Impacts of burrowing sandprawns (*Kraussillichirus kraussi)* on water quality, phytoplankton and pelagic bacterial assemblages

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I understand the meaning of plagiarism is to use another's work and pretend it is one's own, and that it is wrong. I declare that this dissertation was personally produced by myself, and each contribution to this dissertation from the works of others has been cited and referenced.

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ABSTRACT

Coastal environments are among the most threatened ecosystems globally, with water quality degradation constituting a major scientific and management issue that requires addressing. Burrowing sandprawns (*Kraussillichirus kraussi*) have been shown in past research to improve water quality by removing microalgae from the water column. Their burrows are thought to act as biofiltration systems, with water-borne phytoplankton particles being adsorbed onto burrow walls during bi-directional water pumping. However, not much is known about ecological repercussions of this hypothesised mechanism and whether it indiscriminately impacts all microorganisms in the water column. This issue forms the foundation of my research, which aimed to experimentally determine whether potential filtration effects of sandprawns are consistent across pelagic bacterial and phytoplankton assemblages or whether there are any discriminatory responses. Findings demonstrated that increasing sandprawn density did not significantly reduce the abundance of bacterial water quality indicators (*Escherichia coli* and total heterotrophic bacteria). However, sandprawns were found to reduce the abundance of phytoplankton cells. At the end of the experiment, the relative abundance of phytoplankton in the controls were 1.9 times higher relative to the 100% treatment. Similarly, the concentrations of nitrite were 17.7 times higher in controls relative to 100% treatment at the end of the experiment. Furthermore, increasing sandprawn abundance induced a phytoplankton size-based shift from pico- to nano dominance, with nanophytoplankton contributing 17.76% at the beginning of the experiment, but shifting to 58.07% at the end of the experiment in the maximum sandprawn density treatment. Additionally, sandprawn presence had no significant impact on cryptophytes or *Prochlorococcus*-like algal abundance. These results demonstrate that sandprawns disproportionately impact certain groups and influence phytoplankton assemblages beyond biomass decline. These findings are novel as such discriminatory effects on pelagic assemblages have previously not been attributed to endobenthic deposit-feeding ecosystem engineers. This study therefore provides novel insights into mechanisms by which these organisms may alter coastal ecosystems and influence bentho-pelagic coupling processes. This is especially significant when viewed in the context of global change, where understanding the factors that influence phytoplankton dynamics are important for predicting ecosystem functioning under projected climatic conditions. Given the overall top-down impact of sandprawns on phytoplankton, this study supports the idea of sandprawns being effective nature-based tools that can mitigate the global challenge of eutrophication in coastal ecosystems. The results of this study ultimately emphasises the need for protection and conservation of sandprawns (and functionally similar endobenthic engineers) and their habitats from threats such as habitat loss.

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1. INTRODUCTION

Coastal habitats such as mangroves, rocky shores and estuaries play a major role in the functioning of marine ecosystems. Whilst this interface between the land and ocean comprises a small fraction of Earth's total surface area, it provides a wide range of ecosystem services that are vital to human livelihoods (Ward et al., 2020). These services can generally be grouped into four categories, namely provisioning, regulating, cultural and supporting services (Malone and Newton, 2020; United Nations Environment Programme [UNEP], 2006a). Provisioning services include the provision of food and raw materials. Regulating services encompass processes such as climate regulation and coastal flooding prevention, and cultural services include spiritual and recreational benefits provided to humans. Lastly, supporting services (e.g. primary production and nutrient cycling) often involve processes that underpin the capacity of coastal ecosystems to provide all ecosystem services (Malone and Newton, 2020).

While coastlines and their resources and services directly support a large proportion of the human population (Griffin et al., 2003), they are among the fastest degrading ecosystems globally (UNEP, 2006b). These ecosystems are particularly vulnerable as much of the environmental pressures that are experienced do not originate from within coastal zones, but from the catchments that transport water and materials into the coast (Salomons et al., 2014). Anthropogenic processes are the primary drivers of the degradation of these ecosystems and their services, and include overfishing, land use change and pollution (UNEP, 2006b). Humans remain largely water dependent and generate large amounts of waste (e.g. agriculture, chemicals, sewage etc.). These waste products are discharged into coastal waters, thereby rendering them unsafe for human use in some cases, whilst simultaneously impacting ecological processes in recipient ecosystems (Amoatey and Bani, 2011). Wastewater is typically discharged into bodies of water such as estuaries and bays, ideally once treated and rendered environmentally acceptable under local legislation (UNEP, 2016) and is often disposed of directly or indirectly into coastal systems (Griffin et al., 2003). Domestic and industrial effluents are also major sources of pollutants, and in some cases, partially treated wastewater is discharged directly into water bodies (UNEP, 2007). Unfortunately, many areas around the world have inadequate treatment of sewage prior to their release into coastal waters either directly into water bodies or via estuaries. Exposure to this waste is potentially dangerous as it can contain a variety of viral and bacterial pathogens that typically reflect the health of the community from which they derive (World Health Organisation [WHO], 2010). Pollution of fresh water typically impacts coastal waters primarily because of the connectedness of these systems, resulting in most of the pollution entering fresh water systems ending up in coastal waters (Maipa et al., 2001; United Nations [UN]-Water, 2018). The consequences for receiving water bodies is substantial, often resulting in modified and impaired functioning in aquatic ecosystems (Amoatey and Bani, 2011).

Waterborne pathogens from faecal pollution are of particular concern in coastal ecosystems, as they have been strongly associated with infectious diseases globally (WHO, 2010, 2012). Faecal pollution is detected using faecal markers i.e. organisms that indicate the presence of faecal contamination (Ashbolt et al., 2001). Common indicators include faecal coliforms - the bacteria that constitute the typical microbiota found in the gut and faeces of humans and other warm blooded animals. *Escherechia coli* (family Enterobacteriaceae) are gram negative bacteria that are the major species found in the faecal coliform group (New York State Department of Health, 2017). These bacteria exhibit considerable halo-tolerance, surviving salt concentrations up to 60 ‰ (Ogochukwu et al. 2017). Because of their ubiquity, faecal coliforms are often responsible for healthcare issues within communities (European Centre for Disease and Prevention Control, 2016). Although most forms are harmless, some strains (e.g. *E.coli* O157:H7) are pathogenic and produce toxins that can be transmitted to humans, making assessments of loading of these bacteria in coastal waters essential (Aragonés et al., 2016; Leonard et al., 2018; Widmer et al., 2013). The danger of exposure to contaminated coastal water primarily lies in contracting diseases from other pathogens in the water, not necessarily the faecal bacteria itself. Pathogenic bacterial presence is therefore inferred by means of testing for indicator organisms such as coliform bacteria. Faecal coliforms present a reasonable indication of pathogenic bacterial presence as these organisms come from the same source (New York State Department of Health, 2017). For example, the infectious agents responsible for Acute Febrile Respiratory Illness (AFRI) are numerous viral agents including enteroviruses and adenoviruses, which are of human faecal origin (Fleisher et al., 1998). Common indicators of recreational water quality include faecal coliforms such as *E. coli*, pseudomonas and enterococci (Brenniman et al., 1981; Maipa et al., 2001). Although heterotrophic bacteria have no direct association with pathogens or human disease, they are used as indicators for the general microbiological quality of water i.e. the assessment of the microbiological conditions of water relative to animal and human health requirements. (Bartram, 2003, Pachepsky et al. 2018; Van Nevel et al., 2017).

The interaction between the environment and human health should not be underestimated. Exposure to environmental pollution are responsible for a large proportion of diseases globally. According to the World Health Organization (WHO), 23% of all deaths in 2012 were attributed to environmental exposure to air and water pollution that often result in lower respiratory tract infections or diarrhoeal diseases (WHO, 2016). This supports the notion that healthy ecosystems are a strong platform for improved public, community and individual health (WHO, 2016). Based on risk assessments from the WHO and academic research, Shuval (2003) estimated that more than 120 million cases of gastrointestinal disease and 50 million cases of respiratory diseases are caused by recreational use of wastewater-contaminated coastal waters worldwide.

Despite global efforts to improve sanitation frameworks in developing countries (e.g. United Nations and UNICEF, 2013), faecal coliform bacteria have continued to increase over the last two decades (UNEP, 2016). Between 1990 and 2010, faecal coliform bacteria concentrations have increased in almost two thirds of all rivers in Africa. This is largely due to the expansion of sewer systems that discharge untreated wastewater into surface waters (UNEP, 2016). Higher levels of faecal coliforms pose severe threats to human and ecosystem health and therefore constitute a key issue that needs addressing. Furthermore, the greatest increases in exposure to pollutants are predicted to occur in low and lower-middle income countries primarily due to higher populations, economic growth and lack of adequate wastewater treatment systems (UN-Water, 2018). Working towards finding creative solutions to ameliorate the extent of water pollution in coastal ecosystems is therefore critical to achieving sustainable development, healthy ecosystems and community well-being.

Another major water quality issue faced by coastal ecosystems is eutrophication, which is one of the greatest threats to the health of estuarine and marine ecosystems globally (Malone & Newton, 2020). Eutrophication occurs when a water body receives a surplus of nutrients, especially nitrogen and phosphorous, which stimulates the excessive growth of phytoplankton and macrophytes (Cloern, 2001; UNEP, 2006a). In South Africa, satellite remote sensing revealed that between 2002 and 2012, the overwhelming majority (78%) of the country's largest water bodies (such as reservoirs, endorheic pans and coastal lakes) were either eutrophic (20-30 mg.m^3 chlorophyll-a (chl-a)) or hypertrophic (>30mg.m^3 chl-a) (Matthews & Bernard, 2015). Although this process of nutrient enrichment occurs naturally and slowly over time, anthropogenic activities such as fertilizer applications, discharge of human waste and burning fossil fuels have accelerated the movement of nitrogen and phosphorous into coastal ecosystems (Cloern, 2001). The effects of eutrophication are often damaging and can pose severe threats to ecosystem health and value. For example, as organic compounds accumulate and primary production increases, there is an increased respiratory demand for oxygen (Rabalais et al., 2009). This can result in hypoxic or anoxic conditions which have damaging effects on other organisms (e.g. fish stocks) within the ecosystem. It can also result in the loss of biodiversity and algal blooms, some of which may produce toxic substances (UNEP, 2001, 2006a). This ultimately threatens the recreational and ecological value of ecosystems by disrupting the services which they provide, by for example, decreasing fish yields caused by decreasing fish yields caused by oxygen depletion or restrictions on fishing/angling/recreational activity due to algal accumulation (UNEP, 2001).

Although phytoplankton is often detrimental in eutrophic systems due to overgrowth, their role in marine ecosystems is fundamental. Phytoplankton form the base of food webs and are vital components of aquatic ecosystems. Constituting less than 1% of the planet's photosynthetic biomass, they account for more than 45% of the earths yearly net primary production (Field et al., 1998; Katz et al., 2004). These microscopic organisms essentially drive a range of biogeochemical processes in the marine environment including oxygen generation, removal of atmospheric carbon dioxide and nutrient cycling (Benoiston et al., 2017). Therefore given their importance to ocean biology and climate, changes in phytoplankton productivity could significantly influence biodiversity, fisheries and human food supply (NASA, 2010).

With volumes ranging from 0.1 μm^3 in the case of the smallest cyanobacteria to roughly $500\mu m^3$ for the largest diatoms, phytoplankton communities are typically diverse and composed of a continuum of different sized species (Marañón, 2015; Morrissey and Sumich, 2009). A given assemblage could contain several hundred species within one litre, therefore identifying the characteristics of every species is highly impractical. Cell sizes therefore represent a useful approach to describe the structure and function of phytoplankton communities and to understand their roles within ecosystems and biogeochemical cycles (Marañón, 2009). The relative abundance of various size classes of phytoplankton can influence climate processes and the biogeochemical cycling of important elements. For example, it is thought that most of the biomass produced by phytoplankton assemblages dominated by smaller cells is recycled within the euphotic zone, therefore limiting potential contributions to net drawdown of atmospheric CO_2 (Acevedo-Trejos et al., 2015; Falkowski et al., 1998; Marañón, 2015). In contrast, assemblages dominated by larger phytoplankton cells typically result in a greater fraction of production eventually being exported from the euphotic zone. This results in the efficient transportation of carbon to the ocean interior thereby driving the marine carbon pump (Marañón, 2015; Ward et al., 2012).

The role of phytoplankton size composition in determining trophic interactions within food webs is substantial. For example, communities dominated by large cells are typically associated with shorter, direct trophic pathways that support large fish populations (Ward et al., 2012). Likewise, it is well established that size composition of communities significantly influences the feeding ecology of the dominant zooplankton in southern African estuaries (Froneman, 2006). Copepod assemblages for example are generally dominated by omnivores when the dominant phytoplankton are too small to be directly consumed. Conversely, assemblages are dominated by herbivores when phytoplankton are larger (Froneman, 2006; Jang et al., 2010).

The intricate interplay between dispersal, environmental conditions (e.g. nutrient availability and temperature) and interspecific relationships such as predation and competition contributes towards the diverse size structures of phytoplankton assemblages globally (Acevedo-Trejos et al., 2015). These interactions have given rise to biogeographical patterns, where low nutrient regions (e.g. subtropical gyres) are dominated by smaller size classes such as *Synechococcus, Prochlorococcus* and picoeukaryotes. Contrastingly, more productive regions support a greater proportion of larger species such as coccolithophores, diatoms and dinoflagellates (Marañón, 2015; Ward et al., 2012).

Interspecific interactions are known to both directly and indirectly influence changes in phytoplankton size structure. One way in which this is achieved is through size selectivity by grazers when consuming phytoplankton. A study investigating the grazing impact of two copepod species (*Acartia clausi* and *Pseudodiaptomus hessei*) showed that phytoplankton size selectivity was a mechanism used to overcome interspecific competition for food resources (Pagano et al., 2003). Copepods generally feed on a wide range of phytoplankton sizes and are capable of selective feeding based on cell size (Froneman, 2006). Phytoplankton community composition is directly influenced by copepods through grazing selectivity, and indirectly through feeding on specific microzooplanton (e.g. ciliates) that, in turn, feed on smaller phytoplankton size classes (pico and nano sizes) (Fileman et al., 2007; Froneman, 2006).

