Interactive effects of temperature, trophic status and sandprawns (*Kraussillichirus kraussi*) ecosystem engineering on microplankton community structure

Oluwasegun O. Ogunnusi (OGNOLU021)

Supervisor: Associate Professor Deena Pillay

Co Supervisor: Dr. Nicole Dames

Dissertation presented for the degree of Master of Science

Department of Biological Sciences

University of Cape Town

February 2024

PLAGIARISM DECLARATION

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.

Signed: Signed by candidate Date: 26 January 2024

ABSTRACT

Global warming and eutrophication are leading global change stressors that threatened coastal environments. Water quality degradation has gained scientific attention in particular, especially from the perspective of finding nature-based solutions (NBS) to microbiological proliferation. Endobenthic crustaceans (including Southern African sandprawns: *Kraussillichirus kraussi*) and microorganisms have proven to be an effective nature-based tools to improve and monitor coastal water quality respectively. Previous research has proposed that the below-ground burrow systems of *K. kraussi* function as a biofiltration system, whereby active bi-directional water pumping by sandprawns causes phytoplankton cells to be adsorbed onto the walls of their burrows, thus assisting in limiting phytoplankton proliferation associated with eutrophication. However, little quantitative research has been done on the hypothesized biofiltration effects of sandprawns and their burrow systems on microbial assemblages inhibiting the overlying water column. This rationale formed the basis of my research, which aimed to experimentally quantify the effects of common sandprawns on pelagic bacterial assemblages in a global change context. This was achieved using a six-week laboratory mesocosm experiment, with each experimental mesocosm being half-filled with water and sediment collected from Zandvlei Estuary and divided into three treatments of varying natural sandprawn densities (0% (control), 50% and 100%), temperature (13.6^{o}C and 29.4^{o}C) and eutrophication (meso- and eutrophic) levels. At the end of the experiment, water column DNA was collected through a size-fractionated filtration process for DNA extraction followed by 16S rRNA amplicon analysis and metagenomic sequencing was carried out. Results indicate that the bacterial communities from the experiment were dominated by Proteobacteria (84.65%), but Margulisbacteria (0.002%) was the least abundant. Multivariate analysis demonstrated that the interactions between (1) sandprawn density and trophic state and (2) sandprawn density and temperature significantly explained bacterial variance. Notably, increasing sandprawn abundance induced

a pelagic bacterial assemblage shift, with the most discriminating taxa being *Citrobacter freundii* and the Enterobacterales. A significant sandprawn-induced reduction in the abundance of *Escherichia-Shigella coli* was irrespective of the nutrient and temperature levels; these bacteria are indicators of coastal water quality and human health risk. Ecosystem engineering by *K. kraussi* thus reduced abundance of many waterborne pathogens (*E. coli*., *Enterobacter* spp.), but other taxa became more abundant with increasing sandprawn density. Common sandprawns and their habitats should be conserved to assist in averting the proliferation of some waterborne pathogenic microbes (*E. coli*). This study supports the use of sandprawns (and similar endobenthic engineers) as a NBS to control the proliferation of waterborne bacteria. However, further research is necessary to understand the consequences of increasing abundances of some bacterial taxa with sandprawn density.

ACKNOWLEDGEMENTS

This day would not have come to fruition without the intervention and magnanimous of **Mastercard Foundation Scholarship (MCFS)**. I could not have wished for a better scholarship rather than MCFS to have sponsored my master's degree in the best university in Africa, **University of Cape Town (UCT)**. I am deeply grateful for the fully funded financial support. The day I received an acceptance email from my supervisor, **Associate Professor Deena Pillay**, was one of my happiest moments. I am extremely thankful to him for accepting to take me in as one of his master's students. During my research, his constructive criticisms, tutelage, and mentorship were second to none in achieving the research set goals. I can boldly say these trainings I have acquired under his supervisions and teachings have got me closer to my long-term goal of becoming a renowned marine ecologist. To my co-supervisor, **Dr. Nicole Dames**, I am deeply grateful for her consistent support ever since she joins the study till the very end. Her invaluable trainings, teaching, contributions, and constructive criticisms birthed the success of this study. More so, I become more proficient with R-statistics with her help. She is a support system indeed. I would like to extend my appreciation to the staff members of the department of Biological Science **Dr. Emma Rocke** for giving me access to the laboratory space use for DNA extraction, **Bongani Tom** for the equipment provided to facilitate my research, **Anthea Stain** for helping to purchase and order of research equipment and materials needed. To my colleagues, Cheryl Thomas, Abioye Oyatoye and Carla de Cerff who encouraged and assisted me generally, thank you all for making masters an enjoyable experience. Lastly, I would like to extend my deepest gratitude to my parents (Mrs. Fatimo Ogunnusi and Mr. Dayo Ogunnusi), siblings (Abiodun, Doris, Temitope and Samuel Ogunnusi) and friends (Oyatoye Abioye, Oluwaseun Ayinla and Oladimeji Akinwale) for their encouragement, support, endless care and push.

TABLE OF CONTENT

1. INTRODUCTION

Coastal ecosystems are regions of notable ecological diversity and biological productivity (Filipuci, 2011). 17% of humans live in coastal areas less than 10 m above the sea level, but this region accounts for 5% of the earth surface. Globally in addition, two-thirds of the human population lives in less than 100 km of a coast (MEA, 2005; Inman and Brush, 1973; UN, 2017; Hossain et al., 2020). Coastal biotopes such as salt marshes, shellfish reefs, coral reefs, seagrass beds, tidal freshwater and mangrove wetlands are notable characteristics of temperate and tropical coastlines (Waltham et al., 2020). Highly productive coastal ecosystems provide a major benefit to human populations; which results in per capita income being higher in coastal countries when compared to landlocked countries (Gallup et al., 1999; He et al., 2014).

Coastal areas provide essential services to human population and support livelihoods, particularly in terms of supplying protein to billions of impoverished people across the globe. Coasts serve as fish nurseries, offer protection of coastal watersheds, facilitate carbon storage, and are habitat to a wide range of marine animals and plants (Kullenberg, 2010; De Battisti, 2021). They also support vital socio-economic activities such as fisheries, tourism, and agriculture (Flo et al., 2011). Over the years, these systems have been recognized for their provision of cultural services including aesthetic services, whereby people can enjoy and appreciate the natural environment for tourism and recreation purposes (Bowler et al., 2010). About 60 – 68% of global services provided by coastal and marine ecosystems were valued at $141 trillion per year (Costanza et al., 2014).

Currently though, global change is of concern given the negative consequences expected, particularly for coastal resilience through rise in sea level and increasing strength and frequency of storms (Jevrejeva et al., 2012; Levermann et al., 2013; Woodruff et al., 2013; IPCC, 2019; De Battisti, 2021). With high precision, the IPCC (2019) confirmed that a 0.32m increase in

sea levels between 1970 and 2015 within coastal regions was strongly attributed to anthropogenic global change, which was associated with an increase in sea thermal expansion and loss of glacier mass. In addition, increasing human population and activities along coastal areas, (Neumann et al., 2015), subsequently exacerbate global change stressors acting upon coastal systems. Stressors from both climate change and human pressure pose a significant threat to the integrity and resilience of coastal ecosystems, impinging on their ability to deliver their ecosystem services (Hanley et al., 2020). Important services provided include protection of coastal shoreline from storm damage or erosion, as well as transformation of organic waste and carbon sequestration (Gaylard et al., 2020).

Coastal ecosystems are known to be vulnerable and sensitive to environmental change due to ocean and land interaction (frequent exchange of energy and materials) as well as densely population of human along these areas (Lu et al., 2018). Hence, land-based human activities and climate change poses a significant threat to the coastal ecosystems. These challenges or threats include coastal eutrophication, heavy metal pollution, coral reef degradation, coastal exploitation and hypoxia (Halpern et al., 2008; Doney, 2010; Hoegh-Guldberg and Bruno, 2010; Jennerjahn, 2012; Statham, 2012; Halpern et al., 2015). Eutrophication refers to the excessive loading of nutrients such as nitrogen (N), phosphorus (P), and carbon (C) into coastal water bodies (Waycott et al., 2009; Romero et al., 2006; Short and Wyllie-Echeverria, 1996). The processing/breakdown of these nutrients involves different inorganic and organic compounds both in the granulated and dissolved form (Jarvie, 2012), and their interaction with biological (e.g benthic fauna), physical (mixing, tidal flushing, stratification, Robins et al., 2014), and chemical (e.g pH) constituents of the coastal ecosystem. However, excessive nutrient loading is a leading global environmental threat to the ecology of coastal water bodies (Li et al., 2019).

Between 2008 and 2011, 22.6% and 36.8% of rivers and lakes were grouped as eutrophic (20 – 30 µg.l^{-1} chlorophyll-a concentration) or hypertrophic (>30 µg.l^{-1}) (European Commission (EC), 2018). Similarly, in South Africa, between the years 2002 and 2012, a higher percentage (78%) of the largest coastal water bodies were recorded and grouped as eutrophic (Matthews and Bernard 2015). Over the last decades, the escalation of eutrophication in coastal areas has been reported to be primarily driven by growing human populations or activities along its margins, including agriculture, urbanization, fishing, and fertilizer run-off (Ferdie and Fourqurean 2004; Lee et al., 2007 in Mvungi and Pillay, 2019). Agricultural run-off is considered to be the major source of nutrient (nitrogen) pollution into several coastal environments (Duce et al., 2008; Howarth, 2008; Hale et al., 2015). This activity has resulted in critical consequences for ecological integrity and biodiversity in coastal ecosystems (Cloern et al., 2016), mainly the stimulation of phytoplankton production, coastal acidification and hypoxia, which in turn increase microbial respiration (Cai et al., 2011; Gobler and Baumann, 2016). The benthic community consequently deteriorates and decomposes, resulting to the microorganismal growth, which in return depletes oxygen, resulting in anoxic or hypoxic condition (UNEP, 2001; Van Ginkel, 2011). Also, excessive nutrients load into coastal ecosystems alters the supply of energy to upper trophic levels within food webs and the composition of algal species that form the base of food webs (Cloern, 2001).

Global warming is another prominent stressor in coastal systems and is basically driven by an increase in atmospheric greenhouse gas levels (Harley et al., 2006; Orth et al., 2006; IPCC, 2007; Waycott et al., 2009). The heating rate of the planet is alarming, with a prediction of global temperature to increase by 2 - 4°C at the year 2100 (IPCC, 2007; Doney et al., 2012; Mvungi and Pillay, 2019). Changes such as increasing freshwater input, rising sea level, decreased sea-ice extent and altered ocean circulation are linked to elevated temperature in coastal ecosystems (Doney et al., 2012). In addition, changes to ocean circulation with

increased temperature can reduce the concentration of the sea surface oxygen (Keeling et al., 2010). Currently, continual occurrences of these events have been recorded and eventually may exceed the tolerance of organisms to adapt if immediate climatic mitigation promulgations are not enacted. Simultaneously, physiochemical changes in the coastal ecosystem will increase over the next decades (National Research Council, 2011).

All aspects of organismal performance are virtually influenced by temperature (Seidel et al., 2023). According to Doney et al. (2012), changes in coastal ecosystem temperature may modify several aspects of organism ecology/biology including demographic traits (e.g productivity), physiological functioning and behaviours, leading to changes in population sizes, size structure, and spatial range. Shifts or changes in coastal microbial community structure in response to climate change (e.g. warming) have can have a significant effect on ecosystems functioning and marine geochemistry (Seidel et al., 2023). However, structural changes to microbial communities remains unclear in response to climate change (Seidel et al., 2023). Microbial communities within coastal regions are central to nutrient cycles and marine energetics and may act as primary trophic resources and degraders of coastal organic matter. Microbes play a key role in regulating fluxes and production of greenhouse gases such as nitrous oxide, methane, and dimethyl sulfide (Poth and Focht, 1985; Cavicchioli, 2019; Xiao et al., 2017; Zhang et al., 2019). Also, global warming is expected to favour microplanktonic organisms when compared to the macro sizes as thereby alter biogeochemical fluxes such as exportation of particles (Morán et al., 2010). Increased ocean warming and acidification are predicted to increase the extracellular dissolved organic matter released by phytoplankton, which could lead to changes in the microbial loop, possibly causing an increase in the production of microorganisms to the detriment of higher trophic levels (Thornton, 2014). Additionally, warming can weaken iron limitation that nitrogen-fixing cyanobacteria face. This could have a significant effect on the supply of new nitrogen to ocean food webs (Jiang et al.,

2018). However, environmental microbial compositional changes must be carefully interpreted and quantified in response to global change and environmental stressors (Webster et al., 2018; Cavicchioli, 2019).

Whether eutrophication or global warming act independently or interactively, they pose a serious threat to coastal ecology and biodiversity, including structurally complex coastal sedimentary ecosystems (Waycott et al., 2009; Harley et al., 2006; Wyda et al., 2002; Borowitzka et al., 2006; Mvungi and Pillay, 2019). These systems are typically dominated by habitat-forming or bioturbating species, which may also be keystone ecosystem engineers. (Pillay et al., 2010; Hughes et al., 2009; Jones et al., 1994).

Ecosystem engineers play a significant role in the structure and functioning of coastal sedimentary systems as they can physically modify biotic and abiotic matter and thereby alter the availability of resources for other sympatric organisms (Reise, 2002; Bouma et al., 2009; Pillay and Branch, 2011; Pillay et al., 2012; Pillay, 2019). Endobenthic bioturbating crustaceans are arguably the most dominant engineers globally, and locally in South African coastal sedimentary systems (Siebert and Branch 2005a&b; Qwabe, 2019; Holthuis, 1980; Moyo, 2014; Venter, 2019). South African sandprawns (*Kraussillichirus kraussi*) are highly influential ecosystem engineers due to features such as (1) burrows extending up to 1m depth that is unique relative to other ecosystems engineers (2) occurring at a density of about 150 – 200 individuals.m^{-2} in coastal ecosystems and (3) having some of the highest known per capita sediment reworking rates (Cadée, 2001; Berkenbusch and Rowden, 2003; Pillay and Branch, 2011; Pillay, 2019). These features of sandprawn engineering result in noticeable and complex modifications to coastal benthic systems (Pillay et al., 2012).

Bioturbation activities by sandprawns and other endobenthic crustaceans significantly affect benthic fauna and flora, including macrofauna, meiofauna (Branch and Pringle, 1987),

seagrasses, and bacteria (Townsend and Fonseca, 1998). The alteration of the sedimentary ecosystem through the movement of particles and solute by burrowing sandprawn forms one of the major ways driving the development of several microclimates within the ecosystem (Pillay and Branch, 2011). This modification creates diverse habitats for microorganisms including sulfate-reducing groups, anaerobic and aerobic nitrifying species (Bird et al., 2000; Kinoshita et al., 2003). Additionally, the specific retention of organically, fine rich sediments by these ecosystem engineers or the burrow walls stabilization through plant material or mucopolysaccharides, could enhance microbial activities (Aller and Aller, 1986; Aller, 1988, Reichardt, 1988; Steward et al., 1996).

Studies have revealed a significant increase in bacterial numbers with depth of burrow linings when compared to the surface sediments inhabited by the sandprawn *K. kraussi* (Branch and Pringle, 1987). Sediment rapid turnover from burrows to the surface likely limits bacterial colonization on the sediment surface (Pillay and Branch, 2011). Sediments with underdeveloped biofilms are however more susceptible to erosion and prone to resuspension, leading to increased bacterial transport to the overlying water and further diminishing bacterial colonization of sediment (Pillay et al., 2007c). Griffiths et al. (2017) describe or consider this process to be a mechanism that influences benthic-pelagic coupling. Specifically, it describes processes that link the pelagic and benthic components of coastal ecosystems via the movement of nutrients and energy, including suspension feeding and settling of organic matter on the sediment surface. Coastal and estuarine habitats are hotspots for such dynamic interactions where benthic infauna is a vital link connecting the overlying water and benthic systems (Griffiths et al., 2017).

Likewise, environmental temperature plays a key role both in the physiological behaviour and performance of ecosystem engineers, including endobenthic crustaceans (Withers, 1992; Pillay, 2019). Changes in environmental temperature may result in both negative and positive

effects on ecosystem processes (Withers, 1992; Mvungi and Pillay, 2019). This is because endobenthic engineers are predominantly invertebrates that cannot control their body temperature (Withers, 1992). Environmental temperature therefore can significantly influence the bioturbation rate of endobenthic engineers in soft-bottom ecosystems (Pillay, 2019). Maire et al. (2007) reported that elevated temperature increases bioturbation rate of deposit-feeding bivalves' rate. Similarly, bioturbation activity of heart urchins was reported to increase as a result of elevated temperature levels (Hollertz and Duchene, 2001). Activities such as bioturbation, and associated effects of contextual variables such as temperature, will therefore have a consequence for community structure and ecosystem processes, however, little is understood about responses of pelagic systems. In the context of Southern African sandprawns, Venter et al. (2020) showed that sandprawns presence significantly reduce phytoplankton biomass by up to 50% in an urban coastal (estuarine) system. However, little is understood about the bioturbation influence of sandprawns or other endobenthic crustaceans on pelagic microbial community structure in coastal ecosystems.

Estuaries are dynamic environment where the ocean mixes with freshwater; this ecotonal variation contributes significantly to the global marine carbon cycle (Chen et al., 2021; Bauer and Bianchi, 2011). Due to the influx of rich organic matter and inorganic nutrients from rivers, microorganisms in estuaries are more diverse and abundant when compared to the open ocean or freshwater (Hewson and Fuhrman, 2004, Traving et al., 2017). Microbial communities are not only involved in primary production and nitrogen fixation but also primary players in the mineralization and recycling of organic matter (del Giorgio and Cole, 1998). An in-depth understanding of their distribution in estuaries therefore provides insight on their dynamics, processes they mediate (Arrigo, 2005). Also, diverse phylogenetically distinct groups that vary in abundance and distribution are found within complex microbial communities in estuaries (Lisa, 2015). According to Selje and Simon (2003), microbial communities estuaries are well-

studied in the Northern Hemisphere, where typical heterotrophic taxa are found. The dominating phyla includes Proteobacteria and Bacteroidetes, with high occurrences of Alphaproteobacteria at hypersaline condition, and Betaproteobacteria dominating at low salinity (Bouvier and del Giorgio, 2002; Wu et al., 2006; Campbell and Kirchmann, 2013).

