Benthic and pelagic responses to endobenthic bioturbator (*Kraussillichirus kraussi*) density, temperature and eutrophication in a global change mesocosm experiment.

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Thesis presented for the Degree of

DOCTOR OF PHILOSOPHY

in the Department of Biological Sciences

UNIVERSITY OF CAPE TOWN

January 2025

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Acknowledgements

The financial assistance towards this research is hereby acknowledged. Opinions expressed and conclusions arrived are those of the author and not necessarily to be attributed to the funders. Financial support for the overarching project was provided for by the National Research Foundation. The University of Cape Town funded my research by awarding me the Science Faculty PhD Fellowship. I am incredibly grateful to the City of Cape Town for granting me permission to collect materials for my experiment from the Zandvlei Estuary Nature Reserve.

I express my sincere gratitude to my supervisor, Associate Professor Deena Pillay, for his invaluable guidance throughout the course of this research and the tremendous impact he has had on my growth as a student. His knowledge, insights and the many discussions we had enriched my understanding and appreciation of my research. I have been fortunate to have two co-supervisors, Dr Heather Marco and Dr Emma Rocke, to whom I am grateful to for their technical expertise, guidance and support.

It would have been impossible to conduct my experiment without the help of Grégoire Maniel, Carla de Cerff and Abioye Oyatoye. Thank you for the generosity of your time, your attention to detail and for being so vested in the success of this project. I will forever hold dear to me the wonderful friendship we built and the memories of our adventures together as we navigated the challenges of setting up my experiment during a pandemic.

Many thanks go to Pierre Smit, Calvin Hartnick and Sams Aquarium for their assistance with equipment and materials required for my experiment. Melissa Rankin, Cameron Russell and Chelsea Van Rensburgh are thanked for their assistance with field work and data collection.

I am endlessly grateful to my family and friends for their support and encouragement. Special thanks go to Rob Knutsen, Thilo Simadari, Joan Schmidt and Everleen Moodley for boosting my

confidence and always setting me firmly back on track. Heartfelt thanks go to Xan Manne, Liam Murugen and Luke Murugen for their hugs, laughter and refreshing joyfulness. I am incredibly grateful to my mum, Radha, my first teacher, and my dad, Ivan, my biggest fan, for instilling in me a love for reading and encouraging me to study.

I am so grateful to Charlie Thomas for her loyal companionship and the quiet reflections on our many walks together. The unwavering love, understanding and support from Terry Thomas (my rock), Kyle Thomas and Tennille Thomas have been my greatest strength throughout my PhD journey. Thank you for believing in me and giving me the space and time to focus on my research. You are my greatest source of inspiration and motivation, and you mean the world to me.

Abstract

Coastal ecosystems are increasingly being threatened by global change stressors such as eutrophication and warming, which can impair ecosystem functioning and ultimately affect societal well-being. Addressing this challenge, in part, requires identifying and understanding mechanisms that may enhance ecosystem resilience to global change. The axiid crustacean *Kraussillichirus kraussi* (sandprawn) plays a critical role as an endobenthic bioturbator in southern African estuaries and coastal ecosystems, with recent research highlighting its role in mediating bentho-pelagic coupling *via* top-down reductions in phytoplankton biomass, likely through phytoplankton adsorption onto burrow walls during bi-directional water-pumping. This novel finding has the potential to be an important resilience-enhancing mechanism against eutrophication in estuarine and coastal ecosystems locally. However, the robustness of this process, along with other benthic functions provided by sandprawns, is unclear given that they are nested within global change processes such as warming and eutrophication, which have the potential to alter fundamental benthic and pelagic processes mediated by sandprawns. In this context, this thesis addressed critical knowledge gaps in understanding the individual and combined impacts of warming, eutrophication and sandprawn density on ecosystem functions and community dynamics in both pelagic and benthic compartments, using an indoor mesocosm experiment.

Results from Chapter 3 of the thesis revealed that sandprawn water filtration (reduction in phytoplankton biomass) remained robust under simulated warming to levels predicted by the year 2100, with elevated temperatures accelerating reductions in phytoplankton biomass. Sandprawn biofiltration also remained robust under eutrophic conditions, reducing phytoplankton biomass by approximately 74%. Importantly, under eutrophic conditions, sandprawn filtration prevented the development of extreme eutrophy, and prevented a switch to nanoplankton dominance, resulting in even contributions of pico- and nanophytoplankton. Findings thus highlight the potential for

sandprawn water filtration to be an important natural process that can mitigate eutrophication symptoms under future global change conditions. The observed declines in the abundance of subsurface microphytobenthic algal biomass and abundance generally declined with warming, likely due to physiological intolerance of cool-temperate assemblages to elevated temperatures. Sandprawn induced reductions of cyanobacteria may likely enhance ecosystem resilience to coastal eutrophication by limiting harmful cyanobacterial blooms. However, warming-induced declines in microphytobenthic biomass may have important implications for sediment stability and trophic resource availability for benthic feeders under future global change scenarios. Findings from Chapter 5 highlighted the importance of temperature and sandprawn density rather than eutrophication in determining sandprawn bioturbation rates. Sediment boundary roughness (a proxy for bioturbation) increased by 43% at maximum sandprawn density under high temperatures (29.5°C), suggesting robustness of sandprawn physiology to future warming scenarios, particularly within populations inhabiting cool-temperate distribution ranges. Organic matter degradation rates increased under mesotrophic conditions and warming, particularly at high sandprawn densities, but was suppressed under eutrophic conditions, emphasising the importance of minimising nutrient loading to estuaries to prevent impairment of benthic carbon degradation. Lastly, Chapter 6 explored meiofaunal responses to predictor variables, and highlighted exceptionally complex and varied outcomes and the importance of contextual settings in determining responses to global change. Notably however, findings showed that higher sandprawn densities were associated with increased meiofaunal biomass dominance relative to abundance, even under high temperatures and eutrophic conditions (measured using *W*-statistics and ABC curves), suggesting that sandprawn bioturbation and associated benthic ecosystem engineering contributes to less perturbed states that facilitate meiobenthic assemblages.

Overall, findings of this thesis demonstrate the potential for sandprawns to facilitate the development of less disturbed benthic ecosystems, resulting in meiobenthic attributes indicative of stable communities. Findings also indicate the ability of sandprawns to enhance ecosystem resilience by preventing phytoplankton and cyanobacterial blooms and shifts to nanophytoplankton dominance under eutrophic conditions, including under warming scenarios. These findings highlight the potential of sandprawns to be important nature-based processes that can combat global change challenges, while also emphasising the need to conserve and manage sandprawn populations so that they are included in resilience-based ecosystem management.

CHAPTER 1:

INTRODUCTION

1.1 Coastal ecosystems

Estuarine and coastal environments rank among the most diverse and productive ecosystems on Earth, but they face significant threats due to substantial use and the impact of multiple land- and sea-based human activities (Lotze, et al., 2006; Hewitt, et al., 2016). Coastal habitats provide a range of ecosystem services from local to global scales and are vitally important not only ecologically but also economically, culturally and for recreational activities (Snelgrove, et al., 2014). This is particularly true for estuaries, which offer a range of ecosystem services, including food provision, nursery and habitat creation, water filtration, biogeochemical processing, climate regulation and shoreline protection, amongst others (Costanza, et al., 1993; Snelgrove, et al., 2014). The variety and importance of services provided by estuaries have contributed to rapid urbanisation, leading to intensified anthropogenic pressures with subsequent habitat degradation and pollution affecting their functioning (Costanza, et al., 1993; Lotze, et al., 2006; Zhou, et al., 2020; Defeo & Elliott, 2021). Local-scale anthropogenic impacts are further compounded by broader-scale global change, which can heighten ecological stress and threaten the functioning of estuarine habitats, and the provision of essential services to mankind (Costanza, et al., 2014; Schlacher & Wooldridge, 1996; He & Silliman, 2019; Defeo & Elliott, 2021). The combined effect of local- and global scale stressors and potential interactions among them can alter intrinsic ecological processes and jeopardise the resilience of coastal ecosystems. Such outcomes are concerning, since they may drive shifts to undesirable states in the long term (Harley, et al., 2006; Thrush, et al., 2009).

1.2 Global change

Global change is typically driven by high rates of resource consumption by a rapidly growing human population, causing environmental properties and processes to exceed their normal range of variation at unprecedented rates (Vitousek, 1994; Piggott, et al., 2015; Hewitt, et al., 2016). These changes can lead to complex and unpredictable interactions that can affect multiple organisational levels spanning individual taxa, communities and overall ecosystem functioning, with intertwined consequences for human wellbeing (Vitousek, 1994; Breitburg, et al., 1998; Harley, et al., 2006; Rabalais, et al., 2009; Piggott, et al., 2015). While global change effects on ecological processes and properties is undoubtedly complex, there are three main initiators of change. Increasing concentrations of carbon dioxide in the atmosphere has led to global warming, while alterations in biogeochemical cycles, principally increased agricultural inputs of carbon, nitrogen and phosphorus into coastal waters has contributed to eutrophication. Lastly, ongoing anthropogenic land use changes has caused habitat fragmentation, and loss of biodiversity (Fig. 1.1; Vitousek, 1994; Breitburg, et al., 1998).

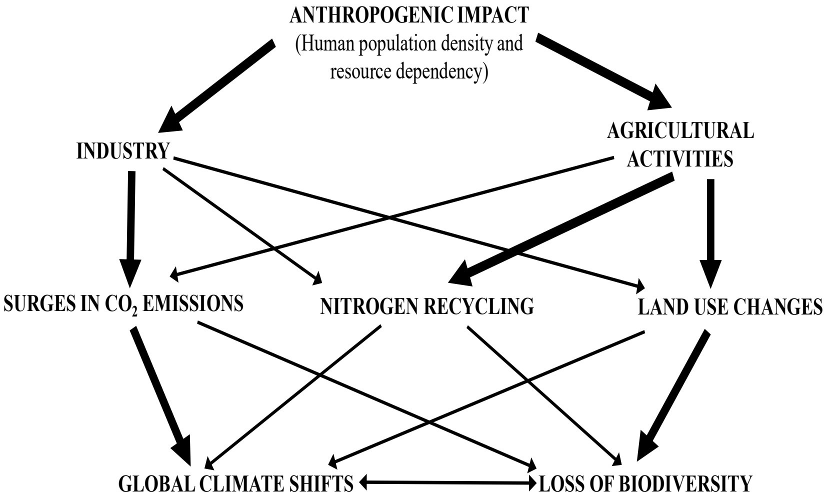

Figure 1.1: The effects of human population growth on global warming, biogeochemistry changes and diversity loss. The wide arrows represent dominant effects (adapted from Vitousek, 1994).

In a global change context, ecosystems are thus exposed to multiple stressors simultaneously, driven ultimately by human activities (Breitburg, et al., 1998) but including natural fluctuations over long time scales (Parmesan, 2006; Harley, et al., 2006). Importantly however, stressors interact with each other, resulting in complex and unpredictable effects being exerted on ecosystem components. For example, when stressors interact synergistically, their combined effect is greater that individual effects of each stressor, leading to stronger ecological consequences that when the same stressors act individually (Folt, et al., 1999; Côté, et al., 2016). In contrast, antagonistic interactions occur when stressors counteract their individual effects, resulting in joint effects being dampened (Folt, et al., 1999; Côté, et al., 2016). Growing concerns are emerging about the capacity of coastal and estuarine ecosystems to withstand the impact of multiple interacting stressors, particularly when interactions are synergistic and erode resilience and stability (Darling & Côté, 2008; Arrigo, et al., 2020). Thus, understanding, disentangling and predicting how multiple stressor interactions affect ecosystem functioning has become a major focus of studies in coastal ecosystems, which in turn underlies management of these ecosystems (Breitburg, et al., 1998; Harley, et al., 2006; Piggott, et al., 2015).

Within coastal ecosystems, estuaries are naturally exposed to dynamic fluctuations in temperature, salinity, chemical composition and biotic assemblages over relatively short time scales; however, their shallow depths make them particularly vulnerable to environmental changes (Costanza, et al., 1993). This argument is taken further in temporarily closed estuaries, given that they are much shallower than permanently open systems. Temporarily closed estuaries are found in South Africa, Australia, Sri Lanka, Uruguay, southeastern Brazil and southwestern India (Perissinotto, 2010; van Niekerk, et al., 2020), as well as in Portugal, and along the coastlines of California and Texas in the United States of America (Lemley, et al., 2021). They make up 71% of the estuaries found in South Africa (Perissinotto, 2010), where the semi-arid climate likely extends periods of mouth closure conditions (Perissinotto, 2010; van Niekerk, et al., 2020). This effect is often intensified by drought and increasing water abstraction for human use. Consistent with global trends, eutrophication is a leading cause of degradation in South African estuaries, with inorganic nutrient inputs from agricultural activities being compounded by organic matter inputs from overloaded and malfunctioning wastewater treatment plants and urban runoff (Adams, et al., 2020). Moreover, the shallow depths (<1.5m) of temporarily closed estuaries (Froneman, 2004) may heighten their vulnerability to the accumulation of contaminants (Adams, et al., 2020) and to rising air temperatures relative to well-flushed, deeper systems (Özen, et al, 2013).

Two prevalent global change pressures within estuarine environments are warming and eutrophication, both of which can alter biological and biogeochemical processes to affect organismal, population and community responses, biodiversity and ecosystem functioning (Vitousek, 1994; IPCC, 2007). At the same time, benthic bioturbators also fundamentally influence the physical, biochemical and biotic properties of mainly sedimentary ecosystems, and thus profoundly influence intrinsic processes such as sediment-water interactions (Heip, 1995). Importantly, warming and eutrophication are likely to determine how bioturbation influences key ecosystem properties and processes, either individually or interactively. To my knowledge, the ecological consequences of these two stressors and bioturbation in influencing key estuarine ecosystem processes, especially bentho-pelagic coupling withing temporarily closed systems, requires clarification. This rationale forms the basis of my PhD thesis, which specifically aimed to experimentally quantify the consequences of warming and eutrophication for key estuarine processes, under varying densities of sandprawns (*Kraussillichirus kraussi* Stebbing, 1900), a key bioturbator in southern African estuaries.

1.3 Eutrophication

Eutrophication is typically described as the increased rate of microalgal primary production and that of other aquatic autotrophic organisms including macroalgae, due to the accumulation of organic matter, nitrogen and phosphorus into a aquatic ecosystems, that changes metabolic resource quantity, quality and traits and potentially ecosystem trophic status (Nixon, 1995).

Eutrophication may result from changes to the physical characteristics of a system (e.g. hydrological cycles), biological interactions (e.g. reduced grazing) or increased terrestrial runoff (Nixon, 1995; Rabalais, et al., 2009). In the 21st century, however, eutrophication is typically linked to anthropogenic activities that mobilise and introduce excessive amounts of inorganic nutrients into ecosystems (Nixon, 1995; Vitousek, et al., 1997; Seitzinger, et al., 2002; IPCC, 2007; Rabalais, et al., 2009). Driven by increasing urbanisation in coastal areas, intensified agricultural practices, industrial development in catchment areas, and increased discharge of sewage and combustion of fossil fuels, coastal eutrophication is a now a global phenomenon that has significant impacts on aquatic biogeochemistry and water quality, with changes propagating to influence biodiversity, and community structure that may cascade to entire food webs (Nixon, 1995; Cloern, 2001; Grall & Chauvaud, 2002; Rabalais, et al., 2009). Simplistically, higher nutrient loadings increase phytoplankton production which, depending on shifts in the phytoplankton community composition, benefits certain primary and secondary consumers that may increase in biomass and abundance (Cloern, 2001; Grall & Chauvaud, 2002). If the productivity of phytoplankton occurs beyond the capacity of the ecosystem to consume phytoplankton, negative effects associated with algal blooms may arise (Rabalais, et al., 2009). Such blooms may lead to reductions in the penetration of light due to shading, (with subsequent loss of submerged aquatic vegetation and reductions in benthic primary productivity), anoxia/hypoxia stemming from higher respiratory and decomposition rates, and fish kills (Nixon, 1995; Cloern, 2001; Grall & Chauvaud, 2002; Rabalais, et al., 2009). Aside from greenhouse gas emissions increasing with eutrophication and contributing to global warming (Nguyen, et al., 2022), eutrophication also increases the potential for opportunistic algae to thrive, and for noxious and harmful algal blooms to form with damaging consequences to the biota of the entire aquatic ecosystem (Rabalais, et al., 2009) and to human health (Cloern, 2001).

In shallow marine ecosystems, approximately 25-50% of the organic matter produced by phytoplankton sinks to the benthos, enhancing organic matter input into the sediment (Cloern, 2001; Rabalais, et al., 2009). The deposition of faecal pellets and eutrophication-induced increases of organic matter from detrital plants and phytoplankton further contribute to eutrophic conditions in the benthos (Heip, 1995; Nixon, 1995; Grall & Chauvaud, 2002). Moderate nutrient enrichment may often lead to increases in benthic abundance, biomass and species richness (Heip, 1995; Grall & Chauvaud, 2002); however, as heterotrophic metabolism and rates of decomposition increase within sediments due to severe eutrophication, rising hypoxic/anoxic conditions may lead to increased prevalence of opportunistic species and overall higher mortality rates among benthic organisms (Heip, 1995; Grall & Chauvaud, 2002). Hypoxia/anoxia can disrupt biogeochemical cycles by increasing the release of phosphorous and nitrogen from sediments, reinforcing eutrophication (Diaz and Rosenberg, 1995). Declining oxygen levels also leads to reduced species diversity and altered behaviour patterns and may impair key ecosystem functions like bioturbation, feeding and burrow ventilation, further affecting the distribution of organic matter and oxygen in sediments (Mermillod-Blondin and Rosenberg, 2006). In addition to causing oxygen depletion, eutrophication can alter benthic community characteristics and shift the composition, distribution and interactions among benthic species (Heip, 1995). In line with this, changes in redox conditions and to the benthic community structure and metabolism may change the direction and magnitude of processes that drive benthic-pelagic coupling, thus affecting the biogeochemistry of the broader ecosystem (Heip, 1995; Grall & Chauvaud, 2002).

Given that persistent eutrophic conditions are a leading cause of coastal water quality decline globally, and its cascading effects on biogeochemical cycles, species composition and the functional diversity of the ecosystem (Cloern, 2001; Rabalais, et al., 2009; Dimitriou, et al., 2017b), it is vitally important that eutrophication is understood, especially in relation to other global change stressors that impact coastal and estuarine ecosystems (Cloern, 2001). Crucially, as a pervasive and escalating global stressor, rising temperatures can significantly impact primary production by phytoplankton under both normal and eutrophic conditions (Rabalais, et al., 2009).

1.4 Warming

Temperature is a key determinant of biological activities, including primary production, resource acquisition and metabolism (Heip, 1995; IPCC, 2007; Madiera, et al., 2012). Natural fluctuations of the Earth's climate systems have determined evolutionary adaptations of species and biogeographic patterns of biota; however, in the past century, unprecedented increases in greenhouse gas emission by human activities has led to accelerated warming on a global scale (Parmesan, 2006; Harley, et al., 2006) and the occurrence of more frequent, persistent and longer-lasting heatwaves (Wedler, et al., 2023). The projected increases in the average global temperature by 1.8 - 4°C by the year 2100 (IPCC, 2007) will incontrovertibly alter the abiotic environment and impact physiological processes and performances of individual organisms, which in turn will influence the dynamics of populations as well as the structure of communities (Harley, et al., 2006; Rabalais, et al., 2009; Colombano, et al., 2021). Depending on the relationships between organismal-level processes, population dynamics and community structure, temperature-driven abiotic changes to the environment will trigger ecological responses such as shifts in biogeographical ranges of species, changes in diversity and productivity, and modifications to trophic interactions (Sanford, 1999; Harley, et al., 2006; Parmesan, 2006; Rabalais, et al., 2009; Colombano, et al., 2021). On a local scale, communities may undergo gradual changes in composition as species with affinities for warmer temperatures become more abundant (Sanford, 1999). Notably, pathogens are favoured by warmer temperatures, signifying that the negative effects of disease are likely to become more severe with warming (Harvell, et al., 2002; Parmesan, 2006). Alongside this, rates of primary production may initially increase with rising temperatures, but are likely to decline with nutrient limitations or reduced light due to self-shading (Rabalais, et al., 2009). Although rising temperatures can initially boost primary production, it also increases the risk of hypoxia by decreasing oxygen solubility in water (Matear and Hirst, 2003). Importantly, warming also heightens the metabolic resource demands of consumers and affects their population dynamics, which may ameliorate or exacerbate the changes in primary production due to grazing effects (Lotze & Worm, 2002). These changes can have immediate impacts on food web interactions, that may propogate through the ecosystem to ultimately cause changes in species diversity, community composition and ecosystem functioning (Sanford, 1999).

The biological impact of high temperatures on organisms varies both within and among species (Somero, 2002; Hughes, et al., 2003; Parmesan, 2006). Given their limited ability to regulate body temperature, ectotherms are especially vulnerable to fluctuations in temperature, making them more susceptible to the negative effects of thermal stress on their physiology, fitness and performance (Kingsolver, et al., 2013). Nevertheless, biological processes generally demonstrate a biphasic response to temperature, where metabolic processes are enhanced as temperature increases to thermal optima, followed by a decline (Willmer, et al. 2000, Sinclair, et al., 2016). This is particularly true for aquatic ectotherms where warming directly enhances metabolic rates and drives higher rates of resource acquisition for example (Clarke & Johnston, 1999; Angilletta Jr., et al., 2002; Angilletta Jr, et al., 2010; Doney, et al., 2012; Schulte, 2015; Harianto, et al., 2018). Bioturbation by benthic ectotherms fulfills an important function in coastal and estuarine environments, and importantly, is temperature-dependent (Cozzoli, et al., 2019). Increased bioturbation has the potential to affect sediment erodibility, sediment mixing, bentho-pelagic exchanges, oxygen penetration and decomposition reactions in the ecosystem, which in turn determines net ecosystem functioning (Zhou, et al., 2023 and references therein). However, high temperatures beyond physiological thresholds can impede bioturbation, negatively affecting their engineering performance and the delivery of essential ecosystem services (Zhou, et al., 2023).

1.5 Ecosystem engineering and bioturbation

Ecosystem engineers physically modify the environment in a variety of ways: for example, corals build reefs and attenuate wave action, seagrass meadows bind sediment and, axiidean shrimp alter the topgraphy of the sediment and increase solute exchange through burrowing and irrigation (Gutiérrez, et al., 2011). These structural alterations cause changes in the abiotic conditions that can feedback to the ecosystem engineer itself, and impact other species, affecting ecological processes and overall ecosystem functioning to varying degrees, including under global change conditions, (Fig. 1.2; Costanza, et al., 1993; Snelgrove, et al., 2014; Gutiérrez, et al., 2011).

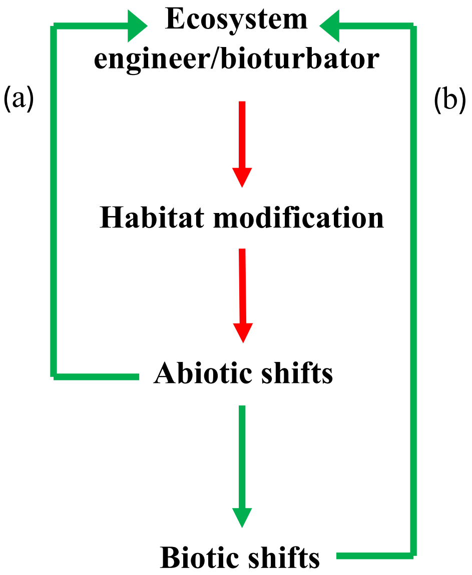

Figure 1.2: Pathways of ecosystem engineering (red arrows) and biotic effects (green arrows), including feedback effects to the engineer driven by (a) abiotic changes and (b) biotic changes (adapted from Gutiérrez, et al., 2011).

Bioturbation in particular, is widely acknowledged as an ecosystem engineering process that significantly influences sedimentary ecosystems, and thereby determines dynamics of biotic and abiotic processes, and hence ecosystem functioning (Meysman, et al., 2006; Maire, et al., 2008; Chen, et al., 2017; Snelgrove, et al., 2014). A critically important outcome of bioturbation is the establishment of a dynamic connection between sedimentary and pelagic environments (Aller, 1982; Meysman, et al., 2006; Bertics & Ziebis, 2009) and its influence on essential ecosystem services such as biogeochemical cycling and energy transfer in food webs is profound (Lohrer, et al., 2004; Meysman, et al., 2006; Maire, et al., 2008; Bertics & Ziebis, 2009; Griffiths, 2017). Through activities such as burrowing, ventilation and foraging, bioturbation drives benthic particle transport and shapes the composition and distribution of communities (Aller, 1982; Meysman, et al., 2006; Bertics & Ziebis, 2009; Chen, et al., 2017). As an example, the globally distributed axiid crustaceans are dominant bioturbators, renowned for their exceptional sediment turnover activities (Cadée, 2001; Pillay & Branch, 2011; Pillay, 2019). They belong to one of the most important group of sediment reworkers with regards to the quantity and rate of sediment they rework as well as to the depth to which they can burrow in coastal and subtidal areas (Cadée, 2001). *Kraussillichirus kraussi* (Stebbing, 1900), is an example of an axiid crustacean that is a dominant ecosystem engineer in southern Africa, renowned for sediment turnover rates of ~12.14 kg.m^{-2}.day^{-1} (Branch & Pringle, 1987; Pillay & Branch, 2011), and is the focal bioturbator in my study.

1.6 The role of *Kraussillichirus kraussi* as an ecosystem engineer

Kraussillichirus kraussi (Stebbing, 1900), commonly known as sandprawn, is an axiidean decapod crustacean (formerly part of the Thalassinidea), known until recently as *Callichirus kraussi* or *Callianassa kraussi* (Branch & Pringle, 1987; Pillay & Branch, 2011). It is found along the southern African coastline from Namibia on the west coast to Mozambique on the east coast (Branch, et al., 2022; Fig 1.3).

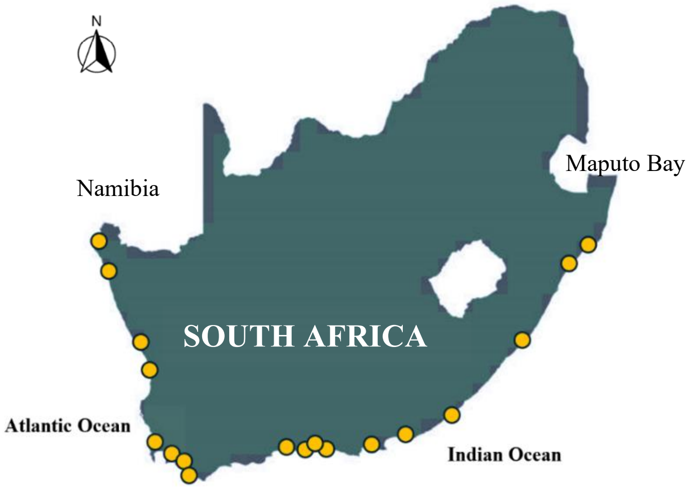

Figure 1.3: Distribution of *Kraussillichirus kraussi* along the southern African coastline (*Kraussillichirus kraussi* (Stebbing, 1900) in GBIF Secretariat (2023). GBIF Backbone Taxonomy. https://doi.org/10.15468/39omei accessed via GBIF.org on 2024-09-23).

K. kraussi is commonly found in the lower reaches of estuaries and sheltered marine habitats (Day, 1981; Branch, et al., 2022), where they may reach densities of ~200 ind.m^{-2} (Branch & Pringle, 1987; Pillay & Branch, 2011). They are permanent residents of sedimentary habitats and influential bioturbators that burrow deeply (>1m) into soft sediments (Branch & Pringle, 1987; Wheatcroft, et al., 1990; Pillay & Branch, 2011). They sift the sediment for food as they burrow (Branch, et al., 2022), constructing niches for other sedimentary organisms (Pillay & Branch, 2011), and consolidating their burrows with fine, richly organic material to stabilise burrow walls (Branch & Day, 1984). *K. kraussi* produce large quantities of organically rich faecal pellets that increase the overall organic content of the sediment (Branch & Pringle, 1987). Their burrow lining behaviour enhances the content of organically rich particulate matter in the sediment and reduces their loss from the ecosystem due to hydrodynamic effects (Branch & Day, 1984).

In the process of burrowing, *K. kraussi* ejects the finer particles of sediment from the burrow to the sediment-water interface, increasing sediment boundary roughness (Fig 1.4), sediment erodibility and resuspension, as well as altering sediment granulometry (Branch & Pringle, 1987; Cadée, 2001; Pillay & Branch, 2011). Sediment resuspension may alter the light conditions by increasing the turbidity of the water (Aller & Dodge, 1974). These ecosystem engineering activities have broad implications for both benthic and pelagic aquatic communities, as they directly affect primary production and food availability (Branch & Pringle, 1987; Pillay & Branch, 2011; Pillay, et al., 2012) which in turn, can indirectly affect the spatial distribution of consumers such as benthic invertebrates, suspension feeders, larvae and epifauna and fish within the ecosystem (Branch & Pringle, 1987; Pillay & Branch, 2011; Pillay, et al., 2012; Henninger & Froneman, 2013).

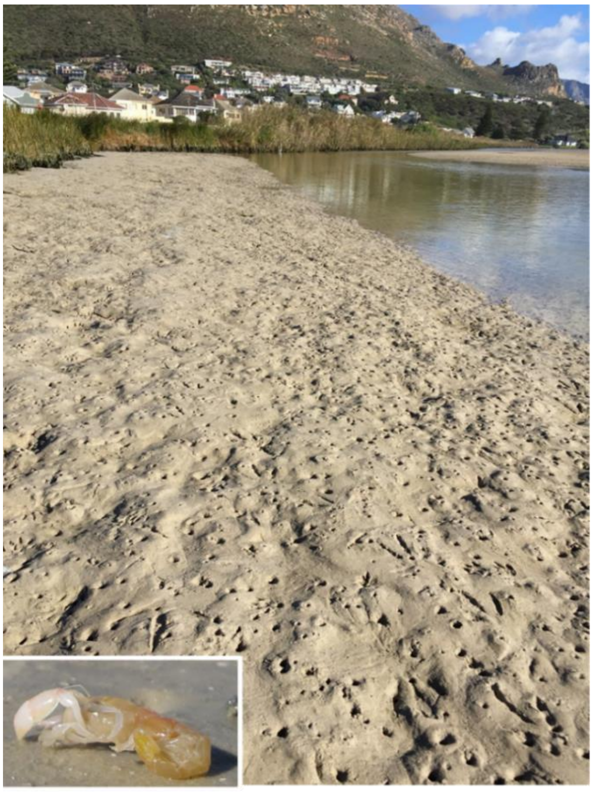

Figure 1.4: Irregular sediment topography created by dense *Kraussillichirus kraussi* (inset) populations in the sandprawn-dominated biotope (Site 1) in the Zandvlei Estuary. Holes on the sediment surface are burrow openings. The main image was taken 3 days after mouth opening, during which drainage into the Atlantic Ocean exposed most of the benthic habitat. Inset and used with permission Olivia Venter.

As *K. kraussi* burrow into the sediment, they often encounter anoxic or hypoxic sediment, which they have adapted to behaviourally, by principally increasing beating rate of their pleopods to irrigate their burrows and draw in oxygenated water (Pillay & Branch, 2011), thereby expanding the oxic surface area of the sediment (Pillay & Branch, 2011). Additionally, the active pumping of overlying waters through their burrow systems can enhance the adsorption of organic matter onto burrow walls (Pillay & Branch, 2011; Venter, et al., 2020) which, coupled with increased oxic conditions in the sediment, stimulates microbial abundance around the burrow linings, positively affecting the decomposition of sedimentary organic matter, particularly in the immediate vicinity of the burrows (Branch & Pringle, 1987). Notably, excessive trampling and pumping of *K. kraussi* for bait causes significant physical disturbance to the sediment: compaction due to trampling collapses their burrows and reduces bioturbation-induced oxygenation, harming the whole ecosystem, while bait collection reduces sandprawn abundance (Pillay & Branch, 2011; Branch, et al., 2022).

A study by Venter, et al. (2020) showed that despite their functional designation as deposit-feeders, sandprawns can impact phytoplankton biomass. Specifically, Venter et al. (2020) showed that sandprawn presence led to a reduction in phytoplankton biomass by ~50% in experimental mesocosms, aligning with long-term estuarine data showing reductions in phytoplankton biomass of ~70% in sandprawn-dominated sediments. This effect was hypothesized to have arisen by phytoplankton adsorption onto burrow walls during bi-directional water pumping. This study highlights the significant influence of bioturbation by *K. kraussi* on the physical and chemical properties of the sediment, as well as on nutrient fluxes, and alludes to the cascading effects of bioturbation on community structure and overall estuarine ecosystem functioning (Branch & Day, 1984; Branch & Pringle, 1987; Pillay & Branch, 2011). The study by Venter et al. (2020) also highlights that there is much to learn about the functional significance of endobenthic crustaceans in coastal ecosystems and puts into context the need for such research, especially in a global change context.

Irrespective of the outcome, enginering effects of *K. kraussi* are fundamentally initiated by sediment and porewater movement, with the magnitude of engineering effects being dependant on local population density (Pillay & Branch, 2011) as well the abiotic environment (Pillay, 2019). A key point though is that as ectotherms, sandprawns are likely to experience changes in energy requirements and metabolic activity under predicted global warming scenarios, which may be influenced by eutrophication (through trophic resource availability or harmful pathogens) that can lead to, as yet, unknown and unpredictable effects on ecosystem functioning (Pillay, 2019). Despite the importance of quantifying such effects on ecosystem multifunctionality in the context of present and predicted global change conditions, research in this area is yet in its infancy (Harley, et al., 2006; Pillay, 2019). While the bioturbation effects of *K. kraussi* on sediment oxygenation and benthic community composition are well-understood (Pillay, 2019), its functional significance and robustness under global change scenarios, such as warming and eutrophication, are under-researched (Venter, et al., 2020). This is despite its ecological and numerical dominance in coastal sediments (Pillay & Branch, 2011).

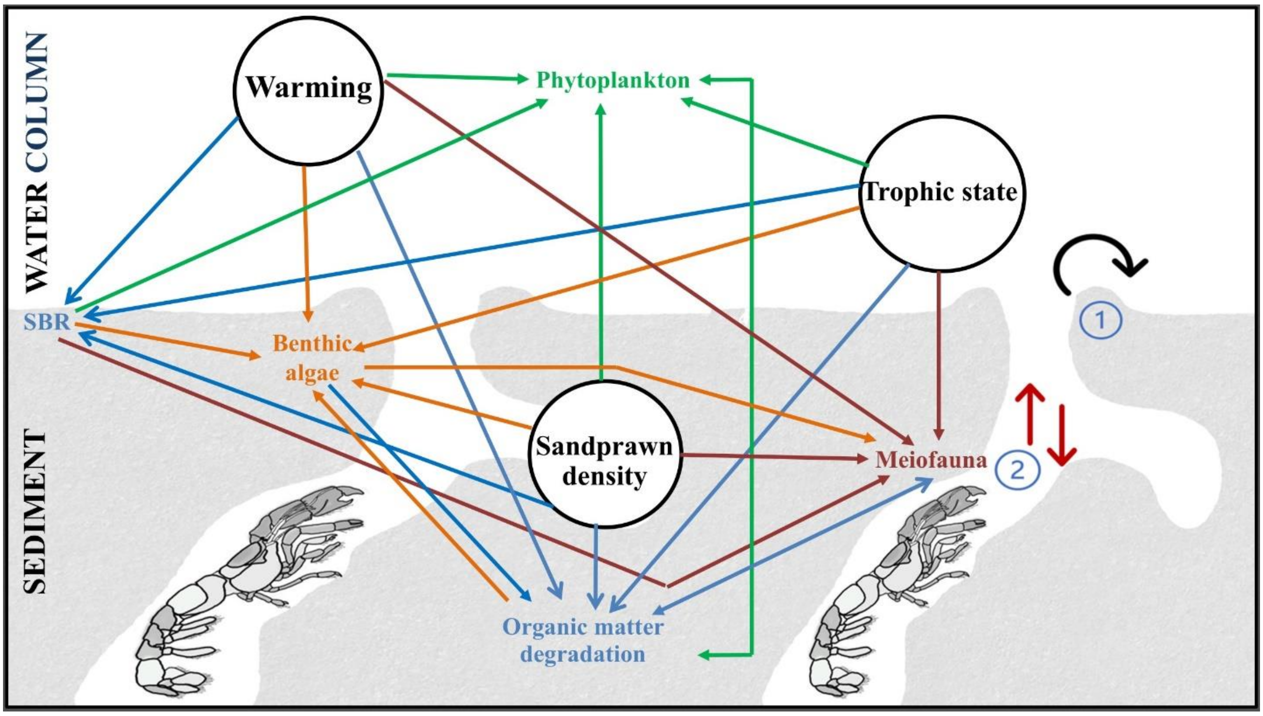

Figure 1.5: The main pathways by which warming and trophic state can influence ecosystem functioning and community structure in sandprawn dominated ecosystems (1 = sediment deposition that influences consumer epibenthic organisms and biogeochemical processes; 2 = bidirectional water pumping that influences particle adsorption on burrow walls, green = the phytoplankton response, orange = benthic microalgal response, blue = ecosystem function response and red = the meiofaunal response). SBR = sediment boundary roughness. Graphical representations of sandprawns in burrows were drawn by Jessica Dawson and used with permission.

In light of these knowledge gaps and to build on the novel findings of Venter, et al. (2020), my study broadly aimed to investigate how warming and eutrophication interact to influence sandprawn bioturbation and the subsequent impact on ecosystem functions and community dynamics in both the pelagic and benthic environment across varying levels of sandprawn density (Fig. 1.5). Given the potential influence of future warming scenarios on the ectothermic response of *K. kraussi*, and in conjuction with trophic state changes induced by eutrophication, I used an experimental mesocosm approach to investigate the individual and joint effects of sandprawn density, eutrophication and warming on ecosystem functioning. Specifically, I measured (1) sandprawn bioturbation by tracking changes to sediment rugosity; and (2) rates of organic matter degradation. Additionally, I investigated the density-dependent water filtration potential of sandprawns by measuring the response of phytoplankton biomass and traits to eutrophication and warming. Finally, to understand how the benthic community responded to these predictors, I measured the responses of benthic microalgal assemblages and meiofauna.

The structure of my thesis is outlined in Figure 1.6. Following this general introduction and literature review, Chapter 2 provides a brief description of the philosophical considerations and rationale underpinning my thesis, while detailing the methodology that was followed. Results from my experiment are reported in Chapters 3 to 6. Chapter 7 concludes my thesis by synthesising the key findings emerging from my study.

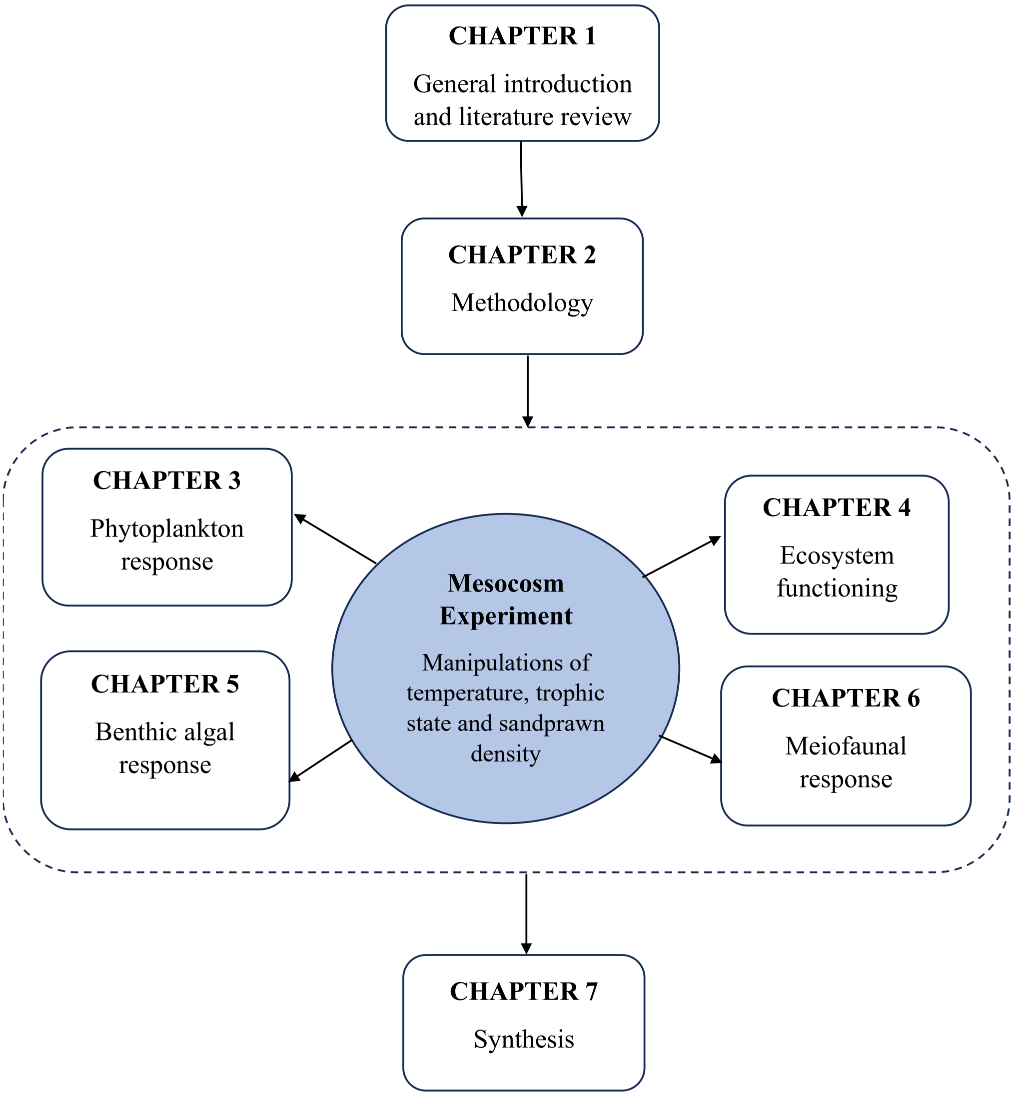

Figure 1.6: Overview of the general structure of my thesis and the chapter topics

CHAPTER 2:

METHODOLOGY

2.1 The philosophical basis of the scientific method

The scientific method is a systematic process that has been singularly instrumental in advancing our understanding of ecological and other natural phenomena (Underwood, 1990; Quinn & Keough, 2002). Applying this framework of logic to unravel ecological complexity involves a sequence of steps: (1) making an observation or identifying a pattern or an anomaly in an observed pattern (Underwood, 1990); (2) providing plausible explanations for the observations and (3) constructing a prediction or hypothesis. Step (2) is also referred to as model development, and oftentimes, competing models/explanations may be proposed (Quinn & Keough, 2002; Underwood, 1990). Prediction and hypothesis construction must be logistically feasible to investigate in terms of temporal and spatial scales and are informed by the researcher's insight and knowledge of existing theories, previous observations and research findings of the system being studied (Underwood, 1990; Underwood, 1991; Quinn & Keough, 2002). Therafter, steps (4) data collection and analysis and (5) critical evaluation of hypotheses or predictions are the final underpinnings of the scientific method (Underwood, 1990; Underwood, 1991).

Testing hypotheses in ecological studies includes three distinct approaches, namely, field comparisons (natural surveys), computational or mathematical modelling, and manipulative experiments (Quinn & Keough, 2002). Field comparisons rely on opportunistic sampling or observations of natural variation within an ecosystem. While field comparisons preserve the scale and timing of natural events and organismal responses, it is challenging to establish proper controls as natural ecosystems are subject to numerous fluctuations of several co-occurring factors (Connell, 1974; Underwood, 1990; Underwood, 1991; Widdicombe, et al., 2010). Thus, the inherent biological complexity that is characteristic of natural ecosystems is difficult to disentangle in field comparisons and makes it difficult to identify the mechanisms and processes involved in how organisms respond to specific and co-occurring pressures

(Breitburg, et al., 1998; Stewart, et al., 2013; Dimitriou, et al., 2017a). It is also difficult to replicate natural conditions which makes it challenging to maintain adequate statistical power in field comparisons (Lawton, 1996; Breitburg, et al., 1998; Schindler, 1998; Cadotte, et al., 2005; Rimmer, et al., 2022). Consequently, field comparisons often only offer partial tests of hypotheses and rely on inferences about causality (Quinn & Keough, 2002). Global change is occuring at an unprecedented rate and it is imperative that solutions for globally urgent ecological problems are proposed within time scales to meaningfully influence policy (Benton, et al., 2007). However, it is difficult to gain a mechanistic understanding of cause-effect relationships using field comparisons and this impedes our ability to accurately forecast the impact of global change on ecosystem functioning and to engage in efforts to mitigate its impact (Underwood, 1990; Benton, et al., 2007).

Computational or mathematical modelling is increasingly being used to make environmental predictions that may not be possible with field comparisons (Benton, et al., 2007; Jackson, et al., 2000; Thieler, et al., 2000; Fulford, et al., 2020). The models rely on existing experimental and empirical data and theoretical understandings of ecological processes. Consequently, the quality and availability of existing data as well as the suite of mechanistic processes included in the model, profoundly influence the predictive power of the model (Thieler, et al., 2000; Stewart, et al., 2013; Fulford, et al., 2020). Mathematical modelling has certain limitations, including the exclusion of critical processes that can oversimplify the system, reliance on inadequate assumptions due to limited understanding of the underlying processes and ecosystem relationships, and the setting of model parameters based on narrow, localised observations (Thieler, et al., 2000). These limitations reduce the model's capacity to offer robust insights into pressing global change issues.

Manipulative experiments theoretically allow researchers greater control by holding a single, or a limited suite of variables constant except the one under investigation, enabling comparisons with a control condition (Connell, 1974; Underwood, 1990; Quinn & Keough, 2002; Widdicombe, et al., 2010). While manipulative experiments lack the full complexity of natural ecosystems, are limited spatially and temporally and may exclude organisms on the basis of size, they are valuable in global change studies (Quinn & Keough, 2002). They facilitate the interpretation of ecosystem responses to specific pressures and can be designed to simulate forecasted conditions not yet encountered in nature (Underwood & Peterson, 1988; Dimitriou, et al., 2017a). In my study, I sought to understand the impact of predicted levels of warming and eutrophication on the bioturbation activities of a key ecosystem engineer (*Kraussillichirus kraussi*) and the concomitant effects on ecosystem functioning. Mesocosms were chosen as the most suitable experimental approach, allowing simultaneous manipulatation of sandprawn density, predicted warming levels to year 2100 and eutrophication. This experimental setup allowed me to evaluate the potential effects of these global change processes on the functioning of coastal systems.

2.2 The role of mesocosm experiments in the context of global change research

Widdicome, et al. (2010) reminds us that all experiments are abstractions from reality, representing a series of compromises, with inherent strengths and weaknesses, and that the approach or experiment used as a means of answering a specific research question holds value if it advances our holistic understanding of an issue.

Among experimental studies, mesocosms have become increasingly important to coastal ecosystem global change research (Schindler, 1998; Underwood & Peterson, 1988; Woodward, et al., 2010; Stewart, et al., 2013; Fordham, 2015). They offer a bridge between the complexity and noise associated with field comparisons and the controlled but often oversimplified nature of microcosm experiments (Lawton, 1996; Stewart, et al., 2013). Mesocosms provide models of simple assemblages and subsets of ecological communities (Widdicombe, et al., 2010) that may incorporate many natural elements of an eccosystem, allowing them to mimic some, albeit not all of the biological processes and interactions that occur in natural systems (Lawton, 1996). Notably, mesocosms enable the manipulation of both abiotic predictors and the biological components of the ecosystem so that cause and effect relationships can be established in experimental setups that can be easily replicated for hypotheses to be tested with statistical rigour (Breitburg, et al., 1998; Schindler, 1998; Cadotte, et al., 2005; Widdicombe, et al., 2010; Woodward, et al., 2010; Stewart, et al., 2013; Fordham, 2015).