Under certain conditions, the abundance of specific phytoplankton taxa can reach levels that pose potential threats to humans and marine organisms (Berdalet et al., 2015). These harmful algal blooms (HABs) have adverse impacts on human wellbeing, primarily through their impacts on coastal ecosystems (e.g. recreation, fisheries) and marine ecosystems as a whole

(Berdalet et al., 2015, Lemley et al. 2020). The common HAB known as red tides is often caused by microalgae such as cryptophytes or dinoflagellates (Anderson et al., 2002; Du Yoo et al., 2017). The 'red tide' dinoflagellate *Karenia brevis*, for example, produces potent marine neurotoxins known as brevetoxins (Flewelling et al., 2005).This toxin has been responsible for a large proportion of fish mortalities and pose threats to humans when toxic aerosols are inhaled or filter-feeding shellfish (that ingest toxins) are consumed (Flewelling et al., 2005). Similarly, Zhang et al. (2015) found a significant association between cyanobacterial blooms and non-alcoholic liver disease in the United States. Cyanobacterial blooms are recognized as an important concern in coastal areas worldwide. These blooms can severely degrade water quality by producing toxins such as anatoxin, nodularin and microcystins (Zhang et al., 2015). The threats posed by these toxins are often of great concern. For example, the neurotoxin β-N-methylamino- L- alanine (BMAA) produced by many genera of cyanobacteria, including *Prochlorococcus,* has been associated with human degenerative diseases (Śliwińska-Wilczewska et al., 2017; Cox et al., 2005). It has been suggested that climate change coupled with eutrophication will promote the proliferation and expansion of harmful cyanobacterial blooms (O'Neil et al., 2011). These studies highlight the importance of developing a multidisciplinary approach that will enhance our understanding of HABs and ultimately mitigate the impacts on human wellbeing and public health (Berdalet et al., 2015). Top-down regulation of phytoplankton could potentially influence the risk of harmful algal bloom proliferation within a given ecosystem with direct consequences for human health. The importance of grazing on phytoplankton community and size structure and its far reaching ecological and biogeochemical consequences, for example, is well established (Charalampous et al., 2021; Grinienė et al., 2016; Jagadeesan et al., 2017).

Despite the variety of species that can interact with phytoplankton, emphasis has been disproportionately directed towards the impact of zooplankton and suspension feeders on phytoplankton and their size structures, thereby largely ignoring other sources of top-down regulation. For example, it has recently been reported that the endobenthic crustacean *Kraussillichirus kraussi* (formerly *Callichirus* & *Callianassa)* significantly impacts microalgal biomass in the water column, despite it being a deposit-feeder (Venter et al., 2020). *K. kraussi*, also known as the common sandprawn, is a burrowing Thalassinidean crustacean typically present in sandy systems such as estuaries in South Africa (Branch et al., 2016). Although two separate infraorders, the Axiddea and Gebiidea, have been proposed for Thalassinideans, this dissertation will make reference to the Thalassinidea as a group for convenience, while acknowledging that the group is paraphyletic (Pillay and Branch, 2011). *K. kraussi* has an extensive distribution within Southern Africa, ranging from Lüderitz Bay in Namibia to the Inhambane region in Mozambique (Branch et al., 2016). These organisms construct deep burrows (>1m) and sift through sandy sediment for food, after which they eject mainly sediment particles from the burrow entrance, thereby creating visible volcano-like mounds on the surface of the sediment (Branch et al., 2016). This 'bioturbation' i.e. the reworking of sediment, is known to profoundly impact ecosystems (Branch et al., 2016), thereby rendering these organisms as effective ecosystem engineers i.e. organisms that modify, maintain or create habitats by directly or indirectly influencing the resources available to other species by causing physical state changes in biotic or abiotic constituents (Jones et al., 1994). *K. kraussi* are considered allogenic engineers as they influence the environment by transforming living or non-living materials from one physical state to another via mechanical means i.e. via their bioturbation. These prawns are often faced with anoxic or hypoxic marine sediment (Pillay & Branch, 2011), which must be opposed through the active irrigation of burrow water (Kristensen and Kostka, 2005). This is accomplished by the beating of their pleopods, which flushes out anoxic waste water and draws in oxygenated water (Pillay and Branch, 2011). This replacement of burrow water essentially results in vital processes such as gaseous exchange, food transport, removal of metabolites and gamete transport (Kristensen and Kostka, 2005).

Burrows influence microbial and geochemical processes primarily by increasing the area of the benthos that meets the overlying water column. It is thought that by actively pumping water through their burrows, the adsorption of organic matter on to mucous- lined burrow walls are enhanced, thereby influencing microbial growth in these areas (Pillay and Branch, 2011). Irrigation also allows for the movement of resources such as microalgae, sediment particles and nutrients between the burrows and overlying water column (Branch and Pringle, 1987; D'Andrea and Dewitt, 2009). Feeding in Thalassinidean is intimately linked with their burrows (Pillay & Branch, 2011). Deposit-feeders such as *K. kraussi* consume the organic material in the sediment or on the burrow walls (Coelho et al., 2000). A variety of food types are consumed, including bacteria, microalgae, and seagrass fragments (Pillay and Branch 2011). These sandprawns are also commonly labelled 'gardeners', since they are known to accumulate algae on their burrow walls thereby increasing their own food supply (Branch and Pringle, 1987; Pillay and Branch, 2011).

Burrowing thalassinideans are among the most significant bioturbators due to the volume of sediment reworked as well as the depth to which they can burrow in both coastal and subtidal areas (Cadée, 2001; Siebert and Branch, 2005). The turnover of sediment by *K. kraussi* is considerable, with populations depositing more than 4.5cm of sediment on the surface per week per square meter (Branch and Pringle, 1987). Their activity is known to greatly influence ecological processes as well as community structure (Pillay and Branch, 2011). Studies have shown that at higher densities of *K. kraussi*, sediment turnover is increased, thereby resulting in the burial of benthic microalgae (Branch and Pringle, 1987; Pillay et al., 2012). This reduction in microalgal biomass by the reworking of sediment was found to reduce the growth *of Liza richardsonii*, a microalgae- consuming fish (Pillay et al., 2012). Similar results were obtained by Pillay et al., (2007), where sandprawn presence was found to reduce food availability for the grazing gastropod *Nqssarius raussianus*. The study included the suspension feeding bivalve *Eumarcia paupercula*, whose food uptake and body condition were found to decline in the presence of sandprawns. This could be partially attributed to the resuspension of sediment particles (through bioturbation) clogging the filtration apparatus of these bivalves (Pillay et al., 2007).

The effects of bioturbation by *K. kraussi* have been well studied in soft sedimentary habitats (e.g. Branch & Pringle, 1987; Pillay et al., 2007; Pillay et al., 2012; Siebert & Branch, 2005). However, the impact of *K. kraussi* (and endobenthic engineers generally) on the overlying water column remains understudied. Coastal and estuarine ecosystems are hotspots for environmental variability, biogeochemical transformations and biological interactions where benthic infauna provide an essential link between the benthos and overlying water (Griffiths et al 2017). Benthic-pelagic coupling refers to the connection between the sediment and overlying water columns through exchanges of nutrients, energy and biomass (Griffiths et al., 2017; Raffaelli et al., 2003). Activities such as bioturbation will therefore have consequences for the overlying water and sediment alike, as exemplified by recent work demonstrating that increasing sandprawn presence may reduce phytoplankton biomass in urban estuarine environments (Venter et al., 2020); specifically, by up to 50% (Figure 1). Sandprawn presence did not, however, significantly impact suspended solid concentrations or increase ammonia concentrations to toxic levels (Venter et al., 2020). The authors attributed the decline in phytoplankton biomass in response to increasing sandprawn densities to the adsorption of phytoplankton cells onto the burrow walls. This mechanism was supported quantitatively by burrow walls having microalgal biomass greater than surface sediments. This suggests that sandprawn burrows function as biofiltration systems - as water is pumped in, materials are adsorbed onto the burrow walls, after which water is flushed out (Figure 2). The findings of Venter et al., (2020) highlight the bioremediation potential of these sandprawns in mitigating eutrophication to a degree, which is a global environmental problem that is predicted to worsen with climate change (Rabalais et al., 2009). The Venter et al. (2020) study formed the foundation for further questions on the functional significance of sandprawns to mitigate eutrophication and declining water quality. This study also opens further lines of questioning into the variety of ways in which sandprawns alter phytoplankton and pelagic assembles more broadly, and influence bentho-pelagic coupling processes.

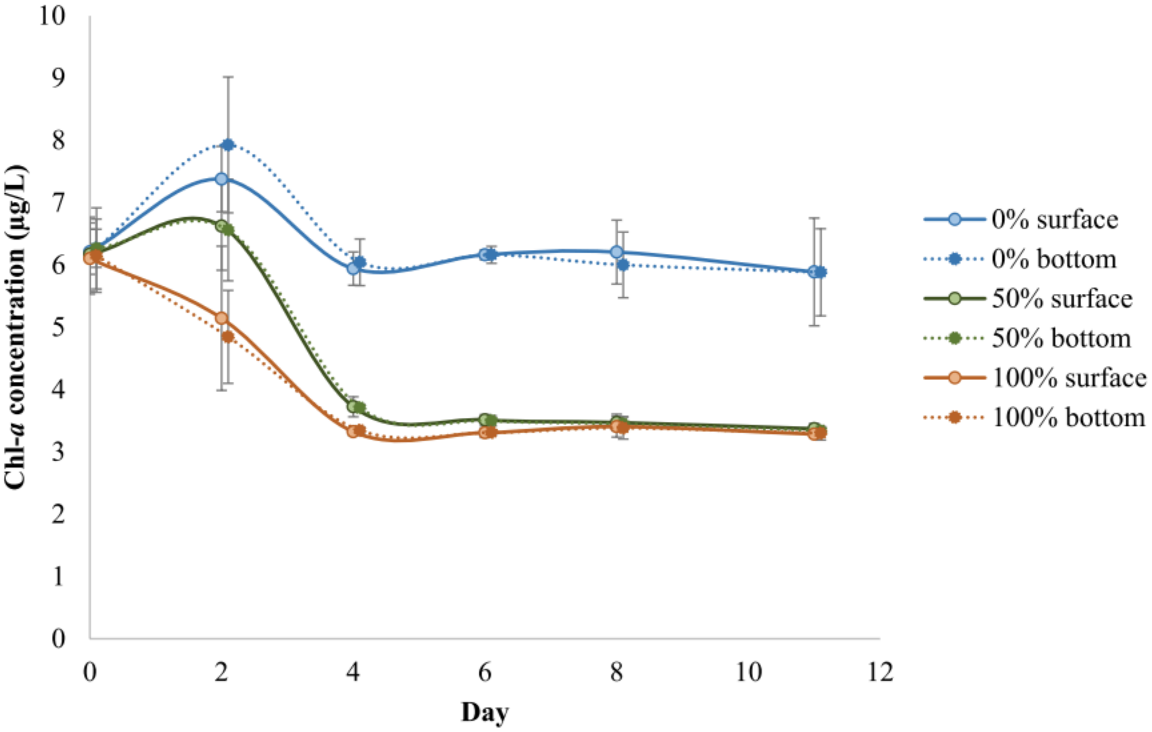

Figure 1: Temporal variability in pelagic chl-a concentrations (means ± SE) among sandprawn density treatments (0% = control; 50% = 6 sandprawns/mesocosm; 100% = 11 sandprawns/mesocosm). Surface and bottom water (20cm) data from the 2-week mesocosm experiment are shown. Taken from Venter et al. (2020).

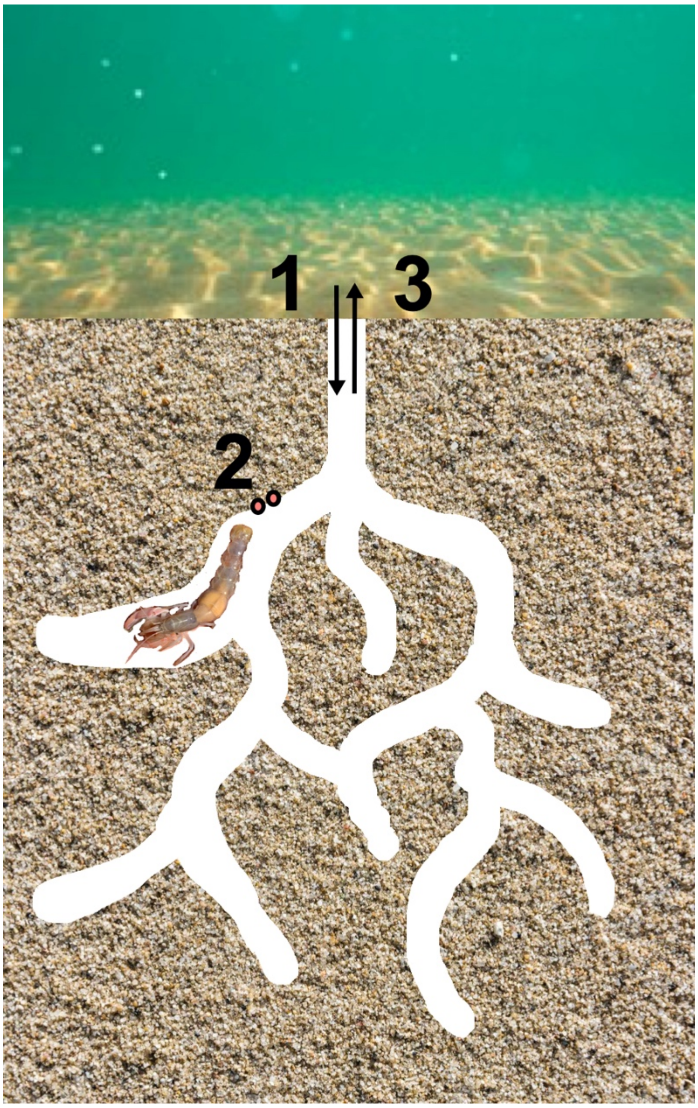

Figure 2: Biofiltration mechanism hypothesized to explain effects of sandprawn (*Kraussillichirus kraussi*) burrow systems on phytoplankton as suggested by Venter et al., (2020). 1: water is drawn into burrow, 2: suspended matter is adsorbed onto burrow wall, 3: water is flushed out of burrow.

Given the increasing importance of improving the condition of our ecosystems, there has been a move towards exploring alternative means of achieving environmental goals (UN-Water, 2018). Inspired by nature, Nature Based Solutions (NBS) essentially use or mimic natural processes with the aim of contributing towards the improved management of water (UN-Water, 2018). Although in its infancy, the potential use of NBS in improving water quality has been recognized. Recent management strategies have utilized the naturally occurring filtration services provided by organisms such as bivalves in addressing water quality degradation (Burge et al., 2016; Coen et al., 2007). Bivalves often exist in dense beds providing necessary structure and habitat for a variety of species and can improve water quality by directly filtering pathogens and suspended matter from the water column (Coen et al., 2007). Nevertheless, not much is known about the effectiveness and general application of biofiltration on pathogen concentrations (Coen et al., 2007). Most of the literature is focused on the use of bivalves as biofilters but the potential of other taxa in influencing the pelagic environment and improving water quality is not well known. Specifically, the impact of endobenthic fauna on ecosystem processes has largely been confined to the benthos with little to no consideration of effects on the overlying water column. By improving our understanding of how endobenthic invertebrates such as *K. kraussi* are impacting their ecosystems, a way is paved forward for potentially harnessing the ecological functions and services they provide in ways that could help manage these ecosystems and prevent further degradation.