Since estuaries are convergences of both freshwater and marine ecosystems, bacteria found in these estuaries are both from the seas and riverine run-off (Matcher et al., 2015). Estuarine bacterial communities are mostly marine species, especially at high tide while at low tide, fresh water taxa from rivers may dominate (Fortunato et al., 2012). Changes in physiochemical parameters often lead to shifts in microbial community structure and microbially-mediated biogeochemical processes within the column of estuarine water and sediment habitats. In addition, sediment microbial community composition is thought to be more complex than the water column but nevertheless significantly influences both the water column and sediment biogeochemical cycles (Huang et al., 2021). It is reported that coastal sediments in urbanized regions have a higher population density of sewage and faecal microbial indicators (Lu and Lu, 2014; Luna et al., 2016) including the presence of antibiotic resistant genes, human pathogens (Liang et al., 2020; Su et al., 2020; Ahmed et al., 2021) and groups associated with particular metabolic processes (Chen et al., 2019). However, in semi-closed coastal ecosystems such as estuaries, (Wang et al., 2007) sediment resuspension may contribute significantly to the variability of microbes and nutrients in the water column (Giani et al., 2001; Pusceddu et al., 2005; Perkins et al., 2014). Besides this, released wastewater is another potential source that supports proliferation of microbes in overlying (pelagic) water (Hylland and Vethaak, 2011).

Coastal areas, including estuaries, support bacterial populations that are larger than in other areas (Pomeroy et al. 1984; Azam and Cho, 1987) due to increased mineral and nutrient inputs such as phosphorus, silicon, and nitrogen from industrial and domestic waste (Nixon 1995; Richardson and Jorgensen, 1996; Mateo-Sagasta et al., 2017). These global human-induced

activities similarly occur in the focal study site of my research – Zandvlei Estuary (Lemley et al., 2019; Venter et al., 2020). Multiple nutrient inputs contribute a mixture of organic/inorganic chemical compounds (Imai et al., 2002; Kontas et al., 2004; Raymond and Spencer, 2015; Wang and Chen, 2018; Malone et al., 2021) and microorganisms, including pathogens (Naidoo and Olaniran, 2014; Buccheri et al., 2019; Numberger et al., 2019), in coastal ecosystems. These inputs have been demonstrated to be pivotal in reducing ecosystems resilience, coastal water quality (Boesch et al., 2001), dissolved oxygen levels (Breitburg et al., 2018), and altering bacterioplankton community structure (Caron et al., 2017; Andersson et al., 2018; Rodríguez et al., 2018; Lønborg et al., 2019). Additionally, the mass mortality and infections of various economically and ecologically important marine species are linked to water-borne pathogenic/disease species of microbes (Sutherland et al., 2010; Ziegler et al., 2016).

The urbanisation and openness of coastal ecosystems facilitates spread of waterborne pathogenic microbes rapidly spread while increases in pollution is ongoing due to insufficient discharge monitoring measures and wastewater treatment (Kalkan and Altuğ, 2020). Particularly, wastewater treatment system inadequacy and poor sanitation play an essential role in pollution and microbial proliferation (Cabelli, 1983; Dufour, 1984; Danulat et al., 2002; WHO, 2003; EPA, 2012). Epidemic diseases emanating from coastal areas could pose a significant threat to human health. A spectrum of illnesses, including skin lesions, acute infections, diarrhoea, and stomach and eye problems induced by pathogenic microorganisms breeding in coastal environments threatens both public health and ecosystems (WHO, 1998; Shuval, 2003; Santoro and Boehm, 2007; Wu et al., 2016). According to WHO (2018) in Kalkan and Altuğ (2020), about 2 million individuals die annually from water-transmitted diseases, with a significant proportion being children under the age of 5 years. Hence, it is vital to routinely monitor coastal water quality from a microbiological perspective to ensure

maintenance of appropriate levels of indicator species and identify conditions that pose a threat to both ecosystem resilience and public health (Kalkan and Altuğ, 2020). Alternatively, nature-based solutions (NBS) have also proven overtime to promote the rehabilitation and conservation of natural or coastal ecosystems. It involves the utilisation of natural processes in artificial, modified, or pristine ecosystems to mimic natural processes that improve water quality. In particular for non-point source (NPS) pollution from agricultural land uses, rehabilitation of ecosystem services can be ensured by adopting NBS to enhance nutrient management and decrease nutrient runoff (Water-UN, 2018, Geronimo et al., 2022). This was evidence in the study of Venter et al. (2020) where endobenthic sandprawns was regarded as a NBS by its effect in reducing phytoplankton biomass by roughly 50% in a eutrophic estuarine system. However, there has been little or no studies of the potential of this specie to be used as NBS to control proliferation of pelagic bacteria. By improving our conception of how endobenthic engineers such as *K. kraussi* influence their ecosystems, a path is facilitated to harness the ecological services and functions they contribute in ways that could assist in reducing further ecosystem degradation and increase ecosystem resilience.

To identify sources of contamination in coastal ecosystems, bacterial indicators are frequently and widely used in ecosystems monitoring (Orel et al., 2022). For example, the abundance and presence of faecal bacteria (e.g enterococci, *Escherichia coli*) are usually detected with a culture-based approach (e.g., Directive, 2006/7/EC) and used to identifying associated health hazards and faecal contamination (Scott et al., 2002). However, this approach is limited in the detection of culturable bacteria that encompass only a small portion of total indigenous bacteria and allochthonous materials (Stewart et al., 2008; Converse et al., 2011). The development of high-throughput sequencing technologies has increased the taxonomic resolution capability in terms of identifying more suitable taxa as indicators of pollution and health risks (reviewed in McLellan and Eren, 2014; Caruso et al., 2016; Cordier et al., 2021).

Objectives of the study

This research aimed to test the relative impact of ecosystem engineering by sandprawn (*K. kraussi*) density, temperature, and eutrophication levels on the pelagic microbial community structure in an estuarine ecosystem. To achieve this goal, metagenomic analysis was used to assess the functional gene composition of pelagic microbial communities experimentally. This approach will provide a broader quantification of the diversity of genes present in estuarine water samples and will further contribute to the growing of knowledge microbial community structure responses to global change stressors.

Specific Objectives

1. To characterize pelagic microbial community structure from estuarine water samples.
2. To determine the main and interactive effects of temperature, eutrophication and sandprawn (*K. kraussi*) density on pelagic microbial assemblages.
3. To investigate the impact of varying temperature, eutrophication, and *K. kraussi* density levels on the pelagic microbial diversity measures.
4. To determine the bacterial taxa that most contribute to variances in pelagic microbial community structure in response to predictors manipulated.

Hypotheses

It is hypothesized that changes in sandprawn density, temperature and trophic state would shape microbial community structure in respect to species abundance, richness, and diversity. This was based on the expectation that each predictor would positively or negatively affect certain microbial taxa depending on their biological traits (e.g., morphology, physiology, nutrition, genetic properties, metabolism, growth, and pathogenesis).

2. MATERIALS AND METHODS

2.1 Ethical clearance

Ethics approval (2019/D1/DP) for the experimental design/methodology was submitted to and approved by the University of Cape Town's Science Faculty Animal Ethics Committee. Permission was obtained from the landowners of Zandvlei Estuary Nature Reserve to collect sediment and sandprawns, *Kraussillichirus kraussi*. A sampling permit was approved from the Department of Environmental Affairs and the Department of Agriculture, Forestry and Fisheries for the collection, transportation, and housing of the sandprawns from the Zandvlei Estuary Nature Reserve for this study.

2.2 Sample collection area

Water, sediment, and sandprawns were collected from the Zandvlei Estuary located near Muizenberg, Cape Town, South Africa (Figure 1). The mean depth of the estuary is 1.4m and is a classified as a seasonal open/closed estuary (Harding, 1994; Venter et al., 2020). Historically, this system has undergone a wide range of anthropogenic modification including construction of a marina in the east, artificial mouth manipulation to control and manage water levels and seasonal dredging of the main channel in the lower reaches (Lemley et al., 2019). The northern part of the estuary is highly eutrophic due to riverine inputs from the surrounding industrial and urban areas, including informal settlements with poor sanitation. This system is kept open mechanically during the high rainfall months (winter season) and opened to the Atlantic Ocean once in a month for about a week during the summer season (Lemley et al., 2019; Venter et al., 2020). The lower reaches are densely populated by sandprawns (density: 176/m^2 – 240/m^2), with the sandprawn habitat accounting for about 4.9% of the total area of this system (Venter et al., 2020).

2.3 Sample collection

The sediment, mesotrophic water and sandprawns (n= ~335), needed for this study were collected from the lower reaches of the Zandvlei Estuary Nature Reserve, while eutrophic water was collected from the upper reaches (Figure 1).

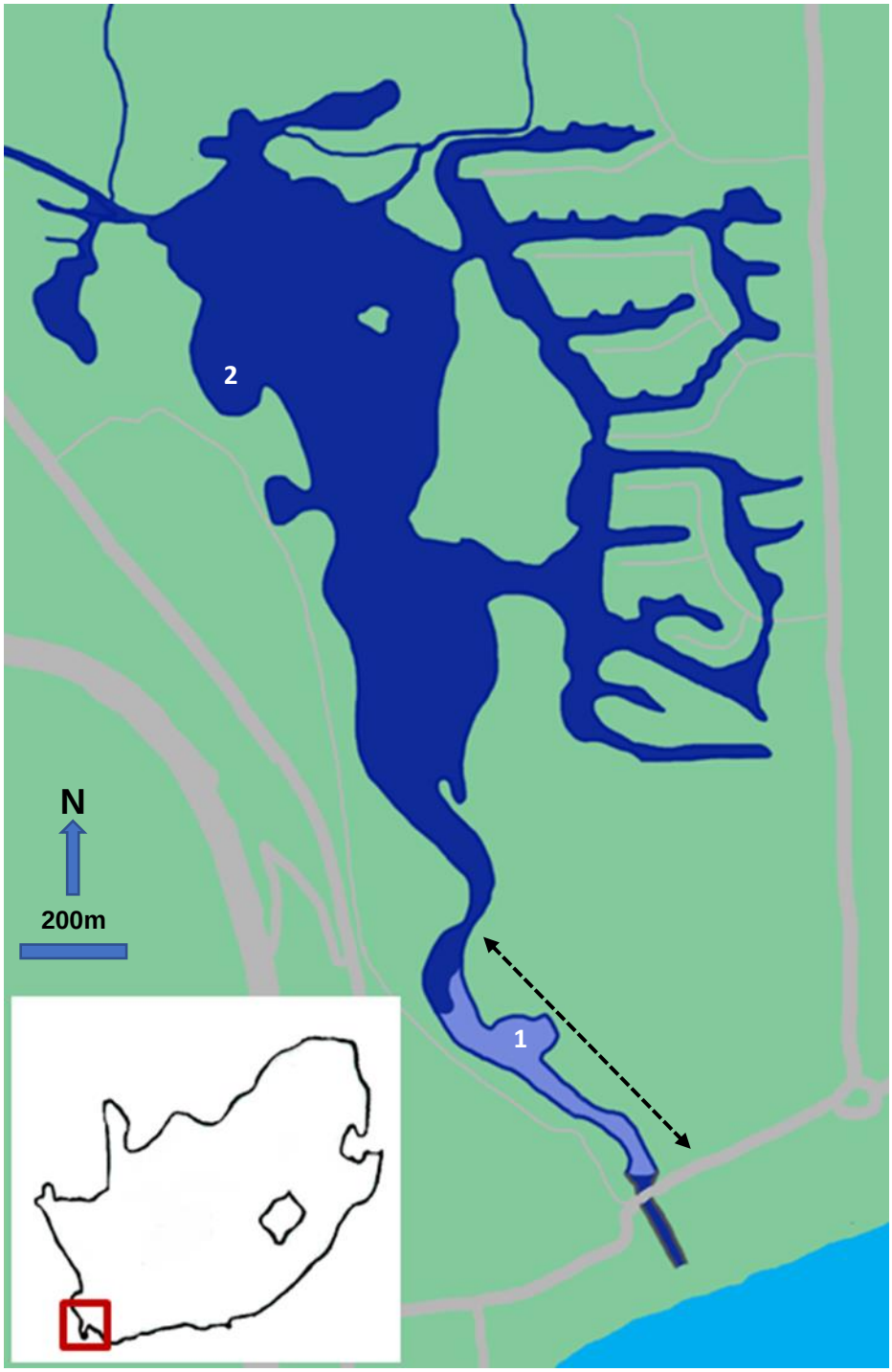

Figure 1: Location of the Zandvlei Estuary within Cape Town, South Africa (inset) showing the positions where samples for the experiment were collected: Site 1 – mesotrophic water, sediment, sandprawns; Site 2 – eutrophic water.

Sediment (primary substrate for the experiment) was collected using a shovel to collect the upper layer (±20cm) and sieved through a 2mm mesh into a bucket. Thereafter, buckets were used to collect water from the estuary for the experiment (mesotrophic water from Site 1; eutrophic water from Site 2; Figure 1). Sandprawns were collected using standard stainless prawn pumps (length = 90cm, diameter = 5cm; Site 1). Egg-carrying females was avoided and only *K. kraussi* between 5mm to 8mm (carapace length) were selected to standardise length. Sandprawns were placed in a moistened layers of newspaper and transported to the aquarium facility in the Department of Biological Sciences, John Day building, at the University of Cape Town (UCT), where the mesocosm experiment was carried out.

2.4 Mesocosm experimental design

A six-week indoor mesocosm experiment was carried out to test the effects of temperature, eutrophication, and varying densities of *K. kraussi* on bacterial assemblages. This aspect was the focus of my MSc dissertation and formed part of a larger project aimed at understanding community-level responses (meiofauna, microphytobenthos, phytoplankton) and ecosystem function (bioturbation, organic matter degradation) to the aforementioned predictor variables.

Three density treatments of *K. kraussi* were used during this experiment: a control with zero (0%) individuals, an intermediate density (50%: 9 individuals per mesocosm), and maximum density (100%: 18 individuals per mesocosm). The maximum density of *K. kraussi* used in the experiment was based on a value of ~200 individuals m^{-2} that can be achieved in South African estuaries and lagoons (Branch and Pringle, 1987). Mesocosms were constructed from glass (8 mm thick) and were 600 mm high, 300 mm long and 300 mm wide).

Naturally eutrophic and mesotrophic water from the Zandvlei Estuary was used in the experiment. This allowed for potential effects of environmental pathogens, especially under eutrophic conditions, to be quantified with respect to sandprawn effects on bacteria. Eutrophic water (chl-a content of >15 µg/l; Harding, 1995; Thornton et al., 1989; salinity = 10ppt; temperature = 19°C) and mesotrophic water (chl-a content of <15µg/l, salinity = 29ppt; temperature = 18 °C) were separately filtered (200µm mesh) into 100L vats and homogenized. Marine salt (Aquamedic) was used to standardize the salinity level of eutrophic water to that of mesotrophic (29ppt). The sieved sediment was added to a depth of 25cm to each mesocosm, followed by the addition of either mesotrophic or eutrophic water (depth = 25cm). The water within each mesocosm was aerated and left for 24hours to settle before taking the initial water quality parameter readings. Sandprawns were introduced into the mesocosms according to their density designations. Each treatment was replicated three times resulting in a total of 36 mesocosms being used in the experiment (Figure 2).

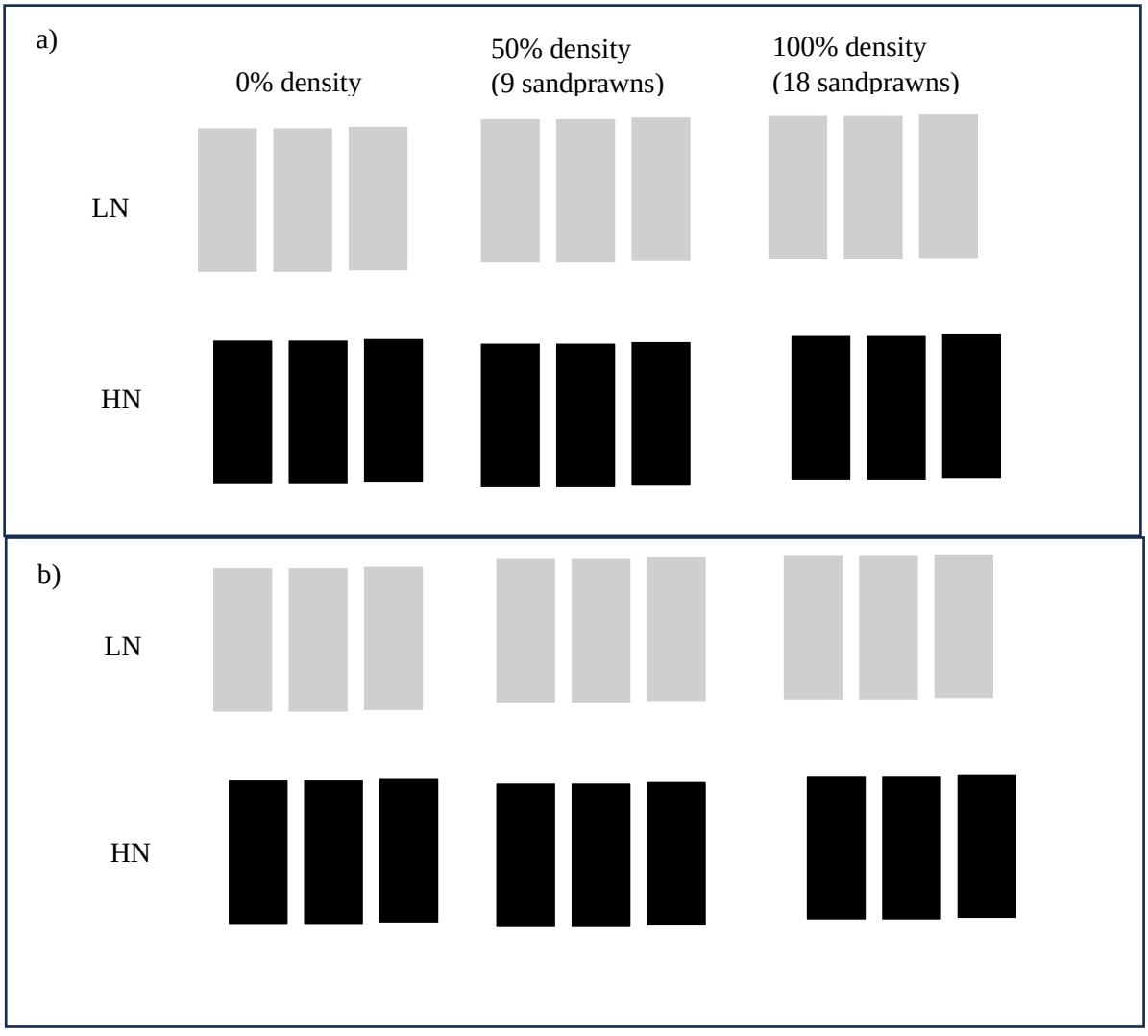

Figure 2: The layout of the mesocosm experiment testing the effect of sandprawn density, low (a) and high (b) temperature, and mesotrophic (LN) and eutrophic (HN) conditions on pelagic bacterial assemblage (note: mesocosm were randomly interspersed to avoid spatial bias and were not clustered by treatment as illustrated).

