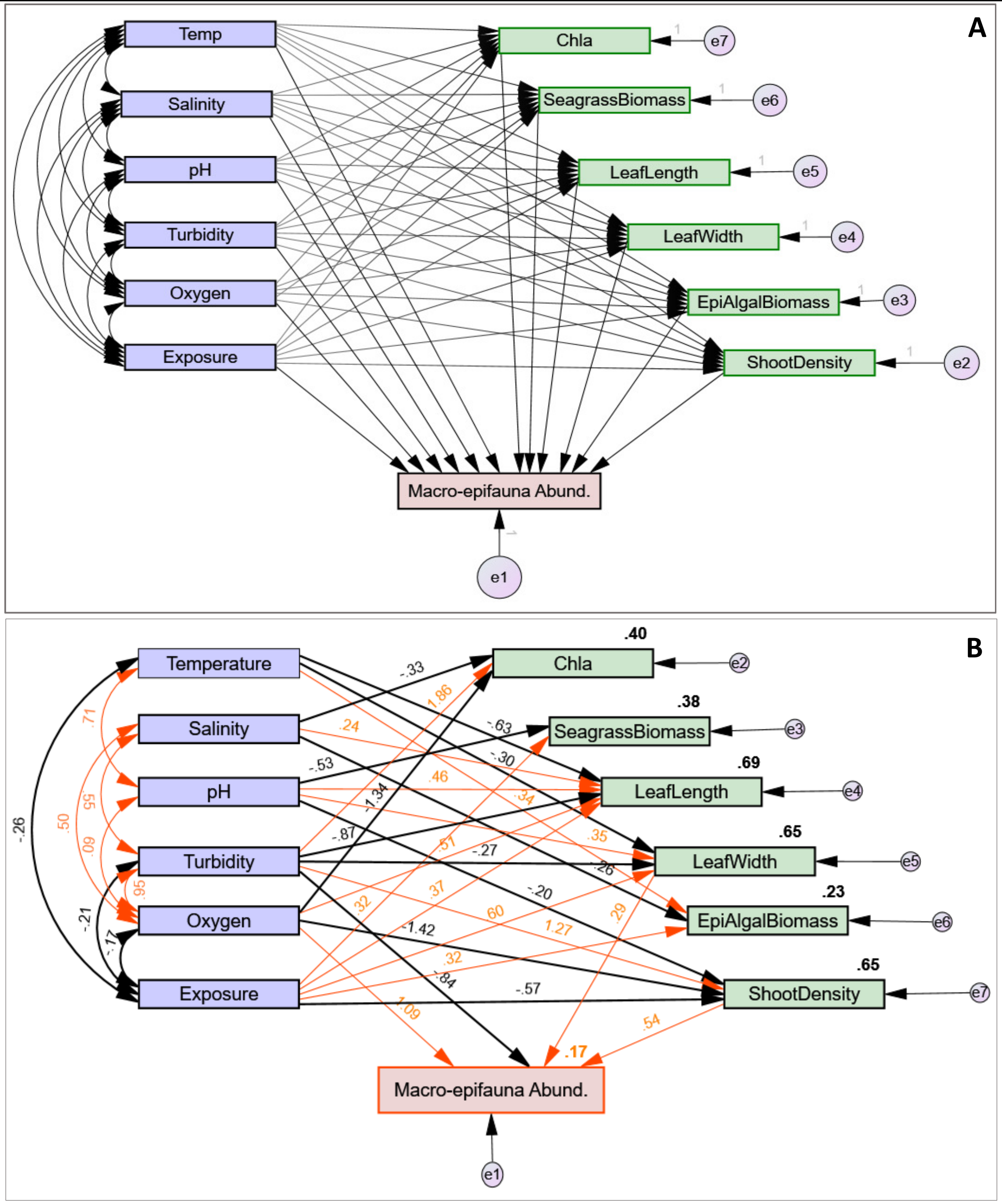


Figure 3.4: (A) Full structural equation model of exogenous environmental variables and endogenous seagrass metrics, chlorophyll *a* and epiphytic algae directly and indirectly influencing macro-epifaunal abundances. Chi-square = 256.66, df = 15, $p < 0.01$, RMSEA = 0.32. See full results table in Appendix III. (B) Standardized coefficients of significant ($p < 0.05$) paths used to compare the strength of direct and indirect effects only are presented (chi-square = 90.66, df = 45, $p = 0.35$, RMSEA = 0.083). For example, if exposure increases by 1SD, shoot density decreases by 0.57 SD. Double-headed arrows indicate correlated environmental variables. Black and orange lines represent negative and positive relationships respectively and "e" is the error term associated with each endogenous variable. R^2 values above endogenous variables denote the variance explained by the model.

Table 3.6: Standardized total, direct, and indirect significant effects of seven environmental and seven seagrass variables on macro-epifaunal abundance (macro-abund) as determined in a structural equation model.

	Exposure	O_2	Turbidity	pH	Salinity	Temp	Shoot density	Leaf Width
Standardized total effects								
Density	-0.57	-1.42	1.27	-0.20	-	-	-	-
Biomass	0.32	-	-	-0.53	-	-	-	-
Leaf Length	0.38	0.51	-0.87	0.46	0.24	-0.63	-	-
Leaf Width	0.60	-	-0.27	0.35	-	-0.30	-	-
Algal biomass	0.32	-	-	-	-0.26	0.34	-	-
Chl *a*	-	-1.34	1.87	-	-0.33	-	-	-
Macro-abund	-0.14	0.32	-0.24	-0.01	-	-0.09	0.54	0.29
Standardized direct effects								
Density	-0.57	-1.42	1.27	-0.20	-	-	-	-
Biomass	0.32	0.00	0.00	-0.53	-	-	-	-
Leaf Length	0.38	0.51	-0.87	0.46	0.24	-0.63	-	-
Leaf Width	0.60	-	-0.27	0.35	0.00	-0.30	-	-
Algal biomass	0.32	-	-	-	-0.26	0.34	-	-
Chl *a*	-	-1.34	1.87	-	-0.33	-	-	-
Macro-abund	-	1.09	-0.85	-	-	-	0.54	0.29
Standardized indirect effects								
Macro-abund	-0.14	-0.77	0.61	-0.01	-	-0.09	-	-

"Total effects" is calculated from the sum of all direct and indirect effects. "Direct effects" refers to the direct effect of one variable on another, and "indirect effects" are the sum of all the variables affecting one variable.

3.4. Discussion

Macro-epifaunal biodiversity in *Zostera capensis* beds in Langebaan Lagoon, as reflected by species diversity and richness, was highest closer to the mouth and in beds below mean low water levels, and declined upstream and in high shore beds. However, species abundances were lower at the mouth and higher at upstream sites. Spatial and temporal differences were significant in influencing macro-epifaunal species abundance which was largely due to abundances of two out of a total 24 species recorded. The observed variation was explained more directly and indirectly by environmental factors than seagrass structural variables. These results are correlative and do not infer direct causal associations driving the observed patterns in macro-epifaunal community structure.

3.4.1. Richness, Abundance and Diversity of Macro-epifauna

Despite fairly stable salinity levels in the lagoon, species richness and diversity displayed a similar pattern of decline from the mouth as observed in typical estuaries where variation in faunal communities are largely influenced by a salinity gradient (Heymans and Baird 1995, Allanson and Baird 1999, Barnes 2010). But unlike typical estuaries, salinity levels in the lagoon increased with distance from the mouth possibly due to evaporation and the lack of distinct inflow of freshwater. Variation in macro-epifaunal community structure is not necessarily attributed directly to salinity but could be an indirect effect since salinity is often correlated with other environmental variables (Yamada et al. 2007). Factors such as current velocity, wave intensity, competition and predation not measured in this study, may account for the unexplained variation in macro-epifaunal community structure.

In a similar enclosed bay with no freshwater input and a relatively high salinity range (22 - 35) in Arcachon, France, macrofauna in beds of another dwarf-eelgrass, *Zostera noltei* showed similar patterns to that observed for Langebaan Lagoon (Blanchet et al. 2004). Species richness was lowest in assemblages furthest from the mouth and at higher tidal levels while abundances were highest at these places. In that study, distance from oceanic waters (km) was used as an environmental factor, and was the main attributor of differences between assemblage groups along with tidal range (Blanchet et al. 2004). Similarly, Barnes and Ellwood (2012) found richness and diversity to be highest in *Z. capensis* beds below mean low water levels in the marine-dominated section of the Knysna estuary, South Africa. In this large estuarine system species richness did not display the classic decline with distance from the mouth, but instead had a stable fauna separated by abrupt changes in areas where salinity levels fell below 30 (Barnes and Ellwood 2012). In Arcachon, species occurring in areas of relatively low salinities were not distinct but rather a subset of those present at the mouth, which was not the case at Langebaan. Several species were found only at sites closer to the mouth and not at Bottelary and Geelbek. In contrast, a stable faunal composition was observed at Knysna despite the wide range in salinity (<5–35) (Barnes and Ellwood 2012). While the observed variation in Knysna was supposedly due to changes in relative abundance of dominant species, or the lack of marine species in upstream localities and upper boundaries of seagrass beds (Barnes and Ellwood 2012), species variation in Langebaan was more likely a result of exposure and tolerance to desiccation (this study), availability of seagrass habitat (Angel et al. 2006) as well as the ability to survive under periods of exposure to sun and wind during low tide (Day 1959).

In Langebaan Lagoon, macro-epifaunal community structure was dominated by grazers i.e. *Siphonaria compressa*, *Fissurella mutabilis* and *Assiminea globulus*. *A. globulus* was the main species recorded at Bottelary and Geelbek and occurred in lower abundances in high shore beds at Oesterval and Klein Oesterval, although high abundances were previously observed at Oesterval in both seagrass and saltmarsh vegetation in the high shore (Day 1959). Regarded as a broad niche generalist and capable of adapting to a range of environmental conditions, this mud snail feeds mainly on bacteria and diatoms found on sediment surfaces and occasionally on epiphytic periphyton, and is strongly averse to prolonged periods of submergence (Angel et al. 2006, Pillay et al. 2009). Gastropods have been documented to dominate many estuarine systems (Allanson and Baird 1999) and Barnes (2013) recorded a dominance of gastropods including 125 times greater average densities of *Assiminea* in upper-estuarine and enclosed sites compared to elsewhere in the Knysna estuary. In Arcachon, seagrass beds at high shore sites and sites further from the mouth were dominated by a single gastropod, *Peringia ulvae* (Blanchet et al. 2004). The prevalence of gastropods in high shore areas is likely attributed to their ability to resist desiccation as well as the upward extension of habitat provided by seagrass and saltmarsh vegetation (Day 1959, Barnes 2013).

High shore sites in Langebaan were also dominated by the pulmonate limpet *Siphonaria compressa* which has been identified as South Africa's most threatened marine invertebrate. Classified as Critically Endangered by the IUCN (Kilburn 1996), this limpet has an extremely narrow habitat range and grows only in *Zostera capensis* meadows in Langebaan Lagoon and the Knysna estuary (Allanson and Herbert 2005). In this study, densities of 100 m^{-2} were recorded at Geelbek and Bottelary and <5 m^{-2} at Oesterval. None were found at Klein Oesterval and Centre Banks, however, Angel et al (2006) found densities between 40-80 individuals per m^2 at the lower edge of seagrass beds at Klein Oesterval over a 30 year period. Analyses of faecal pellets revealed that *S. compressa* primarily feeds on bacteria, diatoms and filamentous algae found on seagrass leaves (Allanson and Herbert 2005), however no direct investigations on feeding have been undertaken.

High abundances of the two dominant grazers *Assiminea globulus* and *Siphonaria compressa* at the same sites suggest a lack of competition and the exploitation of different niches. This hypothesis was explored by Angel et al. (2006) who assessed the interaction between *A. globulus, S. compressa* and *Zostera capensis* cover in relation to exposure during low tide at Langebaan. They found that *S. compressa* thrived on the lower edge of seagrass beds subjected to shorter periods of exposure, and suggested that the virtual absence of the limpet from the upper zone was largely the result of avoiding physiological stress from desiccation - a consequence of its small size and thin shell. Experimental transplants of seagrass into sandbanks saw a proliferation of *S. compressa*, and concluded that confinement of the limpet to beds in the high-shore was suboptimal and essentially due to the restriction of the seagrass beds themselves to expansion further into the low shore due to sediment disturbance by the burrowing sand prawn, *Callichirus kraussi* (previously of the genus *Callianassa)* (Angel et al. 2006). The conclusions of that experiment do not explain the absence of *S. compressa* from low shore beds at Oesterval and Centre Banks in this study. Here, a likely reason is competition with grazing gastropods (Orth et al. 1984) such as *Fissurella mutabilis,* which is less tolerant to desiccation and occurred in high abundances at low shore sites close to the mouth and but not at high shore sites i.e. Geelbek and Bottelary.

In contrast, Angel et al. (2006) recorded high and low densities of *Assiminea globulus* on the upper and lower edges of seagrass beds respectively, and noted a positive correlation with exposure. Once again, this was attributed to disturbance of sediment and reduction of diatoms due to bioturbation, however these snails also appeared to prefer exposure rather than submergence (Angel et al. 2006). This species' preference for more exposed parts of seagrass beds is likely related to the availability of food in the form of sand-dwelling diatoms that are abundant in the high shore zone (G.M. Branch, unpublished data), and therefore occupies a different niche to that of *Siphonaria compressa*. Given that *S. compressa* is assumed to feed essentially on periphyton on seagrass leaves (Allanson and Herbert 2005), there was no evidence to indicate that abundance or zonation of these two species had been influenced by competition (Angel et al. 2006).

Environmental conditions such as a longer tidal emergence period at Bottelary and Geelbek correspond to a higher susceptibility of species to desiccation. This explains the lower species richness at these sites, and the high abundances of the desiccation resistant *A. globulus*, and also attests to the resilience of *S. compressa* to persist in suboptimal conditions. The cushion star *Parvulastra exigua* and keyhole limpet *F. mutabilis* was reported to have completely declined at Klein Oesterval in 2009 (Pillay et al. 2010) but was subsequently recorded in this study. In addition, *Pyura stolonifera*, *Sargartia ornata* and *Oxystele antoni* not documented in the 2009 survey, were also found. These findings attest to the high variability in seagrass ecosystems, while high grazer abundances highlight the trophic significance of grazing in seagrass and intertidal communities generally (Asmus and Asmus 1985).

Species richness and densities recorded in this study were comparable to the survey of macrofauna within seagrass and un-vegetated sandflats at Klein Oesterval carried out in 2009 using the traditional method of digging and sieving (Pillay et al. 2010). That study yielded a total 27 species and while infaunal species (n = 15) were not targeted in the present study, a total of 13 species were documented for that site - nine species were recorded from both surveys, and an additional four were recorded in this study only. These patterns allude to the strengths and weaknesses in sampling techniques, many of which fail to capture various components within the faunal assemblage influenced by processes such as life history stages, succession and colonization (Moura et al. 2008) as well as temporal restrictions related to tidal regime, season and day/night sampling. While traditional methods are considered comprehensive in providing estimates of biodiversity, their value can be exaggerated when weighed against time and logistical constraints. Where such constraints exist, alternative methods including the use of a selection of indicator species in single or multimetric indices, can be useful to assess the ecological status of benthos in marine and estuarine environments (Borja et al. 2011, Dauvin et al. 2016) particularly when resources are limited. Using a subset of species has been found to provide meaningful descriptions on faunal community structure (Kuenen and Debrot 1995, Vellend et al. 2008). Importance should therefore be placed on consistency of methods used as well as regularity of surveys to provide informative results to address research and management needs (Magurran et al. 2010).

3.4.2. Influence of Seagrass Structure and Environmental Variables on Macro-epifaunal Abundance

Variability due to site and season was significant and a seasonal pattern in macro-epifaunal abundance was evident (Figure 3.1). Abundances were highest in summer, but generally decreased in cooler months. Highest abundances occurred in denser seagrass beds at Bottelary and Geelbek, while beds with longer leaves closer to the mouth supported a richer suite of species compared to short-leaved populations, albeit in lower densities. Despite the lack of positive and significant correlations between macro-epifaunal abundance and seagrass metrics, all seagrass variables were selected as important in explaining macro-epifaunal variation with leaf width and length contributing the most to the observed variation (29.78% and 24.39% respectively).

The abiotic environment in Langebaan Lagoon significantly influenced variation in seagrass morphometric parameters (discussed in Chapter Two). Cooler temperatures favoured longer leaves and these provide a greater surface area for epiphytic colonization (Bologna and Heck 1999, Terrados and Medina-Pons 2011). Faunal diversity in seagrass habitats increases with greater plant size and biomass although other factors such as ecological characteristics of fauna can also influence community structure (Heck and Orth 1980, Orth et al. 1984). Given that leaves of different seagrass species differ in area per unit biomass, it has been suggested that plants with greater aboveground foliar biomass provide more shelter, protection and available food than smaller plants with less surface area per biomass (Heck and Orth 1980, Orth et al. 1984). This provides a likely explanation for varying macro-epifaunal abundances at sites with differing seagrass plant sizes and leaf area in Langebaan Lagoon.

Several studies demonstrate similar patterns to that observed in Langebaan. In an assessment of beds with low, medium and high densities of *Zostera marina* in the United Kingdom, infaunal diversity increased with increasing seagrass density, and significant differences in community structure between shoot density ranges were observed (Webster et al. 1998). Similarly, seagrass biomass was a key regulator of macrofaunal diversity, abundance, dominance and trophic arrangement independent of hydrodynamic and sediment properties in Apalachee Bay, Florida (Stoner 1980). In that study, subtidal sites of *Halodule wrightii*, relative abundances of epifaunal polychaetes and amphipods were directly related to seagrass biomass, and abundances of carnivorous polychaetes and suspension feeders increased while omnivorous polychaetes and deposit feeders decreased with seagrass standing crop (Stoner 1980). Likewise, increased habitat complexity as a result of greater seagrass biomass was a key factor in regulating macrofauna in *Zostera capensis* beds in Mozambique (Paula et al. 2001).

In the final structural equation model, seagrass density, leaf width, oxygen and turbidity emerged to have a direct effect on macro-epifaunal patterns in Langebaan Lagoon while other environmental factors played a strong indirect role (Fig. 3.4). Despite the lack of a direct effect of temperature on macro-epifaunal abundance, as well as a low indirect effect (1SD increase in temperature translated to a decrease in macro-epifauna by 0.09SD), temperature had a direct effect on leaf length and width (Table 3.6) and was a significant predictor of five of the six seagrass metrics assessed (Chapter Two: Fig. 2.5). Indirect effects have been shown to influence community structure as significantly as direct effects (Wootton 2002). It is therefore likely that the relationship between declines in macro-epifaunal abundance as observed in the field is as a result of direct physiological effects of temperature, as well as indirect effects on the supporting seagrass ecosystem (Marbà and Duarte 2010).

Smaller, denser morphologies of *Zostera capensis* experienced higher temperatures than their large-leaved counterparts. Seagrass beds at Bottelary and Geelbek had higher shoot and leaf densities from smaller plants with narrow leaves and thus greater leaf areas per biomass. The low species diversity and dominance of two species implies a narrow niche and high degree of acclimation to local environmental conditions at these sites. Edgar and Barrett (2002) determined the greatest influence of variance in species richness examined in 48 estuaries in Tasmania, was linked to tidal height while seasonal variance did not feature as highly as spatial variance, as was evident at Langebaan Lagoon. These authors discovered species richness and faunal biomass to be related to salinity and seagrass biomass especially at low tide and shallow subtidal levels (Edgar and Barrett 2002). Physiological tolerance to environmental stress particularly from exposure has been found to signify a means of escape from hostile effects of predation and competition, more prevalent in diverse communities of temperate seagrass ecosystems (Barnes and Ellwood 2012).

Seagrass habitats have a structuring effect on macro-epifaunal communities. Structurally complex habitats may permit co-existence of a higher number of species by buffering the effects of competition and predation (Menge and Sutherland 1976). These complex habitats can regulate species indirectly by reducing predation since greater options for refuge for prey species are provided, thereby reducing foraging fitness of predators (Menge and Sutherland 1976, Orth et al. 1984, Hammerschlag-Peyer et al. 2013). More refuges are linked to greater prey diversity and have also been found to balance otherwise unstable predator-prey interactions (Menge and Sutherland 1976, Heck and Orth 1980, Orth et al. 1984).

Greater species diversity contributes to improved functioning and resilience of ecosystems (Duffy et al. 2003, Unsworth et al. 2015) while the viability as food of any one species seems to be greatly dependent on the development of significant abundances, since reliability on rare species as a food source is unlikely (Duarte 2000, Balvanera et al. 2006). Maintaining and enhancing biodiversity is therefore a key factor in ensuring ecosystem health and the sustained supply of ecosystem services.

3.5. Conclusions

Macro-epifaunal community structure in *Zostera capensis* beds is regulated more directly by seagrass leaf width and density, and indirectly by environmental variables influencing faunal abundances. The findings from Chapter Two showed temperature as a key factor influencing seagrass metrics with warmer temperatures producing smaller, denser seagrass morphologies. Sustained increases in temperature could therefore lead to the proliferation of smaller structured populations that will support a different fauna to that associated with larger seagrass structure. Further warming could lead to a shift in larger structured populations to a narrow range thereby reducing overall macrofaunal diversity and richness within seagrass ecosystems. Increasing temperatures would also mean greater levels of evaporation. This could see additional shifts towards species that are more tolerant to desiccation. Fluctuation in seagrass abundance could also increase the risk of extinction of *Siphonaria compressa* which is categorized as critically endangered based on its morphological limitations, restricted range and extent and quality of its habitat.

The implications of a loss of macro-epifaunal biodiversity in *Zostera capensis* beds in Langebaan Lagoon implies a loss of productivity, and the output of energy to other trophic levels especially as prey species for fish. The lagoon and adjacent Saldanha Bay support a recreational and industrial fishery, and seagrass habitats are critical to providing food and protection to juvenile fish to sustain these industries.

Further research into testing the effect of specific environmental variables as well as interactions of key macrofaunal species i.e. *Fissurella mutabilis* and *Siphonaria compressa* will aid our understanding of the distributional limitations of these species in *Zostera capensis* habitats. Understanding the interactions that shape macrofaunal population dynamics, life histories and the functioning of communities along with their feedback to environmental change is important and imperative to informing effective management of human activities that affect ecosystems and associated communities.

CHAPTER FOUR

Effects of Temperature on Small and Large-leaved Morphotypes of *Zostera capensis* – A Mesocosm Approach

4.1. Introduction

Shallow water and intertidal communities are characterized by the interaction between physical (tidal regime, temperature, substrate type) and biological (predation, herbivory, competition) factors that determine community structure and boundaries (Marbà et al. 2004, Duarte et al. 2006, Wong et al. 2014). Shallow waters are heated by solar energy and subjected to thermal stress from diurnal and seasonal changes, as well as climatic factors that affect ambient temperatures often far exceeding oceanic levels (Unsworth et al. 2012, Petrou et al. 2013). These temperature changes can produce particularly stressful environments for shallow water communities (Harley et al. 2006, Doney et al. 2012, Unsworth et al. 2012).