The present study aims to improve our understanding of how sandprawns impact the pelagic environment, by investigating their effect on two major components of the water column: bacteria and phytoplankton. Specifically, the aims of the project will be met using a laboratory mesocosm experiment. In terms of phytoplankton, the impact of *K. kraussi* on the abundance of total phytoplankton, nanoplankton, picoplankton, cryptophytes and *Prochlorococcus*-like algae will be investigated. The impact of *K. kraussi* on water quality in terms of faecal pollution will be investigated by exploring responses of contemporary water quality indicators viz. heterotrophic bacteria and *Escherichia coli*. The following alternative hypotheses are tested in this study are to meet the aims of the study:

1. Given the suggested framework of sandprawn burrows acting as biofiltration systems (adsorption of material on burrow walls, Venter et al., (2020)), it is hypothesized that increasing sandprawn density will result in the decline in phytoplankton (including total phytoplankton, nanoplankton, picoplankton, cryptophytes and *Prochlorococcus—like* algae) and bacterial (total heterotrophic bacteria and *E. coli*) abundance.

2. Alternatively, due to the inherent biological trait differences (e.g. size/reproductive rate) between phytoplankton, nanoplankton, picoplankton, cryptophytes, *Prochlorococcus*-like algae, cryptophytes, total heterotrophic bacteria and *E. coli*, certain groups would be differentially affected thereby resulting in differential responses.

2. MATERIALS AND METHODS

2.1 Sample collection Site

Zandvlei Estuary (4°05'S; 18°28'E), from which all material were collected for the mesocosm experiment, is located on the North-Western shore of False Bay in Muizenberg, Cape Town (Figure 3). It is a temporarily open/closed estuary, with a mean water depth of 1.4m (Harding, 1994), it is a relatively shallow system that receives freshwater from the network of rivers that pass through the surrounding urban areas. The system is impacted by periodic sewage spills, which results in the closure of certain areas due to levels of faecal bacteria deemed unsafe for recreational activity (e.g. Ntseku, 2021; Hejana, 2021). This is of particular concern to the public given that following mouth opening, contaminated water is flushed out into one of the city's most popular recreational beaches (Surfers Corner, Figure 3). There have been reports of beach users falling ill (e.g Ntseku ,2021; Hejana, 2021) creating concern for the wellbeing of the greater Zandvlei ecosystem. The estuary mouth is mechanically manipulated and opened to the ocean approximately once per month during summer (low rainfall). However, the mouth is artificially kept open during winter (Venter et al., 2020). The estuary mouth was closed when samples were collected for the study. Dense populations of common sandprawns (*K. kraussi,* up to 176-240 individuals per m^2) are located in the sandflats of the estuary, relatively close to the mouth (Figure 3, Venter et al. 2020). Zandvlei has experienced notable anthropogenic modifications, including the construction of a marina and the canalization of the lower reaches leading to the mouth. The estuary also undergoes regular dredging to prevent the accumulation of sediment on the benthos (e.g. African News Agency, 2015; Staff Reporter, 2020, Figure 4). The system was dredged approximately 4 weeks prior to the onset of the experiment to clear marine sediment near the mouth of the estuary thereby allowing for improved water flow.

Despite water quality concerns, Zandvlei remains of ecological and recreational importance. This is primarily due to the fish nursery function performed by the system, in addition to the habitat provided to a diverse array of animals, including sandprawns, birds and fish (Quick and Harding, 1994).

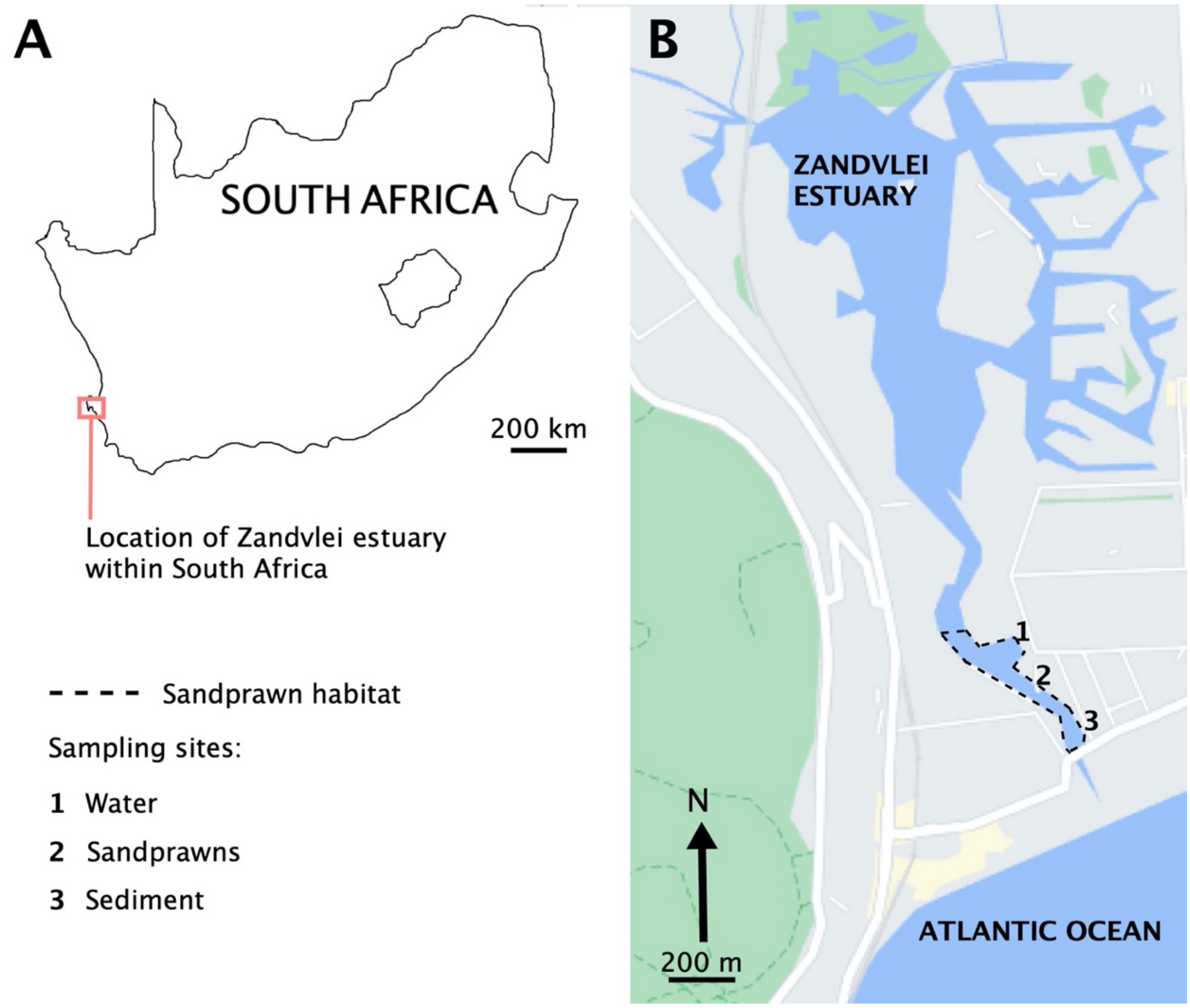

Figure 3: A – Location of the study site, Zandvlei Estuary, within South Africa. B- Zandvlei Estuary Nature Reserve, with locations for sampling sites and sandprawn habitat range shown.

Figure 4: Dredging in Zandvlei Estuary, captured on 27 August 2021 by Carla de Cerff

2.2 Experimental Design

An 18-day mesocosm experiment, modified from Pillay et al., (2012) and Venter et al., (2020), was used to quantify the effects of burrowing sandprawns on phytoplankton and pelagic bacterial assemblages (Figure 5). The experiment comprised 3 treatments with 3 replicates each, based on varying densities of sandprawns. Treatments were 0% (control), 50% and 100% natural sandprawn density, based on the maximum natural sandprawn density of 200 individuals.m^2 (Branch and Pringle, 1987). The number of sandprawns needed per treatment was calculated by scaling the natural sandprawn density down to the surface area of each mesocosm per treatment (50% treatment: 6 individuals/mesocosm; 100% treatment: 12 individuals/mesocosm).

Figure 5: Experimental set-up showing layout of experimental mesocosms in the aquarium facility in the John Day building, University of Cape Town.

The experiment was conducted in an aquarium facility in the John Day Building, University of Cape Town, with a fixed temperature of 15°C and a 12-hour day/night light cycle, which mimics the natural environment. Mesocosms consisted of buckets (0.25m^2, 30cm deep) which were half filled with sediment, and half with water collected from Zandvlei estuary (Figure 2B). Prior to the addition of material to each mesocosm, sediment was sieved through a 2mm mesh to remove large macrofauna. Estuarine water (salinity = 35 ‰, temperature = 15°C) was collected and visible material such as macroscopic algae or plastic removed prior to addition to mesocosms. Sandprawns were collected using stainless steel prawn pumps (length = 90cm, diameter= 5 cm) and transported back to the aquarium facility in loosely placed sheets of damp newspaper. Non-gravid sandprawns longer than 4cm (rostrum to telson) were collected. All relevant permits (including animal ethics approval) were obtained prior to the onset of the experiment. Each mesocosm was individually aerated (Pillay et al., 2012) and left to settle for 1 day before the addition of sandprawns. Since the experiment included the analysis of bacteria, stringent measures were employed to avoid cross-contamination between mesocosms and to ensure sterility. Therefore, all tools used for material and sample collection, including mesocosm components (aeration lines, buckets etc.), were sanitized with a 70% ethanol solution prior to the start of the experiment.

2.3 Sample Preparation

Environmental Variables

Water temperature, pH, conductivity, and oxygen concentration were measured every 3 days for the duration of the experiment (18 days). Temperature and pH were measured using a Crison PH25 pH meter and conductivity was measured using a YSI Ecosense EC300 conductivity meter. Oxygen levels were measured qualitatively using a Sera Oxygen (O_2) Aquarium Test kit. Where probes were used, cross contamination between mesocosms was avoided by collecting a water sample from each mesocosm using a sterile container into which probes were then immediately inserted. Water samples (1 Litre) from each mesocosm were vacuum filtered onto pre-weighed glass fiber filters (0.7μm micro-glass fiber paper, Whatmann) at the end of the experiment. The difference between initial and end weights of each membrane filter was used to determine the weight of suspended solids.

Nutrients

Phosphate (PO_3^{4-}), nitrite (NO^{2-}) and ammonia (NH_3) were measured every 3 days throughout the study period using a photometer (Hanna Instruments Multiparameter Bench Photometer for Aquaculture: HI 83203) along with the necessary reagents for each nutrient (Table 1).

Table 1: Reagents and methods used to measure the concentrations of 3 different nutrient response variables.

Response Variable	Reagent code	Range	Method

Phosphate ($PO_3^{4-)}$	HI 93715-01 0.00	0.00-10.00	Nessler
Nitrite (NO^{2-})	HI 93707-01	0.00-1.15	Diazotization
Ammonia (NH_3)	HI 93713-01 0.00	0.00-2.50	Ascorbic Acid

Biological Response Variables

The biological response variables quantified in this experiment were abundances of phytoplankton, nanoplankton, picoplankton, cryptophytes, *Prochlorococcus*-like algae, *Escherichia coli* (*E. coli*) and total heterotrophic bacteria. Each biological response variable was measured using flow cytometry, which has proven to be a powerful tool for the analysis of aquatic micro-organisms (Manti et al., 2012; Marie et al., 1999). By using a combination of the light scattering and fluorescent properties of individual cells, flow cytometry is a highly sensitive method of quantifying population heterogeneity (Bergquist et al., 2009). By increasing both the number of samples analyzed and of micro-organisms detected, it is considered a precise alternative to traditional microscopic count methods (Manti et al., 2012). A brief background to flow cytometry is presented in Appendix A. Biological response variables were measured every 2-3 days for the duration of the study. At each sampling period, three 5ml water samples were extracted from the surface (i.e. within the top 5cm) of each mesocosm, pooled into a single sample and vortexed to ensure thorough mixing. Since water depth was not found to influence nutrients or chlorophyll-a in an experiment using identical mesocosms (Venter et al., 2020), water samples were only extracted from the surface.

Flow Cytometry Analysis

Flow cytometry facilities within the IDM (Institute of Infectious Disease and Molecular Medicine) at the Faculty of Health Sciences, UCT were used to quantify biological responses to sandprawns. Samples were analyzed using a BD LSR II flow cytometer equipped with an air-cooled argon-ion laser (488 nm, 20mW). A fluorescent 5-5.9µm bead (AccuCount Fluorescent Particles, Spherotech, Lake Forest, IL, USA) with a standard concentration was used to determine absolute cell concentrations, which was accomplished by comparing cellular events to bead events (Gong et al., 2017). Once relevant data were extracted from FlowJo, Microsoft Excel V16.51 was used to calculate the absolute cell concentrations. Similarly, 0.88µm sized fluorescent beads were used as a standard reference to determine the relative cell sizes present in the sample. To account for spectral overlap, compensation was applied where necessary. This is the mathematical correction of removing the fluorescent signal of a fluorochrome from all detectors within the flow cytometer except the one used for the study (Roederer, 2002). Qualitative analyses were completed using FlowJo Software Version 10.7.1.

Phytoplankton (including nanoplankton, picoplankton and cryptophytes)

A 1ml aliquot of the pooled mesocosm water sample was used for analyzing phytoplankton assemblages using flow cytometry. Given the autoflourescent nature of phytoplankton cells due to their photosynthetic pigments, no pre-treatment of samples was necessary (Manti et al., 2012). Fresh samples were therefore analyzed within 40 minutes of collection from each mesocosm and stored in the dark at 4°C prior to analysis. Picoplankton and nanoplankton were measured through their signal emitted on the orange [phycoerythrin (PE): 585/42 band pass] vs. red (PC: 661/16 band pass) fluorescence signals.

E. coli

An immunological labelling technique was used to detect and enumerate *E. coli* cells in water samples. Immunological methods are recognized for their high sensitivity and ability to accurately detect bacterial species (Manti et al., 2010). For this method, a polyclonal fluorescein isothiocyanate (FITC) *E.coli* antibody (Abcam, ab30522) was used to label individual cells. A 1ml aliquot extracted from the pooled mesocosm sample was filtered through a 3µm Polycarbonate (PCTE) membrane filter using a vacuum filtration system. 500µl of filtered sample was then added to 500l 10X phosphate buffered solution (PBS), after which 3 µl of the *E. coli* antibody was added. Samples were left in the dark for roughly 30 minutes before analysis.