The maximum temperature (HT) level used in this experiment was based on the mean summer temperature (25.4℃) close to the sandprawn biotope in the lower reaches of the Zandvlei Estuary plus a predicted 4℃ rise in global temperature by 2100 (Harding, 1994; IPCC 2007; 2019). Water and air temperature was set to ~14.5℃; this was the temperature of low temperature (LT) mesocosms in the experiment. High temperature mesocosms (HT – water temperature ~30℃) was achieved by using aquarium heaters (150W – 300W, Eheim Jager and ViaAqua). Water temperature per mesocosm was monitored daily using a digital thermometer.

During the experiment, sandprawn burrow openings were counted on the sediment surface; sandprawns were added to mesocosms in cases where number of burrow openings declined to maintain desired sandprawn levels (Dumbauld et al., 1996).

2.5 Data collection

Water physio-chemical parameters

Water quality parameters such as salinity, pH, temperature, dissolved oxygen (saturation), and electrical conductivity were measured using the YSI 650 Multiparameter Display System. This was carried out on Day 0 (before sandprawn addition) and every 3 days until the termination of the experiment on Day 15.

Nutrients

40ml of surface water was collected from each mesocosm on Day 0 and then every week after that with the aid of a flexible tube fitted onto a syringe to measure concentrations of ammonium

(NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), and phosphate (PO_4^{3-}). Water samples were kept at -20°C until they were analysed using the Hanna Instruments HI 83203 Multiparameter Bench Photometer and reagents as shown in Table1.

Table 1: *Reagents and methods used to measure nutrients concentrations.*

Response Variable	Reagent code	Range	Method
Phosphate (PO_3^+)	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

Phytoplankton biomass and size

Phytoplankton biomass and size data were obtained as part of the PhD project of Cheryl Thomas (2021). These data were used only to understand the role of phytoplankton changes in influencing bacterial assemblages in relation to predictor variables. Phytoplankton biomass was determined per mesocosm (every 3 days) by quantifying chl-a concentration fluorometrically (Turner Designs Trilogy fluorometer) from surface (5 to 10 cm from surface) water samples. Phytoplankton size (picoplankton and nanoplankton) and abundance were determined using flow cytometry (Faculty of Health Science, University of Cape Town) using the same sampling design as indicated for phytoplankton biomass determination.

Sample collection for metabarcoding analysis

After the end of the experiment, a size-fractionated filtration process was used to collect DNA biomass for library sequencing. A 1L water sample from each of the 36 mesocosms was pre-

filtered through a 200µm mesh. Thereafter, each sample was filtered through a 2µm mesh immediately followed by a polycarbonate (PCTE) membrane filter with a 0.2µm mesh size. Then, each filter was snap frozen in liquid nitrogen and stored at a temperature of -80℃ until further processing (Rocke et al., 2020).

DNA Extraction

A Quick-DNA HMW MagBead kit (Zymo Research) was used to extract DNA samples from the snap frozen membrane filter. The protocol was followed according to manufacturer instructions but was modified from step 1 to 9 to maximise quality and quantity of DNA extracted. The filters were cut into a tube containing beads tube and cetyltrimethyl ammonium bromide (CTAB) was added instead of the recommended phosphate buffered saline (PBS). These tubes were beaten in grinding mill three times at 30 Hz for 1.25 minutes. There after the samples were placed on ice for 30 seconds and centrifuged for 10 minutes in 30 µl of lysozyme. After a 2 hours incubation, 20 µl 10% SDS and 10 µl proteinase K was added which was incubated at 55℃ for 1 hour. During the DNA purification process, 800 µl of Quick-DNA MagBinding Buffer was used instead of 500 µl and the DNA was eluted using 10 µl DNA elution buffer for each sample. The extracted DNA were cleaned up using RNA Clean & Concentrator MagBead (Zymo research) according to a modified protocol as follows: 30 µl of RNA MagBinding buffer was added to the eluted DNA and 200 µl freshly made 70% ethanol (EtOH + Nuclease free water) was used. 12 µl of 10mM Tris-HCl pH8 with 50 mM NaCl was added for the elution of the cleaned DNA samples having a total of 12 µl DNA for each 36 mesocosms. A 199 µl working qubit solution plus 1 µl of each eluted DNA were quantified using a Qubit™ 4 benchtop fluorometer according to their instructions (www.thermofisher.com/qubit).

16S gene region amplification

Amplification of the extracted DNA was done using the 16S Barcoding Kit (SQK-RAB204; Oxford Nanopore Technologies, Oxford, UK) containing the 27F/1492R primer set and Master Mix Q5 Taq.

Primer	Sequence	T_m (℃)	Reference	Target
27F	5′-AGAGTTTGATCMTGGCTCAG	50-52	Lane, 1991	Bacteria
1492R	5′-GGTTACCTTGTTACGACTT	47	Stackebrandt &	Universal
	3′-AAGTCGTAACAAGGTAACC		Liesack, 1993	

F = forward, R = reverse

T_m = 64.9℃ + 41 ℃ × [(number of C′s – 16.4)/N], where N is the length of the primer. (Galkiewicz & Kellogg, 2008)

For samples with low quantities of DNA (0 – 4.48 ng/µl), 10 µl input DNA was added with 12.5 µl Master Mix Q5 Taq, 2.5 µl 16S barcode/primer, providing a total volume of 25 µl. For the samples with higher quantities of DNA (5 – 9.42 ng/µl), 2 µl input DNA was mixed with 8 µl nuclease free water, 12.5 µl Master Mix Q5 Taq and 2.5 µl 16S barcode/primer (total volume of 25 µl). Samples were amplified with an initial incubation step at 95°C for 1 min, followed by 37 cycles with a denaturation step at 95°C for 20 s, an annealing step at 55°C for 30 seconds, an extension step at 72°C for 2 minutes and a final extension step at 72°C for 5 min. Amplification was verified using a 1% agarose gel.

Flow cell preparation and Library sequencing

The sequencing of the 16S genes of the samples was done using the Oxford Nanopore Technology Minion Mk1C device and a platform quality check of the flow cell (R9.4.1, FLO-MIN106D) was carried out prior to flow cell priming. For the first run, 8.14 µl containing genetical materials (DNA), sequencing buffer and library loading beads plus 1.86 µl nuclease free water making a final library of 10 µl, while for the second run 9.87 µl of pooled DNA plus

0.13 µl nuclease free water making a total of 10 µl was prepared according to the manufacturer's recommendation and this sequencing mix was introduced in a gentle drop-wise manner via the SpotOn sample port (Mann, et al., 2021). Flow cell (R9.4.1, FLO-MIN106D) was placed into the MinION for the sequencing and controlled using ONT's MinKNOW software. After the first run, which took 10 hours (18 samples), the flow wash kit (EXP-WSHOO4 from Oxford Nanopore Technologies (ONT), Oxford, UK) was used to wash the flow cells and then reused for the remaining samples (19 – 36) for another 10 hours run.

Processing of Sequenced Data

The genetical raw reads (i.e. HDF5 raw signals) were base-called and de-multiplexed before adapter and primer sequence removal with GUPPY (Version; v2.3.5) software (https://community.nanoporetech.com (ONT, Oxford, UK)) producing fastq files. Bioinformatic analysis of the fastq files was done on the University of Cape Town's ICTS High Performance Computer (hpc.uct.ac.za). Firstly, Nanofilt was used to filter and remove long 16S reads length with a quality score of ≤ 9 (https://github.com/wdecoster/nanofilt; Kai, et al., 2019). Then the filtered fastq files were matched with the SILVA v138.1 reference databases (Quast et al., 2013) and analysed with respect to relative abundance, taxa clustering and taxonomic annotation data with EMU (Curry, et al., 2022) software. This generated a relative abundance operational taxonomic units (OUT) table at the genera level. However, some taxonomic hierarchies of the total bacterial assemblages were unidentified. The unidentified taxa were curated, denoted (X) and filled in manually for statistical analysis purpose. The relative abundances and taxonomic annotations were first imported into an excel spreadsheet and converted into a Comma Delimited file (CSV). Thereafter, these files were imported into Rstudio and converted into a phyloseq object. Species with mean relative abundances below 1% were removed from the dataset. The packages phyloseq (McMurdie and Holmes, 2013), dplyr (https://dplyr.tidyverse.org), tidyr (https://tidyr.tidyverse.org), microbiome

(http://microbiome.github.com/microbiome), gglopt2 (Wickham, et al., 2016), vegan (Oksanen, et al., 2022), reshape2 (Wickham, 2007), psych (https://CRAN.R-project.org/package=psych), DESSeq2 (Love, et al., 2014) were used for data handling (Rosenqvist, et al., 2023).

2.6 Statistical Analysis

The effect of sandprawn densities, trophic state (nutrient) and temperature levels on abiotic (inorganic nutrients data and physio-chemical parameters) response variables were determined using the linear mixed-effects models (LMEMs). The '*lme4*' package (Bates et al., 2015) was employed and fitted by a restricted maximum likelihood (REML) estimation. Since these abiotic data were not strictly temporally independent (Venter et al, 2020), time was used a random factor for these linear mixed-effects models. Graphical presentations including histograms, quantile-quantile (Q-Q) plots and plots of residuals against predicted values were used to check for data normality and homogeneity (Zuur et al., 2009) for model fits evaluation. Sample data that violated model fits were re-fitted using the transformed (log x + 1) data. The significance of main and interactive predictor effects was determined using the "*car*" package (Fox and Weisenberg, 2011), with Type II Wald Chi-Square tests used to generate p values (Mangiafico, 2016) since these were not provided by model outputs. These were carried out using the RStudio (version 2023.03.0: R version 4.1.2, 2021).

Permutational Multivariate Analysis of Variance (PERMANOVA+; Anderson, 2001), an add-on package for the multivariate analysis platform PRIMER (v7; Plymouth Routines in Multivariate Ecological Research) was used to quantify the main and interactive effects of predictors (sandprawn density, temperature & nutrient level) on the bacterial assemblage from the experiment. The DIVERSE function in PRIMER was used to calculate the total bacterial species richness (*S*; number of OTUs), total number of individuals (*N*) and the Shannon-Wiener

diversity index ($H' = -\Sigma Pi$ log [Pi]; where the logs are to the base e; Spellerberg and Fedor, 2003). Treatment-related trends in these indices were visualised using the bar graph of mean values ± standard error. Distance based linear modelling (DISTLM; Legendre and Anderson, 1999; McArdle and Anderson, 2001) was used quantify the influence of environmental variables (water column abiotic and nutrient parameters) in explaining multivariate variability in the bacterial assemblage. However, strong correlated variables such a salinity, pH, and nitrite from the resemblance metrices were removed. One-way Similarity Percentage Analysis (SIMPER) was conducted on the total bacteria assemblage with a 50% cut off contribution to identify which specific group of taxa contributed most to assemblage variation among treatments. A 50% cut-off limit was used since this led to the elimination of taxa that contributed less than 1% to assemblage variability. PERMANOVA was carried out on the order level to determine the significance of main and interactive treatment effects.

A linear model "*lm*" (Chambers, J. M. 1992) was used to determine the effects of sandprawn density, temperature, and nutrient level on the bacterial diversity measures (H', S, N). The "anova" function was applied to the model to obtain significance levels of main and interactive predictor effects. Graphical evaluation of model fits was carried out as indicated for the LMEMs (Zuur et al., 2009). These were carried out using the RStudio (version 2023.03.0: R version 4.1.2, 2021).

3. RESULTS

3.1 Abiotic Variables

Temperature significantly influenced conductivity, dissolved oxygen (DO), pH, salinity, temperature ($p < 0.0001$) and turbidity ($p = 0.006$; Table 3). The interaction between temperature with trophic condition influenced conductivity ($p = 0.05$). Sandprawn density effects on conductivity ($p = 0.002$) and salinity ($p < 0.0001$) were also significant. Trophic condition influenced conductivity ($p < 0.0001$) and salinity ($p < 0.0001$), which was also affected by the interaction between sandprawn density and trophic condition ($p = 0.001$) and $p = 00.017$ respectively). Water temperature for low temperature mesocosms varied between 14 and 15℃ and between 28 and 29.7℃ in high temperature mesocosms. Apart from this, abiotic variable levels were reasonably uniform across the experiment. Conductivity ranged between 44.2±0.2 and 55.4±1.5mS/cm while salinity varied between 28.3±0.3 and 33.6±1.6ppt.. A slight reduction in conductivity and salinity levels were recorded in the presence of sandprawns in the mesotrophic treatment and this occurred mainly towards the end of the experiment. There was an increase in the salinity in high temperature treatments by roughly 5 to 6 ppt during the experiment. pH increased in high temperature treatments by 0.3 to 0.4 units. However, this trend was not evident at low temperature. In the low temperature treatment, a slight variation in oxygen level was recorded but no more than 2% (Table 2; detailed results of abiotic variability are shown in Appendix A).

Table 2: *Spatial and temporal variability in environmental variables (means ± SE) at different sandprawn densities (0% = control; 50% = 9 sandprawns per mesocosm; 100% = 18 sandprawns per mesocosm), temperature (low versus high) and eutrophication (mesotrophic versus eutrophic) over the duration of the experiment*

Treatment	Day	Sandprawn density	Temperature (°C)	Conductivity (mS/cm)	Salinity (ppt)	pH	Turbidity (NTU)	Dissolved Oxygen (%)
Low temperature, mesotrophic	0	0	13.7±0.1	46.3±0.3	30.1±0.2	8±0.1	9±2.9	93.4±1.3
		50	13.6±0.0	45.7±0.2	29.6±0.2	8±0.0	8.7±2.1	94.9±0.1
		100	13.7±0.1	45.7±0.1	29.6±0.1	7.9±0	8.7±1.5	94.6±0.0
	9	0	15.3±0.4	45.7±0.6	29.7±0.4	8.1±0	7.1±0.9	93.8±1.2
		50	14.7±0.1	44.7±0	28.9±0	8.1±0	6.1±0.5	94.3±0.6
		100	15.3±0.4	44.5±0.1	28.5±0.4	8.1±0	6.7±0.9	94±0.7.0
	15	0	15.1±0.3	46.5±0.3	30.2±0.2	8.1±0.0	6.0±0.3	94.5±0.2
		50	14.4±0.1	45.6±0.1	29.5±0.1	8.2±0.1	5.7±0.0	94.1±0.7
		100	14.9±0.3	45.4±0.0	29.5±0.0	8.4±0.3	8.3±1.4	94.5±0.9
High temperature, mesotrophic	0	0	13.9±0.3	46.7±0.2	30.3±0.1	8.0±0.1	8.1±1.3	95.1±0.5
		50	13.8±0.2	46.7±0.3	30.3±0.2	8.0±0.0	10.0±1.6	94.4±0.3
		100	13.8±0.2	46.3±0.6	30±0.4	8.0±0.1	8.8±2.0	95.3±0.7
	9	0	29.0±0.5	53.1±1.3	34.9±0.9	8.4±0.0	6.6±0.4	96.9±0.3
		50	29.1±0.8	51.3±1.1	33.6±0.8	8.4±0.0	6.5±0.4	96.7±0.9
		100	28.7±0.3	48.5±2.0	31.6±1.5	8.3±0.1	6.6±0.1	98.5±1.0
	15	0	29.4±0.3	55.4±1.5	36.6±1.1	8.4±0.0	6.9±0.7	97.3±0.5
		50	29.4±0.7	53.8±1.2	35.4±0.9	8.3±0.0	6.4±0.2	96.9±0.1
		100	28.8±0.1	51.3±2.2	33.6±1.6	8.3±0.0	6.7±0.6	97.2±0.4
Low temperature, eutrophic	0	0	13.7±0.2	45.6±0.1	29.5±0.1	8±0.0	6.2±0.1	95.3±0.1
		50	13.6±0.1	45.3±0.2	29.3±0.2	8±0.0	8.7±1.2	94.9±0.2
		100	13.6±0.1	45.6±0.3	29.5±0.2	8±0.1	8.9±2.1	93.9±1.0
	9	0	14.9±0.1	44.2±0.2	28.6±0.1	8.1±0.0	6±0.1.0	94.3±1
		50	14.5±0.3	43.9±0.4	28.3±0.3	8.1±0.0	5.8±0.0	94.5±0.1
		100	15.5±0.4	44.2±0.2	28.6±0.2	8.1±0.0	5.8±0.1	94.6±0.0
	15	0	14.8±0.2	45±0.2	29.1±0.2	8.1±0.0	6.9±1.0	94.3±0.6
		50	14.3±0.3	44.8±0.4	29.0±0.3	8.1±0.0	5.8±0.1	94.5±0.4
		100	15.1±0.4	45.0±0.3	29.1±0.2	8.1±0.0	6.8±1.0	94.4±0.8
High temperature, eutrophic	0	0	13.6±0.0	45.1±0.2	29.1±0.1	8.0±0.0	10.7±0.0	95.0±0.3
		50	13.5±0.0	45.4±0.0	29.4±0.0	8.0±0.0	8.7±1.6	94.9±0.3
		100	13.5±0.1	45.6±0.3	29.5±0.2	8.0±0.0	9.4±1.8	94.9±0.4
	9	0	29.3±0.8	48.8±0.6	31.6±0.5	8.4±0.1	6.3±0.1	96.6±0.4
		50	29.0±0.2	49.7±0.2	32.4±0.1	8.4±0.0	6.3±0.1	96.5±0.2
		100	28.0±0.2	48.5±0.3	31.6±0.2	8.3±0.0	6.2±0.1	97.8±0.4
	15	0	28.8±0.1	51.5±1.0	33.8±0.8	8.4±0.0	7.0±0.7	97.7±0.0
		50	29.4±0.3	52.9±0.6	34.8±0.4	8.3±0.0	6.2±0.1	96.1±0.3
		100	28.1±0.2	52.0±0.5	34.1±0.4	8.2±0.0	6.2±0.1	97.8±0.9