Our current understanding of the effects of global change stressors on ecosystems has been predominantly influenced by studies focussing on single stressors (Crain, et al., 2008; Widdicombe, et al., 2010; Gunderson, et al., 2016), which may lead to misinterpretations, if these findings are extrapolated to predict the impact of multiple co-occurring stressors on ecosystems (Crain, et al., 2008; Gunderson, et al., 2016). Indoor mesocosms, are less impacted by time and weather constraints and can be constructed from relatively affordable and accessible materials, making them an increasingly popular choice for assessing the vulnerability of biota, community structure, food web dynamics and ecosystem functioning to various manipulations of global change scenarios (Stewart, et al., 2013; Fordham, 2015). Predicting the consequences of global change on species, community structure and ecosystem functioning in natural ecosystems has challenged the scientific community (Stewart, et al., 2013; Fordham, 2015) amid expectations from society and policy makers for knowledge that will guide measures to mitigate the impact of global change on the provision of ecosystem services (Widdicombe, et al., 2010).

The main criticisms that have been levelled against the use of mesocosms is that they lack the complexity of natural ecosystems (Odum, 1984; Lawton, 1996), are limited by ‘wall effects’ due to containment, and that their sizes may limit the number of organisms they can house (Schindler, 1998; Stewart, et al., 2013). Mesocosm experiments also exclude real world processes such as tides, immigration/emigration and the full suite of biological interactions that occur natural environments (Benton, et al., 2007; Widdicombe, et al., 2010). Notably, the extraction of organisms from their natural surroundings may remove them from the environmental stresses that they naturally experience yet imposes experimental stress that could have a significant impact on the response of the organism. This, however, might be reduced by acclimation to the experimental environment before the start of a study (Widdicombe, et al., 2010). Despite their limitations, Benton, et al. (2007) argue that smaller-scale manipulative experiments using mesocosms/microcosms have historically influenced the development of theories and informed replication across broad spatial and temporal scales in various ecological systems, establishing generality and lending credibility for their use in enhancing understanding of processes that impact complex ecosystems.

Notwithstanding that they are closed systems (Urakawa, et al., 2019; Rimmer, et al., 2022), mesocosm experiments benefit from a high degree of experimental control that allows manipulations of variables and offers substantial predictive power in global change studies (Odum, 1984; Stewart, et al., 2013). Thus, mesocosms are becoming increasingly crucial elements in the toolbox of global change studies, particularly where they can be integrated with other, complementary approaches, including field comparisons and modelling (Cadotte, et al., 2005; Stewart, et al., 2013; Fordham, 2015).

Given that no single approach or experiment can explain all the potential impacts of global change on biota, community structure and biological processes (Lauchlan & Keddy, 1997; Widdicombe, et al., 2010), the ultimate value of using mesocosms in my study, lay in the information that emerged on the impact of anticipated temperature increases on the engineering performance a key bioturbator exposed to eutrophic stress.

2.3 The study site

The intermittently closed Zandvlei Estuary (34°05'S; 18°28'E) is the focal ecosystem of this study, from which experimental materials were collected. The estuary is located in False Bay, along the south-western coastline of South Africa, in the City of Cape Town (Fig. 2.1) and is a popular recreational spot within the greater Cape Town area, attracting many vistors for fishing, picnicking and water-based activities (Harding, 1994). During peak holiday season, it is estimated that an average of 2500 people visit the estuary per day (Thornton, et al., 1995). Climatically, the estuary is situated in the winter rainfall region of South Africa and spans an approximate length of 2.5 km and mean depth of 1.4 m (Harding, 1994; Lemley, et al., 2019). The area receives a mean annual rainfall of 890 mm and has well-mixed waters for most of the year (Harding, 1994). The three components of the estuary are a lake in the north, a man-made marina adjacent to the eastern shore and a narrow outlet channel that links to the ocean. The estuary has a relatively small, extensively urbanised catchment area of 92 km^2, used for light industry, forestry, agriculture, commerce, residential space and paved roads (Harding, 1994; Lemley, et al., 2019).

Zandvlei has a long history of anthropogenic manipulation and modification: the Marina da Gama estate was developed on its eastern shores, the lake was deepened to permit recreational boating, and the lower reaches of the estuary has been canalised (Harding, 1994; Thornton, et al., 1995). The water levels are mechanically regulated by periodic dredging and manipulation of a rubble weir in the outlet channel and kept less than 1m above Mean Sea Level (aMSL; Harding, 1994; Quick & Harding, 1994; Hutchings, et al., 2016; Lemley, et al., 2019), This alters the tidal dynamics of the estuary (Thornton, et al., 1995; Hutchings, et al., 2016; Lemley, et al., 2019). The lowering of the rubble weir generally increases salinity in the system by increasing seawater penetration upstream. The mouth of the outlet channel is naturally open during the winter months, mitigating flood risks (Hutchings, et al., 2016;

Lemley, et al., 2019) and closes with the formation of a beach sandbar toward the end of the winter rainfall season (Harding, 1994). In summer, when rainfall is low and salinity increases, the mouth of the estuary is artificially opened on a monthly basis, coinciding with spring high tide, to allow for a greater influx of seawater and to support migration of estuarine and marine species into and out of the system (Hutchings, et al., 2016; Lemley, et al., 2019). Generally, salinity increases in the estuary when the mouth is opened due greater marine influence.

The primary threats and pressures faced by the system stem from pollution, habitat loss from developments such as the marina, and altered flow patterns primarily due to dredging, canalization and manipulations of the mouth (Hutchings, et al., 2016). Periodic sewer and sewer pump station overflows, seepage from septic tanks and overflow from viticulture, agriculture and domestic gardening has led to increased runoff with higher loading of nutrients and bacteria into the system, causing significant decline in estuarine water quality (phytoplankton blooms) and ecosystem functioning (Quick & Harding, 1994; Lemley, et al., 2019). This has resulted in the system being classified as eutrophic (Harding, 1994; Thornton, et al., 1995). Harmful algal blooms, including those of the toxic golden algae *Prymnesium parvum*, associated with fish mortality, have been recorded in the system (Lemley, et al., 2019). Overall, the biotic components are rated as "fair", suggesting a strong potential for recovery if primary threats are effectively addressed. The lower reaches of the estuary are occupied by dense populations ($114/m^2 – 240/m^2$) of the sandprawn *Kraussillichirus kraussi* (Venter, et al., (2020); see Chapter 1, Fig. 1.4).

Despite the substantial anthropogenic modifications, Zandvlei Estuary retains its importance as a functional urban estuary, supporting a popular recreational area with some remaining natural habitat of significant conservation value (Thornton, et al., 1995; Hutchings, et al., 2016). This system serves as an ideal model coastal ecosystem for studying the impacts of multiple urban stressors on ecological processes.

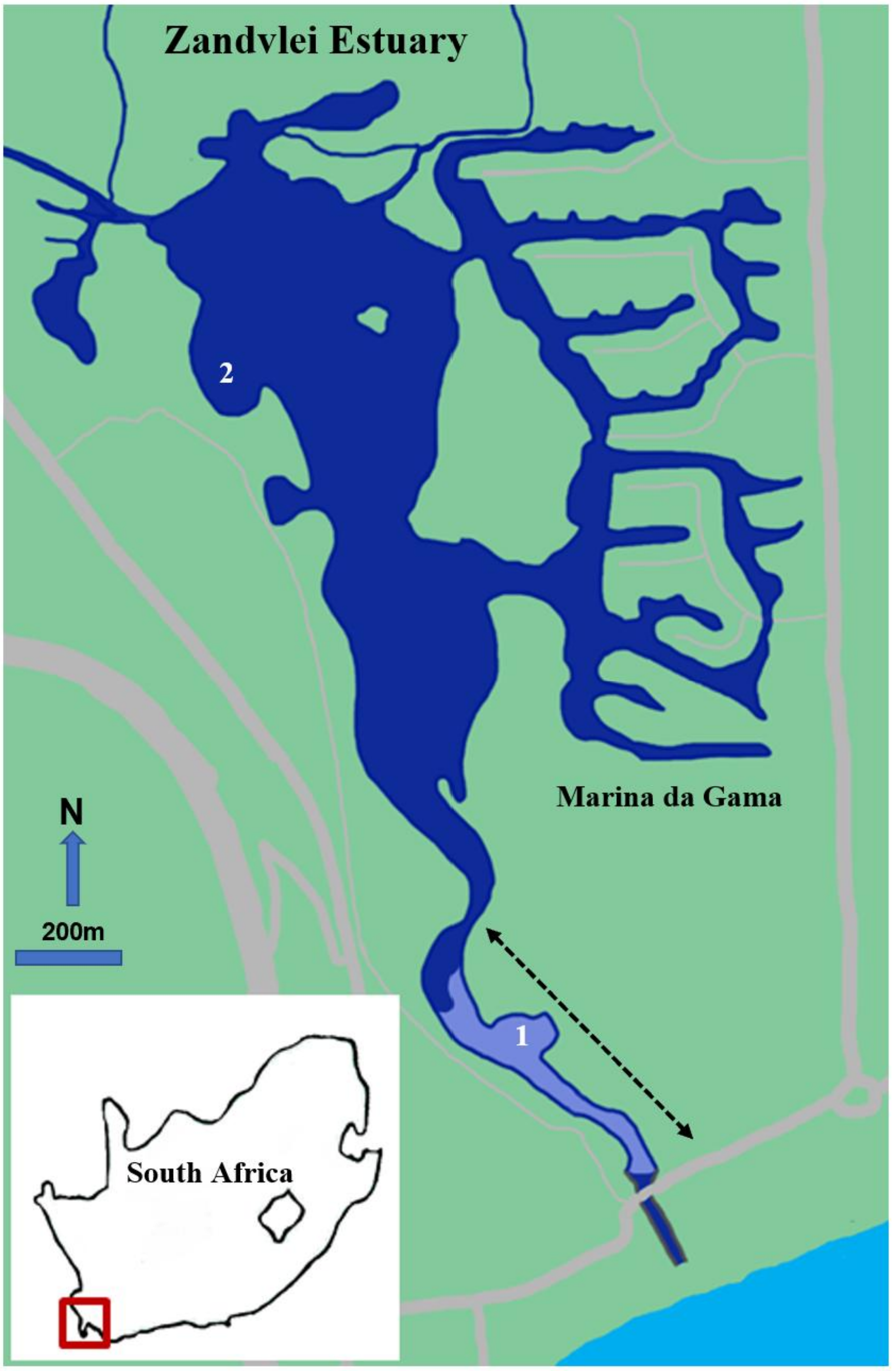

Figure 2.1: Map of the Zandvlei Estuary showing its location within South Africa (inset). The dashed arrow indicates the extent of the sandprawn habitat in the lower reaches. Sandprawns, sediment and mesotrophic water were collected from Site 1 and eutrophic water was collected from Site 2. Maps produced by Jessica Dawson and used with permission.

2.4 Experimental design

A 16-day indoor mesocosm experiment which formed the basis of Chapters 3, 4, 5 and 6, was conducted at the aquarium facilities at the University of Cape Town (UCT), Department of Biological Sciences. The air temperature in the aquarium was set to 15 °C to maintain temperature levels in the low temperature mesocosms in the experiment (explained in detail below) and lighting was scheduled on a 14-hour day followed by a 10-hour night cycle (to simulate natural summer day: night length). Glass mesocosms (thickness: 8 mm, height: 600 mm, length: 300 mm, width: 300 mm) were used to establish 36 independent experimental units for the study (Fig 2.2). The experiment was undertaken between 14-29 November 2020 during national covid lockdowns, and special permission was granted to conduct the experiment. The lockdowns imposed restrictions on the collection of field data to supplement data collected from the experiment.

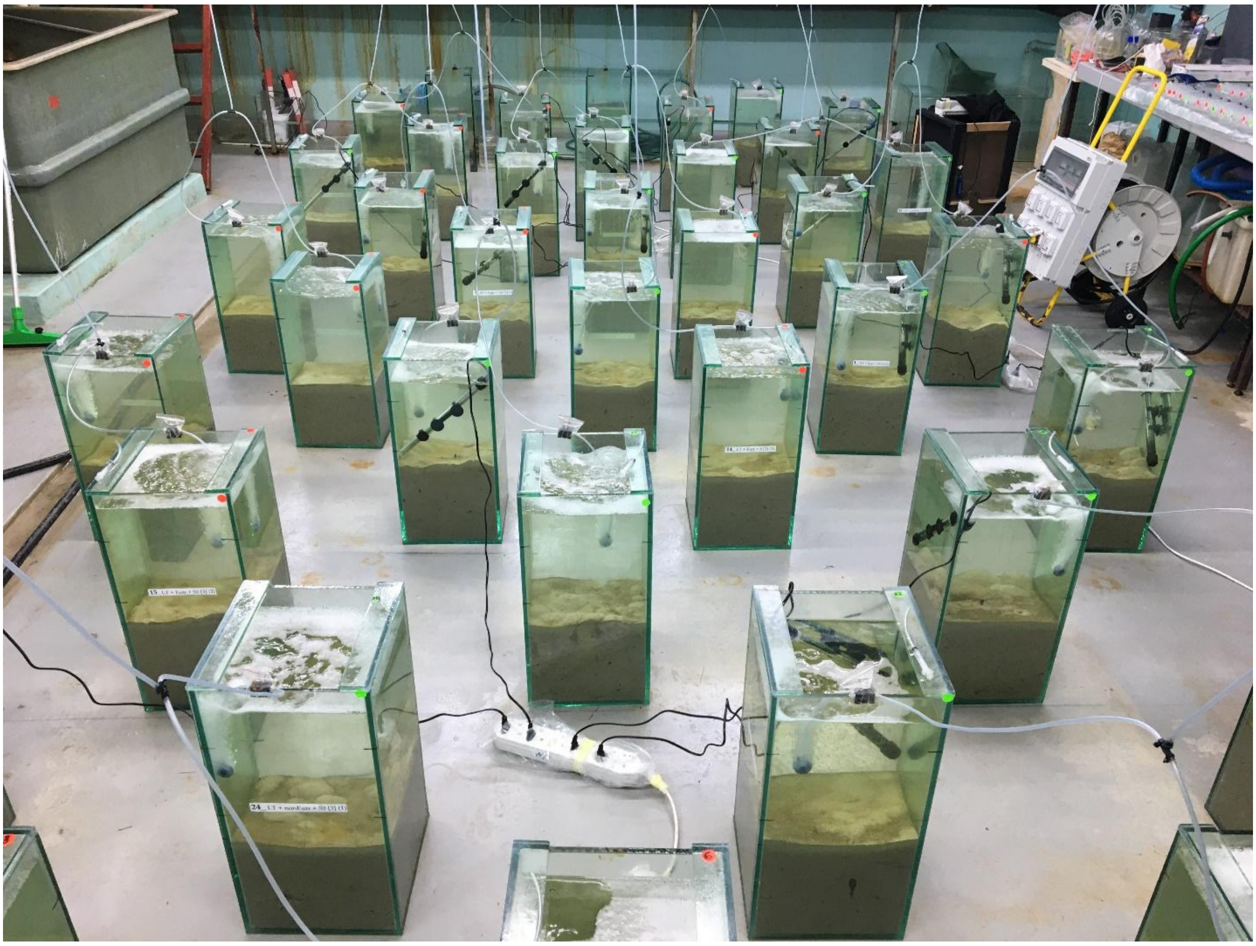

Figure 2.2: Spatial arrangement of mesocosms in the aquarium facility at UCT

The experiment involved manipulating three predictor variables: (1) sandprawn density (three levels: 0% natural density (0 ind/m^2) = no sandprawns, 50% natural density (100 ind/m^2) = 9 sandprawns per mesocosm, 100% natural density (200 ind/m^2) = 18 sandprawns per mesocosm), (2) eutrophication (two levels, mesotrophic (chl-*a* concentration ~ 10 µg/L) versus eutrophic (chl-*a* concentration ~ 15 µg/L) waters (Thornton & McMiIlan, 1989; Thornton, et al., 1995), and (3) temperature (two levels, low ~ 14 °C and high ~ 29 °C), with n = 3. The number of sandprawns used per mesocosm was determined by scaling down the maximum sandprawn densities reported for South African estuaries and lagoons (~ 200 individuals/m^2, Branch & Pringle, 1987) to the area of each mesocosm.

Natural waters were used in the experiment to assess ecological responses to mesotrophic and eutrophic conditions, enabling the inclusion of potential environmental pathogens and toxins to evaluate the robustness of sandprawn filtration effects, particularly under eutrophic conditions. Eutrophic water (chl-a > 15 µg/L; based on classification for semi-arid climates (Harding, 1994, salinity = 10 ppt, temperature = 19°C) was collected from the upper reaches of the estuary (Site 2, Fig. 2.1). Mesotrophic water (chl-a < 15 µg/L, salinity = 29 ppt, temperature = 18°C) was collected from the lower reaches of the system (Site 1, Fig. 2.1), along with sediment and sandprawns. Eutrophic and mesotrophic waters were filtered separately (200 µm mesh) into 100 L vats and homogenized. The water salinity for each trophic treatment was standardized to 29 ppt using marine salt (Aquamedic). Estuarine sediment was sieved (2 mm mesh), to remove macrofauna, before being added to each mesocosm (depth = 25 cm), followed by the addition of either eutrophic or mesotrophic water (depth = 25 cm). This was an important step in ensuring that control sediment was indeed free of sandprawns. Water within each mesocosm was aerated (using airstones that also mixed water; see Fig. 2.2) and allowed to settle for 24 hours before recording initial (Day 0) water quality data. Sandprawns were then introduced into the mesocosms based on designated densities. Non-gravid sandprawns (> 40 mm; rostrum to telson) were collected using stainless steel prawn pumps (length = 900 mm, diameter = 50 mm), placed in loose, moistened layers of newspaper, transported to the aquarium facility and allowed to acclimatise for 2-3 hours before being added to mesocosms.

High water temperature treatments were established using aquarium heaters set to 30 °C, based on maximum recorded temperatures at sites near the sandprawn habitat in the Zandvlei Estuary (25.5 to 26 °C, Harding, 1994) plus a predicted 4°C rise in global temperature by the year 2100 (IPCC, 2007). This temperature level allowed the robustness of sandprawn water-filtration to be assessed at predicted high temperatures, and its potential interaction with eutrophication. Sandprawns allocated to high-temperature treatments were initially placed in water-filled bowls on the surface of the mesocosm water, allowing them to gradually acclimatise to the elevated temperature before being added to the mesocosm. Low temperature treatments were meant to mimic water temperature recorded during winter (Harding, 1994). Throughout the experiment, the number of burrow openings in each mesocosm was monitored, and sandprawns were added where the number of burrow openings decreased so as to maintain density designations (Venter, et al., 2020).

2.5 Monitoring of physico-chemical factors

Water column temperature, electrical conductivity, salinity, pH, turbidity and dissolved oxygen were measured in each mesocosm with a multiprobe (YSI 650 MPI) on Day 0 and every three days thereafter until the experiment concluded. Nutrient concentrations (ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3-}) were measured in each mesocosm on Day 0 and every week thereafter from 40 ml surface water that was collected using a syringe fitted with a flexible tube (diameter = 5 mm). Water samples were stored at -20 °C until analysis using a multiparameter photometer (Hanna Instruments HI 83203).

2.6 Sample collection and processing

The methods used to collect and process samples are presented in each of the data chapters (Chapters 3-6).

CHAPTER 3:

PHYTOPLANKTON AND GLOBAL CHANGE[1]

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[1] Results from this chapter have been published in:
Thomas, C. M., de Cerff, C., Maniel, G. A. V., Oyatoye, A. E., Rocke, E., Marco, H. G., & Pillay, D., 2023. Water filtration by endobenthic sandprawns enhances resilience against eutrophication under experimental global change conditions. *Scientific Reports*, *13*(1), 19067. doi.org/10.1038/s41598-023-46168-y

3.1 Introduction

Phytoplankton are a diverse, heterogenous group of predominantly unicellular photosynthetic organisms that provide fundamental ecosystem services to not only aquatic ecosystems, but to the entire biosphere (Falkowski, et al., 2004; Naselli-Flores & Padisák, 2023). Despite comprising less than 1% of the Earth's photosynthetic biomass, phytoplankton contribute more than 45% to the global net annual primary production (Field, et al., 1998). They serve as the primary source of energy for aquatic ecosystems, due to their role as primary producers (Field, et al., 1998; Winder & Sommer, 2012; Kim, et al., 2020; Naselli-Flores & Padisák, 2023), thereby playing a crucial role in energy transfer through food webs (Naselli-Flores & Padisák, 2023). On a global scale, phytoplankton influence climate regulation, oxygen generation and biogeochemical cycling of macro- and micro-nutrients (Falkowski, et al., 1998; Field, et al., 1998; Winder & Sommer, 2012; Naselli-Flores & Padisák, 2023), including nutrient cycling through the microbial loop (Kim, et al., 2020). In addition, when phytoplankton sink to the bottom of water bodies, they contribute to sediment formation and sustain benthic communities (Naselli-Flores & Padisák, 2023). Some phytoplankton species may produce biologically active toxic compounds, known as phycotoxins, that are harmful to the growth, behaviour, health and population biology of other species and, have been responsible for the deaths of marine organisms and intoxication syndromes in humans, mammals and marine birds (Cembella, 2003).

Phytoplankton size is a fundamental trait that is closely linked to their physiology, influencing their growth, nutrient uptake and interaction with the environment (Cloern & Dufford, 2005; Marañón, 2015). In addition, phytoplankton size structure influences the trophic organisation of assemblages within food webs and, aside from being a key determinant of the efficiency with which energy is transferred to higher trophic levels, also influences the biogeochemical functioning of the ecosystem (Falkowski, et al., 1998; Marañón, 2015). Picoplankton (cells 0.2

< 2 µm in diameter) are not directly accessible to most metazoan consumers, thus, assemblages in which picoplankton dominate support energy transfer through microbial food webs, favouring remineralisation processes and nutrient cycling (Cloern & Dufford, 2005). In contrast, assemblages dominated by larger phytoplankton cells support higher trophic levels within the food web, and due to increased sedimentation rates associated with larger, heavier cells, typically serving as a net sink for CO_2 (Cloern & Dufford, 2005; Marañón, 2015).

Eutrophication is caused by excessive inputs of nutrients into aquatic ecosystems and is a highly problematic anthropogenic stressor impacting coastal zones globally (Vitousek, et al., 1997; Cloern, 2001). It has a deleterious effect on water quality and may trigger extensive ecosystem changes, which may lead to habitat degradation and potentially serious consequences to human health (Vitousek, et al., 1997; Cloern, 2001; Smith & Schindler, 2009; Duarte, et al., 2015). Increased abundance of phytoplankton is an early indication of eutrophication (Kemp, et al., 2005). Notably, eutrophication may alter phytoplankton assemblages and cause a surge in the growth of opportunistic algae, leading to phytoplankton blooms which heightens risks of toxic algal proliferation (Cloern, 2001; Smith & Schindler, 2009; Duarte, et al., 2015). Subsequent increases in oxygen demand due to elevated rates of decomposition may lead to the development of hypoxic/anoxic states that may cause mass mortality of organisms (Cloern, 2001; Rabalais, et al., 2009; Smith & Schindler, 2009; Duarte, et al., 2015). This may alter food web dynamics and initiate trophic cascades with profound implications for biodiversity and ecosystem functioning (Cloern, 2001). Integrating biological processes at a local level (Solan, et al., 2019), which can help prevent or reverse shifts to algal-dominated states has been identified as a tool to reduce the impact of eutrophication in coastal ecosystems (Kemp, et al., 2005; Cloern, 2001; Adams, et al., 2020). For instance, increasing populations of bivalves such as mussels and oysters have been shown to promote phytoplankton consumption and enhance removal of nutrients from the water column, thus mitigating eutrophication (Kellogg, et al., 2014; Petersen, et al., 2014). Managing the ecological impact of human-induced stressors like eutrophication on phytoplankton dynamics is challenging in combination with interacting global change processes (Defeo & Elliott, 2021) as together, these stressors may interact in unpredictable ways to alter phytoplankton community structure, affecting food web dynamics and nutrient cycling (Beardall & Raven, 2004).

Phytoplankton species composition and dynamics are influenced by both bottom-up (resource-driven) and top-down (consumer-driven) processes, and while much focus has been placed on bottom-up controls in efforts to manage eutrophication in coastal and estuarine habitats, the contribution of top-down processes has been less researched (Rodríguez-Gálvez, et al., 2023). This is particularly true for top-down controls driven by endobenthic bioturbators, despite their dominant influence in benthic-pelagic relationships in these ecosystems (Bouma, et al., 2009; Pillay & Branch, 2011). However, a recent study by Venter et al. (2020) revealed that the endobenthic, deposit-feeding sandprawn *K. kraussi*, the focal organism of my study, exerted top-down control on phytoplankton by reducing phytoplankton biomass by nearly 50% in experimental mesocosms, with similar effects observed *in situ* (Venter, et al., 2020). The decline in phytoplankton biomass was linked to bi-directional water pumping during ventilation and irrigation, which likely enhanced phytoplankton subduction into the sediment and entrapment onto burrow walls, driving declines in phytoplankton biomass in the overlying water column (Venter, et al., 2020). Studies by Branch & Pringle (1987) and Papaspyrou, et al. (2005) have found higher levels of chl-*a* relative to the sediment surface near or on the burrow walls of *K. kraussi* and *Pestarella tyrrhena* (also a deposit-feeding Thalassinidean), respectively, lending support to the notion of Venter, et al. (2020) that burrow structures of endobenthic organisms may serve as potential biological filtration systems to enhance the resilience of coastal ecosystems to eutrophication. However, despite its scientific and management importance, research on how effectively sandprawns may perform their water filtration role under future global change conditions is unknown. My study focusses on the impact of two global change processes that have the potential to significantly affect phytoplankton dynamics: predicted increases in global temperatures by the year 2100 (IPCC, 2007) and enhanced levels of coastal eutrophication, and the individual and joint impact of these stressors on the water filtration function provided by sandprawns.

Rising temperatures, up to thermal optima of ectothermic consumers, can accelerate their metabolic rate, increasing energy demands and consumption, thereby intensifying top-down controls on trophic resources within the ecosystem (O'Connor, et al., 2009; Doney, et al., 2012; Carr & Bruno, 2013). However, when temperatures exceed these optima, elevated metabolic rates can induce physiological stress, impair organismal function, increase mortality risk and weaken top-down processes (Seuront, et al., 2019; Wang, et al., 2020). Similarly, enhanced eutrophication can negatively impact ectotherms. For instance, oxygen depletion from accelerated decomposition may intensify physiological stress, while increased occurrence of harmful algal blooms, toxic exudates and pathogenic microbes increase risks of disease and mortality (Cloern, 2001; Harvell et al, 2004; Smith & Schindler, 2009; Kraemer, et al., 2017; Adams, et al., 2020). Furthermore, shifts in phytoplankton assemblages to less edible taxa may reduce trophic resource availability and diminish the ability of consumers to thrive, altering food web interactions and top-down regulation of phytoplankton. (Cloern, 2001; Harvell et al, 2004; Smith & Schindler, 2009; Kraemer, et al., 2017).

To complicate the issue, warming and eutrophication can drive metabolic changes in phytoplankton (Allen, et al., 2005; Doney, et al., 2012). Warming can directly accelerate rates of photosynthesis, thereby increasing resource demand. Without a proportional increase in resource availability, this demand may exceed the metabolic requirements of phytoplankton, resulting in a decline in phytoplankton biomass (Allen, et al., 2005). Conversely, if concentrations of limiting nutrients increase alongside warming, as seen with eutrophication, then phytoplankton biomass may increase, however, this comes with the added complication of shifts towards less palatable phytoplankton taxa (Kraemer, et al., 2017). Given that sandprawns are not specialist filter feeders and therefore not selective in phytoplankton removal, exposure to warming and eutrophication simultaneously could lead to phytoplankton biomass reaching levels that sandprawns may be unable to control effectively, leading to the manifestation of the end-effects of eutrophication in estuarine ecosystems.

In South African estuaries, inorganic nutrient inputs (such as from fertilisers) is intensified further by the discharge of poorly treated organic matter from wastewater treatment facilities, which are beset with poor infrastructure and a lack of operational skills (Adams, et al., 2020). Thus, the combined effect of coastal warming, eutrophication and top-down processes on phytoplankton dynamics are interdependent and complex, creating challenges for predicting future changes to these ecosystems. Without insights into how these interactions may affect phytoplankton communities, and by extension, ecosystem functioning, efforts to mitigate the impact of these global change processes may be ineffective and could result in further degradation of coastal ecosystems and a decline in the goods and services they provide to mankind.

Aims and objectives

Rising temperatures are expected to decrease phytoplankton biomass (Allen, et al., 2005), while eutrophication is anticipated to have the opposite effect, inducing increases to phytoplankton biomass and nanoplankton dominance (Marañón, 2015). To my knowledge, there is limited information on how bioturbation influences these predicted phytoplankton changes and the role that endobenthic bioturbators may have in ameliorating temperature and eutrophication effects on phytoplankton; this is the gap in knowledge that I aim to address in this chapter. In their study on sandprawns, Venter, et al. (2020), showed that sandprawns (*Kraussillichirus kraussi*) can filter out phytoplankton from the water column and suggested that they may have a role to play in counteracting the effects of eutrophication. In this chapter, I intend to build on the research by Venter, et al. (2020) by studying how *K. kraussi* might influence phytoplankton biomass and trait responses in terms of size-class distribution under eutrophic conditions in the context of a warming environment.

I conducted a mesocosm experiment to investigate the effects of sandprawn density, eutrophication and warming on phytoplankton assemblages and to determine whether these effects were antagonistic, additive, or synergistic. I also assessed the resilience of sandprawn filtration under eutrophic and warming conditions, to explore its potential to mitigate phytoplankton blooms linked to high eutrophication levels at the high levels anticipated in future. Additionally, I measured phytoplankton responses in terms of biomass (or abundance) and size, to understand how the top-down effects of sandprawns interacted with eutrophication and warming to influence phytoplankton biomass and traits.

Firstly, I predicted that the ectothermic response of sandprawns (*K. kraussi*) to warming (Pillay, 2019) will boost its water filtration capabilities, enhancing subduction of phytoplankton into the sediment and thus generating proportionally greater declines in chl-*a* from the water column. Secondly, if sandprawn biofiltration is weakened by eutrophication, I predicted that chl-*a* subduction into the sediment will decrease, leading to elevated concentrations of phytoplankton in the water column. My third prediction was that declines in chl-*a* concentrations in the water column will increase with sandprawn density under mesotrophic and current temperatures, and my final prediction was there will be a shift in the algal size-class assemblages in response to warming, eutrophication (Marañón, 2015) and sandprawn density.

3.2 Methodology

Please refer to Chapter 2 for the experimental design for this chapter.

3.3 Data collection

Phytoplankton biomass, represented by chlorophyll-*a* (chl-*a*) concentration, was measured on Day 0 and every three days thereafter until the experiment was concluded. From each mesocosm two 2 mL water samples were collected (depth = 5 cm) and pooled, with chl-*a* concentration determined using fluorometric analysis (Turner Designs Trilogy). Relative phytoplankton cell sizes and absolute abundance thereof were determined by flow cytometry (Faculty of Health Sciences, University of Cape Town) using a BD LSR II flow cytometer equipped with an air-cooled argon-ion laser (488 mm, 20 mW). Forward-scatter (FSC) detected by a photodiode detector with a 488/10 bandpass filter, provided relative cell size data, while side scatter (SSC), detected by a photomultiplier tube (PMT) also fitted with a 488/10 bandpass filter, indicated cell granularity. For absolute cell counts, fluorescent beads (AccuCount Fluorescent Particles, Spherotech, Lake Forest, IL, USA) with a standard concentration were used, with cellular events calibrated against bead events (Gong, et al., 2017; Manti, et al., 2012). Additionally, 0.88 µm sized fluorescent beads served as a standard reference for relative cell sizes. Phytoplankton, naturally auto-fluorescent due to photosynthetic pigments, were identified by their characteristic fluorescence emission spectra (Marie, et al., 1997). Picoplankton and nanoplankton were quantified based on emission signals for orange phycoerythrin (PE): 585/42 bandpass vs. red (PC: 661/16 band pass) fluorescence signals.

On Day 0 (the start of the experiment) and every third day thereafter, three water samples (depth = 5 cm) were randomly collected from each mesocosm with a syringe, pooled to account for within-mesocosm variability, and stored in 2mL cryovials at 4°C in darkness for flow cytometry analysis. The pooled samples were analysed on the day of collection from 1mL aliquots drawn from the pooled sample, vortexed and run at a low flow rate on the BD LSRII with a threshold of ~ 100 000 events or ~ 10 minutes per sample. Flow cytometry data were analysed by gating populations using FlowJo Software (Version 10.7.2; Manti, et al., 2012; Gong, et al., 2017). Population counts of nanophytoplankton and picophytoplankton were converted into concentrations (cells/mL) on Microsoft Excel V16.5 using a standard formula incorporating flow rate and run time of each sample. The proportion of nano- and picophytoplankton was calculated for Day 0 and compared with a mean of values from Day 6 until the experiment concluded on Day 15.

3.4 Data analysis

All data analyses were performed using the data analysis platform R (v4.1.2, 2021; R Core Team, 2019). Linear Mixed-Effects Models (LMEMs) were fitted by restricted maximum likelihood (REML) estimation using the *'lme4'* package (Bates, et al., 2015) to determine the effects of sandprawn density, eutrophication, and temperature on both biotic (chl-*a,* picoplankton and nanoplankton concentrations) and abiotic response variables (physico-chemical and inorganic nutrient data). Time and mesocosm ID were included as random factors in all LMEMs, to account for the temporal dependency of samples (Venter, et al., 2020). Model fits were evaluated graphically using histograms, quantile-quantile (Q-Q) plots and plots of residuals against predicted values to assess normality and homogeneity of variance in the dataset (Zuur, et al., 2009). Where model assumptions were not met, data transformations were applied (chl-*a:* inverse; total phytoplankton and picophytoplankton: $\log_{10;}$ nanophytoplankton: natural log) and the models were re-fitted using transformed data. The 'Anova' function in the *'car'* package was applied to models to determine the significance of main and interactive effects of predictors, given that significance levels for fixed effects were not directly provided in model outputs (Duursma & Powell, 2016).

3.5 Results

Variation in abiotic conditions in the water column among sandprawn densities was minor; where statistically significant effects were found, variance was marginal (Tables 3.1 & 3.2). Variability in salinity was explained by all main predictors as well as the sandprawn density and trophic state interaction. Salinity levels decreased slightly in sandprawn presence in mesotrophic treatments, particularly towards the end of the experiment (Table 3.2). However, at the most, for any specific day of sampling, salinity varied between 36.6 ± 1.1 and 33.6 ± 1.6 SE across increasing sandprawn densities (Table 3.2). Variations in pH was explained by temperature, with values increasing by 0.3 to 0.4 units with rising temperature (Table 3.1).

Water temperature ranged between 14 ± 0.1 SE and 16.5 °C ± 1.2 SE and 26.8 ± 0.4 SE and 29.7 °C ± 0.5 SE in low and high temperature treatments respectively and corresponded with *a priori* designations of levels in the temperature treatment. Salinity increased over the duration of the experiment by 5 to 6 units in high temperature mesocosms; however, this trend was not observed at low temperatures (Table 3.2). A similar trend was observed for dissolved oxygen in high temperature treatments, where percentage saturation increased from initial levels of about 95 % to approximately 100 %. In contrast, oxygen levels in the low temperature treatments fluctuated by no more than 2 % (Table 3.2).

Table 3.1: Results of type II Wald Chi-Square analyses testing the main and interactive effects of predictor variables (sandprawn density, trophic state, temperature) on the abiotic environmental response variables. Statistically significant outcomes are displayed in bold.

Predictor	Abiotic	χ^2	df	*p*-value	pairwise
SP	DO	2.11	2	0.35	
	pH	0.78	2	0.68	
	Salinity	**8.95**	**2**	**0.01**	**100%<0%, p=0.02**
	Temperature	0.59	2	0.74	
	Turbidity	1.16	2	0.56	
TS	DO	0.10	1	0.75	
	pH	0.01	1	0.92	
	Salinity	**11.43**	**1**	**p< 0.001**	**M>E, p= 0.003**
	Temperature	1.38	1	0.24	
	Turbidity	1.98	1	0.16	
T	**DO**	**145.60**	**1**	**p< 0.0001**	**HT>LT, p<0.0001**
	pH	**85.26**	**1**	**p< 0.0001**	**HT>LT, p<0.0001**
	Salinity	**145.85**	**1**	**p< 0.0001**	**HT>LT, p< 0.0001**
	Turbidity	**5.25**	**1**	**0.02**	**HT>LT, p= 0.03**
SP x TS	DO	0.44	2	0.80	
	pH	1.42	2	0.49	
	Salinity	**6.05**	**2**	**0.05**	**0%, 50%, 100% at E < 0% at At, 0% M>E, , p< 0.01**
	Temperature	0.52	2	0.77	
	Turbidity	0.01	2	0.99	
SP x T	DO	0.85	2	0.65	
	pH	5.82	2	0.05	
	Salinity	3.16	2	0.20	
	Temperature	1.22	2	0.54	
	Turbidity	2.04	2	0.36	
TS x T	DO	1.34	1	0.25	
	pH	2.18	1	0.14	
	Salinity	1.35	1	0.25	
	Temperature	0.48	1	0.49	
	Turbidity	0.44	1	0.51	
SP x TS x T	DO	4.88	2	0.09	
	pH	0.52	2	0.77	
	Salinity	1.94	2	0.38	
	Temperature	1.00	2	0.60	
	Turbidity	1.57	2	0.46	

SP = sandprawn density, TS = trophic state (M = mesotrophic, E = eutrophic), T = temperature (HT = high temperature, LT = low temperature). χ^2 = test statistic, df = degrees of freedom, p – value = significance level.

Table 3.2: Spatio-temporal variability in water column temperature (Temp), conductivity (Cond), salinity (Sal), pH, turbidity and dissolved oxygen (DO) levels across temperature, eutrophication and sandprawn density treatments over the 16-day mesocosm experiment. Means ± SE are shown.

Treatment	Day	Sandprawn density	Temp (°C)	Cond (mS/cm)	Sal (ppt)	pH	Turbidity (NTU)	DO (%)
Low temperature, mesotrophic	0	0	13.7±0.1	46.3±0.3	30.1±0.2	8±0.1	9±2.9	93.4±1.3
		50	13.6±0	45.7±0.2	29.6±0.2	8±0	8.7±2.1	94.9±0.1
		100	13.7±0.1	45.7±0.1	29.6±0.1	7.9±0	8.7±1.5	94.6±0
	3	0	14.7±0.3	45.7±0.3	29.7±0.2	8.1±0.1	7.3±1.1	93±2
		50	14.3±0.4	44.1±0.3	28.5±0.2	8±0.1	6.1±0.3	94.4±0.6
		100	14.4±0.2	44.2±0	28.5±0	8.1±0.1	6.1±0.3	93.5±1.9
	6	0	15.1±0.3	45.7±0.4	29.6±0.3	8.1±0	8.3±2.5	93.5±0.2
		50	14.3±0.1	44.8±0.3	28.9±0.3	8.1±0	5.9±0.1	93.5±0.8
		100	14.8±0.3	44.6±0.1	28.8±0	8.1±0.1	6.7±1	94.2±0.6
	9	0	15.3±0.4	45.7±0.6	29.7±0.4	8.1±0	7.1±0.9	93.8±1.2
		50	14.7±0.1	44.7±0	28.9±0	8.1±0	6.1±0.5	94.3±0.6
		100	15.3±0.4	44.5±0.1	28.5±0.4	8.1±0	6.7±0.9	94±0.7
	12	0	15.4±0.3	46±0.3	29.8±0.3	8.2±0.1	5.8±0	93.6±1.9
		50	14.6±0.1	45.1±0	29.2±0	8.1±0.1	5.8±0.1	93.4±1.6
		100	16.5±1.2	44.9±0.2	29.1±0.1	8.2±0.1	5.9±0.2	93.1±1.2
	15	0	15.1±0.3	46.5±0.3	30.2±0.2	8.1±0	6±0.3	94.5±0.2
		50	14.4±0.1	45.6±0.1	29.5±0.1	8.2±0.1	5.7±0	94.1±0.7
		100	14.9±0.3	45.4±0	29.5±0	8.4±0.3	8.3±1.4	94.5±0.9
High temperature, mesotrophic	0	0	13.9±0.3	46.7±0.2	30.3±0.1	8±0.1	8.1±1.3	95.1±0.5
		50	13.8±0.2	46.7±0.3	30.3±0.2	8±0	10±1.6	94.4±0.3
		100	13.8±0.2	46.3±0.6	30±0.4	8±0.1	8.8±2	95.3±0.7
	3	0	30±1	49.3±0.2	32.1±0.2	8.3±0.1	7.8±1.5	97±0.6
		50	28.2±0.8	47.4±0.7	30.8±0.5	8.3±0	6.1±0	95.4±0.3
		100	27.8±0.4	46.1±0.9	29.8±0.6	8.3±0	6.7±0.3	96.8±0.5
	6	0	29.7±0.5	51.5±0.8	33.8±0.6	8.3±0.1	6.7±0.3	96.9±0.6
		50	28.7±0.4	49.5±0.9	32.3±0.7	8.3±0	8.7±1.9	96.6±0.3
		100	28.6±0.2	47.5±1.7	30.8±1.2	8.2±0.1	6.5±0	96±0.8
	9	0	29±0.5	53.1±1.3	34.9±0.9	8.4±0	6.6±0.4	96.9±0.3
		50	29.1±0.8	51.3±1.1	33.6±0.8	8.4±0	6.5±0.4	96.7±0.9
		100	28.7±0.3	48.5±2	31.6±1.5	8.3±0.1	6.6±0.1	98.5±1
	12	0	29±0.3	54.6±1.5	35.8±1.2	8.4±0	6.2±0.1	101.5±1.3
		50	29.5±0.7	52.1±1.1	34.2±0.8	8.3±0	6.4±0.2	98.9±0.5
		100	28.6±0.3	49.8±1.9	32.5±1.4	8.3±0	6.2±0.1	97.5±0.5
	15	0	29.4±0.3	55.4±1.5	36.6±1.1	8.4±0	6.9±0.7	97.3±0.5
		50	29.4±0.7	53.8±1.2	35.4±0.9	8.3±0	6.4±0.2	96.9±0.1
		100	28.8±0.1	51.3±2.2	33.6±1.6	8.3±0	6.7±0.6	97.2±0.4
Low temperature, eutrophic	0	0	13.7±0.2	45.6±0.1	29.5±0.1	8±0	6.2±0.1	95.3±0.1
		50	13.6±0.1	45.3±0.2	29.3±0.2	8±0	8.7±1.2	94.9±0.2
		100	13.6±0.1	45.6±0.3	29.5±0.2	8±0.1	8.9±2.1	93.9±1
	3	0	14±0.1	44.6±0.2	28.8±0.2	8±0	6±0.2	95.8±0.5
		50	14±0.3	43.3±0.2	27.9±0.2	8.1±0.1	5.9±0.1	93.6±1.1
		100	14.9±0.3	43.4±0.2	28.3±0.4	8.2±0.1	5.9±0.1	92.8±0.7
	6	0	14.5±0.1	44.4±0.4	28.7±0.3	8.1±0.1	6.9±0.4	93.8±1.2
		50	14.3±0.4	43.7±0.2	28.2±0.2	8.1±0.1	5.9±0.2	93.4±0.8
		100	15.1±0.4	43.8±0.2	28.3±0.1	8.1±0	6.5±0.5	94.4±0.1

	9	0	14.9±0.1	44.2±0.2	28.6±0.1	8.1±0	6±0.1	94.3±1
		50	14.5±0.3	43.9±0.4	28.3±0.3	8.1±0	5.8±0	94.5±0.1
		100	15.5±0.4	44.2±0.2	28.6±0.2	8.1±0	5.8±0.1	94.6±0
	12	0	14.8±0.1	44.5±0.2	28.8±0.1	8.1±0	5.8±0	95.2±0.1
		50	14.5±0.3	44.4±0.3	28.7±0.2	8.1±0.1	5.7±0	92.2±2
		100	15.5±0.6	44.4±0.3	28.7±0.2	8.2±0.1	5.8±0.1	94.1±0.8
	15	0	14.8±0.2	45±0.2	29.1±0.2	8.1±0	6.9±1	94.3±0.6
		50	14.3±0.3	44.8±0.4	29±0.3	8.1±0	5.8±0.1	94.5±0.4
		100	15.1±0.4	45±0.3	29.1±0.2	8.1±0	6.8±1	94.4±0.8
High temperature, eutrophic	0	0	13.6±0	45.1±0.2	29.1±0.1	8±0	10.7±0	95±0.3
		50	13.5±0	45.4±0	29.4±0	8±0	8.7±1.6	94.9±0.3
		100	13.5±0.1	45.6±0.3	29.5±0.2	8±0	9.4±1.8	94.9±0.4
	3	0	28.8±0.5	46.9±1.1	30.3±0.7	8.3±0	6.6±0.3	95.7±0.6
		50	27.8±0.6	45.7±0.3	29.5±0.2	8.3±0.1	6.5±0.1	97.8±0.9
		100	26.8±0.4	44.9±0.1	29±0.1	8.3±0.1	6.2±0	96.1±0.4
	6	0	29.6±0.7	46.5±1	31.1±0.4	8.4±0.1	7.6±0.7	95.9±0.6
		50	29±0.5	46.6±1.4	36.8±5.5	8.4±0.1	6.6±0.1	96.2±0.5
		100	27.4±0.2	46.9±0.2	30.4±0.1	8.3±0.1	6.3±0	96.1±0.8
	9	0	29.3±0.8	48.8±0.6	31.6±0.5	8.4±0.1	6.3±0.1	96.6±0.4
		50	29±0.2	49.7±0.2	32.4±0.1	8.4±0	6.3±0.1	96.5±0.2
		100	28±0.2	48.5±0.3	31.6±0.2	8.3±0	6.2±0.1	97.8±0.4
	12	0	28.9±0.4	50.1±1	32.7±0.7	8.4±0	6.5±0.1	99±1.4
		50	28.8±0.4	51.8±0.3	33.9±0.3	8.4±0	6.5±0.3	97.1±0.5
		100	27.8±0.2	50.4±0.5	32.9±0.4	8.3±0	6.4±0.2	100.2±2.2
	15	0	28.8±0.1	51.5±1	33.8±0.8	8.4±0	7±0.7	97.7±0
		50	29.4±0.3	52.9±0.6	34.8±0.4	8.3±0	6.2±0.1	96.1±0.3
		100	28.1±0.2	52±0.5	34.1±0.4	8.2±0	6.2±0.1	97.8±0.9

Inorganic nutrient levels in the water column were not affected by variations in sandprawn density and trophic state, but variance in NH_4^+, NO_2^- and PO_4^{3-} levels were influenced by temperature (Table 3.3). Largely, NH_4^+ and NO_2^- levels were elevated in the high temperature treatments, especially towards the end of the experiment (Table 3.4). Lower PO_4^{3-} concentrations were recorded under high temperature, particularly in eutrophic conditions (Table 3.4).

Table 3.3: Results of type II Wald Chi-Square analyses testing the main and interactive effects of predictor variables (sandprawn density, trophic state, temperature) on inorganic nutrient concentrations. Statistically significant outcomes are displayed in bold.

Predictor	Inorganic nutrient	χ^2	df	p-value
SP	Phosphate (PO_4^{3-})	0.10	2	0.95
	Ammonium (NH_4^+)	0.92	2	0.63
	Nitrate (NO_3^-)	4.11	2	0.13
	Nitrite (NO_2^-)	3.24	2	0.20
TS	Phosphate (PO_4^{3-})	0.022	1	0.88
	Ammonium (NH_4^+)	0.04	1	0.84
	Nitrate (NO_3^-)	0.23	1	0.63
	Nitrite (NO_2^-)	0.85	1	0.36
T	**Phosphate (PO_4^{3-})**	**9.57**	1	**0.002**
	Ammonium (NH_4^+)	**8.96**	**1**	**0.003**
	Nitrate (NO_3^-)	0.33	1	0.57
	Nitrite (NO_2^-)	**61.9**	**1**	**<0.0001**
SP x TS	Phosphate (PO_4^{3-})	0.81	2	0.67
	Ammonium (NH_4^+)	0.44	2	0.80
	Nitrate (NO_3^-)	0.25	2	0.88
	Nitrite (NO_2^-)	2.00	2	0.37
SP x T	Phosphate (PO_4^{3-})	0.70	2	0.68
	Ammonium (NH_4^+)	0.66	2	0.72
	Nitrate (NO_3^-)	3.07	2	0.22
	Nitrite (NO_2^-)	**6.32**	**2**	**0.04**
SP x TS x T	Phosphate (PO_4^{3-})	0.85	2	0.65
	Ammonium (NH_4^+)	1.07	2	0.59
	Nitrate (NO_3^-)	0.05	2	0.97
	Nitrite (NO_2^-)	2.30	2	0.32

SP = sandprawn density, TS = trophic state, T = temperature. χ^2 = test statistic, df = degrees of freedom, p – value = significance level.