Total Heterotrophic Bacteria (THB) and Prochlorococcus-like algae

Fluorescence staining was used to identify THB and *Prochlorococcus*-like algae. The nucleic acid stain SYBR Green I (Molecular Probes, Eugene, OR, USA) binds effectively to DNA and clearly identifies heterotrophic bacteria and autotrophic *Prochlorococcus*-like cells in water samples. Furthermore, the sensitivity of flow cytometry allows for the discrimination of these two similar sized groups (Marie et al., 1997). A 1ml aliquot extracted from the pooled mesocosm sample was filtered through a 3 µm PCTE membrane filter, as described for *E. coli* samples. Filtered samples were then fixed with 10µl gluteraldehyde (25% solution) for 10 minutes, stained with SYBR Green I and covered with aluminum foil and left in the dark at room temperature for 20 minutes before analysis by flow cytometry. Heterotrophic bacteria were distinguished from *Prochlorococcus* through their lack of chlorophyll signal (690 nm).

2.4 Statistical Analysis

All statistical analyses were performed using R Studio v1.4.1106 (R Core Team, 2017). For nutrient and biological response variables, data were expressed as percentage change per sampling period relative to the starting values of day 0. This was because despite collection of water from a small area (5 x 5m) *in situ*, initial measurements were variable across mesocosms and treatments before the addition of sandprawns. Secondly, for biological response variables, units needed to be standardized for multivariate testing to assess spatial variability. For environmental variables, the absolute data values were used as values did not vary across mesocosm prior to the start of the experiment.

PCA

Principal components analysis (PCA) was used to qualitatively assess variability in biological response variables in response to sandprawn treatments. PCA was applied for selected sampling days spanning the start (day 3), middle (day 9) and end phases (days 15 & 18) of the experiment using the 'fviz_pca' function in the 'factoextra' package (Kassambra, 2017).
For each PCA plot, vectors were overlaid to show contributions of each planktonic taxon that contributed to multivariate clustering across space (treatments) and time (sampling day).

Models

A modelling approach was used to determine the effect of sandprawn density on response variables. The most suitable model was fitted to each variable based on factors such as the data distribution, type of data (i.e. proportionate or absolute values), method of data collection and adherence to model assumptions. To improve model fit and to ensure model assumptions were not violated, transformations were applied to the data where applicable. The type of model fitted and transformation applied to each variable are specified in Table 2.

For biological response variables (phytoplankton, nanoplankton, picoplankton, cryptophytes, *Prochlorococcus*-like algae, total heterotrophic bacteria, and *E. coli*), models were fitted using the data collected from day 9 onwards. This was based on observations that models fitted using the entire dataset did not reflect temporal trends recorded in graphs for these biological response variables (see section 3.3). During the first 9 days, mesocosms were generally in a state of flux, characterized by high data variability. Given the potential for such variability to impact model outputs and override treatment effects a truncated dataset using values from day 9 onwards were utilized to fit models.

Table 2: Specific models, with distribution in parentheses where applicable and transformations applied to quantify effects of sandprawns on each response variable

Response Variable	**Transformation**	**Model**
Temperature	square root	lmer
pH	square root	lmer
Conductivity	n/a	glmmtmb (gaussian)
Suspended Solids	n/a	lm
Phosphorous	logit	lmer
Nitrite	logit	lmer
Ammonia	logit	lmer
Phytoplankton	n/a	glmmtmb (beta)
Nanoplankton	n/a	glmmtmb (beta)
Picoplankton	n/a	glmmmtb (beta)
Cryptophytes	logit	lmer
Prochlorococcus-like	logit	lmer
Total heterotrophic bacteria	n/a	glmmtmb (beta)
Escherichia coli	n/a	glmmtmb (beta)

Where samples were taken from the same mesocosm over a time series, measurements could not be considered independent. To avoid temporal pseudo replication, mixed effects models were fitted to the data (Crawley, 2007). Sampling days were considered a random effect since measurements were expected to change throughout the duration of the experiment (Venter et al., 2020). As the independent variable, treatment (sandprawn density) was considered the fixed effect. Generalized mixed effects models (GLMs) were fitted using the glmmtmb package, with a beta distribution where proportion data were used, since these data were bound by 0 and 1 (Crawley 2007, Table 2). Linear Mixed Effects model were fitted by restricted maximum likelihood (REML) using the ‘lmer’ command in the ‘lme4’ package (Bates et al., 2015). For the linear model and linear mixed effects models, model validation was evaluated using histograms, quantile-quantile (Q-Q) plots and spread of residuals (Groenewald, 2018). For generalized mixed effects models, the ‘DHARMa’ package was used to evaluate residuals (Hartig, 2021).

To determine the significance of sandprawn density in predicting the variability in each response variable, Analysis of Variance (ANOVA) of models were run using the ‘Anova’ function in the ‘car’ package (Duursma and Powell, 2016). P values were generated with Type II Wald Chi-Square tests, which produce the same results as Types I and III tests if data are balanced (Mangiafico, 2016). To determine within-treatment variability, Tukey post-hoc testing was applied using the ‘emmeans’ package (Lenth, 2016).

3. RESULTS

3.1 Abiotic Variables

Environmental conditions within mesocosms were generally uniform for the duration of the experiment (Table 3). Oxygen level was measured qualitatively and remained uniform at 6mg/L. There was no significant variation in temperature (χ^2= 1.37, df=2, p=0.504, Table 4) and conductivity (χ^2= 3.12, df=2, p=0.210, Table 4) across sandprawn densities. The sandprawn treatment significantly influenced pH (χ^2 =11.17, df=2, p=0.003, Table 4), with significant differences detected between the 100% treatment and 0% control (post-hoc Tukey t=-2.910, df=54, p=0.014) and 100% and 50% treatments (post-hoc Tukey t =2.88, df = 54, p=0.015). With mean measurements between 7.4 ± 0.13 SE and 8.2 ± 0.18 SE, pH did not vary by more than 0.8 units across the duration of the experiment (Table 3). The weight of suspended solids measured at the end of the experiment was significantly different between treatments (F(2,6)=6.59, Table 4), with values in the control and 50% treatment being statistically different (t=-3.59, df=6, 0.026, Table 5), though variability was minor.

3.2 Nutrients

Sandprawn density did not explain significant variation in PO_4^{3-} concentrations (χ^2 =0.144, df=2, p=0.930; Table 4). Density did however explain significant variation in NO_2^- levels (χ^2 =18.59, df=2, p<0.0001), with concentrations in the control being significantly higher than the 50% (t=3.028, df=54, p<0.001, Table 5) and the 100% (t=4.173, df=54, p<0.001, Table 5) treatments. At the end of the experiment, NO_2^- levels were 0.71 ± 0.12, 0.09 ± 0.56 and 0.04 ± 0.69 in the control, 50% and 100% treatment respectively (Table 3). Sandprawn density was also significant in explaining variation in NH_3 concentrations (χ^2 =13.89, df=2, p<0.001, Table

4) with significant differences recorded between the 50% treatment (10.64 ± 0.56) and control (10.15 ± 0.12; $z = 3.687$, $df=54$, $p<0.001$, Table 5).

Table 3: Environmental data (mean ± standard error) measured from mesocosms with varying sandprawn densities. Data shown were measured over the entire experimental period.

Day	Sandprawn Treatment	Temperature (°C)	pH	Conductivity (mg/L)	Oxygen (mg/L)	PO_4^{3-} (mg/L)	NO_2^- (mg/L)	NH_3 (mg/L)
0	0	15.4 ± 0.10	7.7 ± 0.16	39.3 ± 0.28	6.0 ± 0.00	4.43 ± 0.12	0.04 ± 0.00	10.45 ± 0.00
	50	15.3 ± 0.06	7.9 ± 0.07	39.0 ± 0.10	6.0 ± 0.00	2.95 ± 0.33	0.03 ± 0.00	7.71 ± 0 .04
	100	15.3 ± 0.15	7.5 ± 0.15	39.8 ± 0.42	6.0 ± 0.00	1.70 ± 0.84	0.03 ± 0.00	9.64 ± 1.29
3	0	15.4 ± 0.16	8.0 ± 0.05	39.1 ± 0.25	6.0 ± 0.00	2.46 ± 0.98	0.05 ± 0.01	6.08 ± 1.01
	50	15.6 ± 0.06	7.8 ± 0.09	38.6 ± 0.11	6.0 ± 0.00	1.64 ± 1.07	0.05 ± 0.00	5.72 ± 1.26
	100	15.3 ± 0.14	7.4 ± 0.13	38.6 ± 0.22	6.0 ± 0.00	4.65 ± 0.13	0.05 ± 0.01	7.95 ± 0.23
6	0	14.8 ± 0.03	8.1 ± 0.05	36.8 ± 0.15	6.0 ± 0.00	2.79 ± 1.15	0.08 ± 0.02	9.06 ± 0.55
	50	15.3 ± 0.07	8.1 ± 0.16	37.1 ± 0.41	6.0 ± 0.00	2.73 ± 1.12	0.06 ± 0.01	8.79 ± 0.79
	100	15.0 ± 0.12	8.1 ± 0.13	36.7 ± 0.10	6.0 ± 0.00	4.28 ± 1.15	0.05 ± 0.00	7.61 ± 0.28
9	0	14.7 ± 0.15	8.2 ± 0.07	48.5 ± 0.00	6.0 ± 0.00	2.38 ± 1.01	0.13 ± 0.03	9.04 ± 0.72
	50	14.8 ± 0.03	8.2 ± 0.18	40.3 ± 0.07	6.0 ± 0.00	3.17 ± 0.95	0.07 ± 0.01	7.71 ± 1.90
	100	14.1 ± 0.10	8.1 ± 0.05	39.5 ± 0.15	6.0 ± 0.00	2.69 ± 1.02	0.06 ± 0.03	8.43 ± 1.49
12	0	14.4 ± 0.18	8.2 ± 0.04	39.9 ± 0.15	6.0 ± 0.00	2.96 ± 1.33	0.19 ± 0.06	8.83 ± 1.41
	50	13.7 ± 0.23	8.1 ± 0.03	40.8 ± 0.76	6.0 ± 0.00	3.17 ± 1.18	0.08 ± 0.02	9.74 ± 0.84
	100	14.6 ± 0.30	8.1 ± 0.05	39.9 ± 0.30	6.0 ± 0.00	1.91 ± 1.08	0.06 ± 0.01	8.81 ± 0.84
15	0	14.9 ± 02	8.1 ± 0.02	40.3 ± 0.41	6.0 ± 0.00	2.33 ± 1.20	0.34 ± 0.14	9.41 ± 0.63
	50	14.7 ± 03	8.1 ± 0.09	41.7 ± 1.38	6.0 ± 0.00	2.53 ± 1.18	0.07 ± 0.03	9.65 ± 1.64
	100	14.6 ± 03	8.1 ± 0.10	41.1 ± 0.36	6.0 ± 0.00	2.37 ± 0.89	0.05 ± 0.01	11.05 ± 0.03
18	0	14.9 ± 0.17	8.1 ± 0.03	40.3 ± 0.00	4.0 ±0.00	3.13 ± 1.32	0.71 ± 0.28	10.15 ± 0.12
	50	14.4 ± 0.30	8.1 ± 0.07	45.4 ± 1.40	4.0 ±0.00	2.05 ± 0.90	0.09 ± 0.03	10.64 ± 0.56
	100	14.5 ± 0.50	8.0 ± 0.09	41.9 ± 0.48	4.0 ±0.00	2.98 ± 1.32	0.04 ± 0.02	9.69 ± 0.69
18		**Suspended Solids (g/L)**						
	0	0.76 ± 0.02						
	50	0.88 ± 0.02						
	100	0.84 ± 0.03						

Table 4: Results of type II Wald Chi-Square tests testing the effects of treatment (0% (control), 50% and 100% sandprawn density) on response variables in the mesocosm experiment. Results were produced with analysis of variance (ANOVA) of mixed effects models and, in the case of suspended solids, a linear model. Statistically significant outcomes are displayed in bold. *E. coli* – *Escherichia coli*. THB – Total heterotrophic bacteria. Pro-like: *Prochlorococcus*–like algae

	Response Variable	χ^2	**df**	**p-value**
Environmental Variables	Temperature	1.37	2	0.504
	pH	11.17	2	**0.003**
	Conductivity	3.12	2	0.210
	Suspended solids	6.59 (F)	2,6	**0.035**
Nutrients	Phosphate	0.14	2	0.930
	Nitrite	18.59	2	**p<0.0001**
	Ammonia	13.89	2	**p<0.0001**
Biological Variables	Phytoplankton	41.548	2	**p<0.0001**
	Nano	23.405	2	**p<0.0001**
	Pico	40.831	2	**p<0.0001**
	Cryptophytes	5.96	2	0.051
	Pro-like	0.29	2	0.892
	E.coli	5.93	2	0.051
	THB	1.21	2	0.547

Table 5: Results of post-hoc Tukey tests applied to mixed effects models of the nutrients and biological response variables. Statistically significant outcomes are presented in bold.