Table 3: *Results of type II Wald Chi-Square tests of the effects the predictors on the abiotic environmental response variables Statistically significant outcomes are displayed in bold*

	Environmental variables	**χ^2**	**df**	**p-value**
Sandprawn density	Conductivity	**12.6**	**2**	**0.002****
	Dissolved Oxygen (DO)	2.33	2	0.311
	pH	1.15	2	0.563
	Salinity	**20.70**	**2**	**p< 0.0001****
	Temperature	0.59	2	0.743
	Turbidity	1.64	2	0.441
Trophic condition	Conductivity	**24.60**	**1**	**p< 0.0001****
	Dissolved Oxygen (DO)	0.12	1	0.733
	pH	0.02	1	0.902
	Salinity	**26.50**	**1**	**p< 0.0001****
	Temperature	1.38	1	0.239
	Turbidity	2.91	1	0.088
Temperature	Conductivity	**191**	**1**	**p< 0.0001****
	Dissolved Oxygen (DO)	**161**	**1**	**p< 0.0001****
	pH	**125**	**1**	**p< 0.0001****
	Salinity	**338**	**1**	**p< 0.0001****
	Temperature	**933**	**1**	**p< 0.0001****
	Turbidity	**7.49**	**1**	**0.006****
Sandprawn density x trophic condition	Conductivity	**8.13**	**2**	**0.017***
	Dissolved Oxygen (DO)	0.47	2	0.783
	pH	2.10	2	0.350
	Salinity	**14.0**	**2**	**0.001*****
	Temp	0.516	2	0.772
	Turbidity	0.017	2	0.991
Sandprawn density x temperature	Conductivity	1.96	2	0.375
	Dissolved Oxygen (DO)	0.94	2	0.626
	pH	**8.56**	**2**	**0.014***
	Salinity	**7.35**	**2**	**0.025***
	Temp	1.22	2	0.542
	Turbidity	2.93	2	0.231
Trophic condition x temperature	Conductivity	**3.95**	**1**	**0.050***
	Dissolved Oxygen (DO)	1.48	1	0.223
	pH	3.20	1	0.073
	Salinity	3.13	1	0.077
	Temperature	0.48	1	0.490
	Turbidity	0.61	1	0.435
Sandprawn density x trophic condition x temperature	Conductivity	3.33	2	0.189
	Dissolved Oxygen (DO)	5.39	2	0.068
	pH	0.76	2	0.684
	Salinity	4.50	2	0.105
	Temperature	1.00	2	0.605
	Turbidity	2.23	2	0.328

3.2 Nutrients

Temperature level was the only predictor that statistically influenced nutrient levels, significantly explaining variance in phosphate ($p = 0.002$), ammonium ($p = 0.002$) and nitrite concentrations ($p < 0.0001$; Table 5). Generally, mean values of ammonium, nitrate and nitrite were greater in high temperature treatments when compared to the low temperature treatment, especially towards the end of the experiment. However, phosphate concentration was low in high temperature under eutrophic conditions (Table 4). Neither sandprawn density nor trophic conditions (independently or interactively) had a statistically significantly effected on nutrients.

Table 4: *Spatial and temporal variability in inorganic nutrients (means ± SE) at different sandprawn densities (0% = control; 50% = 9 sandprawns per mesocosm; 100% = 18 sandprawns per mesocosm), temperature (low versus high) and eutrophication (mesotrophic versus eutrophic) over the duration of the experiment*

Treatment	Day	Sandprawn density	Phosphate (PO_4^{3-}) (mg/L)	Ammonium ($NH_4^{\pm}$) (mg/L)	Nitrate (NO3-) (mg/L)	Nitrite (NO_2^-) (mg/L)
Low temperature, mesotrophic	0	0	3.1±1.0	5.7±3.4	4.0±4.0	0.1±0.0
		50	4.4±0.3	7.6±1.3	0.0±0.0	0.1±0.0
		100	2.9±0.9	2.7±0.8	0.7±0.7	0.1±0.1
	7	0	3.1±0.6	4.5±3.1	0.7±0.7	0.1±0.0
		50	2.5±0.3	9.2±1.8	0.6±0.6	0.1±0.0
		100	2.5±1.1	10.1±2.2	0.3±0.3	0.1±0.0
	13	0	1.6±0.2	8.4±3.3	0.0±0.0	0.1±0.0
		50	2.7±0.4	2.9±0.4	0.5±0.5	0.1±0.0
		100	3.0±0.2	6.4±3.2	0.0±0.0	0.1±0.1
High temperature, mesotrophic	0	0	3.3±1.4	8.3±1.0	1.0±1.0	0.1±0.0
		50	1.8±0.5	6.6±3.3	0.0±0.0	0.1±0.0
		100	2.7±0.8	7.2±3.4	1.1±0.5	0.1±0.0
	7	0	2.6±1.0	10.2±2.0	0.8±0.5	0.5±0.2
		50	3.2±0.1	10.1±1.4	1.9±1.9	0.4±0.1
		100	2.8±0.6	11.3±0.7	0.1±0.1	0.1±0.0
	13	0	2.0±0.2	11.5±1.1	11±5.4	2.7±0.6
		50	2.3±0.4	6.2±3.0	10.1±3.6	2.0±0.6
		100	1.6±0.6	8.7±3.5	7.0±1.8	1.7±0.5
Low temperature, eutrophic	0	0	3.6±0.5	8.0±3.1	0.0±0.0	0.1±0.0
		50	3.8±0.5	6.8±3.2	1.3±1.3	0.0±0.0
		100	3.7±0.4	4.0±3.3	1.8±1.8	0.1±0.0
	7	0	3.2±0.2	2.7±2.2	1.2±0.8	0.1±0.0
		50	3.2±0.7	6.1±2.1	0.0±0.0	0.1±0.0
		100	3.2±0.7	6.2±2.6	0.0±0.0	0.1±0.0
	13	0	2.7±0.5	10±1.1	2.3±1.4	0.1±0.0
		50	2.3±0.6	6.3±3.2	0.0±0.0	0.2±0.0
		100	2.5±0.5	5.4±2.7	0.0±0.0	0.1±0.0
High temperature, eutrophic	0	0	2.5±1.1	10.3±0.6	0.0±0.0	0.0±0.0
		50	1.8±0.5	5.5±3.6	1.6±1.6	0.0±0.0
		100	0.5±0.3	6.8±3.5	0.4±0.4	0.0±0.0
	7	0	1.9±0.3	10.5±1.0	2.4±1.2	0.3±0.0
		50	2.3±1.0	10.4±0.6	3.5±0.8	0.4±0.1
		100	3.4±0.3	5.2±2.1	1.8±1.0	0.1±0.0
	13	0	1.8±0.4	6.8±3.2	11.8±7.2	3.2±2.0
		50	1.8±0.4	12±0.4	11.1±5.2	3.8±1.1
		100	3.3±0.3	11.8±0.6	1.2±0.7	0.3±0.1

Table 5: *Results of type II Wald Chi-Square tests of the effects of the predictor variables on inorganic nutrients. Results were produced with analysis of variance (ANOVA) of linear mixed effects models. Statistically significant outcomes are displayed in bold*

	Inorganic nutrients	**χ^2**	**df**	**p-value**
Sandprawn density	Phosphates (PO_4^{3-})	0.103	2	0.950
	Ammonium (NH_4^+)	0.983	2	0.612
	Nitrates (NO_3^-)	4.74	2	0.094
	Nitrites (NO_2^-)	3.24	2	0.198
Trophic condition	Phosphates (PO_4^{3-})	0.022	1	0.882
	Ammonium (NH_4^+)	0.045	1	0.831
	Nitrates (NO_3^-)	0.161	1	0.688
	Nitrites (NO_2^-)	0.848	1	0.357
Temperature	**Phosphates (PO_4^{3-})**	**9.57**	1	**0.002****
	Ammonium (NH_4^+)	**9.59**	**1**	**0.002****
	Nitrates (NO_3^-)	0.281	1	0.596
	Nitrites (NO_2^-)	**61.9**	**1**	**<0.0001*****
Sandprawn density x trophic condition	Phosphates (PO_4^{3-})	0.807	2	0.668
	Ammonium (NH_4^+)	0.466	2	0.792
	Nitrates (NO_3^-)	0.269	2	0.874
	Nitrites (NO_2^-)	2.00	2	0.369
Sandprawn density x temperature	Phosphates (PO_4^{3-})	0.769	2	0.681
	Ammonium (NH_4^+)	0.710	2	0.701
	Nitrates (NO_3^-)	3.50	2	0.174
	Nitrites (NO_2^-)	6.32	2	0.042
Trophic condition x temperature	Phosphates (PO_4^{3-})	1.78	1	0.082
	Ammonium (NH_4^+)	0.014	1	0.905
	Nitrates (NO_3^-)	1.95	1	0.162
	Nitrites (NO_2^-)	2.33	1	0.127
Sandprawn density x trophic condition x temperature	Phosphates (PO_4^{3-})	0.848	2	0.654
	Ammonium (NH_4^+)	1.14	2	0.565
	Nitrates (NO_3^-)	0.082	2	0.960
	Nitrites (NO_2^-)	2.30	2	0.316

3.3 Total Bacteria Assemblage

A total of 550 bacterial taxa and 29 phyla were recorded in the bacterioplankton assemblage across all experimental treatments. Proteobacteria was the most abundant Phylum (84.65% of the assemblage) followed by Bacteroidota (5.073%), Firmicutes (2.376%), Planctomycetota (2.263%) while the least abundant was Margulisbacteria (0.002%; Figure 3). PERMANOVA results indicated that there were no statistically significant ($p > 0.05$) main effects of sandprawn density, temperature and trophic condition on the bacterial assemblages (550 taxa) across all experimental mesocosms treatments. However, the interaction between sandprawn density and temperature ($p = 0.027$) and sandprawn density and nutrient level ($p = 0.026$) significantly explained variance in bacterial assemblages in the experiment.

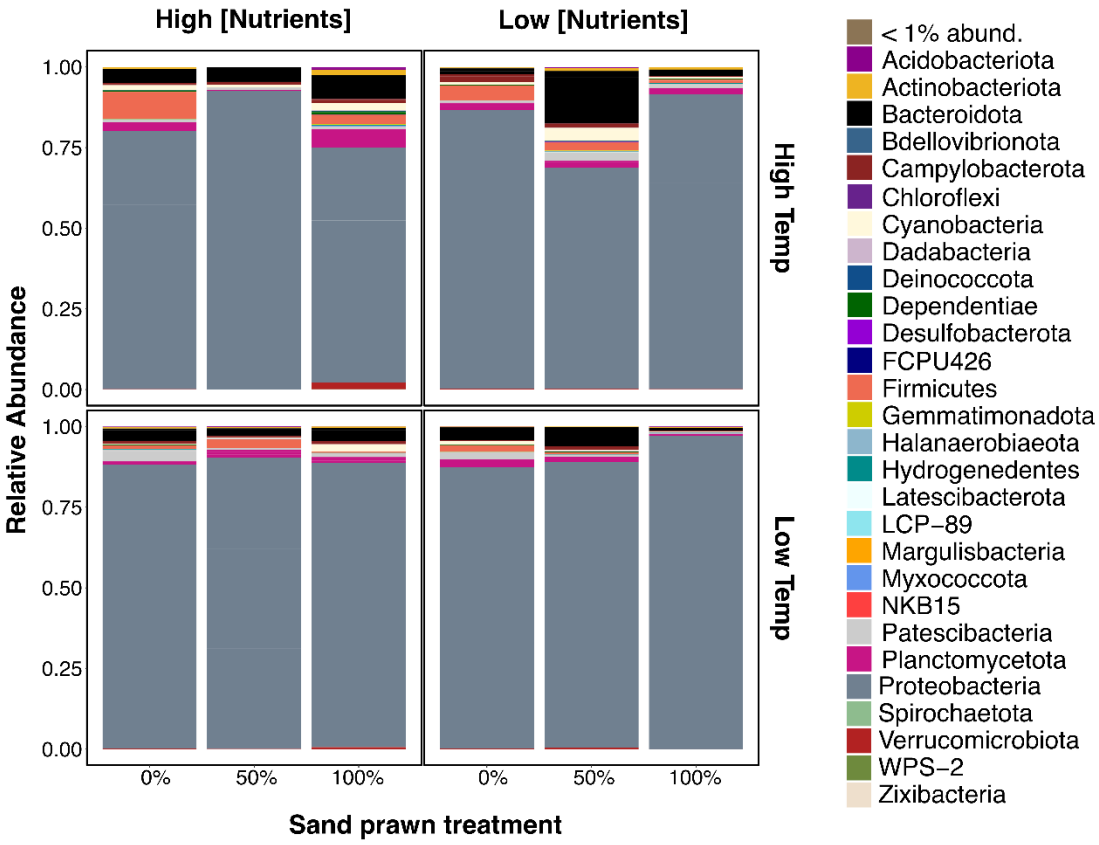

Figure 3: Contribution of Phyla to bacterioplankton assemblages across all experimental treatments. Note: Temp = Temperature, High (Nutrients) = Eutrophic water conditions and Low (Nutrients) = Mesotrophic water conditions.

Table 6: *Results of PERMANOVA analysis testing the main and interactive effects of predictors (sandprawn density, temperature, and trophic state) on the bacterial assemblage composition (550 taxa, based on total operational taxonomic units (OTU)) from the mesocosm experiment. Relative abundance was square root transformed for the analysis with a Bray-Cutis similarity index. The values in bold represent statistical significance. df = degree of freedom, SS = Sums of Squares (Type III partial), MS = Mean square. The output is based on 999 permutations of residuals under a reduced model*

Factors	**df**	**SS**	**MS**	**Pseudo-F**	**p-value**	**Unique perms**
Sandprawn density	2	944.39	472.19	0.64473	0.804	999
Temperature	1	1243.9	1243.9	1.6985	0.116	999
Trophic state	1	542.8	542.8	0.74113	0.593	999
Sandprawn density x Temperature	2	3311.7	1655.9	2.2609	**0.027**	998
Sandprawn density x Trophic state	2	3569.8	1784.9	2.4371	**0.026**	999
Temperature x Trophic state	1	777.78	777.78	1.062	0.331	999
Sandprawn density x Temperature x Trophic state	2	2663.3	1331.6	1.8182	0.069	998
Residual	23	16845	732.39			
Total	34	29562				

SIMPER Analysis of bacterial assemblages

The two-way SIMPER analysis showed that a total of 44 bacterial species contributed most to differentiating bacterial communities among sandprawn densities and temperature levels (based on the significant sandprawn x temperature interaction from PERMANOVA). The genus *Citrobacter* was present in both low and high temperature treatments. *C. freundii* contributed most to differentiating high and low temperature treatments and showed a unimodal response to sandprawn density in the low temperature treatment, but its average abundance increased with increasing sandprawn density in the high temperature treatment. Generally, at low temperature, most taxa that were present in 0% sandprawn treatments were absent at 50% and 100% sandprawn density (species numbers 9-19 & 28-34; Table 7). However, there was also another group of taxa that was absent in 0% sandprawn density mesocosms but become highly abundant in 100% mesocosms (species numbers 20-27; Table 7). In the high temperature treatment, most taxa declined in abundance from 0% to 100% sandprawn density, resulting in total abundance of bacteria generally declining with increasing sandprawn density. However, under low temperature, the decline in total bacterial abundance was less prominent, with a 'U'-shaped response being evident.