The light requirements for photosynthesis in most species of seagrasses are high i.e. 5-35% of surface irradiance, needed largely to support photosynthetic activity to maintain the respiratory needs of the non-photosynthesizing root/rhizome tissue (Dennison 1993, Duarte 2007). These plants are thus restricted to shallow waters (Dennison et al. 1993), where they are often subjected to supra-optimal temperatures (Dunton 1994, Lee et al. 2007, Unsworth et al. 2012). The thermal optima supporting seagrass photosynthesis and growth are species-specific and reflect the geographic range in which a species occurs (Pérez and Romero 1992, Leegood 1995, Masini and Manning 1997). For shallow water habitats, thermal optima are prone to be exceeded during low tides, while seagrass species surviving at upper limits of thermal tolerance may be severely impacted by an increase in average sea temperatures of as much as 2°C (Ralph 1998a, Kaldy et al. 2015). The potential effect of a swift and prolonged shift in environmental regimes from predicted climate change therefore poses a serious threat to seagrasses preventing timely acclimation or migration (Duarte 2002).

Increasing temperatures can create conditions that benefit some genotypes by extending growth periods, and favouring growth rates and reproduction (Ehlers et al. 2008, Bergmann et al. 2010). However, heat stress when sustained for a period above a threshold, results in reduced growth rates and ultimately damage that is often irreversible (Wahid et al. 2007). The rapid reduction in photosynthesis, leaf growth, density and biomass of seagrasses due to thermal damage is well documented (see review in Neilson et al. 2013, Chap. 9), however, recovery is possible in some species if normal conditions are restored in time (Adams and Bate 1994). In photosynthesizing organisms such as algae and seagrasses, ambient temperature influences processes such as nutrient uptake, photosynthesis, respiration, within-plant resource partitioning and leaf senescence (Marsh et al. 1986, Pérez and Romero 1992, Leegood 1995). Photosynthesis increases with increases in temperature reaching a threshold above which photosynthetic efficiency declines as temperature continues to increase (Marsh et al. 1986). The temperature range at which this effect occurs is species-specific, and spatially and temporally influenced (Kübler and Davison 1993). Therefore, initial responses to warming are an increase in photosynthesis in algae and seagrasses, for unlimited growth restrictions, followed by increases in biomass and productivity (Barber and Behrens 1985). Comprehending the effect of temperature on seagrasses and their algal associations would provide important knowledge on the influence of warming on these interactions, as well as the implications on upper trophic levels within the food web.

Seagrass beds provide a physical substrate on which micro- and macroalgae settle. These epiphytes can hamper light absorption in seagrass leaves, thereby reducing the rate of photosynthesis and overall plant fitness (Bologna and Heck 1999, Fong et al. 2000, Michael et al. 2008). Warming along with increases in nutrients through for example eutrophication favours algal growth (Alsterberg et al. 2013, Whalen et al. 2013, Werner et al. 2016, Werner and Matthiessen 2017). This can be problematic in seagrass systems since left unchecked, epiphytic algae can eventually smother seagrass leaves resulting in reduced absorption of nutrients and attenuation of light by seagrasses (Mazzella and Alberte 1986, Silberstein et al. 1986, Short et al. 1995, Hughes et al. 2004).

Seagrasses have developed a range of morphological and biochemical strategies to cope with ecological and environmental variations, and these coping strategies are important to understand given the global decline in seagrasses and their importance in coastal ecosystems. Many seagrass species have developed the ability to acclimate to various stressors, mostly evident in their ability to recover from such conditions (Penhale and Wetzel 1983, , Koch et al. 2007a, Koch et al. 2007b, Lee et al. 2007). Acclimation strategies may include the modification of physical and cellular morphologies, often within a single generation (Tanaka and Nakaoka 2004). Several seagrass species display phenotypic plasticity, where a single species displays multiple growth forms in response to different environments (Bigley and Harrison 1986, Olesen et al. 2002, Schanz and Asmus 2003). The rapid increase in global temperatures pose a challenge to seagrass acclimation and survival since they may face extreme conditions which persist longer than acclimated for (Koch et al. 2013), thereby compromising growth, functioning and associated biodiversity (Eklöf et al. 2012). This highlights the importance of understanding seagrass response and acclimation to increasing temperature.

Despite possessing strategies for acclimation to environmental stress, seagrasses display varying sensitivity to temperature stress which is often species-specific with variations evident within single populations (Bazzaz 1990, Torquemada et al. 2005). Seagrasses living in tropical and upper intertidal habitats are close to their thermal limits and need to up-regulate stress response systems in order to cope with lethal and sub-lethal extremes (Koch et al. 2013). The effects of prolonged or extreme thermal stress are also varied, with observations usually carried out after an event, making it difficult to link physiological responses to mortality (Mayot et al. 2005, Marbà and Duarte 2010, Rasheed and Unsworth 2011). The response to temperature extremes in seagrass systems needs to be understood in order to predict effects of climate change with any certainty.

In the Langebaan Lagoon/Saldanha Bay region mean summer water temperatures have been 3-5^{o}C higher than surrounding areas, likely due to the influence of air temperature and the duration water is retained in the shallow embayment (Smit et al. 2013). Beds of *Zostera capensis* in Langebaan Lagoon display high seasonal variability and a notable decline during summer (Chapter Two, Pillay et al. 2010). In addition, *Z. capensis* displays a large-leaved (LLM) (±20.7cm) and small-leaved (SLM) (±8.6 cm) morphotype (Fig. 4.1), an acclimation to living in low and high intertidal zones respectively. Larger shoots with greater biomass grew in cooler water temperatures in the lower intertidal, while small shoots with narrow leaves occurred in dense beds that have long emergence periods in the high intertidal. Maximum water temperature recorded in the LLM populations was 25.5^{o}C and in the SLM population 28.5^{o}C (Chapter Two). Temperature was the key environmental factor and a consistent predictor of variation in seagrass density, biomass and morphometrics, contributing most variation in leaf length (37.60 %) and aboveground biomass (36.05%) (Chapter Two).

Figure 4.1.: (A) Large-leaved (±20.7cm) and (B) small-leaved morphotypes (±8.6 cm) of *Zostera capensis* from Langebaan Lagoon.

In the study reported in Chapter Two, a considerably greater biomass of epiphytic algae was recorded in LLM populations, while the SLM produced the least epiphytic algae. Environmental variables contributed 29% to the observed variation in algal biomass explained mostly by temperature, chl *a*, turbidity and salinity (Chapter Two: Table 2.4). The high amount of unexplained variability (69%) suggests the existence of other key factors not accounted for in this study possibly driven by nutrients, fluctuation in light levels, grazer preferences etc. Epiphytic algae are a key food source to grazers and increase overall primary production in seagrass habitats (Klumpp et al. 1992, Smit et al. 2005, Lebreton et al. 2011).

In this chapter, I aim to clarify water temperature tolerance of *Zostera capensis* by measuring biomass and morphometric responses of plants in two morphometrically divergent populations after prolonged exposure to four temperature treatments (18, 22, 26 and 30^{o}C). It is predicted that under constant and prolonged exposure to warming, *Z. capensis* will reach or exceed physiological thresholds of tolerance and performance. My aim is to establish the response in seagrass performance (biomass, leaf morphometrics and density) and level of algal fouling to increasing temperature in order to identify different responses in morphologically divergent populations of *Z. capensis*, and predict the potential for expansion of seagrass stands in scenarios of increasing temperature. Because of the vital role of seagrasses in primary production, habitat provision and the support of trophic networks, determining seagrass populations that show greater resilience and tolerance to stress is key to understanding their role in fulling equivalent ecological functions to the species they succeed. Since habitats supporting morphologically distinct populations of *Z. capensis* also support different macro-epifauna (Chapter Three), and long-leaved but not short-leaved populations have shown genetic similarities to populations on the east coast (Phair et al. 2019), expansion of any one type of population would have implications for biodiversity and trophic interactions associated with *Z. capensis* in Langebaan Lagoon.

This is the first experiment testing the effects of water temperature on *Zostera capensis* in Langebaan Lagoon. Arising from findings described in Chapter Two the following three hypotheses are tested: (1) the small-leaved morphotype from Geelbek, which experiences high (>28.5^{o}C) temperatures naturally, will display an increase in seagrass response variables in warmer temperatures (26 and 30^{o}C), while the large-leaved morphotype from Oesterval will sustain or increase seagrass response variables in cooler temperatures (22^{o}C) that will decline as temperature increases. (2) Higher temperatures will favour epiphytic algal growth, therefore more algae will be found on plants growing in 26^{o}C and 30^{o}C treatments and (3) large-leaved morphotypes will experience greater algal fouling than small-leaved morphotypes.

4.2. Methods

The effect of temperature on growth of *Zostera capensis* was investigated in a mesocosm experiment. Seagrass samples from small-leaved morphotypes (SLM, Geelbek) and large-leaved morphotypes (LLM, Oesterval) were subjected to four temperature treatments (18, 22, 26 and 30°C). Eighteen degrees Celsius was chosen as the minimum treatment temperature since sites that experienced between 16-18°C on average produced longest leaves (Chapter Two). The maximum temperature recorded in the lagoon during this study was 28.3°C, while average summer mean temperature in the SLM and LLM populations was 26.2 and 21.8°C respectively (Chapter Two). Average air temperature (which influences shallow water temperatures) over the last 10 years at Langebaan has been 28.41°C ±0.4 SE, with a maximum of 31.10°C (South African Weather Service, unpub. data). Four temperatures consisting 18, 22, 26 and 30°C was thus used in this experiment to cover optimal and maximal temperature ranges experienced by seagrass habitats in Langebaan Lagoon.

4.2.1. Experimental Setup

A temperature treatment comprised three 80L mesocosms (tanks) pumped (Atman PH-2500 and AQUAH$_2$O APH-3000 pump) with filtered aerated seawater from a larger header 120L tank, using an equal release flow system (Fig. 4.2). A fourth tank received surplus water which was pumped back into the header tank creating a re-circulating system that maintained a constant water flow between tanks. Temperatures were adjusted to treatment levels using two 300W aquarium heaters each in the 26°C and 30°C tanks and one each in the 22°C and 18°C tanks. Eight fluorescent tubes (Osram Lumilux Cool White 58 Watt /965) were mounted over each treatment (32 tubes used in total) and provided up to 400 µmol photons m^{-2} s^{-1} on a 12 hour light/dark cycle. Despite being lower than natural sunlight, *Zostera capensis* growth was sustained under these light conditions for up to eight weeks during trial tests of the mesocosm system. Seagrass growth under lower than natural light levels has been achieved in other experimental studies for example, Bergmann et al. (2010) and Kaldy et al. (2017) provided saturating light levels to seagrass shoots at ~200 μmol photons m^{-2} s^{-1} while light levels of up to 400 μmol photons m^{-2} s^{-1} were used in other indoor mesocosms (Koch and Erskine 2001, Abe et al. 2008, Staehr and Borum 2011,Kaldy et al. 2015).

Each tank contained three replicate cores of each morphotype, and three tanks comprised one treatment (n = 9 cores per morphotype per treatment). Weekly exchange with fresh seawater, aerated and adjusted to treatment temperatures, replaced treatment water to maintain sufficient levels of nutrients and inorganic carbon so that seagrass growth requirements could be maintained (Nejrup and Pedersen 2008). Once treatment temperatures were reached, they were maintained for four weeks (after Vergeer et al. 1995).

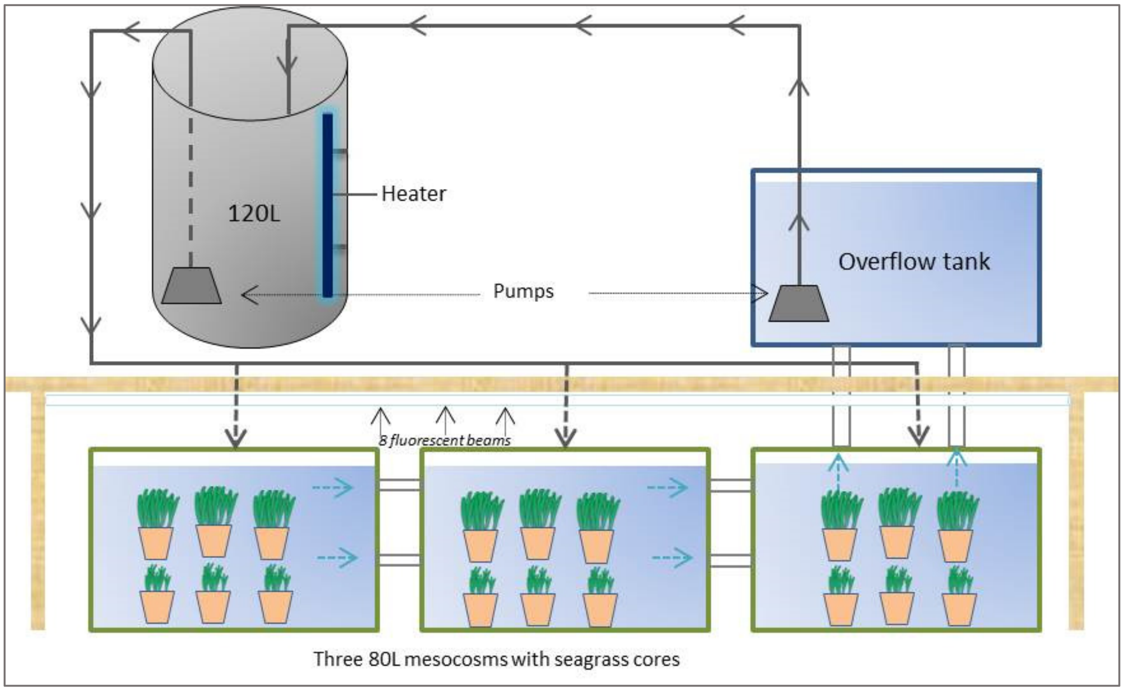


Figure 4.2: Infographic of the experimental setup of one of four treatments comprising a reservoir tank (120L) with aerated seawater heated to treatment temperature levels (18, 22, 26 and 30°C). Water is pumped through an equal release flow through system into three 80L tanks each containing six seagrass replicates– three small-leaved and three large-leaved morphologies.

4.2.2. Seagrass Collection

Thirty six intact seagrass cores (10 cm diameter x 15 cm depth) comprising shoots, roots/rhizomes and sediment were collected from large-leaved (Oesterval) and small-leaved (Geelbek) seagrass beds (72 in total) of similar densities during spring low tide (refer to map for site location in Chapter Two: Fig. 2.1). Each core was placed in a plant pot lined with a plastic bag and stored in a large bin during transportation to the laboratory. Upon arrival, all visible epiphytic fauna were removed and epiphytic algae gently scraped off all leaves with the back of a scalpel blade. Separating shoots and disentangling roots/rhizomes resulted in breakage, loss of leaves and overall damage to the plants. Instead, it was considered important to maintain intact above and belowground biomass, which implied that shoot numbers varied between cores and averaged 20±4.1 SE for LLM and 83 ±3.2 SE for SLM.

Ambient temperatures ranged from 19.2 to 21.9^{o}C at large-leaved sites and from 21.9 to 24.2^{o}C at sites with small-leaved morphotypes. Plants were therefore acclimatized at 20^{o}C which was considered a reasonable temperature for the two morphotypes to acclimate to while preventing thermal shock. Experiments were carried out during peak austral summer where water temperature ranges of 18 to 24^{o}C in a day were previously recorded at small-leaved sites, indicating these plants are able to tolerate a temperature drop of up to 4^{o}C. Also, 18-20^{o}C was used during eight and ten week experimental trials during summer, with a favourable response from the two morphotypes. Plants were placed in aerated seawater tanks and maintained for five days under saturating light conditions (~400 µmol photons m^{-2} s^{-1}) (Nejrup and Pedersen 2008). Thereafter, temperatures were adjusted by 1^{o}C per day (after Koch et al. 2007) until 18, 22, 26, and 30^{o}C treatment temperatures were reached.

4.2.3. Seagrass Response Variables

Pre-treatment measurements of shoot and leaf densities, leaf length and width and above and belowground biomass were taken for each morphology from three cores in each tank at the start of the experiment using methods described below.

Unfortunately, pre-treatment epiphyte biomass could not be obtained. On termination of the experiment, all cores were harvested, removed of sediment and the roots/rhizomes separated from shoots. Most of the leaves had detached from the shoots during this process, and the number of leaves per shoot could not be accurately estimated. Therefore, total number of shoots and leaves per core (0.0079m^2) were counted. Thereafter, the length and width of 10 intact leaves per core (90 leaves per treatment) were measured. Each leaf was then placed in a petri dish with seawater and algal epiphytes scraped off with the back of a scalpel until leaves were visibly clean. Petri dishes were then drained onto filter paper and dried to constant dry weight at 60^{o}C to obtain algal biomass per leaf area (after Fong et al. 2000, Carruthers et al. 2001). Seagrass leaves and roots were also dried at 60^{o}C to constant dry weight (~12 – 24 hours) to obtain above and belowground biomass.

4.2.4. Statistical Analyses

Data were visually assessed using histograms and q-q plots. Data were transformed (log x +1) where necessary if assumptions for normality (Shapiro-Wilk's test) and homogeneity of variance (Levene's Test) were not met. To test the effects of four temperature treatments on *Zostera capensis*, two-factor analysis of variance (ANOVA) mixed models with seagrass response variables as dependent variables, 'treatment' as a fixed factor and 'tank' as a random factor nested within 'treatment', were performed using SPSS (IBM SPSS Statistics 25). This was followed by *a posteriori* (Tukey HSD) tests to assess differences between treatments. Given the existing high variation between morphometrics in small and large-leaved morphotypes, as well as differences in their initial densities and biomass, results are reported to compare response variables pre and post treatment within each morphotype and not between them. Thereafter, overall responses to temperature are discussed. Results are presented as values per core area (i.e. 0.0079m^2) and are significant at $\alpha < 0.05$.

4.3. Results

4.3.1. Large-leaved morphotype

Average shoot densities of large-leaved morphotypes were higher in 26°C tanks pre-treatment (Fig. 4.4A) and did not differ significantly between treatments (Table 4.1). Pre-treatment densities remained similar after treatment in 22 and 26°C temperatures, but differed significantly from densities in 18 and 30°C treatments (Fig. 4.4A). An equivalent result was observed for leaf densities given their high correlation (Spearman $Rs = 0.89$, $p < 0.001$) with shoot densities, however treatment effects on leaf densities were significant ($F_{4,10} = 4.65$, $p = 0.02$). Because the number of leaves per shoot could not be accurately estimated, and due to the difference in significance of shoot and leaf densities in response to temperature treatments, the observed variation in leaf densities are assumed to be mainly the result of number of leaves per shoot. Post hoc tests revealed leaf densities between all treatments to differ significantly except between 22 and 26°C (Fig. 4.4B).

Leaf lengths increased in 18°C treatments from pre-treatment measurements, and remained similar pre and post treatment in 22°C temperatures, but declined in warmer treatments (26 and 30°C) (Fig. 4.4C), and revealed significant treatment effects ($F_{4,10} = 13.24$, $p = 0.001$). Variation in leaf lengths between pre, 18 and 22°C treatments were not significant, however leaf lengths in 26°C treatments were significantly different to pre-treatment lengths, as well as between 18 and 30°C treatments. Post hoc analyses also found leaf lengths to be similar in 22 and 26°C treatments, while 30°C produced the shortest leaves (Fig. 4.4C). This pattern was not reflected in leaf width where pre and post treatment measurements remained largely similar for all treatments except the warmest (30°C) (Fig. 4.4D). The effect of treatment on leaf width was not significant (Table 4.1). This result was explained mainly through variation observed in leaf width in the warmest treatment that differed significantly from pre- and 18°C treatment values, but remained similar in 22 and 26°C treatments (Fig. 4.4D).

Exposure to supra-optimal temperatures (30°C) caused extensive decline in aboveground biomass (AGB) (Fig. 4.4E) however treatment effects were not significant (Table 4.1). Post hoc analysis revealed AGB to differ significantly between 18°C and the other three treatments. Variation in AGB was similar at 22 and 26°C, while both differed significantly to AGB at 30°C (Fig. 4.4E). In contrast, treatment effects on belowground biomass (BGB) was significant ($F_{4,10} = 5.03$, $p = 0.02$), although pre-treatment levels of BGB did not differ significantly after treatment in 18 and 26°C (Fig. 4.4F). Post hoc tests revealed significant variation in BGB at 30°C compared to the other temperature treatments, along with a severe decline in BGB post warming (Fig. 4.4F).

Average biomass of epiphytic macroalgae per leaf area was highest at 18°C and declined with warming (Fig. 4.4G). Despite significant differences between 18°C and the other three treatments (Fig. 4.4G), the main effect of temperature was not significant on variation in epiphyte biomass (Table 4.1).

Tank effects were significant for all seagrass metrics measured ($p < 0.05$; Table 4.1).

4.3.2. Small-leaved morphotype

Shoot densities of small-leaved morphotypes (SLM) showed a similar response to temperature as that of large-leaved morphotypes. Initial average shoot densities were generally higher compared to post-treatment densities (Fig. 4.4A), although differences between treatments were not significant (Table 4.1). Pre-treatment shoot densities in 18, 22 and 26°C were equivalent to post treatment measurements, and differed significantly from densities in 30°C treatments (Fig. 4.4A). On the other hand, treatment effects were significant on leaf densities ($F_{4,10} = 7.93$, $p < 0.01$). As with LLM, variation in leaf densities in SLM is assumed to be mainly as a result of number of leaves per shoot though exact estimates of this cannot be provided. Pre-treatment leaf densities showed an overall decline post-treatment varying significantly between all treatments except 22 and 26°C (Fig. 4.4B).

Leaf lengths in SLMs increased in 18 and 26°C treatments from pre-treatment lengths but were similar in 22°C post treatment, while the warmest treatment (30°C) produced shorter leaves (Fig. 4.4C). Leaf lengths showed significant treatment effects ($F_{4,10} = 11.68$, $p = 0.001$). This was due to variation in pre and post-treatment leaf lengths in 26 and 30^{o}C, as well as between lengths at 18^{o}C and the two warmer treatments (Fig. 4.4C). Pre and post treatment measurements in leaf width remained largely the same in all treatments except 30^{o}C, and increased in 18^{o}C compared to pre-treatment levels (Fig. 4.4D), producing significant treatment effects ($F_{4,10} = 3.23$, $p = 0.05$). This was mainly explained by variation in leaf width between 30^{o}C and the other three treatments (Fig. 4.4D).