Table 3.4: Spatio-temporal variability in pelagic nutrient (phosphate, ammonium, nitrate, nitrite) concentrations across temperature, eutrophication and sandprawn density treatments over the 15-day mesocosm experiment. Means ± 1SE are shown.

Treatment	**Day**	**Sandprawn density**	**Phosphate (PO_4^{3-}) (mg/L)**	**Ammonium ($NH_4^{\pm}$) (mg/L)**	**Nitrate (NO_3^-) (mg/L)**	**Nitrite (NO_2^-) (mg/L)**
Low temperature, mesotrophic	0	0	3.1±1.0	5.7±3.4	4.0±4.0	0.1±0.0
		50	4.4±0.3	7.6±1.3	0.0±0.0	0.1±0.0
		100	2.9±0.9	2.7±0.8	0.7±0.7	0.1±0.1
	7	0	3.1±0.6	4.5±3.1	0.7±0.7	0.1±0.0
		50	2.5±0.3	9.2±1.8	0.6±0.6	0.1±0.0
		100	2.5±1.1	10.1±2.2	0.3±0.3	0.1±0.0
	13	0	1.6±0.2	8.4±3.3	0.0±0.0	0.1±0.0
		50	2.7±0.4	2.9±0.4	0.5±0.5	0.1±0.0
		100	3.0±0.2	6.4±3.2	0.0±0.0	0.1±0.1
High temperature, mesotrophic	0	0	3.3±1.4	8.3±1.0	1.0±1.0	0.1±0.0
		50	1.8±0.5	6.6±3.3	0.0±0.0	0.1±0.0
		100	2.7±0.8	7.2±3.4	1.1±0.5	0.1±0.0
	7	0	2.6±1.0	10.2±2.0	0.8±0.5	0.5±0.2
		50	3.2±0.1	10.1±1.4	1.9±1.9	0.4±0.1
		100	2.8±0.6	11.3±0.7	0.1±0.1	0.1±0.0
	13	0	2.0±0.2	11.5±1.1	11±5.4	2.7±0.6
		50	2.3±0.4	6.2±3.0	10.1±3.6	2.0±0.6
		100	1.6±0.6	8.7±3.5	7.0±1.8	1.7±0.5
Low temperature, eutrophic	0	0	3.6±0.5	8.0±3.1	0.0±0.0	0.1±0.0
		50	3.8±0.5	6.8±3.2	1.3±1.3	0.0±0.0
		100	3.7±0.4	4.0±3.3	1.8±1.8	0.1±0.0
	7	0	3.2±0.2	2.7±2.2	1.2±0.8	0.1±0.0
		50	3.2±0.7	6.1±2.1	0.0±0.0	0.1±0.0
		100	3.2±0.7	6.2±2.6	0.0±0.0	0.1±0.0
	13	0	2.7±0.5	10±1.1	2.3±1.4	0.1±0.0
		50	2.3±0.6	6.3±3.2	0.0±0.0	0.2±0.0
		100	2.5±0.5	5.4±2.7	0.0±0.0	0.1±0.0
High temperature, eutrophic	0	0	2.5±1.1	10.3±0.6	0.0±0.0	0.0±0.0
		50	1.8±0.5	5.5±3.6	1.6±1.6	0.0±0.0
		100	0.5±0.3	6.8±3.5	0.4±0.4	0.0±0.0
	7	0	1.9±0.3	10.5±1.0	2.4±1.2	0.3±0.0
		50	2.3±1.0	10.4±0.6	3.5±0.8	0.4±0.1
		100	3.4±0.3	5.2±2.1	1.8±1.0	0.1±0.0
	13	0	1.8±0.4	6.8±3.2	11.8±7.2	3.2±2.0
		50	1.8±0.4	12±0.4	11.1±5.2	3.8±1.1
		100	3.3±0.3	11.8±0.6	1.2±0.7	0.3±0.1

Phytoplankton biomass was explained by each of the main predictor variables and, except for the interaction between sandprawn density and temperature, by all combinations of predictor interactions (Table 3.5). From Day 3 until the end of the experiment, phytoplankton biomass was consistently suppressed in sandprawn presence when compared to controls, regardless of temperature or eutrophication treatment (Fig. 3.1). By the end of the experiment, phytoplankton biomass was similar in sandprawn presence across all temperature and trophic state treatments

(Fig. 3.1). The greatest reduction in phytoplankton biomass in sandprawn presence relative to controls occurred under low temperature, eutrophic conditions, with a decline of 74% recorded by the end of the experiment (Fig. 3.1C). Smaller declines in phytoplankton biomass in sandprawn presence was recorded under low temperature, mesotrophic (43%; Fig. 3.1A) and high temperature, eutrophic conditions (59%; Fig. 3.1D) compared to controls by the conclusion of the experiment. The smallest declines in phytoplankton biomass in sandprawn presence relative to controls was recorded under high temperature, mesotrophic conditions (32%; Fig. 3.1B) at the end of the experiment. Upon termination of the experiment, phytoplankton biomass in control mesocosms at low temperatures increased by approximately 32% beyond initial levels recorded on Day 0 in both mesotrophic (Fig. 3.1A) and eutrophic (Fig. 3.1C) treatments. In contrast, at high temperatures, phytoplankton biomass in control mesocosms remained similar to initial levels, with no detectable increase over time (Figs. 3.1B & 3.1D).

Table 3.5: Results of type II Wald Chi-Square analyses testing the effects of the predictor variables (sandprawn density, trophic state, temperature) on phytoplankton response variables. Statistically significant outcomes are displayed in bold.

	Plankton variable	**χ^2**	**df**	**p-value**
SP	**Chl-*a***	**197.18**	**2**	**< 0.0001**
	Nanoplankton	**55.97**	**2**	**< 0.0001**
	Picoplankton	**5.82**	**2**	**0.05**
TS	**Chl-*a***	**74.52**	**1**	**< 0.0001**
	Nanoplankton	**81.40**	**1**	**< 0.0001**
	Picoplankton	3.18	1	0.07
T	**Chl-*a***	**31.32**	**1**	**< 0.0001**
	Nanoplankton	2.33	1	0.13
	Picoplankton	1.16	1	0.28
SP x TS	**Chl-*a***	**8.06**	**2**	**0.02**
	Nanoplankton	**9.12**	**2**	**0.01**
	Picoplankton	2.44	2	0.29
SP x T	Chl-*a*	0.54	2	0.76
	Nanoplankton	**7.85**	**2**	**0.02**
	Picoplankton	3.31	2	0.19
SP x TS x T	**Chl-*a***	**12.41**	**2**	**0.002**
	Nanoplankton	0.62	2	0.73
	Picoplankton	1.66	2	0.44

SP = sandprawn density, TS = trophic state, T = temperature. χ^2 = test statistic, df = degrees of freedom, p – value = significance level).

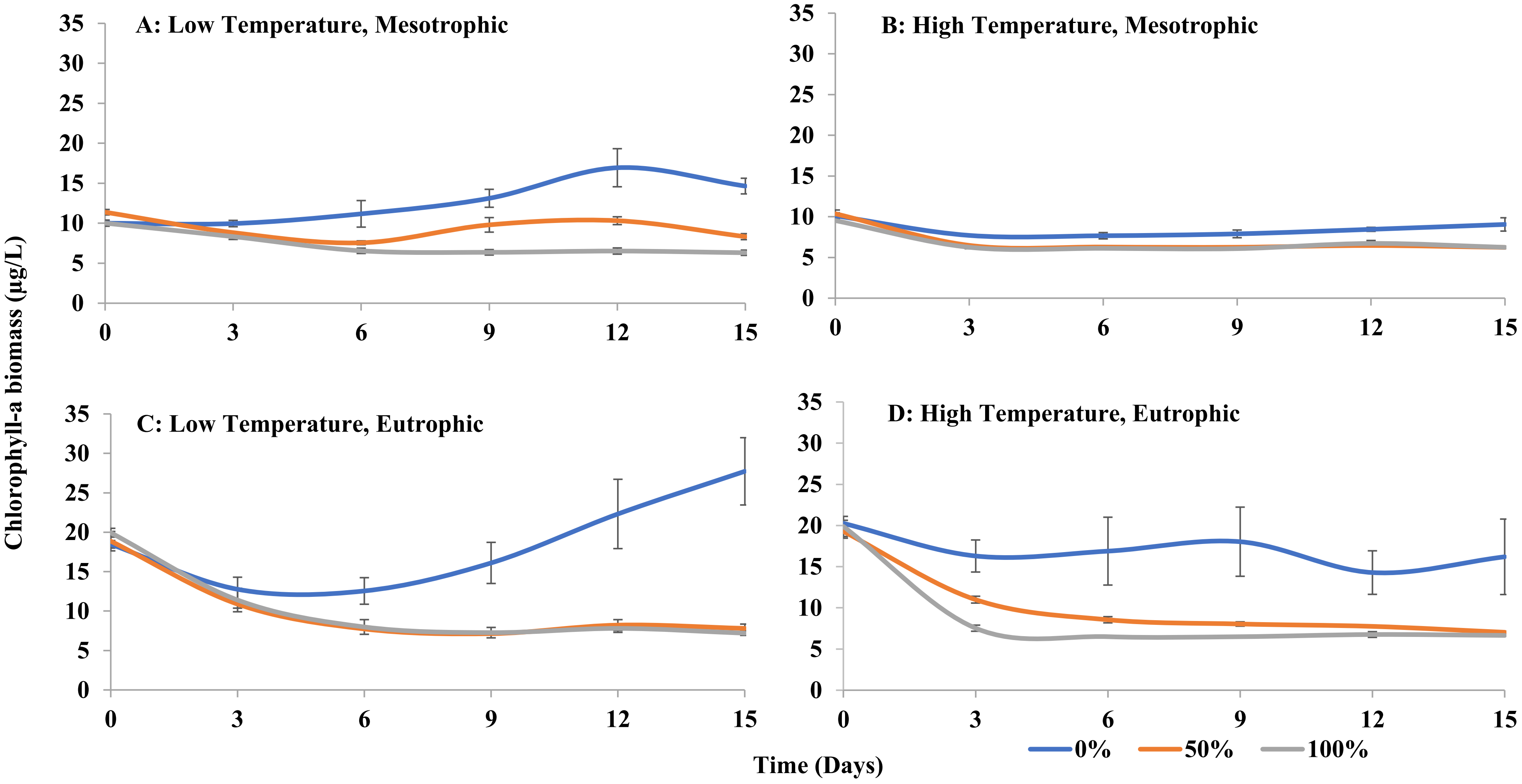

Figure 3.1: Spatio-temporal variability in chl-*a* biomass (mean ± SE) in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%).

Variations in nanophytoplankton abundance was explained by sandprawn density and trophic state, individually as well as by the interaction between (1) sandprawn density and trophic state and (2) sandprawn density and temperature (Table 3.5). By comparison, variance in picophytoplankton abundance was not accounted for by any of the predictors tested (Table 3.5). Overall, the effects of predictor variables on picophytoplankton abundance were unclear, unlike those observed for nanophytoplankton (Figs. 3.2 and 3.3). In mesotrophic conditions, nanophytoplankton abundance showed minimal variation across varying sandprawn densities. Under eutrophic conditions, on the other hand, nanophytoplankton abundance in control mesocosms was almost three times higher by the end of the experiment relative to mesotrophic mesocosms. In addition, under eutrophic conditions, nanophytoplankton abundance was suppressed in sandprawn presence of by approximately three times relative to controls (Fig. 3.3).

Over the duration of the experiment, eutrophication and sandprawn abundance induced shifts in the composition of phytoplankton assemblages (Fig. 3.4). At the start of the experiment, proportional contributions of picophytoplankton and nanophytoplankton to total phytoplankton abundance were similar and remained largely unchanged in mesotrophic mesocosms throughout the experiment, regardless of temperature (Fig. 3.4). However, under eutrophic conditions, nanoplankton was more dominant at the start of the experiment (Day 0; 65 - 79%); and this trend persisted in control mesocosms to the end of the experiment (Days 6 – 15; 65-73% nanophytoplankton). However, the presence of sandprawns reduced the dominance of nanophytoplankton under eutrophic conditions (54%-65%) by the end of the experiment, leading to more balanced contributions of pico- and nanophytoplankton (Fig. 3.4).

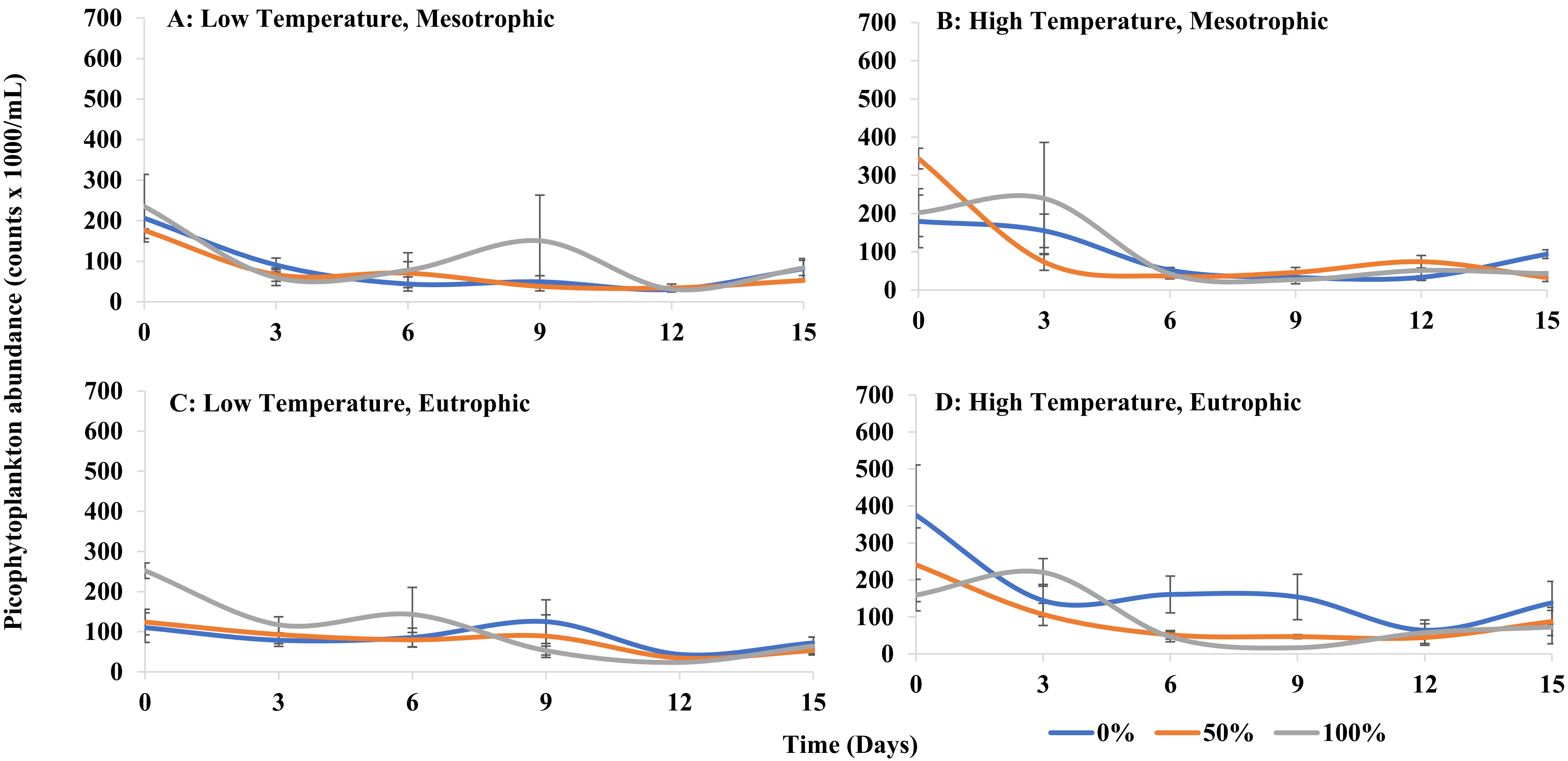

Figure 3.2: Spatio-temporal variability in picophytoplankton abundance (mean ± SE) in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%).

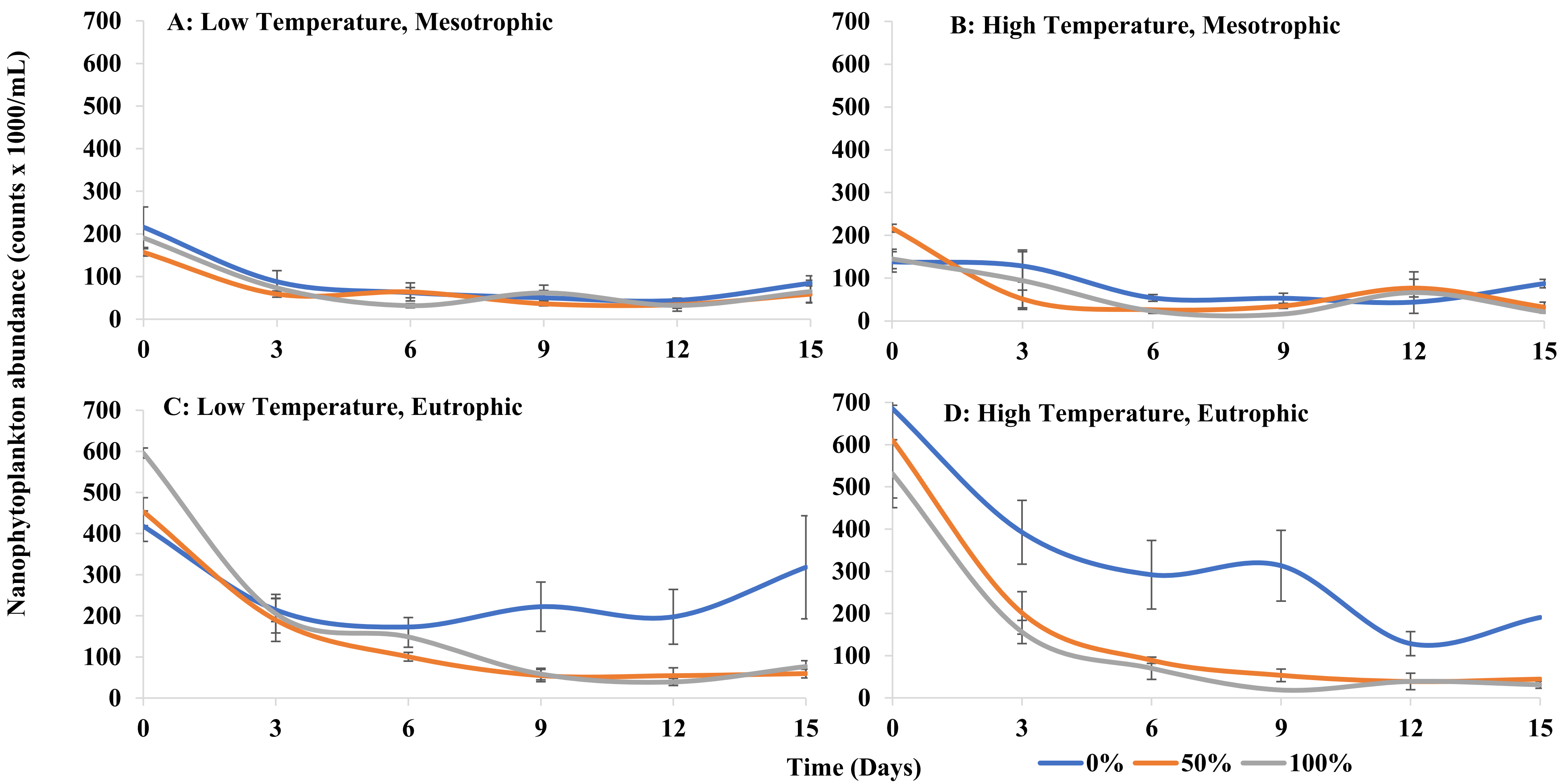

Figure 3.3: Spatio-temporal variability in nanophytoplankton abundance (mean ± SE) in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%).

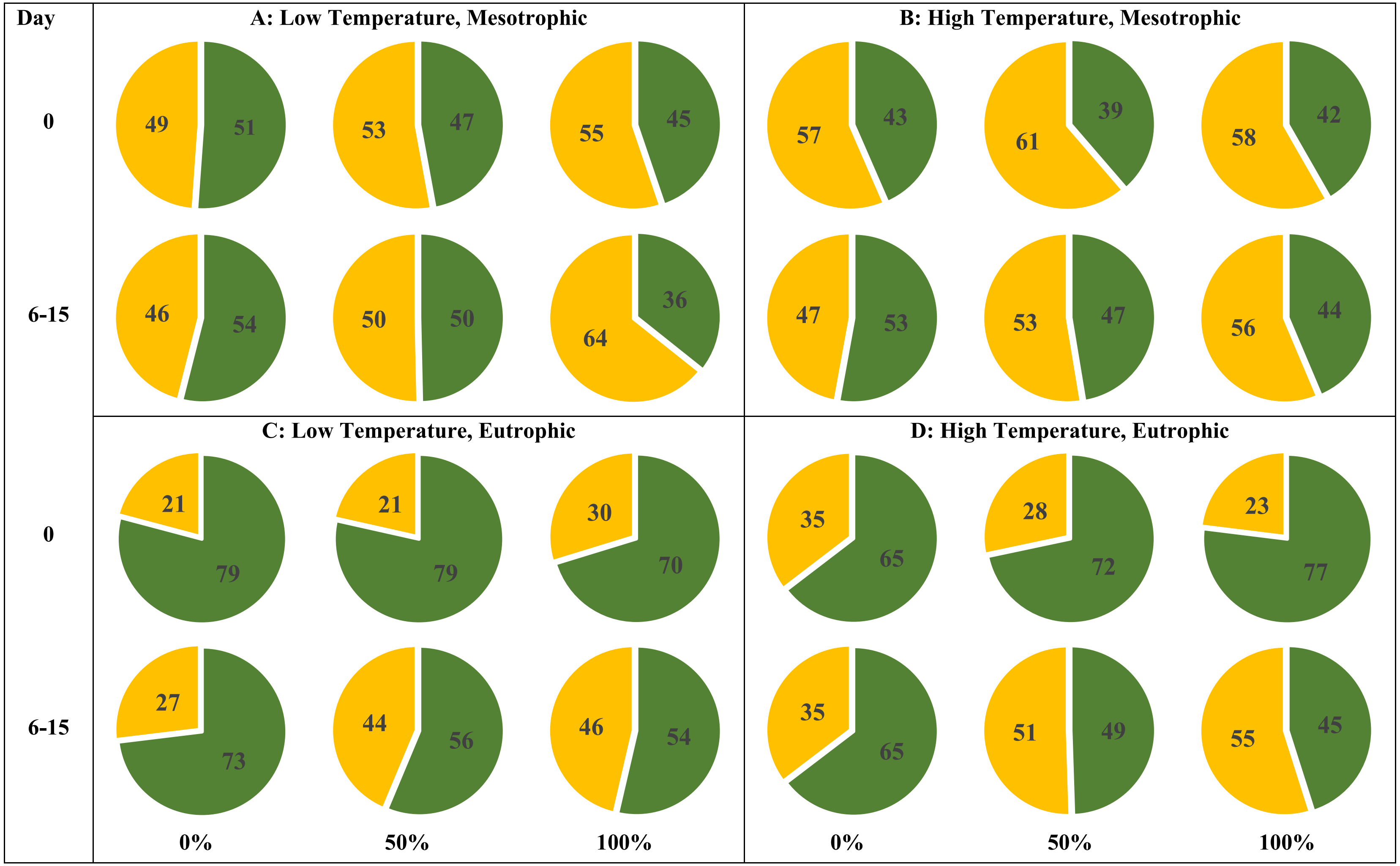

Figure 3.4: Changes in proportions of pico- (yellow) and nanophytoplankton (green) from the start of the experiment (Day 0) to Days 6-15 (averaged) in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms across varying sandprawn densities (0%, 50% and 100%).

3.6 Discussion

More rapid declines in pelagic chl-*a* concentrations in the presence of sandprawns with warming were recorded in my study and supported my prediction that the ectothermic response of sandprawns to elevated temperatures will enhance phytoplankton filtration. Moreover, observations of similar declines in chl-*a* concentrations in eutrophic mesocosms indicted that sandprawn biofiltration was not impaired by eutrophication. My prediction that maximum density will lead to greater declines in pelagic chl-*a* concentrations had minimal support: by the end of my experiment, declines in chl-*a* were roughly the same at medium and maximum sandprawn densities, though in the high temperature, eutrophic treatment, more rapid declines were observed at maximum sandprawn density. In addition, I observed that nanoplankton dominance was suppressed in the presence of sandprawns, even under eutrophic conditions, partially supporting my prediction (as there was no significant temperature effect) that phytoplankton sizes will be altered by the predictor variables in my study. Overall, my findings suggest that water-filtration by endobenthic sandprawns could play a vital role in preventing shifts towards eutrophic and hypertrophic conditions by limiting phytoplankton blooms, even at high temperature conditions, and by preventing shifts to nanoplankton dominance. My results highlight the importance of actively conserving sandprawn populations, and their habitats as part of management strategies to ameliorate current and future coastal eutrophication.

Upon termination of my experiment, I observed a steady reduction in phytoplankton biomass in sandprawn presence, regardless of temperature or eutrophication levels (Fig. 3.1), suggesting that the upper levels of warming and eutrophication did not impair the water filtration capabilities of the sandprawns in the mesocosms. Notwithstanding the fact that sandprawn density was maintained by replacement, declines in biofiltration rates were not observed, reinforcing my suggestion that sandprawn performance remained resilient under the experimental conditions. Eutrophication has been linked to an increase in the abundance of diseased organisms in marine ecosystems (Harvell et al, 2004), with outbreaks of a fecal-enteric microbe in the Florida Keys (Patterson, et al., 2002), aspergillosis (Kim & Harvell, 2002; Bruno, et al., 2003), and the occurrence of black band disease in corals (Kuta & Richardson, 2002) linked to nutrient enrichment and declining water quality. Notably, in my experiment, metagenomic analysis of water column microbial assemblages taken from my experimental system for a separate study, showed that eutrophic waters had double the abundance of bacteria compared to mesotrophic treatments, along with a corresponding doubling in the abundance of Enterobacterales, a group that includes known pathogenic taxa (NICD, 2023; Supplementary Table S1). At the start of my experiment, the phytoplankton biomass in eutrophic mesocosms was almost double that of the mesotrophic mesocosms and, by the end of my experiment, the shift towards hypertrophic states (> 30ug/L, Matthews & Bernard, 2015) may have impaired sandprawn water filtration, especially as sandprawns are not obligate filter-feeders (Pillay & Branch, 2011). However, the significant reductions in phytoplankton biomass under the eutrophic and hypertrophic conditions in sandprawn presence suggests resilience in their water filtration capabilities, even under the potentially damaging effects of toxins, pathogens, and elevated phytoplankton concentrations.

The high temperature treatment, which was approximately 4 degrees above the maximum temperatures recorded near the sandprawn habitat in the Zandvlei Estuary, did not impair sandprawn water filtration in my experiment. Visually, warming appeared to enhance sandprawn water filtration rates; for instance, in mesocosms containing sandprawns at normal temperatures, the decline in phytoplankton biomass reached a plateau (point at which no further reductions were evident) by Day 6 (Fig 3.1A & C). However, with warming, saturation was reached by Day 3 (Fig 3.1B & D). Comparable findings have been reported for urchins, with enhanced top-down control on macroalgae at elevated temperatures being associated with increased metabolic demand (Carr & Bruno, 2013). The resilience of sandprawn water filtration to high temperatures in my study may be reflective of their widespread geographical distribution along the South African coastline, spanning both sub-tropical and cool temperate climates (Branch, et al., 2016). With particular reference to sandprawn biotopes, mean summer water temperatures of 28.4°C and 25.5 - 26 °C have been recorded in Durban Bay (sub-tropical east coast, Pillay, et al., 2008) and the Zandvlei Estuary (cool-temperate southwest coast, Harding, 1984; Chapter 2, Fig. 2.1), respectively. Therefore, the effectiveness of sandprawn water filtration up to temperatures as high as 29.5 °C in my experiment, offers evidence that the water filtration function of sandprawns is resilient to projections of warming scenarios in the future, at least in the context of populations within cool-temperate climates facing predicted temperature changes up until 2100 (IPCC, 2007).

Eutrophic conditions have been associated with trait-shifts in phytoplankton, with larger size classes often dominating (Jiang, et al., 2019). This is attributed to their competitive advantage in nutrient uptake and transfer efficiency (Marañón, 2015). In my experiment, eutrophic conditions in sandprawn absence prompted a shift towards nanoplankton dominance; however, sandprawn presence had a moderating effect, facilitating a balance between pico- and nanophytoplankton concentrations under eutrophic conditions (Fig 3.4). Suspension feeders have been observed to select particles for ingestion based on size and other traits (Schlacher & Wooldridge, 1996; Griffen, et al., 2004; Ward & Shumway, 2004; Safi & Hayden, 2010). However, to my knowledge, the observed size-based shifts in phytoplankton, induced by sandprawns under eutrophic conditions in my experiment, have not been previously reported for any deposit- or filter feeding endobenthic crustacean (Pillay & Branch, 2011). Notably, these size-based shifts in phytoplankton, whether driven by eutrophication or sandprawn activity, may have significant implications for pelagic food web interactions and trophic structure, as phytoplankton size-class strongly influences the size and consumer traits of food webs (Jiang, et al., 2019 & refs therein).

The observed declines in phytoplankton biomass with sandprawn presence in my study likely resulted from consumption. In support of this assertion, stable isotope analysis identified particulate organic matter (POM) as a major component of the diet of sandprawns in the Gamtoos Estuary (Eastern Cape, South Africa, Schlacher & Wooldridge, 1996). Since sandprawns are not obligate filter feeders (Pillay & Branch, 2011), declines in phytoplankton likely occurs through the adsorption of cells onto burrow walls, followed by sorting and ingestion. This notion was supported by the study of Venter et al. (2020), in which it was observed that phytoplankton declines in the water column in sandprawn presence corresponded to an enrichment of chl-*a* in burrow walls in relation to sediment surfaces. The phytoplankton filtration potential of endobenthic burrows is further underscored by estimates suggesting that a 1 m length of burrow of *Upogebia pugettensis*, a filter-feeding endobenthic crustacean, can indirectly filter up to 70% of total phytoplankton compared to direct filtration (Griffen, et al., 2004). The filtration potential of deposit-feeding endobenthic crustaceans could be even greater, considering their burrowing depths, which can reach 3.5 - 4m (Pemberton, et al., 1976; Zeibis, et al., 1996). In addition to consumption as the main factor influencing phytoplankton biomass, some observed effects in my study may also stem from abiotic changes caused by sandprawn activity, as sandprawn density was statistically linked with changes in pH and salinity (individually and/or interactively). However, the relatively small magnitude of these abiotic changes suggest that they were not likely to be the primary drivers of phytoplankton responses in my experiment.

Phytoplankton biomass responses to eutrophication and warming in my experiment revealed notable patterns that may provide insights into how coastal ecosystems could respond to interactions between future warming and eutrophication scenarios. Eutrophic waters had twice the chl-*a* biomass of mesotrophic waters, which was consistent with previous studies in which high nutrient loading was linked to phytoplankton growth (Kemp, et al., 2005; Smith & Schindler, 2009; Adams, et al., 2020). However, over time, eutrophic mesocosms at low temperature reached hypertrophic levels (Fig. 3.1C; chl-*a* > 30 ug/L; Matthews & Bernard, 2015) in the absence of sandprawns, while warming in mesotrophic treatments reduced phytoplankton biomass, causing chl-*a* levels to decline relative to initial conditions (Fig 3.1B). In the absence of sandprawns under eutrophic and warming conditions, chl-*a* levels increased (Fig. 3.1D), but did not reach the high levels recorded at low temperatures. These results suggest that warming exerted an antagonistic effect, counteracting phytoplankton proliferation induced by eutrophication. The suppressive role of warming detected in my study aligns with findings from open ocean ecosystems, where, despite large-scale, regional increases in phytoplankton biomass, most observational and modelling evidence suggests warming has generally reduced phytoplankton biomass and productivity (Lewandowska, et al., 2014), likely by affecting nutrient supply through stratification and/or through altering plankton metabolism (Lewandowska, et al., 2014).

3.7 Concluding remarks

Estuarine phytoplankton provide fundamental ecosystem services, influencing climate regulation, oxygen generation, food web dynamics and energy transfer, as well as biogeochemical cycling of nutrients in the ecosystem (Falkowski, et al., 2004; Naselli-Flores & Padisák, 2023). Research on the interactions between top-down process, eutrophication and warming in these highly valuable ecosystems is scarce and limits our ability to predict how shifts in phytoplankton assemblages may affect ecosystem functioning. My study highlights the potential of sandprawns to counter eutrophication, even under anticipated future warming conditions.

My findings, along with evidence of the resilience of endobenthic crustaceans to stressors such as hypoxia/anoxia, high sulphide levels and hypercapnia (Pillay & Branch, 2011), suggests that their biological and functional traits can play a vital role in combating future eutrophication-induced coastal degradation under future warming scenarios. To optimise the ecosystem benefits of endobenthic burrowing crustaceans and strengthen coastal resilience to eutrophication in future, it is essential to prioritise protection of urban sandprawn populations at local-scales, and to effectively manage endobenthic crustacean populations at a global level.

CHAPTER 4:

BENTHIC MICROALGAE[2]

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[2] Results from this chapter have been published in:
Thomas, C.M., Maniel, G.A.V., Gilmour, K., Oyatoye, A.E., Marco, H.G., Pillay, D., 2024. Microphytobenthic responses to endobenthic bioturbator density, temperature and eutrophication in a global change mesocosm experiment. *Journal of Experimental Marine Biology and Ecology*, 581, p.152066. doi.org/10.1016/j.jembe.2024.152066.

4.1 Introduction

Benthic microalgae or microphytobenthos (MPB) is a major component of coastal sedimentary ecosystems, wherein it inhabits the upper few centimetres of sediment (Pinckney & Zingmark, 1993; MacIntyre, et al., 1996; Miller, et al., 1996; Kromkamp, et al., 2003; Vieira, et al., 2013; Hope, et al., 2020; Méléder, et al., 2020). Benthic microalgae are well adapted to the variable conditions of these environments, which are characterised by strong physical, chemical and biological gradients (MacIntyre, et al., 1996; Miller, et al., 1996; Herman, et al., 2001; Kromkamp, et al., 2003; Bauer, et al., 2013; Vieira, et al., 2013; Hope, et al., 2020). The vertical distribution of MPB within sediment depends on their migration within the upper millimetres of the sediment and on the extent of sediment mixing facilitated by waves, currents, and benthic macrofauna (MacIntyre, et al., 1996). In sediments that experience low-energy waves and high organic matter content, MPB may be restricted to the upper oxygenated layer (MacIntyre, et al., 1996). However, in well-mixed and bioturbated, sandy sediments, MPB could be distributed deeper into the sediment (>10cm; Fielding, et al., 1988; MacIntyre, et al., 1996; Jesus, et al., 2009).

Diatoms are usually the most abundant microalgal component of the MPB community in intertidal estuaries and coastal areas (Vieira, et al., 2013; Ribeiro, et al., 2020), followed by cyanobacteria, green algae and flagellates (Pinckney & Zingmark, 1993; MacIntyre, et al., 1996; Miller, et al., 1996; Kromkamp, et al., 2003; Hope, et al., 2020; Me´ le´der, et al., 2020). MPB functions as a trophic resource of considerable significance in estuarine food webs (Underwood & Kromkamp, 1999), wherein it also influences water quality by stabilising sediments and reducing turbidity and mediating nutrient cycling and bentho-pelagic coupling. (Miller, et al., 1996; Hope, et al., 2020; Richard, et al., 2023).

MPB also has a key role in primary productivity (Kromkamp, et al., 2003; Hope, et al., 2020) and can be responsible for a significant fraction of total estuarine primary production (Underwood & Kromkamp, 1999; Sundbäck & Miles, 2000; Perissinotto, et al., 2002; Kromkamp, et al., 2003; Jones , et al., 2017). MPB productivity often equals or even surpasses that of phytoplankton in the overlying water column (Underwood & Kromkamp, 1999; Sundbäck & Miles, 2000; Perissinotto, et al., 2002; Kromkamp, et al., 2003; Jones , et al., 2017) and serves as a readily available high quality, labile trophic resource (Rizzo, et al., 1996) for higher consumers and for secondary production (Kromkamp, et al., 2003; Werner & Matthiessen, 2013; Como, et al., 2014; Jardine, et al., 2015; Dalu, et al., 2018; Daggers, et al., 2020; Hope, et al., 2020). It is worth noting that MPB can also contribute as a trophic resource for suspension feeders, but the extent is influenced by sediment turnover activities of burrowing organisms, which, by enhancing sediment erodibility, suspends benthic microalgae into the water column (Pillay, et al., 2007; Pillay, et al., 2012). In the absence of bioturbators, resuspension of benthic microalgae is limited, and therefore do not serve as a major component in the diet of suspension feeders (Herman, et al. 2001). This has led to a suggestion that benthic microalgae are predominantly consumed within the sediment where they are produced when bioturbation is negligible (Herman, et al. 2001). Nonetheless, primary production by MPB together with that of pelagic phytoplankton, results in estuaries and coasts being highly productive ecosystems on a global scale (MacIntyre, et al., 1996; Sundbäck , et al., 2000; Kromkamp, et al., 2003; Hope, et al., 2020).

Intertidal and shallow subtidal estuarine sediments are crucial nursery and feeding ground for heterotrophs at higher trophic levels, including wading birds, fish and crustaceans. Abundance of such consumers is typically facilitated by the availability of food resources in the form of benthic invertebrates (Kromkamp, et al., 2003; Hope, et al., 2020; Kromkamp, et al., 2020), which, in turn, rely on diverse MPB assemblages as a primary trophic resource

(Kromkamp, et al., 2003; Hope, et al., 2020). Given that MPB biomass contributes to benthic primary productivity; biotic disturbances such as bioturbation and top-down grazing pressure that influence MPB biomass indirectly influences benthic primary productivity (MacIntyre, et al., 1996).

Photosynthesis and nutrient assimilation by MPB play a significant role in modulating nutrient recycling and bentho-pelagic coupling at the sediment-water interface (Sundbäck , et al., 2000; Sundbäck & Miles, 2000; Kromkamp, et al., 2003). In some coastal ecosystems, photosynthetic oxygen production by MPB can enhance the availability of oxygen and increase its penetration into the sediment to influence nitrification and denitrification processes (Rizzo, et al., 1996; An & Joye, 2001). Under favorable light conditions, photosynthetic-induced nutrient assimilation by MPB reduces the availability of recycled nutrients for pelagic photosynthetic demand, while ensuring that the system, on the whole, remains productive (Grenz, et al., 2021). As MPB assimilates porewater dissolved inorganic nitrogen (DIN) and directly takes up DIN from the water column, it has the potential to attenuate nitrogen efflux from the sediment to the overlying water, thus significantly reducing pelagic photosynthesis (Tobias, et al., 2003). Notably, the degree to which MPB modulates benthic biogeochemical processes is contingent on different attributes of MPB such as its community composition, functional diversity, species richness, abundance and evenness (Thrush, et al., 2017). Aside from these factors, the size-class structure of the MPB assemblage is also an important trait that is intrinsically linked to their physiology and influences their growth and interactions with the environment (Marañón, 2015).

MPB plays a central role in the biostabilisation of sediments (Kromkamp, et al., 2003; Hope, et al., 2020; Herman, et al., 2001), consequently contributing to the improvement of water quality (MacIntyre, et al., 1996; Kromkamp, et al., 2003; Hope, et al., 2020). Biostabilisation of sediments occurs as MPB (mainly diatoms) secrete mucilaginous extracellular polymeric substances (EPS; MacIntyre, et al., 1996; Sundbäck , et al., 2000; Kromkamp, et al., 2003; Bellinger, et al., 2009; Malarkey, et al., 2015) that form a cohesive biofilm on the sediment surface, effectively binding sediment particles, including pollutants (Yallop, et al., 2000; Hope, et al., 2020). Biofilms therefore stabilise the sediment and reduces the degree to which the sediment is resuspended, thereby mitigating erosion (MacIntyre, et al., 1996; Miller, et al., 1996; Kromkamp, et al., 2003; Malarkey, et al., 2015) and indirectly enhances light availability and water quality (Hope et al., 2020). EPS, which is readily consumed (and secreted) by bacteria, may determine the composition and bioactivity of the bacterial community, thereby indirectly influencing functions such as decomposition reactions and nutrient recycling (Yallop, et al., 2000; Herman, et al., 2001).

Various biotic and abiotic factors determine the spatial and temporal dynamics of MPB biomass and structure (Yong, et al., 2022). In terms of biotic determinants, grazing is a well-studied top-down process that alters MPB assemblages in coastal sediments (Miller, et al., 1996; Pillay, et al., 2009; Pillay, et al., 2010). MPB is consumed by the macrobenthos (> 500 µm size class), including crustaceans, bivalves, and polychaete worms; meiofauna (500 µm - 63 µm size class) and microfauna (< 63 µm size class) such as bacteria, fungi and protozoa. Not only do grazers regulate the abundance of MPB, they also serve as critical intermediate trophic linkages to fish, birds and other higher consumers (Miller, et al., 1996).

Bioturbation is also a well-studied biogenic process that influences MPB biomass and composition, yet several knowledge gaps prevail (Richard, et al. 2023), especially in the context of endobenthic bioturbators. This type of bioturbation has the potential to increase sediment erosion, particle resuspension and overall sedimentary instability in coastal systems (Pillay, 2019). Besides disrupting sediment stability, bioturbation-driven sediment turnover buries MPB, depriving it of light and nutrients essential for photosynthesis, consequently having a negative effect on MPB biomass and productivity (Pillay, et al., 2012). Then again, bioturbation also influences sediment pore-water characteristics and nutrient fluxes, which can enhance the productivity of MPB (Hope, et al., 2020; Pascal, et al., 2022), given that these nutrients may be limiting in some cases. Thus, depending on the extent of sediment destabilisation and nutrient mobilisation, bioturbators can have either net positive or negative effects on MPB biomass and productivity (Giorgini, et al., 2019).

Nutrient enrichment linked to eutrophication, can alter both the biomass and composition of MPB to a large degree (Kemp, et al., 2005). Phytoplankton blooms induced by nutrient enrichment may reduce the productivity of certain MPB taxa due to shading effects (Kemp, et al., 2005; Hope, et al., 2020). However, in shallower conditions, nutrient enrichment might stimulate MPB productivity while also shifting MPB composition, as taxa respond differently to specific nutrients that may otherwise be limiting (de Jonge, 1990; Hillebrand & Sommer, 1997). Pathogens and pollutants linked to eutrophic conditions (Cole, et al., 2011) may also bind to both MPB and the EPS they secrete to hinder the growth, photosynthetic activity and community composition of MPB (Hope, et al., 2020).

Warming can similarly alter coastal MPB biomass and productivity (Cartaxana, et al., 2015). Rising temperatures can induce switches in MPB composition by favouring taxa with higher thermal tolerance, while negatively impacting those less adapted to temperatures that surpass their thermal optima (Cartaxana, et al., 2015). Below thermal optima, increasing temperature can stimulate MPB metabolism, growth and productivity (Blanchard, et al., 1996; Vieira, et al., 2013; Cartaxana, et al., 2015). However, if the level of warming exceeds species-specific thermal optima, it may result in declining MPB biomass and productivity (Beardall & Raven, 2004; Hicks, et al., 2011; Hillebrand, 2011). Apart from this, increasing temperature also influences trophic interactions, food web structures, the metabolism and bioturbation activity of ectothermic consumers (IPCC, 2007), all of which impacts MPB biomass and assemblage and adds to the complexity of mechanisms mediating MPB response to warming.

For example, heightened ectothermic metabolism linked to warming increases food consumption and enhances grazing pressure on MPB, decreasing their biomass and reducing their productivity (Zhang, et al., 2020). Additonally, temperature-induced increases in bioturbation may reduce MPB biomass by increasing sediment instability, while temperature-induced increases in nutrient fluxes from enhanced eutrophication may have the opposite effect, supporting MPB growth (Pillay, 2019).

The contribution of MPB to estuarine productivity, biogeochemical processes and sediment stability, as well as their strong links with higher consumers have established MPB as central to benthic interaction networks and coastal ecosystem functioning (MacIntyre, et al., 1996; Hope, et al., 2020). A thorough understanding of the processes that influence the composition and dynamics of MPB is thus essential due to the diverse ecological functions they mediate in sedimentary ecosystems. Specifically, an understanding of how MPB communities respond to anthropogenic pressures, such as global change, including eutrophication, is key to managing and protecting coastal ecosystems sustainably and preserving essential ecosystem services. However, in the context of global change, research on the individual and interactive (synergistic, antagonistic or additive) effects of global change stressors on MPB dynamics is limited (Alsterberg, et al., 2011; Hardison, et al., 2013).

Aims and objectives

Research to date has generally treated MPB as a single entity, without probing the contributions of key component groups such as diatoms, cyanobacteria and green algae in MPB variability. This has, to a degree, led to knowledge gaps regarding MPB group contributions to the functioning of sedimentary systems, including under global change scenarios (Armitage, et al., 2009; Herman, et al., 2001). The exposure of coastal ecosystems, especially estuaries, to a combination of global and regional stressors, is widely reported in literature (Defeo & Elliott, 2021); yet experimental studies investigating the impact of global change on these areas have neglected to include the effect of global change on MPB, and it's components, in these ecosystems (Alsterberg, et al., 2011; Hardison, et al., 2013). Simultaneously, axiid crustaceans that inhabit extensive areas of coastal sediment and form a major component of sheltered bays and estuaries worldwide, have the potential to influence MPB community structure and hence its contribution to ecosystem functioning (Pillay, 2019).

In the context of warming and eutrophication-induced nutrient-enrichment, I sought to quantify the role of a key bioturbator in determining spatial changes in MPB biomass and composition. This chapter specifically aimed to quantify the net effects of increasing temperature, eutrophication level and varying densities of a South African endobenthic sandprawn (*Kraussillichirus kraussi*) on the biomass, assemblage composition and size-class structure of estuarine MPB assemblages. The secondary goal was to understand whether sandprawn effects, eutrophication and warming interacted antagonistically, additively, or synergistically to influence MPB biomass and community composition.

In terms of the MPB response to warming, I predicted that increasing temperature would (1) decrease the biomass of epibenthic diatoms, cyanobacteria and green algae as the elevated metabolic demands of microalgal cells induced by warming may not be met, resulting in declines in microalgal biomass and size (Clarke & Fraser, 2004; Allen, et al., 2005); (2) promote cyanobacterial biomass given previous research indicating that cyanobacteria thrive over other taxa under higher temperatures (Van der Grinten, et al., 2005; Hillebrand, 2011; Cartaxana, et al., 2015); (3) cause a shift to subsurface pico-sized microalgal components due to increased rates of cell division reported for this group under increasing temperature (Vieira, et al., 2013); (4) cause declines in epibenthic MPB biomass with increasing sandprawn density due to temperature-induced stimulation of bioturbation burying microalgae, depriving them of light and nutrients required for photosynthesis (Armitage, et al., 2009; Pillay and Branch, 2011; Pillay, et al., 2012; Pillay, 2019).

I also predicted that eutrophic conditions would (1) stimulate epibenthic biomass of diatoms, cyanobacteria and green algae, due to associated increases in nutrient levels (de Jonge, 1990; Hillebrand & Sommer, 1997; Werner & Matthiessen, 2013) and (2) induce a shift in the composition of the subsurface size-class microalgal assemblage towards larger nano- sized cells due to the nutrient-rich conditions associated with eutrophication (Cermeño, et al., 2005)

Regarding sandprawn density, I predicted that increasing sandprawn density would (1) reduce the biomass of epibenthic diatoms, cyanobacteria and green algae due to sediment turnover activities associated with bioturbation. This prediction was based on past research indicating that *K. kraussi,* through increased sediment turnover, reduces MPB biomass (Pillay, et al., 2012). The MPB community response to the interaction between warming, eutrophication and sandprawn density is multifaceted and complex and predictions on how epibenthic biomass and subsurface size-class assemblage would respond were therefore not made.

4.2 Methodology

Please refer to Chapter 2 for the experimental design for this chapter.