		Estimate	**Std. Error**	**z value**[1]	**Pr (> |z|)**
Phosphate	100% - 0%	0.100	0.276	0.362	0.930
	50% - 0%	0.024	0.278	0.086	0.996
	50% - 100%	-0.076	0.275	-0.267	0.959
Nitrite	100% - 0%	1.033	0.181	5.713	**<0.001**
	50% - 0%	0.815	0.181	4.508	**<0.001**
	50% - 100%	-0.218	0.181	-1.205	**0.456**
Ammonia	100% - 0%	0.123	0.085	1.533	0.275
	50% - 0%	0.302	0.082	3.678	**<0.001**
	50% - 100%	0.173	0.079	2.163	0.078
Phytoplankton	100% - 0%	-0.766	0.119	-6.444	**<0.001**
	50% - 0%	-0.260	0.106	-2.457	**0.037**
	50% - 100%	0.506	0.123	4.111	**<0.001**
Nano-plankton	100% - 0%	-0.458	0.098	-4.693	**<0.001**
	50% - 0%	-0.087	0..088	-0.991	0.582
	50% - 100%	0.371	0.099	3.747	**0.00051**
Pico-plankton	100% - 0%	-0.686	0.107	-6.389	**<0.001**
	50% - 0%	-0.224	0.093	-2.396	0.043
	50% - 100%	0.462	0.112	4.135	**<0.001**
Cryptophytes	100% - 0%	0.103	0.161	0.640	0.799
	50% - 0%	-0.277	0.161	-1.720	0.207
	50% - 100%	-0.380	0.161	-2.360	0.056
***Prochlorococcus*-like algae**	100% - 0%	-0.101	0.25	-0.405	0.914
	50% - 0%	-0.105	0.25	-0.422	0.909
	50% - 100%	-0.004	0.25	-0.017	0.999
Total Heterotrophic Bacteria	100% - 0%	-0.256	0.234	-1.093	0.518
	50% - 0%	-0.138	0.227	-0.609	0.815
	50% - 100%	0.118	0.237	0.499	0.872
E.coli	100% - 0%	0.297	0.240	1.238	0.431
	50% - 0%	-0.304	0.254	-1.216	0.444
	50% - 100%	-0.605	0.249	-2.432	**0.039**

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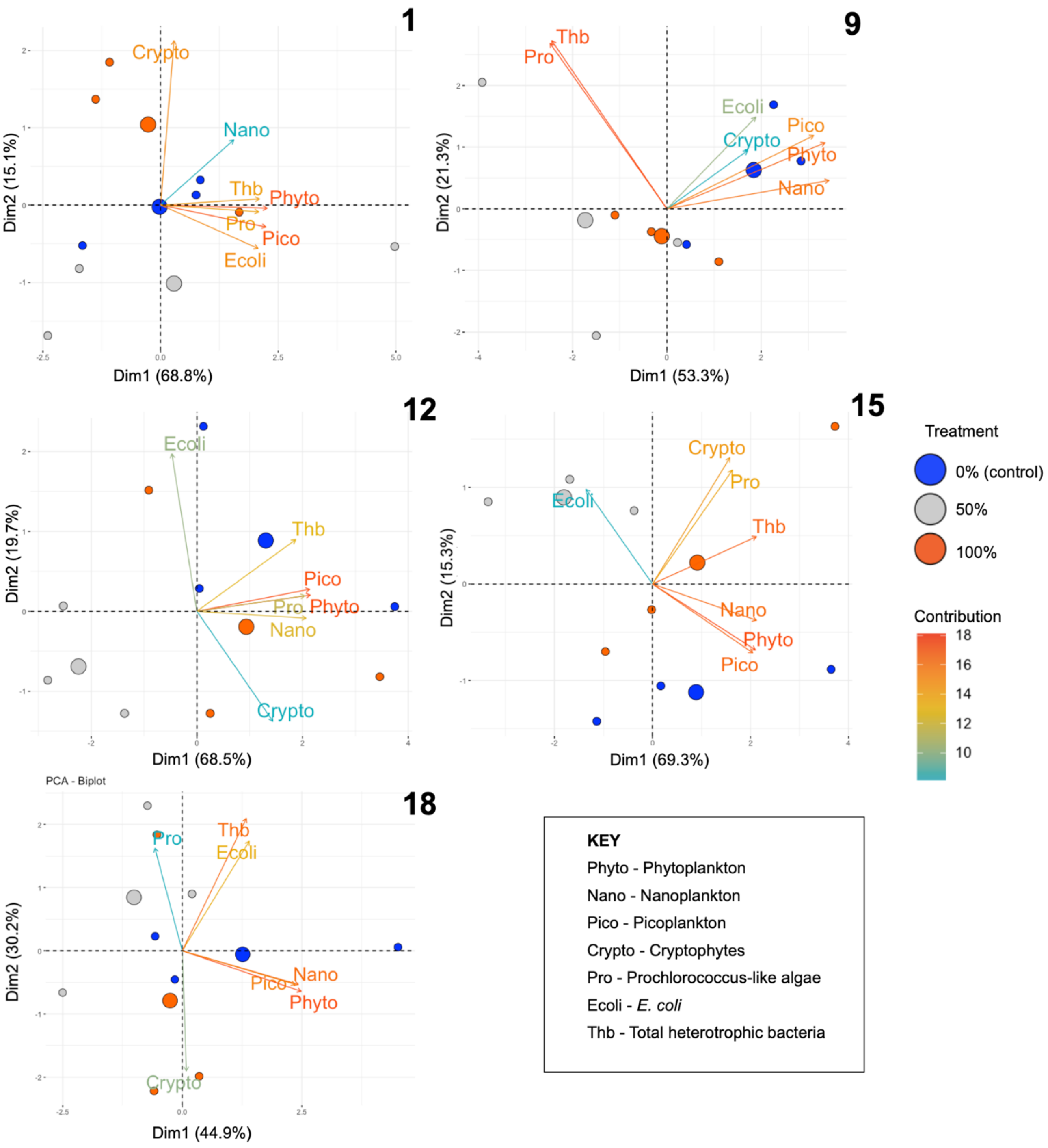
[1] T ratio is shown for Cryptophytes and nutrients

3.3 Biological Response Variables

Principal component analyses (PCA) for selected days illustrate how the contributions of bacteria and phytoplankton groups to sandprawn density change with time (Figure 6). On day 1 of the experiment, samples from controls were positioned between the 100% and 50% density treatments (Figure 6, Day 1). However, from day 9 onwards samples were generally more dispersed with clustering according to treatment. This was particularly evident for the control, which progressed from its initial position to being distinctly separate from other treatments (Figure 6). It was also evident that over time, phytoplankton, nanoplankton and picoplankton contributed more to differentiating controls from sandprawn inclusion treatments. The proportion of total variance explained by the 1st and 2nd axes (Dim1 and Dim2) of the principal components were high for all days, with the minimum proportion of variance explained being 74.4% (Day 9).



KEY

Phyto - Phytoplankton

Nano - Nanoplankton

Pico - Picoplankton

Crypto - Cryptophytes

Pro - Prochlorococcus-like algae

Ecoli - *E. coli*

Thb - Total heterotrophic bacteria

Figure 6: Principal Components Analysis of biological response variables in an 18 day mesocosm experiment with treatments of 0% (control), 50% and 100% sandprawn densities. Plots are numbered according to days of sampling. Each sample is represented by a smaller circle and larger circles represent the treatment centroid. Proportion of the variance explained by the axes Dim1 and Dim2 are displayed on each PCA. The contribution colour scale illustrates the contribution made by each variable to overall variability in bacterial and phytoplankton assemblages in response to sandprawn treatments over time

Prokaryotic assemblage:

The fluorescence emitted by the cell populations provides an indication the composition of the population. Fluorescence emitted by the stained cell populations varied temporally and between treatments. This is indicated by a shift in fluorescence peaks (i.e., fluorescence intensity with the highest cell counts) towards weaker intensities (leftward shift) over time (Figure 7A, B and C). Furthermore, differences between treatments are evident in secondary peaks on Day 18 (Figure 7C), with fluorescence histograms displaying downward contractions from the 100% sandprawn treatment to the controls.

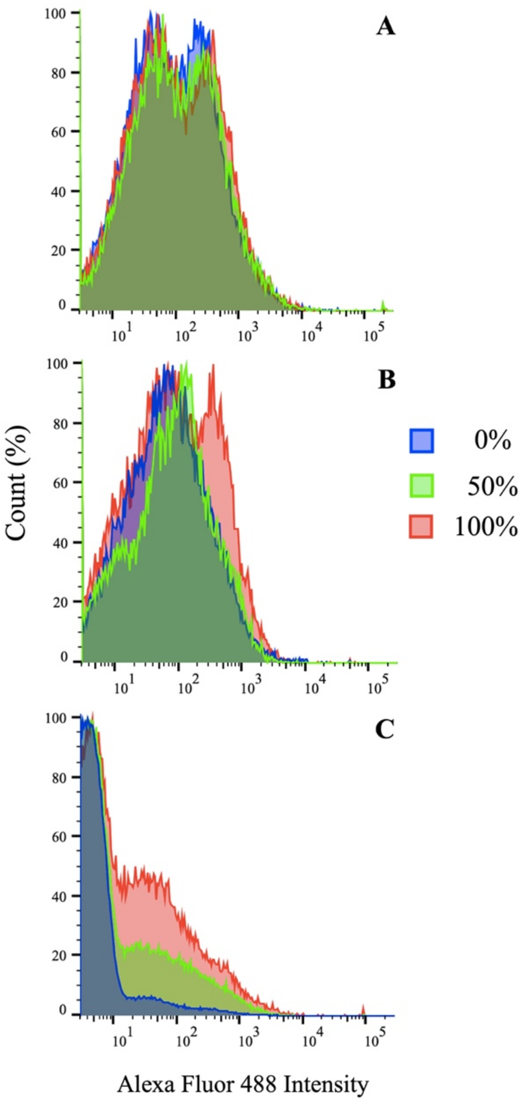


Figure 7: Histograms comparing the fluorescence intensity emitted by cells smaller than 0.88µm in the compensated Alexa-Fluor 488 Channel of SYBR Green I stained samples. Comparisons between 0% (control), 50% and 100% sandprawn density treatments for days 0 (A), 9 (B) and 18 (C) are shown. Blue, green, and red colours on histograms indicate sandprawn treatments. Count (%) is the proportion of the cell counts relative to each days maximum.

F

Prochlorococcus*-like algae, cryptophytes, total heterotrophic bacteria, and *Escherichia coli

Sandprawn density did not significantly explain variation in the relative abundance of pelagic *Prochlorococcus*-like algae (χ^2=0.29, df=2, p=0.892, Table 4) and total heterotrophic bacteria ($\chi^{2=}$1.207, df=2, p=0.546, Table 4) over the duration of the experiment. Trends for both variables were temporally and spatially erratic (Figures 8 and 9). Sandprawn density was marginally insignificant in predicting relative abundance of cryptophytes (χ^2 = 5.96, df = 2, p=0.051, Table 4) and *E.coli* (χ^2 = 5.93, df=2, p=0.051, Table 4). For cryptophytes, relative abundance peaked in the 50% sandprawn treatment (Figure 10) and in the 100% sandprawn treatment for *E.coli* (Figure 11).

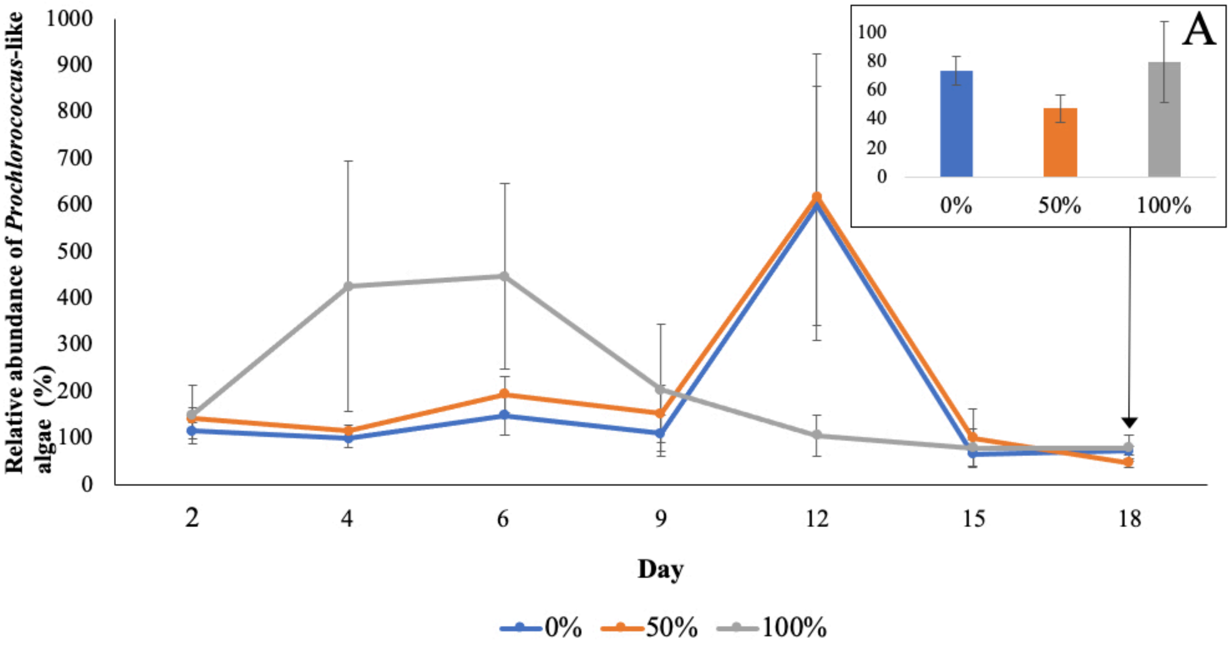


Figure 8: Mean ± 1SE of the change in relative abundance of Prochlorococcus-like algae among 0% (control), 50% and 100% sandprawn density treatments. Inset (A) Proportion change (mean ± 1SE) in phytoplankton abundance among sandprawn treatments at the end of the mesocosm experiment.

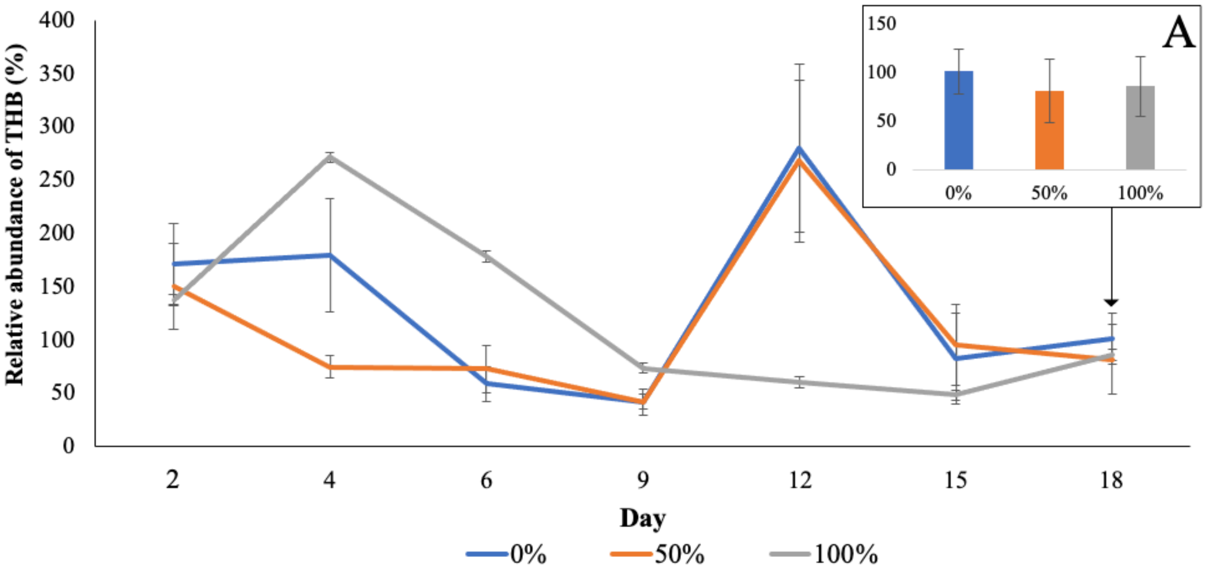


Figure 9: Mean ± 1SE of the change in relative abundance of total heterotrophic bacteria (THB) among 0% (control), 50% and 100% sandprawn density treatments. Inset (A) Proportion change (mean ± 1SE) in THB abundance among sandprawn treatments at the end of the mesocosm experiment.