Aboveground biomass (AGB) of SLM increased at 22^{o}C compared to pre-treatment estimates and remained similar at 18 and 26^{o}C, but declined significantly in supra-optimal temperatures (Fig. 4.4E). 'Treatment' did not produce a significant effect (Table 4.1), and post hoc analysis revealed no significant variation in AGB pre and post-treatment in all treatments except 30^{o}C. However, variation between 18^{o}C and the other three treatments was significant (Fig. 4.4E). Similar variations were observed in AGB in 22 and 26^{o}C treatments. Belowground biomass (BGB) increased at 26^{o}C compared to pre-treatment measurements, but remained largely equivalent in all treatments except the warmest which produced the lowest BGB (Fig. 4.4F). Post hoc tests showed significant variation in BGB in pre and post 22 and 30^{o}C treatments, while significant differences were observed between 18 and 22^{o}C, as well as 22 and 26^{o}C. All treatments differed significantly to the 30^{o}C treatment (Fig. 4.4F).

Leaf scrapings produced no algae from SLM leaves in 18, 26 and 30^{o}C treatments while 0.0003 g dry weight mm^{-2} of algae was collected from leaves at 22^{o}C (Fig. 4.4G). Given the lack of pre-treatment measurements, variation in epiphytic algal biomass pre and post treatment cannot be determined, however a general deficiency in epiphytes on SLM leaves was observed during seasonal field measurements (Chapter Two). Epiphyte biomass in LLM was highest at 18^{o}C and declined substantially in warmer treatments with the lowest biomass recorded at 30^{o}C (Fig. 4.4G). Post hoc analyses showed epiphyte biomass to be similar in 22, 26 and 30^{o}C treatments but differ significantly from biomass at 18^{o}C (Fig. 4.4G).

All SLM seagrass metrics showed significant experimental tank effects ($p < 0.05$; Table 4.1).

Table 4.1: ANOVA results on the effect of treatment (fixed factor: four temperature levels) and tank (random factor nested within treatment) on shoot and leaf densities, leaf length and width, above (AG) and below ground (BG) biomass and epiphytic algal biomass of two morphotypes (large and small-leaved) of *Zostera capensis* seagrass. Effect size of each variable on the observed variation is represented by partial Eta^2 values. Significant effects are based on p =/< 0.05 and in bold.

Variable	**Factor**	Large-leaved			Small-leaved		
		$F_{4,10}$	***P***	**Partial Eta^2**	**$F_{4,10}$**	***P***	**Partial Eta^2**
Shoot density	Treatment	2.10	0.16	46%	0.73	0.59	23%
	Tank(Treatment)	158.76	**0.00**	78%	47.29	**0.00**	53%
Leaf density	Treatment	4.65	**0.02**	65%	7.93	**0.00**	76%
	Tank(Treatment)	135.88	**0.00**	76%	27.62	**0.00**	39%
Leaf length	Treatment	13.24	**0.00**	84%	11.68	**0.00**	82%
	Tank(Treatment)	3.84	**0.00**	8%	12.29	**0.00**	22%
Leaf width	Treatment	2.29	0.13	48%	3.43	**0.05**	58%
	Tank(Treatment)	2.24	**0.02**	5%	26.79	**0.00**	39%
AG biomass	Treatment	1.06	0.42	30%	1.19	0.37	32%
	Tank(Treatment)	34.64	**0.00**	44%	30.88	**0.00**	42%
BG biomass	Treatment	5.03	**0.02**	67%	4.69	**0.02**	65%
	Tank(Treatment)	5.35	**0.00**	11%	4.16	**0.00**	9%
Epi-algal biomass	Treatment	1.22	0.36	31%	2.98	0.10	53%
	Tank(Treatment)	13.07	**0.00**	23%	8.45	**0.00**	17%

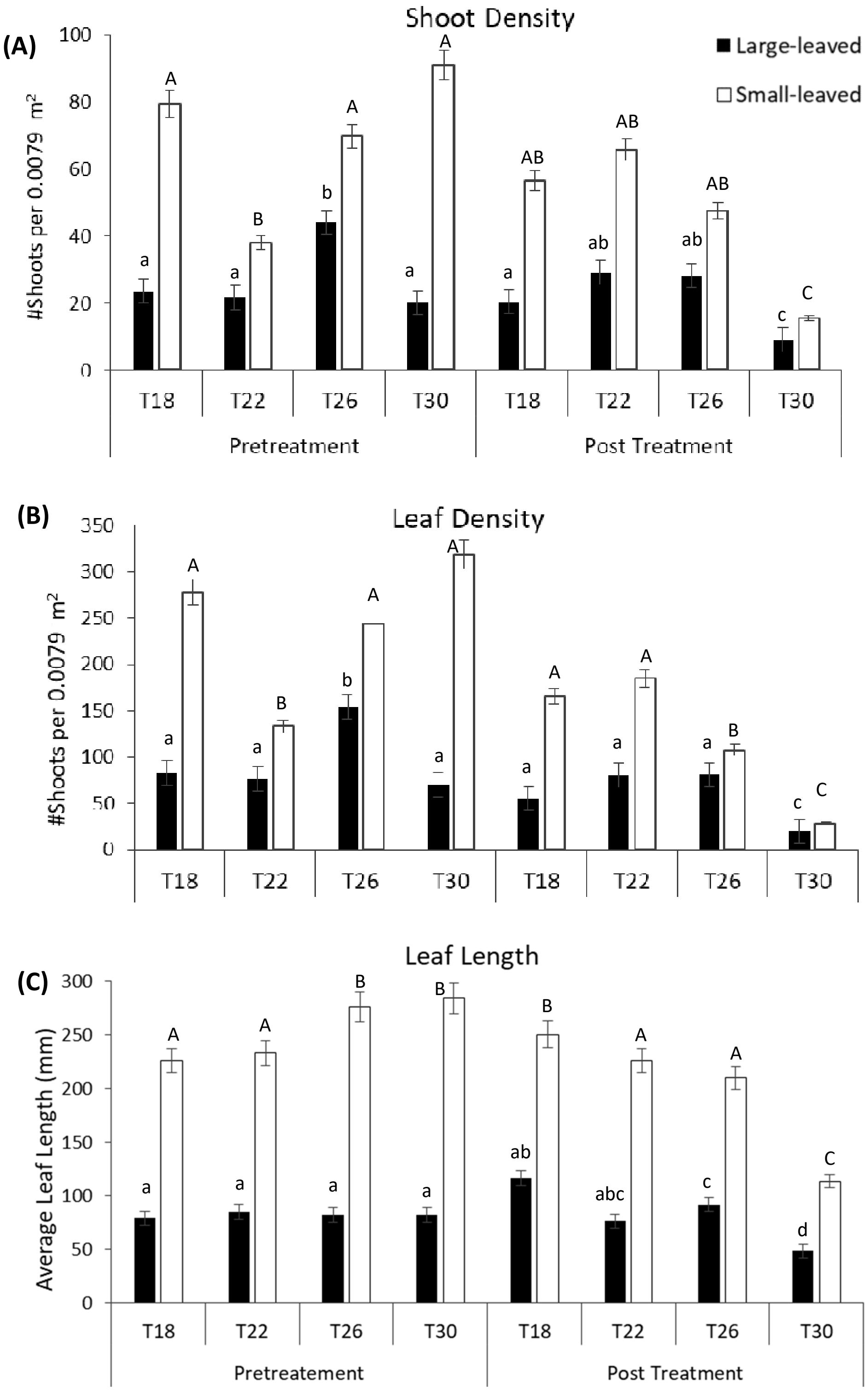


Figure 4.4: Variation in shoot (A) and leaf densities (B), leaf length (C), leaf width (D), aboveground (E) and belowground biomass (F), and epiphytic algal biomass (G) of small-leaved (white bars) and large-leaved (black bars) morphotypes of *Zostera capensis* pre and post treatment in 18, 22, 26 and 30°C temperatures. Means ±1SE are presented. Letters represent homogenous means not significantly different (p >0.05) when compared in a Tukey HSD test.

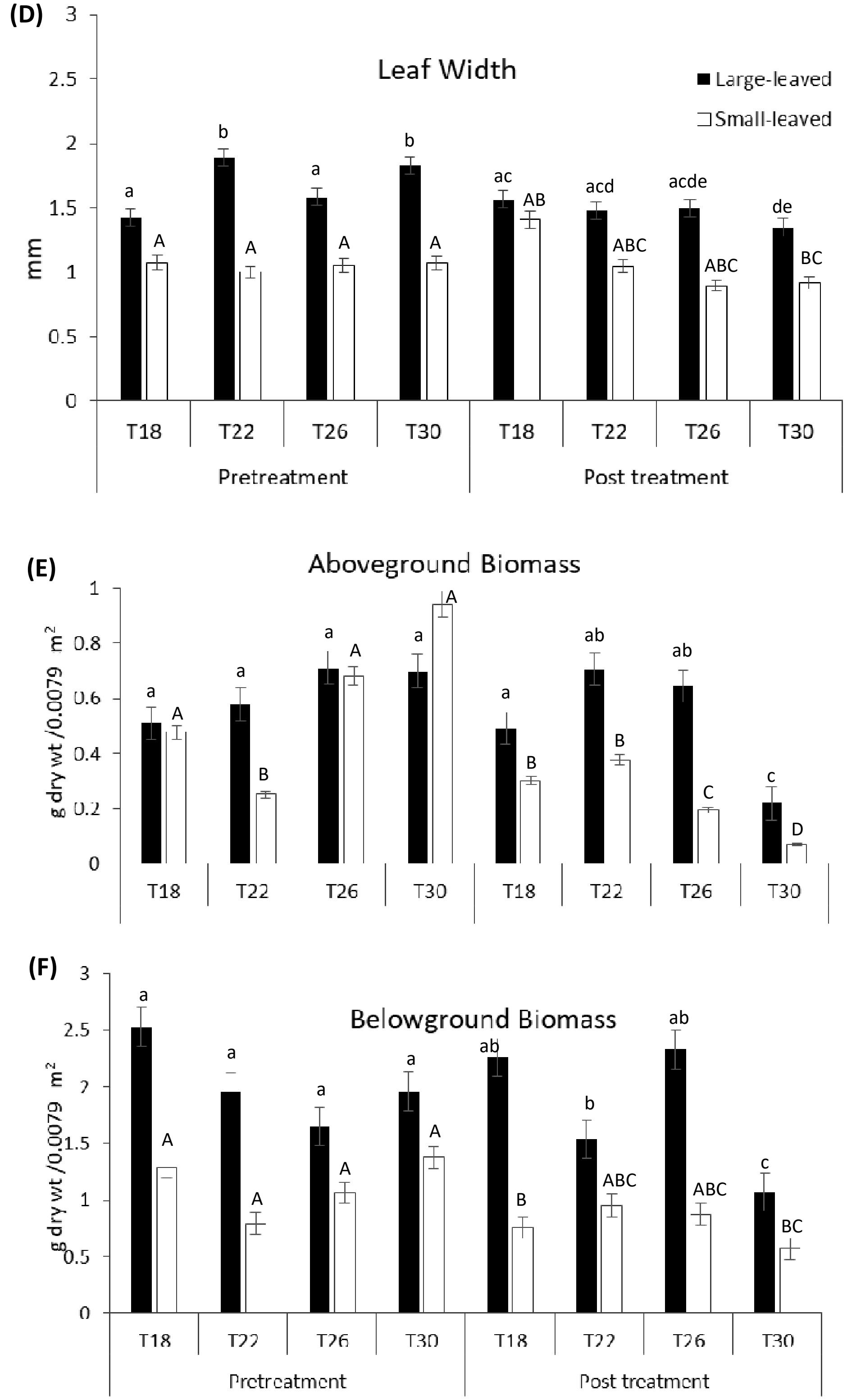


Fig. 4.4. Continued…

(G)

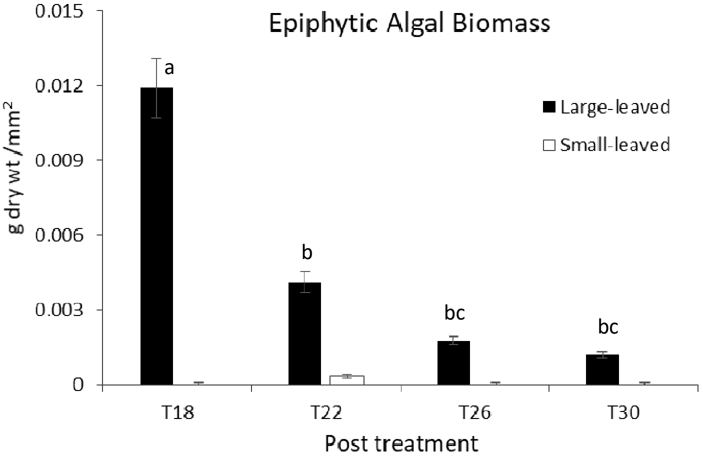


Fig. 4.4 continued…

4.4 Discussion

The two morphologically divergent populations of *Zostera capensis* displayed similar responses following prolonged exposure to four temperature treatments (18, 22, 26 and 30^{o}C), indicating a propensity for acclimating to local conditions. There was a general increase in density, leaf size and biomass in the two morphotypes at 22^{o}C and a decline in these variables in warmer temperatures. The first hypothesis that the small-leaved morphotype (SLM) which tolerates an average summer temperature of 28.5^{o}C, will display an increase in seagrass metrics in warmer (26 and 30^{o}C) temperatures, can be rejected since shoot/leaf densities and leaf size were highest at 18^{o}C and 22^{o}C and declined under further warming. Secondly, the prediction that seagrass response variables of the large-leaved morphotype (LLM) will remain largely unchanged or increase at 22^{o}C and decline at 26^{o}C can be accepted for all variables except leaf width and belowground biomass (BGB). In LLM treatments leaf width had declined at 22^{o}C compared to pre-treatments levels, while BGB was highest at 26^{o}C. Lastly, higher temperatures did not favour epiphytic algal growth as hypothesized, since algae declined with increasing temperature in LLM and were only recorded in small amounts at 22^{o}C in SLM.

4.4.1. Morphological Responses to Temperature

The observed increase in seagrass response variables in warmer temperatures (22 and 26^{o}C) can be attributed to a response to elevated levels of photosynthesis and respiration shown to encourage seagrass growth when temperatures are close to optimum levels (Zimmerman et al. 1989, Campbell et al. 2006). However, uptake of nitrogen and carbon as well as protein synthesis and partitioning of resources may occur at different temperature optima, thus limiting growth (Leegood 1995) which explains the observed response in supra-optimal (30^{o}C) temperature treatments. The duration of exposure to light and temperature has been linked to seasonal changes in seagrass biomass particularly in sublittoral species (Chapter Two, Erftemeijer and Herman 1994). Apart from these environmental factors, seagrasses growing intertidally are exposed to distinctive stresses from extreme high and low temperatures which alternate between water (submerged) and air (emerged) (Yabe et al. 1995). Furthermore, elevated water temperatures from for example heat waves and El Niño events have been shown to trigger an acute stress response in seagrasses, with subsequent reductions in biomass (Seddon and Cheshire 2001, York et al. 2013). The lengthening of leaves in SLM treatments could be the result of a reallocation of energy towards leaf growth in the absence of desiccation stress which has been linked to short, narrow leaf morphologies (Shafer et al. 2007). For intertidal seagrasses to survive in their fluctuating environments a degree of flexibility in their development is essential, and morphological acclimation along with biomass re-distribution are often an efficient means of achieving this.

Similar responses to suboptimal temperatures have been reported in other temperate systems. For example, *Zostera marina*, in Odense Fjord, Denmark, exhibited lower photosynthetic rates and growth in supra-optimal temperatures (25-30^{o}C), which also saw greater seagrass mortality, while growth and photosynthesis were reduced at low temperatures (5^{o}C) without resulting in plant deaths (Nejrup and Pedersen 2008). Temperature optima for *Z. marina* growth and photosynthesis appeared to be between 10-20^{o}C. In the same way, Höffle et al. (2011) recorded a significant effect of temperature on *Z. marina* shoot survival and aboveground growth in treatment temperatures of 21 and 27^{o}C and recorded an average 68% mortality in the higher compared to lower temperature treatments. In Korea, *Z. marina* growth was inhibited above 20^{o}C, and optimal temperatures for growth was recorded between 15-20^{o}C (2005). In South Africa, Edgcumbe (1980) cultivated *Z. capensis* under laboratory conditions and observed high vegetative and reproductive growth in the range of 15-20°C, and decreases in growth rates at >26°C. Highest biomass and leaf length of *Z. capensis* were recorded at cooler temperatures (average 15-18°C) (Chapter Two) in Langebaan Lagoon, which supports both Setchell's (1943) and Edgcumbe's (1979) claims that optimum temperatures for *Z. capensis* growth is between 15-20°C.

The negative effect of temperature on density, leaf size and biomass of *Zostera capensis* at 26°C was an unexpected outcome since warming has been shown to increase photosynthesis and subsequent plant growth (Campbell et al. 2006, Lee et al. 2007, Olsen et al. 2018). A positive response to warmer (i.e. 26°C) temperatures was therefore expected particularly for SLMs. The observed response suggests the temperature threshold for photosynthesis in *Zostera capensis* had been exceeded, and along with other factors for example nutrient supply, could have led to the observed decrease in plant performance and productivity (Leegood 1995, Ralph 1998a). When temperature thresholds are exceeded, respiration increases relative to photosynthesis thus reducing the ratio of photosynthesis to respiration, and as a consequence growth is limited, observed especially at the peak of summer (Marsh et al. 1986, Leegood 1995, Lee et al. 2005).

An increase in belowground biomass (BGB) at 26°C in LLM treatments compared to SLM was also unexpected given that SLMs have been reported to be better adapted to tolerate warm temperature (McMillan 1984, Pérez-Lloréns and Niell 1993). The low aboveground biomass (AGB) but higher BGB recorded in LLM at 26°C treatments implies a negative carbon balance and possible limitations on carbon uptake, further contributing to reduced growth rates (Marsh et al. 1986). Modification of internal carbon levels along with significant reductions in shoot size has been suggested as a morphological response to heat stress (Touchette et al. 2003). Seagrass growth is therefore severely reduced at upper thermal limits, particularly in temperate species, since elevated temperature directly affects growth and productivity (Hoeffle et al. 2011, Niu et al. 2012, Collier and Waycott 2014), which explains the high biomass and leaf size at 22°C and the substantial loss of shoots at 30°C. *Zostera* populations at Langebaan can therefore be expected to decline considerably when exposed to prolonged temperatures greater than 22°C.

4.4.2. Responses in Epiphytic Algal Biomass

Environmental variables accounted for 29% of variation in epiphyte biomass in field observations, with temperature emerging as a significant predictor of algal epiphyte biomass (Chapter Two). However, other factors were likely responsible for the unexplained variation like nutrient availability and grazing (Prado et al. 2007, Jaschinski and Sommer 2011) especially given the high grazer abundances in SLM at Geelbek where no algae were recorded (Chapter Three). Despite experimental conditions being controlled for this factor since grazers were removed before the experiment commenced, and algae scraped off leaves from the two morphotypes, algal growth on SLM leaves were negligible while the higher biomass recorded in LLM post treatment implied adequate conditions for algal growth. The low abundance of algae in SLM was therefore unexpected and implies an influence of other factors not accounted for in this study.

Van Montfrans et al. (1984) proposed several factors responsible for controlling species composition and productivity of epiphytic algae. These included chemical interactions between epiphytes and seagrass leaves along with host-specific relationships, leaf morphology, age, growth rate and availability of algal propagules (Orth and van Montfrans 1984, van Montfrans et al. 1984). Any of these factors individually or combined could be accountable for the lack of algal growth on SLM. Another likely factor is the influence of compounds such as condensed tannins and phenolic acids used in defence to enhance leaf toxicity to grazing (Pohnert 2004, Lane and Kubanek 2008, Ross et al. 2008), and which may in turn inhibit settlement of algae directly or indirectly. This assumption is explored further in Chapter Five.

Hasegawa (2007) noted that biomass of primary producers switched from that of eelgrass in spring and early summer to epiphytic algae in late summer and autumn in Akkeshi-ko Estuary, Japan. There, changes in epiphytic algal biomass on leaves of *Zostera marina* were attributed to the dominant diatoms changing from highly adhesive to filamentous species, or those with less adhesiveness, a likely consequence of grazing. In my study, algal epiphytes were not individually identified to levels of either species or functional groups, and post treatment comprised mainly of green algae (Chlorophyta), which is usually associated with eutrophic or elevated nutrient conditions (Lapointe et al. 2004, Borowitzka et al. 2006). Some filamentous algae were observed on seagrass leaves before they were cleaned in preparation for the experiment. Competition for nutrients and light between seagrass and epiphytic algae may have also contributed to the observed result in algal biomass in my study, since the heightened photosynthetic rates in seagrass in warmer temperatures require greater nutrients and light (Hasegawa et al. 2007, Ruesink 2016).

4.4.3. Artefacts of Mesocosm Experiments

Mesocosm experimental studies have been successfully used across a wide range of applications and systems (Grantham et al. 2012), most especially in providing an understanding of responses to isolated test factors while controlling for effects of other variables (Short 1987). Despite their usefulness in providing key information, mesocosms are prone to design artefacts that must be considered when extrapolating results to real world scenarios (Graney et al. 1989). One common artefact in enclosed experimental structures is the prevalence of fouling or wall growth (Chen et al. 1997), which can be controlled by frequent cleaning of mesocosm walls (e.g. Brussaard et al. 2005). Given the mesocosm used in this study was a simplified representation of a natural lagoon environment, several mitigation measures were put in place to improve the integrity and design of the model system. For example, fluorescent light beams were constructed directly above the mesocosms to provide equal levels of light to all tanks. Furthermore, an equal release flow-through system facilitated homogenous mixing and flow rates across tanks. Tank walls were cleaned once a week to reduce excess fouling and potential light reduction, along with competition for nutrients with seagrasses and their epiphytes (Valentine et al. 2006). Additionally, water was exchanged and seagrass pots repositioned weekly to further reduce tank artefacts. Despite clear responses of seagrasses to experimental temperature treatments, and the measures applied to reduce experimental artefacts, tank effects were still observed - the exact reasons for which is largely unknown. Nonetheless, this study provides an approximation of the effect of a single environmental factor (i.e. temperature) at the population level, and can be considered a likely response in natural conditions, while allowing for methodological limitations.

4.5. Conclusions

Both large-leaved and small-leaved morphotypes of *Zostera capensis* thrived in cooler temperatures (22^{o}C) displaying appropriate responses to optimum temperatures for growth and productivity. However, a severe decline in *Z. capensis* samples was observed after prolonged exposure to high (26-30^{o}C) temperatures, consistent with observed seasonal trends (Chapter Two). In addition, LLM had significantly higher biomass of epiphytic algae compared to SLM. Fouling from epiphytic algae hampers growth and could further stress LLM under supra-optimal temperature conditions. Future global warming predicts a rise in seawater temperature which could result in the migration of LLMs to even lower intertidal zones than which they presently occur at Langebaan Lagoon, provided environmental conditions are conducive. This would result in longer leaves and sparser meadows, as seagrasses adapt to lower light levels from longer submergence, with likely increases in epiphyte loads. The high diversity of grazing macro-epifauna associated with large-leaved populations (Chapter Three) might offset the increase in algal fouling. This hypothesis will be tested in the following chapter.