4.3 Data collection

Upon termination of the experiment on Day 16, a BenthoTorch (BTo 09-145, bbe-Moldaenke GmbH Schwentinental, Germany) was used to measure biomass of three epibenthic microphytobenthic (MPB) algal groups: cyanobacteria (blue-green algae), diatoms and green algae. Four replicate BenthoTorch measurements were made per mesocosm and pooled for subsequent analyses. The BenthoTorch is a fluoroprobe designed for the rapid *in situ* quantification of green algae, cyanobacteria and diatom biomass on substrates (bbe-Moldaenke, 2013). It operates as a Pulse-Amplitude Modulated (PAM) fluorometer, emitting light pulses at four wavelengths (470, 525, 610 and 700 nm) and records the responses of the benthic algae at 690 nm. The biomass of the different algal groups, as separate measures of chlorophyll-*a*, is calculated with inbuilt algorithms and quantified per square centimetre (bbe-Moldaenke, 2013; Carpentier, 2013; Kahlert and McKie, 2014). Light-emitting diodes (LEDs) discharge light pulses causing excitation of cell pigments of alga, causing them to fluoresce, which was used in turn to quantify the different algal group chlorophyll-a concentrations (bbe-Moldaenke, 2013; Carpentier, 2013; Kahlert and McKie, 2014; Harris & Graham, 2015).

While the BenthoTorch is an innovative tool that provides *in situ* data rapidly, it has received some criticism in the literature for its lack of accuracy when measuring MPB proportions, particularly where biofilms are thick or where light levels and temperature are not easily controlled (Rosero-Lopez et. al., 2021). In the context of my study, where the sediment used was well bioturbated by sandprawns, biomass of biofilm components is typically low (Pillay, et al., 2019). Therefore, the BenthoTorch was considered an appropriate tool to measure MPB biomass in the current study.

The proportion of the nano and pico size classes to the subsurface MPB assemblage was determined using flow cytometry (FCM) technology (described in Chapter 3). At the end of the experiment on Day 16, five sediment cores (depth = 5cm), were collected per mesocosm using a corer (internal diameter = 2cm). Extracted cores were pooled and fixed (4% paraformaldehyde) and frozen at -80°C prior to further analysis. Methods to extract and quantify nano- and pico- size classes of the MPB from the sediment samples were adapted from Tso & Taghon (1997) and Vignola, et al. (2018). Prior to extraction of the MPB algae from the sediment, samples were defrosted at room temperature (20°C) and then transferred to a beaker. Thereafter, 20mL filtered, autoclaved seawater was added to the beaker and the sediment swirled (30 times) with residual sediment from the sample container being rinsed with filtered (GFF, Whatman), autoclaved seawater. Particles were allowed to settle for 5-10 seconds, followed by the supernatant being decanted into a second beaker. This process was repeated five times after which the pooled decanted supernatant was filtered (200µm) into a third beaker. 2mL sub-samples of the filtered supernatant, which contained the extracted MPB algae, were used to quantify nano- and pico-phytoplankton abundance of the MPB assemblage using flow cytometry (Vignola, et al., 2018).

4.4 Data analysis

All multivariate analyses were run using PRIMER-e (Plymouth Routines in Multivariate Ecological Research) v.6.1.18 (Clarke et al, 2015) and PERMANOVA+ v1.0.8 (Anderson et al, 2008) statistical packages. Univariate data analyses (Linear Mixed Effects Models (LMEM) and Linear Fixed Effects Models (LFEM)) were performed using the RStudio data analysis platform (R Core Team, 2019. Where model assumptions were violated, models were re-fitted. Transformations used and models fitted to response data are shown in Table 4.1.

Table 4.1: Statistical analyses performed on response variables (microphytobenthic (MPB) epibenthic community composition, epibenthic biomass, and subsurface size class assemblage) in relation to predictors (SP: sandprawn density, T: temperature, TS: trophic state).

Response variable	Predictors	Statistical Tool	Analyses	Transformation
Epibenthic MPB community composition	SP, TS, T, + interactions	PRIMER-e	PERMANOVA	Fourth Root
Epibenthic MPB biomass	SP, TS, T, + interactions	RStudio	LFEM	
• Diatoms				
• cyanobacteria				Inverse Log$_{10}$
Green algae (non-parametric)			Excluded	
Subsurface MPB size class assemblage (nano- and pico-phytoplankton counts)	SP, TS, T, + interactions	RStudio	LFEM	None

4.5 Results

Epibenthic MPB community composition. Multivariate analyses revealed that sandprawn density (PERMANOVA, pseudo-$F_{2,\ 136.8}$ = 4.20, p = 0.015, Table 4.2) and temperature (PERMANOVA, pseudo-$F_{1,\ 462.5}$ = 4.20, p = 0.001) were the only predictors to significantly influence epibenthic MPB assemblages (cyanobacteria, diatoms and green algae). Eutrophication was insignificant at explaining variation in epibenthic MPB assemblage (PERMANOVA p = 0.157), as were interactions among sandprawn density, temperature and eutrophication (Table 4.2).

Table 4.2: Summary statistics for PERMANOVA and pairwise tests (where relevant) assessing the influence of sandprawn density, temperature, trophic conditions, and their interactions on epibenthic MPB assemblage structure. Significant p-values are indicated in **bold.** SP: sandprawn density, T: temperature (HT= high temperature, LT=low temperature), TS: trophic state (M = mesotrophic, E = eutrophic).

Source	**df**	**SS**	**Pseudo-F**	**p-value (PERMANOVA)**
SP	**2**	**136.83**	**4.20**	**0.015** **(50% ≠ 100%, Pairwise p=0.028)**
T	**1**	**462.45**	**28.41**	**0.001** **(LT ≠ HT, pairwise = 0.0001)**
TS	1	36.34	2.23	0.157
SP x T	2	43.35	1.33	0.276
SP x TS	2	9.77	0.30	0.808
T x TS	1	9.19	0.57	0.552
SP x T x TS	2	18.84	0.580	0.632

Responses of individual epibenthic taxa. Deconstruction of the epibenthic MPB assemblages into the three main taxa, showed several trends. Firstly, cyanobacteria and diatoms accounted for the majority of the total epibenthic MPB biomass, albeit that variance for both taxa was notably high under high temperature conditions (Figs. 4.1 & 4.2). For cyanobacterial biomass, temperature was the most significant individual predictor of variability ($F = 65.87$, $df = 1$, $p = 0.0001$, Table 4.3) followed by sandprawn density ($F = 7.48$, $df = 2$, $p = 0.003$). Within-temperature comparisons indicated a significant suppression of cyanobacterial biomass at high compared to low temperatures (pairwise, $p < 0.0001$). Increasing sandprawn density generally elicited declines in mean cyanobacterial biomass with the strongest decline evident between control and maximum sandprawn density (100% < 0%, pairwise, $p = 0.003$) and a secondary decline from intermediate to maximum density (100% < 50%, pairwise, $p = 0.03$). While no significant interaction between sandprawn density and temperature was detected (Table 4.3), it is notable that strongest declines in cyanobacterial biomass with increasing sandprawn density occurred at low temperature. At high temperature, biomass declines with increasing sandprawn density was weaker under eutrophic conditions, and almost non-existent under mesotrophic conditions. Likewise, temperature significantly explained variability in diatom biomass ($F = 58.99$, $df = 1$, $p < 0.0001$) with high temperature inducing a suppression of biomass (pairwise, $p < 0.0001$, Fig. 4.2). Sandprawn effects on diatom biomass were insignificant ($F = 2.26$, $df = 2$, $p = 0.091$), but declines in mean biomass were evident as sandprawn density increased from 0% to 50% and 100% (pairwise, $p = 0.034$). Green-algal biomass was the lowest of the epibenthic MPB components measured and exhibited high variability, resulting in treatment trends being vague (Fig. 4.3). Trends for total epibenthic MPB biomass were similar to those observed for diatom biomass, but with sandprawn effects being significant ($F = 4.77$, $df = 2$, $p = 0.018$, Fig. 4.4). Specifically, increases in sandprawn density from 0% to 100% and 50% to 100% elicited declines in biomass (pairwise, $p = 0.034$).

As with cyanobacteria and diatoms, high temperature led to a suppression of total epibenthic MPB biomass ($F = 86.00$, $df = 1$, $p < 0.0001$, pairwise, $p < 0.0001$).

Table 4.3: Results of the linear fixed effects models testing for differences in biomass of epibenthic MPB (total MPB, diatoms, cyanobacteria) between the different levels of sandprawn density, temperature and trophic states and their interactions. Statistically significant outcomes are displayed in **bold**. SP: sandprawn density, T: temperature (HT= high temperature, LT=low temperature), TS: trophic state (M = mesotrophic, E = eutrophic).

*Green algae were not normally distributed (due to low sample sizes) and were excluded from linear models.

	Response variable	**F-Value**	**df**	**p-value**	**pairwise**
SP	**Total microalgae**	**4.77**	**2**	**0.018**	**100% < 50% & 0%, p = 0.034**
	Diatoms	2.66	2	0.091	
	Cyanobacteria	**7.48**	**2**	**0.003**	**50% > 100%, p = 0.03** **0% >100%, p = 0.003**
TS	Total microalgae	0.84	1	0.369	
	Diatoms	0.03	1	0.869	
	Cyanobacteria	0.13	1	0.719	
T	**Total microalgae**	**86.00**	**1**	**p < 0.0001**	**LT>HT, p < 0.0001**
	Diatoms	**58.99**	**1**	**p < 0.0001**	**LT>HT, p < 0.0001**
	Cyanobacteria	**65.87**	**1**	**p < 0.0001**	**LT>HT, p < 0.0001**
SP x TS	Total microalgae	0.77	2	0.476	
	Diatoms	0.47	2	0.630	
	Cyanobacteria	0.13	2	0.883	
SP x T	Total microalgae	1.72	2	0.199	
	Diatoms	1.93	2	0.167	
	Cyanobacteria	1.03	2	0.372	
TS x T	Total microalgae	0.84	1	0.368	
	Diatoms	1.63	1	0.214	
	Cyanobacteria	1.50	1	0.233	
SP x TS x T	Total microalgae	0.20	2	0.818	
	Diatoms	0.06	2	0.943	
	Cyanobacteria	1.99	2	0.159	

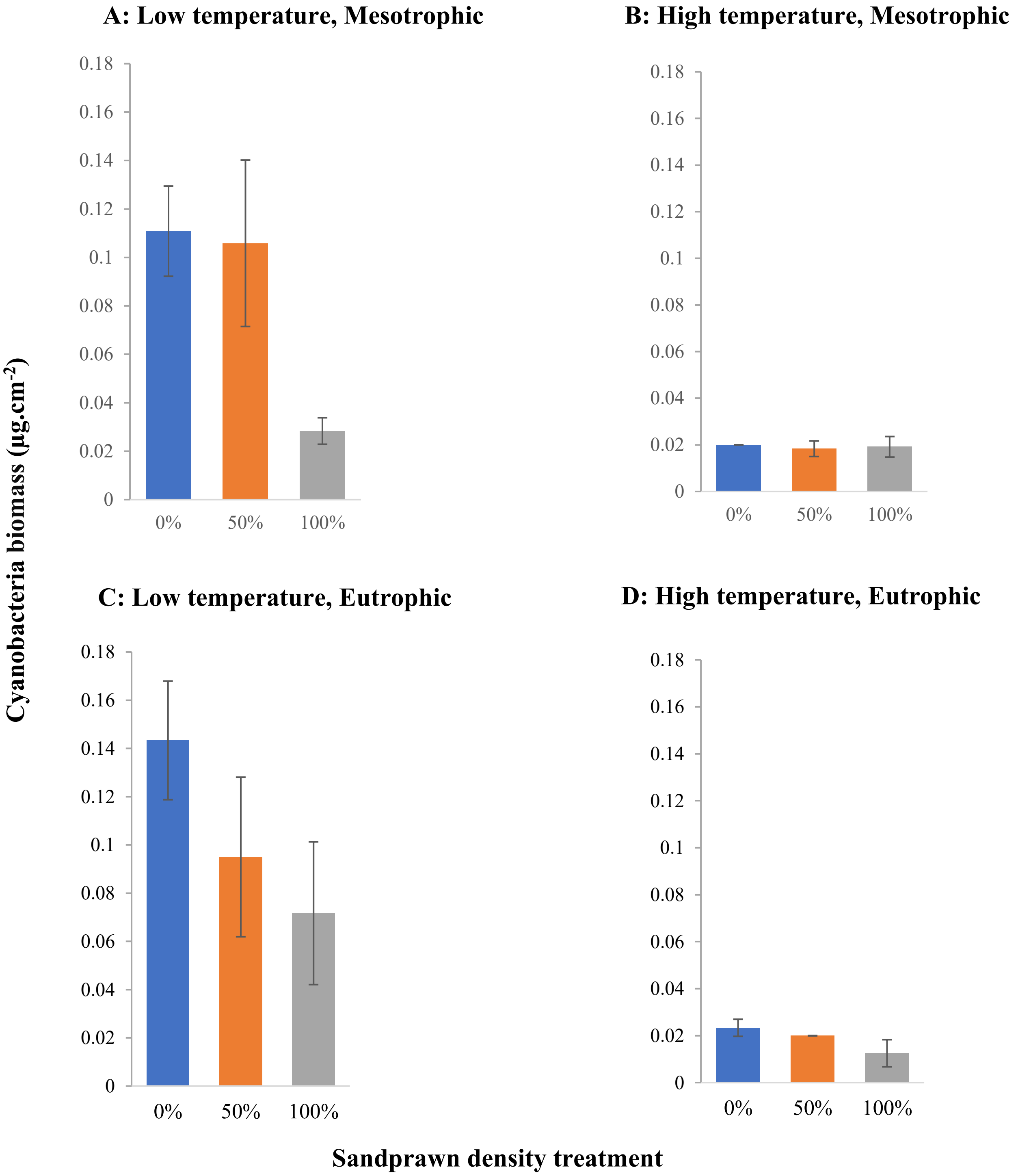


Figure 4.1: Spatial variability (mean ± SE) of epibenthic cyanobacteria in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%).

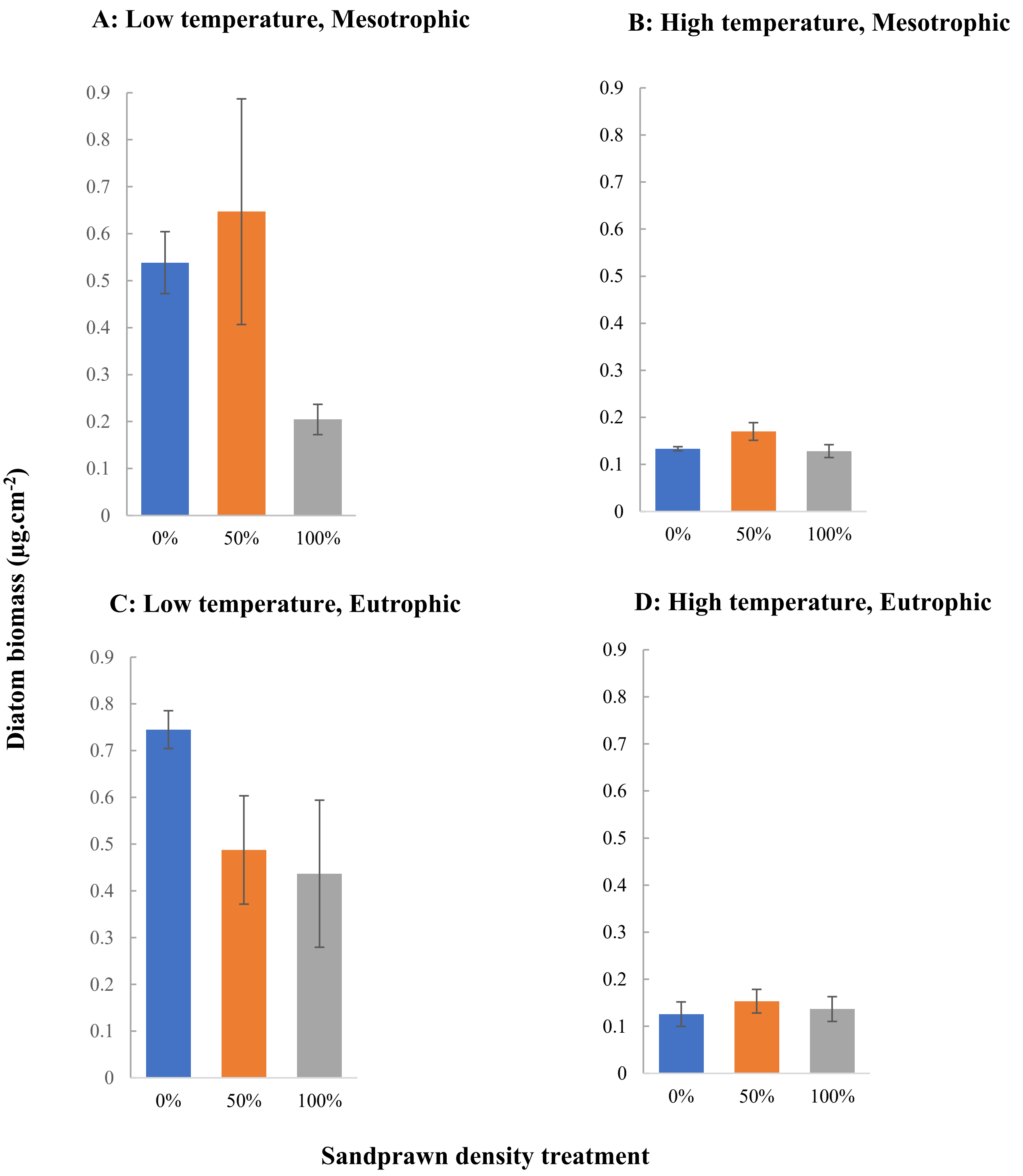


Figure 4.2: Spatial variability (mean ± SE) of epibenthic diatoms in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%).

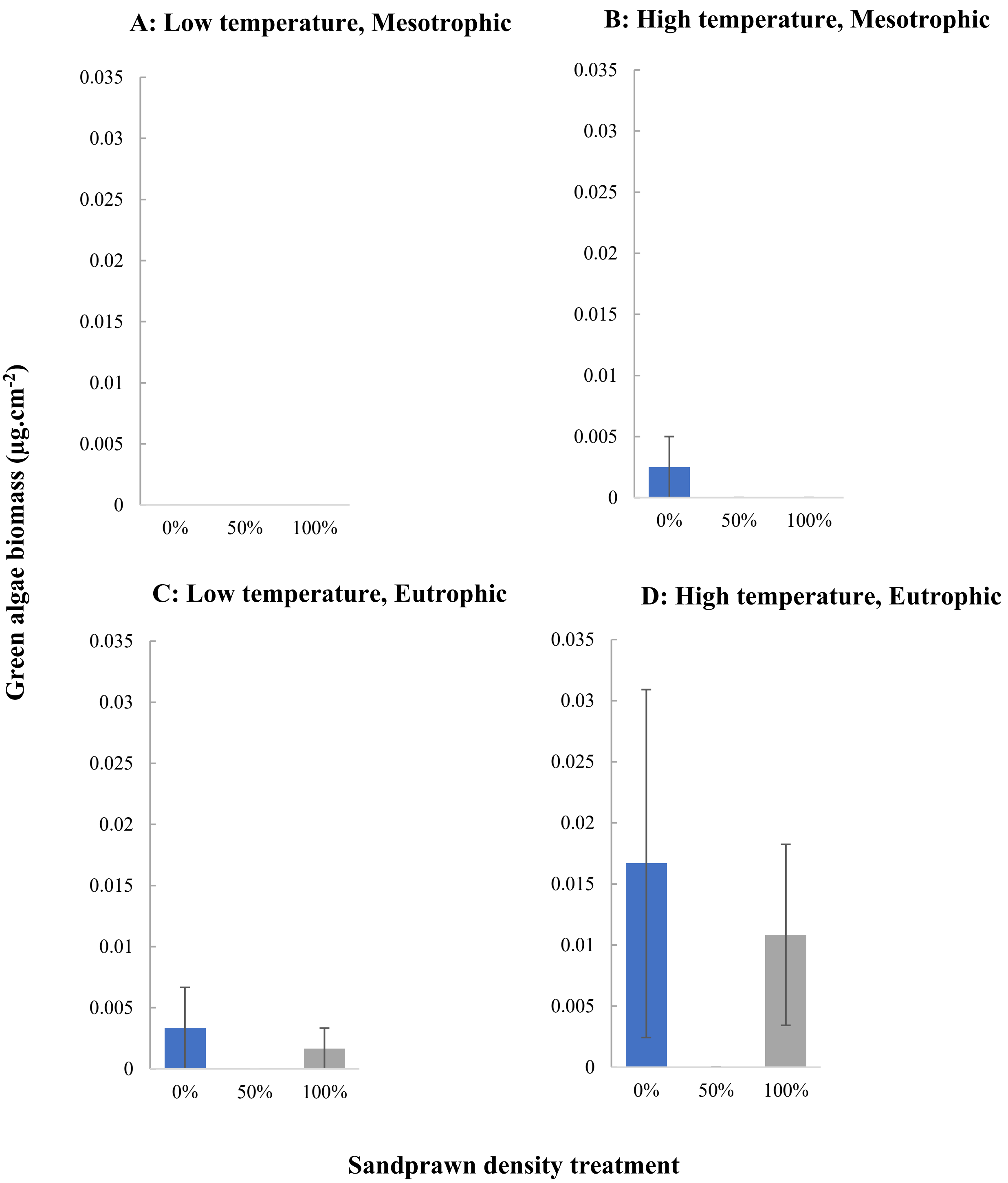


Figure 4.3: Spatial variability (mean ± SE) of epibenthic green algae in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%).

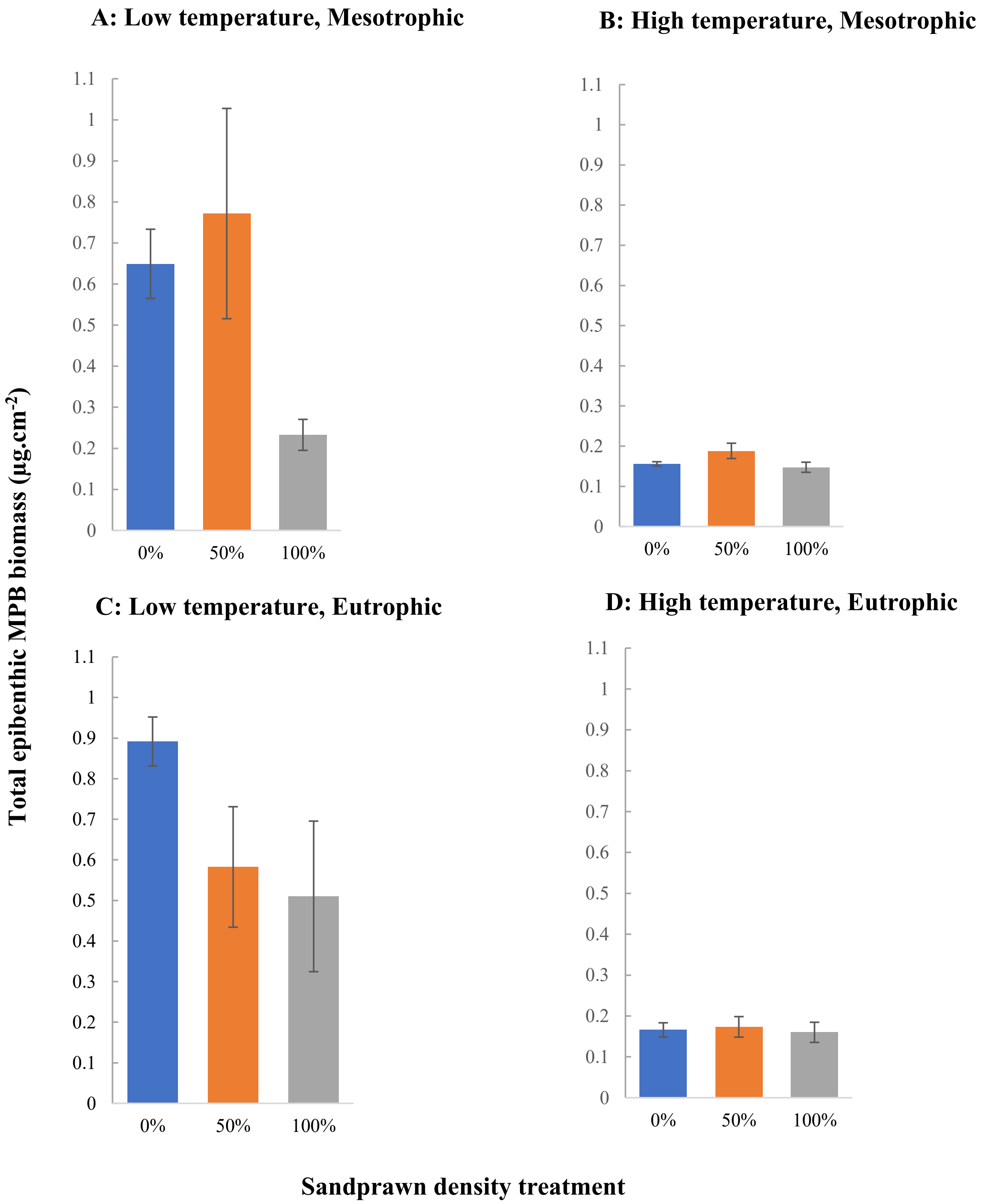


Figure 4.4: Spatial variability (mean ± SE) of total epibenthic MPB in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%).

Subsurface MPB size classes. In terms of size classes, pico- sizes were more abundant than nano- sizes in all treatments (Fig. 4.5) but proportions of pico- and nano sizes remained consistent across temperature, trophic state and sandprawn treatments. Trophic state and temperature significantly predicted variations in the mean abundance of nano-, pico-sized and total subsurface MPB algae (Table 4.4), with trends for nano- and pico-sized classes being similar to those of total subsurface MPB algae (Figs. 4.6 - 4.8). Mean subsurface MPB algal abundances were higher under eutrophic conditions (total: $F = 6.73$, $df = 1$, $p = 0.016$, pairwise, $p = 0.016$; nano-: $F = 6. 87$, $df = 1$, $p = 0.015$, pairwise, $p = 0.015$; and pico-sized: $F = 5.31$, $df = 1$, $p = 0.03$, pairwise, $p = 0.03$) and low relative to high temperatures (total: $F = 11.3$, $df = 1$, $p = 0.003$, pairwise, $p = 0.003$; nano-: $F = 14.2$, $df = 1$, $p = 0.001$, pairwise, $p = 0.001$; and pico-sized: $F = 5.31$, $df = 1$, $p = 0.03$, pairwise, $p = 0.03$). Sandprawn density effects on total subsurface MPB abundance and abundances of pico- and nano-size MPB were not significant statistically. However, visually, mean abundances of total subsurface MPB algae increased with increased sandprawn densities in all treatments with the exception of the high temperature, eutrophic treatment, where the highest abundance was recorded in the absence of sandprawns (Fig. 4.8). Similar trends with increasing sandprawn density were recorded for pico-sized MPB (Fig. 4.7) algae while trends for nano-sizes were less variable (Fig. 4.6).

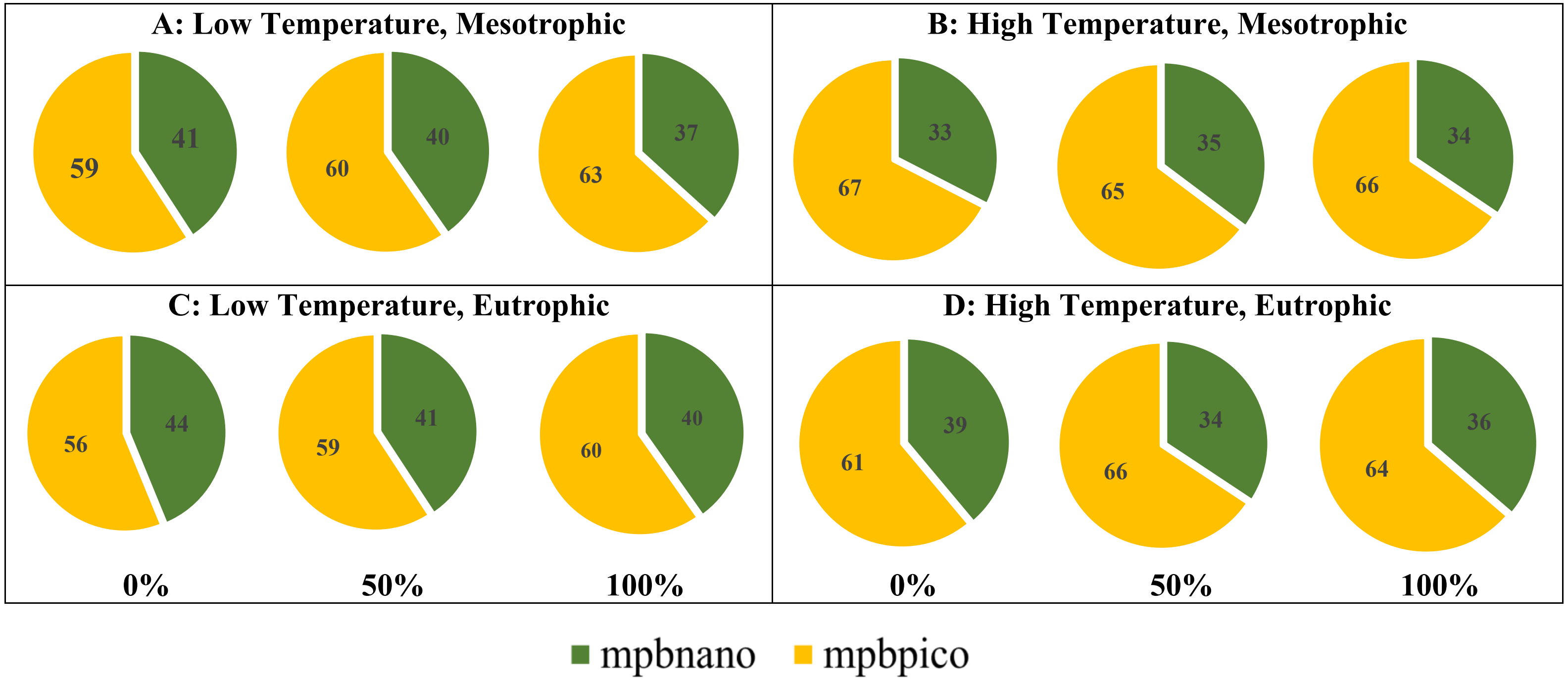


Figure 4.5: Proportions of subsurface MPB pico- and nano-sized algae in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms across varying sandprawn densities (0%, 50% and 100%). mpb nano = microphytobenthic nano-size algae, mpb pico = microphytobenthic pico-size algae.

Table 4.4: Results of linear fixed effects models testing for differences in abundance of subsurface MPB algal size classes (total, nano- and pico-size classes) between the different levels of sandprawn density, temperature and trophic states and their interactions. Statistically significant outcomes are displayed in **bold**. SP: sandprawn density, T: temperature (HT= high temperature, LT=low temperature), TS: trophic state (M = mesotrophic, E = eutrophic); mpb tot = total subsurface microphytobenthic algae, mpb nano = microphytobenthic subsurface nano-size algae, mpb pico = microphytobenthic subsurface pico-size algae.

	Response variable	**F-Value**	**Df**	**p-value**	**pairwise**
SP	mpb tot	0.719	2	0.497	
	mpb nano	0.252	2	0.779	
	mpb pico	1.25	2	0.304	
TS	**mpb tot**	**6.73**	**1**	**0.016**	**E>M, p = 0.016**
	mpb nano	**6.87**	**1**	**0.015**	**E>M, p = 0.015**
	mpb pico	**5.31**	**1**	**0.030**	**E>M, p = 0.030**
T	**mpb tot**	**11.3**	**1**	**0.003**	**LT>HT, p = 0.003**
	mpb nano	**14.2**	**1**	**0.001**	**LT>HT, p = 0.001**
	mpb pico	**7.24**	**1**	**0.013**	**LT>HT, p = 0.013**
SP x TS	mpb tot	1.35	2	0.277	
	mpb nano	1.29	2	0.294	
	mpb pico	1.05	2	0.364	
SP x T	mpb tot	0.39	2	0.682	
	mpb nano	0.17	2	0.847	
	mpb pico	0.52	2	0.602	
TS x T	mpb tot	1.01	1	0.324	
	mpb nano	0.36	1	0.556	
	mpb pico	1.37	1	0.253	
SP x TS x T	mpb tot	0.97	2	0.393	
	mpb nano	0.74	2	0.486	
	mpb pico	0.87	2	0.434	

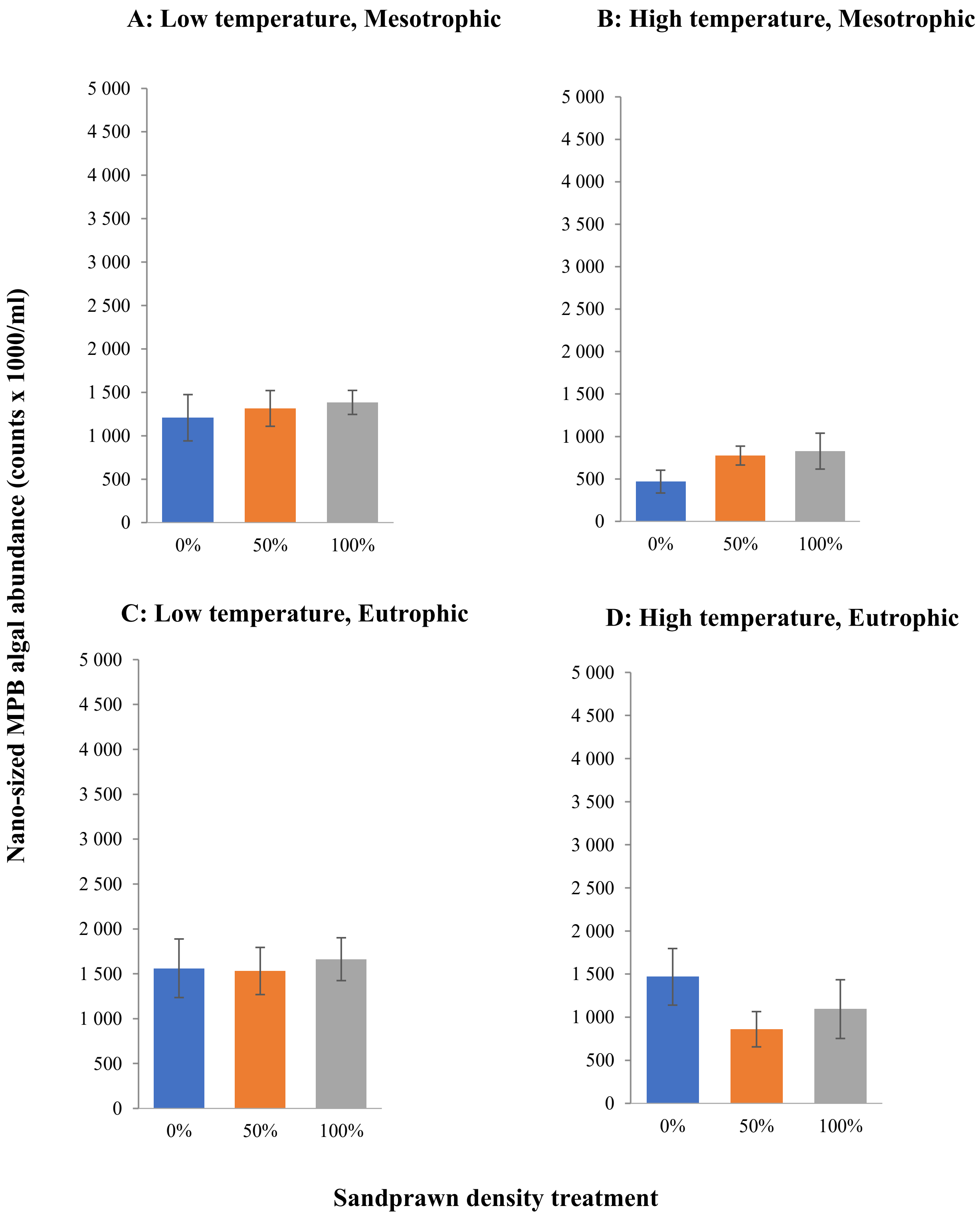


Figure 4.6: Spatial variability (mean ± SE) of nano-size class MPB algae in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%).

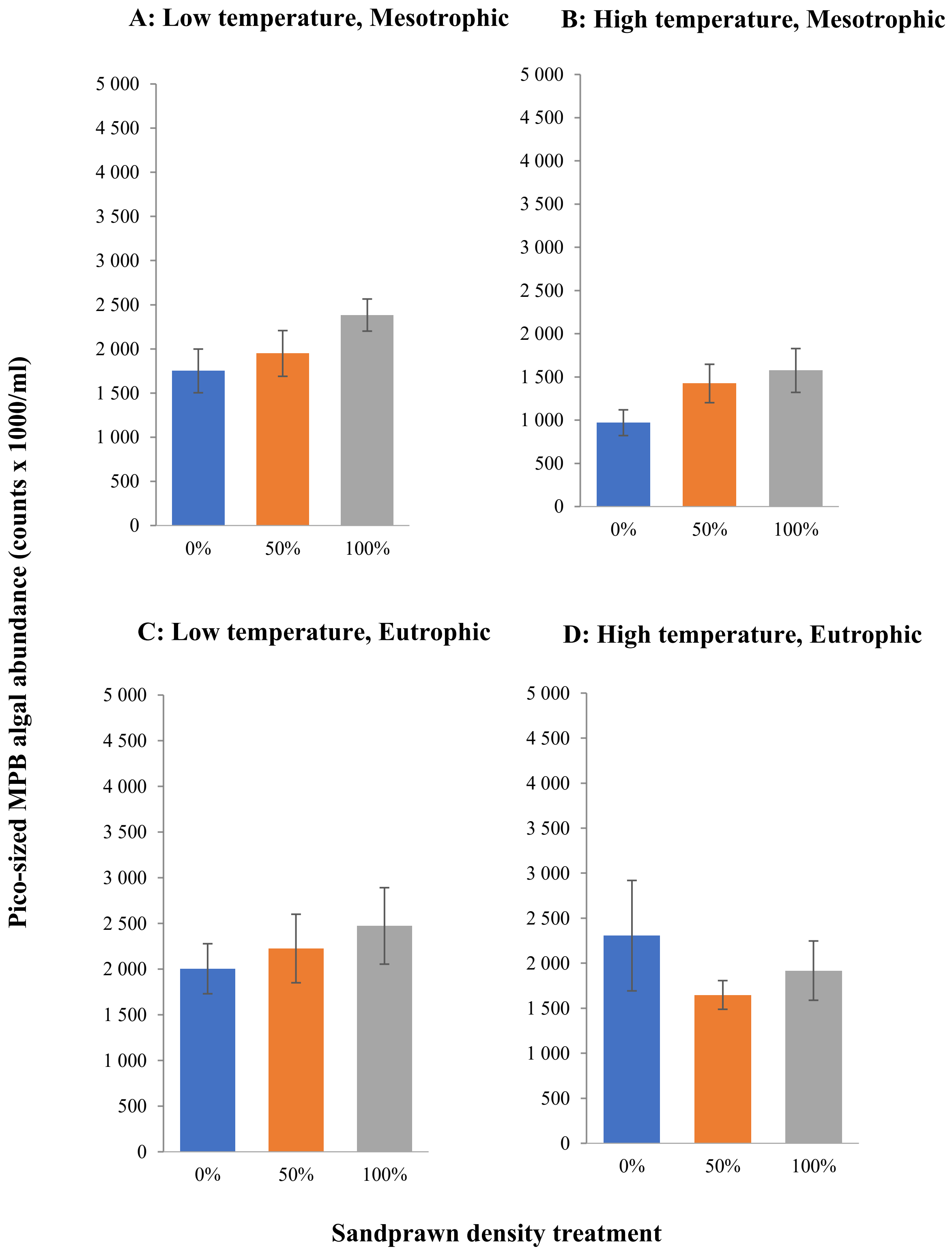


Figure 4.7: Spatial variability (mean ± SE) of pico-size class MPB algae in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%).

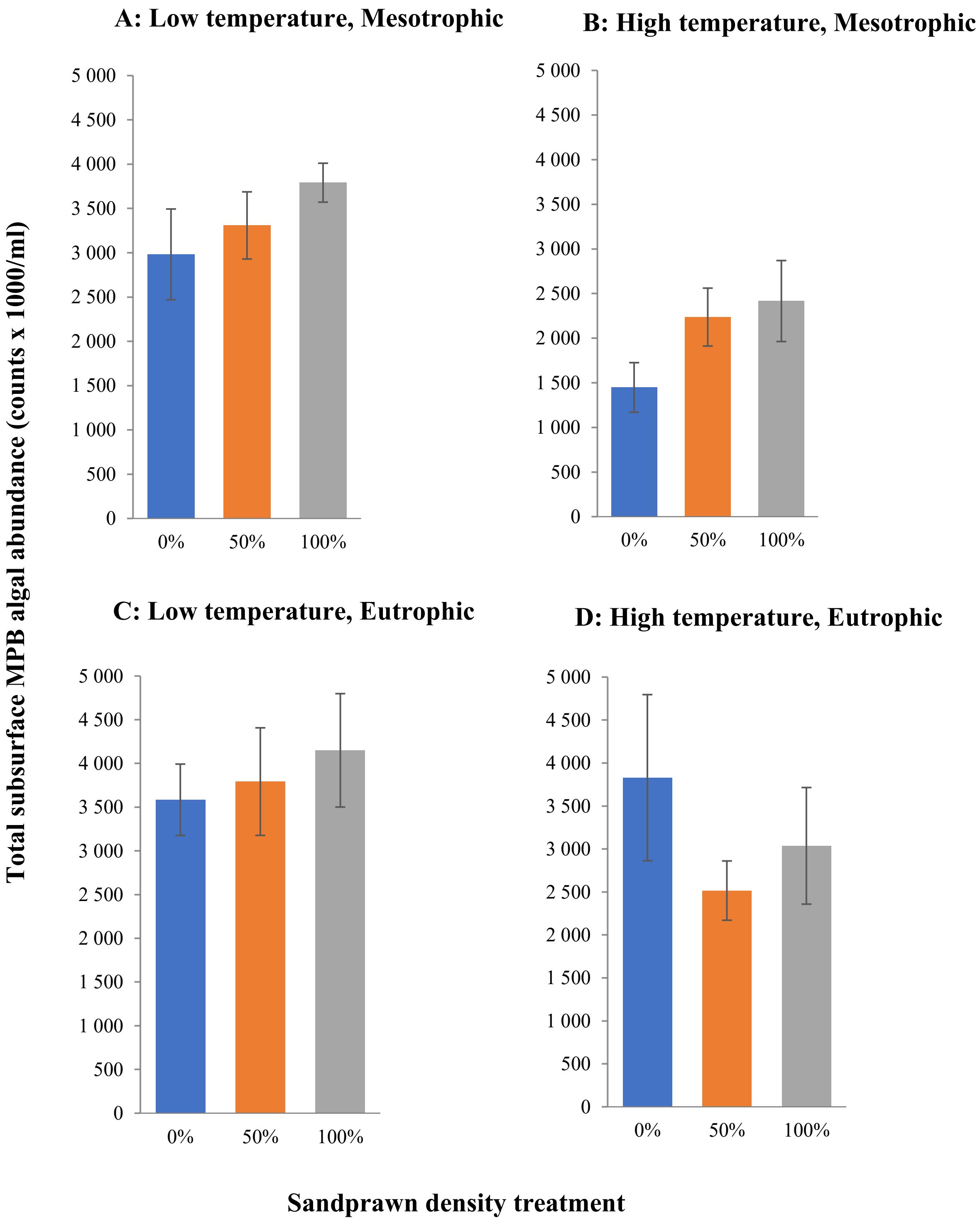


Figure 4.8: Spatial variability (mean ± SE) of total subsurface MPB algal size classes in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%).

4.6 Discussion

In this study, temperature and sandprawn density significantly influenced epibenthic microphytobenthic (MPB) algal total and cyanobacteria biomass individually, without significant interactive effects. Diatoms dominated the epibenthic MPB assemblage, with biomass ranging between 0.75 and 0.13 ug.cm^{-2} across all mesocosms (Fig. 4.2). Green algae exhibited high data variability, with overall biomass levels much lower relative to those of diatoms and cyanobacteria (Fig. 4.3). In terms of size-classes of subsurface MPB, the abundance of pico-size classes was significantly explained by temperature and trophic conditions, with abundance ranging between 2472 and 9704 counts x 1000/ml (Figs. 4.5 & 4.7). While both trophic state and temperature significantly influenced the abundance of total subsurface MPB, pico- and nano-sized cells, trends were not strong but suggestive of increased mean abundance at low relative to high temperatures. However, despite significant statistical effects of trophic state and temperature on pico- and nano-sizes, their proportions in the subsurface MPB assemblage showed minimal variation across treatments. Lastly, there was no evidence of sandprawns influencing absolute or relative abundances of pico- and nano-sizes. The dominance of pico-sized individuals within the subsurface MPB assemblage, irrespective of the treatment effect, suggests an adaptive benefit of smaller sized MPB cells in the Zandvlei Estuary, probably due to their larger surface-to-volume ratios (Vieira, et al., 2013) being conducive to increasing photosynthetic rates.

As predicted, warming led to declines in mean total epibenthic MPB biomass and that of cyanobacteria and diatoms (where declines of 79.6% and 72.3% were recorded, respectively). Declines in mean abundances of pico- (by 23%) and nano- (by 36.6%) sized components of the subsurface MPB assemblage with warming were also apparent. Notably, the variability in epibenthic total MPB, cyanobacteria and diatom biomass that was apparent at low temperatures across sandprawn densities was reduced at high temperatures, suggesting a general homogenising effect of warming on the epibenthic MPB groups (Figs. 4.1, 4.2 & 4.4). Studies by Hillebrand (2011) using a microcosm experiment, Hicks et al. (2011) and Defew, et al. (2004), based on estuarine field studies, similarly documented suppressed MPB biomass at high temperatures.

Similarly, Cartaxana et al. (2015) reported a suppressive effect of increasing temperature on MPB biomass using NDVI (Normalised Differential Vegetation Index) and chl-*a* biomass as proxies. In the same study, increasing temperature reduced maximum photosynthetic electron transport efficiency and light utilization coefficients (Cartaxana et al. 2015), suggesting an impairment of photosynthetic electron transport and energy capture and ultimately photosynthetic capacity at the high temperature regimes used (Vieira, et al., 2013; Béchet, et al., 2017). Cartaxana et al. (2015) also reported that the temperature-induced reduction in MPB biomass was specifically associated with a decline in relative abundance of diatoms, aligning with my findings. However, the increase in relative abundance of cyanobacteria as reported by Alsterberg, et al. (2011); Hillebrand (2011), and Cartaxana et al. (2015) with greater temperature, contrasts with our findings, but is supported by Van der Grinten et al. (2005) and Sommer & Lengfellner (2008).

The decrease in mean diatom and cyanobacterial biomass under high temperatures in my experiment suggests that the species pool that comprise the epibenthic MPB groups may have been physiologically intolerant to increasing thermal conditions, possibly surpassing their temperature tolerance threshold (Béchet, et al., 2017). This is likely given that the Zandvlei Estuary, from which all experimental material was collected for my study, is situated within the cool-temperate biogeographical zone of the Western Cape of South Africa. Metabolic theory may also explain the suppressive effect of temperature on MPB observed in this study (Clarke & Fraser, 2004). According to metabolic theory, increasing temperatures increase cellular metabolic rates, thereby elevating the demand for rate-limiting resources. If resource availability remains constant, the elevated metabolic demands caused by warming may not be met, resulting in declines in microalgal biomass and size (Allen, et al., 2005). This mechanism has been used to explain some of the declines in phytoplankton biomass observed in open ocean and lake ecosystems (Lewandowska et al, 2014, Kraemer, et al., 2017) with rising temperature, but it may also apply to benthic microalgae.

My prediction that eutrophic conditions would enhance the biomass of the epibenthic MPB taxa and cause shifts in the composition of the subsurface size-classes were not upheld in this experiment. This finding contradicts that of Agatz, et al. (1999), who reported an increase in benthic diatom biomass as a consequence of eutrophication enhancing nutrient availability. Alsterberg, et al. (2011) also investigated the effects of nutrient enrichment in shallow-water sediments using a semi-outdoor flow-through mesocosm facility and likewise reported an increase MPB biomass with nutrient enrichment. Interestingly, the nitrogen and phosphorous levels in my experiment were not elevated in eutrophic waters (versus mesotrophic conditions) in proportion to phytoplankton biomass, which was almost double in eutrophic relative to mesotrophic mesocosms (see Chapter 3). This suggests that a significant proportion of nutrients may have been absorbed by the enriched phytoplankton community under eutrophic conditions, leading to increased nutrient competition between phytoplankton and MPB. As a result, nutrient-limitation may have occurred, which potentially restricted the bottom-up stimulation of MPB biomass at eutrophic levels in my experiment. The lack of proportional changes in subsurface MPB size classes under eutrophic conditions (Fig 4.5) may also relate to nutrient limitation induced by phytoplankton proliferation.