***Table 7**: SIMPER analysis (50% cut off) showing bacterial taxa that contributed most to the variance among different sandprawn densities and temperature levels. Taxonomic names with X represent unidentified names. The letters in bracket represents the bacterial order names, which are shown at the end of the table. SN = species number.*

		Low Temperature (LT)			**High Temperature (HT)**		
S/N	***Genus specie***	**0%**	**50%**	**100%**	**0%**	**50%**	**100%**
1	*Citrobacter freundii* (E)	5391.50	11926.20	6261.17		1303.83	15242.67
2	*Mitochondria* X (Rk)		6867.40	850.50			
3	*Citrobacter pasteurii* (E)	756.00	2819.60	4392.33	1287.00	10000.83	
4	*Roseivirga* X (Cy)		3821.80				
5	Rhodobacteraceae X (Rh)		935.60				
6	*Enterobacter* X (E)	4705.83	8482.40	828.67			
7	*Citrobacter* X (E)	1990.50	434.60	1389.17	1450.83	2384.67	
8	*Escherichia-Shigella* X (E)	4128.00	1604.40	1674.17			
9	*Klebsiella* X (E)	3491.50		1428.67			
10	*Clostridium* X (Cl)	5776.33					
11	*Serratia marcescens* (E)	5493.50			1709.33		
12	*Kluyvera georgiana* (E)	2355.00			1422.67		
13	*Citrobacter braakii* (E)	2804.83			12317.17	3605.33	5874.33
14	*Pseudomonas* X (P)	1554.17					
15	Thiotrichaceae X (T)	1972.50					
16	*Enterobacter cloacae* (E)	621.00		2603.50	13566.33	3766.17	
17	*Escherichia-Shigella coli* (E)	2488.17			1649.33		
18	*Raoultella planticola* (E)	3054.00		12832.50	6117.83		
19	*Lentibacter* X (Rh)	3158.00					
20	*Alkanindiges* X (P)			5010.33	816.83		
21	*Erythrobacter* X (Sm)			1671.83			
22	*Klebsiella aerogenes* (E)			4585.00			
23	*Polycyclovorans* X (S)			1120.00		1940.00	
24	*Oleiphilus messinensis* (P)			620.67			
25	*Legionella* X (L)			643.00			
26	*Citrobacter werkmanii* (E)			1629.17			
27	Chloroplast X (Ch)			1018.00			
28	*Delftia acidovorans* (Bk)	1000.00					
29	*Marivita lacus* (Rh)	1272.33					
30	Legionellaceae X (L)	612.33				5332.33	
31	*Klebsiella pneumoniae* (E)	413.17			1981.17		
32	*Anaerobacillus* X (B)	695.67					
33	*Yangia* X (Rh)	407.67					
34	*Serratia* X (E)	451.33					
35	*Enterobacter kobei* (E)					10024.17	
36	*Pantoea* X (E)					3365.17	
37	*Aestuariicoccus* X (Rh)				2090.50		
38	*Edwardsiella ictaluri* (E)				1537.67		
39	*Kluyvera* X (E)				1037.67		
40	Gracilibacteria X (C)				2927.33		
41	*Enterobacter lignolyticus* (E)				593.17		
42	*Paraglaciecola* X (E)				847.50		
43	*Mf105b01* X (Pv)				375.67		
44	*Marivita cryptomonadis* (Rh)				611.33		
	Total	**54593.33**	**36892**	**48558.68**	**52339.33**	**41722.5**	**21117**

*__Note:__ Enterobacterales (E), Burkholderiales (Bk), Bacillales (B), Candidatus (C), Rhodobacterales (Rh), Cytophagales (Cy), Clostridiales (Cl), Pseudomonadales (P), Sphingomonadales (Sm), Salinisphaerales (S), Mf105b01 (Mf)

SIMPER Analysis of bacterial assemblages contd.

For the trophic state treatment, a total of 52 bacterial species contributed most to differentiating bacterial communities among sandprawn densities and trophic states (based on the significant sandprawn x trophic state interaction from PERMANOVA). The bacterial species *Citrobacter freundii*, *C. pasteurii* and *Enterobacter cloacae* were present in both mesotrophic and eutrophic treatments. *C. freundii* contributed most to differentiating mesotrophic and eutrophic treatments and showed a unimodal response to sandprawn density in the mesotrophic treatment, but its average abundance increased with increasing sandprawn density in the eutrophic treatment. Generally, in mesotrophic conditions, most taxa that were present in 0% sandprawn treatments were absent at 50% and 100% sandprawn density (species numbers 1-21 & 23-24; Table 8). However, there was also another group of taxa that were absent in 0% sandprawn density mesocosms but become abundant in 100% mesocosms (species numbers 29-46; Table 8). In the mesotrophic treatment, most taxa declined in abundance from 0% to 100% sandprawn treatments, resulting in total abundance of bacteria generally declining with sandprawn density. However, with eutrophic conditions, total abundance decline was negligible, with a 'negative U'-shaped response recorded.

***Table 8**: SIMPER analysis (50% cut off) showing bacterial taxa that contributed most to the variance among different sandprawn densities and trophic state. Taxonomic names with X represent unidentified names. The letters in bracket represents the bacterial order names, which are shown at the end of the table. SN = species number.*

		Mesotrophic (LN)			Eutrophic (HN)		
S/N	***Genus specie***	**0%**	**50%**	**100%**	**0%**	**50%**	**100%**
1	*Serratia marcescens* (E)	6741.83					
2	*Enterobacter* X (E)	3504.50		714.83	2234.00	1350.50	
3	*Citrobacter braakii* (E)	13780.67	4272.80		1341.33		
4	*Escherichia-Shigella coli* (E)	3221.50			916.00		
5	*Citrobacter* X (E)	1206.17	1669.00	3041.33	2235.17	1356.00	
6	*Pseudomonas* X (P)	1567.00					
7	*NS3a* X (F)	1334.83	507.33				
8	*Raoultella planticola (*E)	3525.67			5646.17		
9	*Klebsiella aerogenes* (E)	1571.67					
10	*Citrobacter pasteurii* (E)	786.50	6283.60	1596.00	1256.50	7114.17	
11	*Enterobacter lignolyticus* (E)	1407.17		545.83			
12	*Klebsiella pneumoniae* (E)	1663.33					
13	*Raoultella* X (E)	618.00	15100.00				
14	Holosporaceae X (H)	2033.00					
15	*Marivita lacus* (Rh)	1182.33					
16	*Roseibacterium* X (R)	953.50					
17	*Limnobacter* X (B)	587.00					
18	*Kluyvera georgiana* (E)	1839.00				5313.67	
19	EV818SWSAP88 X (EV)	456.67					
20	*Edwardsiella ictalurid* (E)	1162.83					
21	*Marivita* X (R)	1009.83					
22	*Enterobacter cloacae* (E)	8799.83	5376.00	2580.33	5387.50	1424.67	
23	B2M28 X (B2)	455.83					
24	*Kluyvera* X (E)	770.17					
25	*Citrobacter freundii* (E)		6975.00	5953.00	1207.17	5429.83	15550.83
26	Legionellaceae X (L)		3452.80			2529.17	
27	*Polycyclovorans* X (Sm)		2023.40	1356.83			
28	*Roseivirga* X (Cy)		2799.40			1955.50	
29	Chloroplast X (Ch)			2256.33			
30	*Escherichia-Shigella* X (E)			1690.83	4214.33	1297.50	
31	*Kluyvera georgiana* (E)			3639.67	1938.67		
32	*Citrobacter werkmanii* (E)			1651.50			
33	WCHB1-41 X (W)			1446.00			
34	*Acinetobacter* X (P)			1488.50			
35	*Erythrobacter* X (Sm)			1318.50			
36	Mitochondria X (Rk)			863.83		9004.83	
37	*Oleiphilus messinensis* (P)			557.67			
38	OM190 X			454.00			
39	*Salmonella enterica* (E)			344.33			
40	*Klebsiella* X (E)			334.00	4489.83		
41	Rhodobacteraceae X (Rh)			1139.33		352.50	
42	Thiotrichaceae X (T)			1351.50			
43	*Marinobacter* X (P)			751.67			
44	*Marivita lacus* (Rh)			1278.67			
45	Methyloligellaceae X (Rb)			316.33		538.83	
46	*Alkanindiges* X (P)				995.83		6751.83
47	*Enterobacter kobei* (E)					9969.33	
48	*Pantoea* X (E)					3600.00	
49	NS11-12 X (S)					4674.50	
50	Alteromonadaceae X (E)				553.17	1011.83	
51	Mf105b01 X (Pv)				474.50		
52	*Clostridium* X (Cl)				2016.67		
	Total	**60178.83**	**48459.33**	**36670.81**	**34906.84**	**56922.83**	**22302.66**

Note: Enterobacterales (E), Flavobacteriales (F), Burkholderiales (Bk), Legionellales (L), Pseudomonadales (P), Rickettsiales (RK), Rhodobacterales (Rh), Cytophagales (Cy), Sphingomonadales (Sm), Rhizobiales (Rb), Salinisphaerales (S), B2M28 (B2), Chloroplast (Ch), Parvibaculales (Pv), Holosporales (H), Burkholderiales (B), WCHB1-41 (W), Thiotrichales (T), EV818SWSAP88 (EV)

Total bacterial biodiversity measures

Visually, abundance, richness and diversity appeared to respond in a unimodal pattern to increasing sandprawn densities, with most metrics peaking at 50% sandprawn density (Figure 4). However, according to the linear model output, total bacterial diversity measures were not significantly ($p > 0.05$) influenced by any main or interactive predictors effect. The 50% cut off simper analysis bacterial (62 taxa) diversity measure chart show a unimodal trend to increasing sandprawn density. Species diversity (H´) and total individual (N) peaked at 50% and 100% sandprawn density, respectively. However, total species peaked around the 50% sandprawn density treatment (Figure 5). The total individuals were significantly influenced by temperature ($p = 0.01$). Temperature and trophic state influenced ($p = 0.04$) the species diversity. However, total species were not significantly ($p > 0.05$) influenced by any main or interactive predictors effect.

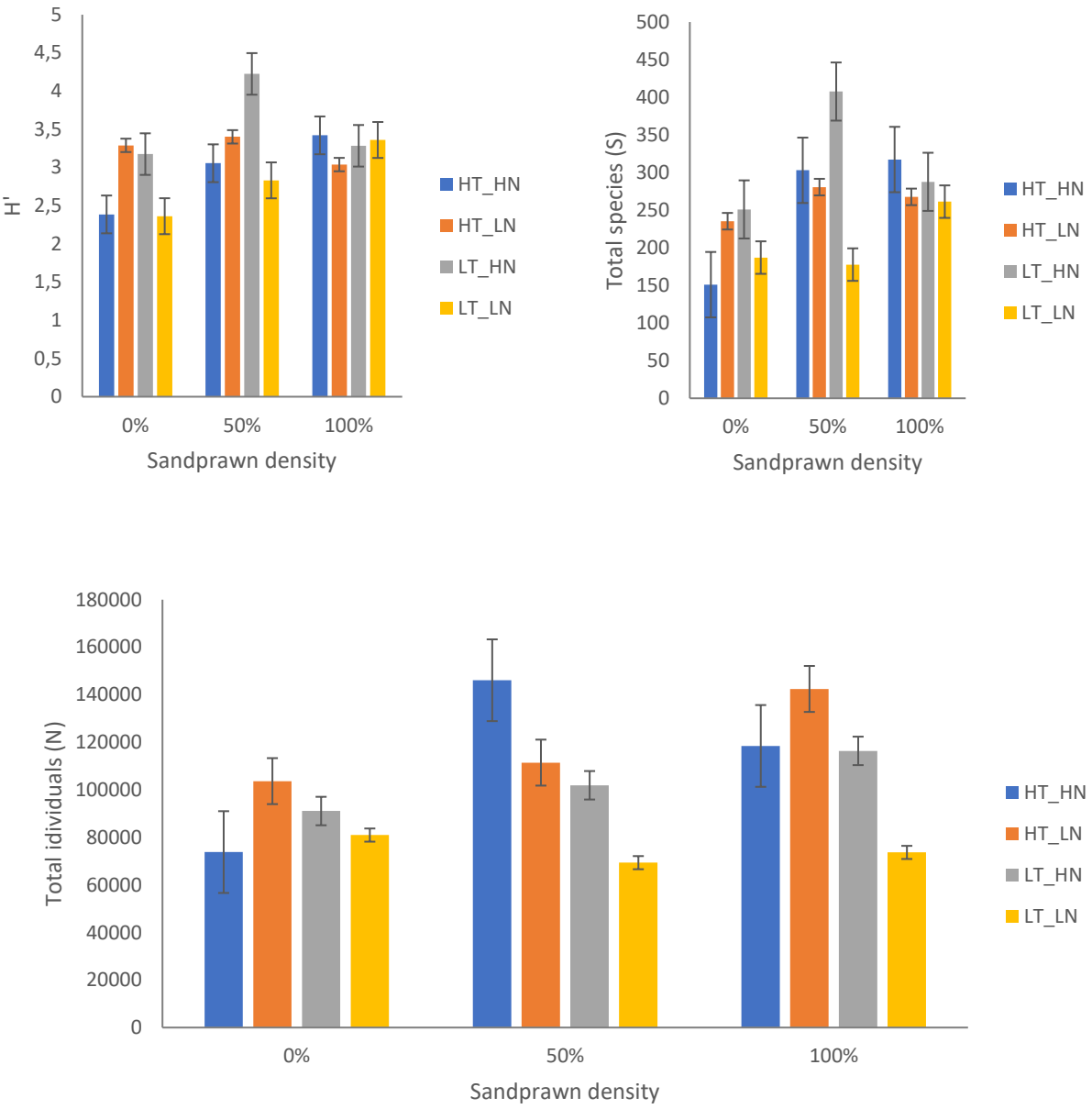

Figure 4: Variation in species diversity (H'), total mean species (S) and total mean individuals (N) (mean ± SE) across 0%, 50% and 100% sandprawn treatment. The full bacterial assemblage comprising 550 taxa was used in the analysis. Legends: HT_HN = High temperature, Eutrophic; HT_LN = High Temperature, Mesotrophic; LT_HN = Low Temperature, Eutrophic; LT_LN = Low Temperature, Mesotrophic.

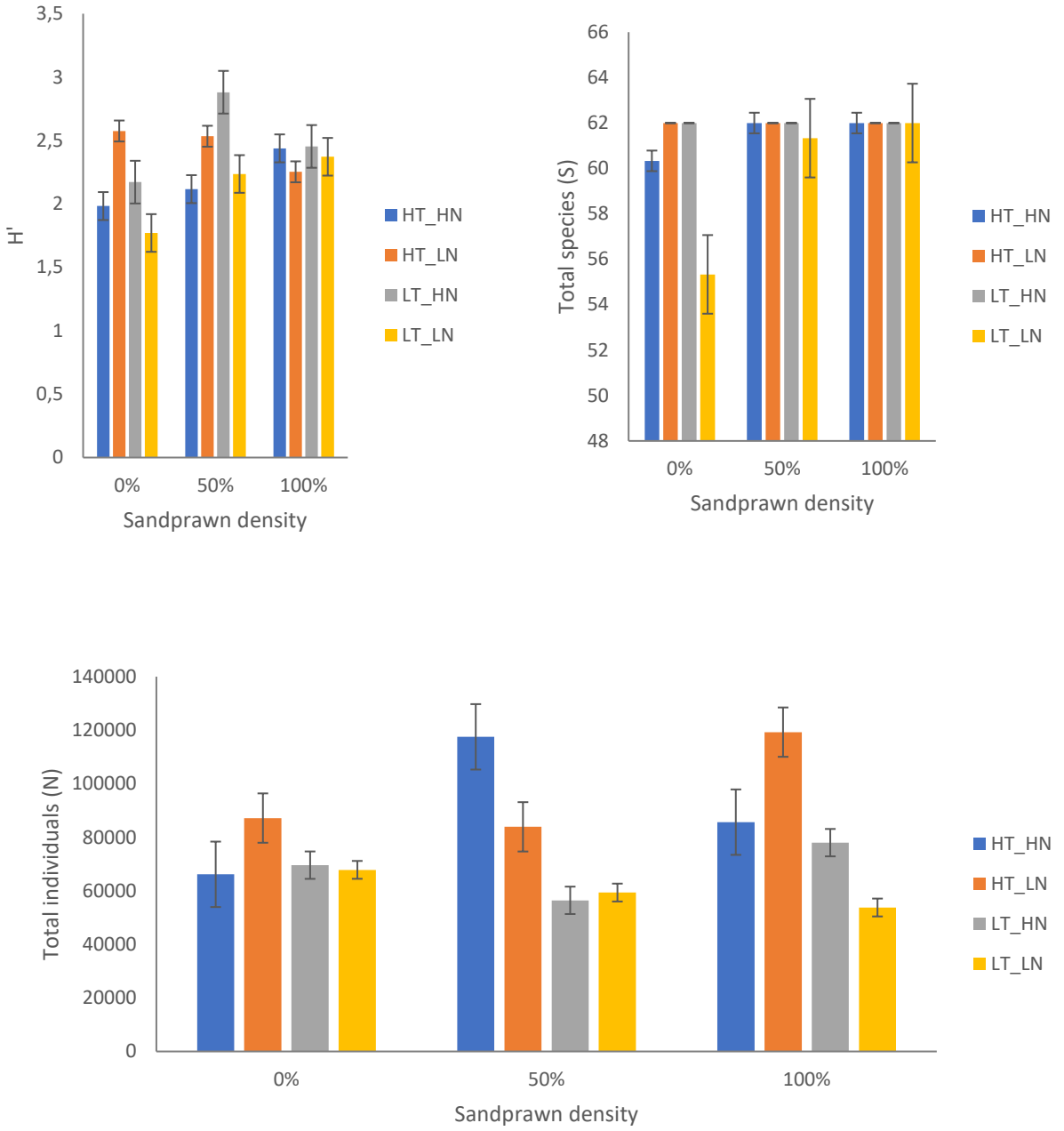

Figure 5: Variation in species diversity (H'), total mean species (S) and total mean individuals (N) (mean ± SE) across 0%, 50% and 100% sandprawn treatment. The 50% simper cut off bacterial assemblage comprising 62 taxa was used in the analysis. Legends: HT_HN = High temperature, Eutrophic; HT_LN = High Temperature, Mesotrophic; LT_HN = Low Temperature, Eutrophic; LT_LN = Low Temperature, Mesotrophic.