An alternate prediction is the transformation of LLM populations to SLM by developing shorter shoots and leaves (leaf lengths of LLM had reduced in warmer treatments) to cope with thermal stress. Algal settlement on SLM was almost non-existent, and implies potentially lower primary productivity for grazing invertebrates in these populations, thereby supporting a lower faunal diversity. The predicted rise in global air temperatures could ultimately drive SLM populations living in upper thermal limits, to extinction. High thermal stress has been shown to enhance seagrass vulnerability to disease and fouling and could result in further susceptibility to decline and loss.

Thermal stress symptoms as observed for *Zostera capensis* subjected to supra-optimal temperatures, implies a tolerance threshold and vulnerability to ecophysiological impairment when seawater temperatures reach atypically high levels. Despite having a broad thermal range that extends from temperate to tropical waters, low tolerance to supra-optimal temperatures implies a narrow tolerance range and detrimental effects on photosynthesis, growth and productivity should episodic increases in temperature exceed 26^{o}C. The ability of *Z. capensis* to inhabit a wide latitudinal range and persist in both tropical and temperate environments attests to its ability to adapt to local environmental conditions.

Research into additional physiological strategies in *Zostera capensis* to acclimate and cope with stress from warming is important. Mitigation measures to prevent further loss of *Z. capensis* in Langebaan should include reducing disturbance, improving water quality in the adjacent bay, and possibly transplanting seagrasses into other areas of the lagoon. Such measures have been shown to lower stress and build resilience in other seagrass ecosystems and may provide a similar benefit to seagrass populations in Langebaan Lagoon.

CHAPTER FIVE

Top-Down Effects of Grazing on the Ecophysiology of Two Morphotypes of *Zostera Capensis* under Warming - A Mesocosm Experiment

5.1. Introduction

5.1.1 Effects of Temperature on Seagrasses

Global mean surface temperature of the Earth has risen by 0.6-0.8°C since 1880 (Griggs and Noguer 2002), and by 2100 the earth's atmosphere is predicted to warm by a further 3-4°C along with subsequent warming of sea surface temperatures (Domingues et al. 2008, Cheng et al. 2017, Durack et al. 2018). Temperature affects photosynthesis and respiration in seagrasses (Dunton 1994, Larkum et al. 2006, Lee et al. 2007), by controlling the balance of carbon fixed and consumed, while the photosynthetic apparatus and pathways remain highly sensitive to temperature (Leegood 1995, Ralph 1998a, Niu et al. 2012). Rapid reduction in photosynthesis has therefore been observed when temperatures exceed optimum thresholds (Pérez and Romero 1992, Leegood 1995, Ralph 1998a) affecting plant performance and productivity. While direct influences of temperature on growth and metabolism of seagrasses is relatively well known (Campbell et al. 2006), field and experimental studies have provided greater clarity on thermal thresholds and tolerance mechanisms for some algal and seagrass species (reviewed by Koch et al. 2013 for tropical and subtropical seagrasses), but remain unexplored in other seagrass species.

Variations in seagrass morphology, both genetic and/or environmentally induced, include acclimation to cope with supra-optimal temperatures (Peralta et al. 2000, Pilon and Santamaría 2002, Ehlers et al. 2008). Genetic variability within populations in response to thermal stress may be a key factor in a population's ability to adapt to climate change (Ehlers et al. 2008, Reusch 2014). The experimental influence of temperature on morphology and macronutrient content (carbon, nitrogen and phosphorus) of *Zostera marina*, documented significant reductions in shoot size and modification of internal carbon and nitrogen levels, and suggested a morphological response to heat stress (Touchette et al. 2003). McMillan (1984) established a tolerance (measured as survival after treatment) to supra-optimal temperatures in several tropical seagrasses growing in shallow intertidal sites. Narrow-leaved plants of the genus *Halodule* were found to be more resistant to heat stress by possessing more green leaf material compared to wide-leaved plants (McMillan 1984). Narrow-leaved morphotypes of *Z. noltei* have been shown to tolerate higher temperatures by reducing CO_2 compensation points and having higher photosynthetic rates relative to their large-leaved counterparts (Pérez-Lloréns and Niell 1993).

Morphological adjustments to cope with stress such as reduced plant size and narrow/small leaves, infer a cost to growth (Holopainen and Gershenzon 2010, Lattanzio et al. 2012, Neilson et al. 2013), while the allocation of resources to acclimate to heat stress implies a constraint on plant fitness (Bekaert et al. 2012, Neilson et al. 2013). Morphological acclimation in *Zostera capensis* in response to environmental conditions have been described in Chapter Two. What remains to be explored is whether different morphotypes have also developed different rates of photosynthesis, nutrient and secondary metabolite configurations to cope with heat stress. Small-leaved populations may attain different quantities of nitrogen, carbon and secondary metabolites to cope with environmental stress, since resources are channeled towards defense rather than growth. Large-leaved populations on the other hand, which invest in growth might be less defended and therefore more susceptible to diseases and fouling. Understanding a species' physiological and chemical response to warming and the consequences thereof on their trophic associations is important for our ability to understand and mitigate the effects of climate change.

5.1.2. Seagrass Response to Epiphytes and Fouling

Studies on the bioactivity of seagrass extracts and metabolites have mainly investigated their toxicity to herbivory such as variations in response to grazing by sea urchins and fish (Vergés et al. 2007a, 2007b), as well as microbial settlement and attack (Steele et al. 2005, Lane and Kubanek 2008, Trevathan-Tackett et al. 2015). Interesting findings have emerged from studies on the antifouling potential of zosteric acid, a phenolic acid found in the genus *Zostera* (Todd et al. 1993), while other research has focused on compound variations, especially those influenced by season and/or the environment (Vergeer et al. 1995). Secondary metabolite production can increase under warming attributed to the higher rates of biosynthesis in elevated temperatures (Holopainen and Gershenzon 2010). In some cases, increases in secondary metabolites are a response to thermal stress, likely due to their anti-oxidative function, and ability to scavenge reactive oxygen species. Elevated levels of secondary metabolites have been recorded in land plants exposed to heat stress, however, a study on *Zostera marina* did not reveal a similar trend. Instead, concentrations of phenolic compounds were reduced with increasing temperature (Vergeer et al. 1995).

Phenolic compounds produced in response to thermal stress could hinder settlement of epiphytes thereby limiting the protection epiphytes might provide such as shading. The likelihood of these functions acting out concurrently and how they influence seagrass growth has remained unexplored. In this chapter I investigate the physiological and chemical response to warming in two phenotypically divergent populations of *Zostera capensis* with the aim of understanding intrinsic abilities that might explain acclimation strategies to cope with heat stress. I further examine the role of grazing in controlling fouling epiphytes and its subsequent effects on *Z. capensis*.

5.1.3. Influence of Grazing in Seagrass Habitats

Growing evidence implicates species interactions as a key determinant of a community's response to climate change, since the effect on one trophic level can trigger effects on other levels (Harley 2011, Zarnetske et al. 2012). The size of this top-down effect may be influenced by warming temperatures as a consequence of altering herbivore and plant distribution and abundance (Short et al. 2016), primary productivity (Harley et al. 2012, Collier and Waycott 2014) and consumption rates (O'Connor 2009). Characteristics that influence a plant's susceptibility to herbivory for example secondary metabolite production (Gong and Zhang 2014) and internal nutrient configurations (i.e. C:N ratios) (Touchette et al. 2003) may also be affected by warming. The extent to which temperature influences interactions between plants and herbivores implies an indirect effect of temperature on multiple species, which in turn may distort the direct negative impacts of temperature (O'Connor 2009), and further confound single species predictions of temperature effects (Connell et al. 2011).

Primary production from seagrasses and algae is strongly regulated by herbivory in coastal habitats (Bakker et al. 2016a, 2016b). Macroalgae provide a vital food source to a large proportion of grazing herbivores (mainly amphipods, gastropods, isopods and decapods) associated with seagrasses, many of which feed heavily on epiphytic algae (Orth et al. 1984, Jernakoff et al. 1996, Smit et al. 2005, Lebreton et al. 2011). These grazers in turn are food for predators, highlighting key top-down processes that regulate dynamics in coastal food webs (Heck et al. 2000). Most mesocosm experiments show warming to generate stronger top-down control of primary production due mostly to consumer respiration rates being faster than photosynthetic rates (O' Connor 2009, Kordas et al. 2011, Gilbert et al. 2016).

Greater effort is being placed on understanding the effects of eutrophication and the cascading effects of the removal of top predators (mainly fish) on seagrass habitats, as the threat from coastal nutrient enrichment increases (Baggett et al. 2010, Eriksson et al. 2011, Short et al. 2011, 2016). In many cases, grazing counteracts the negative effects of eutrophication by controlling algal fouling, with a subsequent positive response in seagrass performance, as described in a meta-analysis of 35 studies (Hughes et al. 2004). Sieben et al. (2011) investigated the effects of nutrient enhancement and the exclusion of predatory fish in a caging experiment in the Baltic Sea. They reported that even though meso-predatory fish abundances had increased, total herbivore abundance was not altered, but rather shifted in dominance to gastropods, while amphipod abundances were reduced by up to 60%. Under nutrient enrichment, this shift in community composition did not reduce algal biomass which had increased (Sieben et al. 2011). Similarly, in Florida Bay, USA epiphyte loads in *Thalassia testudinum* beds increased under low levels of added nutrients, while supplements of up to 60% of grazers did not alter productivity of algae or seagrass (Peterson et al. 2007). These studies imply that additions in nutrient levels may not increase palatable epiphytes, but rather enhance epiphytic species (e.g. coralline algae) that are more resistant to grazing (Peterson et al. 2007), while grazer communities in some habitats might experience changes that don't affect algal biomass (Sieben et al. 2011). Observations of grazer, seagrass and algal interactions under increasing temperatures appears to be species specific (Garthwin et al. 2014), and need to be explored in specific seagrass populations.

Another experimental investigation of the effects of grazing under warming and ambient scenarios showed grazing did not control warming-induced algal growth, since grazers (i.e. *Littorina littorea*, *Gammarus locusta* and *Rissoa* sp.) fed mostly on more palatable, out-competing *Ulva* spp. allowing less palatable red and brown algal taxa to proliferate (Eklöf et al. 2012). Transformation of the epiphyte community to a combination of epiphytes and drift algal mats, which could not be controlled by grazers and further impacted by predation on the key grazer most effective in controlling algae, resulted in a shift of dominance from a seagrass (*Zostera marina*) to an algal system (Eklöf et al. 2012). A similar mesocosm experiment investigated the combined effects of warming and acidification and found no direct effect of CO_2 enrichment, but strong and positive combined effects of warming and acidification on microalgae in the absence of grazers (Alsterberg et al. 2013). Here, top-down control by mesograzers was shown as a key factor in negating direct and indirect effects of acidification and warming, which also highlighted the direct influence of climate change being as significant as indirect effects (Alsterberg et al. 2013).

Strong top-down control was also supported in an *in situ* warming experiment that demonstrated the negative effects of the exclusion of a predatory fish on algal biomass strengthened by warming (Svensson et al. 2017). In that study, algal growth was favoured via a different mechanism whereby smaller invertivorous fish abundance had reduced because of their physiological intolerance to the heated temperatures, thus driving larger fish to target grazing invertebrates. Here, warming appeared to have restructured a coastal food web from four trophic levels to three, and not by direct changes in herbivore abundance but due to a predator-driven alteration of herbivore behaviour (Svensson et al. 2017), and demonstrates a climate-driven effect on trophic interactions that can alter seagrass systems as we know it.

Competitive algal epiphytes can increase under elevated temperatures (Werner and Matthiessen 2017), and their overgrowth is likely to hamper seagrass growth and productivity (Fong et al. 2000, Hays 2005). Control of algae by grazers or through inhibition of settlement by seagrasses, therefore serves a key role in reducing fouling and alleviates competitive stress on seagrasses. This could in turn increase seagrass resilience and the ability of seagrass ecosystems to resist stress and recover from degradation or loss (Unsworth et al. 2015). Reducing stress from fouling may allow a seagrass meadow to withstand further stress since photosynthesis, respiration and overall plant fitness can still be maintained (Baden et al. 2012).

In Langebaan Lagoon, west coast of South Africa, *Zostera capensis* exhibits a small-leaved (SLM) and large-leaved (LLM) morphology which corresponds with its position on the shore. The LLM occurs in lower intertidal and subtidal areas, while the SLM forms dense meadows in the high intertidal (Chapter Two). In high intertidal seagrass populations narrow leaves and shorter shoots appear to be an acclimation to minimise water loss (Shafer et al. 2007), while high shoot densities are suggested to induce self-shading and cooling, and protect plants from excess UVB (Chapter Two, Björk et al. 1999, Huong et al. 2003, Boese et al. 2005;). Larger leaves are better able to reach and absorb sunlight and are an acclimation strategy of submerged populations subjected to lower light levels (Tanaka and Nakaoka 2004, York et al. 2013). Morphologically distinct populations of *Z. capensis* support a unique community of associated macro-epifauna in Langebaan Lagoon (Chapter Three), which highlights the need to investigate local scale variability in seagrass populations.

In this chapter, I investigate the physiological, chemical and nutrient responses in two morphotypes (or leaf sizes) of *Zostera capensis* to simultaneous warming and grazing, in a mesocosm experiment. The grazing function is provided by a gastropod (limpet) that does not appear to directly feed on seagrasses but on periphyton, micro and macroalgae growing on seagrass leaves. No direct observations on *S. compressa* feeding have been previously documented however, *Zostera* leaves especially from Geelbek had the highest limpet densities (Chapter Three) and the least biomass of epiphytic algae (Chapter Two) assumed to have been grazed down by *S. compressa*. In addition, this limpet lives exclusively on *Z. capensis* leaves and was found in both large- and small-leaved populations, and is therefore considered most suitable to fulfil the grazing function in this study. The extent to which warming and grazing influence seagrass fitness are also explored in order to understand their implication for shaping morphology under heat stress. Fitness cost is determined by statistically assessing photosynthetic rate, seagrass biomass, nutrient (carbon and nitrogen) content, and secondary metabolite (phenolics) abundance of small-leaved and large-leaved morphotypes under high (30^{o}C) temperature conditions. I further investigate the extent to which grazing explains variation in biomass of epiphytic algae, and hypothesize that the small-leaved morphotype produces greater quantities of phenolic compounds than the large-leaved morphotype at higher temperatures, making the former better able to tolerate heat stress. Lastly, I hypothesise that nutrient levels will be largely driven by temperature and this will differ between leaf sizes. The following key questions are investigated:

1. To what extent do temperature and/or grazing influence photosynthetic rate, morphometrics and epiphytic algae in small and large-leaved morphotypes of *Zostera capensis*? Is there an apparent effect of grazing in mitigating heat stress in *Z. capensis*?
2. How do temperature and/or grazing affect the uptake and storage of nutrients and production of phenolics in *Z. capensis*? Is there a relationship between seagrass leaf phenolics, carbon and nitrogen, and does this differ under different temperature treatments for each morphotype?

5.2. Methods

5.2.1. Experimental Setup

The mesocosm described in Chapter Four was used in this experiment with a few modifications. Increased water volume was achieved by replacing the 80L overflow tank with a 120L tank. Also, instead of four temperature treatments (18, 22, 26 and 30^{o}C) used previously, two temperatures were used here. In the previous chapter, growth parameters in *Zostera capensis* were highest at 22 and 26^{o}C for the two morphotypes, therefore an average temperature of 24°C (unheated) was used as the lower temperature while 30°C (heated) was used as the higher temperature in this experiment.

For each temperature treatment eight seagrass cores (experimental units), each in its own pot, representing four each of small-leaved (SLM) and large-leaved (LLM) morphotypes (also referred to as leaf size), with grazers present (GP) and grazers absent (GA), were contained within one 80L tank. This was replicated for a total of three 80L tanks connected through an equal release flow through system. Aerated seawater was re-circulated by pumping (AquaH$_2$O APH-3000) treatment water from one 120L reservoir tank across the three 80L tanks into a second reservoir tank (Fig 5.1). Water was heated to treatment levels using two 300W aquarium heaters. Two mesocosm systems were created for each temperature treatment (24 and 30°C), containing a total of 48 leaf size/grazer experimental units.

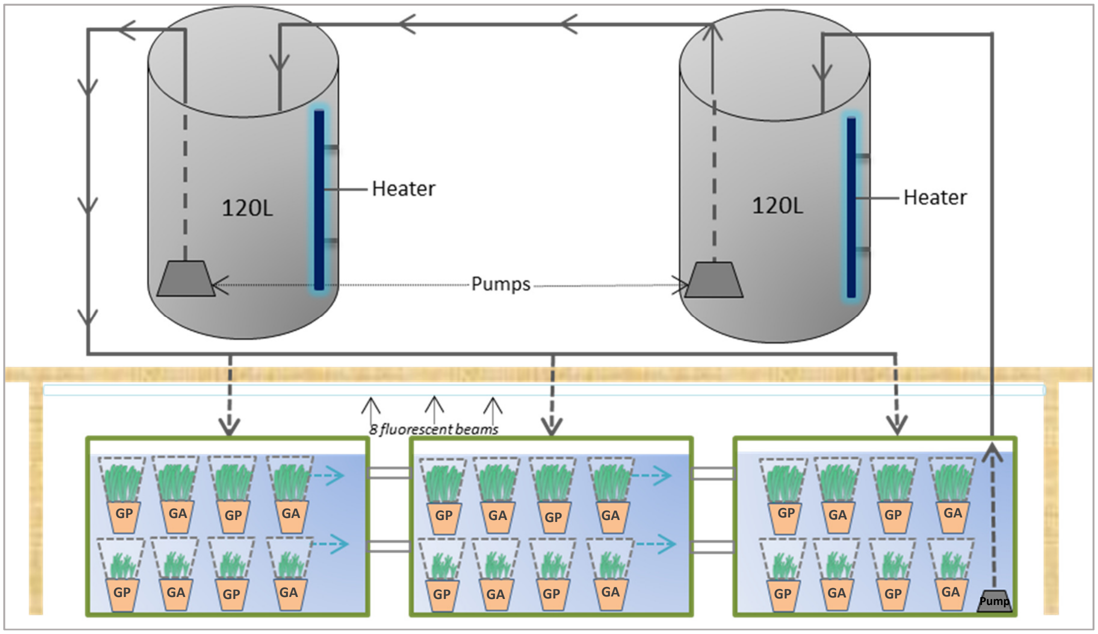


Fig. 5.1: Experimental setup of one mesocosm system comprising two 120L reservoir tanks with aerated seawater heated to treatment temperature levels (24 or 30°C). Treatment water was then pumped through a re-circulating, equal release flow through system into three 80L tanks each containing eight grazer/leaf size experimental units: four small-leaved with grazers present (GP)/grazers absent (GA), and two large-leaved with GP/GA. Arrows indicate direction of flow. Light levels were maintained from eight fluorescent beams placed above the mesocosm system.

A third of the seawater, first adjusted to treatment temperatures, was replaced every three days to maintain sufficient levels of nutrients and inorganic carbon to sustain growth, and a complete water change was performed once a week (Nejrup and Pedersen 2008). Eight fluorescent tubes (Osram Lumilux Cool White 58 Watt /965) providing up to 400 µmol photons $m^{-2} s^{-1}$, were placed above each treatment on a 12 hour light/dark cycle. Although irradiance levels were much lower than natural levels of sunlight, during trial testing of the experimental setup, *Zostera capensis* was found to sustain growth under these conditions. Similar light levels (400 µmol photons $m^{-2} s^{-1}$) were reported to support seagrass growth in several other experiments (Koch and Erskine 2001, Abe et al. 2008, Staehr and Borum 2011, Kaldy et al. 2015). Water temperature, light levels (measured mid-way between the sediment and leaf canopy in three plant pots on the outer and middle sections of each tank) and salinity were measured daily using a thermometer, light and salinity meters respectively. Plant pots were randomly repositioned once a week. An aquarium test kit was used weekly to measure seawater nitrate, nitrite, ammonia and phosphate, in order to monitor fluctuations in levels of these elements.

5.2.2. Grazers

The pulmonate siphonariid limpet, *Siphonaria compressa*, provided the grazing effect in this experiment. *S. compressa* and has been reported to feed on surface epiphyton (Allanson and Msizi 2010), and occurred in seagrass beds at both Oesterval and Geelbek in low (0.62 m^{-2}) and high (119.40 m^{-2}) abundances respectively (Chapter Three), however no direct examination of the limpets diet was performed. As a pulmonate limpet, *S. compressa* possesses a lung cavity as well as a secondary gill (Hodgson 1999, Allanson and Msizi 2010), which allows it to respire in and out of water. Therefore, a tidal emergence period of 4.5-5 hours was simulated once a day during the light cycle to provide a period of air exposure to the limpets and as a result to the seagrasses. This was achieved by pumping seawater out of the mesocosm into a separate tank until the bases of the seagrass shoots were exposed. Water was aerated and held under treatment temperature conditions then pumped back into the system after ±five hours.

Siphonaria compressa grows to a maximum length of 4.5 mm, and given its mobility, needed to be contained within each experimental unit. Therefore plastic open-top cages first covered with a fine mesh, then folded into a tube and fastened with cable ties, were placed around each plant pot to allow unrestricted water flow along with the exchange of nutrients and algal propagules while preventing grazers from escaping. Grazer densities were maintained by taking the average density per m^2 recorded at Geelbek and Oesterval which equated to five individuals per experimental unit (0.0079 m^2) (Chapter Three). To ensure the grazing effect was maintained throughout the experiment, limpets were located daily within each core, and adjusted to maintain abundances that may have been altered by mortality or loss (escape). Individuals were collected from the field every two weeks along with fresh seagrass plants, and kept alive in an aerated aquarium.

5.2.3. Preparation of Seagrasses

Intact seagrass cores (10 cm diameter x 15 cm depth) comprising shoots, roots/rhizomes and sediment were collected during peak growing season i.e. austral summer (January), during spring low tide at Langebaan Lagoon. Cores were collected from each of two sites, Oesterval and Geelbek (refer to map in Chapter Two: Fig. 2.1), representing large-leaved (LLM) and small-leaved (SLM) morphotypes respectively, and were taken from beds with similar shoot densities averaging 18 ±3.0SE for LLM and 86 ±5.9 SE for SLM. Each core was contained in a plant pot (10 cm diameter) lined with a plastic bag, then placed in a large box for transportation to the aquarium at the University of Cape Town.