With regards to the influence of endobenthic bioturbators, I predicted that increasing sandprawn density would reduce the biomass of epibenthic diatoms, cyanobacteria, green algae and hence total microalgae, due to the disturbance and instability resulting from increased sediment turnover (Pillay & Branch, 2011). This prediction was statistically supported in my experiment, since (1) sediment turnover (measured as SBR) generally increased with sandprawn density (see Chapter 5) and (2) mean biomass of total epibenthic MPB (Fig. 4.4) and cyanobacteria (Fig. 4.1) declined with sandprawn density. However, these effects appeared to be specific to the low temperature conditions of this study. In Langebaan Lagoon on the west coast of South Africa, annual sediment turnover rates of 12.14 $kg.m^{-2}.day^{-1}$ have been reported for sandprawns (Branch & Pringle, 1987; Fielding, et al., 1988). This estimate demonstrates the high sediment manipulation and turnover rates of sandprawns, and is a primary mechanism through which they, and other endobenthic crustaceans, suppress benthic microalgal communities (Cadée, 2001; Pillay & Branch, 2011) given that they do not feed on MPB directly (Pillay et al., 2011). Data for surface sediment boundary roughness (Chapter 5, Fig. 5.3) indicated a clear density-dependent increase in surface sediment rugosity across all sandprawn density treatments. The recorded decline in mean epibenthic diatom, cyanobacterial and total MPB biomass with increasing sandprawn density is thus likely driven by sediment processes, with sediment turnover burying MPB cells and causing cell mortality through light and nutrient deprivation (Branch & Pringle, 1987; Pillay & Branch, 2011). Secondarily, sandprawn-occupied benthic habitats, which generally have lower microbial biofilm levels (Fig 4.9; Pillay et al., 2007), tend to experience greater sediment erodibility (Paterson & Hagerthey, 2001), thereby increasing MPB resuspension. It is important to note, however, that even though MPB biomass declined under high bioturbation in my experiment, previous studies (Needham, et al., 2011) suggest that photosynthetic efficiency can increase under such conditions of improved oxygenation and may partially offset biomass losses. This warrants further investigations in future studies.

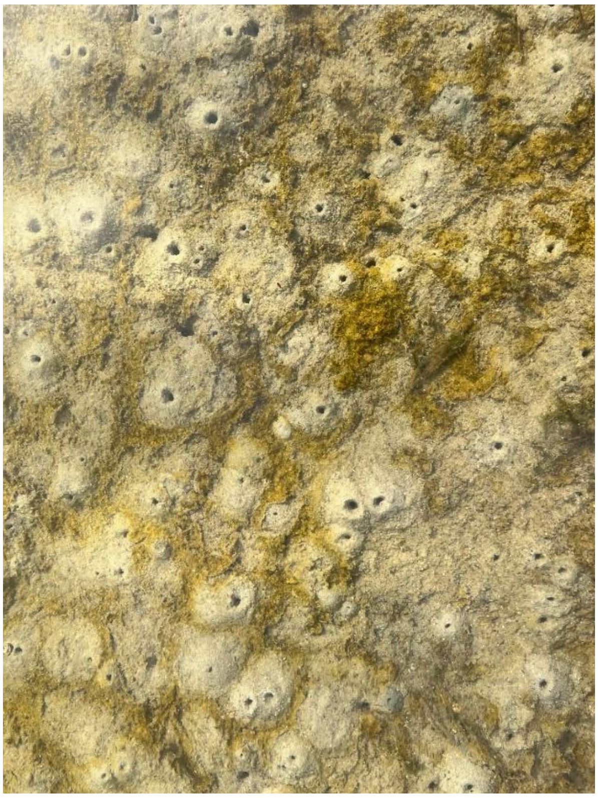


Figure 4.9: Surface of the benthos in the sandprawn biotope (Site 1) in the Zandvlei Estuary. Newly turned over sediment from sandprawn burrows with limited microphytobenthic biofilm development.

While predicted sandprawn-induced declines in mean diatom biomass was apparent, especially at the low temperature conditions of this experiment (Fig. 4.2), statistically, only declines in total epibenthic MPB and cyanobacteria with increasing sandprawn density was significant (Table 4.3). These results are similar to those recorded by Pillay et al. (2007) in an *in-situ* inclusion/exclusion experiment. In the above-mentioned study, chl-*a* biomass was reduced by approximately four times in sandprawn presence in experimental cages relative to controls. Likewise, chl-*a* biomass decreased in the presence of sandprawns in a laboratory experiment (Pillay, et al. 2012). In both of these studies, three sediment cores (diameter = 2 cm and 10 cm, respectively) were collected from each experimental unit and pooled. From this level of replication, the declines in MPB biomass described above were statistically detectable.

In my experiment, I collected four BenthoTorch measurements per experimental mesocosm that were pooled; however, high variance resulted in the detection of sandprawn effects on diatoms being weakened. I therefore recommend using larger sample sizes when employing optical tools to measure biomass of microphytobenthic biofilm components, as this technique might be more sensitive to small-scale (cm-scales) variability than traditional methods based on sediment chl-*a* biomass extraction and quantification. However, increasing sample sizes in similar future research is feasible given the rapidity of data acquisition with the BenthoTorch. In contrast to experimental findings reporting suppressive effects of sandprawns on microalgal biomass (Pillay et al. 2007; Pillay et al. 2012), Henninger and Froneman (2013) did not detect sandprawn effects on MPB. It is noteworthy though that the average density of sandprawns was 43 ind.m^{-2} in the Henninger and Froneman (2013) study, which was lower than the densities used by Pillay et al. (2007), which often exceeded 100 ind.m^{-2}. In my study, sandprawn density per mesocosm was determined by scaling down the maximum sandprawn numbers of ~200 ind.m^{-2} documented for South African estuaries and lagoons (Branch & Pringle, 1987). These disparities in study outcomes most likely stem from contextual variability among studies, such as differences in environmental conditions, and the density and traits of endobenthic crustaceans and responding assemblages (Pillay & Branch, 2011; Pillay, 2019).

A secondary goal of this chapter was to understand whether sandprawn effects, eutrophication and warming interacted antagonistically, additively, or synergistically to influence MPB biomass and community composition. No statistically significant interactions were detected among predictors, suggesting that antagonistic, additive or synergistic interactions were negligible determinants of MPB community structure.

4.7 Concluding remarks

Overall, my experiment demonstrated that sandprawns have the potential to limit MPB proliferation likely due to their sediment turnover activities. In the field, as well as in mesocosms containing sandprawns in my study, patches of sediment turned over by sandprawns appear cleaner and less colonised by microalgae (Fig. 4.9), which aligns with the epibenthic MPB data in my experiment that showed decreases in mean diatom, cyanobacteria and total MPB biomass (albeit at low temperature) with increasing sandprawn density. My results also suggest that warming in future scenarios may suppress MPB biomass, either due to physiological intolerance of cool-temperate microalgal assemblages or to nutrient limitations brought on by elevated temperature-induced metabolic demands. However, there is the potential for MPB cells to adapt, provided that warming occurs at a slow pace. These findings have implications for ecosystem productivity under future warming scenarios particularly in light of the significant role that MPB plays in benthic primary productivity and in structuring food webs in coastal ecosystems. Secondary consequences of warming-induced declines in productivity of diatoms, in particular, may include reduced sediment stability stemming from associated declines in EPS production and a reduction in the amount of food available for epibenthic feeders. Therefore, future research in this field needs to probe more deeply the consequences of rising temperature in MPB assemblages, especially in southern African temporarily closed estuaries, under an expanded set of abiotic conditions to that used in my experiment. Detailed assessments of benthic microalgal community composition and functional traits (where possible) can assist in advancing local understanding of warming-induced shifts in MPB assemblages.

My results also suggest that epibenthic microalgal assemblages were unaffected by eutrophication, though this might relate to nutrient limitations manifesting under experimental conditions. However, under natural estuarine conditions, where nutrient inputs may not be limiting, rising temperature may increase microalgal biomass, possibly shifting dominance to cyanobacteria (Havens, 2008; Kraemer, et al., 2017). Increasing human populations in coastal areas (Defeo & Elliott, 2021), coupled with global change processes acting in concert with local-scale anthropogenic pressures, are likely to increase the intensity and frequency of blooms of benthic cyanobacteria Catherine, et al., 2013). Importantly, the high temperatures and eutrophic conditions of my experiment did not inhibit sandprawn turnover activities, suggesting that if microalgal biomass were to increase *in situ* in response to eutrophication or warming, sandprawn bioturbation could limit biofilm formation and the development of cyanobacterial mats. Many cyanobacterial genera demonstrate optimal growth rates and bloom potential at higher temperatures; hence global warming can significantly contribute to their expansion and persistence (Paerl & Otten, 2013). Cyanobacterial dominance can be harmful from environmental, organismal, and human health perspectives (Paerl & Otten, 2013). Cyanobacterial blooms cause a loss of water clarity, which depresses aquatic macrophytes, negatively affecting fish and invertebrate habitats (Havens, 2008; Paerl & Otten, 2013). When cyanobacterial blooms decompose, it leads to oxygen depletion that cause fish and benthic invertebrate kills (Havens, 2008). Moreover, cyanobacterial blooms outcompete beneficial phytoplankton leading to changes in the taxonomic structure of benthic assemblages and food web interactions (Havens, 2008; Paerl & Otten, 2013). Many harmful cyanobacterial taxa produce toxic secondary metabolites (cyanotoxins) that can cause serious, acute poisoning in aquatic organisms and humans that affect the liver, pancreas, digestive, and nervous systems when ingested, with the potential to lead to organismal death (Paerl & Fulton III, 2006; Havens, 2008; Catherine, et al., 2013). Previous research in the Diep River Estuary has highlighted concerns about reduced sediment oxygenation potential due to a nearly 70% decline in sandprawn stocks (Viskich, et al., 2016). However, my study indicates that such declines in sandprawn density may also increase the potential for benthic cyanobacterial proliferation. Collectively, past studies and my current research underscore the need to manage sandprawn populations and prevent their decline to maintain their beneficial functions, such as sediment oxygenation, water filtration, and regulation of cyanobacterial proliferation. To my knowledge this is the first experimental study to demonstrate the role of sandprawns in preventing harmful cyanobacterial blooms and alludes to the possibility that sandprawns (and other endobenthic bioturbators) may have value as nature-based solutions to mitigate the harmful effects of eutrophication under current and future global change scenarios, thereby sustaining ecosystem resilience against local coastal and global change stressors.

CHAPTER 5:

ECOSYSTEM FUNCTIONING

5.1 Introduction

Coastal ecosystems are highly valued for their provision of ecosystem services that benefit humankind (Millennium Ecosystem Assessment, 2005a; Costanza, et al., 2014; Carrasco De La Cruz, 2021; De Battisti, 2021). They are also amongst the most threatened ecosystems in the world due to resource exploitation pressures resulting from escalating human population densities (Millennium Ecosystem Assessment, 2005a; IPCC, 2007; Granek et al, 2009; Hewitt, et al., 2016; Carrasco De La Cruz, 2021; De Battisti, 2021; Rillig, et al., 2023;). However, exploitation pressures are nested within broader scale global change stressors, resulting in joint interactions that can impact biological processes, biodiversity and ecosystem functions. Such interactions, especially when acting synergistically, can induce profound and potentially irreversible changes to ecosystems (Carrier-Belleau, et al., 2021).

Ecological processes such as primary and secondary productivity, nutrient fluxes, rates of decomposition (Loreau, et al., 2001; Sekercioglu, 2010; Cardinale et al, 2012; Zhang, et al., 2022;) and habitat formation through ecosystem engineering, are important components of a natural ecosystem (Jones, et al., 1994; Cardinale et al, 2012; Correia & Lopes, 2023). Ecosystem functioning refers to the combined effects of biotic and abiotic processes that occur within an ecosystem and play a crucial role in the provision of ecosystem services that contribute significantly to human well-being (Gamfeldt, et al., 2008; Costanza, 2012; Zhang, et al., 2022). Ecosystem services encompass: (1) provisioning services, which supply products such as food, fuel and water; (2) regulating services, such as flood control, protection from human diseases, and water purification; (3) supporting services, such as primary production, habitat provision, and nutrient cycling; and (4) cultural services, offering recreational, spiritual, and religious benefits (Millenium Ecosystem Assessment, 2005b; Granek et al, 2009; Sekercioglu, 2010; Costanza, 2012; Tomimatsu, 2013). Given the reliance of human societies on coastal ecosystems, effective ecological management is imperative to ensure their sustainable functioning (Tomimatsu, et al., 2013).

Biodiversity, including the complex interactions occurring among species, is crucial in ecosystem functioning (Malhi, et al., 2020), with key species being of particular importance due to their disproportionately large influence over ecological processes (Cardinale, et al., 2006; Hale & Koprowski, 2018). In marine and coastal benthic environments, key bioturbators, such as axiid crustaceans like sandprawns, powerfully influence ecosystem functioning through their bioturbating activities (Branch & Pringle, 1987; Jones, et al., 1994; Cadée, 2001; Meysman, et al., 2006; Maire, et al., 2008; Pillay & Branch, 2011; Chen, et al., 2017; Hale & Koprowski, 2018; Pillay, 2019). For the South African sandprawn *Kraussillichirus kraussi*, sediment turnover rate has been estimated to be of 12.14 kg.m^{-2}.day^{-1}, or 4.4 ton.m^{-2}.day^{-1}, which highlights the magnitude of ecosystem transformation by these organisms (Branch & Pringle, 1987). Given the profound effect of bioturbation on ecosystem processes, quantifying bioturbation activity is of utmost importance in unravelling the complex biological mechanisms that regulate benthic ecosystem functioning. This assumes added importance under global change conditions since such conditions can impact the physiology and behaviour of bioturbators and hence their sediment reworking behaviour (Maire, et al., 2006; Maire, et al., 2008).

Current and anticipated global change is predicted to substantially affect organismal function and population persistence, potentially placing at risk the ecosystem manipulation activities of key engineers, with indirect consequences for ecosystem functioning (Millenium Ecosystem Assessment, 2005b; Jax, 2010; Sekercioglu, 2010; Cardinale et al, 2012; Cheng et al, 2015; Malhi, et al., 2020; Leuzinger & Rewald, 2021; Hong, et al., 2022; Zhang, et al., 2022; Correia & Lopes, 2023). Warming can increase the metabolism of ectothermic organisms like sandprawns, affecting sediment turnover activities and other associated processes (Maire, et al., 2007). Warming-induced (or other global change drivers) depression of organismal function can compromise population fitness and genetic variation, increasing the risk of extinction (Staudt, et al., 2013; Bozinovic & Pörtner, 2015). However, species can lose their ability to fulfil their functional roles even before facing extinction (Meysman, et al., 2006). Warming can also influence ecosystem functioning by causing changes to organismal distributions (e.g. bioturbators and those nested within bioturbated ecosystems), ecosystem productivity, biogeochemical process rates, and the ways in which these properties interact with other natural and anthropogenic stressors (Staudt et al., 2013; Bozinovic & Pörtner, 2015).

Eutrophication also has repercussions for ecosystem functioning that extend beyond the occurrence of phytoplankton blooms and the proliferation of opportunistic and toxic algae (Cloern, 2001; Smith & Schindler, 2009). Eutrophication initiates hypoxia/anoxia, food-web alterations, and biodiversity losses, disrupting ecosystem functioning and the delivery of essential services (Cloern, 2001; Smith & Schindler, 2009). Additionally, the release of pathogenic microbes, harmful algae and toxins resulting from algal blooms, heightens the incidence of disease or mortality (including bioturbators) in the ecosytem, impacting biodiversity and ecosystem functioning (Harvell, et al., 2004; Thomas, et al., 2023). Shifts in trophic resource quality and quantity in response to eutrophication also alter ectotherm performance (Auer, et al., 2018; Ramachandran, et al., 2021), potentially increasing the rate at which food is consumed (Barrios-O'Neill, et al., 2019). In a warming scenario, eutrophication effects can be amplified as higher metabolic demands related to ectothermy may increase trophic resource demand (Maire, et al., 2007; Barrios-O'Neill, et al., 2019; Pillay, 2019).

Bioturbators, including sandprawns, contribute significantly to ecosystem functioning (Lohrer, et al., 2004; Solan, et al., 2004b; Maire, et al., 2006; Meysman, et al., 2006; Maire, et al., 2008; Bertics & Ziebis, 2009; Pillay & Branch, 2011; Griffiths, 2017; Pillay, 2019), especially when density, rate of resource manipulation and spatial distribution are great (Pillay

& Branch, 2011; Pillay, 2019). Bioturbation is of global importance (Maire, et al., 2008) since (1) bioturbation changes the depth-distribution of organic matter and increases the quantity and quality of food for deposit feeders in sediments (Shull, 2019); (2) nutrient fluxes increase as a consequence of bioturbation, leading to elevated rates of benthic primary production (Maire, et al., 2007; Shull, 2019); and, (3) bioturbation directly stimulates microbial activity and organic matter degradation by improving the oxic environment of the sediment (Branch & Pringle, 1987; Mermillod-Blondin & Rosenberg, 2006; Maire, et al., 2007; Dimitriou, et al., 2017b).

Another key aspect of coastal sedimentary ecosystems is that biogeochemical dynamics are a major determinant of ecological processes, but biogeochemical reactions are in turn, directly or indirectly affected by organic matter degradation (Aller, 1982; Arndt, et al., 2013), which is profoundly influenced by bioturbation (Branch & Pringle, 1987; Maire, et al., 2007; Dimitriou, et al., 2017b). The presence of key bioturbators enhances oxygen penetration and shifts redox conditions to favour oxic reaction pathways and microbial metabolism (Aller, 1982; Meysman, et al., 2006; Na, et al., 2008; Bertics & Ziebis, 2009; Kristensen, et al., 2012; Fusi, et al., 2022). Consequently, the biogeochemical properties of the sediment is modified in three main ways: Firstly, through activities like egestion, burrow construction and feeding, bioturbators mechanically increase the subduction and surface area of organic detritus for decomposition (Aller, 1982), creating various niches that foster unique microbial assemblages within microhabitats in the sediment (Branch & Pringle, 1987; Meysman, et al., 2006; Kristensen, et al., 2012). Feeding activities play a vital role in stimulating decomposition of organic matter as grazing maintains rapid growth of microbial populations. Burrowing activities loosen the sediment, improving its porosity and permeability (Pillay, 2019), thus facilitating pore-water flow and influencing solute exchange in sediments (Zeibis, et al., 1996).

Secondly, bioturbators actively counteract anoxia within their burrows by flushing with overlying waters (Meysman, et al., 2006; Bertics & Ziebis, 2009; Kristensen, et al., 2012). Endobenthic crustaceans have been reported to pump up to 3450 ml. day^{-1} of water through their burrows, representing a considerable flow of water through the sediment (Branch & Pringle, 1987). The fact that these burrows can extend several meters (in some cases, 2-3m) deep into the sediment (Zeibis, et al., 1996; Na, et al., 2008) suggests that advective curents can be generated at considerable depths into the anoxic zone of the sediment (Na, et al., 2008), highlighting the powerful influence bioturbators can have on advective irrigation and ventilation of sediments (Bertics & Ziebis, 2009; Pillay, 2019).

Through these various mechanisms, bioturbation enhances the availability of the essential reagents and conditions for microbial metabolism, influencing microbial activity and rates of organic matter degradation (Aller, 1982; Meysman, et al., 2006; Na, et al., 2008; Kristensen, et al., 2012). Given that the rate of bioturbation is directly dependent on the density, feeding and behaviour of dominant bioturbators, environmental parameters such as food availabilty and temperature likely play important roles in sediment reworking activities and bioirrigation (Gerino, et al., 1998; Maire, et al., 2007; Mermillod-Blondin, 2011). Global change factors, such as warming and eutrophication, influence bioturbation (Solan, et al., 2004b); and, an understanding of this impact is particularly important in light of the role of bioturbation in ecosystem functioning and the delivery of essential services to humans (Solan, et al., 2004b).

Aims and objectives

The first objective of this chapter was to quantify sediment boundary roughness (SBR), a proxy for bioturbation, under warming and eutrophication conditions at varying densities of the sandprawn *K. kraussi*. Given previous studies showing how changes in temperature could modify SBR (Farrell, et al., 2023) and the effect of temperature on ectothermic metabolism (Maire, et al., 2007) it was predicted that SBR would increase with temperature, eutrophication and sandprawn density.

Eutrophic environments typically experience high carbon input and it is therefore important to understand how organic matter is degraded, especially in the context of global change. The second objective of this chapter was therefore to assess the relative role of microbially- vs infaunally- (microbes + meiofauna + sandprawns) mediated organic matter degradation (OMD) under warming and eutrophication conditions at varying *K. kraussi* densities. This was accomplished by quantifying changes to depth related alterations to the OMD profile mediated by microbes vs infauna. In light of previous studies clarifying the mechanisms by which temperature, eutrophication and bioturbation drive the breakdown of organic material in sediments (Abdelrhman & Cicchetti, 2012; Chen, et al., 2017), assessments were made on whether (1) OMD would be greater when mediated by infauna compared to microbes because of a greater joint consumer effect (microbes + meiofauna + sandprawns), (2) rates of OMD would increase with sandprawn density as a consequence of increased bioturbation-induced oxygenation and possibly its influence on microbial abundance, (3) the positive effects of warming on bioturbation and microbial metabolism would increase rates of OMD, and (4) OMD in the upper layers of the sediment would be greater due to greater availability of oxygen (Fusi, et al., 2022). It was difficult to speculate how OMD would respond to eutrophication as OMD could decrease due to the presence of harmful toxins and pathogenic bacteria in eutrophic waters. Alternatively, with the higher microbial loading and quantity of carbon substrate that is associated with eutrophication, organic matter degradation could be stimulated.

5.2 Methodology

Please refer to Chapter 2 for the experimental design for this chapter.

5.3 Data collection

Sediment boundary roughness (SBR) was measured as a proxy of sediment reworking (bioturbation) by sandprawns, based on non-destructive methods adapted from Solan, et al. (2016) where, SBR was measured as the difference in maximum and minimum level of sediment per experimental mesocosm. Photographs per side of each mesocosm (4 in total per mesocosm) were taken daily from the start of the experiment for the first 7 days and thereafter every alternate day until the experiment was terminated. Photographs were taken using an iPhone 6s mounted onto a wooden frame (H = 60cm; W = 30cm, D = 40.5cm). A light source (Eurolux Model FS82) was fitted over the top of the frame and a black cloth was used to cover the frame and mesocosm when taking photos (to standardize lighting). The captured images were saved in JPEG format and subsequently analysed quantitatively using ImageJ (Version 1.53) software (Schneider, et al., 2012). A scale line was drawn across the known length (30cm) of one side of the mesocosm, using the original level of the sediment (Day 0, before sandprawns were added) as a reference point (Fig 5.1). The minimum and maximum levels of the sediment surface in photographs were visually identified, and the quantitative height values determined by placing the cursor over these points and reading the y-axis value from the status bar in ImageJ. The range of elevations in the sediment-water interface was then calculated for each side of the mesocosm and the values averaged to provide one SBR value per mesocosm per day of data collection. For consistency, SBR measurements were made on sediment peaks and troughs directly against the glass sides.

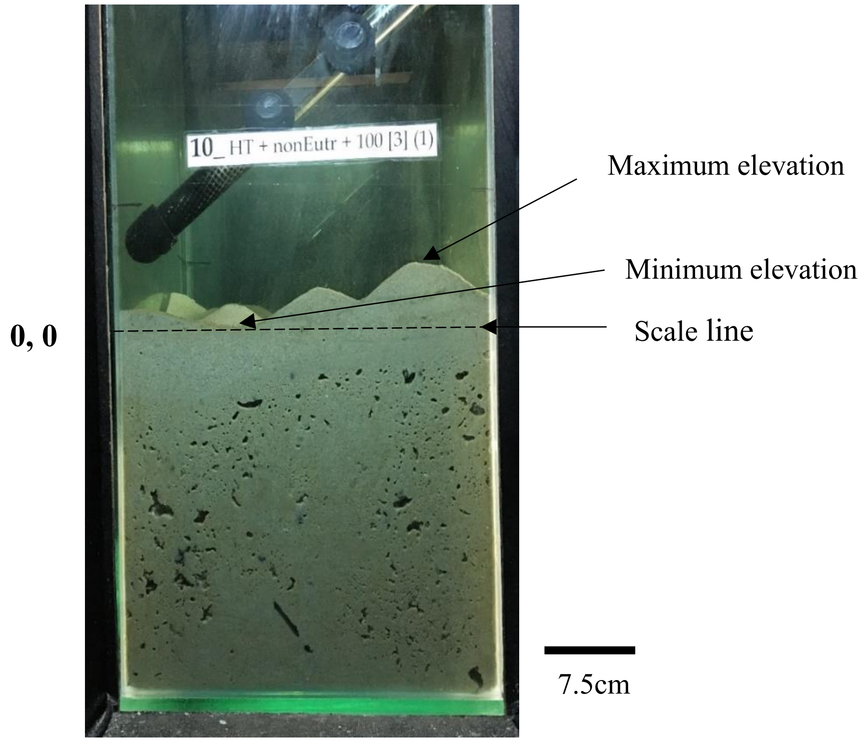


Figure 5.1: Image of one side of a mesocosm showing the scale line (---) and elevations of the sediment-water interface.

Rapid organic matter assays (ROMA), adapted from O'Maera, et al. (2018) were employed to quantify organic matter degradation rate in response to treatment effects. Briefly, this method involved the deployment of known quantities of carbon substrate into sediment and then determining changes in quantity after a fixed time period. The data derived from ROMA provided information on the influence of biological activity on sediment organic matter degradation (O'Maera, et al., 2018). Each ROMA plate was constructed using 1.5cm thick, clear acrylic sheets cut to 18cm x 12cm blocks. The bottom of each ROMA plate was bevelled to facilitate its deployment into the sediment and a cable tie was attached to the top of the plate for ease of removal. Wells (diameter = 1cm, depth = 0.9cm, 3cm apart) were bored into each ROMA plate in four vertical rows at distances of 1, 3, 5, 7, 10, and 15 cm from the top of each plate (Fig 5.2). The volume of each well was 0.9ml and was determined by filling with water from a 1ml syringe (O'Maera, et al., 2018).

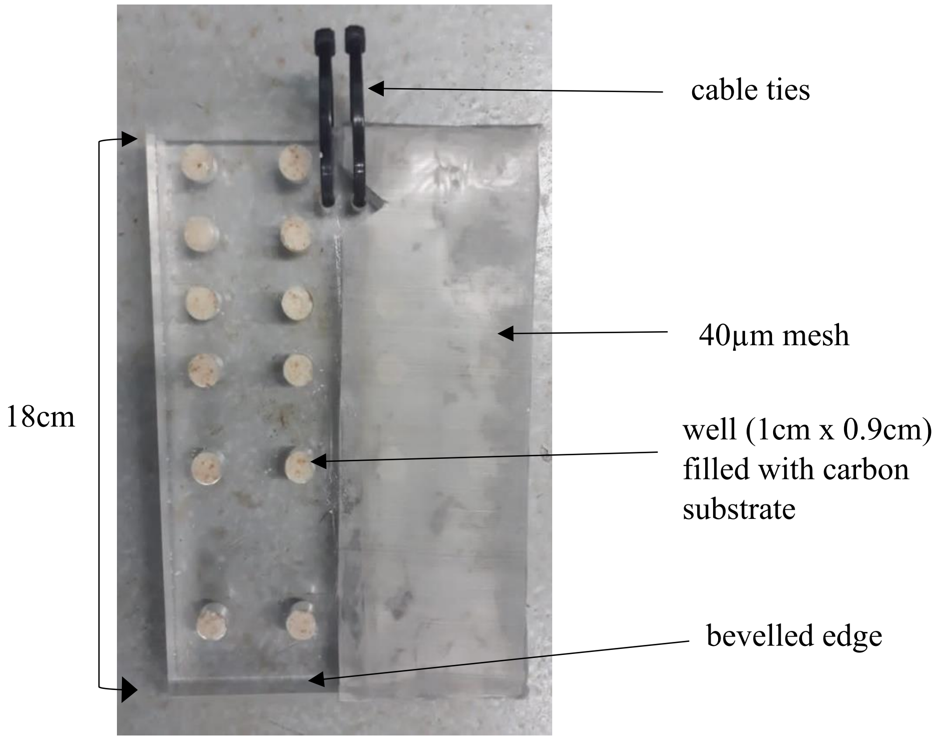


Figure 5.2: Design of ROMA plate used to quantify carbon degradation rate in response to different temperatures (low, high), trophic state (mesotrophic, eutrophic) and varying sandprawn densities (0%, 50% and 100%).

Each well per ROMA plate was filled with a 0.029gC/ml mixture of food grade agar, microcrystalline cellulose (CAS #9004-34-6 MW of (162.1)n; Formula $(C_6H_{10}O_5)_n$; Thermofisher), and powdered bran flakes (Kelloggs All Bran flakes) ground with a coffee grinder (Mellerware, Aromatic). To prepare the media, 10g agar, 21.66g cellulose and 13.2g bran were added to 500ml of deionised water and heated until the mixture began to thicken. Wells were filled with the warm mixture flush with the surface of the ROMA plate. Care was taken to ensure that air bubbles were not trapped in the wells. Once filled, two columns of wells per ROMA plate were covered with a mesh (40µm), held in place with cyanoacrylate adhesive (Aqua World), while the other two rows were left unmeshed. This allowed for predominantly microbial (<40µm) and infaunal (microbes + meiofauna + macrofauna (40-2000 µm) + sandprawns effects on organic matter degradation to be distinguished. ROMA plates were stored until deployment (for a period not exceeding 4 hours) in the aquarium facility.

A single ROMA plate was deployed in the centre of each mesocosm, prior to the addition of sandprawns, by pushing it into the sediment until the top was 1cm below the sediment surface. Each ROMA plate was deployed for 15 days following which they were carefully lifted vertically out of the mesocosms by pulling on the attached cable tie. Deposits of sediment on the plate was gently rinsed off and the plates were stored at 4°C prior to analysis (within 24 hours).

Following the method outlined in O'Maera, et al. (2018), organic matter degradation was measured as the change in agar volume in each well per ROMA plate by the termination of the experiment. Each well was filled with 0.9ml de-ionised water (unfilled cell volume) flush with the plate surface using a 1ml syringe. The volume of water remaining in the syringe was used as an approximation of agar/carbon mixture within each well. Carbon consumption was calculated using the formula: $C = \frac{(V_0 - V_F)(\frac{0.026gC}{V_0})}{d}$

Where, C = rate of carbon consumption (g of carbon per day)

V_0 = initial volume of agar/carbon mixture (0.9 ml)

V_F = volume of agar remaining after the deployment period (ml)

d = duration of deployment (days)

The rate of degradation of organic matter at the surface of the sediment was then calculated using the following formula: $C = C_0 e^{-kz}$

Where, C = rate of organic matter degradation at any given depth

C_0 = organic matter degradation rate at the sediment surface

k = extinction coefficient

z = depth

The value k was derived from the slope of the linear regression of C (ln) in relation to depth of the wells.

5.4 Data analysis

Linear Mixed Effects Models (LMEM) and Linear Fixed Effects Models (LFEM) were performed using RStudio v 4.1.2, 2021 data analysis platform (R Core Team, 2019) as previously described. Where model assumptions were violated, models were re-fitted. Transformations used and models fitted to response data are shown in Table 5.1. The effect of infauna and microbes on organism matter degradation were not explicitly included in the model to limit predictors given the data sample size. This approach also prevented reporting of interaction terms from becoming too complex.

Table 5.1: Statistical analyses performed on response variables (SBR: sediment boundary roughness, OMD: organic matter degradation, C0: organic matter degradation rate at the sediment surface, C: rate of organic matter degradation at any given depth) in relation to predictors (SP: sandprawn density, T: temperature, TS: trophic state).

Response variable	Predictors	Statistical Tool	Analyses	Transformation
SBR	SP, T, TS + interactions	RStudio	LMEM	Square Root
OMD (microbial +infaunal)				
• C_0	SP, T, TS + interactions	RStudio	LFEM	Cube Root
• C	SP, TS, T, depth, + interactions	RStudio	LFEM	Cube Root

5.5 Results

Sandprawn density ($\chi^2 = 121.82$, df = 2, $p < 0.0001$) and temperature ($\chi^2 = 4.90$, df = 1, p =0.03) significantly explained variability in SBR (Table 5.2). Specifically, higher sandprawn densities were associated with greater SBR values compared to lower densities (p< 0. 01, Table 5.2, Fig. 5.3) and mean SBR was greater under high temperature conditions (pairwise, p=0.04) in comparison to low temperatures. At high temperatures, the distinction in SBR between the 50%

and 100% sandprawn densities was more pronounced when compared to low temperatures; and at 50% sandprawn density, SBR was similar between high and low temperatures (Fig. 5.3). SBR was unaffected by trophic state ($p = 0.06$), which was visually apparent at both high and low temperatures (Fig. 5.3).

Notably, in the mesocosms without sandprawns, there was barely any evidence of sediment turnover indicating that the sandprawns used in this study was the only bioturbator in the mesocosms (Fig. 5.3).

Table 5.2: Results of linear mixed effects models testing the effects of the predictor variables on SBR. Statistically significant outcomes are displayed in **bold**. SP: sandprawn density, T: temperature (HT= high temperature, LT=low temperature), TS: trophic state (M = mesotrophic, E = eutrophic).

	χ^2	df	p-value	Pairwise comparisons
SP	**121.82**	**2**	**<0.0001**	**0%<50%<100% (p< 0.01)**
T	**4.90**	**1**	**0.03**	**HT>LT (p=0.04)**
TS	3.62	1	0.06	
SP x T	4.28	2	0.12	
SP x TS	0.36	2	0.84	
TS x T	2.38	1	0.12	
SP x T x TS	0.19	2	0.91	

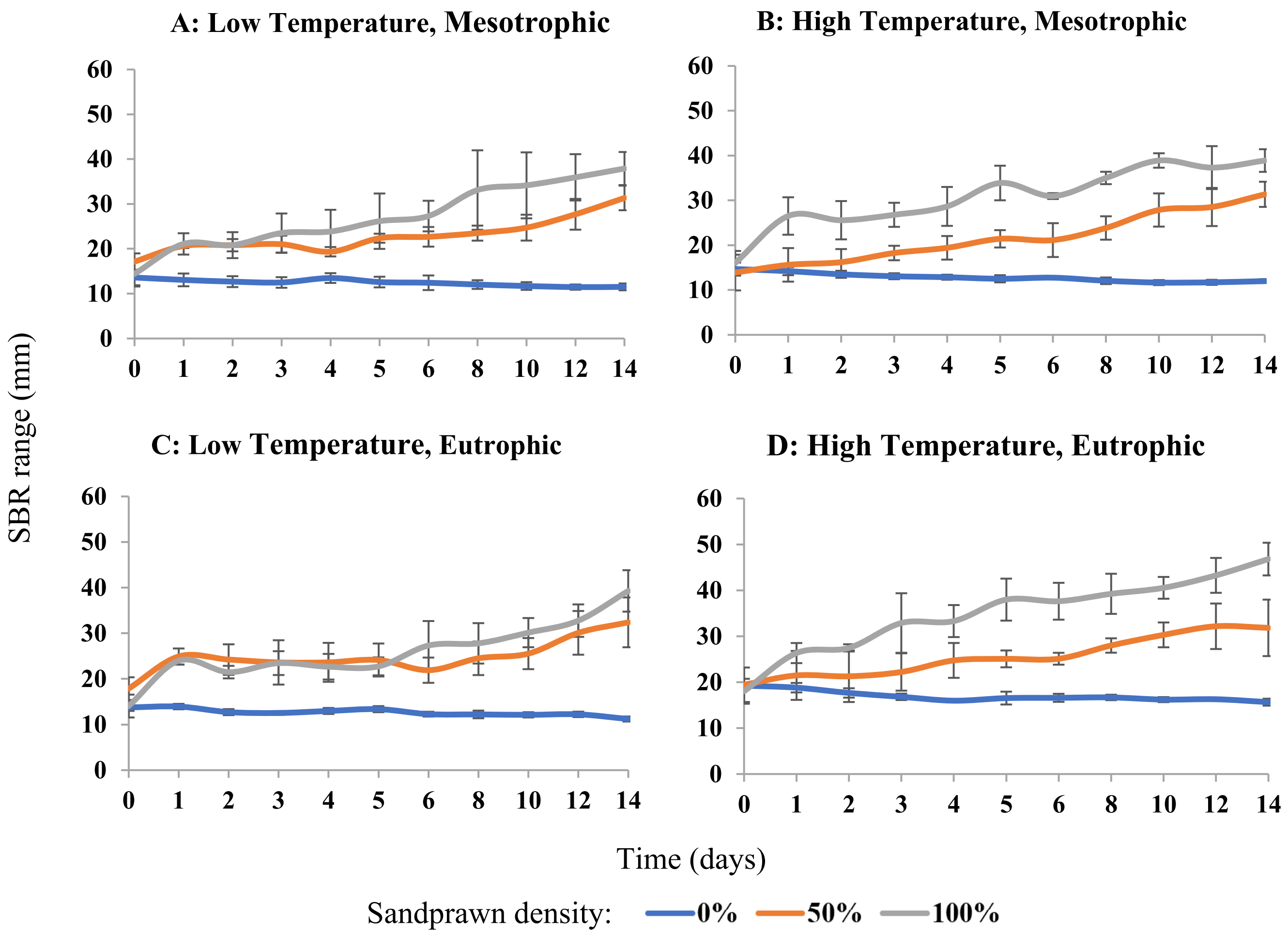


Figure 5.3: Spatio - temporal variability in SBR (mean ± SE) in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%).

Carbon degradation at the sediment surface (C_0) mediated by infauna (microbes + meiofauna + sandprawns) increased significantly at higher temperatures (F = 8.35, df = 1, p = 0.004, Table 5.3). Both microbially- (F = 5.31, df = 2, p = 0.0006 and infaunally- (F = 3.68, df = 2, p = 0.03) mediated C_0 was significantly influenced by the interaction between sandprawn density and trophic state.

Table 5.3: Results of linear fixed effect models testing for differences in microbially- and infaunally-mediated rates of carbon consumption on the sediment surface (C_0) between different levels of sandprawn density, temperature and trophic states and their interactions. Statistically significant outcomes are displayed in **bold**. SP: sandprawn density, T: temperature (HT= high temperature, LT=low temperature), TS: trophic state.

	Response variable	**F-Value**	**df**	**p-value**	**pairwise**
SP	Microbial	0.75	2	0.47	
	Infaunal	0.82	2	0.44	
TS	Microbial	0.33	1	0.57	
	Infaunal	0.27	1	0.61	
T	Microbial	0.0003	1	0.99	
	Infaunal	**8.35**	**1**	**0.004**	**LT < HT, p = 0.004**
SP x TS	**Microbial**	**5.31**	**2**	**0.0006**	No significant terms
	Infaunal	**3.68**	**2**	**0.03**	No significant terms
SP x T	Microbial	2.45	2	0.09	
	Infaunal	0.02	2	0.98	
TS x T	Microbial	1.83	1	0.18	
	Infaunal	0.40	1	0.53	
SP x TS x T	Microbial	0.32	2	0.73	
	Infaunal	1.52	2	0.22	

Carbon degradation within the sediment (C) mediated microbially was influenced by temperature ($F = 7.10$, $df = 1$, $p < 0.01$, Table 5.4) and by the interactions between sandprawn density x temperature ($F = 5.44$, $df = 2$, $p < 0.01$), sandprawn density x trophic state ($F = 8.42$, $df = 2$, $p < 0.001$) and, temperature and depth ($F = 3.1$, $df = 5$, $p = 0.01$). Infaunally-mediated C was explained by depth ($F = 2.45$, $df = 5$, $p = 0.04$) and by the interactions between sandprawn density and trophic state ($F = 4.58$, $df = 2$, $p = 0.01$), and temperature and depth ($F = 3.25$, $df = 5$, $p < 0.01$).

Table 5.4: Results of linear fixed effect models testing for differences in microbial- and infaunal-mediated rates of carbon consumption (C) at increasing depths (D) within the sediment across different levels of sandprawn density, temperature and trophic states and their interactions. Statistically significant outcomes are displayed in **bold**. SP: sandprawn density, T: temperature (HT = high temperature, LT = low temperature), TS: trophic state (M = mesotrophic, E = eutrophic), D: depth.

	Response variable	**F-Value**	**df**	**p-value**	**pairwise**
SP	Microbial	0.19	2	0.83	
	Infaunal	2.28	2	0.11	
TS	Microbial	0.31	1	0.58	
	Infaunal	0.38	1	0.54	
T	**Microbial**	**7.10**	**1**	**p < 0.01**	**LT< HT, p = <0.01**
	Infaunal	0.93	1	0.34	
D	Microbial	1.11	5	0.36	
	Infaunal	**2.45**	**5**	**0.04**	No significant terms
SP x TS	**Microbial**	**8.42**	**2**	**p < 0.001**	**M: 50%SP < 100%SP, p < 0.05** **50% SP: M < E, p = 0.03**
	Infaunal	**4.58**	**2**	**0.01**	**E: 50%SP < 0%SP, p < 0.05**
SP x T	**Microbial**	**5.44**	**2**	**p < 0.01**	**100%SP: LT< HT, p< 0.001** **100%SP at LT < 0%SP at HT, p < 0.05**
	Infaunal	1.78	2	0.17	
SP x D	Microbial	0.64	10	0.78	
	Infaunal	0.57	10	0.84	
TS x T	Microbial	0.40	1	0.53	
	Infaunal	0.03	1	0.87	
TS x D	Microbial	0.59	5	0.71	
	Infaunal	0.49	5	0.78	
T x D	**Microbial**	**3.10**	**5**	**0.01**	**HT: 1cm >15cm, p < 0.05** **1cm at HT > 1cm, 7cm, 10cm at LT, p < 0.05**
	Infaunal	**3.25**	**5**	**p < 0.01**	**HT: 1cm >7cm & 10cm, p < 0.01** **1cm at HT > 3cm & 7cm at LT, p < 0.05**
SP x TS x T	Microbial	0.64	2	0.53	
	Infaunal	2.89	2	0.06	
SP x TS x D	Microbial	0.72	10	0.70	
	Infaunal	0.26	10	0.99	
SP x T x D	Microbial	0.55	10	0.85	
	Infaunal	1.15	10	0.33	
T x TS x D	Microbial	1.61	5	0.16	
	Infaunal	0.46	5	0.81	
SP x T x TS x D	Microbial	1.09	10	0.37	
	Infaunal	1.07	10	0.39	

The two main trends that emerged from the analysis indicates (1) that the infauna facilitated higher levels of carbon degradation in comparison to microbes only (Figs 5.4 & 5.5); (2) carbon degradation facilitated both by microbes (Fig. 5.4) and infauna (Fig. 5.5) was most pronounced when maximum sandprawn density occurred at mesotrophic conditions and high temperatures. Notably, this pattern largely continued down to a depth of 15cm, which represented the maximum depth at which measurements of carbon degradation were conducted.

Microbially-mediated organic matter degradation

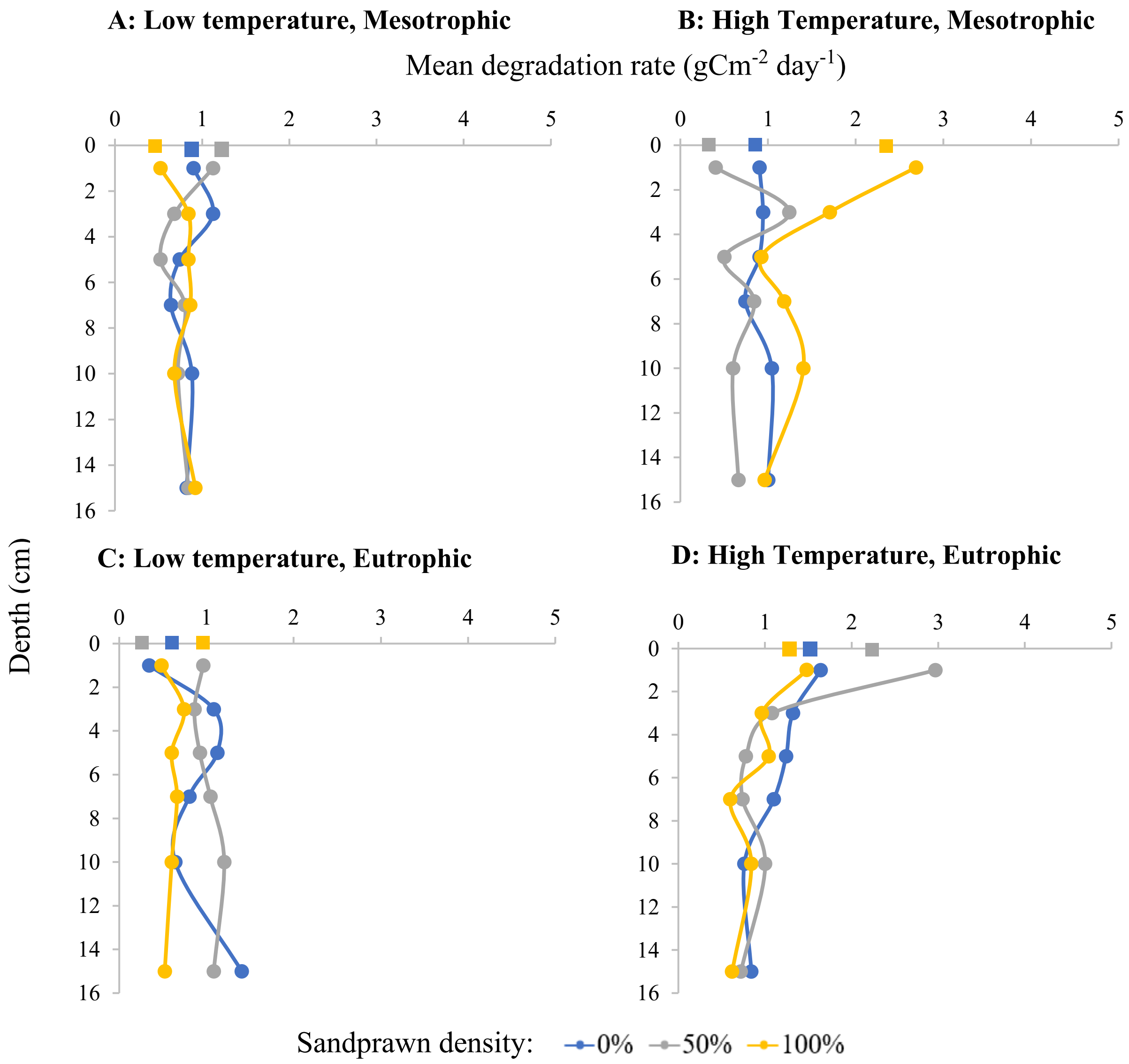


Figure 5.4: Spatial variability in sediment organic matter degradation (OMD) with depth (cm) mediated microbially in A (low temperature, mesotrophic), B (high temperature, mesotrophic),

C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%). C_0 values are represented by squares.