Distance-based linear model (DistLM)

DistLM marginal testing indicated that temperature, turbidity, phosphate, ammonium, and nitrate did not individually explain the variance in bacterial assemblages. Also, sequential testing indicated that none of the 5 combined environmental variables significantly ($p > 0.05$) explained variance in the bacterial assemblages (Table 9).

Table 9: *Results of distance-based linear modelling (DITLM) showing results of main (marginal test) and cumulative (sequential test) effects of abiotic and biotic environmental variables in explaining variance in the bacterial (550 taxa) assemblage across all mesocosm treatments*

Environmental Variable	**Adj. R^2**	**SS(trace)**	**F_{Pseudo}**	**p-value**	**Proportion of variance**	**Cumulative Variance Explained**	**Res.df**
			Marginal test				
Temperature		3137.6	0.932	0.577	0.027		
Turbidity		4447.4	1.337	0.075	0.039		
Phosphate		2347	0.692	0.937	0.021		
ammonium		3182.4	0.946	0.569	0.028		
Nitrate		3844	1.149	0.218	0.034		
			Sequential tests				
+Temperature	0.027	3137.6	0.932	0.572	0.027	0.027	33
+Turbidity	0.062	3891.7	1.162	0.222	0.034	0.061	32
+Phosphate	0.088	3051.4	0.908	0.608	0.027	0.088	31
+Ammonium	0.114	2889.5	0.856	0.717	0.025	0.114	30
+Nitrate	0.145	3556.4	1.056	0.337	0.031	0.145	29
Specified solution							
R^2	RSS	No.Variables	Selections				
0.14471	97678	5	All				

***Note:** Statistically Significant (p-values <0.05) Adj. R^2 = Adjusted R^2, SS(Trace) = Sums of squares of trace statistic, F_{Pseudo} = Pseudo F-statistic, p-value = Probability value by permutation, Res. df = Residual degrees of freedom.

Order-level trends

PERMANOVA analysis run at the order (152 orders) level indicated that bacterial community composition was significantly influenced by the interaction between sandprawn density and trophic state ($p = 0.031$; Table 10). Of all the bacterial orders, one-way SIMPER analysis (predictors: sandprawn density x trophic conditions; 50% cut-off) revealed that Enterobacterales contributed the most to the difference of the bacterial order level in both mesotrophic and eutrophic treatments. The average abundance of Enterobacterales decreased from 64568.59 to 40357.92 with increasing sandprawn density in mesotrophic treatments while the opposite occurred in eutrophic mesocosms from 40357.92 to 62295.72 with increasing sandprawn density (Table 11).

Table 10: *PERMANOVA analysis result of the main and interactive effect of different sandprawn densities, temperature, and nutrient levels on the total bacteria assemblages at the order level*

Factors	**df**	**SS**	**MS**	**Pseudo-F**	**p-value**	**Unique perms**
Sandprawn density	2	40428	20214	0.97894	0.462	995
Temperature	1	16763	16763	0.81182	0.612	998
Trophic state	1	13208	13208	0.63966	0.789	999
Sandprawn density x Temperature	2	46089	23045	1.116	0.303	998
Sandprawn density x Trophic state	2	71727	35863	1.7368	**0.031**	996
Temperature x Trophic state	1	17732	17732	0.85874	0.559	999
Sandprawn density x Temperature x Trophic state	2	36840	18420	0.89206	0.588	998
Residual	23	0.0000475	20649			
Total	34	0.0000720				

Table 11: *SIMPER analysis with a 50% low cut off identifying the average abundance of bacterial orders that contributed most to variance in bacterial assemblages at the order level between the interaction of different sandprawn densities and trophic state (as determined by PERMANOVA)*

	Mesotrophic (LN)			**Eutrophic (HN)**		
Order	**0%**	**50%**	**100%**	**0%**	**50%**	**100%**
Enterobacterales	64568.59	56449.57	40357.92	40357.92	46963.40	62295.72

4. DISCUSSION

This research investigated the interactive and individual influences of ecosystem engineering by sandprawns (*Kraussillichirus kraussi*), temperature and eutrophication (trophic state) on pelagic bacterial assemblages. The broader goal was to contribute to understanding microbial community-level responses to global change, as well as factors that determine changes in bacterial species composition in the Zandvlei Estuary, and similar urban systems. I hypothesized that changes in sandprawn density, temperature and trophic state would shape microbial community structure in respect to species abundance, richness, and diversity. This was based on the expectation that each predictor would positively or negatively affect certain microbial taxa depending on their biological traits. Overall, understanding consequences of the predictors investigated on pelagic microbes is timely as they are known to play a vital role in biogeochemical processes such as coastal carbon and nitrogen cycling (Fuhrman, 2009).

4.1 Physical Environment

Abiotic conditions were generally uniform in the experiment across sandprawn density. This result is related to the findings of Venter et al. (2020) where abiotic variability across mesocosms and sandprawn treatments were minimal. This is expected in an indoor mesocosm experiment because the environment is largely controlled and not subjected to external variation. However, there were cases where abiotic variation was detected statistically, but these were generally minor. In mesotrophic treatments for example, conductivity and salinity levels were reduced slightly the presence of sandprawn towards the end of the experiment. Hence, it is plausible that the significant statistical effect detected for sandprawn density on conductivity and salinity during this experiment stems from marginal temporal variation that occurred towards the end of the experiment.

Generally, nutrient variables varied slightly across sandprawn treatment levels. However, concentration of ammonium, nitrate and nitrite were greater in some cases under high temperatures. Venter et al. (2020) reported that concentrations of pelagic ammonium was greatest in mesocosms relative to other nutrients. This is not surprising given that ammonium is the most available of the dissolved nitrogen specie for exchange between water and sediment (Pillay and Branch, 2011). Varying temperature levels are recognised as an important climatic factor that influences the functioning and structuring of coastal ecosystem (Brierley and Kingsford, 2009). Increase in temperature can impact coastal biogeochemistry, which includes change in microbially driven reactions. This could lead to changes in benthic infaunal activity, with temperature having the potential to alter bioturbation and bioirrigation and thereby modify nutrient fluxes between sediments and the water column (Statham, 2012). These mechanisms may explain why phosphate, ammonium, and nitrate concentrations were significantly affected by temperature in this experiment.

4.2 Bacterial Assemblages

Overall, the bacterial assemblages reported in this study reflect estuarine conditions from which they were collected, which in turn are known to be temporarily fluctuating and sometimes nutrient-rich (MacArthur & Wilson, 1967; Song et al., 2017). The most dominant bacterial phylum detected in this study was the Proteobacteria. This is similar to the findings of Matcher et al. (2018), who reported Proteobacteria and Bacteroidetes to be the dominant bacterial phyla recorded from three different Southern African estuaries (Kowie Estuary, Kariega Estuary and Sunday River Estuary). Separately, Kirchman et al. (2005) reported Proteobacteria (alpha and beta) as one of the most dominant bacterial groups in the Delaware Estuary (USA). In addition, Cheng et al. (2019) reported a 52.2% relative abundance of Proteobacteria, in a eutrophic lake (Lake Chaohu located in China). Proteobacteria are recognised as meditators of coastal ecosystem processes such as carbon and nutrient cycling (Ghosh and Bhadury, 2019; Davies

et al., 2011). Margulisbacteria were the least dominant bacterial phyla in this study. Ohore et al. (2023) reported these bacteria in surface waters of the Rongjiang River Estuary (China) and are considered a rare microbial group that is responsible for nutrient cycling, and in maintaining estuarine resilience and community stability. It has been suggested that members of this group may respond promptly to environmental perturbations (Xu et al., 2021). The presence of Margulisbacteria in the current study reflected the dynamic nature of the urbanised Zandvlei Estuary, from which material for the experiment was obtained. .

Variance in pelagic microbial community composition in the current study was affected by the interaction between (1) sandprawn densities and temperature and (2) sandprawn densities and trophic state. Importantly, the main effects of predictors were not detected, and sandprawn effects in particular evident through interactions with temperature and trophic state, implying that trophic and thermal context determine sandprawn effects on the bacterial assemblages. Regarding trophic state, increased concentrations of inorganic and organic nutrients has been shown to promote the growth of bacterial resulting in higher bacterial variance and shifts in community composition (Garnier et al., 1992; Goñi-Urriza et al., 1999; Jordaan et al., 2019). The northern reaches of my study site – the Zandvlei estuary, are highly eutrophic and are influenced by inputs stemming from poor sanitation infrastructure, informal settlements, and riverine discharge from surrounding areas (Harding, 1994; Venter et al., 2020).

One-way SIMPER analysis identified bacterial species that differentiated the low and high temperature treatments. While several species discriminated this treatment, *Citrobacter freundii* contributed most to the dissimilarity. However, when considering sandprawn effects in conjunction with temperature (based on the significant sandprawn x temperature interaction identified by PERMANOVA), the average abundance of *C. freundii* increased with increasing sandprawn density in high temperature the treatment (28 to 29℃; Table 2). Generally, *Citrobacter* spp. are mesophilic in nature but have been reported to be well adapted to higher

temperatures (50 - 70°C) (Droffner et al., 1995), with reports of optimal growth at a temperature of 37°C. (Rogers et al., 2016). These bacteria can also be found in variety of environments such as water, soil, human clinical samples and digestive tracts of animals (Frederiksen et al., 2003; Frederiksen et al., 2005; Cabral, 2010). In contrast to the high temperature treatment, a unimodal trend for *C. freundii* abundance was recorded in the low temperature treatment with increasing sandprawn density.

In terms of high temperature and effects on bioturbation, studies have shown increasing sediment reworking rate by deposit feeders. Maire et al. (2007) reported an increased rate of sediment expulsion by a deposit-feeding bivalve (*Abra ovata*) under elevated temperature level. This was also reported for by Berkenbusch and Rowden (1999) who quantified the rate of sediment expulsion rate of *Biffarius* (as *Callianassa*) *filholi* over a span of 12 months in a New Zealand intertidal sandflat. The result from their research showed a significant correlation between temperature and bioturbation activities of *B. filholi*. Specifically, sediment expulsion was greater in summer when compared to winter season. Similarly, Rowden et al. (1998) reported a higher level of sediment reworking by *Callianassa subterannea* during the summer season but inactivity in springtime at a low temperature of 7°C. From my results, *C. freundii* and *C. braakii* were positively affected by increasing sandprawn density, particularly in the high temperature treatment. This might have been due to increased sediment turnover and erodibility induced by sandprawns causing suspension of these species into the water column. Bacteria, larvae, and diatoms that colonise sediment are known to be expelled and swept into the water column with high bioturbation (Pillay et al., 2007). Studies have shown that sediment erodibility via bioturbation by deposit feeders can disrupt sediment stabilizers such as bacteria, diatoms, and the extracellular polymeric substances (EPSs) they secrete (Widdows et al., 2000; de Deckere et al., 2001). Alternatively, if *C. freundi* and C. *braakii* are not suspended into the water with increasing sandprawn density, they may benefit from trophic resources that are

resuspended such as microalgae. In addition, the active water pumping through bi-directional water exchange between the benthic and pelagic zones (Volkenborn et al., 2010; Moyo et al., 2017) oxygenates the mesocosm at smaller scales (cm; minutes), which could have positively influenced *C. freundii* and *C. braakii* abundance.

Overall, there was a declining trend of most taxa with increasing sandprawn density but more strongly in the high temperature treatment. This could be as a result of strong filtration power by sandprawns and strengthened by high temperature. Venter et al. (2020) previously reported an approximate 50% decrease of phytoplankton (chl-*a*) biomass in the overlying mesocosm waters in the presence of sandprawn, which was linked biofiltration activities of sandprawn. Burrow walls had greater levels of microalgal biomass than sediment surfaces, suggesting that walls were trapping phytoplankton cells. A similar mechanism might explain the declining trend recorded for bacteria with increasing sandprawn density. Alternatively, these declines could be related to interspecific interactions (e.g. competition) driven by increases in abundance of species such as *C. freundi* and *C. braaki* with sandprawn density. A significant portion of the taxa that displayed declining trends with sandprawn density were from the phylum Enterobacterales. Among other taxa that decline or disappeared with increasing sandprawn density are *Echeriscia* and *E. coli*. These taxa are recognised as faecal indicator organisms (FIO) and their presence in coastal ecosystems indicate the presence of potentially pathogenic microbes which has been positively linked to organic matter loading in the water column (Howell et al., 1996). They have also been reported as effective water quality and human health risk indicators in coastal and estuarine ecosystems (Hassard et al., 2016). In my study, some *Echeriscia* and *E. coli* strains were eradicated in the water column with increasing sandprawn density. It is possible these taxa were filtered during the biofiltration activities of sandprawns from the water column (Venter et al., 2020). Alternatively, *C. freundii* top-down

(consumption of *E. coli*) or competitive (*C. freundii* outcompeting *E.coli*) effects may also have played a role.

Some bacterial taxa that were absent at low temperature were present at high temperature. Taxa such as *Edwardsiella ictaluri*, *Kluyera* sp., *Enterobacter lignolyticus*, *Paraglaciecola* sp, *Aestuariicocus* sp. and *Marivita cryptomonads* were recorded in the 0% sandprawn density at high temperature. Thermal conditions have been reported to significantly affect the physiology and behaviour of *Edwardsiella* (Leung et al., 2022). Du et al. (2007) reported that *Edwardsiella* incubated in a seawater at 4℃ were viable but not culturable. However, the reverse occurred when these bacteria were incubated at 26℃. In addition, *E. lignolyticus* are classified has mesophilic and can be viable in an optimum temperature of 30℃ (DeAngelis et al., 2011; Ashburner et al., 2000). Thus, the set of bacteria that were recorded in the high temperature treatment were likely tolerant to the higher temperatures in the experiment.

Previous research (Harding, 1994) has characterised the Zandvlei estuary as eutrophic, mainly due to high sewage discharge and riverine inputs spanning from informal settlements. Eutrophic conditions could explain the high abundance of *C. freundii* in my experiment, especially in the eutrophic treatment. These bacteria are known to be nutrient-loving and aerobic (Jung and Park, 2015; Wu et al., 2019). In the mesotrophic treatment, most of the bacterial species were recorded under 0% and 100% sandprawn density. The most dominant species were from the order Enterobacterales such as *Serratia marcescens*, which has been reported from raw sewage water (Dacayo et al., 2019; Xu et al., 2012).

Overall, total microbial abundance under the mesotrophic and eutrophic treatment declined with increasing sandprawn density. However, the abundance decline in mesotrophic treatment was more significant. This effect could be due to water pumping and bacteria being adsorbed them onto the burrow walls, leading to a reduction in bacterial abundance in the overlying

water column (Pillay, 2019). Previous studies have argued the fact that burrow walls constructed by thalassinideans are a diverse niche for microbes such as anaerobic denitrifying and aerobic nitrifying bacteria and sulphate-reducing groups (Bird et al., 2000; Kinoshita et al., 2003). More so, the stabilization of burrow walls with the aid of plant materials or mucopolysaccharides as well as the selective retention of fine organically rich sediments by some thalassinideans may positively influence the proliferation (or adsorption) of microbes (Aller and Aller, 1986; Aller, 1988; Reichardt, 1988; Steward et al., 1996). Branch and Pringle (1988) reported reduction in bacteria numbers on surface sediment while there was an increased in bacteria density with the depth of *Kraussillichirus kraussi* burrow linings using a direct counting method. Increased productivity in the eutrophic treatment is likely to have facilitated the unimodal response of bacterial abundance to increasing sandprawn density. At 50% density, nutrients likely favoured bacterial growth, but at 100% sandprawn density, filtration probably outweighed bacterial growth.

Environmental parameters such as total organic carbon, dissolved oxygen, turbidity, salinity, and temperature can determine the fate of microbial communities. They often dictate the functional groups within an ecosystem and the phylogenetic diversity of microbial assemblages (Ohore et al., 2023). Previous studies had shown the impact of temperature gradients on the degradation activities of microbes and microbial community composition, which indirectly contributed to gradients in organic pollutant distributions in aquatic ecosystems (Gao, 2021; Li et al., 2013; Warrior and Carder, 2005). Hou et al. (2013) reported that anaerobic ammonium oxidation (anammox) bacterial biodiversity from Yangtze estuary (China) were significantly influenced by temperature and organic carbon availability. However, in my study, none of the three predictors effects (sandprawns, temperature and trophic state) had a significant influence on the total pelagic bacterial diversity metrices. There was evidence of changes in species composition in response to interactions between sandprawns and temperature and sandprawns

and eutrophication, based on SIMPER and PERMANOVA. However, opposing effects of treatments on tolerant and intolerant taxa meant that as some taxa increased in response to a predictor, others decreased, thus resulting in neutral diversity metric responses. In the case of sandprawn density, some species such as *C. freundii*, *C. braakii*, *Citrobacter pasteurii* increased in abundance while others like *E. coli* decreasing or disappearing.

To understand the determinants of variance in bacterial assemblages in my experiment, Distance-Based Linear Modelling (DistLM) was applied. Generally, none of the predictor variables considered in this study explained variance in the bacterioplankton community. This suggests that changes in microbial community structure or composition experienced in this study may be attributed to environmental parameters or events that were outside of those considered in the DistLM. In addition, the R^2 values were small, suggesting that the input variables were weak predictors (Table 9).