Seagrass metrics from three cores for each morphotype were measured to record pre-treatment quantities (average number of shoots were 34.5 and 68.5 in LLM and SLM respectively), however initial estimates of carbon, nitrogen and phenolics were not obtained. Of the remaining cores, all visible epiphytes were removed and epiphytic algae gently scraped off all leaves with the back of a scalpel blade. Plant pots were then fitted with the open-top mesh-covered cages and transferred into aerated tanks containing 20^{o}C seawater to acclimatize for one week (Nejrup and Pedersen 2008). Temperatures were adjusted by 1^{o}C per day to allow for gradual acclimation to treatment temperatures (Koch et al. 2007). Once treatment temperatures (unheated: 24°C and heated: 30°C) were reached, seagrass plants were maintained under treatment conditions for six weeks. Algal overgrowth was frequently removed from the walls of the mesocosm in an effort to reduce additional stress on seagrasses from competition for nutrients.

Experimental testing of the effects of warming and grazing was determined over a total experimental period of six weeks. Other experiments that measured photosynthetic responses to heat stress in seagrasses varied in treatment times from 1-5 hours (Seddon and Cheshire 2001, Torquemada et al. 2005, Campbell et al. 2006) to 3-6 weeks (Pilon and Santamaría 2002, Koch et al. 2007, Eklöf et al. 2012, Alsterberg et al. 2013). Several studies evaluated concentrations of condensed tannins and phenolic acids following grazer treatments after 2-4 weeks (Pavia and Toth 2000a, 2000b, Steele and Valentine 2012, 2015) in seaweeds and seagrasses. A six week treatment period was therefore selected for this study, to allow for adequate grazer response time, while still maintaining suitable conditions for plant growth.

5.2.4. Seagrass Performance Metrics

5.2.4.1. Chlorophyll Fluorescence

An underwater Pulse Amplitude Modulated (PAM) fluorometer (Opti-Sciences 5p+ Portable Fluorometer) was used to measure fluorescence from seagrass leaves in each mesocosm. When light (i.e. photons) is absorbed by a photosynthetic molecule such as chlorophyll, its energy causes the rapid displacement of electrons, thereby energising or exciting the molecule. The excited chlorophyll molecule reverts back to a stable state through a process of de-excitation, resulting in a discharge of energy primarily used to propel photosynthesis, while a lesser portion is responsible for the generation of heat and fluorescence (Beer et al. 2014). Fluorescence is inconsequential to the photosynthesis process, but is rather a consequence of de-excitation-derived energy that is not channelled into photosynthesis (Schreiber et al. 1988). There is therefore, a negative correlation between photosynthetic and fluorescence yields, thus enabling the use of fluorescence measurements to determine photosynthetic rates. The photosynthetic output per photon absorbed by photosynthetic pigments, is described as quantum yield (Beer et al. 2014). The measure of quantum yield is based on the movement of electrons through Photosystem II (PSII) which is measured by the PAM, since this provides an estimation of the proportion of photon energy photosynthetically converted to carbohydrates.

The photosynthetic transfer of electrons through PSII or effective quantum yield (Genty et al. 1989) was measured using the equation:

$Y = (F_m' - F_s) / F_m' = \Delta F / F_m'$

where,

Y = effective quantum yield

F_m' = fluorescence that is not dark-adapted (i.e. the pre-photosynthetic fluorescent state with minimum fluorescence),

F_s = the initial level of fluorescence created by actinic (photosynthesis-causing) light (expressed as F_0 when dark adapted).

ΔF = $(F_m' - F_s)$

The measure of quantum yield (Y) was used since it is an efficient light adapted test that can be done in steady-state light conditions, and measures the proportion of quantity of light used in photochemistry in PSII, a component of the photosynthetic apparatus with the highest sensitivity to temperature (Ralph 1998b), as opposed to just light absorbed by PSII-associated chlorophyll (Silva et al. 2009). Y is sensitive to plants under stress (Beer and Björk 2000), because it provides a normalised ratio associated with achieved photosynthetic efficiency that correlates with integration of carbon, and is directly related to linear transport of electrons (Genty et al. 1989).

Chlorophyll fluorescence was measured in submerged plants pre-treatment (n = 30 leaves), and after five weeks in treatment between 10am – 12pm. Measurements were taken on the second youngest seagrass leaf under constant light levels (350-400 µmol photons m^{-2} s^{-1}), by holding the optic fibre measuring end of the instrument ±10 mm away from the bottom third of the leaf at a 60^{o} angle (Björk et al. 1999). Quantum yield was estimated from five leaves per pot in each temperature, grazer and leaf size treatment (n = 120).

5.2.4.2. Seagrass Morphometrics and Epiphyte Biomass

On termination of the experiment, seagrass cores were gently rinsed of all sediment, placed in labelled plastic bags, and stored in a freezer at -20°C for later analysis. Thereafter, total number of surviving shoots and leaves were counted, and roots/rhizomes were separated from shoots. Leaf length (tip to base) and the average of three width measurements (tip, mid and base), of up to five intact leaves per core were measured and multiplied to obtain leaf area. Each leaf was then placed in a pre-weighed petri dish with water, and epiphytic algae were scraped into the dish with the back of a scalpel until leaves were visibly clean. Petri dishes were placed in a drying oven at 60°C till constant dry weight was attained and weighed to obtain algal biomass per leaf area (Fong et al. 2000, Carruthers et al. 2001). Leaves as well as roots/rhizomes were also dried to constant dry weight at 60°C (~12 hours for above- and ~14 hours for belowground biomass) and weighed to obtain seagrass biomass.

5.2.5. Phenolic Acid Measurements

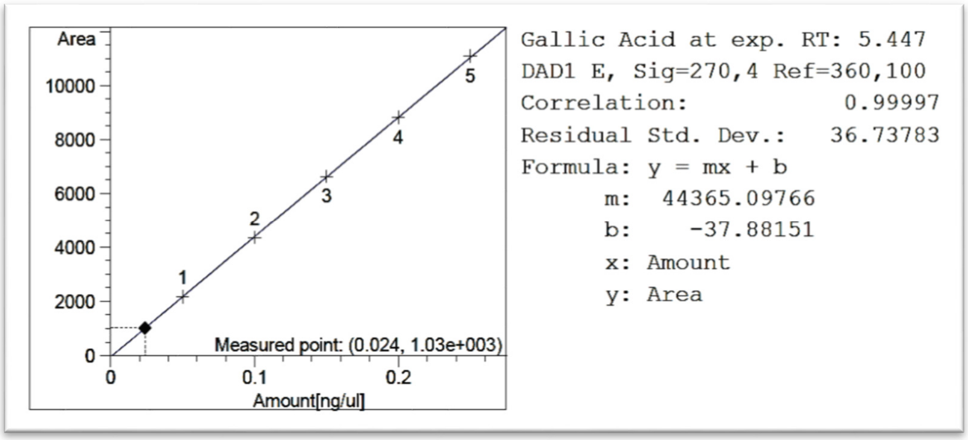
5.2.5.1. Preparation of crude extracts

One hundred milligrams of leaf material was needed to prepare assays for phenolic analyses, and a further 10 mg was required for carbon and nitrogen assessments. On termination of the experiment, several cores/experimental units (EUs) in the heated (30°C) treatment did not contain adequate quantities of aboveground biomass and could not meet the requirements for carbon, nitrogen and gallic acid assessment at the experimental unit level. However, leaf material representing each temperature/leaf morphotype/grazer combination was obtained and analysed in triplicate (n= 8). Seagrass leaves were freeze-dried (lyophilised) for 72 hours, weighed (to obtain aboveground biomass), then milled to a fine powder using a Retsch MM200 mixer mill.

Assay preparation was adapted from Cuny et al. (1995) and Sauvesty et al. (2013), where 10 mg of lyophilised seagrass leaf material was extracted in 5mL of aqueous methanol (20% Millipore water and 80% analytical grade methanol), covered and incubated at 4°C for 30 minutes with constant mixing. Thereafter, samples were covered and placed on a shaker at room temperature overnight. Four hundred microliters (µL) of cold Millipore water was then added and samples subjected to sonication for 15 minutes. They were then centrifuged at 13 000 g for 15 minutes. Five hundred microliter aliquots were decanted into pre-weighed Eppendorf tubes (1.5 mL) and dried down for eight hours in a speed vac on medium heat in order to concentrate the samples. Tubes were then re-weighed to calculate the soluble mass. The equation: $C_1V_1=C_2V_2$, where C represents concentrate and V volume, was used to calculate the dilution factor that yielded a concentrate of 10 mg/mL, and each mass was re-suspended accordingly. Only HPLC (high performance liquid chromatography)-grade solvents were used. A Millipore water purification system producing analytical grade water was used throughout the extraction process.

5.2.5.2. HPLC analysis

Quantification and separation of phenolic acids in dried crude extracts of *Zostera capensis* was performed using an HPLC (Quaternary Agilent Technologies 1200 Series) comprising a liquid chromatography system and a column chamber thermostatically controlled. Separations were carried out at 20°C on a Hypersil GOLD C12 column. The UV spectral range was 200-400 nm and the run time was 24 mins. Data were assimilated using the 3D software ChemStation for Liquid Chromatography. The following phenolic acids were used as standards against which to quantitatively determine phenolic acid concentrations in seagrass samples: ferulic, gallic, gentisic, p-coumaric, sinapic, and transcinnamic acids, each in amounts of 0.1, 0.2, 0.0.4 and 0.05 mg/mL. Using a calibration curve that generated a linear regression coefficient of 0.99 (Fig. 5.2), peak area measurements at the corresponding spectra for each standard were taken, and measured against each sample to determine quantities of that standard. In the end only gallic acid was present in the samples. Gallic acid is commonly used as a comparative standard when measuring plant phenolics (Dai and Mumper 2010). Data were extracted for each sample and expressed as mg/100g dry sample.



```
Gallic Acid at exp. RT: 5.447
DAD1 E, Sig=270,4 Ref=360,100
Correlation:              0.99997
Residual Std. Dev.:    36.73783
Formula: y = mx + b
      m:  44365.09766
      b:    -37.88151
      x: Amount
      y: Area
```

Figure 5.2: Example of a calibration curve for gallic acid prepared in ChemStation, based on elution rates from five standards producing a linear regression correlation coefficient close to 1.

5.2.6. Carbon and Nitrogen Assessments

Measures of carbon and nitrogen in seagrass leaves were provided by the Stable Light Isotope Laboratory in the Archaeology Department of the University of Cape Town. Lyophilized and milled leaf material (weighed to an accuracy of 1µg) was combusted in a Flash 2000 organic elemental analyser, and the gases passed through a Delta V Plus isotope ratio mass spectrometer (IRMS) via a Conflo IV gas control unit (Thermo Scientific, Bremen, Germany). All standards used were calibrated against International Atomic Energy Agency standards. Nitrogen was expressed as a percentage value relative to atmospheric nitrogen, while the percentage value of carbon was calculated relative to the standard Pee-Dee Belemnite. The corresponding ratio of C and N was then calculated for each sample.

5.2.7. Statistical Analyses

Data were tested for homogeneity of variances (Levene's test) and normality (Shapiro-Wilk's test) prior to analyses. The effects of temperature (pre-treatment, unheated: 24°C and heated: 30°C), leaf size (small vs large-leaved morphotypes) and grazing (presence vs absence) on leaf area, shoot densities, total biomass, above/belowground biomass ratio, effective quantum yield (Y) and epiphytic algal biomass were assessed. Temperature, leaf size and grazing were set as fixed factors and 'tank' was assigned as a random factor nested under 'temperature' in a linear mixed-effects ANOVA (IBM SPSS Statistics 25) followed by post hoc (Turkey HSD) tests to identify differences between temperature treatments. Given the naturally high variation between small and large-leaved morphotypes, and differences in initial densities and biomass, morphotype response to temperature/grazing treatments was assessed rather than direct differences between leaf sizes.

A non-parametric pairwise analysis (Wilcoxon signed-rank test) was performed to test the main effects of temperature, leaf size and grazing on seagrass carbon, nitrogen and phenolics i.e. gallic acid. This is a paired test that is used to compare two related samples, and tests the hypothesis that the two sets of observations from one population differ from another population in location i.e. median or mean, and is a suitable test for small sample sizes (Quinn and Keough 2002). Data are presented as means ± 1 standard error except for C and N and gallic acid where total amounts are presented.

Seagrass response variables are expressed as values per core area (i.e. 0.0079m^2). Factors are considered significant at $p < 0.05$.

5.3. Results

5.3.1. Seagrass Performance Metrics

Compared to pre-treatment measures, 33% of shoots remained in heated and 90% in unheated treatments (Fig 5.3A). Shoot densities severely declined in heated (30^{o}C) treatments compared to unheated (24^{o}C) and pre-treatment densities, and the effect of treatment temperatures was highly significant (Table 5.1). In heated treatments, shoot densities ranged from 10-18 and 15-30 per core (0.0079m^{-2}) in large-leaved (LLM) and small-leaved (SLM) morphotypes respectively, and from 31-38 (LLM) and 52-70 (SLM) in unheated treatments. Shoot densities had greatly declined under heating, however shoots were present in all cores at the end of the experiment. Conversely, leaf densities i.e. aboveground biomass were greatly reduced and no leaves were present in some cores. In heated treatments, leaf densities ranged from 0-12 in LLM and 2-13 in SLM treatments. Tukey tests revealed significant differences between pre-treatment and heated, as well as between unheated and heated treatment densities, but not between pre- and unheated treatments. Both LLM and SLM plants had higher shoot densities in unheated treatments when grazers were present versus absent, while in heated treatments, LLM had lower densities when grazers were present compared to absent, and SLM had lower densities when grazers were absent than in grazed treatments (Fig. 5.3A). Grazing had no significant effect on shoot densities ($F_{1,37} = 0.93$, $p = 0.34$), neither did the interactions between temperature and grazing ($F_{1,37} = 0.35$, $p = 0.56$), nor temperature, grazing and leaf size ($F_{1,37} = 0.35$, $p = 0.56$). Tank effects on shoot densities were significant (Table 5.1).

Temperature effects on leaf area was significant ($F_{2,15} = 964.61$, $p < 0.01$) due mainly to differences between pre- and unheated treatments contrasted with heated treatments (Fig. 5.3B). In both heated and unheated treatments, leaf area of LLMs were slightly higher in grazed versus ungrazed treatments, while SLMs had higher leaf area in ungrazed as opposed to grazed treatments (Fig. 5.3B). The effect of grazing was not significant ($F_{1,37} = 1.47$, $p = 0.23$) however, the interactions between temperature and grazing, temperature and leaf size as well as grazing and leaf size were significant (Table 5.1).

Total biomass of LLMs in unheated treatments had increased from pre-treatment levels, but this was not the case for SLMs (Fig. 5.3C), although biomass between pre- and unheated treatments for the two morphotypes did not differ significantly (Tukey HSD, $p = 0.87$). Seagrass biomass had substantially declined in heated compared to pre- and unheated treatments and was significantly affected by temperature ($F_{2,19} = 521.35$, $p < 0.01$). The effect of temperature on each morphotype was also significant ($F_{2,37} = 28.69$, $p < 0.01$). In unheated treatments, biomass from the two morphotypes was higher in grazed compared to ungrazed treatments, but remained relatively equal in heated treatments (Fig 5.3C). The effect of grazing on total biomass was significant ($F_{1,37} = 18.94$, $p < 0.01$), along with the interaction between temperature and grazing ($F_{1,37} = 24.43$, $p < 0.01$), but not grazing and leaf size ($F_{1,37} = 3.48$, $p = 0.07$). The interaction of all main effects (temperature, leaf size and grazing) significantly affected variation in total seagrass biomass (Table 5.1).

Despite significant treatment effects on total biomass, no effects of treatment factors were observed on above/belowground biomass (ABB) ratios (Table 5.1). ABB ratios had increased for the two morphotypes in heated and unheated treatments from pre-treatment levels (Fig. 5.3D). Tukey comparisons established differences between pre- and post-treatment ABB ratios to be significant ($p = 0.01$) but not between unheated and heated treatments ($p = 0.96$).

Effective quantum yield (Y) as a measure of photosynthetic rate was higher in both LLM and SLM plants in treatment compared to pre-treatment states (Fig. 5.3E). While the main effect of temperature was not significant (Table 5.1), post hoc comparisons revealed significant differences between initial and post-treatment photosynthesis (Tukey HSD, $p < 0.001$) but no differences between heated and unheated treatments ($p = 0.58$). On the other hand, the interaction between temperature and leaf size did produce a significant effect ($F_{2,37} = 4.25$, $p = 0.02$). Although Y was generally higher in unheated/grazed treatments for the two morphotypes, but lower for LLMs in heated/grazed treatments, grazing did not have a significant effect on Y ($F_{1,37} = 0.15$, $p = 0.70$), while the interactions between grazing and temperature, and grazing and leaf size were significant ($F_{1,37} = 5.15$, $p = 0.03$ and $F_{1,37} = 5.15$, $p = 0.03$ respectively). Tank effects were also found to significantly influence Y (Table 5.1).

Treatment effects on epiphytic algal (EA) biomass per leaf area were not significant (Table 5.1). Despite the substantial increase in EA biomass post-treatment from initial quantities, post hoc analyses did not find significant differences between treatments ($p > 0.05$). Significant differences in EA biomass between morphotypes was expected given the high variation in leaf size, however this was not the case ($F_{1,37} = 2.14$, $p = 0.15$). Temperature and tank effects on EA biomass accounted for model variations of 22% and 20% respectively, while variation attributed to grazing was 0.02% (Table 5.1). EA biomass was greater in heated compared to unheated treatments for the two morphotypes (Fig. 5.3F). In unheated treatments, EA biomass was higher in grazed as opposed to ungrazed treatments, while the opposite was observed in heated treatments, however grazing did not significantly affect EA biomass (Table 5.1). In addition, there was a slightly higher average loss of grazers (limpets) in heated compared to unheated treatments for both LLM (1.03/day and 0.97/day in heated and unheated respectively) and SLM (1.05/day in heated and 0.98/day in unheated) (Fig. 5.4), however differences in limpet loss between temperature treatments were not significant ($t = -1.45$, $p = 0.15$).

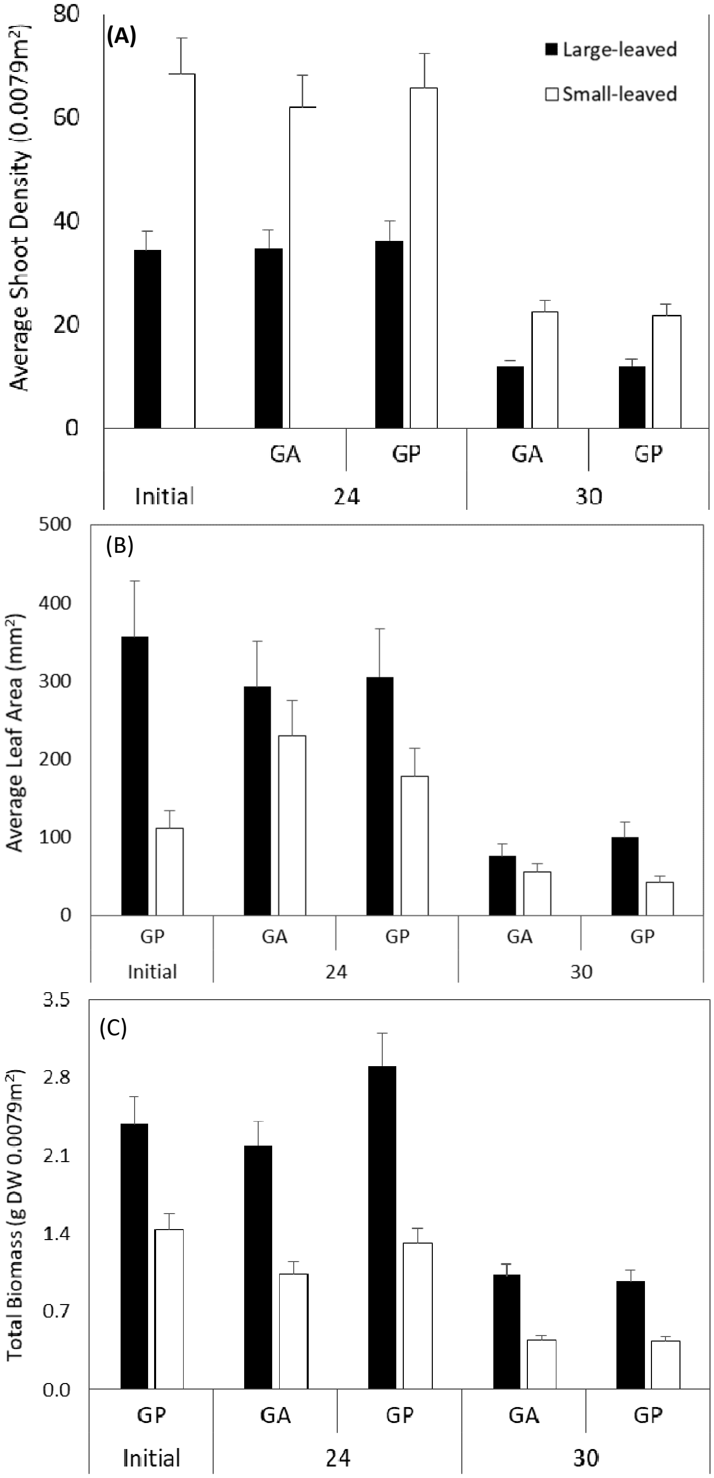


Figure 5.3: Mean ± 1SE of (A) average shoot densities (0.0079m^2), (B) leaf area (mm^2), (C) total biomass (g dry weight/0.0079m^2) small-leaved (white bars) and large-leaved (black bars) morphotypes of *Zostera capensis*, pre- (initial) and post treatment in unheated (24) and heated (30) temperature and grazing treatments. GA = grazers absent and GP = grazers present.