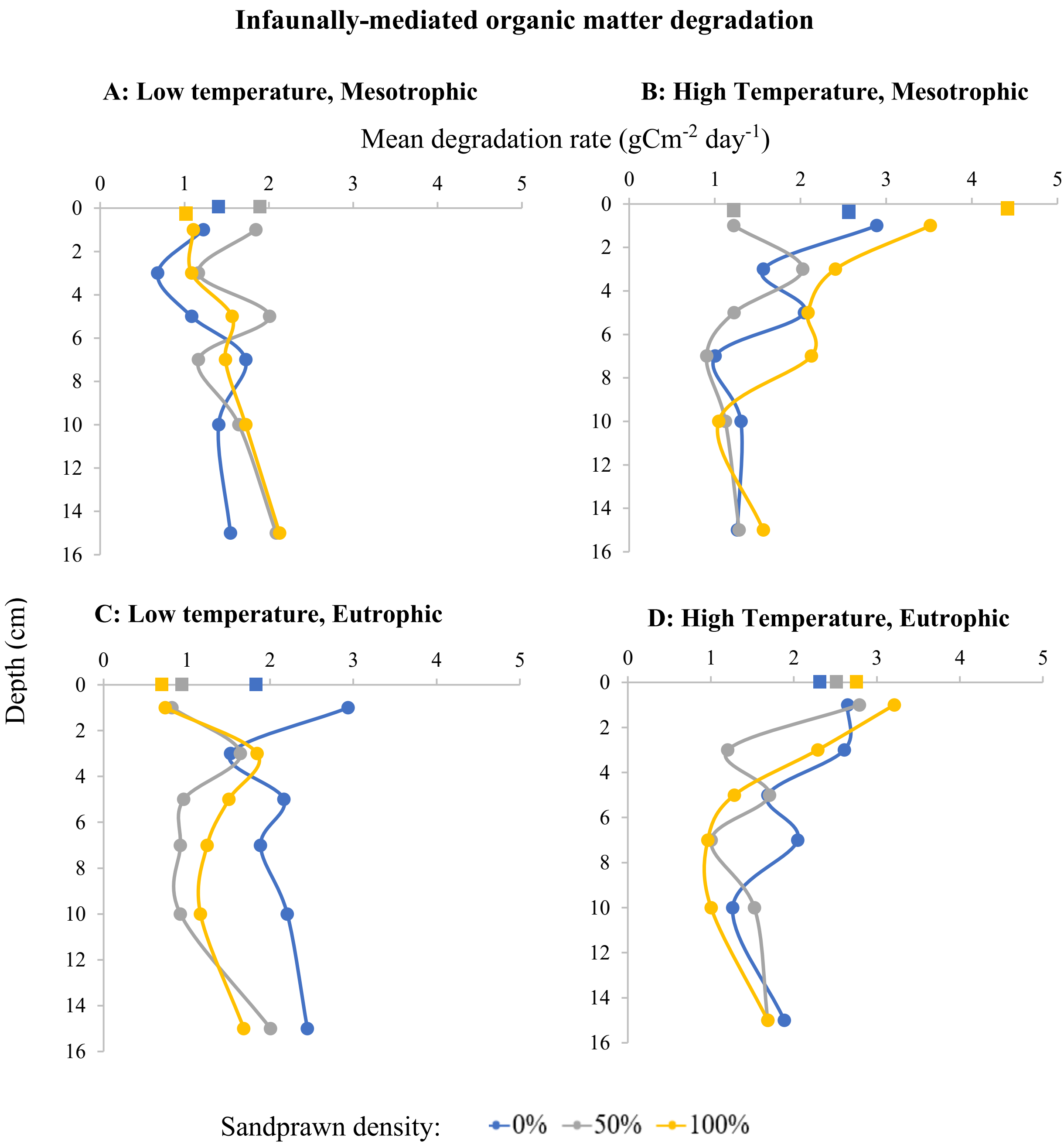


Figure 5.5: Spatial variability in sediment organic matter degradation (OMD) with depth (cm) mediated by infauna in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%). C_0 values are represented by squares.

5.6 Discussion

As predicted, SBR increased with sandprawn density in all treatments (Fig. 5.3); however, there was also evidence that SBR was also influenced by temperature. For the duration of the experiment, elevated temperatures elicited increases in SBR (Fig. 5.3) mainly through increases in sandprawn sediment turnover rates, likely through heightened metabolic activity. From the start of the experiment up until Day 5, SBR at 100% sandprawn density and at high temperatures increased by 43%, compared to the 5% increase at low temperatures. Generally, under low temperatures, SBR increases occurred later in the experiment. The temperature-induced changes to SBR recorded in my experiment are supported by prior research (White & Klahr, 1987; Berkenbusch & Rowden, 2003; Pillay 2019), and can be explained by an increase in feeding rate of sandprawns in response to warming-induced elevation of metabolic activity (Zhang, et al., 2020). The diminished SBR at low temperatures, even under eutrophic conditions with high phytoplankton biomass (Fig. 5.3C), suggests that temperature had a greater influence on SBR than eutrophication and resource availability.

Consistent with the findings of this study, White & Klahr (1987) reported increased rates of sediment reworking with increasing temperature in *Stylodrilus heringianus,* an oligochaete species dominating Lake Michigan. White & Klahr (1987) linked the increased rates of sediment reworking to accelerated rates of consumption in response to greater metabolic activity induced by elevated temperature. Interestingly, in the experimental enclosures, the oligochaetes exhausted almost all their dietary trophic resources (sedimentary organic particles and bacteria) with increasing temperature (White & Klahr, 1987). Maire, et al. (2007) conducted an experiment similar to the current study, to measure sediment reworking rates in *Abra ovata*, a deposit-feeding bivalve that dominates Mediterranean lagoons. Their investigation involved quantifying sediment reworking during winter (low temperature) and summer (high temperature) periods, under experimentally manipulated levels of trophic resource (phytodetritus) supply, in a laboratory experiment. Their results indicated a higher proportion of sediment reworking in summer (~93%) compared to winter (~16%). Additionally, their experiment highlighted that variance in reworking of sediment between food supply treatments were evident only during the initial stages of the summer experiments, with sediment reworking being lowest for treatments with the highest concentrations of food, and vice versa. These findings imply that, in terms of controlling sediment reworking of *A.ovata*, the influence of temperature outweighed that of food availability. The findings by Maire, et al. (2007) align with the findngs of this study regarding temperature effects on sediment reworking by sandprawns. However, concerning eutrophication, this study predicted an increase in SBR with eutrophication due to greater resource availability in the form of phytoplankton (refer to Chapter 3 for phytoplankton data); however, there was no conclusive evidence to support this prediction, in contrast to the findings of Maire, et al. (2007).

A comparison of SBR trends between the low and high temperature regimes of this study could be clearly distinguished at maximum sandprawn density (Fig 5.3). These observed trends for SBR to elevated temperatures in the current study suggests a tolerance of sandprawns to the high temperature conditions of this experiment. Given the extensive geographical distribution of sandprawns in both cool temperate and in sub-tropical regions along the South African coastline, (Branch, et al., 2016), it is highly plausible that findings for SBR reflect a high thermal plasticity in sandprawns. For instance, in Durban Bay (sub-tropical east coast) and in the lower reaches of Zandvlei Estuary (Chapter 2, Fig. 2.1), mean summer water temperatures of 28.4°C (Pillay, et al., 2008) and between 25.5 and 26 °C (Harding, 1994) have been recorded in the sandprawn habitats. In this experiment, the sandprawn induced SBR response to temperatures as high as 29.5 °C offers promising indications of its adaptive capabilities to future warming scenarios, particularly within populations inhabiting cool-temperate distribution ranges and considering projected temperature changes up to 2100 (IPCC, 2007).

The impact of global change on the physiology of ectothermic organisms, including key bioturbators, can induce large changes to the structure of sedimentary habitats, with cascading impacts on local biodiversity, and sediment ecosystem functioning (Cardinale et al, 2012). Considering the fundamental importance of decomposition reactions in benthic ecosystem functioning, and its dependence on the end-products of bioturbation, this chapter also assessed how rates of organic matter degradation (OMD) compared when mediated by infauna (microbes + meiofauna + macrofauna (40-1000µm) + sandprawns) vs microbial organisms only, in the context of simulated warming, eutrophication and at varying sandprawn densities. Sediment organic matter degradation appeared visually greater when mediated by the infauna in comparison to microbes only. For instance, OMD mediated by infauna ranged between 0.7 gCm^{-2} day^{-1} to 3.5 gCm^{-2} day^{-1} (Fig 5.5) while OMD mediated by microbes ranged between 0.5 gCm^{-2} day^{-1} to 2.9 gCm^{-2} day^{-1} (Fig. 5.4). In a comparable *in-situ* experiment conducted by O'Maera, et al. (2018), rates of OMD were compared in three types of marine habitats: a mangrove ecosystem, intertidal mudflat and a cockle bed. Irrespective of the habitat, their findings likewise indicated higher rates of OMD in the presence of infauna (~4 gCm^{-2} day^{-1} to ~22 gCm^{-2} day^{-1}) when compared to rates of OMD mediated by microbes only (~0gCm^{-2} day^{-1} to ~5gCm^{-2} day^{-1}). It is probable that the movements and bioturbation effects from the infauna could have cumulatively promoted the conditions necessary for the degradation of organic matter in the current study and that of O'Maera, et al. (2018). It is also evident that the rates of OMD reported in the current study were considerably lower than those reported in the experiment conducted by O'Maera, et al. (2018). This may be a consequence of the differing stocks of organic carbon and benthic microbes, meiofauna and macrofauna typically present in the sediment type. For instance, mangroves are known to be rich in organic carbon (Alongi, 2012), whereas the sandy sediment sourced from the sandprawn-biotope in Zandvlei Estuary for this study, has low organic content (Harding, 1995). Furthermore, differences in experimental settings between O'Maera, et al.'s (2018) *in-situ* study and the indoor mesocosm setup of the current study, could have contributed to the disparity in OMD rates between the two experiments. In the current study, it would have been inevitable that there would be significant disturbance to the sediment during the collection procedure and transport from the Zandvlei Estuary to the aquarium facility, which housed the mesocosms for the duration of the experiment. This disturbance might have led to alterations in infaunal and microbial assemblages, whereas such disruptions would have been less problematic in the field study conducted by O'Maera, et al. (2018).

The contribution to sediment reworking by infauna apart from sandprawns was negligible in this study, as demonstrated in the mesocosms without sandprawns. Therefore, the observed patterns of sediment turnover primarily stemmed from the bioturbation activity of sandprawns (Fig 5.3). However, contrary to expectations, sandprawn density was not significant in explaining rates of OMD in the current study. Previous studies have shown that bioturbators play an important role in stimulating OMD, in both terrestrial (Meysman, et al., 2006; Sofo, et al., 2020) and marine (Aller, 1982; Meysman, et al., 2006; Na, et al., 2008; Kristensen, et al., 2012; Fusi, et al., 2022) environments. This occurs because bioturbation improves ventilation and advective irrigation of the burrows, renewing the supply of oxygen and reagents required for decomposition (Zeibis, et al., 1996; Aller, 1982; Meysman et al, 2006; Kristensen, et al., 2012). In this way bioturbation ulitmately modifies the biogeochemistry of the sediment (Zeibis, et al., 1996; Aller, 1982; Meysma et al, 2006; Kristensen, et al., 2012) and promotes the development of functional niches for OMD pathways (Booth, et al., 2023), despite the mechanical disturbance to the sediment that is a consequence of sediment turnover (Aller, 1982). However, it is important to note that the oxygenation effect of infaunal bioturbators is may be localised in some cases depending on factors such as sediment grain size and proximity to burrows.

Aligned with the ectothermic response envisaged for sandprawns, expectations were that warming would generally increase rates of OMD (Arndt, et al., 2013), since sandprawns were present in two-thirds of mesocosms. However, in this study, warming was significant in only elevating microbially-mediated rates of OMD singly and in interaction with maximum sandprawn density. This suggest either community level shifts in the microbial assemblage in the heated mesocosms and in 100% sandprawn density mesocosms, or joint stimulation of top-down effects of bacteria by sandprawns and temperature (Hall, et al., 2008). Interestingly, Aller (1982) proposed that feeding and mechanical disturbance by macrobenthos may stimulate the growth phase of microbial populations that mediate decomposition, which in the context of this study, could explain why with warming, high densities of sandprawns increased rates of microbially-mediated decomposition reactions. In future warming scenarios, the results of the current study suggests that sandprawns may lead to enhanced carbon breakdown and recycling. However, further research is needed to to test this outcome in laboratory and field studies.

The response of OMD to eutrophication in this study was unclear and was significantly explained only in interaction with sandprawn density. While trends could not be clearly deciphered, both microbially- and infaunally- mediated rates of OMD appeared greatest when sandprawn density was at its maximum under warm, mesotrophic conditions (Fig 5.5). It is conceivable that, relative to the eutrophic treatments, mesotrophic mesocosms harboured fewer pathogens and toxins (Supplementary Table S1), creating a more favourable environment for organic matter degradation. Future research should determine whether eutrophic conditions cause shifts in benthic microbial assemblages due to greater pathogenic taxa that may not play a role in carbon degradation. It is also possible that high concentrations of phytoplankton may be subducted from the water column into the sediment under eutrophic conditions by sandprawns (inferred from Fig 3.1, Chapter 3) and that phytoplankton cells were targeted for consumption instead of the carbon in the ROMA plates. For infaunal-mediation, rates of OMD were greater at 50% sandprawn density under eutrophic conditions. It could be that at 50% density, sandprawns were able to attenuate eutrophic stress sufficiently and facilitate rates of organic matter degradation (Booth, et al., 2023); however, further studies are necessary to reveal whether the effect reported is truly a sandprawn-density mediated process or background noise.

Rates of OMD along the depth profile of the ROMA plate was nuanced in this study, and not uniform across all treatments (Figs.5.4 & 5.5). While depth was an insignificant predictor of OMD when mediated by microbes, depth emerged as a significant (albeit relatively weak) predictor of OMD mediated by infauna. Interestingly, in conjunction with warming, OMD decreased with depth for both infaunally- and microbially- mediated rates of OMD (Table 5.4), perhaps reflecting depth related shifts in microbes and infauna. In the investigation conducted by O'Maera, et al (2018), rates of OMD decreased clearly with depth regardless of the type of marine sediment, a trend that was linked to the depth of oxygen penetration into the sediment.

5.7 Concluding remarks

The results presented in this chapter demonstrate that warming and an increased density of sandprawns led to greater bioturbation (as SBR), which was presumably mediated by an ectotherm (sandprawn) metabolism and the need for trophic resource acquisition. The SBR response to warming in the current study, suggests that sandprawns were tolerant to the experimental temperature conditions, albeit for a two-week period. These findings imply that sandprawns may maintain their functional significance in providing essential ecosystem services (e.g. oxygenation through bioturbation) even at temperatures predicted to 2100.

However, further research is necessary to assess their robustness and response to prolonged warming conditions, and possibly simulated heatwaves.

The results for OMD indicate that, under mesotrophic conditions, a temperature increase will facilitate organic matter degradation at greatest sandprawn density. Importantly though, eutrophic conditions seem to nullify the above-mentioned effect. While this chapter was not fully able to mechanistically explain this outcome, shifts in trophic resources (e.g. subduction of phytoplankton into the benthos) and microbial assemblage structure may have contributed to a dampening of OMD in ROMA plates. Quantifying changes in particulate organic matter (POM) across treatments may have shed light on these processes, particularly in the context of eutrophication and phytoplankton subduction into the sediment. Future research should include measurements of POM so that organic matter dynamics can be more comprehensively understood. Nevertheless, the implication is that if sandprawn-dominated habitats become eutrophic, the rate of organic matter degradation might decline. This finding also suggests that reducing eutrophication in estuaries is crucial, so that their organic matter degradation functions are not compromised.

CHAPTER 6:

MEIOFAUNAL RESPONSE

6.1 Introduction

Meiofauna (or meiobenthos) represent a unique and diverse assemblage of the smallest metazoans (<500µm), adapted to thrive in the upper layer of aquatic sediments where microbial activity is high and there are steep gradients in solutes (McIntyre, 1969; Hulings & Gray, 1971; Rysgaard, et al., 2000; Balsamo, et al., 2012; Traunspurger & Majdi, 2017; Schratzberger & Ingels, 2018; Álvarez-Castillo, et al., 2023). Meiofauna are the most abundant size class in the benthos (Coull, 1999), defined by sizes ranging between 44 µm and 500 µm (Giere, 2009; Urban-Malinga, 2014). Their microscopic size is an evolutionary advantage that enables them to inhabit the interstitial environment of sediments (Hulings & Gray, 1971; Urban-Malinga, 2014; Schratzberger & Ingels, 2018). Meiofauna are vital to ecosystem functioning as they help decompose detritus, recycle nutrients and establish trophic links with higher-level consumers, particularly macrofauna (Coull, 1999).

Permanent meiofauna are distinguished as being fully benthic species that remain in the meiofaunal size-range for their whole life-span (Urban-Malinga, 2014; Traunspurger & Majdi, 2017). They are often dominated by nematodes, rotifers and harpacticoid copepods with ostracods, cladocerans, gastrotrichs, foraminifera and microturbellarians also common in some habitats (Traunspurger & Majdi, 2017). Temporary meiofauna are classified as those species that start off as meiofauna but grow into macrofauna or emerge out of the benthos during their lifespan (McIntyre, 1969; Urban-Malinga, 2014; Traunspurger & Majdi, 2017). They are typically dominated by the larvae and juvenile stages of species that, as adults, belong to the macrofauna (Hulings & Gray, 1971; Giere, 2009; Traunspurger & Majdi, 2017). Meiofauna may also be classified as mesobenthic (true interstitial forms), epibenthic (surface-dwelling) or endobenthic (shallow-burrowing, Raffaelli, 1987; Giere, 2009). Mesobenthic forms are confined to the sediments whilst epi- and endo-benthic forms are more dependent on sediment water content and exhibit greater motility (Raffaelli, 1987).

Despite limited dispersive potential (Schmid-Araya, et al., 2002; Giere, 2009; Urban-Malinga, 2014; Álvarez-Castillo, et al., 2023), meiofauna are ubiquitous in soft-sediment-dominated ecosystems (Mevenkamp, et al., 2018) such as estuaries (Coull, 1999). Importantly, the size and shape of the sediment grains directly determines the spatial and structural conditions of the interstitial habitat, affecting the pore-water volume and the surface area for meoifaunal colonisation (Swedmark, 1964; Giere, 2009). These factors indirectly regulate food supply and oxygen concentrations, which, in turn, influences meiofaunal abundance and assemblage (Swedmark, 1964; Coull, 1999; Giere, 2009; Zhang & Hu, 2019; Álvarez-Castillo, et al., 2023). There is compelling evidence that meiofauna play a significant role in ecosystem functioning (Aller & Aller, 1992; Giere, 2009; Urban-Malinga, 2014; Zeppilli, et al., 2015; Álvarez-Castillo, et al., 2023), specifically influencing biogeochemical processes and the flow of energy in benthic ecosystems (Zeppilli, et al., 2015). They have charactersitically high turnover rates, which leads to meiofaunal decomposition products rapidly becoming part of the microbial loop to eventually contribute to biomass production by higher trophic levels and/or be remineralised for primary production (Coull, 1999; Schmid-Araya, et al., 2002).

Regarding their influence on biogeochemical processes, meiofauna act as micro bioturbators, agitatating and irrigating the sediment to influence solute transport, oxygen dynamics and reaction rates (Aller & Aller, 1992; Coull, 1999; Giere, 2009). Effective meiofaunal bioturbators are ostracods, nematodes and, particularly at the surface, harpacticoid copepods (Giere, 2009). Meiofauna also influence sediment stability (Urban-Malinga, 2014) by either secreting mucus to stabilise the sediment or conversely destabilise and increase the erodibility of the sediment through locomotor activities and/or by consuming large amounts of microalgae, particularly the EPS-secreting diatoms (Heip, 1995; MacIntyre, et al., 1996; Yallop, et al., 2000; Kromkamp, et al., 2003; Bellinger, et al., 2009; Urban-Malinga, 2014; Malarkey, et al., 2015) Meiofauna additionally graze on bacterial populations, which in moderation, keeps bacterial populations in a state of logarithmic growth (Aller, 1982; Giere, 2009). By stimulating bacterial growth, meiofauna ultimately enhance remineralisation and nutrient regeneration (Aller, 1982; Urban-Malinga, 2014; Traunspurger & Majdi, 2017). Specifically, with regards to decomposition, meiofauna enhances the aerobic degradation of organic matter by mechanically breaking down detritus, excreting nutrients into the environment for microbial use, and producing mucus that promotes bacterial growth (Aller & Aller, 1992; Heip, 1995).

Historically, meiofauna have been commonly excluded from food web studies leading to knowledge gaps in interpreting food web complexity and patterns (Schmid-Araya, et al., 2002). Yet, meiofauna serve as trophic intermediaries between the microbial/detrital organisms and higher trophic levels, contributing to the transfer of energy in sediments, and benthic-pelagic coupling by linking the sediment with the water column through nutrient cycling (Coull, 1999; Schmid-Araya, et al., 2002; Giere, 2009). Meiofauna are immensely diversified in their trophic habits and modes, feeding as suspension feeders, deposit feeders, detritivores (both phytodetritus from phytoplankton blooms and aged detritus), predators, parasites, and grazers of vascular plants, bacteria and benthic algae (particularly diatoms and the extracellular polymeric substances (EPS) they secrete, which are their main food source) (Swedmark, 1964; Coull, 1999; Urban-Malinga, 2014; Traunspurger & Majdi, 2017). Interestingly, meiofauna are able to actively seek out food particles of preferrred size, shape and quality relative to their body size (Warwick & Clarke, 1984). Meiofauna are, in turn, preyed upon by higher level consumers such as adult bottom-feeding fish and the juveniles of several fish species, benthic macroinvertebrates and epifauna, and various shorebirds (Coull, 1999; Schmid-Araya, et al., 2002; Urban-Malinga, 2014; Traunspurger & Majdi, 2017).

The complexity and interactions among abiotic and biotic factors, including temperature, salinity, oxygenation levels, sedimentary processes and food availability, impact the spatial and temporal distribution of the meiofauna (Swedmark, 1964; Giere, 2009; Urban-Malinga, 2014; Zhang & Hu, 2019; Álvarez-Castillo, et al., 2023) and their buffering conferring capacity to the ecosystem (Bongers & Ferris, 1999). Given their limited dispersive and migration potential, meiofauna are highly vulnerable to global change and interacting stressors which can heighten their exposure and response to ecological stress (Coull, 1999; Defeo & Elliott, 2021).

As coastal and intertidal areas are naturally exposed to high fluctuations in temperature, meiofauna that inhabit these areas are generally eurythermal (Swedmark, 1964; Giere, 2009). Ordinarily, an increase in temperature within thermal limits of these ectothermal species is related to shorter life cycles, increased population growth, faster development time and increased food assimilation (Mevenkamp, et al., 2018). Nothwithstanding this, there is a high probability that meiofauna can be vulnerable to more extreme fluctuations of temperature that surpass their upper thermal limits that might pose additional stress on their functioning (Madiera, et al., 2012). Of particular concern is the predicted increase in mean water temperature of aquatic ecosystems (IPCC, 2007) potentially affecting environmental factors such as ecosystem productivity, anoxia levels, and bioturbation (Coull, 1999). Should these changes trigger differential responses among individual species, the composition, abundance and biomass of the meiofaunal assemblage and its functioning are at risk with potential repercussions for the ecosystem (Aller & Aller, 1992; Coull, 1999; Sarmento, et al., 2017; Álvarez-Castillo, et al., 2023). For example, studies have shown that warming significantly affects the abundance and diversity of meiofaunal assemblages (Zeppilli, et al., 2015 and references therein) and has also altered the body size structure of meiofaunal populations, disrupting top-down control, with repercussions to benthic food-web stability (Jochum, et al., 2012).

Eutrophication enhances primary production and results in a substantial influx of organic matter into the sediment (Heip, 1995). Meiofaunal assemblages occupy a low level in trophic webs and are extremely sensitive to eutrophication (Urban-Malinga, 2014; Álvarez-Castillo, et al., 2023). They are also affected by eutrophication-induced hypoxia/anoxia within the sediment (Heip, 1995; Carriço, et al., 2013), often enhanced by warming (Giere, 2009). Initially, meiofauna, and other endobenthic organisms such as macrofauna and bacteria may benefit from eutrophication, resulting in increased abundance and biomass (Heip, 1995). The likelihood also exists for meiofaunal diversity to decrease with a corresponding shift in species composition (Pearson & Rosenberg, 1978; Beukema, 1991). It is highly likely that such changes in meiofaunal communities may directly affect biomass production or sediment processes, or indirectly alter the structure of other benthic size classes (Schratzberger & Ingels, 2018). Ultimately, these changes to the endobenthic community, may affect bottom-up control (resulting from increased productivity, alleviating resource limitation), or it may impact top-down control (due to changes to top-predator abundance), giving rise to trophic cascades (Widboom & Elmgren, 1988) that might also hold ramifications for the stability of the benthic food web and ultimately ecosystem functioning and resilience (Sarmento, et al., 2017; Schratzberger & Ingels, 2018).

Macrofaunal bioturbators are influential in determining the physico-chemical and biological attributes of aquatic sediments and thereby modifying the benthic community structure through coupled predation and sediment disturbance (Widdicombe & Austen, 1999; Urban-Malinga, 2014). Moreover, bioturbation creates oxic and anoxic microniches, which is often the basis of the patchy meiofaunal distribution (Giere, 2009). Ultimately, bioturbation affects the abundance, composition and distribution of meiofauna in complex ways: disturbance and destabilisation of the sediment can have a negative impact while bioturbation-enhanced oxygenation and subduction of organic matter to the sediment can have a positive effect (Heip, 1995; Giere, 2009; Pillay & Branch, 2011). Relative to volume, meiofauna have a relatively large surface area and mostly high oxygen demands, which makes the oxygen supply to the habitat the predominant abiotic factor influencing the presence of meiofauna at any given time (Giere, 2009). In addition, the intricate three-dimensional pattern of tubes that are created in the sediment by macrofaunal bioturbators extend the habitat space for meiofauna, serving as hot spots for them and colonies of bacteria (Branch & Pringle, 1987; Heip, 1995; Pillay & Branch, 2011; Urban-Malinga, 2014). While much is known about bioturbation, little is understood about the role of bioturbators as top-down regulators (trophic and non-trophic pathways) of meiofaunal assemblages (Pillay & Branch, 2011), or their potential to increase resilience against global change (Warwick, et al., 1990).

Due to their high abundance, small size and short life cycles, meiofaunal responses to environmental change are excellent indicators of benthic ecosystem health (Sandulli & Giudici, 1989; Zeppilli, et al., 2015). Their shorter generation times allow for early detection of disturbances relative to macrofaunal species, which have comparatively longer lifespans and slower changes in community structure (Carriço, et al., 2013). Challenges to meiofaunal research include their small size, making identification and counting difficult and time-consuming, their high spatial and temporal variability, and limited accessibility to taxonomic literature on meiofauna (Giere, 2009; Balsamo, et al., 2012). Nonetheless, technological advancements in electronic identification keys and molecular appraches to identification now facilitate greater use of meiofauna in ecological studies (Giere, 2009).

Meiofauna play key roles in nutrient recycling and organic matter degradation, significantly influencing sedimentary processes (Urban-Malinga, 2014). Despite their potential to enhance our understanding of trophic dynamics and ecosystem functioning (Carriço, et al., 2013; Urban-Malinga, 2014; Traunspurger & Majdi, 2017; Álvarez-Castillo, et al., 2023), meiofauna are a neglected component in benthic ecosystem research compared to microbes and macrofauna, including in Southern Africa (Widboom & Elmgren, 1988; Coull, 1999; Kennedy & Jacoby, 1999; Pillay & Perissinotto, 2009; Carriço, et al., 2013; Zeppilli, et al., 2015; Sarmento, et al., 2017; Traunspurger & Majdi, 2017).

Aims and objectives

Crucially, with global change, the effects of localised pressures, such as eutrophication and bioturbator loss/distribution shifts, are likely to be exacerbated when interacting with other global change stressors such as warming (IPCC, 2007; Jochum, et al., 2012; Carriço, et al., 2013; Urban-Malinga, 2014; Traunspurger & Majdi, 2017; Álvarez-Castillo, et al., 2023), yet the consequences for meiofaunal assemblage remains largely unknown; with attempts to understand such effects initiated only recently (Widboom & Elmgren, 1988; Kennedy & Jacoby, 1999; Álvarez-Castillo, et al., 2023; Mohammad, et al., 2024). This research is highly relevant, where issues such as interactions between global change processes and bioturbator loss/declines have not been quantified, to the best of my knowledge. In this context, the objectives of this chapter are to evaluate the effects of warming, eutrophication and sandprawn density on the meiofaunal composition, abundance and biomass distribution in estuarine sediments.

I predicted that:

(1) Increasing sandprawn density will suppress meiofaunal abundance and biomass due to increased sediment disturbance (rapid sediment turnover, Branch & Pringle, 1987). Notably, according to Branch and Pringle (1987), *K. kraussi* is unlikely to consume meiofauna, making predation an improbable cause for variations in meiofaunal abundance and biomass.

(2) Alternatively, sandprawn-induced phytoplankton subduction into the sediment (mainly burrow walls; Venter, et al., 2020) may enhance the supply of trophic resources (Widboom & Elmgren, 1988; Heip, 1995) and increase meiofaunal abundance and biomass.

(3) Meiofaunal abundance and biomass will either increase with eutrophication due to the greater availability of food resources (phytoplankton, benthic microalgae; Raffaelli, 1987) or decrease if toxic effects associated with eutrophication (pathogenic bacteria) compromises survival of the organisms.

(4) Meiofaunal taxa will have differential responses to warming and eutrophication at the varying densities of sandprawns used in this study, as meiofaunal taxa likely differ in their tolerance to these conditions (Raffaelli, 1987).

(5) Diversity and richness of taxa will be reduced with warming and eutrophication due to the disappearance of the more sensitive taxa (Pusceddu, et al., 2007).

6.2 Methodology

Please refer to Chapter 2 for the experimental design for this chapter.

6.3 Data collection

Meiofauna community composition and abundance: Upon termination of the experiment on Day 15, five sediment cores (2cm diameter x 1cm deep) were collected from each of the 36 mesocosms to measure the responses of benthic meiofaunal assemblages to the predictor variables. Established methods used previously in South African experiments and surveys (Nozais, et al., 2005; Pillay & Perissinotto, 2009; Bownes & Perissinotto, 2012; Dawson, et al., 2020) were followed, with cores being collected from the interior of each mesocosm to avoid edge effects. Each sediment core was preserved in an ethanol-Rose Bengal solution (70%) until the meiofauna were identified. Prior to identification, each sample of sediment was sieved through a 500μm mesh (to remove macrofauna and debris) followed by a 45μm mesh (to isolate the meiofauna). On completion of the extraction, meiofauna were sorted, enumerated, and identified to the lowest possible taxonomic level using a compound microscope (Leica DM 500 compound microscope 100x, fitted with a Leica ICC50 camera).

Meiofaunal biomass. Biomass of dominant taxa (those that cumulatively contributed ~85% to the meiofaunal assemblage) was calculated by measuring the wet weight (A&D, GH-202 Balance) of each taxon (a maximum of 30 organisms) from each treatment and placing them in a desiccator (30 minutes). The biomass per organism was calculated by dividing the batch biomass by the number of organisms in the batch. Total biomass per dominant taxon was determined by multiplying individual biomass by the abundance of organisms.

6.4 Data analysis

Multivariate analyses: All multivariate analyses were run using PRIMER-e (Plymouth Routines in Multivariate Ecological Research) v.6.1.18 (Clarke & Gorley, 2015) and Permanova+ v1.0.8 (Anderson, et al., 2008) statistical packages, as previously described.

The Bray-Curtis similarity coefficient was used to produce resemblance matrices from transformed (log x+1) abundance data, from which non-metric multidimensional scaling ordinations (nMDS) were produced for visual assessments of trends (Clarke, et al., 2014). PERMANOVA (permutational multivariate analysis of variance; 3-way crossed design) was used to assess whether the predictors (sandprawn density, temperature, and trophic condition), singly and interactively influenced meiofaunal assemblages (Anderson, et al., 2008), with pairwise tests being used for inter- and intra- treatment comparisons. DISTLM (distance based linear modelling) was used to identify environmental variables (averaged over the duration of the study) that best accounted for variability in meiofaunal assemblages (Legendre & Anderson, 1999; McArdle & Anderson, 2001). Preliminary assessments of data distribution and multi-collinearity among the environmental predictors were visually evaluated using draughtsman plots. Where required, the environmental data were log, square root or fourth root transformed, with inter-correlated variables being removed from the dataset to improve model parsimony (Table 6.1). The 'forward' selection procedure using the adjusted R^2 criterion was used to fit the model, while predictor variable effects on meiofauna assemblages were visualised using the dbRDA (distance-based redundancy analysis) routine (Anderson, et al., 2008). The DIVERSE function was used to calculate the following community descriptors for meiofaunal assemblages: total abundance (*N*), species richness (*S*, expressed as total number of taxa), Pielou's evenness (*J',*), and the Shannon-Wiener diversity index (*H'*, log to the base e).

Table 6.1: Transformations and correlations of environmental variables used in the DISTLM analysis.

Environmental variable	**Transformation**	**Correlations**
Temperature		
Sediment boundary roughness		
Salinity		Conductivity
pH		
Ammonium		
Nitrite	Square root	Nitrate
Chl-a	Log_{10}	
Nanoplankton		Total phytoplankton
Picoplankton		
Microphytobenthic nanoplankton		Total microphytobenthic algae & microphytobenthic picoplankton
Green algae		
Cyanobacteria	Fourth root	Diatoms
Reflectance (excl)		

Univariate data analyses: Univariate data analyses were performed using the RStudio data analysis platform (R Core Team, 2019), as previously described. Linear Fixed Effects Models (LFEM) were applied to determine whether meiofaunal abundance, richness, Pielou's evenness and the Shannon-Wiener diversity index, as well as abundance and biomass of dominant taxa were affected by sandprawn density, temperature, and trophic states. Where model assumptions were violated, the model was re-fitted on transformed data (Table 6.2).

Table 6.2: Statistical analyses performed on meiofaunal community descriptors (abundance, species richness, Pielou's evenness and the Shannon-Wiener diversity index) and abundance and biomass of dominant taxa in relation to predictors (SP: sandprawn density, T: temperature, TS: trophic state).

Response Variable	**Predictors**	**Statistical Tool**	**Analyses**	**Transformation**
Meiofaunal Community Descriptor:	SP, T, TS + interactions	RStudio	LFEM	
Abundance				
Species Richness				
Evenness				
Shannon Diversity Index				
Dominant Taxa:	SP, T, TS + interactions	RStudio	LFEM	
NEMATODA				
Nematodes sp.1				Log_{10}
Nematodes sp.2				Square root
ANNELIDA				
Polychaeta sp.1				Square root
Sipunculid sp.1				Square root
FORAMINIFERA				
Foraminifera sp.1				Square root
ARTHROPODA				
Harpacticoida sp.1				Square root
Ostracoda sp.1				Square root

6.5 Results

Meiofaunal community response. A total of 31 meiofaunal taxa was recorded at the end of the experiment (Table 6.3), with nematode sp.1 (contribution = 25.5%), polychaete sp.2 (contribution = 15.1%), foraminifera sp.1 (contribution = 11.6%), nematode sp.2 (contribution = 9.8%), harpacticoid copepod sp.1 (contribution = 8.5%), ostracod sp.1 (contribution = 7.6%) and sipunculid sp.1 (contribution = 6.7%) cumulatively accounting for 85% (abundance) of the overall meiofaunal assemblage .

Table 6.3. Mean abundance (± SE) of meiofauna taxa (ind.core^{-1}) in the different temperature, trophic condition and sandprawn density treatments. Dominant taxa are represented in **bold.** SP: sandprawn density, T: temperature (LT: low temperature, HT: high temperature), TS: trophic state (M: mesotrophic, E: eutrophic).

	LT, M			**LT, E**			**HT, M**			**HT, E**		
Dominant taxa	**0**	**50**	**100**	**0**	**50**	**100**	**0**	**50**	**100**	**0**	**50**	**100**
NEMATODA												
1. Nematode sp.1	**23.4±11.6**	**33.1±12**	**15.6±1.4**	**20.9±5.1**	**27.7±11.7**	**19.5±7.9**	**8.5±0.9**	**3.4±0.6**	**8.5±2.6**	**7.5±0.6**	**4.3±1**	**6.5±2.3**
2. Nematode sp.2	**4.3±1.1**	**5.3±1.8**	**4.9±1.6**	**6.4±3.6**	**6.6±2.3**	**6.7±5**	**4.2±1.7**	**2.2±0.5**	**7.4±3.3**	**5.7±1.7**	**5.4±0.3**	**9.7±4.5**
ANNELIDA												
3. Polychaeta sp.1	–	0.3±0.3	0.9±0.9	–	–	0.1±0.1	–	–	–	0.3±0.3	0.2±0.2	–
4. Polychaeta sp.2	**8.7±3.2**	**12.7±3.9**	**4.5±1.4**	**7.9±2.7**	**8.9±3.9**	**5.7±2.1**	**11.6±5.1**	**4±1.5**	**4.9±1.9**	**9.3±1.4**	**19.9±8.5**	**8.3±3.5**
5. Polychaeta sp.3	–	–	0.1±0.1	–	–	0.1±0.1	–	–	–	–	0.1±0.1	–
6. Polychaeta sp.4	0.1±0.1	–	0.1±0.1	–	–	0.1±0.1	–	–	–	–	–	–
7. Oligochaeta	0.9±0.2	4.9±1.6	2.6±0.6	1.9±0.4	2.7±0.8	3.1±1.3	1.4±0.3	0.5±0.2	0.9±0.3	1.5±0.5	1.6±0.6	1.9±0.2
8. Sipunculid sp.1	**7.9±2.4**	**6.3±3.4**	**7.6±1.6**	**5.5±0.6**	**4.5±0.5**	**6.2±2.8**	**0.9±0.3**	**0.9±0.2**	**1.9±0.7**	**1.5±0.8**	**0.4±0.1**	**3.5±0.5**
9. Sipunculid sp.2	0.8±0.2	0.4±0.2	0.8±0.6	1.9±0.5	1.1±0.6	1.6±0.2	0.1±0.1	0.1±0.1	0.3±0.3	0.1±0.1	–	0.5±0.3
FORAMINIFERA												
10. Foraminifera sp.1	**7.1±0.6**	**5.3±0.7**	**5.9±1.2**	**4.5±1**	**5.7±1.5**	**6.4±1**	**11.4±2.2**	**10±1.5**	**7.7±1.1**	**6.7±1.5**	**5.3±0.2**	**5.3±0.5**
11. Foraminifera sp.2	0.7±0.4	1±0.2	1±0.5	0.5±0.2	1.3±0.4	0.9±0.2	2.3±0.6	2.4±1.2	1.4±0.1	2.3±1.1	1.1±0.4	0.8±0.2
12. Foraminifera sp.3	0.8±0.1	0.5±0.2	0.3±0.2	0.2±0.1	0.9±0.2	1.1±0.6	0.4±0.3	0.9±0.3	0.4±0.1	1.7±0.5	1.4±0.8	1.1±0.3
13. Foraminifera sp.4	–	0.1±0.1	–	–	–	–	0.1±0.1	–	–	0.1±0.1	–	–
14. Foraminifera sp.5	0.7±0.2	0.7±0.5	1.2±0.5	0.5±0.3	0.4±0.2	0.3±0.1	1.2±0.5	0.8±0.4	0.4±0.1	1.2±0.2	0.8±0.4	3.1±1.3
15. Foraminifera sp.6	0.3±0.1	0.6±0.2	0.1±0.1	0.1±0.1	0.3±0.3	0.1±0.1	0.5±0.3	0.2±0.2	0.1±0.1	0.3±0.1	–	0.3±0.2
16. Foraminifera sp.7	2.7±1.3	1.8±0.3	0.7±0.4	1.3±0.5	2.2±1	1.8±0.3	2.2±0.6	2.5±0.4	2.1±0.2	1.5±0.3	1.9±1.2	2.5±0.5
17. Foraminifera sp.8	0.2±0.1	0.1±0.1	–	0.1±0.1	0.3±0.3	0.1±0.1	0.2±0.2	0.1±0.1	0.3±0.1	0.1±0.1	0.1±0.1	0.1±0.1
18. Foraminifera sp.9	–	–	–	0.1±0.1	0.1±0.1	–	0.3±0.1	–	0.1±0.1	–	–	–
19. Foraminifera sp.10	0.3±0.1	0.2±0.2	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0.5±0.1	0.2±0.2	0.3±0.2	0.3±0.3	0.2±0.1
20. Foraminifera sp.11	–	–	–	–	–	0.1±0.1	–	0.2±0.2	–	–	–	–
ARTHROPODA												
21. Harpacticoida sp.1	**6.3±2.8**	**3.6±1.9**	**3.1±1**	**4±2.4**	**1.4±0.5**	**12.6±7.6**	**1±0.8**	**2.9±1**	**8.5±6.5**	**4.1±2.4**	**3.5±1.2**	**8.7±2.9**
22. Harpacticoida sp.2	–	–	0.2±0.2	–	–	0.1±0.1	–	–	–	–	–	–
23. Ostracoda sp.1	**3.9±1.5**	**8.3±2.8**	**11.7±3**	**3.5±1**	**3.5±0.6**	**4.9±1.8**	**1.4±0.7**	**1.4±0.3**	**1.6±1.4**	**3.9±1.4**	**2.3±0.5**	**7±3.1**
24. Ostracoda sp.2	0.3±0.2	0.4±0.2	–	–	0.1±0.1	0.1±0.1	0.3±0.1	0.4±0.1	0.8±0.4	0.5±0.2	0.2±0.1	0.3±0.1
25. Arachnida	–	–	–	–	–	–	–	–	–	–	0.1±0.1	–
MOLLUSCA												
26. Gastropod sp.1	0.3±0.1	–	0.1±0.1	–	–	–	–	–	–	–	0.1±0.1	–
27. Gastropod sp.2	0.1±0.1	–	–	–	–	0.1±0.1	–	–	0.1±0.1	–	–	–
PLATYHELMINTHES												
28. Rhabdocoela	–	–	0.1±0.1	–	0.2±0.1	0.5±0.3	–	0.1±0.1	0.1±0.1	0.1±0.1	–	0.2±0.1
OTHER												
29. unid. sp.1	0.1±0.1	0.1±0.1	–	–	0.2±0.1	–	0.2±0.2	0.1±0.1	–	0.1±0.1	–	0.1±0.1
30. unid. sp.2	–	0.1±0.1	–	–	0.1±0.1	–	–	–	–	–	–	–
31 unid. sp.3	–	–	–	–	0.1±0.1	–	–	0.1±0.1	–	–	–	–

Temperature (PERMANOVA, pseudo-$F_{1,\,2814}$ = 9.82, p = 0.001, Table 6.4), and the temperature x trophic state interaction (PERMANOVA, pseudo-$F_{1,\,782}$ = 2.73, p = 0.006) significantly influenced meiofaunal assemblages. Specifically, at high and low temperatures, meiofaunal assemblages differed between mesotrophic and eutrophic conditions (pairwise, $p < 0.01$).

Table 6.4. Summary statistics for PERMANOVA and pairwise tests (where relevant) assessing the influence of sandprawn density, temperature, trophic conditions and their interactions on meiofaunal assemblages. Significant p-values are indicated in **bold.** SP: sandprawn density, T: temperature (HT= high temperature, LT=low temperature), TS: trophic state (M = mesotrophic, E = eutrophic).

Source	**df**	**SS**	**Pseudo-F**	***p*-value (PERMANOVA)**
SP	2	777	1.36	0.159
T	**1**	**2814**	**9.82**	**0.001** **LT≠HT, pairwise, *p* = 0.001**
TS	1	457	1.59	0.118
SP x T	2	785	1.37	0.172
SP x TS	2	331	0.58	0.916
T x TS	**1**	**782**	**2.73**	**0.006** **For E&M, LT ≠HT, pairwise, *p* = 0.001;** **At HT, M≠E, pairwise, *p* = 0.017**
SP x T x TS	2	530	0.92	0.574

nMDS ordinations visually supported the distinction of meiofaunal assemblages between low and high temperature conditions (Fig. 6.1).

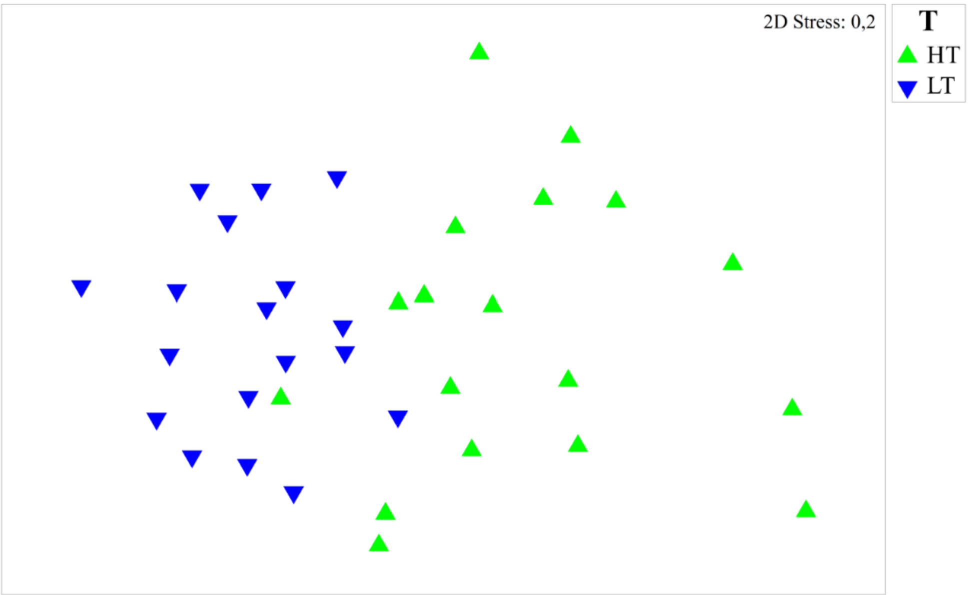


Figure 6.1: Non-metric multidimensional scaling ordination (nMDS) illustrating spatial variation in meiofaunal assemblage (abundance data) between high and low temperature conditions. T: temperature (HT= high temperature, LT=low temperature).

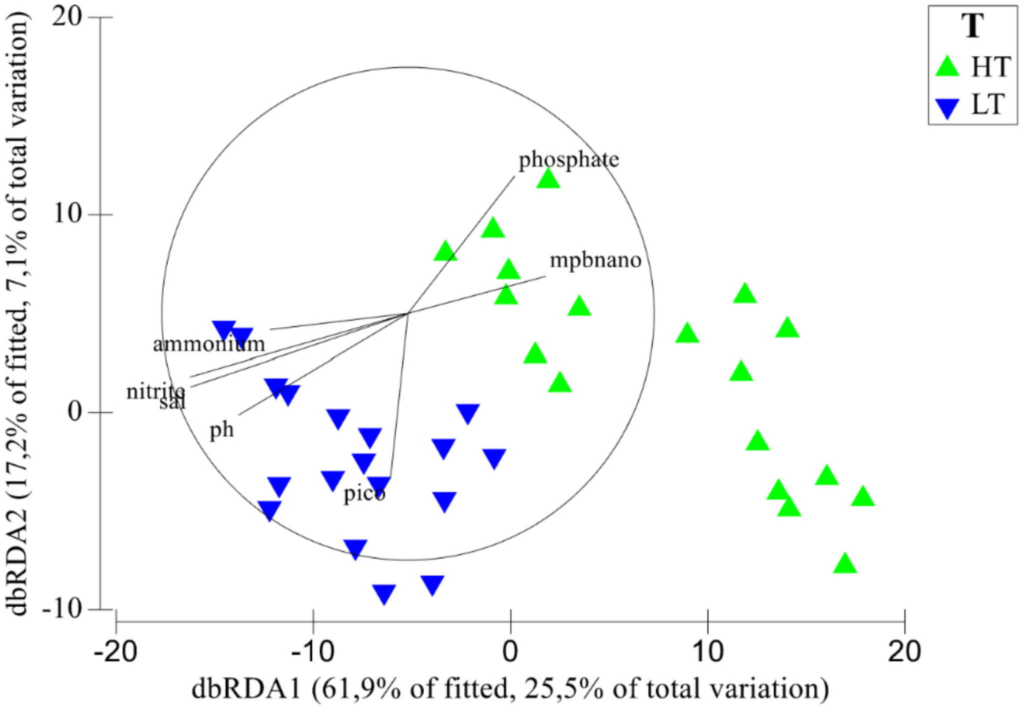
Marginal tests showed that variability in meiofaunal assemblages was significantly explained by inorganic nutrients [nitrite (18%, p = 0.001), ammonium (8%, p = 0.004) and phosphate (7%, p = 0.018), Table 6.5], as well as by salinity (18%, p = 0.001), temperature (17%, p = 0.001), pH (11%, p = 0.001) and microphytobenthic nanoplankton abundance (8%, p = 0.004), which was correlated with total microphytobenthic algae and microphytobenthic picoplankton (Table 6.1). Sequential tests indicated that nitrite concentration, which correlated with nitrate concentration, and picoplankton abundance (p=0.001, Table 6.5) significantly influenced meiofaunal variability, along with the non-significant influence of salinity, sediment boundary roughness, nanoplankton, ammonium and phosphate. These predictor variables collectively explained 41% of the variation in meiofaunal assemblages (adjusted $R^2 = 0.265$).