To the best of my knowledge, most of the previous research (Papaspyrou et al., 2005; Laverock et al., 2010) regarding changes in microbial assemblages in reflex to burrowing thalassinidean has focused on the benthic microbial community structure, with little or no attention on overlying water microbial assemblages. The results of my study emanated from considering the potential for thalassinids to influence bacterial assemblages occupying the overlying water. Enterobacterales contributed more than 50% to the variance of bacterial taxa recorded from this study. The decrease in relative abundance of the Enterobacterales in response to increasing sandprawn densities under mesotrophic treatment suggests a negative effect of grazing or biofiltration pressure by sandprawns. Sandprawn gut analyses have identified bacterial as a source of food for deposit feeders such as *K. kraussi* (Harris et al., 1991), however, it is possible that these bacteria may have been trapped onto the burrow walls of *K. kraussi*. Studies have reported that *Callianassa* burrow walls are traps or hotspot for pelagic microbes (Dobbs and Guckert 1988; Pillay and Branch, 2011). Through the estimation of phospholipid fatty acid

(PLFA) and phospholipid phosphate assays, Dobbs and Guckert (1988) reported a greater biomass of bacteria in the burrow lining of *Sergio* (as *Callianassa*) *trilobata* when compared to the sediment surface. Similarly, Papaspyrou et al. (2005) demonstrated an increase in bacterial abundance in the burrow walls of the Callianassid *Pestarella tyrrhena* relative to the adjacent sediments. Thus, the negative influence of sandprawns on Enterobacterales in moderately eutrophic water could be the result of biofiltration and consumption. However, under eutrophic conditions, Enterobacterales numbers increased with sandprawn density, possibly due to trophic resource availability stimulating reproductive rates, which in turn overcame potential biofiltration and consumptive effects of sandprawns. Interestingly, Berkenbusch and Rowden (1999) recorded that at high productivity, feeding rate of *Biffarius* (as *Callianassa*) *filholi* was reduced as well as sediment turnover rate. In my study, greater productivity could have reduced sandprawn feeding and water pumping rate, thereby reducing top-down effects on Enterobacterales.

Potentially, the low sandprawn sediment turnover rate with greater resource availability (eutrophic treatment) may have been associated with increased excretion. This mechanism could have also played a role in the increased average abundance of Enterobacterales in the eutrophic treatment. Frankenberg et al. (1976) have drawn attention to the trophic significance of faeces of burrowing endobenthic crustaceans, as a substrate for bacterial colonisation. Specifically, organically-rich faecal pellets are extruded from the burrows and deposited onto the sediment surface (Branch and Pringle, 1987). Such faecal extrusions in the eutrophic treatment may have supported the proliferation of Enterobacterales in this treatment. Ohore et al. (2021a&b) suggested that aquatic nutrient compositions are major drivers shaping or influencing aquatic microbial community structure. This corroborates the findings of Custodio et al. (2023) who reported a higher abundance of Enterobacterales in Tipicocha Lagoon (Peru), which was determined by fish farming activities and loading of nutrients. More so, studies have

revealed that surplus or unconsumed food favours a selected group of bacterial that can use up this available resource or selectively eradicate specific bacterial from the bacterial assemblage (Long et al., 2021; Hu et al., 2017). Thus, nutrient availability via increased faecal matter production by sandprawns may explain increases in Enterobacterales abundance with increasing sandprawn density under eutrophic conditions.

Conclusion

Based on the findings of this study, it was evident that temperature, eutrophication level and sandprawns did not independently influence pelagic microbial community structure. However, the interaction between two predictors (sandprawn x temperature; sandprawn x trophic state) were responsible for determining pelagic microbial community structure.

Only *C. freundii* was found consistently in both low and high temperature treatments, generally displaying increases in population sizes with increasing sandprawn density. This suggests that this species are resilient to both varying temperature levels used and any top-down sandprawn effects related to biofiltration and consumption. Overall, some bacterial species were resilient to the sandprawn top-down activities while others were eliminated from samples. Total pelagic bacteria abundance showed declining trends with increasing sandprawn density in the mesotrophic treatment, while under eutrophic conditions, the response was unimodal.

One of the pronounced effects of increasing sandprawns density in my experiment was the reduction in *E. coli* abundance irrespective of the nutrients or temperature levels. *E. coli* is classified as a faecal bacterium that is deliberately or accidentally introduced into the aquatic ecosystems through wastewater discharge, surface run off, direct defecation, discharge from sewage treatment plants and water drainage from agricultural land (Hassard et al., 2016). These activities are very similar to the anthropogenic activities occurring within the Zandvlei Estuary catchment, where the materials for this study was collected. The abundance of *E. coli* has been

reported to effectively signal water quality and human health risk in aquatic/coastal ecosystems (Hassard et al., 2016). However, my findings suggest that endobenthic crustaceans such as sandprawns could be an effective nature-based tool to control the proliferation of *E. coli*. Hence, these endobenthic crustaceans and their habitats need to be protected considering the ecological service they provide in filtration of *E. coli*. This in turn translates into improved water quality and can assist in preventing human diseases outbreaks, while building estuarine resilience.

5. REFERENCES

Ahmed, W., Gyawali, P., Hamilton, K. A., Joshi, S., Aster, D., Donner, E., Simpson, S. L., & Symonds, E. M. (2021). Antibiotic resistance and sewage-associated marker genes in untreated sewage and a river characterized during baseflow and stormflow. *Frontiers in Microbiology*, ***12***, 632850.

Aller, J. Y., & Aller, R. C. (1986). Evidence for localized enhancement of biological associated with tube and burrow structures in deep-sea sediments at the HEEBLE site, western North Atlantic. *Deep Sea Research Part A. Oceanographic Research Papers*, ***33*****(6)**, 755-790.

Aller, R. C. (1988). Benthic fauna and biogeochemical processes in marine sediments: the role of burrow structures. *Nitrogen cycling in coastal marine environments*, 301-338.

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral ecology*, ***26*****(1)**, 32-46.

Andersson, A., Brugel, S., Paczkowska, J., Rowe, O. F., Figueroa, D., Kratzer, S., & Legrand, C. (2018). Influence of allochthonous dissolved organic matter on pelagic basal production in a northerly estuary. *Estuarine, Coastal and Shelf Science*, ***204***, 225-235.

Arrigo, K. R. (2005). Marine microorganisms and global nutrient cycles. *Nature*, 437:349-355.

Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., Davis, A. P., Dolinski, K., Dwight, S. S., Eppig, J. T., Harris, M. A., Hill, D. P., Issel-Tarver, L., Kasarski, A., Lewis, S., Matese, J. C., Richardson, J. E., Ringwald, M., Rubin, G. M., & Sherlock, G. (2000). Gene ontology: tool for the unification of biology. *Nature genetics*, ***25*****(1)**, 25-29.

Azam, F., & Cho, B. C. (1987). Bacterial utilization of organic matter in the sea. In M. Fletcher (Ed.), Ecology of microbial communities (pp. 261–268). Cambridge: Cambridge University Press.

Bates, D., Kliegl, R., Vasishth, S., & Baayen, H. (2015). Parsimonious mixed models. arXiv preprint arXiv:1506.04967.

Bauer, J. E., & Bianchi, T. S. (2011). 5.02—dissolved organic carbon cycling and transformation. *Treatise on estuarine and coastal science*, ***5***, 7-67.

Berkenbusch, K., & Rowden, A. A. (1999). Factors influencing sediment turnover by the burrowing ghost shrimp *Callianassa filholi* (Decapoda: Thalassinidea). *Journal of Experimental Marine Biology and Ecology*, ***238*****(2)**, 283-292.

Berkenbusch, K., & Rowden, A. A. (2003). Ecosystem engineering—moving away from'just-so'stories. *New Zealand Journal of Ecology*, 67-73.

Bird, F. L., Boon, P. I., & Nichols, P. D. (2000). Physicochemical and microbial properties of burrows of the deposit-feeding thalassinidean ghost shrimp *Biffarius arenosus* (Decapoda: Callianassidae). *Estuarine, Coastal and Shelf Science*, ***51*****(3)**, 279-291.

Boesch, D. F., Brinsfield, R. B., & Magnien, R. E. (2001). Chesapeake Bay eutrophication: Scientific understanding, ecosystem restoration, and challenges for agriculture. *Journal of environmental quality*, ***30*****(2)**, 303-320.

Borowitzka, M. A., Lavery, P. S., & Van Keulen, M. (2006). Epiphytes of seagrasses. In: Larkum, A. W. D., Orth, R., & Duarte, C., editors. Seagrasses: *Biology*, *Ecology and Conservation. Springer*, Netherlands. Pp 441- 461.

Bouma, T. J., Olenin, S., Reise, K., & Ysebaert, T. (2009). Ecosystem engineering & biodiversity in coastal sediments: posing hypotheses. *Helgoland Marine Research*, ***63*(1)**, 95-106.

Bouvier, T. C., & del Giorgio, P. A. (2002). Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. *Limnology and oceanography*, ***47*(2)**, 453-470.

Bowler, D. E., Buyung-Ali, L. M., Knight, T. M., & Pullin, A. S. (2010). A systematic review of evidence for the added benefits to health of exposure to natural environments. *BMC public health*, ***10*(1)**, 1-10.

Bowman, A. W., & Azzalini, A. (2003). Computational aspects of nonparametric smoothing with illustrations from the sm library. *Computational statistics & data analysis*, ***42*(4)**, 545-560.

Branch, G. M., & Pringle, A. (1987). The impact of the sandprawn *Callianassa kraussi* Stebbing on sediment turnover & on bacteria, meiofauna, & benthic microflora. *Journal of Experimental Marine Biology & Ecology*, ***107*(3)**, 219-235.

Breitburg, D., Levin, L. A., Oschlies, A., Grégoire, M., Chavez, F. P., Conley, D. J., Garçon, V., Gilbert, D., Gutiérrez, D., Isensee, K., Jacinto, G.S., Lumburg, K., Montes, I., Naqvi, S. W. A., Pitcher, G. C., Rabalais, N. N., Roman, M. R., Rose, K. A., Seibel, B. A., Telszewski, M., Yasuhara, M., & Zhang, J. (2018). Declining oxygen in the global ocean and coastal waters. *Science*, ***359*(6371)**, eaam7240.

Brierley, A. S., & Kingsford, M. J. (2009). Impacts of climate change on marine organisms and ecosystems. *Current biology*, ***19*(14)**, R602-R614.

Buccheri, M. A., Salvo, E., Coci, M., Quero, G. M., Zoccarato, L., Privitera, V., & Rappazzo, G. (2019). Investigating microbial indicators of anthropogenic marine pollution by 16S and 18S High-Throughput Sequencing (HTS) library analysis. *FEMS Microbiology Letters*, ***366*****(14)**, fnz179.

Cabelli, V. J. (1983). *Health effects criteria for marine recreational waters* (Vol. 600, No. 1-31). Health Effects Research Laboratory, Office of Research and Development, US Environmental Protection Agency.

Cabral, J. P. (2010). Water microbiology. Bacterial pathogens and water. *International journal of environmental research and public health*, ***7***(10), 3657-3703.

Cadée, G. C. (2001). Sediment dynamics by bioturbating organisms. In *Ecological comparisons of sedimentary shores* (pp. 127-148). *Springer*, Berlin, Heidelberg.

Cai, W. J., Hu, X., Huang, W. J., Murrell, M. C., Lehrter, J. C., Lohrenz, S. E., Chou, W., Zhai, W., Hollibaugh, J. T., Wang, Y., Zhao, P., Guo, X., Gundersen, K., Dai, M., & Gong, G. C. (2011). Acidification of subsurface coastal waters enhanced by eutrophication. *Nature geoscience*, ***4*****(11)**, 766-770.

Campbell, B. J., & Kirchman, D. L. (2013). Bacterial diversity, community structure and potential growth rates along an estuarine salinity gradient. *The ISME journal*, ***7*****(1)**, 210-220.

Caron, D. A., Gellene, A. G., Smith, J., Seubert, E. L., Campbell, V., Sukhatme, G. S., Seegers, B., Jones, B. H., Lie, A. A., Terrado, R., Howard, M. D. A., Kudela, R. M., Hayashi, K., Ryan, J., Birch, J., Demir-Hilton, E., Yamahara, K., Scholin, C., Mengel, M., & Robertson, G. (2017). Response of phytoplankton and bacterial biomass during a wastewater effluent diversion into nearshore coastal waters. *Estuarine, Coastal and Shelf Science*, ***186***, 223-236.

Caruso, G., Azzaro, M., Caroppo, C., Decembrini, F., Monticelli, L. S., Leonardi, M., Maimone, G., Zaccone, R., & La Ferla, R. (2016). Microbial community and its potential as descriptor of environmental status. *ICES Journal of Marine Science*, ***73*****(9)**, 2174-2177.

Cavicchioli, R. (2019). A vision for a 'microbcentric'future. *Microbial biotechnology*, ***12*****(1)**, 26-29.

Cavicchioli, R., Ripple, W. J., Timmis, K. N., Azam, F., Bakken, L. R., Baylis, M., Behrenfeld, M. J., Boetius, A., Boyd, P. W., Classen, A. T., Crowther, T.W., Danovaro, R., Foreman, C. M., Huisman, J., Hutchins, D. A., Jansson, J. K., Karl, D. M., Koskella, B., Mark Welch, D. B., Martiny, J. B. H., Moran, M. A., Orphan, V. J., Reay, D. S., Remais, J. V., Rich, V. I., Singh, B. K., Stein, L. Y., Stewart, F. J., Sullivan, M. B., van Oppen, M. J. H., Weaver, S. C., Webb, E. A., & Webster, N. S. (2019). Scientists' warning to humanity: microorganisms and climate change. *Nature Reviews Microbiology*, ***17*****(9)**, 569-586.

Chambers, J. M., & Hastie, T. J. (1992). Linear models. Chapter 4 of statistical models in S. *Wadsworth & Brooks/Cole*, *1992*.

Chen, J., McIlroy, S. E., Archana, A., Baker, D. M., & Panagiotou, G. (2019). A pollution gradient contributes to the taxonomic, functional, and resistome diversity of microbial communities in marine sediments. *Microbiome*, ***7***, 1-12.

Chen, Q., Chen, F., Gonsior, M., Li, Y., Wang, Y., He, C., Cai, R., Xu, J., Wang, Y., Xu, D., Sun, J., Zhang, T., Shi, Q., Jiao, N., & Zheng, Q. (2021). Correspondence between DOM molecules and microbial community in a subtropical coastal estuary on a spatiotemporal scale. *Environment International*, ***154***, 106558.

Cheng, H., Cheng, L., Wang, L., Zhu, T., Cai, W., Hua, Z., Wang, Y., & Wang, W. (2019). Changes of bacterial communities in response to prolonged hydrodynamic disturbances in the eutrophic water-sediment systems. *International Journal of Environmental Research and Public Health*, ***16*****(20)**, 3868.

Cloern, J. E. (2001). Our evolving conceptual model of the coastal eutrophication problem. *Marine ecology progress series*, ***210***, 223-253.

Cloern, J. E., Abreu, P. C., Carstensen, J., Chauvaud, L., Elmgren, R., Grall, J., Greening, H., Johansson, J. O. R., Kahru, M., Sherwood, E. T., & Xu, J. (2016). Human activities & climate variability drive fast-paced change across the world's estuarine–coastal ecosystems. *Global change biology*, ***22*****(2)**, 513-529.

Converse, R. R., Piehler, M. F., & Noble, R. T. (2011). Contrasts in concentrations and loads of conventional and alternative indicators of fecal contamination in coastal stormwater. *Water Research*, ***45*****(16)**, 5229-5240.

Cordier, T., Alonso-Sáez, L., Apothéloz-Perret-Gentil, L., Aylagas, E., Bohan, D. A., Bouchez, A., Chariton, A., Creer, S., Frühe, L., Keck, F., Keeley, N., Laroche, O., Leese, F., Pochon, X., Stoeck, T., Pawlowski, J., & Lanzén, A. (2021). Ecosystems monitoring powered by environmental genomics: a review of current strategies with an implementation roadmap. *Molecular Ecology*, ***30*****(13)**, 2937-2958.

Costanza, R., De Groot, R., Sutton, P., Van der Ploeg, S., Anderson, S. J., Kubiszewski, I., Farber, S., & Turner, R. K. (2014). Changes in the global value of ecosystem services. *Global environmental change*, ***26***, 152-158.

Curry, K. D., Wang, Q., Nute, M. G., Tyshaieva, A., Reeves, E., Soriano, S., Wu, Q., Graeber, E., Finzer, P., Mendling, W., Savidge, T., Villapol, S., Dilthey, A., & Treangen, T. J.

(2022). Emu: species-level microbial community profiling of full-length 16S rRNA Oxford Nanopore sequencing data. *Nature Methods*, ***19*****(7)**, 845-853.

Custodio, M., Peñaloza, R., Ordinola-Zapata, A., Peralta-Ortiz, T., Sánches-Suárez, H., Vieyra-Peña, E., Dela Cruz, H., & Alvarado-Ibáñez, J. (2023). Diversity of enterobacterales in sediments of lagoons with fish farming activity and analysis of antibiotic resistance. *Toxicology Reports*, ***10***, 235-244.

Dacayo, R. C., Rosana, A. R. R., & Villegas, L. C. (2019). Isolation and characterization of *Serratia marcescens* NBL 1001 bacteriophages from sewage water. *Journal of Nature Studies*, *18*(2), 92-107.

Danulat, E., Muniz, P., Garcia-Alonso, J., & Yannicelli, B. (2002). First assessment of the highly contaminated harbour of Montevideo, Uruguay. *Marine pollution bulletin*, ***44*****(6)**, 551-576.

Davies, T. W., Jenkins, S. R., Kingham, R., Kenworthy, J., Hawkins, S. J., & Hiddink, J. G. (2011). Dominance, biomass, and extinction resistance determine the consequences of biodiversity loss for multiple coastal ecosystem processes. *PLoS One*, ***6*****(12)**, e28362.

De Battisti, D. (2021). The resilience of coastal ecosystems: A functional trait-based perspective. *Journal of Ecology*, ***109*****(9)**, 3133-3146.

De Deckere, E. M. G. T., Tolhurst, T. J., & De Brouwer, J. F. C. (2001). Destabilization of cohesive intertidal sediments by infauna. *Estuarine, Coastal and Shelf Science*, ***53*****(5)**, 665-669.