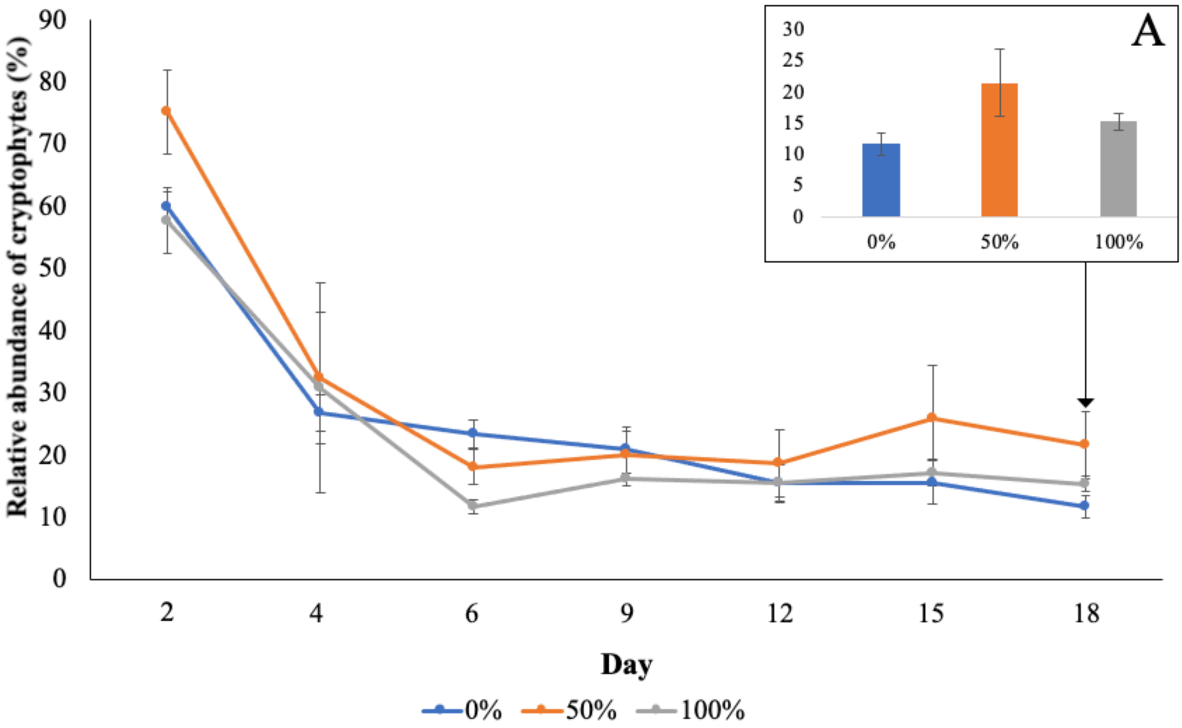


Figure 10: Mean ± 1SE of the change in relative abundance of cryptophytes among 0% (control), 50% and 100% sandprawn density treatments. Inset (A) Proportion change (mean ± 1SE) in cryptophytes abundance among sandprawn treatments at the end of the mesocosm experiment.

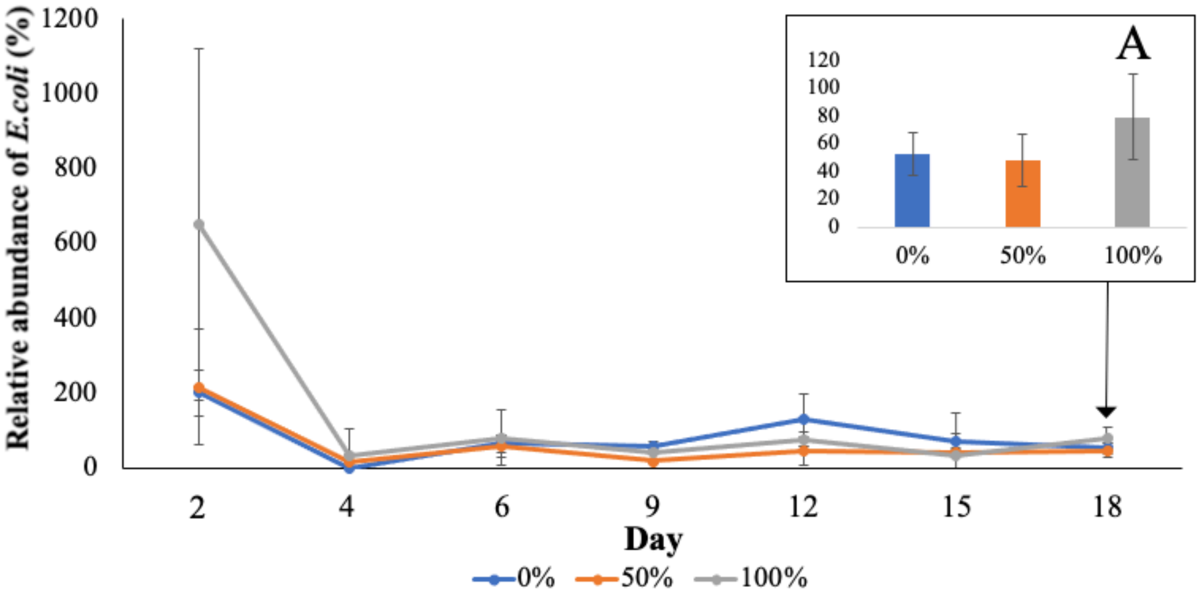


Figure 11: Mean ± 1SE of the change in relative abundance of *Escherichia coli* among 0% (control), 50% and 100% sandprawn density treatments. Inset (A) Proportion change (mean ± 1SE) in *E.coli* abundance among sandprawn treatments at the end of the mesocosm experiment.

Total Phytoplankton

There was a reduction in phytoplankton cells across all mesocosms regardless of treatment (Figure 12). However, at the end of the experimental period, the 100% treatment had the largest decline in total phytoplankton relative to controls. Consequently, upon the termination of the experiment the control contained the largest proportion of phytoplankton cells (8.1%) relative to starting conditions (Figure 12). This was followed by the 50% treatment which retained 5.8% and the 100% treatment which recorded 4.3% of its phytoplankton cells relative to starting conditions. Sandprawn density was a significant predictor of total phytoplankton abundance (χ^2=41.55, df=2, $p<0.0001$, Table 4), with significant differences detected across all treatments (post hoc Tukey, $p < 0.05$; Figure 12, Table 5).

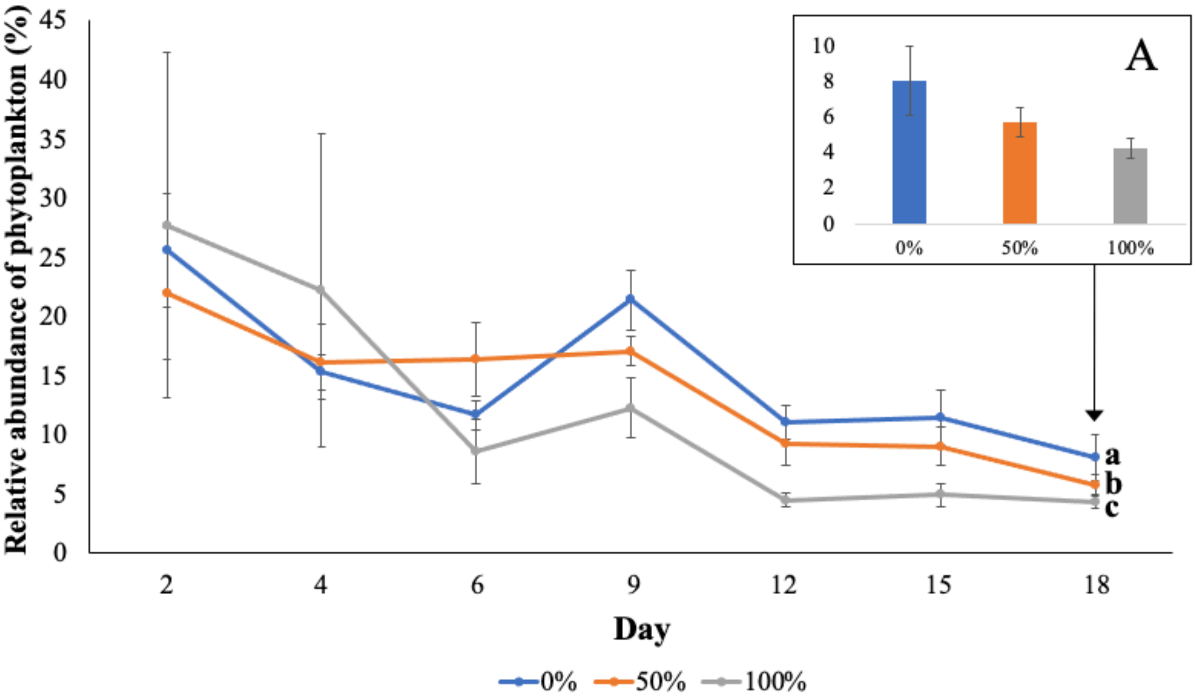


Figure 12: Mean ± 1SE of the change in relative abundance of phytoplankton among 0% (control), 50% and 100% sandprawn density treatments. Letters a, b and c denote significant differences (post hoc Tukey $p<0.05$) between treatments. Inset (A) Proportion change (mean ± 1SE) in phytoplankton abundance among sandprawn treatments at the end of the mesocosm experiment.

Nanoplankton

Sandprawn density was significant in explaining variation in nanoplankton abundance ($\chi^2=23.405$, df=2, $p<0.0001$, Table 2). As with phytoplankton, there was an overall decline in nanoplankton abundance over time (Figure 13). The 100% sandprawn treatment had the largest decline, resulting in a final abundance of 13.59% relative to initial conditions, followed by the 50% treatment, in which abundance was 15.67% (Figure 13). At the end of the experiment, the control nanoplankton abundance was 16.32%. Nanoplankton abundance in the control differed significantly from the 100% treatment (post hoc Tukey $p<0.0001$, Table 3), whereas the 50% and 100% treatments were insignificantly different marginally ($p=0.051$, Table 3). The 50% and control treatments were statistically indistinguishable ($p = 0.58$, Table 3).

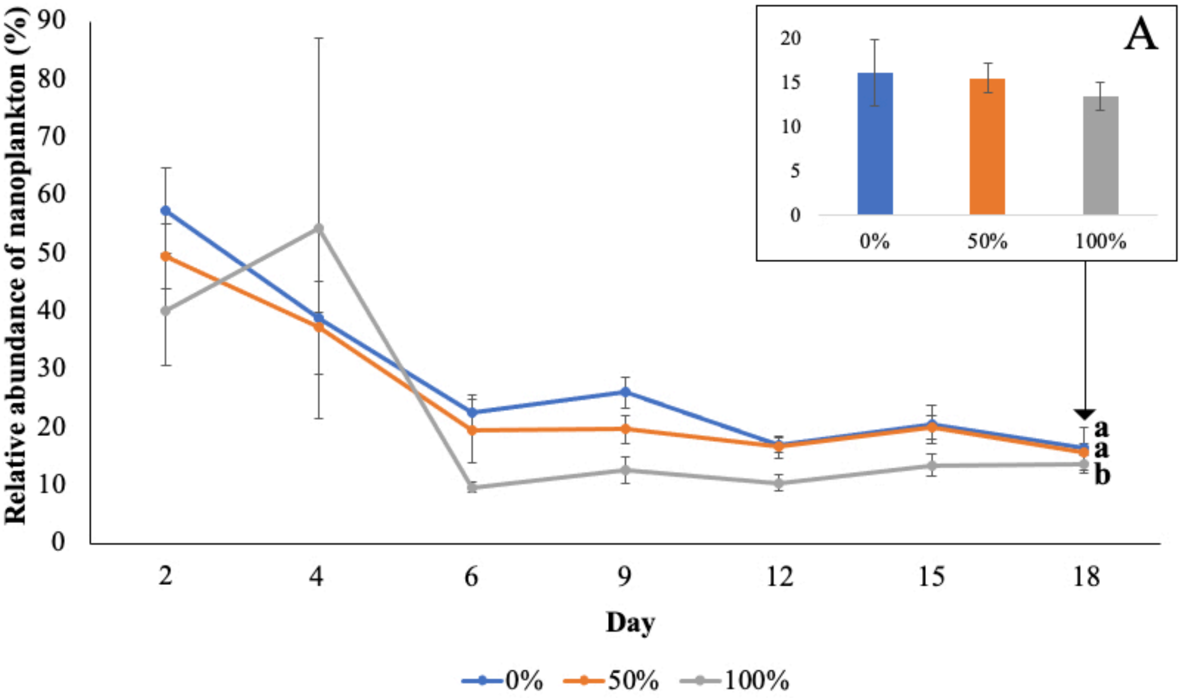


Figure 13: Mean ± 1SE of the change in relative abundance of nanoplankton relative to starting conditions (Day 0) in the water column among 0% (control), 50% and 100% sandprawn density treatments. Letters a and b, created by Tukey pairwise comparisons, represent significant differences ($p<0.05$) between treatments; Inset A: Proportion change (mean ± 1SE) in nanoplankton abundance among sandprawn treatments at the end of the mesocosm experiment.

Picoplankton

Sandprawn density was a significant predictor of changes in picoplankton relative abundance ($\chi^2=40.83$, df=2, $p<0.0001$, Table 4). Trends for picoplankton followed those for phytoplankton and nanoplankton, with the control having highest relative abundance of picoplankton at the end of the experiment (5.57%, Figure 14), followed by the 50% (3.00%) and 100% treatments(2.13%). Significant differences in relative abundance of picoplankton were detected among all sandprawn treatments ($p<0.05$, Table 5).