Table 6.5. Results of DISTLM analysis showing individual and joint environmental predictors of variance in meiofaunal assemblages (abundance data) across varying levels of temperature, sandprawn densities and trophic conditions.

Marginal tests				Sequential tests					
	Pseudo-F	*p*-value	Prop.		AdjR^2	Pseudo-F	*p*-value	Prop.	Cumul.
nitrite	**7.89**	**0.001**	**0.21**	**+nitrite**	**0.18**	**8.89**	**0.001**	**0.21**	**0.21**
salinity	**8.86**	**0.001**	**0.21**	**+pico**	**0.24**	**3.30**	**0.001**	**0.07**	**0.28**
temp	**8.39**	**0.001**	**0.20**	+salinity	0.25	1.53	0.128	0.03	0.31
pH	**5.12**	**0.001**	**0.13**	+SBR	0.26	1.49	0.158	0.03	0.34
mpbnano	**3.63**	**0.004**	**0.10**	+nano	0.26	1.20	0.323	0.03	0.37
ammonium	**3.40**	**0.002**	**0.09**	+ammonium	0.26	1.04	0.396	0.02	0.39
phosphate	**2.69**	**0.010**	**0.07**	+phosphate	0.27	1.02	0.415	0.02	0.41
pico	1.58	0.069	0.04						
chl-*a*	1.39	0.183	0.04						
nano	1.12	0.314	0.03						
cyano	0.68	0.754	0.02						
SBR	0.74	0.678	0.02						

temp=temperature, mpbnano=microphytobenthic nanoplankton, pico=picophytoplankton, nano=nanophytoplankton, cyano=cyanobacteria, SBR=sediment boundary roughness.

dbRDA axes highlighted the environmental variables that contributed most to influencing meiofaunal assemblage variation between high and low temperature treatments. Under low temperature conditions, levels of nitrite, ammonium, salinity, pH and picophytoplankton, were visually greater, while phosphate and microphytobenthic nanoplankton levels were greater at high temperatures (Fig. 6.2).



sal = salinity, mpbnano = microphytobenthic nanoplankton, pico = picophytoplankton,

Figure 6.2: dbRDA ordination of meiofaunal assemblage data (based on Bray-Curtis abundance data after log x + 1 transformation) at high and low temperature conditions with vector overlays of environmental variables that best explained community variance. T: temperature (HT= high temperature, LT=low temperature).

Meiofaunal community descriptors. Temperature had a significant main effect on total meiofaunal abundance (F= 7.54, df = 1, *p* = 0.011, Table 6.6), and Pielou's evenness (F = 5.06, df = 1, *p* = 0.034). Meiofauna was more abundant at low temperatures (pairwise, *p* = 0.01, Fig. 6.3A), yet more evenly distributed, from a statistical point of view, at high temperatures (pairwise, *p* = 0.03, Fig. 6.3).

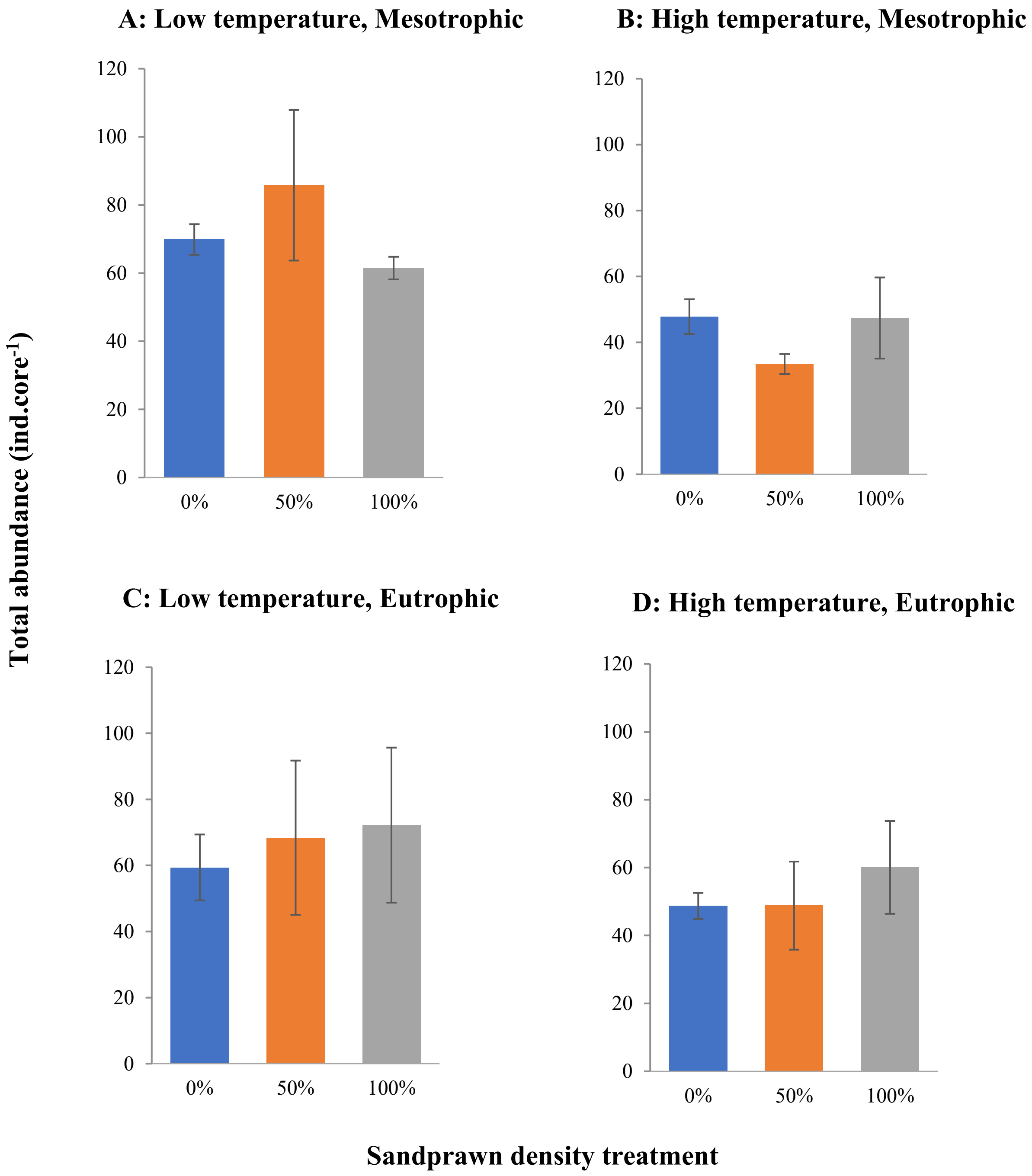


Figure 6.3: Spatial variability in total meiofaunal abundance (mean ± SE) in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%).

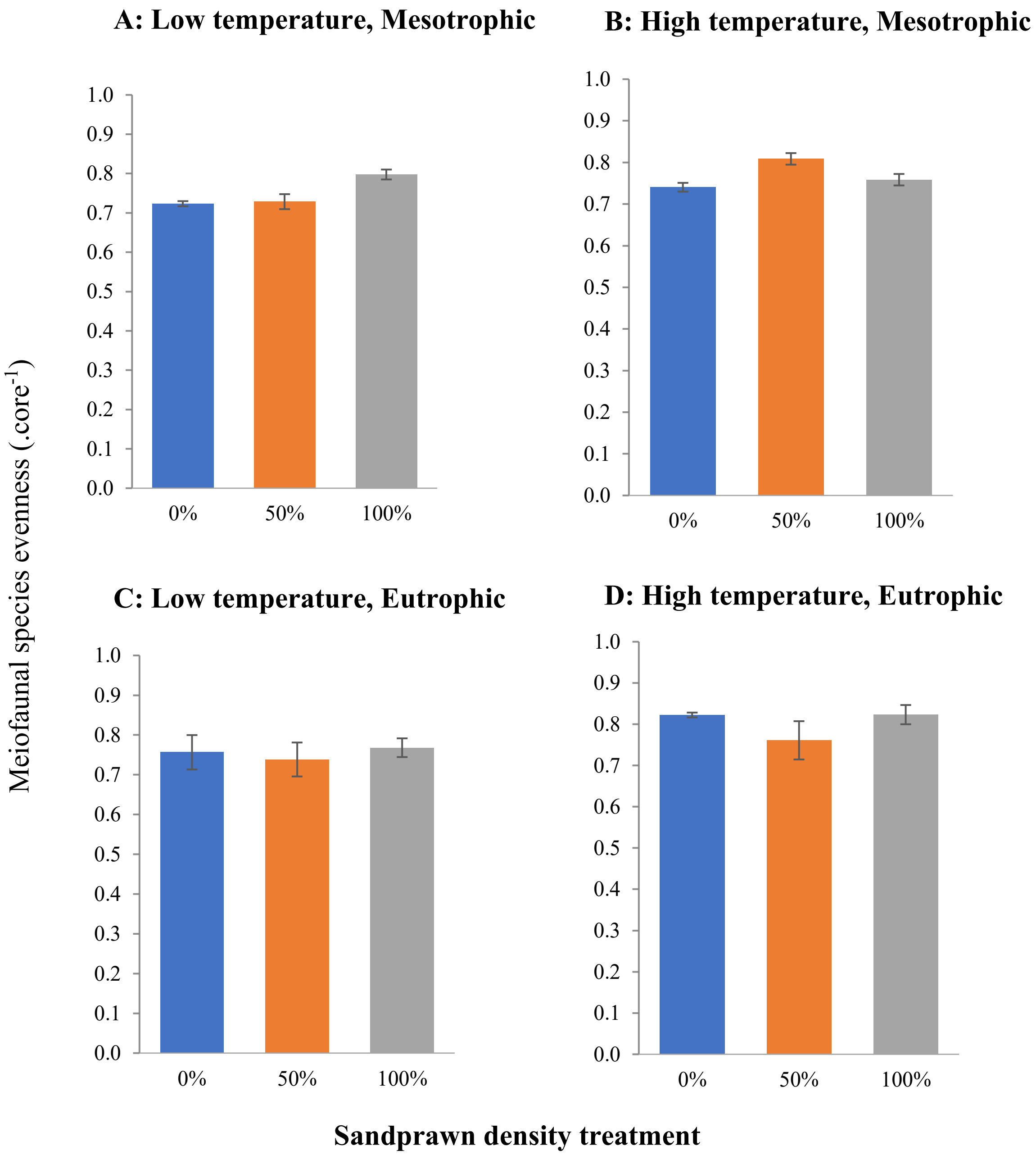


Figure 6.4: Spatial variability in meiofaunal species evenness (mean ± SE) in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%).

Table 6.6. Results of linear fixed effects models (LFEM) testing for differences in meiofaunal diversity indices between the different levels of sandprawn density, temperature and trophic condition treatments and their interactions. Significant p-values are indicated in **bold.** SP: sandprawn density, T: temperature (HT= high temperature, LT=low temperature), TS: trophic state (M = mesotrophic, E = eutrophic).

	Community descriptor	**F**	**df**	***p*-value**
SP	Abundance	0.08	2	0.922
	Pielou's Evenness	1.45	2	0.254
	Species Richness	0.07	2	0.937
	Shannon Index	1.91	2	0.170
T	**Abundance**	**7.54**	**1**	**< 0.011** **(LT>HT, pairwise, *p* = 0.01)**
	Pielou's Evenness	**5.06**	**1**	**0.034** **(LT< HT, pairwise, *p*=0.03)**
	Species Richness	0.45	1	0.507
	Shannon Index	3.39	1	0.078
TS	Abundance	0.06	1	0.807
	Pielou's Evenness	1.54	1	0.222
	Species Richness	0.08	1	0.775
	Shannon Index	1.20	1	0.285
SP x T	Abundance	0.81	2	0.458
	Pielou's Evenness	0.77	2	0.476
	Species Richness	0.45	2	0.641
	Shannon Index	0.45	2	0.644
SP x TS	Abundance	0.39	2	0.679
	Pielou's Evenness	2.18	2	0.135
	Species Richness	**3.69**	**2**	**0.040** (no significant terms)
	Shannon Index	**3.40**	**2**	**0.050**
T x TS	Abundance	0.93	1	0.344
	Pielou's Evenness	0.92	1	0.347
	Species Richness	0.08	1	0.775
	Shannon Index	1.34	1	0.252
SP x T x TS	Abundance	0.33	2	0.723
	Pielou's Evenness	2.26	2	0.126
	Species Richness	**4.86**	**2**	**0.017** (no significant terms)
	Shannon Index	**6.32**	**2**	**0.006** (no significant terms)

Species richness (F = 4.86, df = 2, p = 0.017) and the Shannon-Wiener diversity index (F = 6.32, df = 2, p = 0.006) were significantly explained by the three-way interaction between sandprawn density x temperature x trophic state. However, clear distinctions between the levels of the predictors were not evident from pairwise analyses (Table 6.6). Visually, at low temperature, species richness (Fig 6.5 C) and the Shannon-Wiener diversity index (Fig 6.6 C; to a lesser degree) displayed a linear increase with increasing sandprawn density under eutrophic conditions. However, for species richness, this trend was reversed under mesotrophic conditions (Fig 6.5A), while a marginal increase was observed for the Shannon-Wiener diversity index (Fig 6.6A). At high temperatures, similar trends were observed for species richness and the Shannon-Wiener diversity index in response to increasing sandprawn density, wherein weak unimodal patterns recorded under mesotrophic conditions (Fig 6.5B & Fig. 6.6B) were reversed under eutrophic conditions (Fig 6.5D & Fig. 6.6D).

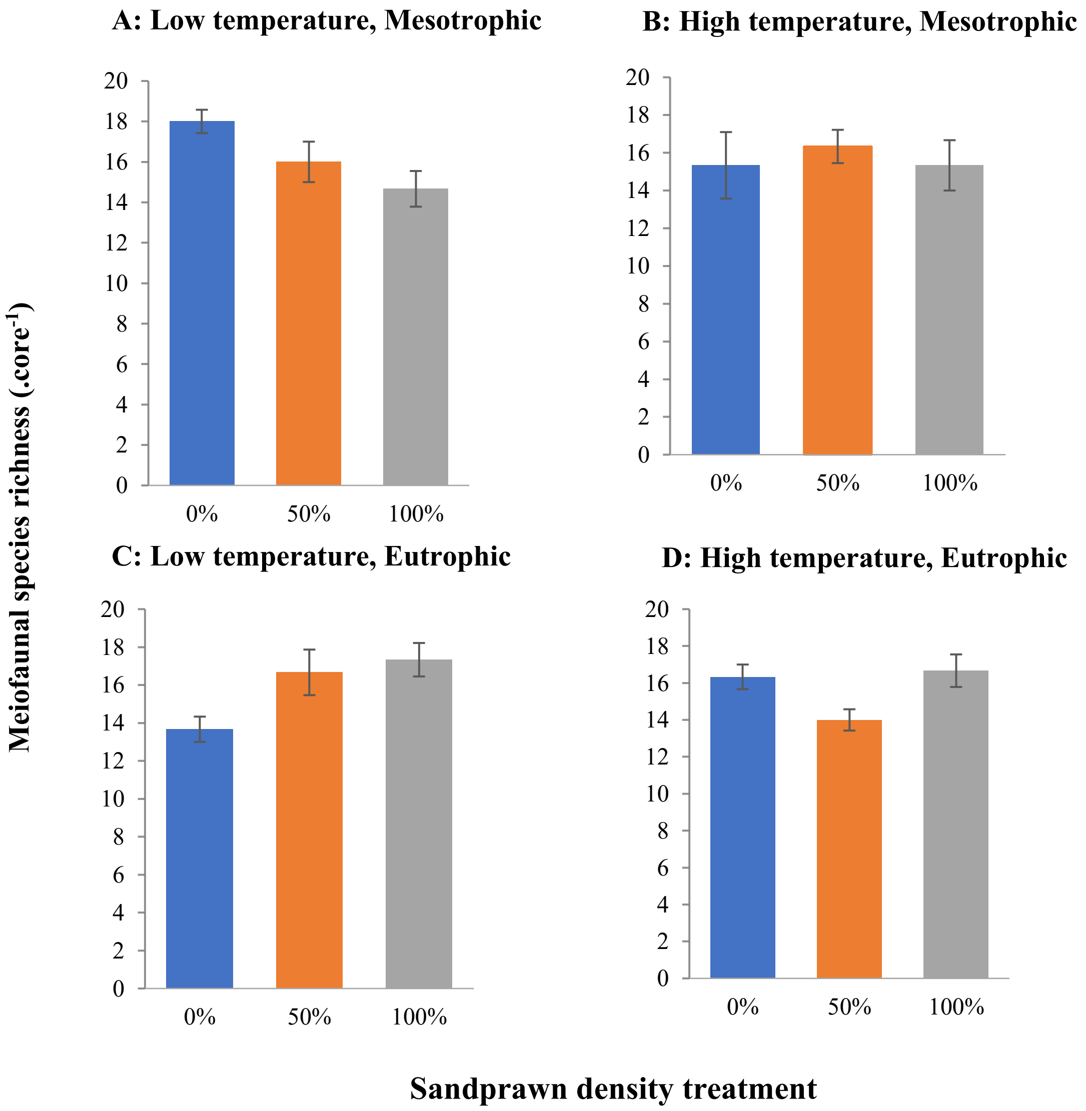


Figure 6.5: Spatial variability in meiofaunal species richness (mean ± SE) in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic)

and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%).

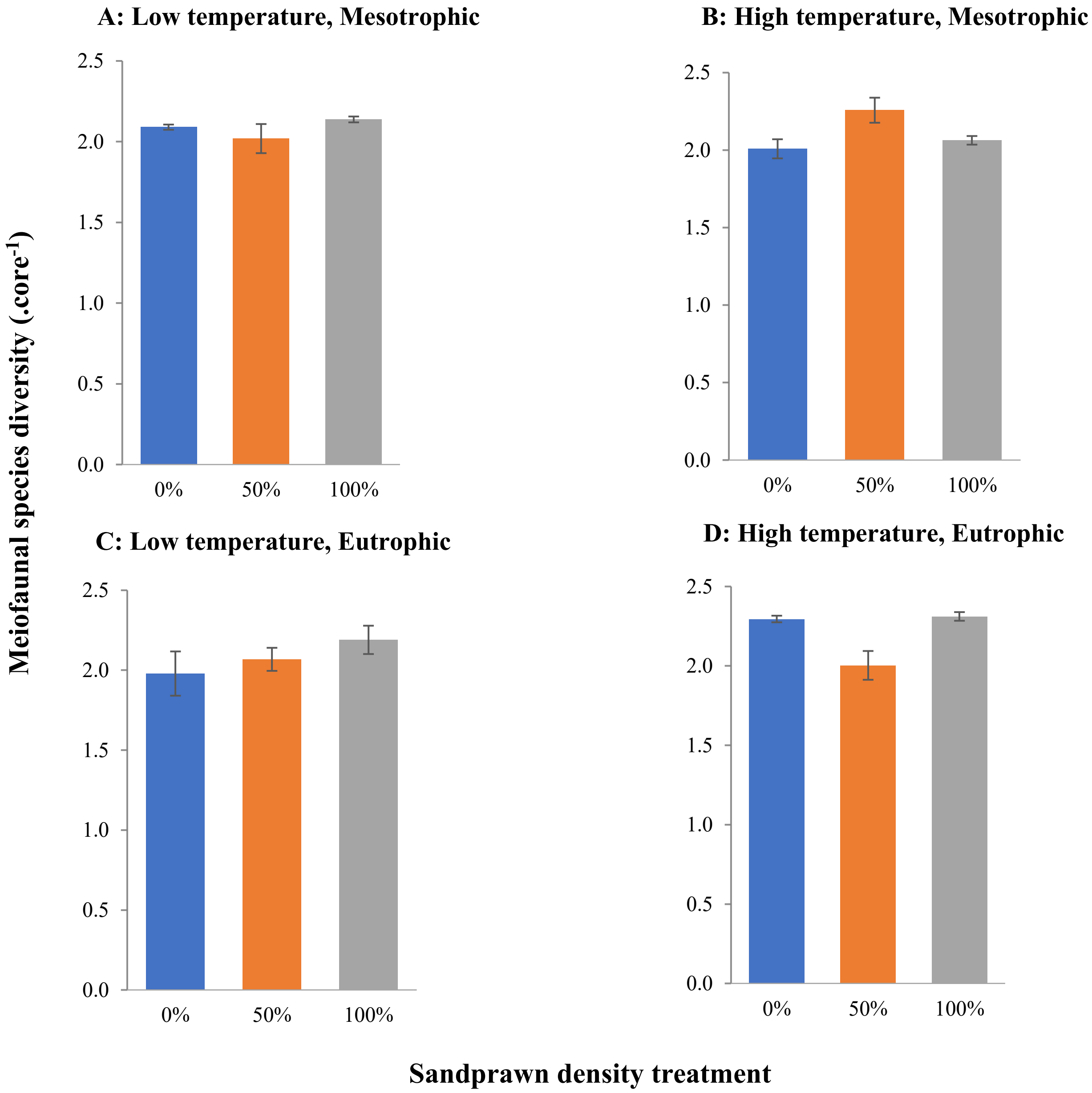


Figure 6.6: Spatial variability in meiofaunal species diversity (mean ± SE) in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%).

Responses of the dominant taxa. Regarding abundance, temperature singularly induced significant reductions in the total abundance of dominant taxa (F = 8.62, df = 1, $p = 0.007$, Table 6.7, Fig. 6.7) and, in terms of individual taxa, only that of Sipunculid sp.1 (F = 51.56, df = 1, $p < 0.0001$, Fig 6.8). With regards to the other dominant taxa, the abundance of Nematode sp1, Foraminifera sp.1 and Ostracoda sp.1 were significantly influenced by two factorial interactions. Firstly, the interaction between temperature and sandprawn density significantly affected the abundance of Nematode sp.1, with low temperatures generally supporting a higher abundance of Nematode sp.1 (F = 4.52, df = 1, $p = 0.022$), with the unimodal response to sandprawns at low temperature being reversed at high temperature (Fig. 6.8). In addition, the interaction between temperature and trophic state significantly impacted Foraminifera sp.1 and Ostracoda sp.1, where under mesotrophic conditions, Foraminifera sp.1 was more abundant at high temperatures (F = 5.68, df = 1, $p = 0.025$), with the pattern being reversed for Ostracoda sp.1 (F = 11.23, df = 1, $p = 0.003$). Variations in abundance of Nematode sp.2, Polychaeta sp.2 and Harpacticoida could not be statistically explained and, while visually, the abundance of Nematode sp.2 was elevated under eutrophic condition, graphical trends were generally erratic and difficult to decipher for these taxa.

For biomass, generally, sandprawn density, trophic state and temperature influenced variations in total and individual biomass of the dominant taxa. However, responses were inconsistent due to the prominence of two and three factor interactions. For total biomass, the sandprawn density, temperature and trophic state interaction was significant (F = 17.8, df = 2, $p < 0.0001$, Table 6.7). Variations in the biomass of four (Nematode sp.1, Sipunclid sp.1, Foraminifera sp.1, and Harpacticoida sp.1) of the seven dominant taxa were also significantly explained by the three-way interaction, however the pairwise outcomes for the interaction were too cumbersome to report. In general, total biomass was relatively higher in the absence of sandprawns under normal conditions (low temperatures and mesotrophic conditions; $p <$

0.001), with biomass reduced with increasing sandprawn density (Fig. 6.9A). With a switch to eutrophic conditions, biomass diminished, and the pattern of linear decline with increasing sandprawn density was no longer present (Fig. 6.9). Under high temperature, mesotrophic conditions (Fig 6.9B) and low temperature, eutrophic conditions (Fig. 6.9C), biomass responses to increasing sandprawn density shifted to being unimodal.

Significant two-way interactions were recorded between sandprawn density and temperature for Nematode sp.2 (F = 16.3, df = 2, $p < 0.0001$, Table 6.7), and between trophic state and temperature for Polychaeta sp.2 (F = 6.48, df = 1, $p = 0.018$). Generally, the biomass of Nematode sp.2 was higher at low relative to high temperatures; however, at low temperatures, biomass decreased with increasing sandprawn density, while at high temperatures, this trend was reversed (Fig. 6.10). The biomass of Polychaeta sp.2 was highest under normal (low temperature, mesotrophic) conditions (Fig 6.10). However, significant declines in the biomass of Polychaeta sp.2 were observed under low temperatures as trophic state shifted from mesotrophic to eutrophic conditions (p = 0.026, Table 6.7) and under mesotrophic conditions as temperature increased (p = 0.006). Individually, increasing temperature (p = 0.007) and eutrophication (p = 0.009) significantly depressed the biomass of Ostracoda sp.1.

Table 6.7. Results of LFEM testing for differences in mean biomass and abundances of the dominant meiofaunal taxa between different levels of sandprawn density, temperature and trophic condition treatments and their interactions. Significant p-values are indicated in **bold.** SP: sandprawn density, T: temperature (HT= high temperature, LT=low temperature), TS: trophic state (M = mesotrophic, E = eutrophic).

	Dominant taxa	df	Mean Abundance			Mean Biomass		
			F	*p*-value	Pairwise	F	*p*-value	Pairwise
SP	Nematode sp.1	2	1.24	0.309		**13.9**	**0.0001**	**0%>50%, $p < 0.001$** **0%>100%, $p = 0.017$**
	Nematode sp.2	2	0.87	0.431		**3.91**	**0.034**	**0%>100%, $p = 0.028$**
	Polychaeta sp.2	2	2.16	0.138		**14.1**	**< 0.0001**	no significant terms
	Sipunculid sp.1	2	3.15	0.061		**8.03**	**0.002**	**0%>100%, $p = 0.001$**
	Foraminifera sp.1	2	0.93	0.410		0.84	0.446	
	Harpacticoida sp.1	2	2.86	0.077		**4.64**	**0.020**	**0%<50%, $p = 0.016$**
	Ostracoda sp.1	2	3.46	0.050		0.37	0.692	
	TOTAL	2	0.07	0.937		**6.54**	**0.005**	**0%>100%, $p = 0.005$** **50%>100%, $p = 0.039$**
T	Nematode sp.1	1	**64.54**	**<0.0001**	**LT>HT, $p < 0.0001$**	**68.6**	**< 0.0001**	**LT>HT, $p < 0.0001$**
	Nematode sp.2	1	0.001	0.971		**28.5**	**< 0.0001**	**LT>HT, $p < 0.0001$**
	Polychaeta sp.2	1	0.53	0.473		**7.09**	**0.014**	**LT>HT, $p = 0.014$**
	Sipunculid sp.1	1	**51.56**	**<0.0001**	**LT>HT, $p < 0.0001$**	**82.1**	**< 0.0001**	**LT>HT, $p < 0.0001$**
	Foraminifera sp.1	1	**7.11**	**0.014**	**LT<HT, $p < 0.014$**	0.14	0.711	
	Harpacticoida sp.1	1	0.23	0.637		0.80	0.379	
	Ostracoda sp.1	1	**8.78**	**0.007**	**LT>HT, $p < 0.007$**	**8.67**	**0.007**	**LT>HT, $p = 0.007$**
	TOTAL	1	**8.62**	**0.007**	**LT>HT, $p < 0.007$**	**26.7**	**< 0.0001**	**LT>HT, $p < 0.0001$**
TS	Nematode sp.1	1	0.29	0.593		**16.23**	**<0.001**	**M>E, $p = 0.001$**
	Nematode sp.2	1	1.72	0.202		**7.93**	**0.009**	**M>E, $p < 0.01$**
	Polychaeta sp.2	1	1.09	0.306		3.15	0.089	
	Sipunculid sp.1	1	0.24	0.630		**13.03**	**0.001**	**M>E, $p = 0.001$**
	Foraminifera sp.1	1	**10.0**	**0.004**	**M>E, $p < 0.004$**	**16.31**	**<0.001**	**M>E, $p < 0.001$**
	Harpacticoida sp.1	1	0.74	0.397		**6.50**	**0.018**	**M>E, $p = 0.018$**
	Ostracoda sp.1	1	0.28	0.602		**8.10**	**0.009**	**M>E, $p = 0.009$**
	TOTAL	1	0.04	0.849		**42.10**	**< 0.0001**	**M>E, $p < 0.0001$**
SP x T	Nematode sp.1	2	**4.52**	**0.022**	**0%&100%LT>50%HT, $p < 0.001$** **50%LT>0%&100%HT, $p < 0.01$** **100%LT>100%HT, $p = 0.038$**	**5.10**	**0.014**	**LT: 0% > 50%&100%, $p < 0.01$** **0%LT>0%, 50%, 100%HT, $p < 0.0001$** **50%LT>100%HT, $p = 0.002$** **100%LT>50%HT, $p < 0.001$**
	Nematode sp.2	2	0.84	0.446		**16.3**	**< 0.0001**	**0%&50% LT>100%LT, $p < 0.05$** **0%LT>0%, 50%, 100%HT, $p < 0.001$** **50%LT>0%&50%HT, $p < 0.05$**
	Polychaeta sp.2	2	0.02	0.985		2.45	0.108	
	Sipunculid sp.1	2	0.95	0.402		0.64	0.534	

	Foraminifera sp.1	2	1.38	0.272		0.35	0.706	
	Harpacticoida sp.1	2	1.11	0.347		**10.6**	**<0.001**	**0%&100%LT>0%HT, *p* < 0.05** **50%LT<50%HT, *p* = 0.047** **HT:0%<50%, *p*<0.001 & 50% > 100%, *p* = 0.015**
	Ostracoda sp.1	2	0.93	0.408		0.42	0.662	
	TOTAL	2	0.70	0.506		**4.66**	**0.020**	**LT:0%>100%, *p* < 0.01** **0%LT>0%, 50% &100%HT, *p* < 0.01** **50%LT>0%&100%HT, *p* < 0.05**
SP x TS	Nematode sp.1	2	0.04	0.964		**11.3**	**<0.001**	**0%M>50%&100%M, *p* < 0.001** **0%M>0%, 50%&100%E, *p* < 0.01**
	Nematode sp.2	2	0.01	0.993		1.24	0.306	
	Polychaeta sp.2	2	1.00	0.384		2.30	0.122	
	Sipunculid sp.1	2	0.31	0.734		**5.32**	**0.012**	**M, 0%>E, 0%, 50%&100%, *p* < 0.01.** **For M, 0%>100%, *p* < 0.001**
	Foraminifera sp.1	2	1.18	0.325		0.32	0.730	
	Harpacticoida sp.1	2	0.93	0.409		**3.74**	**0.039**	**0%M<50%M, *p* < 0.01** **50%M>0%, 50%&100%E, *p*< 0.05**
	Ostracoda sp.1	2	0.74	0.489		1.70	0.204	
	TOTAL	2	0.27	0.766		3.12	0.062	
T x TS	Nematode sp.1	1	0.04	0.837		3.45	0.075	
	Nematode sp.2	1	0.03	0.862		1.31	0.264	
	Polychaeta sp.2	1	2.41	0.134		**6.48**	**0.018**	**At LT, M>E, *p* = 0.026** **At M, LT>HT, *p* = 0.006**
	Sipunculid sp.1	1	1.68	0.208		0.22	0.647	
	Foraminifera sp.1	1	**5.68**	**0.025**	**M&E, LT<M, HT, *p* < 0.05** **At HT, M>E, *p* < 0.003**	2.17	0.153	
	Harpacticoida sp.1	1	0.26	0.612		**5.10**	**0.033**	**M, HT>E, HT, *p* = 0.012**
	Ostracoda sp.1	1	**11.23**	**0.003**	**For M, LT>HT, *p* < 0.001**	4.06	0.055	
	TOTAL	1	0.85	0.366		**10.4**	**0.004**	**For M, LT>HT, *p* < 0.0001** **At LT, M>E, *p* < 0.0001** **LT, M>HT, E, *p* < 0.0001**
SP x T x TS	Nematode sp.1	2	0.60	0.557		**12.5**	**0.0002**	
	Nematode sp.2	2	0.05	0.948		3.23	0.057	
	Polychaeta sp.2	2	2.23	0.129		1.76	0.193	
	Sipunculid sp.1	2	0.62	0.545		**4.13**	**0.029**	
	Foraminifera sp.1	2	0.41	0.666		**19.7**	**< 0.0001**	
	Harpacticoida sp.1	2	1.08	0.356		**5.02**	**0.015**	
	Ostracoda sp.1	2	1.80	0.187		1.79	0.188	
	TOTAL	2	0.45	0.646		**17.8**	**< 0.0001**	

Note: Pairwise testing outcomes are not indicated for the SP x T x TS interactions due to the complexity of this interaction. General patterns are discussed in-text to simplify the table.

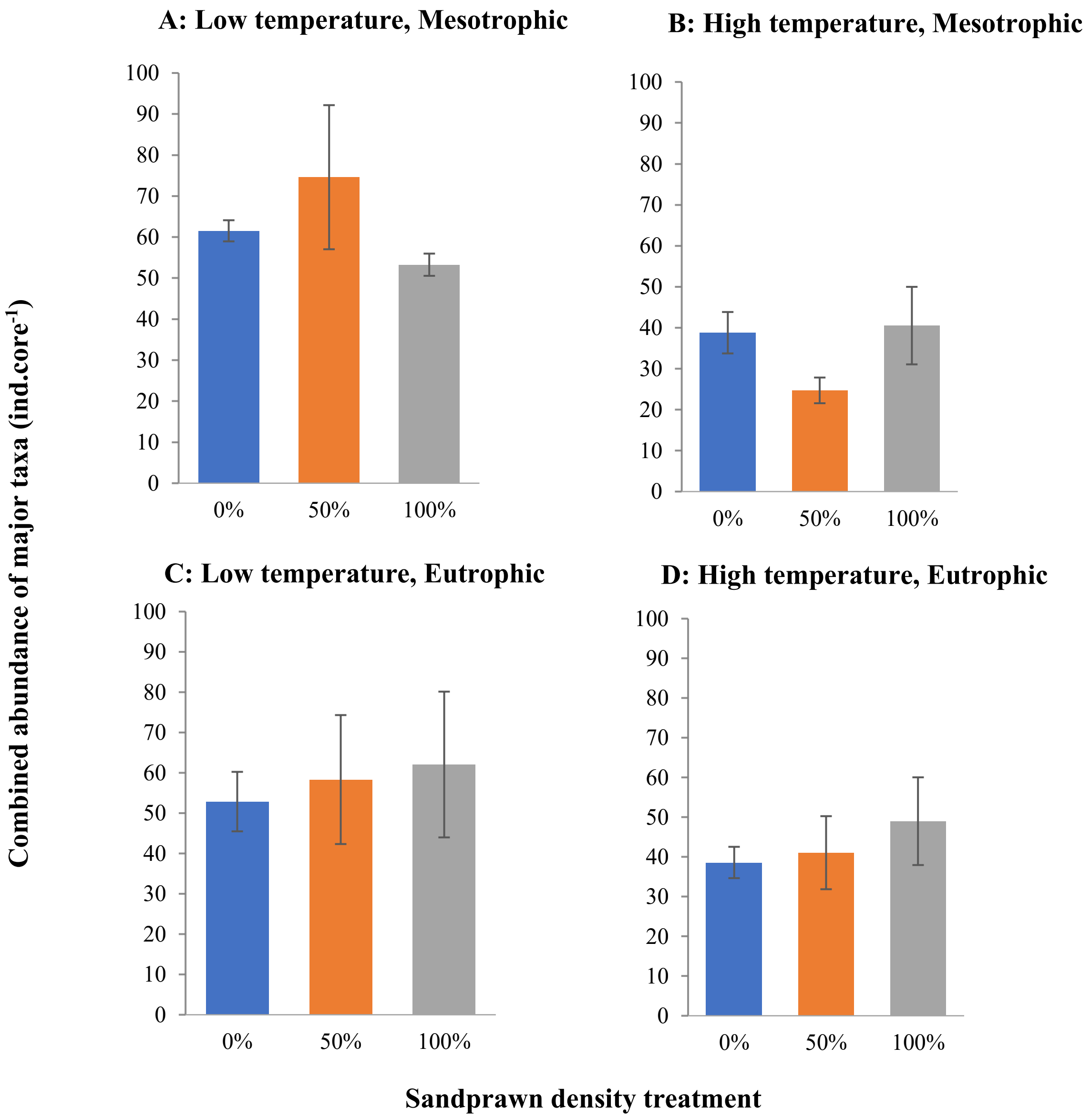


Figure 6.7: Spatial variability in mean (± SE) combined abundance of the dominant meiofaunal taxa in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%).

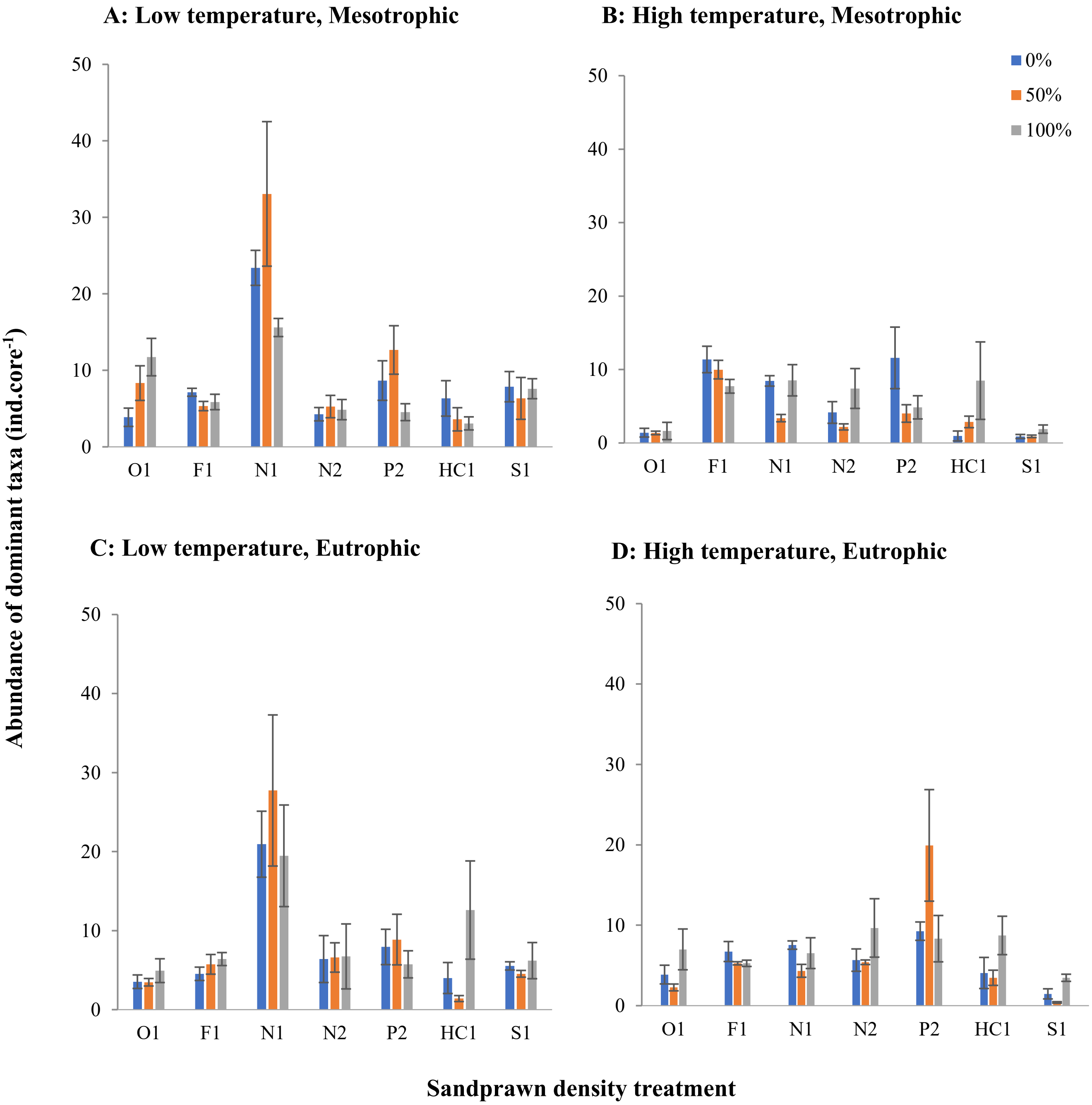


Figure 6.8: Spatial variability in mean (± SE) abundance of the dominant meiofaunal taxa in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%). O1= Ostracoda sp.1, F1=Foraminifera sp.1, N1= Nematode sp.1, N2= Nematode sp.2, P2= Polychaeta sp.2, HC1= Harpacticoida sp.1, S1= Sipunculid sp.1.

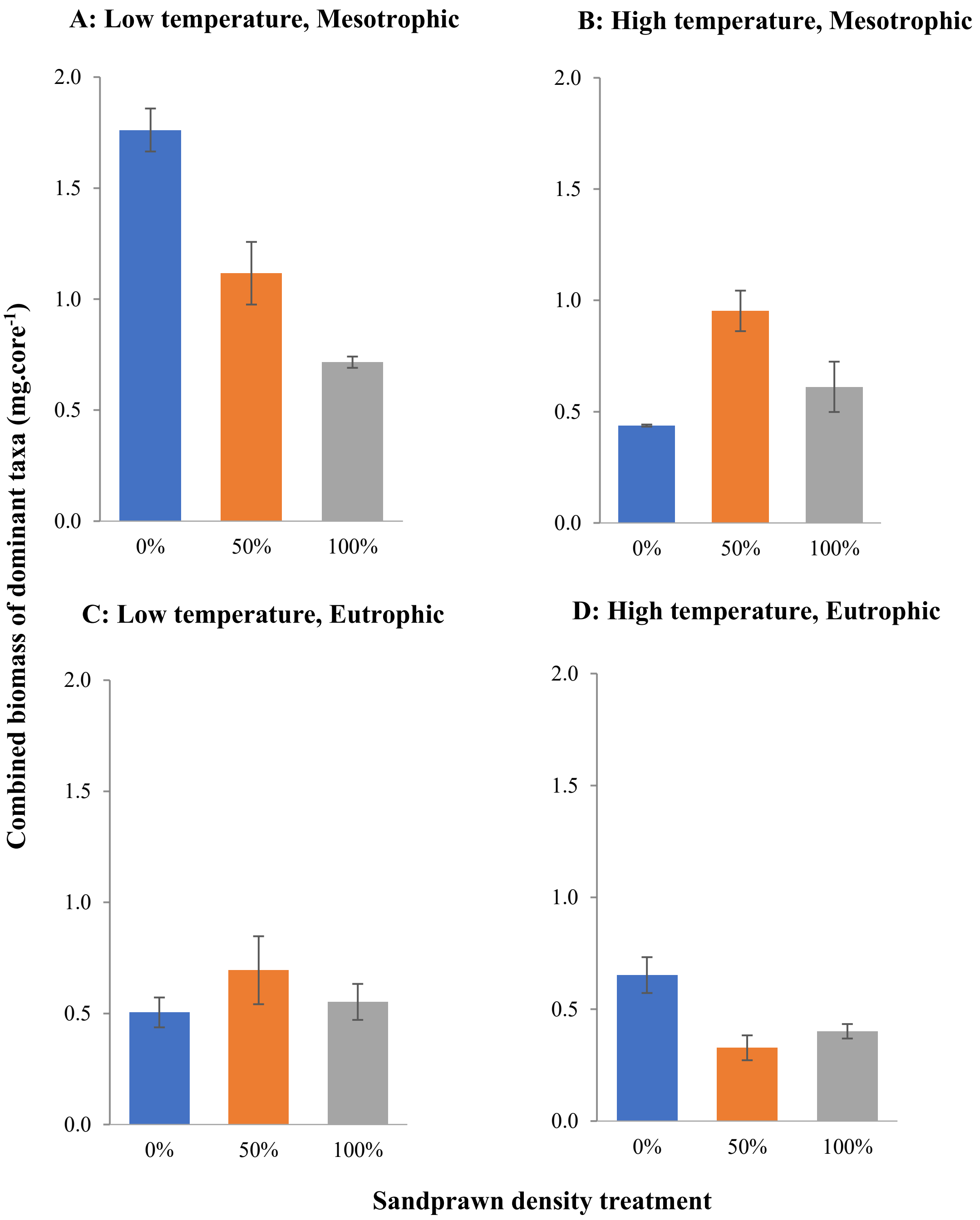


Figure 6.9: Spatial variability in e mean (± SE) biomass of the major meiofaunal taxa in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%).

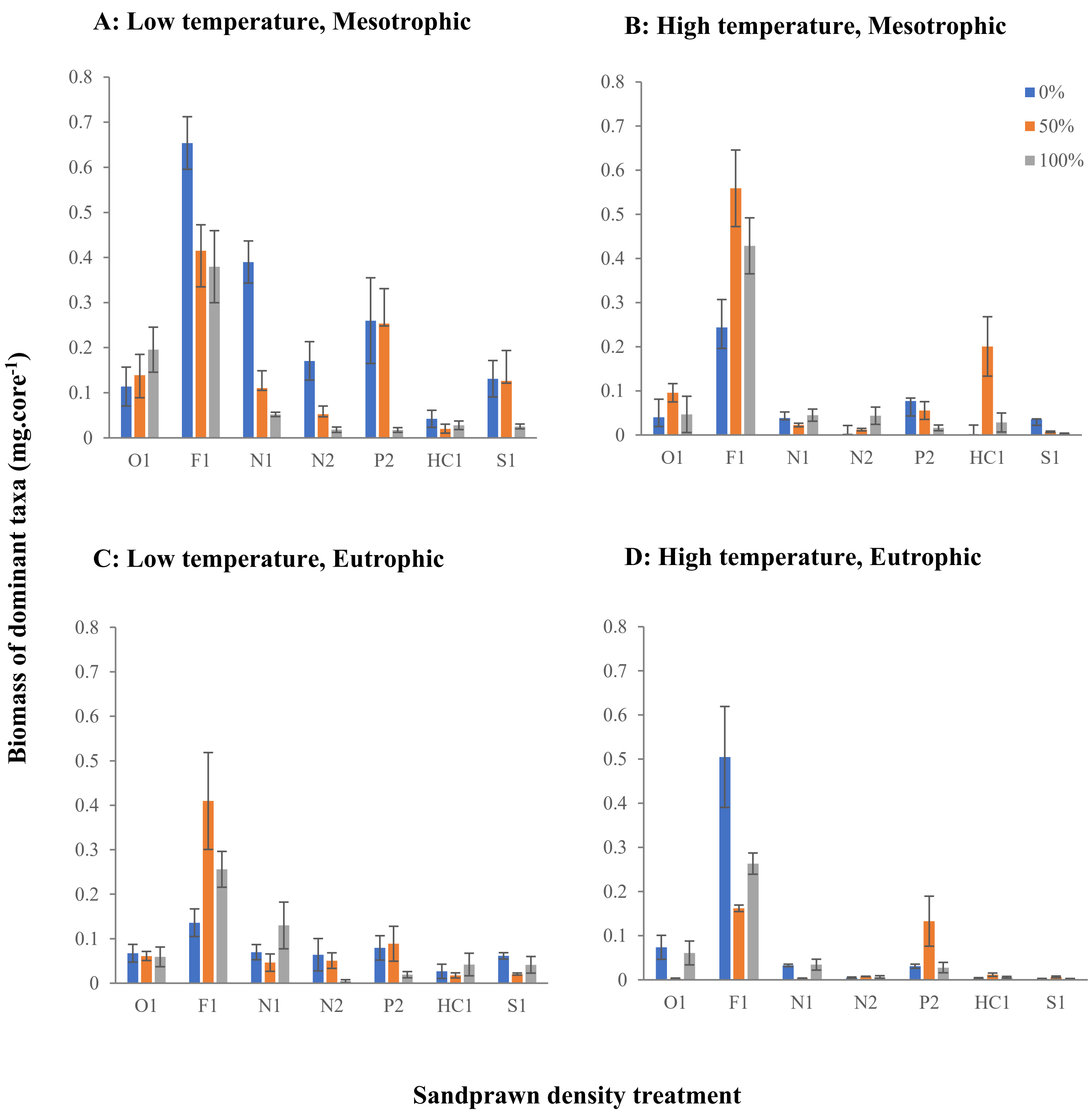


Figure 6.10: Spatial variability in mean (± SE) biomass of the major meiofaunal taxa in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%). O1= Ostracoda sp.1, F1=Foraminifera sp.1, N1= Nematode sp.1, N2= Nematode sp.2, P2= Polychaeta sp.2, HC1= Harpacticoida sp.1, S1= Sipunculid sp.1.