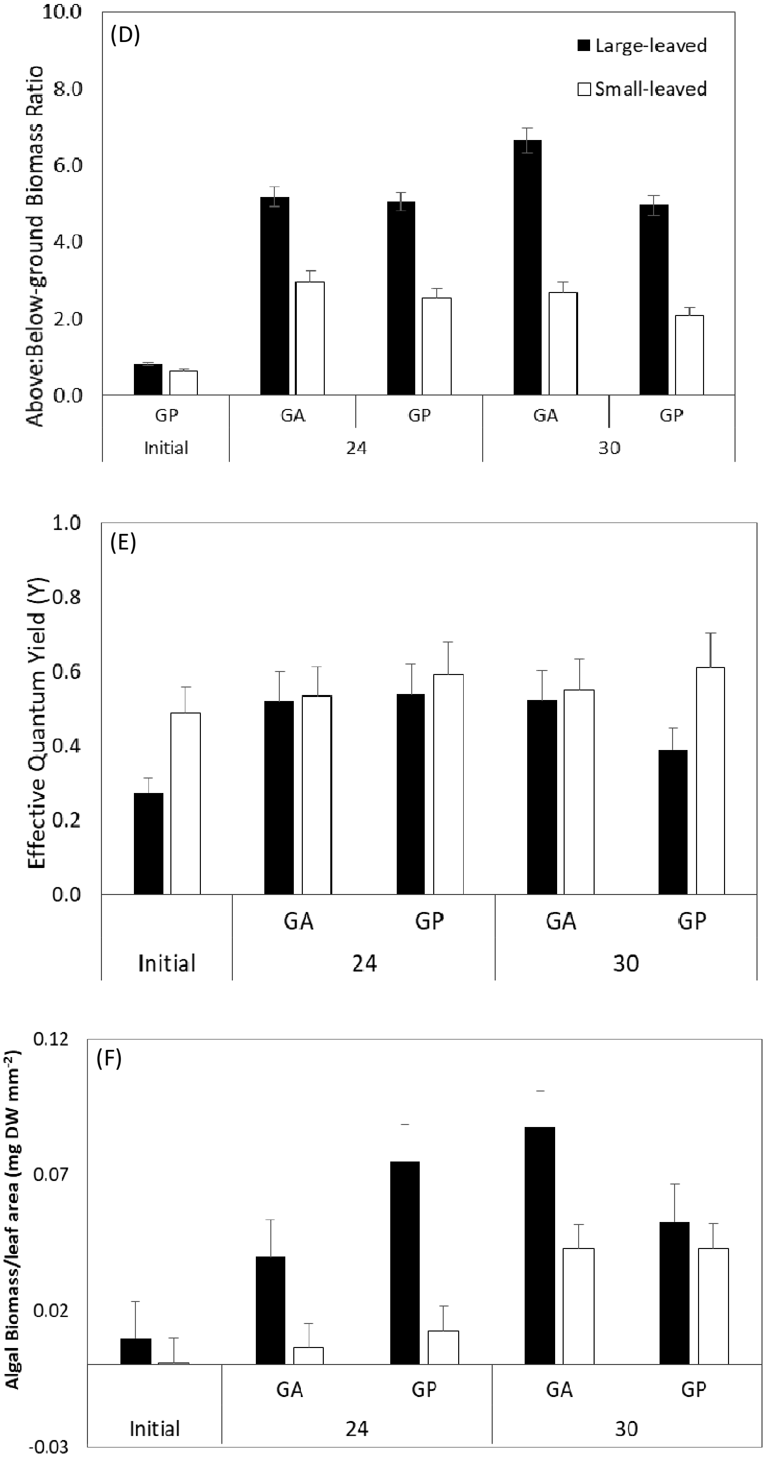


Figure 5.3 Continued: Mean +1SE of (D) above: belowground biomass ratio, (E) effective quantum yield (Y), and (F) algal biomass per leaf area (mg dry weight mm^{-2}) in small-leaved (white bars) and large-leaved (black bars) morphotypes of *Zostera capensis*, pre- (initial) and post treatment in unheated (24) and heated (30) temperature and grazing treatments. GA = grazers absent and GP = grazers present.

Table 5.1: Three factor ANOVA testing effects of temperature (pre, heated and unheated), leaf size (small vs large) and grazing (presence vs absence) on shoot density, leaf area, total biomass, above/belowground biomass ratio, effective quantum yield, and algal biomass of *Zostera capensis*. Effect size is represented by partial Eta². *P* values < 0.05 are in bold.

Variable	**Factor**	**df**	***F***	***P***	**Partial Eta²**
Shoot density	Temperature	2,6	132.99	**<0.01**	97.7%
	Grazing	1,37	0.93	0.34	2.5%
	Temp x Grazing	1,37	1.05	0.31	2.8%
	Temp x Leaf Size	2,37	27.31	**<0.01**	59.6%
	Grazing x Leaf Size	1,37	0.08	0.78	0.2%
	Temp x Leaf Size x Grazing	1,37	0.35	0.56	0.9%
	Tank (Temperature)	5,37	2.88	**0.03**	28.0%
Leaf area (mm^2)	Temperature	2,15	964.61	**<0.01**	99.2%
	Grazing	1,37	1.47	0.23	3.8%
	Temp x Grazing	1,37	4.51	**0.04**	10.9%
	Temp x Leaf Size	2,37	35.59	**<0.01**	65.8%
	Grazing x Leaf Size	1,37	18.83	**<0.01**	33.7%
	Temp x Leaf Size x Grazing	1,37	1.32	0.26	3.4%
	Tank (Temperature)	5,37	0.38	0.86	4.9%
Total biomass (g dry weight $0.0079m^{-2}$)	Temperature	2,19	521.35	**<0.01**	98.2%
	Grazing	1,37	18.94	**<0.01**	33.9%
	Temp x Grazing	1,37	24.43	**<0.01**	39.8%
	Temp x Leaf Size	2,37	28.69	**<0.01**	60.8%
	Grazing x Leaf Size	1,37	3.48	0.07	8.6%
	Temp x Leaf Size x Grazing	1,37	5.15	**0.03**	12.2%
	Tank (Temperature)	5,37	0.28	0.92	3.7%
Above:below ground biomass	Temperature	2,8	3.36	0.09	46.3%
	Grazing	1,37	1.64	0.21	4.2%
	Temp x Grazing	1,37	0.62	0.43	1.7%
	Temp x Leaf Size	2,37	1.19	0.32	6.0%
	Grazing x Leaf Size	1,37	0.12	0.74	0.3%
	Temp x Leaf Size x Grazing	1,37	0.38	0.54	1.0%
	Tank (Temperature)	5,37	1.26	0.30	14.5%
Effective quantum yield	Temperature	2,6	3.19	0.11	51.7%
	Grazing	1,37	0.15	0.70	0.4%
	Temp x Grazing	1,37	5.17	**0.03**	12.3%
	Temp x Leaf Size	2,37	4.25	**0.02**	18.7%
	Grazing x Leaf Size	1,37	6.57	**0.01**	15.1%
	Temp x Leaf Size x Grazing	1,37	2.41	0.13	6.1%
	Tank (Temperature)	5,37	3.45	**0.01**	31.8%
Epiphytic algal biomass (mg dry weight mm^{-2}).	Temperature	2,7	0.98	0.42	22.1%
	Grazing	1,37	0.01	0.93	0.0%
	Temp x Grazing	1,37	1.12	0.30	3.0%
	Temp x Leaf Size	2,37	0.25	0.78	2.7%
	Grazing x Leaf Size	1,37	0.01	0.93	0.4%
	Temp x Leaf Size x Grazing	1,37	0.78	0.38	3.5%
	Tank (Temperature)	5,37	1.82	0.13	19.7%

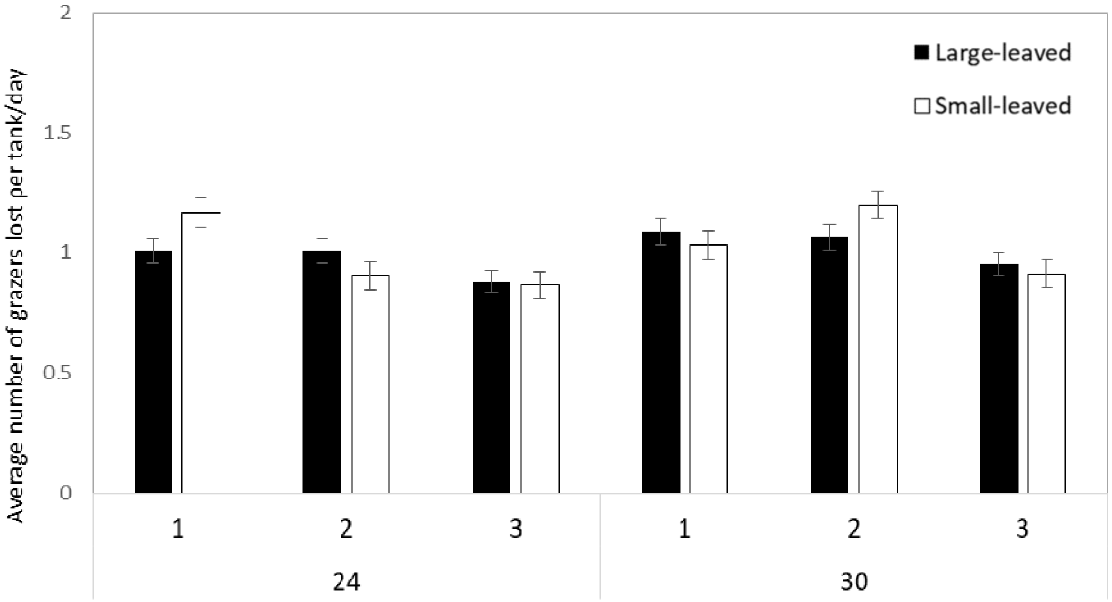


Figure 5.4: Average (±1SE) grazers/limpets lost per day per tank (1, 2 and 3) over the duration of the experimental period (42 days) in large- and small-leaved morphotypes of *Zostera capensis*, subjected to 24 and 30°C treatments. Averages are based on numbers of individuals not accounted for out of five per core ($0.0079 m^2$). Mean difference in grazer loss between temperature treatments was not significant ($t = -1.45$, $p = 0.15$).

5.3.2. Phenolics, Carbon and Nitrogen

The percentage of carbon (C) and nitrogen (N) in *Zostera* leaves was higher in SLM compared to LLM plants in both heated and unheated treatments (Fig. 5.5 A&B). LLM contained more C in heated treatments compared to unheated, while C remained relatively similar in SLM in unheated and heated treatments (Fig. 5.5 A). Generally, there were no marked changes in C between grazed (SLM = 37.36, LLM =36.01) and non-grazed (SLM = 40.37, LLM = 34.74%) treatments. In addition, C did not show a significant response to the main treatments effects (Table 5.2A). On the other hand, N differed significantly ($W = 10$, $p = 0.03$) between SLM and LLM, but showed no effect of temperature and grazing (Table 5.2B). N was higher in LLM in heated compared to unheated treatments, but showed an opposite trend in SLM where leaves contained more N in unheated as opposed to heated) treatments (Fig. 5.5B). C:N ratios were greater in unheated treatments compared to heated, and also higher in LLM compared to SLM plants

(Fig.5.5C). The relationship of C to N showed different responses for each leaf size and temperature treatment (Fig. 5.6A).

Leaf size significantly influenced gallic acid (GA) content in *Zostera* ($W = 10$, $p = 0.03$), while temperature and grazing had no significant effect (Table 5.2D). SLM contained higher levels of GA compared to LLM across temperature and grazing treatments (Fig. 5.5D). In SLM, GA levels were marginally higher in unheated (0.003mg/100g) compared to heated (0.002mg/100g) treatments, a pattern also observed for LLM (Fig. 5.5D). There were no correlations between GA content and epiphytic algal biomass, Y, C or C:N, however, GA was negatively and significantly correlated with total seagrass biomass (Spearman $R_s = -0.7$, $p < 0.001$) and positively correlated with N (Spearman $R_s = 0.8$, $p < 0.05$).

Table 5.2: Non-parametric Wilcoxon signed-ranks test for paired samples to compare differences in gallic acid (mg/100g dry wt), carbon (%) and nitrogen (%) in leaves of *Zostera capensis* under temperature (24 and 30^{o}C), leaf size (small and large-leaved) and grazer (present/absent) treatments. Significant differences (p <0.05) are in bold.

Variable	**Factor**	***W***	***p***
(A) % Carbon	Temperature	15.00	0.49
	Leaf Size	14.00	0.34
	Grazing	15.00	0.49
(B) % Nitrogen	Temperature	18.00	1.00
	Leaf Size	10.00	**0.03**
	Grazing	17.00	0.89
(C) C:N Ratio	Temperature	18.00	1.00
	Leaf Size	10.00	**0.03**
	Grazing	18.00	1.00
(D) Gallic Acid (mg/100g)	Temperature	14.50	0.34
	Leaf Size	10.00	**0.03**
	Grazing	15.50	0.47

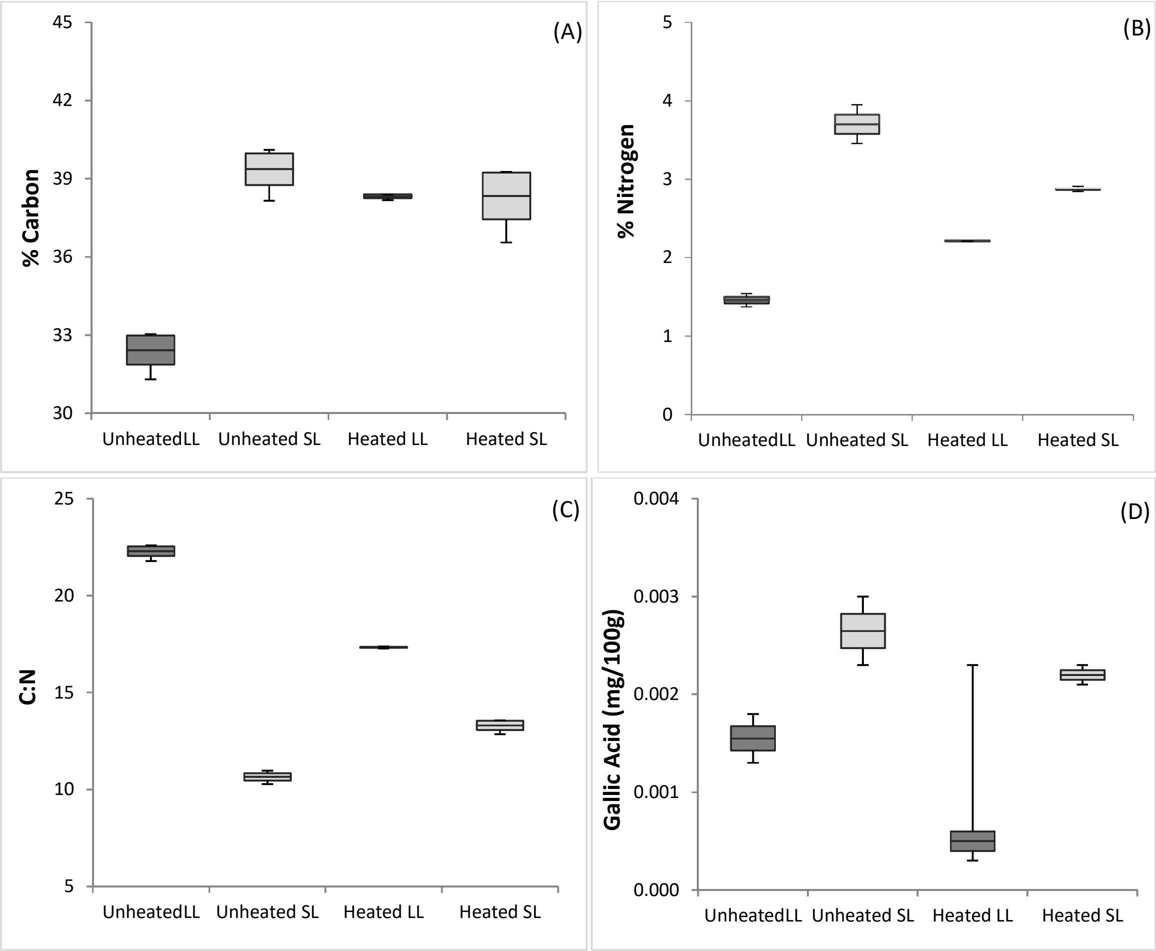


Figure 5.5: Mean, minimum and maximum values presented for (A) % carbon, (B) % nitrogen, (C) C:N ratio and (D) gallic acid (mg/100g) in large (LL) and small (SL) leaves of *Zostera capensis* after subjection to heated (30°C) and unheated (24°C) treatments.

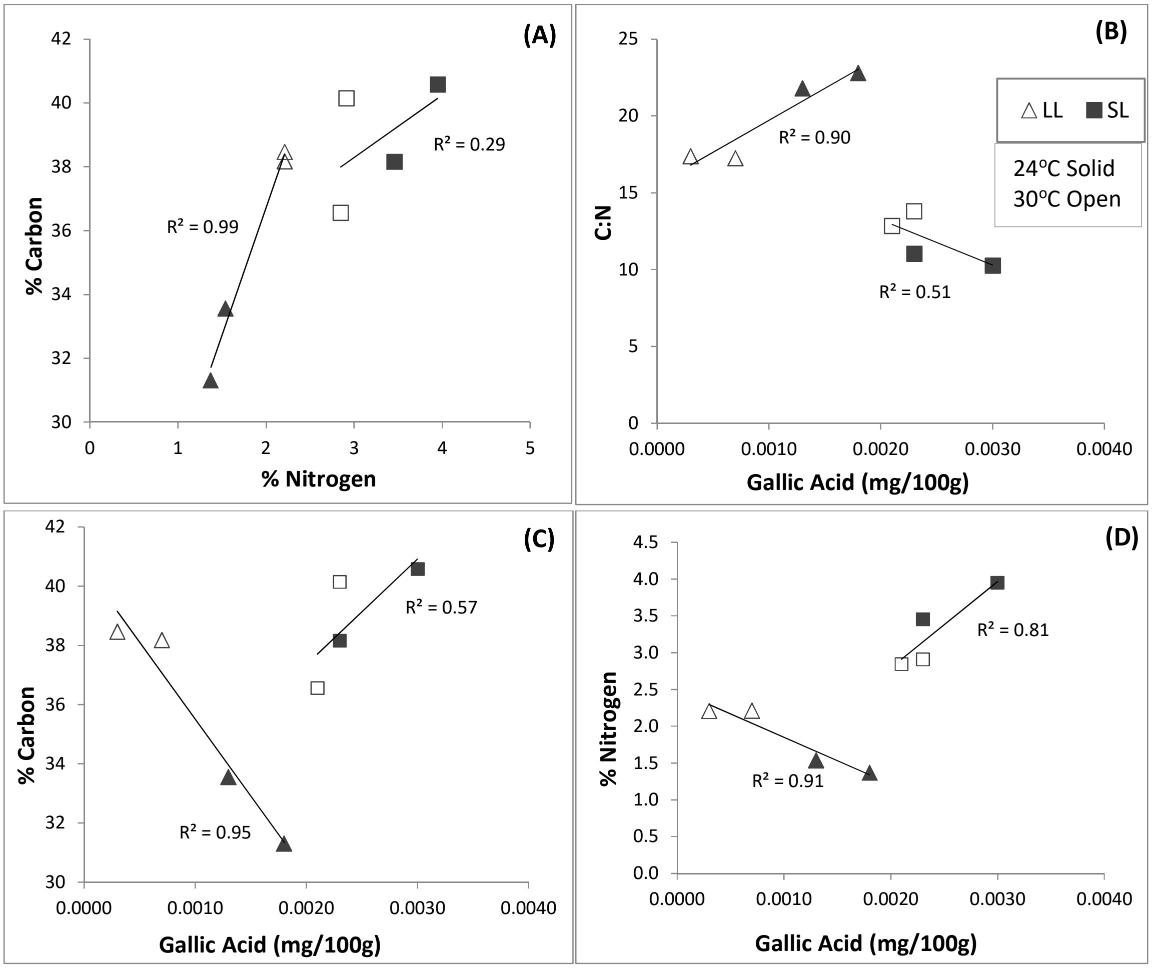


Figure 5.6: Relationships between carbon and nitrogen (A), gallic acid and C:N (B), gallic acid and carbon (C) and gallic acid and nitrogen (D) in leaves of large-leaved (triangles) and small-leaved (squares) morphotypes of *Zostera capensis* after six weeks in 24 (solid) and 30°C (open) temperature treatments. Linear trajectories and corresponding R^2 values are presented.

5.4. Discussion

In this chapter, I investigated the extent to which temperature and grazing explained variation in response parameters of *Zostera capensis* and of algal epiphytes, and hypothesized that a small-leaved morphotype (SLM) would perform better (greater photosynthetic rates, shoot densities and secondary metabolites) compared to a large-leaved morphotype (LLM) under warming conditions. A mesocosm experimental approach was used because it was best suited to examine the grazing effects of a small (<4.5mm) gastropod under controlled temperature conditions.

Temperature and its interaction with leaf size significantly influenced photosynthesis. Photosynthetic rates were higher in LLMs in unheated treatments, but decreased under warming, while photosynthesis increased slightly in SLMs in warmer treatment conditions. The initial response of increasing photosynthesis as temperature increases, has been well documented in both algae and seagrasses and is followed by increases in biomass and productivity (Barber and Behrens 1985). This is supported by the higher total biomass in unheated treatments for the two morphotypes. This increase in photosynthesis with a corresponding increase in temperature however, reaches a threshold where photosynthesis declines as temperatures continue to rise (Lee et al. 2005). A corresponding decline in metabolic rate coupled with heightened respiration that eventually supersedes photosynthesis (Kim et al. 2012) could explain the overall reduction in productivity and biomass in the heated treatments (Fig. 5.3C), as plant resources are possibly used to contend with the heat stress.

Epiphytic algae generally increased under warmer temperature conditions confirming the effect of warming in favouring algal growth (Alsterberg et al. 2013, Werner and Matthiessen 2017). This may have played a role in the observed lower photosynthetic rates since not only do algae compete with seagrasses for nutrients and light (Short et al. 1995) but epiphytes have been shown to create a biofilm on seagrass leaves that reduces the diffusion rates of nutrients and CO_2 thus hampering photosynthesis (Nelson and Lee 2001, Borowitzka et al. 2006). However, the influence of biofilm on photosynthesis, and the extent to which epiphytic algae compete with *Zostera capensis* for water column nutrients remains to be assessed.

Algal biomass was generally higher in unheated treatments with grazers present. A possible explanation for this might be due to the entrapment of microalgae in the mucus trail produced by limpets. A mucus or slime trail is secreted by gastropods to essentially aid in locomotion, but also acts as an area of organic enrichment providing nutrition to while also trapping bacteria, diatoms and algae thus enhancing algal growth (Davies and Beckwith 1999). In heated treatments on the other hand, grazers appeared to have controlled algal biomass which was reduced in grazed compared to non-grazed treatments however, the effect of grazing on the observed variation in epiphyte biomass was negligible. Given the lack of direct evidence of the precise autotrophic constituents that *Siphonaria compressa* feeds on, rendering a cautious interpretation of these results, as a small-sized (4.5mm) grazing gastropod it is assumed to feed on epiphyton (Allanson and Msizi 2010). Anatomically, *S. compressa* may have been unable to control more fast growing green macroalgae which has been shown to rapidly cover leaf surface areas under warming conditions (Eklöf et al. 2012), and at the same time lacked the means to feed on tougher brown and red algae. Nonetheless, warmer conditions had clearly intensified algal growth despite a reduction in seagrass shoots and this could be problematic for *Z. capensis* under future warming scenarios given the potentially stressful effects of algal fouling on seagrasses (Nelson and Lee 2001, Raun and Borum 2013).