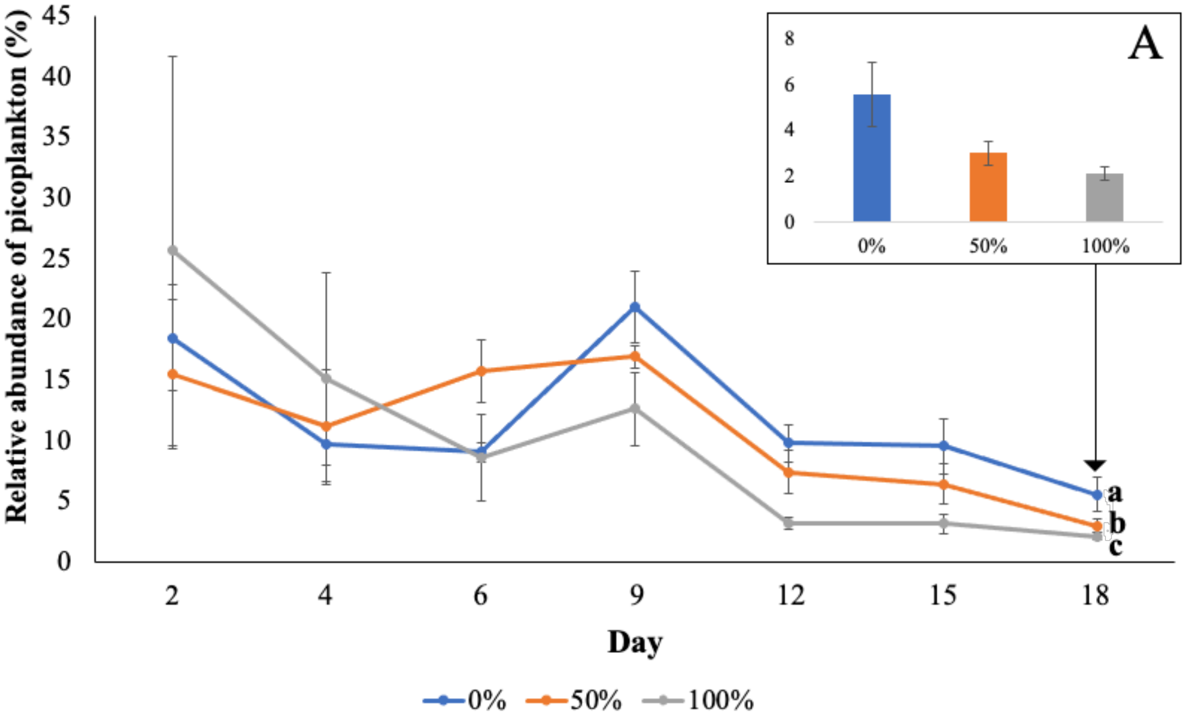


Figure 14: Mean ± 1SE of the change in relative abundance of picoplankton relative to starting conditions (Day 0) in the water column among 0%, 50% and 100% sandprawn density treatments. Letters a, b and c created by Tukey pairwise comparisons, represent significant differences ($p<0.05$) between treatments; Inset A: Proportion change (mean ± 1SE) in picoplankton abundance among sandprawn treatments at the end of the mesocosm experiment.

Figure 15: Proportions of pico- and nanoplankton across varying densities of sandprawns (0%, 50% and 100%). on days 0 (A), 9 (B) and 18 (C) of the mesocosm experiment.

At the start of the experiment, phytoplankton in experimental mesocosms were picoplankton-dominated (80.06% to 82.24%) (Figure 15A). This pattern persisted midway through the experiment (day 9; Figure 15B), with picoplankton dominating (77.72%– 81.47%). However, at the termination of the experiment (Day 18, Figure 15C), phytoplankton in the sandprawn treatments (50% and 100%) contained higher proportions of nanoplankton relative to starting conditions and controls. On day 18, nanoplankton accounted for 56.83% and 58.07% of phytoplankton in the 50% and 100% sandprawn treatments. In the controls however, nanoplankton contributed 41.57% to the phytoplankton assemblage

4. DISCUSSION

Results from the mesocosm experiment indicated that sandprawn presence elicited differential impacts on selected pelagic microscopic organisms. This is evident from the substantial influence sandprawns had on phytoplankton relative to bacteria, with the study demonstrating a decline in phytoplankton abundance with increasing sandprawn densities. While bacterial abundance was not statistically different between treatments, the qualitative data (Figure 7) suggest that sandprawns may influence the composition of prokaryotic assemblages, but subtly. Furthermore, findings show that selectivity in responses extends beyond the broad categories of phytoplankton and bacteria, with groups within phytoplankton being differentially impacted. Notably, picoplankton was differently affected in comparison to nanoplankton, resulting in a shift in phytoplankton size class from pico- to nanoplankton.

Generally, abiotic variables were uniform in the experiment across sandprawn treatment levels, but there were exceptions. Nitrite levels were influenced by sandprawn treatment levels, with levels in the controls increasing over time relative to mesocosms with sandprawns. In the controls, mean nitrite levels were 0.71 ± 0.12 mg/L compared to 0.09 ± 0.56 mg/L and 0.04 ± 0.69 mg/L for the 50 and 100% at the end of the experiment. Ammonia levels were also influenced by sandprawn treatment levels, with significant differences, although minor, found between the 50% and 100% treatments (10.64 ± 0.56 mg/L and 10.15 ± 0.12 mg/L respectively). Similarly, sandprawn levels were found to significantly impact pH, which also had a minor variation among treatments, not exceeding 0.8 units. Trends for ammonia and pH were similar in that most of the variation between treatments were recorded at the beginning of the experiment with values becoming more uniform as the mesocosms settled over time (Table 3). Therefore, it is plausible that the significant differences detected by the statistical tests for pH and ammonia stem from the variation that occurred at the initial stages of the experiment related to variance in establishment times for benthic and pelagic habitats (and associated microbial assemblages). Furthermore, the lack of a consistent pattern in pH and ammonia levels across sandprawn treatments (controls, 50% & 100%) suggests that significant differences detected in pH and ammonia were not sandprawn induced, and unlikely to have played a major role (if any) in influencing the biological response variables. Similarly, variation in concentrations of suspended solids across the sandprawn treatment were minor, and with differences detected between the 50% (0.88 ± 0.02 g) and 0% (0.76 ± 0.02 g) treatments only. As with ammonia and pH, the minor variation and lack of a consistent pattern between treatments suggests that significant differences detected may not have been a sandprawn treatment effect solely.

Nitrite concentrations remained relatively uniform in + sandprawn treatments, but values progressively increased in the controls with time, which could be due to a number of interrelated processes. Stief et al. (2002) found that net nitrite production occurs mainly in the suboxic zone of marine sediments. Since activities such as burrow irrigation increase oxygen levels within the sediment (Laverock et al., 2010), the lack of burrows in the controls could result in larger anoxic patches, thus providing a greater suboxic/anoxic area in which nitrite can be produced.

Nitrification (the reduction of nitrogen compounds to nitrate and nitrite) and denitrification (the reduction of nitrite to gaseous nitrogen) are bacterially-mediated processes (Howe et al., 2004). Therefore, any effects on bacterial activity will likely affect nitrification and denitrification and ultimately the nitrogen cycle (Howe et al., 2004). The uniformity of nitrite in the + sandprawns treatments and its progressive increase in the controls suggest that sandprawns may facilitate colonization and proliferation of microbes that utilize nitrite, thereby preventing its accumulation. This agrees with Howe et al. (2004), who found that the presence of burrowing mudshrimp *Upogebia deltaura* significantly increased both denitrification rates (i.e nitrite utilization) and coupled nitrification-denitrification rates. The mechanisms suggested for increasing microbially-mediated denitrification rates are the extension of the sediment-water interface, increased oxygenation and enhanced solute transport provided by burrowing shrimp (Howe et al., 2004).

Chen and Gu (2017) investigated the impact of infaunal burrows on the structure and abundance of selected prokaryotes involved in nitrification and denitrification processes. Specifically, burrows in mangrove sediments were associated with lower concentrations of nitrite - as found with *Kraussillichirus kraussi* in the present study. Two nitrite-dependent prokaryotes (i.e. they reduce nitrite to nitrogen gas) were studied: denitrifying anaerobic methane oxidation (n-damo) bacteria and anaerobic ammonia-oxidizing (anammox) bacteria. Infaunal burrows were found to significantly increase the abundance of n-damo bacteria in bioturbated areas. Whilst the abundance of anammox bacteria was not influenced by burrows, the diversity of the community was greatly enhanced. This research therefore raises the possibility that in my experiment, *K. kraussi* presence, and their extensive below-ground burrow systems, could promote n-damo and/or anammox bacterial assemblages, thereby stimulating the processes linked to utilization of nitrite.

Whether sandprawns are enhancing anammox and/or n-damo bacteria should be researched in greater detail in future studies - especially given the importance of these processes as tools for the treatment of wastewater, where the removal of nitrogen is of great importance to prevent eutrophication and improve water quality (Van Kessel et al., 2018). At levels high enough

(>1mg/L), nitrite is also known to be toxic to humans and its accumulation in the water column and sediment could be detrimental to sediment-dwelling macro-invertebrates (Stief et al., 2002). The results obtained indicate that sandprawns can prevent the accumulation of nitrite in the water column, most likely through their indirect impacts on the bacterial assemblages. Interestingly, the finding of nitrite concentration being reduced in the presence of sandprawns contrasts with findings of Venter et al. (2020), who found no significant impact of sandprawns on nitrate concentrations in the water column. This contrast in experimental outcomes could be explained by differences in duration of the experiments, since my experiment ran for 7 days longer than that of Venter et al. (2020), which ran for 11 days. This is supported by the temporal trends in nitrite levels recorded in my study, given that larger increases in nitrite concentrations were recorded in the controls from day 9 onwards (Table 3). The differences in trends for nitrite concentrations and could also be explained by contextual differences between experiments. While the Venter et al. (2020) and my experiments were based on the same design (with the experimental duration being the exception) and with ecological materials for the experiment originating from the Zandvlei Estuary, both experiments were run at two different times (2019 versus 2020). The microbial assemblages present in the systems as well as the sediment characteristics, could therefore have been different between experimental periods, which could ultimately explain differences in trends for nitrite recorded between the experiments.

K. kraussi are primarily considered deposit feeders. Feeding is intimately linked with their burrows, with burrow morphological characteristics such as subsurface chambers and surface mounds being typically indicative of deposit feeding (Pillay and Branch, 2011). The presence of surface mounds indicates that sediment is actively processed and is generally a clear indication that the species feeds on sediment-bound material by processing large amounts of sediment (Griffis and Suchanek, 1991). It has, however, been strongly argued in past research that *K. kraussi* are filter feeders. Specifically, fatty acid and stable isotope analysis indicated that *K. kraussi* relied predominantly on marine-derived particulate organic matter (POM) as a trophic resource and only utilized benthic microalgae when pelagic food resources were in low supply (Antonio and Richoux, 2014). Similarly, stable isotope analysis demonstrated that the main source of carbon present in the tissue of *K. kraussi* is derived from suspended POM (Schlacher and Wooldridge, 1996). In both cases, the authors concluded that *K. kraussi* are suspension feeders due to the unequivocal tracer evidence that their main food sources were derived from the water column, as with other suspension feeders. However, this conclusion does not take their lifestyles and taxonomy into account and does not consider the mechanism by which POM is obtained. Venter et al. (2020) demonstrated a near 50% reduction in phytoplankton biomass in the presence of sandprawns, but with burrow walls having greater microalgal biomass relative to surface sediment and that of controls lacking sandprawns. The Venter et al. (2020) study reconciles arguments regarding the feeding mode of *K. kraussi* (deposit vs suspension feeding) by providing clarity on the mechanism by which these deposit feeders may impact pelagic resources. Specifically, the Venter et al. (2020) study indicates that biofiltration, i.e. the adsorption of pelagic phytoplankton cells on the burrow walls, by the sandprawns and their burrows, underpins the observed influence of sandprawn presence on the overlying water column. This mechanism (biofiltration) likely explains the main effects recorded in my study, with phytoplankton cell counts being reduced in the presence of sandprawns, and size classes shifting from pico- to nano-dominance.

The findings emanating from my experiment regarding the differential impact of macrofauna on bacteria and microalgae are similar to those of Pillay et al. (2009), though the functional characteristics of the predictor organism and response assemblages differ. Specifically, grazing by starfish *Parvulastra (Patiriella) exigua* on sandflat communities resulted in a reduction of benthic microalgal biomass, but a facilitation of bacterial abundance. The authors suggested that the differential impact on microalgae vs bacteria is most likely due to the relative regeneration rates and nutritional value of these resources. Dividing 1 to 6 times a day, bacteria are known to have considerably faster regeneration rates than microalgae (Hayes, 1964 as cited in Pillay et al., 2009). They are therefore able to withstand a greater grazing pressure, and rapid cell division allows bacterial populations to react faster to any disturbances to their surroundings (Hargrave, 1970). Whilst gut content analyses have identified bacteria as food sources for deposit feeders such as *K. kraussi* (Harris et al., 1991), bacteria have typically been viewed as a minor component of their diets (Pillay et al., 2009). The importance of bacteria in the diets of *K. kraussi* therefore remains unclear. Although studies on functionally similar endobenthic deposit feeders are not available (to my knowledge), it is possible that the differential impact of sandprawn treatments on phytoplankton vs bacteria observed in this study are due to similar mechanisms as described above in Pillay et al. (2009). The starfish *P. exigua* and sandprawn *K. kraussi* are functionally different macrofauna, with starfish being epibenthic grazers and sandprawns endobenthic deposit feeders (Branch and Pringle, 1987; Pillay et al., 2009). Nevertheless, comparisons between the starfish study and the present one can be drawn given that both animals exploit a common trophic resource i.e. microalgae, and consequently cause their removal from their respective habitats.

Changes in microbial or prokaryotic assemblages in response to burrowing thalassinidean species have been reported previously (e.g. Laverock et al., 2010; Papaspyrou et al., 2005), although these studies have focused on how burrow microhabitats elicit distinct effects on sedimentary microbial assemblages. To date, research in the field of prokaryotic assemblages and endobenthic macrofauna have focused on the benthic assemblages and processes, with little consideration of the overlying water column. While in my study it was qualitatively observed that sandprawns could be influencing pelagic prokaryotic assemblages (Figure 7), this was not supported statistically, as evident in the non-significant impact of sandprawn treatment levels on total heterotrophic bacteria and *E. coli* (Table 4). Nevertheless, the observed results suggest a need for further research in this area, especially given qualitative trends indicating subtle shifts in prokaryotic assemblages induced by increasing sandprawn density (Figure 7), along with cases in which responses were marginally non-significance (e.g. *E.coli*). Whilst the technique used to assess the prokaryotic structure was able to discriminate cells on the basis of the amount and type of nucleic acids present (Gasol and del Giorgio, 2000), specialized analyses such as gene sequencing (e.g. Laverock et al., 2010) would provide a more comprehensive analysis of how prokaryotic assemblages are changing in response to sandprawns.