Cumulative abundance-biomass plots of meiofaunal communities based on dominant taxa (Fig. 6.11) revealed that, in most cases, the 100% sandprawn density treatment had greater cumulative biomass versus abundance relative to other sandprawns density treatments. This was supported by the *W*-statistics, which generally increased with increasing sandprawn density (Fig. 6.11). The exception to this trend was the high temperature, eutrophic treatment, where the W-statistic did not increase uniformly with increasing sandprawn density relative to the other treatments, with biomass generally being greater than abundance.

A: Low temperature, Mesotrophic

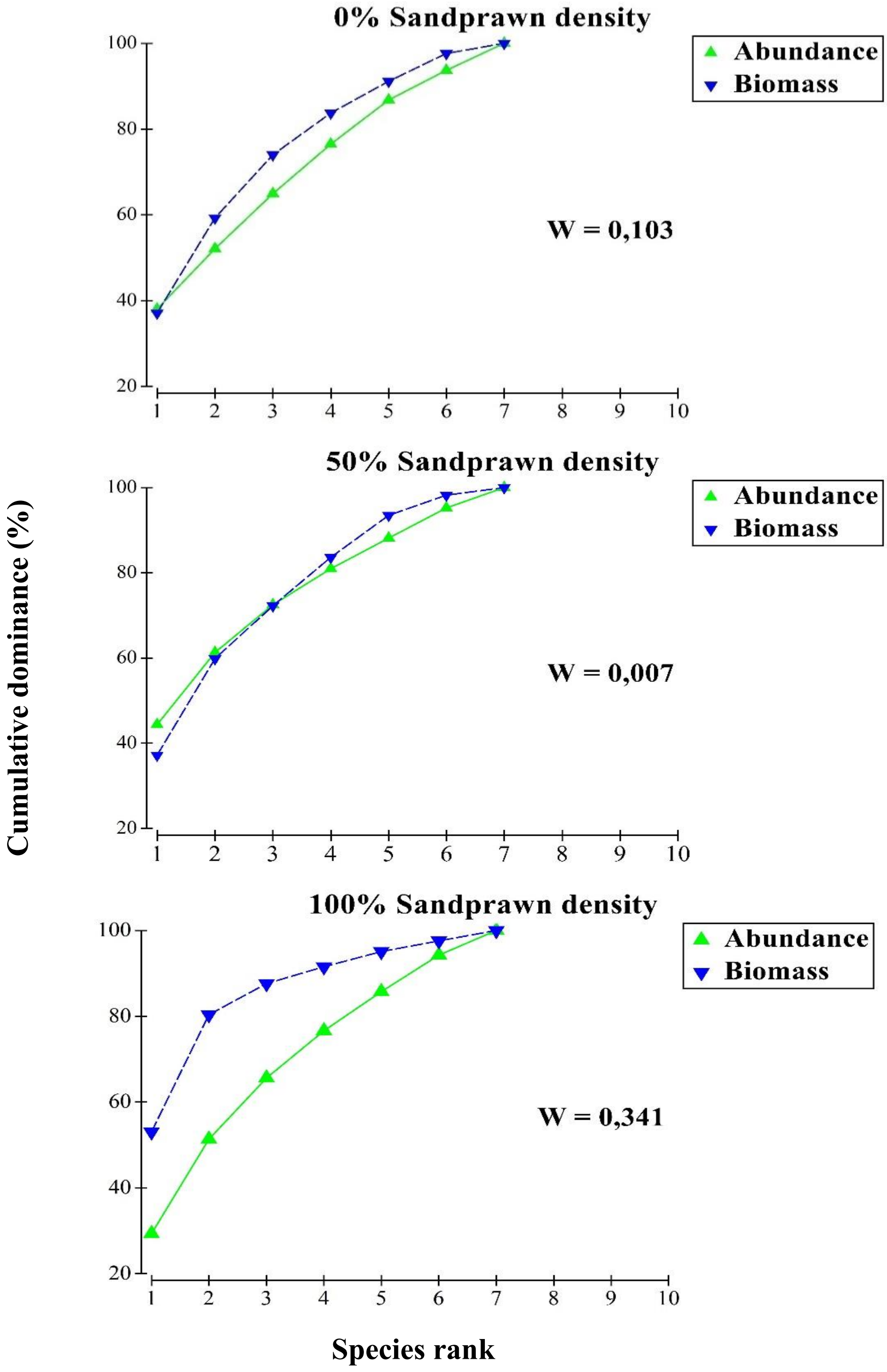


Figure 6.11: Abundance-biomass cumulative dominance plots showing ranked species abundance (green symbols) and biomass (blue symbols) for the dominant meiofaunal taxa in A (low temperature, mesotrophic), B (high temperature, mesotrophic), C (low temperature, eutrophic) and D (high temperature, eutrophic) mesocosms at varying sandprawn densities (0%, 50% and 100%).

B: High temperature, Mesotrophic

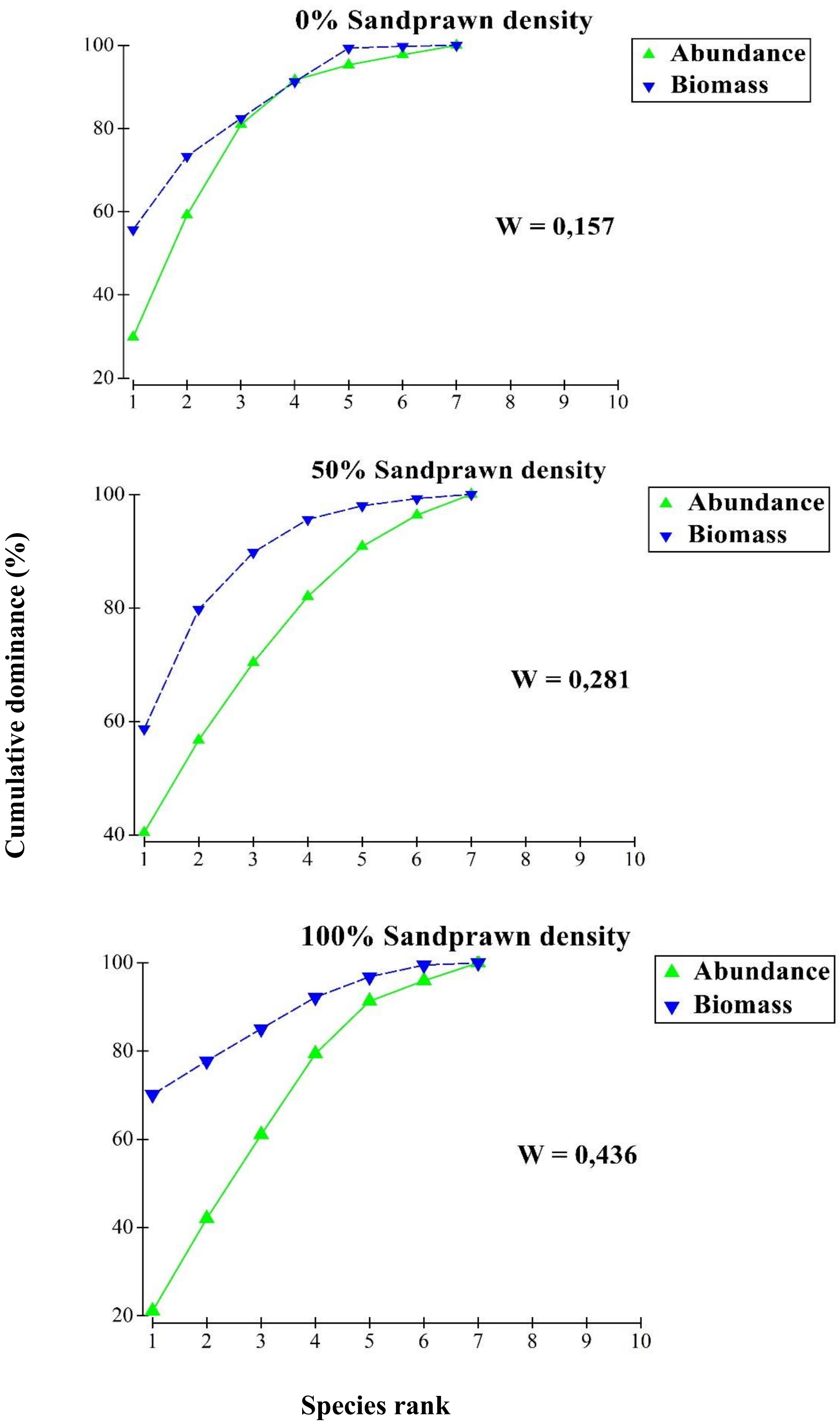


Figure 6.11 continued.

C: Low temperature, Eutrophic

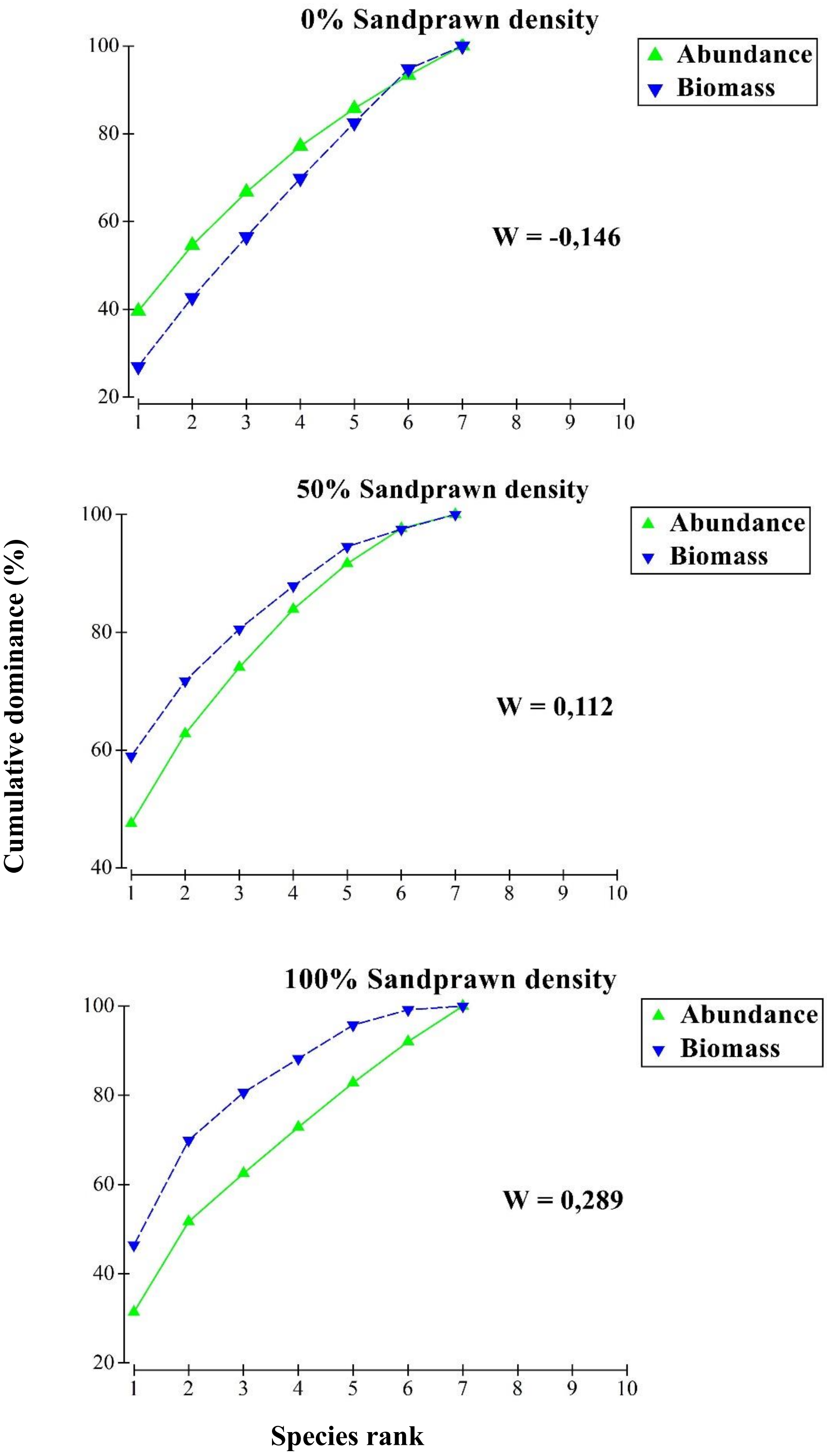


Figure 6.11 continued.

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D: High temperature, Eutrophic

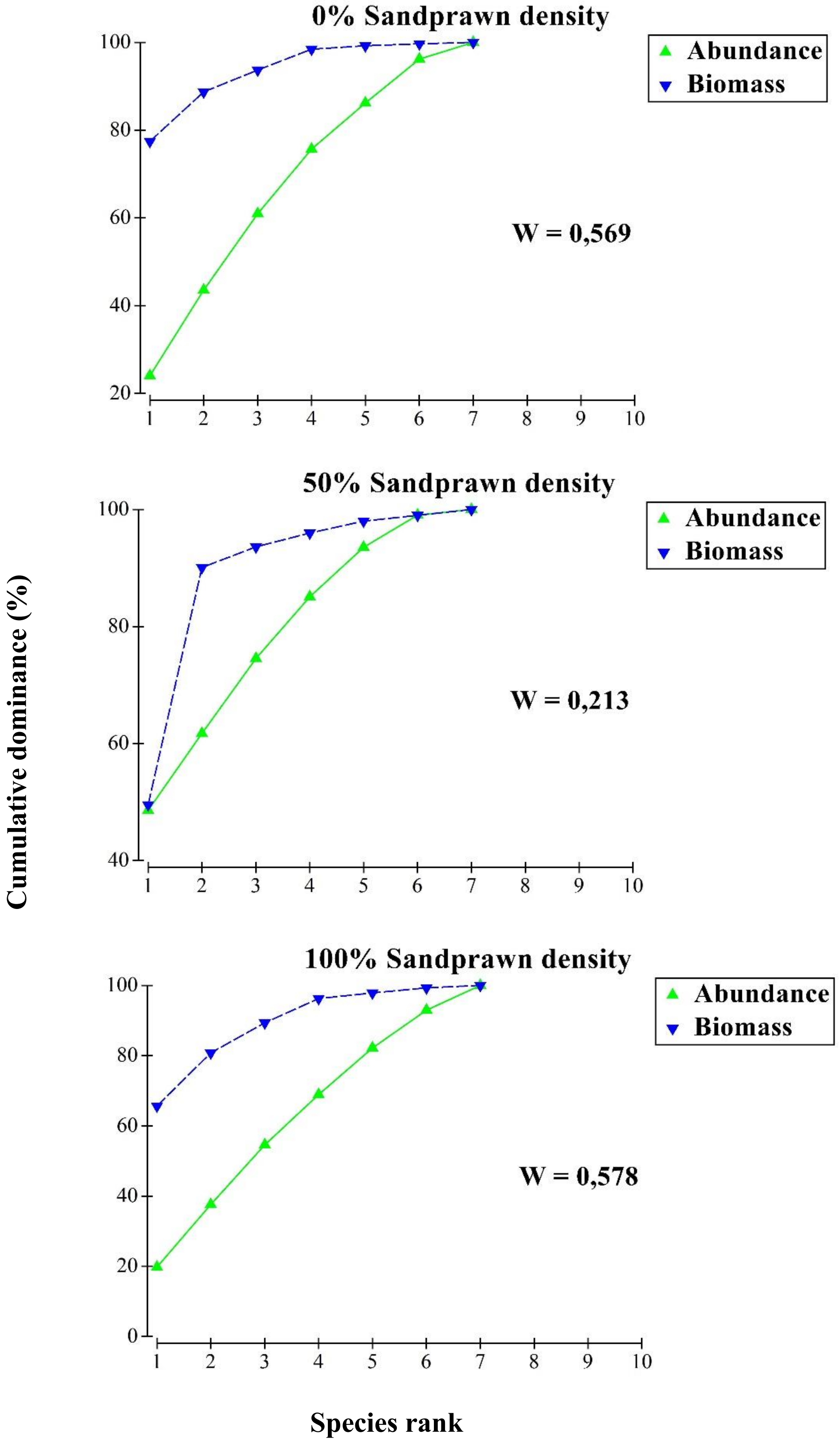

Figure 6.11 continued.

6.6 Discussion

The distribution and coexistence of species in an environment relies on the interaction between abiotic (including physical, chemical, climatic, and consumable abiotic resources) and biotic factors (such as trophic resource availability, competition, predation and mutualism) that defines the conditions, or potential niche space available to species, and determine tolerances to prevailing and changing environmental conditions (Colwell & Rangel, 2009). With reference to meiofauna, this interaction influences variation in community structure, which in turn may affect the ecosystem functions and processes that they influence (Giere, 2009, Pillay, 2019; Álvarez-Castillo, et al., 2023). Given the context of global change (IPCC, 2007) and the role that meiofauna play in ecosystem functioning, the objective of this chapter was to deepen understanding of the changes in meiofauna assemblages induced by warming, eutrophication and varying sandprawn density.

Overall, nematodes dominated the entire meiofaunal assemblage in terms of abundance, collectively accounting for 35.3% of the assemblage, followed by foraminiferans (21.1%), polychaetes (15.5%), harpacticoid copepods (8.5%), ostracods (8.0%) and sipunculids (7.8%). Gastropods, mites and platyhelminths were also present but poorly represented (<1%). With regards to the seven dominant taxa, contrasting results were obtained for biomass and abundance contributions. For example, even though Nematode sp.1 was the most abundant of the dominant taxa (30.1%), it contributed only 11.2% to the total biomass, ranking third in terms of biomass. In contrast, Foraminifera sp.1 had the highest gravimetric contribution at 50.5%, followed by Polychaeta sp. 2 (12.1%). Ostracoda sp.1 contributed 11.0%, Sipunculid sp.1 5.3%, Nematode sp.2 5.0% and Harpacticoid sp.1 4.9% to the total biomass of the dominant taxa. Consistent with the findings in my study, nematode abundance in meiofaunal assemblages have been widely reported in literature for estuarine ecosystems in South Africa

(Orren, et al., 1981; Nozais, et al., 2005; Bownes & Perissinotto, 2012) and across the globe (Ólafsson & Elmgren, 1997; Tita, et al., 2002; Rodríguez, et al., 2003; Gingold, et al., 2013; Álvarez-Castillo, et al., 2023; Mohammad, et al., 2024) as well as in mangroves (Zeppilli, et al., 2018) and marine habitats in general (Dobbs & Guckert, 1988; Giere, 2009; Zeppilli, et al., 2013). Contrasting contributions and patterns of meiofaunal abundance and biomass have also been reported in several habitats in response to changing abiotic contexts, with trophic conditions (related to the quantity and quality of sediment organic matter) generally explaining patterns (Dobbs & Guckert, 1988; Zeppilli, et al., 2013; Zeppilli, et al., 2018). However, other factors, such as the physico-chemical properties of the sediment and biological interactions, may also play a role, with the relative significance of trophic and abiotic processes being contextually dependent on the unique interplay among them over time and space (Zeppilli, et al., 2018; Álvarez-Castillo, et al., 2023).

PERMANOVA analysis showed that temperature and the interaction between temperature and trophic state were the main predictors of the meiofaunal community (Table 6.4). Sandprawn density was statistically inconsequential in predicting shifts in meiofaunal community structure either individually or interactively. DISTLM analysis further showed that variability in nitrite and nitrate (by way of its correlation with nitrite, Table 6.1) and picoplankton, best sequentially explained variance in the meiofaunal community statistically, with minor contributions by salinity, sediment boundary roughness (SBR), nanoplankton, total phytoplankton (by way of its correlation with nanophytoplankton), ammonium and phosphate. Interestingly, the proportion of meiofaunal community variance explained by these variables was 41%, implying that 59% of variance was accounted for by variables that were not measured. Such variables may include biological interactions including within-community predation and competition, and reproductive behaviour (Fleeger & Decho, 1987; Steyaert, et al., 2003; Giere, 2009).

My prediction that meiofaunal abundance and biomass may be suppressed by increased sandprawn density was not supported in my study. However, in interaction with temperature and trophic state, sandprawn density did influence meiofaunal biomass and abundance. For the biomass response, the three-way interaction between sandprawn density, temperature and trophic state was statistically significant and induced complex changes to the total biomass of the dominant taxa and to certain dominant (Nematode sp.1, Foraminifera sp.1, Harpacticoida sp.1 and Sipunculid sp.1). However, the general pattern was that total biomass declined at high relative to low temperatures, with shifts from mesotrophic to eutrophic conditions inducing a similar response. Notably, under low temperature mesotrophic conditions, increasing sandprawn density led to a decline in total meiofaunal biomass as predicted, but this trend was dampened under high temperature and eutrophic conditions, and shifted to a unimodal pattern for the low temperature eutrophic treatments and high temperature mesotrophic treatments (Fig. 6.9). Biomass of Nematode sp.2 was significantly influenced by the interaction between sandprawn density and temperature where, at low temperatures, biomass decreased linearly with increasing sandprawn density, while a reversal in this trend was observed at high temperatures, where there was also evidence of a temperature-induced depression in the biomass of this taxon (Fig. 6.10). Regarding meiofaunal abundance, significant effects were only observed for Nematode sp.1, the most dominant taxa in my study, involving the interaction between sandprawn density and temperature. At low temperature, the abundance of Nematode sp.1 increased with medium sandprawn density relative to controls, followed by a decrease as sandprawn density increased to maximum levels. This trend was reversed at high temperatures (Fig. 6.10).

The differential responses of meiofaunal biomass and abundance to varying sandprawn densities, in interaction with temperature and trophic state in my experiment, emphasises the need to understand the environmental contexts that can potentially alter responses of meiofaunal assemblages to bioturbation (Pillay 2019). Interestingly, studies on the response of benthic communities to ecosystem engineering by *K. kraussi* across various locations in South Africa has produced varying outcomes, leading to the postulation that bioturbation may differentially generate net amensalistic, neutral or facilitative effects on benthic community structure in response to changing environmental contexts (Pillay 2019). Amensalism may be induced mainly when physical sediment disturbance overrides facilitation effects, leading to reductions in infaunal abundance (Brenchley, 1981; Austen & Widdicombe, 2006; Giere, 2009; Pillay, 2019). On the other hand, net facilitative effects may arise from habitat creation by endobenthic crustaceans, including secondary consequences such as enhanced sediment oxygenation, and rapid subduction and elevated availability of trophic resources (Giere, 2009; Pillay, 2019). In South Africa, Branch and Pringle (1987) showed that increased sandprawn density induced a decline in meiofaunal numbers in Langebaan Lagoon, situated along the western coast, likely in reponse to sediment disturbance. In contrast, a study by Henninger & Froneman (2013) in the Kasouga estuary situated in the south-eastern coast of South Africa, showed that sandprawns had a neutral effect on infaunal community structure. Widdicombe & Austen (1999) observed similarities between the macrofaunal and meiofaunal responses to different types and densities of bioturbators. In Durban Bay, which is situated on the sub-tropical east coast coast of South Africa, Pillay, et al. (2007a) found that while bioturbation by *K. kraussi* had an amensalistic effect on the abundance of surface-dwelling macrofauna, it had no effect on macrobenthic infauna. In my study, the meiofaunal responses to *K. kraussi* density varied with trophic and thermal conditions, likely as a consequence of shifts in the balance between physical disturbance of the sediment and facilitative effects such as enhanced oxygenation. Specific traits unique to meiofauna and macrofauna may explain some the above-mentioned differences in responses, but contextual factors within each organism group could have also been influential (Pillay 2019).

Building on the finding by Venter, et al., (2020), which showed that phytoplankton subduction into the sediment is linked to increasing sandprawn density (via increased burrow wall chl-*a* biomass), I alternatively predicted that higher sandprawn densities would strengthen bottom-up regulation, resulting in positive effects on meiofaunal abundance and biomass. In my study, phytoplankton biomass declined in the presence of sandprawns regardless of temperature and trophic state (see Chapter 3), and I anticipated that increased provision of trophic resources to meiofauna may lead to increases in their abundance. Moreover, I also expected that the combination of eutrophication and increasing sandprawn density would further amplify the supply of trophic resources into the sediment, mitigating the amensalistic effects of bioturbation on meiofauna (Pillay, 2019), leading to facilitative effects on meiofanal abundance. However, my results did not align with my expectations, as increased sandprawn density had a neutral effect on meiofaunal abundance and biomass, regardless of trophic state. The alternative expectation of a net facilitative effect of sandprawns on meiofaunal abundance or biomass additionally stemmed from subduction of phytoplankton into the sediment positively affecting the microbial community, thereby indirectly boosting meiofaunal biomass and abundance, given that meiofauna consume microbes (Austen, et al., 1998; Giere, 2009). However, in my study, sandprawn density had a neutral effect on rates of organic matter degradation (see Chapter 5), indicating that increases to sandprawn density may not have influenced microbial activity and abundance.

Findings from Chapter 4 of my study may shed some light on the lack of facilitative effect of sandprawns on meiofaunal abundance or biomass. In this chapter, I found that while sandprawns decreased the biomass of epibenthic diatoms and cyanobacteria under low temperatures, they did not affect the abundances of the pico- and nano-sized sub-surface microphytobenthic (MPB) classes, regardless of temperature and trophic condition. The implication therefore is that, while phytoplankton biomass was reduced in the presence of sandprawns, there was no evidence of increased MPB biomass within subsurface sediments, suggesting limited increase in MPB biomass to drive meiofaunal increases.

Contrary to my prediction, eutrophication alone did not significantly influence meiofaunal abundance or biomass. Instead its influence emerged primarily through interactions with temperature, and induced differential responses in the dominant taxa, likely based on their individual tolerance levels to the varying combinations of eutrophication with temperature. For instance, the abundance of Foraminifera sp.1 was suppressed by combinations of high temperature and eutrophication, but increased under mesotrophic, low temperature conditions (Fig 6.8, Table 6.7), while for Ostracoda sp.1, high temperatures in combination with mesotrophic conditions limited abundance. Notably, even though smaller polychaetes are known for being opportunistic in eutrophic sediments (Heip, 1995), the biomass of Polychaeta sp.2 declined under the joint influence of warming and eutrophication (vs low temperature mesotrophic conditions), suggesting that this taxon may be negatively impacted by simultaneous increases in temperature and eutrophication. Widboom & Elmgren (1988) found that meiofauna did not respond positively to nutrient inputs and, suggested that in addition to the availability of trophic resources, biotic interactions may also play a role in structuring meiofaunal communities. Mitwally & Fleeger (2013) also found that the response of meiofauna was inconsistent and variable to nutrient enrichment and suggested that environmental factors and biotic interactions may play a significant part in the bottom-up response of meiofauna. These findings highlight the unpredictable and complex nature of meiofaunal responses to eutrophication, both alone and in interaction with warming. While some taxa are favoured under these conditions, others are adversely affected, potentially leading to shifts in community dynamics and ecosystem functioning over time. This underscore the necessity of accounting for contextual dependencies when evaluating how biotic assemblages respond to interacting global change drivers (Pillay, 2019).

My findings supported my prediction that dominant taxa would respond differentially to manipulations of the predictor variables depending on the specific tolerance levels of taxa. Temperature influenced the abundance of most of the dominant taxa, however, its effects varied depending on interactions with sandprawn density and trophic state. For example, the abundance of Nematode sp.1 displayed a unimodal response to increasing sandprawn density at low temperatures which was reversed at high temperatures (Fig. 6.8); Foraminifera sp.1 was more abundant in mesotrophic conditions when temperature levels were elevated; while the abundance and biomass of Ostracoda sp.1 was generally suppressed with warming and eutrophication, indicating a vulnerability of this taxon to the combination of these stressors. In terms of biomass, variations in dominant taxon responses were influenced by interactions between temperature, sandprawn density and trophic state (for Nematode sp.1, Sipunclid sp.1, Foraminifera sp.1 and Harpacticoida sp.1), sandprawn density and temperature (for Nematode sp.2) and temperature and trophic state (for Polychaeta sp.2; Fig 6.10). Overall the total biomass of the dominant taxa declined under normal (low temperature, mesotrophic conditions) as sandprawn density increased (Fig. 6.9). However, the biomass responses of individual taxa often showed inconsistent patterns (Fig. 6.10) with multiple two- and three way interactions between varying levels of temperature, trophic state and sandprawn density, complicating interpretations and generalisable predictions.

Typically, diversity and richness of taxa are lower in stressed and polluted environments due to the exclusion of more sensitive species (Pusceddu, et al., 2007). It is on this basis that I predicted that diversity and richness of the meiofaunal assemblage may be lower with warming and eutrophication, which may be potential meiofaunal stressors. However, the lack of strong individual effects of variations in temperature and trophic state on richness and diversity of the total meiofaunal assemblage suggest that levels of the predictors used in my study were insufficient to depress these community metrics. Interestingly, the diversity and richness of the meiofaunal assemblage was significantly influenced by interactions between sandprawn density, trophic state and temperature. For species richness at low temperatures, increasing sandprawn density had an amensalistic effect under mesotrophic conditions (Fig. 6.5A) and a facilitative effect under eutrophic conditions (Fig. 6.5C). In contrast, at high temperatures, this trend appeared to neutralise for both trophic states when sandprawn density increased (Figs. 6.5 B & D). Trends in species diversity mirrored those for species richness (Fig. 6.6), with the exception of the low temperature, mesotrophic treatment (for which diversity change appeared neutral). While it is possible that extending the duration of my experiment for a longer time period could have revealed more distinct trends, Ambrose (1991) postulated that significant effects of macro-infaunal organisms on meiofauna are usually recorded in less than two weeks of the start of an experiment, which suggests that the 16-day duration of my study was likely sufficient to capture the net effects of bioturbation on meiofaunal community metrics. Nevertheless, my results suggests complex richness and diversity responses that were likely determined by the relative impact of physical disturbance to the sediment caused by bioturbation *versus* the benefits thereof (enhanced trophic resource supply; improved oxygenation; Austen & Widdicombe, 2006; Giere, 2009; Pillay, 2019) in conjunction with meiofaunal taxon-specific physiological tolerances to changes in trophic state and temperature. These results further emphasise the necessity of considering broader contextual dependencies when assessing the impact of bioturbation on benthic ecosystems (Pillay, 2019)

The ‘abundance biomass comparison’ (ABC) method is often used to detect the impact of natural, physical, biological and human-induced disturbances on assemblages of organisms based on *r*- and *k*-selection theory and provides a snapshot of the condition of the assemblage at any point in time or space (Warwick, 1986; Warwick, 2008). The underlying idea is that ranked species abundance and biomass are plotted on the same set of axes and their relative positions compared (Warwick, 2008). In undisturbed conditions, biomass curves are positioned above the abundance curve, indicating a dominance of larger species that are *K*-selected (Fig. 6.12 (a); Warwick, 2008). In contrast, disturbed assemblages are characterised by *r*-selected species with low biomass but high abundance, resulting in the abundance curve being above the biomass curve (Fig. 6.12 (c); Warwick, 2008). With moderate disturbance, the biomass and abundance curves may intersect, indicating co-existance between large competitive dominants and smaller opportunistic species (Fig. 6.12 (b); Warwick, 1986; Warwick, 2008). Furthermore, such visual comparisons can be aided quantitatively by the *W*-statistic, which is determined by subtracting the cumulative abundance values from the biomass values per ranked species (Warwick, 2008). For unperturbed assemblages, the *W*-statistic is strongly positive, perturbed assemblages have negative *W*-statistics, and moderately perturbed assemblages have *W*-statistics close to zero (Warwick, 2008).

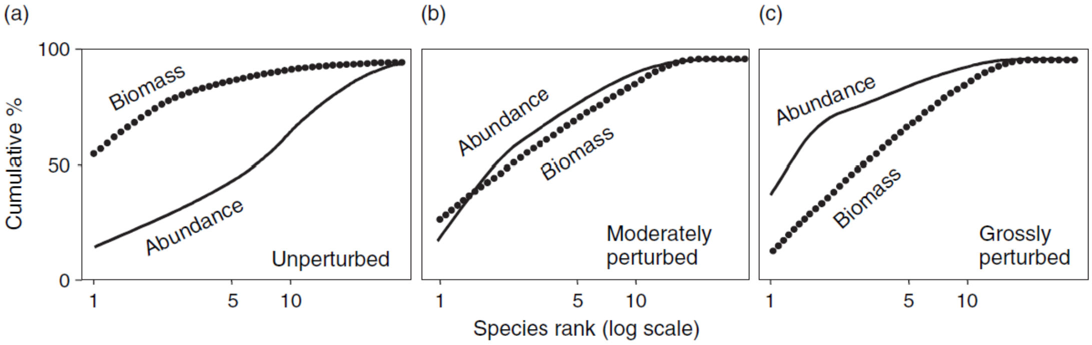

Figure 6.12: Hypothetical cumulative abundance and biomass curves (ABC) showing (a) unperturbed, (b) moderately perturbed, and (c) grossly perturbed conditions (Warwick, 2008).

I used the ABC method to compare how the meiofaunal assemblage in my study responded to manipulations in temperature, trophic condition and sandprawn density. The ABC plots showed that, overall, the biomass curve was more elevated relative to the abundance curve when sandprawn density was at its maximum, compared to the treatments where sandprawns were either at intermediate density or absent from mesocosms (Fig. 6.11). The only scenario showing a strongly disturbed meiofaunal assemblage occurred at the low temperature, eutrophic treatment in the absence of sandprawns (Warwick, 1986), suggesting that sandprawns may play a facilitative role for meiofaunal assemblages, by reducing stressful conditions. Notably, in the high temperature, eutrophic treatment, the W-statistic reached its highest value of 0.578 when sandprawns were at maximum density, suggesting even under warming and eutrophic stress, high sandprawn density contributes to a less perturbed meiofaunal assemblage. Such effects might arise due to sediment oxygenation by sandprawns, provision of trophic resources, (for example through phytoplankton subduction), and greater refuge provision (via burrow structures) from stressor effects. These results suggest that the presence of sandprawns at their maximum density may act as a buffer to create conditions that reduce stress, leading to ranked biomass contributing more than abundance. This effect seems to be prevalent under eutrophication and future warming conditions and therefore may contribute to enhancing ecosystem resilience to predicted stress. To my knowledge, this adds to the evidence of Venter, et al. (2020) and Thomas, et al. (2023), pointing to the value of sandprawns as a nature-based tool to counter global change associated with coastal eutrophication. This additionally highlights the functional significance of sandprawns as ecological facilitators in benthic ecosystems in the context of meiofaunal assemblages.

6.7 Concluding remarks

Variations in abiotic contexts have contributed to variance in meiofaunal responses to biological disturbance across studies, making generalisable scientific insights difficult (Ambrose, 1991; Austen, et al., 1998). In summary, my findings showed that predicting meiofaunal community-level and taxon-specific responses to variations in sandprawn density was complicated by variations and categorical interactions in temperature, trophic state and sandprawn density, thereby emphasising the necessity of accounting for contextual dependencies when evaluating ecological responses to global change processes. An additional limitation of this study was the relatively low level of taxonomic resolution used for meiofauna. This was due to the limited expertise in meiofaunal taxaonomy, but the lack of finer taxonomic resolution may have contributed to the complexity of findings and high data variance on occasion.

A consistent trend that did emerge from my study, however, was that high sandprawn densities facilitated meiofaunal stability and shifts to biomass-dominance, including under high temperatures and eutrophic conditions. This suggests a potential role for sandprawns as a key nature-based solution to mitigate the impacts of eutrophication under future warming scenarios. Given this finding, further research on the combined effects of multiple stressors and the bioturbation roles of dominant ecosystem engineers such as sandprawns is crucial, as such species have the potential to profoundly influence the sedimentary communities due to their engineering functions dampening stressful conditions. Future studies should therefore focus on developing understanding of the traits of benthic assemblages and bioturbators and their physiological tolerances to stressors. Insights from such research could then inform strategies for managing and conserving biodiversity and ecosystem functioning in habitats that are dominated by key endobenthic bioturbators such as sandprawns, in the context of increasing resilience to global change.

CHAPTER 7:

SYNTHESIS

Synthesis

The unprecedented increase in both human population and consumption in the Anthropocene has led to an escalation in the diversity and intensity of environmental stressors impacting species, ecological processes and ecosystem functioning (Sanderson, et al., 2002). Intensified levels of fossil-fuel combustion, agricultural activities, industrial processes and deforestation have increased emissions of greenhouse gases and accelerated warming globally (Chapin III, et al., 2000; Parmesan, 2006; Harley, et al., 2006), with average global temperatures expected to increase by 1.8 - 4°C by the year 2100 (IPCC, 2007). The worldwide rise in terrestrial nutrient runoff has resulted in eutrophication driving substantial ecological changes in estuaries and coastal habitats (Cloern, 2001). Aside from influencing the metabolic rates of species, both warming and eutrophication influence primary productivity, triggering potential cascading changes to water quality, trophic interactions and biodiversity (Heip, 1995; Cloern, 2001). Biodiversity, including both species and functional dimensions, influences how ecosystems function and maintain internal regulatory processes, as species or functional units contribute uniquely to intrinsic ecosystem functions, with biological interactions such as predation, competition and mutualism enhancing ecosystem functionality, efficiency and stability (Chapin III, et al., 2000). In some ecosystems, the activities of single species are key in determining the organisation, functionality and stability of ecosystems; these species are referred to as keystone species or keystone engineers (Paine, 1969). The axiid crustacean, *Kraussillichirus kraussi* (sandprawn), a dominant bioturbator in estuaries in southern Africa, has been identified as such a key engineer, principally due to its exceptional sediment turnover activites (sediment turnover = 12.14 $kg.m^{-2}.day^{-1}$, or 4.4 $ton.m^{-2}.day^{-1}$, Branch & Pringle, 1987) that structure sedimentary habitats, influence bentho-pelagic coupling, primary productivity, porewater characteristics and the spatial distribution of the benthic community (Branch & Pringle, 1987; Pillay & Branch, 2011).

Despite growing global awareness, the ecological and societal consequences of cumulative anthropogenic stressors for ecosystem functioning are poorly understood. Such knowledge limitations, coupled with ineffective human behavioural change, economic policies and political strategies to mitigate environmental degradation, are causes of concern (Chapin III, et al., 2000; Hall, et al., 2001). Globally, identifying processes that enhance ecosystem resilience to global change remains a critical yet complex priority for research that is essential for safeguarding ecosystem functions and services that sustain human well-being (Urrutia-Cordero, Ekvall, & Hansson, 2016). This need forms the foundation of resilience-based ecosystem management, emphasising the importance of identifying, managing, and conserving aspects of biological diversity that contribute to ecosystem resilience (Briske, et al., 2008; Suding & Hobbs, 2009; Sasaki, et al., 2015) in the face of key challenges such as coastal hardening (e.g. canalization), habitat destruction from coastal development, pollution and bait collecting (Venter, et al., 2020). In this context, the findings from my study provide novel evidence supporting the role of sandprawns as key ecosystem engineers with potential to enhance ecosystem resilience against global change stressors, including eutrophication and warming. Sandprawns were shown to contribute substantially to ecosystem functionality through bioturbation (sediment reworking), regulating phytoplankton proliferation through biofiltration and limiting nanophytoplankton dominance, which were novel findings. Additionally, the consistent trend of meiofaunal biomass-over-abundance dominance at high sandprawn density, even under eutrophic and future high temperature scenarios, highlights their capacity to ameliorate stress for meiofaunal assemblages, and points to the resilience-conferring potential of sandprawns in estuarine habitats. However, findings also caution that eutrophication in sandprawn-dominated habitats can suppress organic matter degradation rates, potentially undermining ecosystem services and functionality. Importantly, the general robustness of sandprawns to stressors such as hypoxia, hypercapnia and high sulphide levels

(Pillay & Branch, 2011), emphasise their functional potential in mitigating future coastal degradation caused by eutrophication in future warming scenarios. However, it goes without saying that findings need to be interpreted within the limitations of *in situ* experimental approaches, as identified in Chapter 2. Despite study limitations, however, results emphasise the importance of managing sandprawn populations to preserve critical ecosystem functions and increase coastal resilience. Beyond their use as bait organisms, greater societal awareness of the ecological significance of sandprawns could also elevate their relevance to society, as even trampling of sandprawn habitats during recreational activity or bait collecting, damages sandprawn habitats and their burrows, and need to be evaluated for their impacts on sandprawn populations and associated functions (Pillay & Branch, 2011; Branch, et al., 2022).

The mesocosm approach that underpinned this thesis provided the only platform to simultaneously manipulate sandprawn density, eutrophication and warming levels predicted to the year 2100 – an undertaking that would not have been feasible or practical using a field comparative or experimental approach (Benton, et al., 2007). Furthermore, this controlled mesocosm setup facilitated the isolation of predictor variables, albeit within a simplified representation of an estuarine ecosystem, which is inherently complex and variable (Day, 1981; Branch, et al., 2016). The mesocosm approach was also well-suited for studying processes in a microtidal, temporarily closed system like the Zandvlei Estuary, where shallow depths and restricted flow, especially under closed conditions, significantly influence ecosystem processes (Froneman, 2004; Perissinotto, 2010).

While the mesocosm approach proved invaluable for simulating, isolating and examining interactions among predictors under controlled conditions, these experiments provide subsets of environmental conditions. For instance, mesocosm experiments exclude key processes such immigration/emigration and the full range of biological interactions and environmental variability found naturally, while introducing experimental stress that may influence organism responses (Benton, et al., 2007; Widdicombe, et al., 2010). The size constraints of mesocosms also limited support of a diverse range of organisms, which may be constrained by “wall effects”due to containment (Schindler, 1998; Stewart, et al., 2013).Thus the confined nature of mescosms may have amplified the effect of sandprawn processes that were measured such as water filtration (Chapter 3), the SBR response (Chapter 5) and MPB (Chapter 4).

The fully factorial design of my mesocosm experiment also necessitated a compromise on replication (n=3) due to logistical constraints, such as aquarium space and resources. Nonetheless, the design of the experiment, which allowed for comprehensive testing of main and interaction effects, was appropriate for the research questions posed and yielded biologically meaningful and statistically robust results. Notably though, in the Meiofauna Chapter (Chapter 6), the three way factorial design with three levels of sandprawn density (0%, 50% and 100%) , two levels of trophic state (mesotrophic and eutrophic) and two levels of temperature (high and low), resulted in complicated reporting of interaction , with indication of high variances in the data. These limitations, as stressed previously, necessitate caution in interpreting and generalising resultsand highlight the need for validating findings through further testing under an expanded set of field and modelling studies. However, given that manipulative experiments using mesocosms and microcosms have historically contributed to the advancement of ecological theories and models, the use of mesocosms in my study is a valuable and relevant method that can advance understandings of global change and sandprawn-mediated processes and their impact on estuarine ecosystem functioning (Benton, et al., 2007).

Looking ahead, further research should expand on the foundation built in this PhD by quantifying long-term resilience of sandprawns, especially to extremes of warming, eutrophication and multi-stressor conditions. To improve understanding of the thermal responses of sandprawns, I recommend that future studies aim to utilise high resolution temperature data, including extreme heat events, to understand thermal stress responses and concomitant effects on functions such as water filtration. Similarly, tolerance to eutrophication-induced hypoxia needs to be evaluated on sandprawn physiology and functions provided. Complementary approaches, such as *in situ* experimentation in sandprawn-dominated estuaries, or comparisons across gradients at broader spatial scales, offer a valuable means of capturing the effects of episodic and long- term nutrient pulses and temperature extremes within a natural environment on sandprawn-mediated functions and services. In addition, the use of intact sediment cores needs to also be explored in future studies, to more closely approximate field sediment conditions in the mesocosm studies, so that more natural sedimentary and biological structures can be used in experiments. To support interpretations of depth-related rates of OMD and benthic community responses more comprehensively, it is recommended that future research include measurements of sediment grain size, particulate organic matter (POM) levels, porewater nutrient and benthic oxygen levels.

Again, it is worth emphasising that despite limitations, this research has provided valuable insights on the functional significance of sandprawn in a global change contact, offering new perspectives on water filtration, MPB dynamics and meiofaunal community change, amongst others. The significance of findings is that globally, leveraging the filtration capabilities of endobenthic crustaceans like sandprawns offers a promising nature-based solution to enhance the resilience of coastal ecosystems against eutrophication. To harness the resilience-enhancing functions of sandprawns, proactive measures are essential to conserve their populations and habitats including strong public education campaigns. Strategies to reduce nutrient inputs into estuarine systems remain a cornerstone of addressing coastal eutrophication but should be complemented by sustainable management of bioturbator populations. Protecting, and where necessary, restoring these key species habitats, can result in their ecological contributions being harnessed to confer a degree of resilience to ecosystem functioning in the face of global change.

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SUPPLEMENTARY MATERIAL

Supplementary Table S1: Results of SIMPER analysis identifying taxa that contributed most (70% cumulatively) to differentiating bacterial assemblages between mesotrophic and eutrophic waters at the end of the 16-day mesocosm experiment. Bacterial taxa and 16S gene abundances were quantified from single 1L water samples from control mesocosms (low temperature, no sandprawns). Samples were sequenced using a Minion Mk1C (Oxford Nanopore Technologies) and analysed using EMU v3[1] using the SILVA v1.38.1 database. SIMPER was carried out in PRIMER v7 (Plymouth Routines in Multivariate Ecological Research). Bacterial orders are presented in parentheses next to genus names. The data originated from the MSc research of Oluwasegun Ogunnusi and is used with permission.

Taxon	**Mesotrophic**	**Eutrophic**				
	Ave Abundance	**Ave Abundance**	**Ave Dissimilarity**	**Diss/ SD**	**Contribution (%)**	**Cumulative (%)**
Citrobacter braakii (Enterobacterales)	454.67	24179.67	13.19	0.70	16.04	16.04
Enterobacter cloacae (Enterobacterales)	10120.67	17012	10.21	0.95	12.41	28.44
Raoultella planticola (Enterobacterales)	10318	1917.67	4.83	0.77	5.87	34.31
Citrobacter werkmanii (Enterobacterales)	86	6475	2.42	0.73	2.94	37.25
Pseudomonas x. (Pseudomonadales)	4982.33	132.33	2.36	0.66	2.87	40.12
*Delftia*_ x. (Burkholderiales)	45.67	5525.67	2.06	0.67	2.50	42.62
*Gracilibacteria*_ x. (Candidatus)	736.67	5118	1.96	0.84	2.39	45.01
Klebsiella pneumoniae (Enterobacterales)	790	3172.33	1.41	1.31	1.71	46.72
*Klebsiella*_ x (Enterobacterales)	2595.67	128.67	1.34	0.67	1.62	48.34
*Stenotrophomonas*_ x (Xanthomonadales)	99.33	3050.67	1.24	1.23	1.51	49.85
*Aestuariicoccus*_ x (Rhodobacterales)	1700.33	2480.67	1.22	1.09	1.48	51.34
Kluyvera georgiana (Enterobacterales)	2610	235.33	1.19	0.84	1.45	52.79
Escherichia-Shigella coli (Enterobacterales)	833.33	2465.33	1.17	0.90	1.42	54.21
*Holosporaceae*_ x (Holosporales)	19.33	3105	1.16	0.68	1.40	55.61
Gammaproteobacteria x (Enterobacterales)	192.67	3066	1.13	0.72	1.38	56.99
Serratia marcescens (Enterobacterales)	615.67	2803	1.13	1.48	1.37	58.36
Citrobacter pasteurii (Enterobacterales)	2176.67	397.33	1.06	0.84	1.28	59.65
Edwardsiella ictaluri (Enterobacterales)	1330.33	1745	1.05	0.95	1.28	60.93
Stenotrophomonas maltophilia (Xanthomonadales)	286.33	2096.33	1.05	0.86	1.28	62.20
*Serratia*_ x (Enterobacterales)	2297.33	36.33	1.03	0.67	1.25	63.45
*Enterobacter*_ x (Enterobacterales)	1931.67	133.67	0.90	0.68	1.09	64.55
Enterobacter kobei (Enterobacterales)	107.67	1703.67	0.86	0.69	1.05	65.59
*Mitochondria*_ x (Rickettsiales)	1788	118.33	0.80	0.70	0.97	66.56
NS3aNS3a x (Flavobacteriales)	66.33	2055	0.79	0.94	0.96	67.52
*Marivita*_ x (Rhodobacterales)	224	1386.33	0.71	0.76	0.87	68.38
Marivita lacus (Rhodobacterales)	352.67	1463.33	0.71	0.85	0.86	69.24
*Citrobacter*_ x (Enterobacterales)	1385	1516.67	0.66	1.12	0.81	70.05
Mean bacterial abundance	48146.34	93519.33				
Mean Enterobacterales abundance	37845.35	66987.67				

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