Non-grazed treatments contained higher levels of gallic acid (GA) compared to grazed treatments for the two leaf sizes, although there was no significant influence of grazing on GA. This result however, is consistent with those of Steele and Valentine (2015) who investigated the experimental effects of herbivory by the gastropod, *Crepidula ustulatulina* and the isopod, *Paracerceis caudata* on phenolic production in *Thalassia testudinum* and *Halodule wrightii*. They found phenolic concentrations to be mostly lower in grazed treatments of *T. testudinum* than in non-grazed controls. They further noted that gastropod and isopod grazing led to a decrease in concentration of some phenolic acids by ~40-50% in *T. testudinum* and suggested that plant perceptions with regards to the cost of growth versus defence could possibly explain this outcome (Steele and Valentine 2015). Mesograzer identity was also important in eliciting a chemical response to grazing in some seagrass species (Steele and Valentine 2015). Similarly, Darnell and Heck (2013) reported lower concentrations of phenolics in *T. testudinum* leaves grazed by parrotfish than those that were not grazed. In another example, the deterrent effects of phenolic compounds from *Posidonia oceanica* was investigated on sea urchins, fish and the gastropod, *Cerithium vulgatum* (Vergés et al. 2007a). The results of that study indicated that while some consumers were deterred by the extracts with varying degrees of inhibition, *C. vulgatum*, was unaffected. As suspected for *S. compressa*, *C. vulgatum* fed mostly on micro and filamentous algae since it is unable to graze on tougher seagrass leaves due to its delicate chitinous teeth and buccal muscles (Steneck and Watling. 1982), and was therefore immune to the deterrent effects of phenolic acids.

The grazing effect in this chapter was provided by five individual limpets per experimental unit, reflecting average abundances recorded *in situ*. And while *S. compressa* thrives at Geelbek where maximum water temperatures of 28.5^{o}C have been recorded, epiphyte biomass was barely detected on seagrass leaves suggesting strong top-down control (Chapter Two). Experimental limpet densities may have been unable to control the rapid algal (green, red and brown varieties) production under warming, and implies the need for multiple species with adequate grazing capacities to maintain top-down control of algal epiphytes in seagrass habitats as global temperatures continue to rise (Eklöf et al. 2012, Alsterberg et al. 2013, Werner and Matthiessen 2017).

Greater photosynthetic output, above that needed for growth and metabolism, results in carbohydrates being stored as secondary metabolites (Holopainen and Gershenzon 2010) however, photosynthetic output in LLM under heating appears to have been channelled into maintaining primary production i.e. above/belowground biomass as opposed to secondary production. This is further evident in the higher carbon (C) content in LLM under heating. The opposite was observed for SLM under warming where higher photosynthetic rates did not result in greater biomass, but more secondary products evidenced in the higher gallic acid (GA) content. A similar finding was recorded in measurements of GA in leaves of SLM and LLM *in situ*. In a single summer survey of three beds in each morphotype, greater amounts of GA were recorded from SLM, while GA was only recorded in one out of three LLM beds (see Appendices IV and V). A possible theory is that these differences reflect an acclimative strategy to cope with heat stress, where LLM are likely adapted to less or brief stressful environments and therefore continue to support structural growth, while SLM have adapted to prolonged stress and consequently store resources to be channelled towards growth when conditions are favourable again.

The findings from my study reflect that documented by Vergeer et al. (1995) where lower concentrations of phenolics in *Zostera marina* leaves was recorded from warmest temperature treatments. Similarly, Ravn et al. (1994) found concentrations of rosmarinic and caffeic acids in *Z. marina* field collections to be lower in warmer months but higher in spring in Øresund, Sweden. This was in contrast to the findings of Harrison and Durance (1989) who reported lower levels of phenolics in cooler months and increases in concentrations at the end of the warm growing season in Roberts Bank, British Columbia. A likely explanation for these patterns could be that these populations are at the thresholds of their geographic ranges where summer appears to be more stressful for one population of *Z. marina* (Harrison and Durance 1989) and winter more stressful for others (Ravn et al. 1994, Vergeer et al. 1995). Both respiration and photosynthesis are influenced by temperature which in turn influences the balance of C and N in plants. In addition, the enzymatic biosynthesis of phenolics is directly impacted by temperature, and each enzyme can have different optimum temperatures (Waterman and Mole 1994). This is further evidenced in the different responses in each morphotype of *Z. capensis* to warming, and highlights the phenotypic acclimation of this seagrass to cope with heat stress.

The carbon-nutrient balance (CNB) hypothesis applies to traits of resistance such as chemical defence in plants and could explain the observed patterns in C, N and GA levels in *Zostera capensis*. It was initially proposed by Bryant et al. (1983) to determine the effects of shading and N on phenotypic variation in the production of secondary metabolites. The CNB hypothesis states that if plant carbohydrate concentrations exceed that which is required to maintain growth, the excess C will be invested in defence i.e. secondary metabolites, in low N and high light conditions. The hypothesis further proposes that growth is limited more than photosynthesis when nutrient levels are low, thereby allowing for nutrient-limited plants to store carbohydrates which increase plant C:N ratios. Excess carbohydrates are then channelled into C-based secondary metabolites (Bryant et al. 1983). The opposite is then inferred for high nutrient conditions where growth is prioritised, and as a result C:N ratios decrease along with C-based secondary metabolites, while the accumulation of excess N may be allocated to N-based secondary metabolite production (Bryant et al. 1983). Resistance variations observed in phenotypes of *Z. capensis* could therefore be the result of varying concentrations of nutrients available for growth and secondary metabolite production.

Studies on macroalgae, aquatic and terrestrial plants have identified the link between phenolic production and excess organic C as compared to N, corroborating the theory that phenolics are produced when N is limited (Bryant et al. 1983, Buchsbaum et al. 1990, Herms and Mattson 1992). This could then explain the low phenolic content in LLM since C levels had increased under warming while GA concentrations had reduced. The inverse relationship observed between N and GA in LLM was also observed in *Zostera marina* where concentrations of phenolics and N were inversely related following a mesocosm experiment with different substrata (Buchsbaum et al. 1990). Increasing levels of N have been known to promote growth and reproduction as well as survival in some seagrasses and algae, while growth rates are often severely reduced when N is limited (Lapointe 1984, Williams and Ruckelshaus 1993, Invers et al. 2004, Li et al. 2008). Nitrogen levels had increased under warming in LLM implying that N may have been limited in my experiment.

A plant's fitness is strongly correlated with its capacity to accumulate and store resources, and SLM displayed greater fitness compared to LLM based on its ability to store C, N and GA under warming. Natural selection has influenced patterns in allocation of resources so as to maximise growth and fitness in different environments, within genetic limitations (Stearns 1989). The production of secondary metabolites requires an extensive allocation of energy by seagrasses (Ravn et al. 1994), therefore the temporal and spatial distribution of metabolites within and between plants, often vary in ways that are adaptive. Since C is seldom limited relative to N, the direct cost of allocation of resources towards C-based defence chemicals remains unclear, however, considerable costs could be related to the synthesis, storage and breakdown of compounds (Hay 1988). Production and maintenance of the unique enzymes required to generate and store defence compounds might require greater energy cost than producing the compounds themselves (Hay 1988). Plants or specific parts of plants that are at highest risk are often best defended by the allocation of chemical compounds to only those particular parts of value, such as for growth and reproduction, as evident in the highest concentrations of chemical compounds found in youngest leaves of *Posidonia oceanica* (Agostini et al. 1998). This strategy influences within plant growth patterns, while variations in chemistry between conspecific plants as well as plant populations contributes to between population growth variability (Duffy and Hay 1990, Gong and Zhang 2014), a trait to be further explored in *Zostera capensis* populations.

Gallic acid levels were significantly different between morphotypes of *Z. capensis* in in heated and unheated treatments, with SLM plants containing higher levels of GA compared to LLM. Between-plant variation in specific secondary metabolites as well as their concentrations have been observed in individual seaweeds growing only meters apart, but also in populations of plants from different habitat types (Paul and Hay 1986, Paul and van Alstyne 1992). In one example, variation in the type and concentration of compounds was suggested to be as a result of seaweeds growing on reef-slope habitats being more susceptible to intense herbivory which therefore produced greater quantities of more potent defensive chemicals compared to populations of the same species in moderate herbivory habitats (Hay 1988, Paul and Hay 1986). Ragan and Glombitza (1986) observed salinity and tidal exposure to alter phlorotannin levels in brown seaweeds, generating variability among plants along an exposure gradient, while concentrations of secondary metabolites in the red algae, *Neorhodomela larix*, growing in tide pools were reported to be double that of plants growing intertidally (Temporal et al. 1982). The observed variation in GA concentrations between *Z. capensis* morphotypes and populations is therefore not atypical.

Phenotypic plasticity has been recognized as a key factor in plant acclimation since plasticity can induce the most adaptive phenotype in response to a particular environment, thereby stifling the effects of variation in resources both spatially and temporally (Stearns 1989, West-Eberhard 1989, Farrar and Williams 1991, Sultan 1995). Plasticity traits in plants are displayed in leaf size and area, biomass production, growth rates, ratio of shoots to roots, nutrient uptake rates and production of secondary metabolites (this study, Bryant et al. 1983, Sultan 1995). Despite these sometimes radical physical and biochemical alterations to maximise function in unfavourable or resource-deficient environments, phenotypic plasticity may not reflect limitations in growth, and has been contested to represent true adaptation (Stearns 1989, Sultan 1995). The two morphotypes of *Zostera capensis* were not observed to co-occur at the same sites at Langebaan, and average leaf sizes and shoot densities were consistently different between Oesterval and Geelbek (Chapter Two) giving no indication that the morphological expressions were not solely as a consequence of local environmental conditions. Phenotypic acclimations as observed in *Zostera capensis* in Langebaan Lagoon may well have given rise to alterations of the genome (Phair et al. 2019) and reflect permanent adaptive traits however these differences remain to be explicity resolved.

Phenotypic plasticity in secondary metabolism, may be as a response to limited resources, and represents the interaction of environment and genotype. It also represents the key genetic variation required for evolutionary adaptation of phenotypic plasticity (Stearns 1989, Berenbaum 1995, Sultan 1995). For a response to be deemed adaptive rather than a subsidiary and passive expression in response to environmental conditions, changes in the plant need to be genetically based (Smith-Gill 1983). This has not been resolutely established for the case of secondary metabolism, since variability in secondary metabolism is correlated with the balance in plant C and N levels (Bryant et al. 1983, Herms and Mattson 1992).

Balancing C and N requires a highly regulated biosynthetic process prompted by environmental cues, rather than inadvertent responses to variations in environment (Lavola et al. 2000, Touchette and Burkholder 2000, 2007). Phenotypic response to environmental variation may buffer the effects of other pressures such as herbivory and fouling, which favour particular configurations in C allocation for each environmental scenario. For widely distributed plants that cover multiple habitats, different pressures of selection that vary in importance, may facilitate the evolution towards a stable polymorphism that balances allocation of resources to growth and differentiation. These populations may therefore be more resilient to stress and extreme environmental change.

5.5. Conclusions

In this chapter, I investigated the photosynthetic, morphometric and nutrient responses in two morphologically different populations of *Zostera capensis* to simultaneous warming and grazing, and further explored the influence of temperature and grazing on algal epiphytes. Despite the low replication in phenolic abundance measurements, this study presents the first recording of specific phenolics i.e. gallic acid (GA) in the leaves of *Z. capensis*. A previous study measured total phenolics in *Z. capensis* from Shimo la Tewa (Kenya) and Durban (South Africa) without specifying the exact compounds present (McMillan et al. 1980). Future research on phenolics in *Z. capensis* would benefit from larger sizes of experimental units so that adequate quantities of seagrass biomass can be assessed. In addition, measurements taken at multiple time scales would be useful to test if treatment length is a limiting factor, since other studies that measured seagrass and seaweed phenolics, did so after two and four weeks in treatment (Pavia and Toth 2000a, 2000b, Steele and Valentine 2012, 2015).

While morphometric responses to warming/grazing treatments were generally similar in the two morphotypes with the exception of biomass and photosynthetic rate, responses in nutrient and secondary metabolites differed. GA, C and N were higher in small-leaved morphotypes (SLM) compared to large-leaved morphotypes (LLM). These findings suggest variation in phenotypic responses of *Zostera* *capensis*, and provide insight into their chemical and physiological responses to heat stress. Variations observed in C, N and GA production in morphologically divergent populations imply differing acclimation and potentially adaptation strategies to cope with stressors, including fouling and grazing.

A plant's fitness has been strongly correlated with its capacity to attain and store nutrients demonstrating a greater fitness in SLM populations of *Zostera capensis*. The energy requirements of chemical defence imply a divergence of nutrients away from growth and reproduction, and suggests that in the absence of stress from herbivory or environment factors, populations may produce less defence chemicals and display enhanced growth. This rationale can be applied to the varying populations of *Z. capensis* in Langebaan Lagoon. Environmental conditions sustained by seagrasses at Geelbek (Chapter Two) could explain their higher concentrations of secondary metabolites. The energy requirements in response to stress from high irradiance, desiccation and grazing appear to be at the cost of growth in high shore populations.

The results of this study suggest that *Zostera capensis* meadows and the associated ecosystem services they provide will be negatively affected by the continuing rise in summer temperatures and temperature extremes. *Z. capensis* populations at Oesterval are currently not subjected to the same environmental conditions, and energy assigned to chemical defence is lower (this study, Appendix IV and V). The larger form variation is evidence that more energy is allotted to growth. *Z. capensis* beds at Oesterval support a greater diversity of grazers compared to Geelbek (Chapter Three) but have been reduced to fewer patches (pers. observation). Given the low concentration of phenolic compounds in meadows at Oesterval, this population is likely to be less resilient and more susceptible to stressors such as competition for example with *Gracilaria*, fouling, herbivory and disease. Further investigation to unravel the extent to which phenolics influence other stressors in the seagrass ecosystem in Langebaan Lagoon will be highly valuable in predicting further causes of variation and declines in the population.

Phenotypic and genotypic diversity may provide varying responses in maintaining the functioning of ecosystems, and prove to be a key factor for acclimation and adaptation to climate change. Further understanding of population adaptations of *Zostera capensis* will benefit from investigations on genetic responses to stress, and observations from transplant experiments that document phenotypic responses across its tropical and temperate ranges.

CHAPTER SIX

General Discussion

In a rapidly changing world where environmental trends deviate further away from long-established patterns, understanding strategies that allow habitats to persist under sub-optimal conditions is critical for effective management of human impacts, and the maintenance of ecosystem services. This has been essential for seagrasses which provide coastal protection, and a key habitat and nursery area for species of importance to society (Jackson et al. 2001, Vonk et al. 2008, De la Torre-Castro et al. 2014). Seagrass ecosystems are highly variable and are on a global decline (~7% per annum) due to natural and anthropogenically-induced pressures (Orth et al. 2006, Duarte 2009, Waycott et al. 2009). Understanding the resilience, adaptability and capacity to persist under stress is therefore essential to ensuring ongoing functioning and productivity of these habitats.

In South Africa, the dwarf eelgrass, *Zostera capensis* forms monospecific populations supporting important estuarine and coastal habitats. Seagrass habitats have been declining, and in some cases completely lost, from several estuaries around the coast (van Niekerk et al. 2012, Adams 2016). A remaining seagrass ecosystem is found in Langebaan Lagoon, a marine protected area on the west coast of South Africa. Decadal observations (Pillay et al. 2010) as well as more recent distributional assessments (Adams 2016) have identified high variation in seagrass cover in the lagoon. A deeper understanding of the spatial and temporal factors influencing seagrass population distribution at the lagoon scale was needed, along with understanding the associated faunal communities that define this system. Further investigations on trophic interactions particularly when seagrasses are under stress, were also important to understanding resilience in *Zostera* populations, with particular interest in adaptations and plasticity under predicted warming scenarios. The findings from this study have established a foundation on which to further research and monitoring of *Zostera* habitats in Langebaan Lagoon as well as seagrass ecosystems worldwide.

6.1.1. Distribution of Seagrass Ecosystems

Variations in seagrass performance parameters (densities, biomass and leaf morphometrics) were significantly influenced by the interaction of seasonal and local environmental factors (particularly exposure, temperature and turbidity) as described in Chapter Two, and highlighted the effects of supra-optimal (summer) temperatures on seagrass growth. Although these results are correlative and do not demonstrate causation, which is determined primarily through experimental observations, they are commonly observed in temperate seagrass ecosystems that display seasonal variability, where seagrass growth peaks in spring/early summer and declines in peak summer and winter (Duarte 1989, Olesen and Sand-Jensen 1994, Watanabe et al. 2005,). The harmful effects of heat stress on biological and physiological processes in seagrasses are well documented (Short et al. 1999, Pergent et al. 2014, Duarte et al. 2018). Understanding variation, determining thresholds, and within and between population responses to a changing environment is fundamental to our knowledge of ecosystem function, and key to how we respond to ecosystem shifts.

Identifying optimal temperature ranges that influence seagrass growth is an important factor in understanding seasonal drivers of patterns in performance. For *Zostera capensis* this range is between 15-20^{o}C, measured by Edgcumbe (1980) in laboratory cultures, and is similar to that of other temperate seagrasses (Lee et al. 2007). Variables such as nutrients and light are also critical to photosynthesis and growth with unique optimal ranges, and combined with temperature, are important factors influencing variation in seagrass distribution (Lee et al. 2007, Kim et al. 2012). Growth and distribution of seagrasses are therefore dependent on a narrow range of optimal conditions. The adaptability of seagrasses to grow under these varying conditions is a key feature of their capacity to acclimate (Duarte et al. 2006, Lee et al. 2007) however, global warming is expected to bring about shifts in temperature regimes beyond the thresholds of many seagrass species (Jordà et al. 2012, Olsen et al. 2012, Koch et al. 2013, Collier and Waycott 2014,).

Seagrass growth is highly influenced by site specific conditions and interactions between abiotic and biotic factors (Lee et al. 2007, Kim et al. 2012) that largely determine their phenotypic expressions. In Langebaan Lagoon, *Zostera* beds closer to the lagoon mouth had a distinct morphology (long, wide leaves in sparse beds), that differed from populations closer to the head of the lagoon (small, narrow leaves in dense beds). These form variations are likely related to emergence (i.e. the period when plants are exposed to air during low tide) since large-leaved populations have been shown to occur at subtidal and deeper intertidal sites while small-leaved populations occupy higher intertidal zones (Pérez-Lloréns and Niell 1993, Peralta et al. 2000, Cabaço et al. 2009). Large upright leaves are difficult to flex downwards in order to remain moistened by sediment pore water, while high shore populations form denser stands with overlapping leaves that minimise desiccation (Björk et al. 1999) – coping strategies observed in *Zostera* populations in Langebaan.

The ability to adapt and/or acclimate to particular environments is an important evolutionary trait and key indicator of a species resilience and ability to function in sub-optimal conditions. While adaptation refers to an immutable genetic state, acclimation, otherwise known as plasticity, is a short-term, immediate response that allows a population to maintain function only to revert to its original state when environmental conditions are optimal (Merilä and Hendry 2014). However, in the case where sub-optimal conditions prevail, phenotypic expressions can lead to alterations at the genome level (Duarte et al. 2018). An assessment of genomic variation in small and large-leaved morphotypes of *Zostera capensis* in Langebaan Lagoon found the two populations to differ in frequency variation of outlier loci and nucleotide diversity although not significantly so (Phair et al. 2019). Within site variation showed populations to be highly clonal with low genomic diversity (Phair et al. 2019). Gene flow between populations in the lagoon are assumed to be high (Phair et al. 2019) and morphotypic differences between high and low shore populations are likely attributed to phenotypic responses rather than genetically driven. The genetic basis for the observed acclimation strategies in *Zostera* remain to be confirmed. Since most variation and decline have been observed in large-leaved populations, these plastic expressions provide insight into the adaptability and coping strategies of *Zostera,* and the importance of understanding intrinsic variability that maintains productivity and functioning.

Although there were rarely more than five *Zostera capensis* beds at Klein Oesterval that collectively produced an average seasonal coverage in the lagoon of 16.04% (similar to that reported by Pillay et al. 2010), shoot density and aboveground biomass were similar to that of beds at Oesterval which often had >20 beds and greater (21.31%) average seagrass cover (Chapter Two). This observation suggests that even though some sites have low seagrass cover (attributed to possible human disturbance, Pillay et al. 2010), productivity per meter square may still be on par with sites with higher coverage.

6.1.2. Biodiversity in Seagrass Ecosystems

Given the high primary productivity in seagrass habitats from seagrasses and their algal epiphytes (Hillman et al. 1995, Rasheed et al. 2008), macrofaunal communities are often dominated by grazing invertebrates that feed on periphyton, micro- and macroalgae attached to seagrass leaves (Blanchet et al. 2004, Barnes 2013). The structuring influence of seagrasses is derived from the fact that plants with greater foliar biomass provide more shelter, protection and available food than plants with less surface area per unit biomass (Heck and Orth 1980, Orth et al. 1984). Seagrass structure (biomass, density and leaf size) is a significant determinant of macro-epifaunal community structure, which is further influenced directly and indirectly by environmental factors (Chapter Three). While environmental effects might result in direct changes in macro-epifauna physiology and behaviour, indirect influences occur via environmental effects on seagrasses themselves (Chapter Two).

The temporal and spatial ‘net effect’ of environmental conditions can be partitioned into direct and indirect effects using structural equation modelling (SEM) (Chapter Three). SEM results revealed shoot density and leaf width to produce strong direct effects on macro-epifaunal abundances along with turbidity and oxygen. Indirectly, temperature, pH and exposure negatively affected macro-epifaunal abundance through their direct effects on leaf width and shoot densities. Despite the absence of a direct effect of temperature on macro-epifaunal abundance, temperature had a direct effect on seagrass structure and was a significant predictor of five of the six seagrass metrics measured (Chapter Two). Indirect effects have been shown to influence community structure as significantly as direct effects (Wootton 2002), and it is likely the relationship between variability in macro-epifaunal abundance in Langebaan Lagoon is a result of both a direct physiological effect of temperature as well as the indirect effect of temperature on the supporting seagrass ecosystem (Marbà and Duarte 2010).

In Langebaan Lagoon, large-leaved (LLM) populations closer to the mouth supported a higher species diversity and richness and generally low abundances of macro-epifaunal invertebrates than small-leaved (SLM) populations. The gastropod, *Assiminea globulus* and the critically endangered endemic limpet, *Siphonaria compressa* were dominant in SLM beds. These are mainly desiccation resistant species that can survive at high intertidal sites, which supported a lower species richness and diversity compared to low intertidal seagrass habitats (Chapter Three). Understanding the influence of environmental variables on seagrass habitats, and the consequent influence on associated macrofauna is important especially with regards to *S. compressa* which is contained in only two localities in South Africa – the Knysna estuary and Langebaan Lagoon (mainly Geelbek and Bottelary). *S. compressa* is closely associated with *Z. capensis* abundance and approached extinction twice when seagrass cover had all but disappeared (Angel et al. 2006).