It was hypothesized that sandprawns would differentially impact groups of biotic particles over others in the water column. This was observed for phytoplankton, as indicated by the larger effect magnitude of sandprawns on picoplankton relative to nanoplankton. The findings regarding the decline in total phytoplankton agree with Venter et al. (2020), who reported a decline in pelagic microalgal biomass by roughly 50% in the presence of sandprawns. With higher levels of microalgae recorded on burrow walls, and considering the deposit-feeding nature of sandprawns, Venter et al. (2020) ascribed the observed filtration effect due to adsorption of microalgae on burrow walls. This mechanism is also the most likely the driver behind the differential effects on pico- and nanoplankton recorded in my study. Whilst plankton particle adsorption onto burrow walls has not been studied in endobenthic crustaceans yet, analogous mechanisms that drive particle selection have been comprehensively studied for suspension and deposit feeding bivalves. These studies generally attribute particle selection to morphological characteristics specific to filtration appendages of bivalves (e.g. Safi and Hayden, 2010; Ward and Shumway, 2004). For example, it was found that particle selection by suspension-feeding bivalves can occur through interactions between the carbohydrates present on the surface of microalgal cells and lectins lining the filtration organs. It has been suggested that the carbohydrates act as recognition molecules for the lectins, and this allows for selection of certain particles. Additionally, Rosa et al., (2017) demonstrated that various surface properties of microalgal cells drive selection by two bivalve species. It was found that wettability (capability of liquid to maintain contact with a solid surface) was the most important characteristic in predicting particle selection by the blue mussel *Mytilus edulis*, and surface charge was the most important for the eastern oyster *Crassostrea virginica*. These ideas and findings are applicable to burrow walls of sandprawns and other endobenthic crustaceans, since burrow walls and their biochemical constituents such as extracellular polymeric substances, likely function as adhesive traps for particulate organic matter (Chen et al., 2020). This is an idea put forward by Kinoshita et al. (2003) for suspension feeding endobenthic crustaceans, but is applicable to deposit-feeders given the amount of time spent on water pumping through burrows (Volkenborn et al., 2010). It is possible that characteristics of burrow walls (e.g. organic coating) and interaction with pelagic material drive the retention of certain phytoplankton cells, perhaps for the reasons mentioned above. However, further research is required to improve our understanding the role of burrow traits in phytoplankton selection.

While burrow wall traits are likely the primary mechanism driving differential selection of phytoplankton cells, it is possible that consumption of phytoplankton of particular size ranges by sandprawns may be a secondary driver of the effect of sandprawns on picoplankton recorded in my experiment. While studies on size-based shifts in pelagic microalgae due to endobenthic crustaceans are non-existent (as far as I am aware), size-based discrimination of benthic particles has been reported for other thalassinidean crustaceans. When examining the behavior of *Upogebia omissa*, it was reported that these crustaceans select smaller particles when feeding. Detailed behavioral observations indicated that smaller particles were collected by resuspending the sediment and keeping the 1st and 2nd pereiopods separated to allow larger, heavier particles to pass through. Individuals would then clasp their pereiopods together to form a setal net on which smaller particles were retained. Food was then carried towards the mouth with the aid of the 3rd maxillipeds (Coelho et al., 2000). Given that these shrimp are considered both deposit and suspension feeders, and that the technique described involves the resuspension of sediment and not suspension feeding, it is possible that in my experiment, the technique described for *U. omissa* or a variation thereof, is utilized by *K. kraussi* as a secondary mechanism for preferential selection of picoplankton.

The potential selection of phytoplankton on the basis of size by *K. kraussi* (picoplankton in my experiment) is in agreement with predictions from the optimal foraging model for deposit feeders (Taghon et al., 1987), which predicts that the smallest particles should always be ingested. This is predicted in view of the higher surface area: volume ratio of smaller cells; smaller particles theoretically represent more food per unit volume and are energetically more profitable for deposit feeders (Taghon et al., 1978; Stamhuis et al., 1998). This has been demonstrated in other callianassid species (*Callianassa subterrenea, Biffarius arenosus* and *Trypea austaliensis*) that have also been described as optimal foragers (Stamhuis et al., 1998; Stapleton et al., 2001). Comparisons of sediment grain size between the stomach/gut contents and surrounding sediment of *C. subterrenea* revealed that these deposit feeders actively reject particles larger than 70 μm and ingest 12-30 μm sized particles in the largest quantity (Stamhuis et al., 1998). This was also found for *Biffarius arenosus* and *Trypaea australiensis,* where most of the particles ingested were smaller than the majority of available particles in the habitat (Stapleton et al., 2001).

The fact that *Prochlorococcus*-like algae and cryptophytes were not significantly impacted by sandprawns suggest that they are not selectively adsorbed onto burrow walls or consumed preferentially by sandprawns off burrow walls. Alternatively, it is possible that they are, in fact, being removed from overlying waters but are able to withstand such losses through compensatory mechanisms. Members of the genus *Prochlorococcus* are known to have a competitive advantage regarding the uptake of nutrients such as nitrogen, largely due to their small sizes (Chisholm, 1992). Furthermore, in marine waters, they reach reasonably high divisions in the order of 1 division per day (Partensky et al., 1999). These properties could provide them with a competitive advantage in the environment over other microalgal producers, causing them to surmount potential top-down process, including those induced by sandprawns due to a filtration and/or grazing effect. Similarly, cryptophytes could also have traits that compensate for potential top-down disturbances to their community caused by sandprawns. For example, certain cryptophyte species are known to be mixotrophic (e.g. Marshall and Laybourn-Parry, 2002; Yoo et al., 2017), which entails individuals showing plasticity in switching between autotrophy and heterotrophy (Crane and Grover, 2010). Whilst the physiological response of cryptophytes to changing conditions are likely to be species specific (Lewis et al., 2013), it has been suggested that the exploitation of mixotrophy by cryptophyte communities is a mechanism that increases fitness (Marshall and Laybourn-Parry, 2002). This dietary plasticity could confer cryptophytes with a competitive edge over other phytoplankton groups in my experiment and could explain the non-significant impact of sandprawns on this community.

Study implications and conclusion

While my study has shed significant light on processes initiated by endobenthic ecosystem engineers, it is important that findings be interpreted in the context of study limitations. Due to the global COVID-19 pandemic and associated national lockdowns instituted to curb viral spread, it was not possible to obtain field data to validate findings from my mesocosm experiment. Despite this limitation, results obtained are meaningful representations of processes occurring in the Zandvlei Estuary (the focal system in this study) and other estuarine habitats dominated by *K. kraussi*. The majority of South Africa's estuaries are temporarily open/closed systems (James et al., 2007), which are typically closed for significant periods of time (Perissinotto et al., 2010). This is largely due to South Africa's semi-arid climate, but is compounded by the increasing demand and abstraction of fresh water for human use (Breen, 2001). Under closed conditions tidal action is generally non existent, therefore water flow or movement is at a minimum. Furthermore, geomorphological characteristics of South African estuaries are such that these systems are shallow and rarely exceed depths of 2m (Harrison, 2004). These combinations of factors create conditions under which filtration effects by sandprawns are likely to manifest in the water column of systems similar to that of Zandvlei, given the dominance of shallow estuarine conditions with minimal water flow for extended periods of time.

Additionally, laboratory mesocosm experiments are key scientific tools used to study ecosystems and processes nested therein, in an attempt to create models to explain natural complexity (Howe et al., 2004). *Ex situ* experimentation is especially useful for assessing otherwise intractable (outside of a mesocosm) research problems. Whilst mesocosms are never exact replicas of natural environments, these simplified models allow for rigorous control of conditions outside of predictor variables of interest, and have played a key role in the development of ecological theory by acting as a foundation for further investigation (Benton et al., 2007). Mesocosm experiments can additionally accelerate understanding of environmental problems and ultimately facilitate the development of practical solutions.(Benton et al., 2007). Some ecological questions cannot be answered currently without mesocosm experiments due to logistical impracticalities. Such is the case in my study, where effects of sandprawn density on phytoplankton assemblages and other pelagic microbes cannot be rigorously addressed to demonstrate causality *in situ*. Lastly, some of the results obtained in my experiment are in agreement with Venter et al., (2020), whose mesocosm experiment results indicated a decline in chlorophyll-a in response to sandprawns presence.

Findings from this study, albeit limited due to its *ex situ* experiment nature, may have implications that are broader than endobenthic ecosystem engineering and bentho-pelagic coupling, and present opportunities for new research questions. This is the case in the field of carbon sequestration research. Specifically, the amount of carbon sequestered from the atmosphere by phytoplankton is thought to increase with phytoplankton size, and is therefore lowest in ecosystems dominated by picoplankton (Richardson, 2019). Although microplankton are thought to account for most oceanic production, it has recently been suggested that nanoplankton play a more important role in net community production than previously thought (Juranek et al., 2020). Lomas and Moran (2011) found that both picoplankton and nanoplankton contribute significantly towards carbon export in the Sargasso Sea, but nanoplankton accounted for the highest proportions of total particulate organic carbon (POC) exported. It was found that nanoplankton contributed to total POC export in roughly equal proportion to its autotrophic biomass, as opposed to e.g., cyanobacteria, whose contribution to POC export was significantly lower at roughly one tenth of its autotrophic biomass. Similarly, Juranek et al. (2020) recorded elevated rates of net community production across the North Pacific subtropical-subpolar transition zone, which is dominated by nanoplankton. Given these points, the sandprawn-induced shift to larger nanoplankton in my experiment may indicate an increase in the amount of carbon sequestered in sandprawn dominated habitats, by virtue of a shift from picoplankton dominance. This, of course, is based on the untested assumption that findings recorded in my experiment can be extrapolated to real world conditions. However, this issue is worth investigating in future research, given the potential for sandprawns to indirectly increase ecosystem blue carbon value i.e. the organic carbon captured and stored by coastal ecosystems (Macreadie et al., 2019). Blue carbon is of particular global interest since it focuses on the potential to mitigate the impacts of climate change whilst simultaneously achieving ecological benefits such as improved coastal health (Macreadie et al., 2019). However, the idea that sandprawns could increase the amount of carbon sequestered from the atmosphere should also be viewed in the context of the sandprawn-induced decline in phytoplankton recorded in my experiment and that of Venter et al. (2020). By decreasing the total amount of phytoplankton in the water column, carbon sequestration rates could be reduced in sandprawn habitats, though there might be some unknown effect of a community switch to nanoplankton dominance (e.g. Basu and Mackey 2018).

Until recently, research in the area of endobenthic crustacean ecosystem engineering has largely been confined to understanding effects on benthic ecosystem properties, processes and biotic assemblages including microbial activity, sediment granulometry or meiofaunal community composition (e.g. Laverock et al., 2010; Stamhuis et al., 1998; Stapleton et al., 2001). Whilst the significance of bentho-pelagic coupling by endobenthic deposit-feeders has been recognized, it has largely centered around the flux of materials, especially nutrients, between benthic and pelagic environments. Findings of my experiment build on the work of Venter et al. (2020), that added novel perspectives on existing bentho-centric paradigms by reporting the clear impact of sandprawn filtration on chlorophyll-a. The findings of my mesocosm experiment are an extension of the Venter et al. (2020) study, indicating the importance of endobenthic macrofauna in driving bentho-pelagic processes by causing subtle shifts in phytoplankton community traits beyond biomass declines. By demonstrating that biofiltration by sandprawns disproportionately impacts certain groups, this study provides novel insights into the mechanisms by which these engineers may alter coastal ecosystems. Size-based shifts in phytoplankton assemblages (or any pelagic group) has not previously been attributed to deposit feeding engineers and highlights the need for further research in this area to understand the mechanisms driving particle retention. This is especially important when viewed in the context of climate change, where understanding the processes that determine phytoplankton and prokaryotic communities are especially important for predicting the future functioning of coastal ecosystems under projected climatic conditions (Laufkotter et al., 2015).

In a wider context, my findings indicate that endobenthic crustaceans such as sandprawns could be considered as effective nature-based solutions in coastal ecosystems. This is due to their role in improving water quality by limiting phytoplankton abundance and preventing the accumulation of nitrite in the water column, which ultimately improves ecosystem resilience against the global challenge of eutrophication. This is especially important given the global distribution of endobenthic axiid and gebiid crustaceans (Pillay and Branch, 2011). The results of this study encourage the protection of sandprawns and functionally similar species in their respective environments. Their habitat conservation and protection from e.g. bait collection is critical, especially given the significance of their ecosystem services to both human and ecosystem health.

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6. APPENDICES

6.1 Appendix A

A brief overview of flow cytometry

Once a sample is run through a flow cytometer, the cell suspension is injected into a stream comprised of a sheath fluid. The principles underlying hydrodynamic focusing position cells in single file within a narrow stream. Consequently, individual cells are passed through an excitation source that is typically a laser beam of an appropriate wavelength. Once the laser beam contacts the cell, light is scattered in different directions and any fluorescent molecules present on the cell's surface (autoflourescent or induced fluorescent molecules) will emit light at a given wavelength. These data are collected by a complex system of filters and detectors and are converted into electrical signals that are ultimately processed by computers and prepared for analysis. The light scattering properties provide information on cell size and internal complexity, whereas fluorescent data enable the identification of fluorescently labelled cells and the investigation of population compositions based on pigment emissions (Bergquist et al., 2009; Manti et al., 2012; Gong et al., 2017).

6.2 Appendix B

Table 6: Mixed effects models. P-values are available for generalized mixed effects models fitted with beta distribution. Linear mixed effects models were fitted to logit transformed variables (cryptophytes and Prochlococcus-like algae), and do not generate p-values. Significant results are displayed in bold.

		Estimate	**Std. Error**	**z value**	**Pr (> |z|)**
	(Intercept)	-1.9730	0.2203	-8.956	**<0.0001**
Phytoplankton	50%	-0.2600	0.1058	-2.457	**0.014**
	100%	-0.7660	0.1189	-6.444	**<0.0001**
	(Intercept)	-3.90881	0.07437	-52.56	**<0.0001**
Nanoplankton	50%	-0.45818	0.0974	-4.69	**<0.0001**
	100%	-0.08743	0.08821	-0.99	0.322
	(Intercept)	-1.9781	0.2088	-9.472	**<0.001**
Picoplankton	50%	-0.1250	0.1908	-2.062	**0.0392**
	100%	-0.3934	0.1828	-0.684	0.4941
				t value	
	(Intercept)	-1.2370	0.3302	-3.747	**0.00707**
Cryptophytes	50%	0.2771	0.1611	1.720	0.09116
	100%	-1.030	0.1611	-0.640	0.52518
	(Intercept)	-4.4486	0.2640	-16.849	**<0.0001**
Prochlorococcus-like algae	50%	0.1054	0.2500	0.422	0.687
	100%	0.1012	0.2500	0.405	0.675
	(Intercept)	-1.8412	0.1930	-9.538	**<0.0001**
Total Heterotrophic Bacteria	50%	-0.1379	0.2267	-0.609	0.543
	100%	-0.2564	0.2345	-1.093	0.274
	(Intercept)	-4.7517	0.3520	-13.499	**<0.0001**
E.coli	50%	-0.3083	0.2537	-1.216	0.224
	100%	0.2971	0.2400	1.238	0.216