Another interesting finding in my study was the occurrence of the cushion star *Parvulastra exigua* and limpet *Fissurella mutabilis* which were *previously* reported to have completely declined at Klein Oesterval (Pillay et al. 2010). A further three species (*Pyura stolonifera*, *Sargartia ornata* and *Oxystele antoni*) not previously documented (Pillay et al. 2010) were also found at this site in my study. These results elucidates the importance of seagrass structure on faunal diversity, and the likelihood of recruitment of macrofauna into re-established seagrass habitats where seagrass cover had effectively been lost. This study further highlights the importance of undertaking regular surveys of seagrass habitats to acquire a clearer understanding of variability and macrofaunal community dynamics.

6.1.3. Influence of Temperature on Seagrass Ecosystems

Empirical data described in Chapters Two and Three provide narratives on some of the key relationships that underpin seagrass variability in Langebaan Lagoon. Mesocosm experiments were a logical next step. These enclosed experimental systems provide a compromise between laboratory microcosms and macrocosms found in nature (Odum 1984), and allowed for specific environmental variables to be manipulated – a feat not always possible in field experiments. I examined morphological, physiological and biochemical responses to different temperatures in an indoor mesocosm experiment to understand fitness in morphologically divergent populations of *Zostera capensis* (Chapter Four). Greater plasticity plays a key role in a population's resilience and ability to persist under stressful conditions (Sultan 1987, 1995, Stearns 1989), and this theory was explored experimentally in *Z. capensis*. I tested the hypothesis that small-leaved morphotypes show better performance (aboveground biomass, leaf morphometrics and density) and therefore greater resilience under heat stress than large-leaved morphotypes, in order to predict the potential for expansion of seagrass stands in Langebaan Lagoon in scenarios of increasing temperature.

Generally, performance parameters increased at 22°C with significant declines observed in supra-optimal (26°C and 30°C) temperatures in the two morphotypes (Chapter Four). The hypothesis was refuted based on the seagrass parameters measured in the first experiment since the two morphotypes displayed similar responses in biomass and leaf size following prolonged exposure to four temperature treatments (18, 22, 26 and 30°C). However, the hypothesis was supported in the second mesocosm experiment (Chapter Five), which showed that small-leaved morphotypes had higher photosynthetic rates as well as a greater propensity to store resources (C and N) transcending their ability to contend with heat stress compared to large-leaved forms. These findings allude to this seagrass's ability to adapt to local environmental conditions.

Despite a broad thermal range of many species of *Zostera* that extend from temperate to tropical waters, their temperature optima are lower than that of other tropical seagrass species, observed by the substantial declines at supra-optimal temperatures (Lee et al. 2007, Kim et al. 2012, 2016). Edgcumbe's (1980) measurements of growth in *Z. capensis* that reported declines above 26°C were observed from subtropical populations north of Durban, South Africa. This suggests that regardless of its tropical range, *Z. capensis* tolerance to thermal stress is limited, potentially confining populations to specific habitat and environmental contexts (Paula et al. 2001).

Seagrass species are able to up-regulate their stress responses to cope with lethal and sub-lethal extremes (Koch et al. 2013). The effects of prolonged or extreme thermal stress on seagrasses are varied, with observations usually carried out after an event, making it problematic to link physiological responses to mortality (Mayot et al. 2005, Marbà and Duarte 2010, Rasheed and Unsworth 2011). My study provides a key insight into the response to prolonged heating of a broad ranging seagrass living on the (western) edge of its distributional range, and its likely response to climatic related stressors such as heatwaves. High thermal stress in seagrasses can potentially compromise ecosystem integrity as it increases their vulnerability to other stressors such as pathogens and herbivory (Vergeer et al. 1995).

6.1.4. Temperature Effects on Trophic Interactions

A general observation was that small-leaved morphotypes displayed greater fitness compared to large-leaved plants based on their ability to store resources (C and N) when heated (Chapter Five). In addition, the higher photosynthetic rates in SLM imply that these populations are better able to maintain productivity compared to LLM populations under thermal stress. Low concentrations of phenolic compounds in seagrasses as well as their propensity to algal fouling suggest lower resilience and thus greater susceptibility to, for example, disease (Grignon-Dubois and Rezzonico 2012, Sieg and Kubanek 2013). Large-leaved populations may therefore be at higher risk of decline compared to their small-leaved counterparts. This has indeed been observed in Langebaan Lagoon, where the most seagrass variability and loss have been reported for LLM populations closer to the lagoon mouth, while variation in SLM populations remain negligible (Pillay et al. 2010; Adams 2016, Chapter Two). LLM populations support a different suite of macro-epifauna than SLMs, and the loss of LLM populations is therefore likely to lead to changes in biodiversity and trophic structures associated with seagrass habitats in the lagoon.

The presence of the putative grazer, *Siphonaria compressa,* produced a significant and positive effect on total seagrass biomass, while the interaction of grazing and temperature also had significant positive effects on leaf area and effective quantum yield (Chapter Five). Positive effects of grazing on seagrass performance have been reported for several seagrass species particularly in mitigating the effects of temperature (Hughes et al. 2004, Baggett et al. 2010, Short et al. 2011, 2016) and these effects were marginally supported experimentally in this study. The extent to which grazer presence influenced responses in *Zostera capensis* morphotypes was largely as a consequence of variation in biomass and leaf sizes. Investigations into the reciprocal effects of seagrass structure and grazing on morphotypes separately might further elucidate the observations in this experiment.

The presence of the grazing limpet did not significantly influence epiphyte biomass however, despite studies alluding to *Siphonaria compressa*, feeding on epiphyton (Allanson and Msizi 2010), there is a lack of direct evidence that this limpet feeds exclusively on algae and not on seagrasses, so these results should be interpreted with caution. Epiphytes generally proliferated under warming conditions for the two morphotypes despite the presence of the limpet and particularly in unheated treatments. A possible explanation for this is the organic enrichment provided by mucus secreted by gastropods to aid in locomotion, but which inadvertently trap bacteria, diatoms and algae (Davies and Beckwith 1999), thus supporting algal growth. Moreover, grazer stocking densities (five) per experimental unit may have been insufficient to control the unpredicted extent of algal growth in heated treatments. Examples of increases in grazer stocking densities that were ineffectual in reducing algal biomass have been previously reported (Peterson et al. 2007), while warming has been documented to strengthen (O'Connor 2009) and weaken grazing effects (Eklöf et al. 2012). Follow up investigations on grazing effects need to explore the use of alternate and multiple grazer species to further clarify the influence of grazing on epiphytes associated with *Z. capensis*.

While similar trends in seagrass patterns, macro-epifaunal diversity as well as phenotypic strategies to cope with environmental stress, have been observed in other temperate systems (Olesen and Sand-Jensen 1994, Thom et al. 1995, Barnes

2010), they cannot be extrapolated to all systems, and investigations that enhance understanding of ecosystems at local scales is crucial to informing mitigation and restoration measures. The findings of this study establish the importance of understanding patterns of variability in seagrass habitats as well as a population's adaptive and acclimatory strategies in response to thermal stress. Resolving the physiological, morphological and biochemical responses to temperature stress, further informs patterns in distribution and productivity, which are vital for important habitats if we are to prepare for and adapt to change in our natural environments, while ensuring important ecosystem services are maintained.

6.2. Recommended Future Research

This research provides critical understanding of the ecology of the seagrass habitats in Langebaan Lagoon, and forms a foundation on which to structure future research elsewhere. The decline in seagrass meadows in many estuaries and on coastlines denotes shifts in ecosystem stability and the likelihood of seagrass recovery into those ecosystems can inform research and restoration efforts.

Additional questions that could provide further understanding of factors influencing seagrass distribution, growth and decline include the influence of sediment particle size and movement in the lagoon. This is especially important in systems subjected to human disturbance from trampling, dredging, and excavation of bait species, as well as natural disturbance from bioturbators (e.g. sandprawns) that impact sediment dynamics and compete with seagrasses for space (Siebert and Branch 2006). Effects on seagrass habitats as a result of smothering and potential competition with macroalgae such as the red gracilarioid alga (Rothman et al. 2009) should also be investigated.

The proposed expansion and economically-driven developments in Saldanha Bay are an imminent threat to the integrity of the Langebaan Lagoon system. Future developments include the expansion of aquaculture to include oyster, mussels and finfish farms which is likely to increase contaminants, organic enrichment, and the introduction of alien species into the lagoon (Griffiths et al. 2009). Further proposed developments include the construction of an oil pipeline as well as urban and tourism infrastructure (Clark et al. 2016). Establishing a monitoring programme designed to detect change at appropriate temporal and spatial scales has thus become paramount, and is a key requirement to inform environmental impact assessments and management plans.

Monitoring protocols should include *in situ* instruments such as loggers and CTD's (Conductivity Temperature Depth) that continually measure key variables like temperature, salinity, pH and oxygen, along with traps to assess sediment dynamics. Regular column and pore water monitoring of nutrients are also essential. Evaluations of biotic variables that include population estimates of infauna and epifauna, should be carried out biannually using standard methods for seagrass, fauna and sediment, while assessments of higher trophic levels can include trawl surveys, visual counts and catch records. Remote surveys including the use of aircraft and drones as well as satellite and bathymetric LiDAR (Light Detection and Ranging) can deliver a high sampling frequency and extensive geographic coverage of data long-term (Proença et al. 2017). These corroborated with field observations can provide high resolution information (Valle et al. 2014) and a greater understanding of variability in seagrass systems.

My study has provided an understanding of key ecological factors influencing seagrass habitats, and highlights the importance of identifying characteristics and acclimation strategies that allow habitats to persist under climate change, to maintain productivity and support biodiversity. These results can assist in ensuring the functioning of seagrass ecosystems, and the safeguarding of services they provide to society.

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Appendix I: Pearson 2-tailed correlations between seagrass morphological metrics and environmental variables differentiating responses based on a 3 month seasonal average and 1 month lag preceding seagrass sampling. Correlations significant at α < 0.05 are in bold.

	3 Month seasonal average						1 Month lag					
	Temp	Sal*	Turb**	pH	O_2	Chl *a*	Temp	Sal*	Turb**	pH	O_2	Chl *a*
Seagrass biomass	**-0.53**	**-0.25**	**-0.23**	**-0.53**	**-0.28**	**-0.46**	**-0.45**	-0.09	**-0.23**	**-0.40**	**-0.37**	**-0.45**
Shoot density	0.15	0.21	**0.67**	-0.24	**0.49**	**0.31**	0.08	**0.30**	**0.59**	**-0.34**	**0.31**	0.19
Blade density	0.18	0.19	**0.61**	-0.17	**0.41**	**0.25**	0.06	**0.25**	**0.52**	**-0.26**	**0.39**	0.11
Leaf length	**-0.51**	**-0.55**	**-0.53**	-0.02	**-0.47**	**-0.50**	**-0.37**	**-0.46**	**-0.44**	0.08	**-0.58**	**-0.42**
Leaf width	**-0.41**	**-0.47**	**-0.56**	-0.09	**-0.53**	**-0.56**	**-0.37**	**-0.39**	**-0.51**	0.02	**-0.61**	**-0.50**
Epi-algal biomass	0.05	-0.05	-0.18	0.04	**-0.24**	-0.14	-0.01	0.08	-0.20	-0.04	-0.14	-0.19

*Sal = Salinity
**Turb = Turbidity

Appendix II: Mean ±1SE of seagrass morphological metrics and epiphyte biomass collected from five sites in Langebaan Lagoon, across four seasons. Seasonal and yearly totals are also provided.

	Spring	**±SE**	**Summer**	**±SE**	**Autumn**	**±SE**	**Winter**	**±SE**	**Yearly Total**
Shoot Density (m^{-2})									
Centre Bank	247.26	19.50	80.21	6.46	256.77	28.41	239.79	16.91	195.44
Klein Oesterval	418.64	34.51	251.25	42.02	299.00	30.88	355.02	29.51	343.50
Oesterval	667.94	37.13	323.86	45.49	182.48	10.34	444.42	36.12	412.47
Bottelary	929.04	112.05	431.42	65.28	800.23	62.73	816.57	68.35	744.31
Geelbek	1098.04	58.12	177.11	17.99	1006.50	48.04	795.90	34.96	769.39
Seasonal Total	653.08		243.55		457.51		527.41		480.11
Leaf Length (mm)									
Centre Bank	242.01	11.43	343.68	14.22	343.45	11.25	226.39	7.22	289.87
Klein Oesterval	116.95	7.03	119.81	10.74	199.94	21.84	213.47	11.94	156.03
Oesterval	144.41	8.16	184.56	12.81	223.15	14.09	209.13	9.82	190.61
Bottelary	92.08	5.78	75.33	3.96	157.39	8.88	130.93	6.68	113.93
Geelbek	55.86	2.66	50.83	1.44	81.61	3.20	87.92	3.13	69.06
Seasonal Total	135.52		167.39		197.36		176.80		167.86
Leaf width (mm)									
Centre Bank	2.12	0.04	2.18	0.03	2.12	0.04	1.80	0.02	2.08
Klein Oesterval	1.58	0.05	1.32	0.06	1.66	0.08	1.73	0.03	1.58
Oesterval	1.53	0.03	1.70	0.06	1.90	0.05	1.70	0.05	1.71
Bottelary	1.35	0.07	1.11	0.03	1.27	0.03	1.21	0.04	1.24
Geelbek	1.04	0.03	0.98	0.02	1.09	0.04	1.04	0.02	1.04
Seasonal Total	1.54		1.52		1.65		1.52		1.56
Aboveground biomass (g dry wt m^{-2})									
Centre Bank	10.12	1.11	3.96	0.32	18.01	1.48	8.94	0.84	9.45
Klein Oesterval	7.02	0.93	3.88	0.89	10.36	1.56	11.61	1.34	8.04
Oesterval	11.41	0.95	6.31	0.60	7.84	0.69	12.26	0.47	9.72
Bottelary	10.04	1.59	2.46	0.34	10.86	0.77	8.67	0.74	8.01
Geelbek	4.97	0.55	0.69	0.22	6.77	0.53	4.72	0.26	4.29
Seasonal Total	9.10		3.76		9.60		9.74		8.13
Epiphytic algal biomass (g dry wt m^{-2})									
Centre Bank	7.61	1.39	2.25	0.81	0.45	0.21	0.00	0.00	3.17
Klein Oesterval	1.53	0.84	4.27	1.15	0.64	0.22	0.33	0.13	1.67
Oesterval	4.82	0.87	1.86	0.68	1.01	0.28	0.00	0.00	1.93
Bottelary	0.81	0.55	7.87	2.33	0.00	0.00	0.00	0.00	2.17
Geelbek	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Seasonal Total	3.44		2.64		0.55		0.04		1.73

Appendix III: Results from structural equation modelling examining the effects of six environmental factors on six seagrass metrics and macrofauna abundance. One model path is represented on each row, with arrows indicating the direction of effect. Each line indicates one path in the model, with arrows showing direction of effect. Significant path coefficients ($p = 0.05$) are in bold. Chi-square of the full model = 256.66, df = 15, p = <0.001, RMSEA = 0.32. Stand. est.: standardized estimate; unstand. est.: unstandardized estimate; C.R: Critical Ratio.

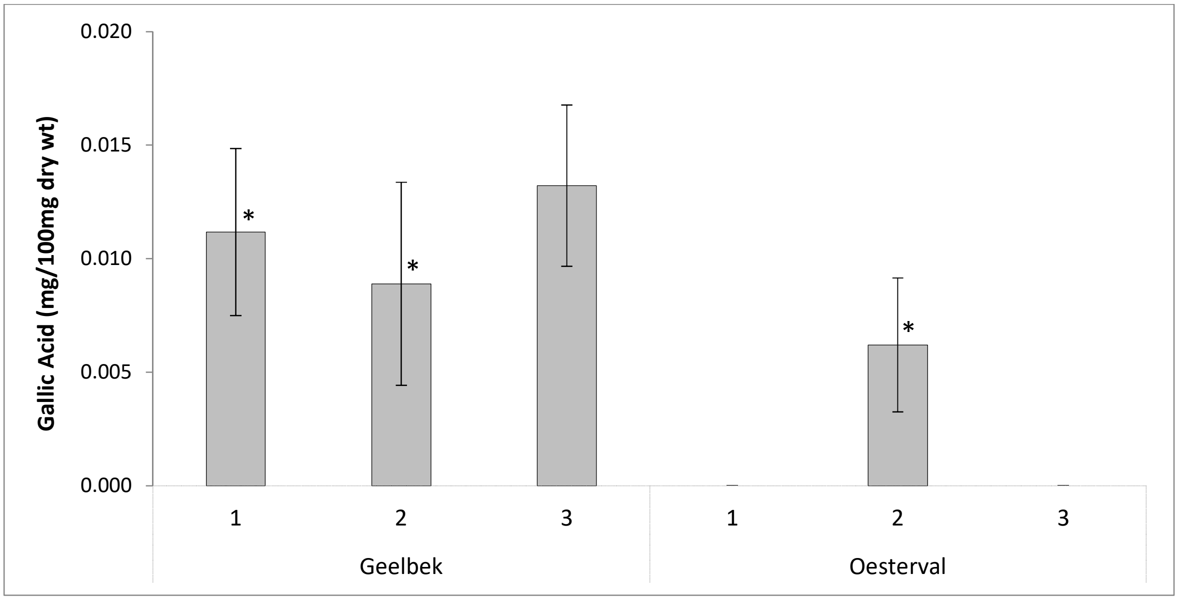
Model Path			**Stand. Est.**	**Unstand. Est.**	**SE**	**C.R.**	***P***
Shoot Density	←	Temperature	0.31	0.048	0.694	0.446	0.655
Seagrass Biomass	←	Temperature	-0.019	-0.215	0.012	-1.59	0.112
Leaf Length	←	Temperature	-13.954	-0.645	2.188	-6.378	**<0.001**
Leaf Width	←	Temperature	-0.03	-0.28	0.011	-2.686	**0.007**
Epi-Algal Biomass	←	Temperature	0.022	0.329	0.011	2.005	**0.045**
Chlorophyll a	←	Temperature	-0.044	-0.14	0.044	-1.001	0.317
Shoot Density	←	Salinity	0.211	0.011	1.594	0.132	0.895
Seagrass Biomass	←	Salinity	0.076	0.293	0.027	2.779	**0.005**
Leaf Length	←	Salinity	15.566	0.245	5.023	3.099	**0.002**
Leaf Width	←	Salinity	0.028	0.088	0.026	1.079	0.281
Epi-Algal Biomass	←	Salinity	-0.054	-0.277	0.025	-2.167	**0.03**
Chlorophyll a	←	Salinity	-0.287	-0.31	0.101	-2.85	**0.004**
Shoot Density	←	pH	-9.638	-0.234	3.887	-2.48	**0.013**
Seagrass Biomass	←	pH	-0.222	-0.394	0.066	-3.336	**<0.001**
Leaf Length	←	pH	61.585	0.445	12.245	5.029	**<0.001**
Leaf Width	←	pH	0.203	0.295	0.063	3.23	**0.001**
Epi-Algal Biomass	←	pH	0.005	0.011	0.061	0.078	0.938
Chlorophyll a	←	pH	0.231	0.114	0.246	0.939	0.348
Shoot Density	←	Turbidity	2.856	1.251	0.502	5.694	**<0.001**
Seagrass Biomass	←	Turbidity	-0.007	-0.22	0.009	-0.8	0.424
Leaf Length	←	Turbidity	-6.802	-0.885	1.58	-4.304	**<0.001**
Leaf Width	←	Turbidity	-0.024	-0.63	0.008	-2.968	**0.003**
Epi-Algal Biomass	←	Turbidity	0.008	0.351	0.008	1.054	0.292
Chlorophyll a	←	Turbidity	0.215	1.923	0.032	6.787	**<0.001**
Shoot Density	←	Oxygen	-2.469	-1.427	0.369	-6.695	**<0.001**
Seagrass Biomass	←	Oxygen	0	-0.001	0.006	-0.002	0.998
Leaf Length	←	Oxygen	3.071	0.528	1.162	2.643	**0.008**
Leaf Width	←	Oxygen	0.01	0.335	0.006	1.624	0.104
Epi-Algal Biomass	←	Oxygen	-0.006	-0.361	0.006	-1.117	0.264
Chlorophyll a	←	Oxygen	-0.124	-1.463	0.023	-5.32	**<0.001**
Seagrass Biomass	←	Exposure	0.107	0.261	0.034	3.105	**0.002**
Leaf Length	←	Exposure	37.751	0.377	6.318	5.975	**<0.001**

Model Path			**Stand. Est.**	**Unstand. Est.**	**SE**	**C.R.**	***P***
Leaf Width	←	Exposure	0.291	0.586	0.032	9	**<0.001**
Epi-Algal Biomass	←	Exposure	0.105	0.339	0.032	3.323	**<0.001**
Chlorophyll a	←	Exposure	-0.234	-0.16	0.127	-1.848	0.065
Shoot Density	←	Exposure	-16.975	-0.570	2.005	-8.464	**<0.001**
Macro-epifauna Abund.	←	Shoot Density	2.616	0.735	0.518	5.049	**<0.001**
Macro-epifauna Abund.	←	Seagrass Biomass	-2.104	-0.008	30.287	-.069	0.945
Macro-epifauna Abund.	←	Leaf Length	0.069	0.065	0.164	0.417	0.676
Macro-epifauna Abund.	←	Leaf Width	28.969	0.136	32.103	0.902	0.367
Macro-epifauna Abund.	←	Epi-Algal Biomass	-4.553	-0.013	32.873	-0.139	0.89
Macro-epifauna Abund.	←	Chlorophyll a	-3.249	-0.045	8.196	-0.396	0.692
Macro-epifauna Abund.	←	Exposure	35.371	0.333	18.308	1.932	0.053
Macro-epifauna Abund.	←	Temperature	3.164	0.138	4.46	0.709	0.478
Macro-epifauna Abund.	←	Salinity	9.61	0.143	9.394	1.023	0.306
Macro-epifauna Abund.	←	pH	12.062	0.082	24.819	0.486	0.627
Macro-epifauna Abund.	←	Turbidity	-10.357	-1.273	3.726	-2.779	**0.005**
Macro-epifauna Abund.	←	Oxygen	9.008	1.462	2.578	3.495	**<0.001**

Appendix IV: Results of a pilot study to assess the occurrence of phenolic acids in *Zostera capensis* leaves. Means ±1 SE amounts of gallic acid (mg/100mg dry wt) measured from three beds (100 g wet wt per bed) each at Geelbek (small-leaved morphotype) and Oesterval (large-leaved morphotype) in summer are presented.

Bed	**Geelbek**	**Oesterval**
1	0.011 ±0.004	0.000
2	0.009 ±0.005	0.006 ±0.003
3	0.013 ±0.004	0.000

Appendix V



Appendix V: Average amounts of gallic acid extracted from leaves of *Zostera capensis* in three beds (1-3) each at Geelbek (small-leaved morphotype) and Oesterval (large-leaved morphotype) in one sampling event. Error bars represent ± 1 standard error. Asterisks denote beds with homogenous means as determined by a Dunnet's T3 *post hoc* test.