DeAngelis, K. M., D'Haeseleer, P., Chivian, D., Fortney, J. L., Khudyakov, J., Simmons, B., Woo, H., Arkin, A. P., Devenport, K. W., Goodwin, L., Chen, A., Ivanova, N., Kyrpides, N. C., Mavromatis, K., Woyke, T., & Hazen, T. C. (2011). Complete genome

sequence of "*Enterobacter lignolyticus*" SCF1. *Standards in genomic sciences*, ***5*(1)**, 69-85.

Del Giorgio, P. A., & Cole, J. J. (1998). Bacterial growth efficiency in natural aquatic systems. *Annual Review of Ecology & Systematics*, ***29*(1)**, 503-541.

Deppeler, S. L., & Davidson, A. T. (2017). Southern Ocean phytoplankton in a changing climate. *Frontiers in Marine Science*, ***4***, 40.

Dobbs, F. C., & Guckert, J. B. (1988). *Callianassa trilobata* (Crustacea: Thalassinidea) influences abundance of meiofauna and biomass, composition, and physiologic state of microbial communities within its burrow. *Marine Ecology Progress Series*, ***45*(1-2)**.

Doney, S. C. (2010). The growing human footprint on coastal and open-ocean biogeochemistry. *science*, ***328*(5985)**, 1512-1516.

Doney, S. C., Ruckelshaus, M., Emmett Duffy, J., Barry, J. P., Chan, F., English, C. A., Galindo, H. M., Grebmeier, J. M., Hollowed, A. B., Knowlton, N., & Polovina, J., (2012). Climate change impacts on marine ecosystems. *Annual review of marine science*, ***4***, 11-37.

dplyr: a grammar of data manipulation. https://dplyr.tidyverse.org (2022).

Droffner, M. L., Brinton Jr, W. F., & Evans, E. (1995). Evidence for the prominence of well characterized mesophilic bacteria in thermophilic (50–70°C) composting environments. *Biomass and Bioenergy*, ***8*(3)**, 191-195.

Du, M., Chen, J., Zhang, X., Li, A., Li, Y., & Wang, Y. (2007). Retention of virulence in a viable but nonculturable *Edwardsiella tarda* isolate. *Applied and Environmental Microbiology*, ***73*(4)**, 1349-1354.

Duce, R. A., LaRoche, J., Altieri, K., Arrigo, K. R., Baker, A. R., Capone, D. G., Cornell, S., Dentener, F., Galloway, J., Ganeshram, R. S., Geider, R. J., Jickells, T., Kuypers, M. M., Langlois, R., Liss, P. S., Liu, S. M., Middelburg, J. J., Moore, C. M., Nickovic, S., Oschlies, A., Pedersen, T., Prospero, J., Schlitzer, R., Seitzinger, S., Sorensen, L. L., Uematsu, M., Ulloa, O., Voss, M., Ward, B., and Zamora, L. (2008). Impacts of Atmospheric Anthropogenic Nitrogen on the Open Ocean. *Science*, ***320*****(5878)**, 893–897.

Dufour, A. P. (1984). Health effects criteria for fresh recreational waters. *(No Title)*.

Dumbauld, B. R., Armstrong, D. A., & Feldman, K. L. (1996). Life-history characteristics of two sympatric thalassinidean shrimps, *Neotrypaea californiensis* and *Upogebia pugettensis*, with implications for oyster culture. *Journal of Crustacean Biology*, ***16*****(4)**, 689-708.

EU Commission (EC). (2018). Report from the Commission to the Council and the European Parliament on the Implementation of Council Directive 91/676/EEC Concerning the Protection of Waters against pollution caused by Nitrates from Agricultural Sources Based on Member State Reports for the Period 2008–2011. *EU Commission: Brussels, Belgium*.

Ferdie, M., & Fourqurean, J. W. (2004). Responses of seagrass communities to fertilization along a gradient of relative availability of nitrogen and phosphorus in a carbonate environment. *Limnology & Oceanography*, ***49*****(6)**, 2082-2094.

Filipuci, I. (2011, September). The effects of environmental stressors on coastal fish: in situ and experimental approach. *Littoral*.

Flo, E., Garcés, E., Manzanera, M., & Camp, J. (2011). Coastal inshore waters in the NW Mediterranean: physicochemical & biological characterization & management implications. *Estuarine, Coastal & Shelf Science*, ***93*****(4)**, 279-289.

Fortunato, C. S., Herfort, L., Zuber, P., Baptista, A. M., & Crump, B. C. (2012). Spatial variability overwhelms seasonal patterns in bacterioplankton communities across a river to ocean gradient. *The ISME journal*, ***6*****(3)**, 554-563.

Fox, J., & Weisberg, S. (2011). An R companion to applied regression. Sage. *Thousand Oaks*.

Frankenberg, D., Coles, S. L., & Johannes, R. E. (1967). The potential trophic significance of Callianassa major fecal pellets. *Limnology and Oceanography*, ***12*****(1)**, 113-120.

Frederiksen W. Genus *Citrobacter*. In: Brenner D. J., Krieg, N. R., Staley, J. T., editors. *Bergey's Manual of Systematic Bacteriology.* 2nd ed. Part B. Vol. 2. Springer; New York, NY, USA: 2005. pp. 651–656.

Frederiksen W., & Søgaard P. The genus *Citrobacter*. In: Dworkin M, Falkow S, Rosenberg E, editors. *The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community*. 3th ed. Springer-Verlag; New York, NY, USA: 2003. electronic release 314,

Fuhrman, J. A. (2009). Microbial community structure & its functional implications. *Nature*, ***459***:193-199.

Galkiewicz, J. P., & Kellogg, C. A. (2008). Cross-kingdom amplification using bacteria-specific primers: complications for studies of coral microbial ecology. *Applied and environmental microbiology*, ***74*****(24)**, 7828-7831.

Gallup, J. L., Sachs, J. D., & Mellinger, A. D. (1999). Geography and economic development. *International regional science review*, ***22*****(2)**, 179-232.

Gao, K. (2021). Approaches and involved principles to control pH/pCO2 stability in algal cultures. *Journal of Applied Phycology*, ***33*****(6)**, 3497-3505.

Garnier, J., Servais, P., & Billen, G. (1992). Bacterioplankton in the Seine River (France): impact of the Parisian urban effluent. *Canadian Journal of Microbiology*, ***38*****(1)**, 56-64.

Gaylard, S., Waycott, M., & Lavery, P. (2020). Review of coast and marine ecosystems in temperate Australia demonstrates a wealth of ecosystem services. *Frontiers in Marine Science*, ***7***, 453.

Geronimo, F. K., Guerra, H., Jeon, M., Reyes, N. J., & Kim, L. H. (2022). Influence of bacterial community to the water quality performance of urban stormwater nature-based solutions. *Ecological Engineering*, ***183***, 106761.

Ghosh, A., & Bhadury, P. (2019). Exploring biogeographic patterns of bacterioplankton communities across global estuaries. *MicrobiologyOpen*, ***8*****(5)**, e00741.

Giani, M., Boldrin, A., Matteucci, G., Frascari, F., Gismondi, M., & Rabitti, S. (2001). Downward fluxes of particulate carbon, nitrogen, and phosphorus in the north-western Adriatic Sea. *Science of the Total Environment*, ***266*****(1-3)**, 125-134.

Gobler, C. J. & Baumann, H. (2016). Hypoxia and acidification in marine ecosystems: Coupled dynamics & effects on ocean life. *Biology Letters*, ***12***, 20150976.

Goñi-Urriza, M., Capdepuy, M., Raymond, N., Quentin, C., & Caumette, P. (1999). Impact of an urban effluent on the bacterial community structure in the Arga River (Spain), with special reference to culturable Gram-negative rods. *Canadian journal of microbiology*, ***45*****(10)**, 826-832.

Griffiths, J. R., Kadin, M., Nascimento, F. J., Tamelander, T., Törnroos, A., Bonaglia, S., Bonsdorff, E., Brüchert, V., Gårdmark, A., Järnström, M., & Kotta, J. (2017). The

importance of benthic–pelagic coupling for marine ecosystem functioning in a changing world. *Global change biology*, ***23*****(6)**, 2179-2196.

Hale, R. L., Grimm, N. B., Vörösmarty, C. J., & Fekete, B. (2015). Nitrogen and phosphorus fluxes from watersheds of the northeast US from 1930 to 2000: Role of anthropogenic nutrient inputs, infrastructure, and runoff. *Global Biogeochemical Cycles*, ***29*****(3)**, 341-356.

Halpern, B. S., Frazier, M., Potapenko, J., Casey, K. S., Koenig, K., Longo, C., Lowndes, J. S., Rockwood, R. C., Selig, E. R., Selkoe, K. A., & Walbridge, S. (2015). Spatial and temporal changes in cumulative human impacts on the world's ocean. *Nature communications*, ***6*****(1)**, 1-7.

Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V., Micheli, F., d'Agrosa, C., Bruno, J. F., Casey, K. S., Ebert, C., Fox, H. E., Fujita, R., Henemann, D., Lenihan, H. S., Madin, E. M. P., Perry, M. T., Selig, E. R., Spalding, M., Steneck, R., & Watson, R. (2008). A global map of human impact on marine ecosystems. *science*, ***319*****(5865)**, 948-952.

Hanley, M. E., Bouma, T. J., & Mossman, H. L. (2020). The gathering storm: optimizing management of coastal ecosystems in the face of a climate-driven threat. *Annals of botany*, ***125*****(2)**, 197-212.

Harding, W. (1994). Water quality trends and the influence of salinity in a highly regulated estuary near Cape Town, South Africa. *South African Journal of Science*, ***90*****(4)**, 240-246.

Harley, C. D., Randall Hughes, A., Hultgren, K. M., Miner, B. G., Sorte, C. J., Thornber, C. S., Rodriguez, L. F., Tomanek, L. & Williams, S. L. (2006). The impacts of climate change in coastal marine systems. *Ecology letters*, ***9*****(2)**, 228-241.

Harris, J. M., Seiderer, L. J., & Lucas, M. I. (1991). Gut microflora of two saltmarsh detritivore thalassinid prawns, *Upogebia africana* and *Callianassa kraussi. Microbial ecology*, ***21***, 277-296.

Hassard, F., Gwyther, C. L., Farkas, K., Andrews, A., Jones, V., Cox, B., Brett, H., Jones, D. L., McDonald, J. E., & Malham, S. K. (2016). Abundance and distribution of enteric bacteria and viruses in coastal and estuarine sediments—A review. *Frontiers in microbiology*, ***7***, 1692.

He, Q., Bertness, M. D., Bruno, J. F., Li, B., Chen, G., Coverdale, T. C., Altieri, A. H., Bai, J., Sun, T., Pennings, S. C., Liu, J., Ehrlich, P. R., & Cui, B. (2014). Economic development and coastal ecosystem change in China. *Scientific reports*, ***4*****(1)**, 5995.

Hewson, I., & Fuhrman, J. A. (2004). Richness and diversity of bacterioplankton species along an estuarine gradient in Moreton Bay, Australia. *Applied and environmental microbiology*, ***70*****(6)**, 3425-3433.

Hoegh-Guldberg, O., & Bruno, J. F. (2010). The impact of climate change on the world's marine ecosystems. *Science*, ***328*****(5985)**, 1523-1528.

Hollertz, K., & Duchêne, J. C. (2001). Burrowing behaviour & sediment reworking in the heart urchin Brissopsis lyrifera Forbes (Spatangoida). *Marine Biology*, ***139*****(5)**, 951-957.

Holthuis, L. B. (1980). FAO species catalogue (Vol. 1, pp. 1-261). Fao.

Hossain, M. S., Gain, A. K., & Rogers, K. G. (2020). Sustainable coastal social-ecological systems: how do we define "coastal"?. *International journal of sustainable development & world ecology*, ***27*****(7)**, 577-582.

Hou, L., Zheng, Y., Liu, M., Gong, J., Zhang, X., Yin, G., & You, L. (2013). Anaerobic ammonium oxidation (anammox) bacterial diversity, abundance, and activity in marsh

sediments of the Yangtze Estuary. *Journal of Geophysical Research: Biogeosciences*, ***118*****(3)**, 1237-1246.

Howarth, R. W. (2008). Coastal nitrogen pollution: a review of sources and trends globally and regionally. *Harmful algae*, ***8*****(1)**, 14-20.

Howell, A. B., Vorsa, N., Marderosian, A. D., & Foo, L. Y. (1998). Inhibition of the adherence of P-fimbriated *Escherichia coli* to uroepithelial-cell surfaces by proanthocyanidin extracts from cranberries. *New England Journal of Medicine*, ***339*****(15)**, 1085-1086.

Hu, A., Ju, F., Hou, L., Li, J., Yang, X., Wang, H., Mulla, S. I., Sun, Q., Bürgmann, H., & Yu, C. P. (2017). Strong impact of anthropogenic contamination on the co-occurrence patterns of a riverine microbial community. *Environmental microbiology*, ***19*****(12)**, 4993-5009.

Huang, S., Sherman, A., Chen, C., & Jaffé, P. R. (2021). Tropical cyclone effects on water and sediment chemistry and the microbial community in estuarine ecosystems. *Environmental Pollution*, ***286***, 117228.

Hughes, A. R., Williams, S. L., Duarte, C. M., Heck Jr, K. L., & Waycott, M. (2009). Associations of concern: declining seagrasses and threatened dependent species. *Frontiers in Ecology and the Environment*, ***7*****(5),** 242-246.

Hylland, K., & Vethaak, A. D. (2011). Impact of contaminants on pelagic ecosystems. *Ecological impacts of toxic chemicals*, 212-224.

Imai, A., Fukushima, T., Matsushige, K., Kim, Y. H., & Choi, K. (2002). Characterization of dissolved organic matter in effluents from wastewater treatment plants. *Water research*, ***36*****(4)**, 859-870.

Inman, D. L., & Brush, B. M. (1973). The Coastal Challenge: Fragile ribbons which border our land require more understanding, new technology, and resolute planning. *Science*, ***181*****(4094)**, 20-32.

IPCC (2007). Climate Change: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Working Group I Contribution to the Fourth Assessment Report of the IPCC.

IPCC. (2019). Summary for policymakers. In Pörtner, H. O., Roberts, D. C., Masson-Delmotte, V., Zhai, P., Tignor, M., Poloczanska, E., Mintenbeck, K., Nicolai, M., Okem, A., & Petzold, J. (2019). IPCC Special Report on the Ocean and Cryosphere in a Changing Climate, IPCC Intergov. *Panel Clim. Chang*.

Jarvie, D. M. (2012). Shale resource systems for oil and gas: Part 2—Shale-oil resource systems.

Jennerjahn, T. C. (2012). Biogeochemical response of tropical coastal systems to present and past environmental change. *Earth-Science Reviews*, ***114*****(1-2)**, 19-41.

Jevrejeva, S., Moore, J. C., & Grinsted, A. (2012). Sea level projections to AD2500 with a new generation of climate change scenarios. *Global and Planetary Change*, ***80***, 14-20.

Jiang, H. B., Fu, F. X., Rivero-Calle, S., Levine, N. M., Sañudo-Wilhelmy, S. A., Qu, P. P., Wang, X.W., Pinedo-Gonzalez, P., Zhu, Z., & Hutchins, D. A. (2018). Ocean warming alleviates iron limitation of marine nitrogen fixation. *Nature Climate Change*, ***8*****(8)**, 709-712.

Jones, C. G., Lawton, J. H., & Shachak, M. (1994). Organisms as ecosystem engineers. *Oikos*, 373-386.

Jordaan, K., Comeau, A. M., Khasa, D. P., & Bezuidenhout, C. C. (2019). An integrated insight into the response of bacterial communities to anthropogenic contaminants in a river: A case study of the Wonderfonteinspruit catchment area, South Africa. *PloS one*, ***14*****(5)**, e0216758.

Jung, J., & Park, W. (2015). Acinetobacter species as model microorganisms in environmental microbiology: current state and perspectives. *Applied microbiology and biotechnology*, ***99***, 2533-2548.

Kai, S., Matsuo, Y., Nakagawa, S., Kryukov, K., Matsukawa, S., Tanaka, H., Iwai, T., Imanishi, T., & Hirota, K. (2019). Rapid bacterial identification by direct PCR amplification of 16S rRNA genes using the MinION™ nanopore sequencer. *FEBS open bio*, ***9*****(3)**, 548-557.

Kalkan, S., & Altuğ, G. (2020). The composition of cultivable bacteria, bacterial pollution, and environmental variables of the coastal areas: An example from the Southeastern Black Sea, Turkey. *Environmental monitoring and assessment*, ***192*****(6)**, 356.

Keeling, R. F., Körtzinger, A., & Gruber, N. (2010). Ocean deoxygenation in a warming world. *Annual review of marine science*, ***2***, 199-229.

Kinoshita, K., Wada, M., Kogure, K., & Furota, T. (2003). Mud shrimp burrows as dynamic traps and processors of tidal-flat materials. *Marine Ecology Progress Series*, ***247***, 159-164.

Kirchman, D. L., Dittel, A. I., Malmstrom, R. R., & Cottrell, M. T. (2005). Biogeography of major bacterial groups in the Delaware Estuary. *Limnology and oceanography*, ***50*****(5)**, 1697-1706.

Kong, W. (2020). Evaluation and Optimization of Methods for the DNA Extraction from Drinking water.

Kontas, A., Kucuksezgin, F., Altay, O., & Uluturhan, E. S. İ. N. (2004). Monitoring of eutrophication and nutrient limitation in the Izmir Bay (Turkey) before and after wastewater treatment plant. *Environment International*, ***29*****(8)**, 1057-1062.

Kullenberg, G. (2010). Human empowerment: Opportunities from ocean governance. *Ocean & Coastal Management*, ***53*****(8)**, 405-420.

Lane, D. J. 1991. 16S/23S rRNA sequencing, p. 115-147. In E. Stackebrandt and M. Goodfellow (ed.), Nucleic acid techniques in bacterial systematics. John Wiley & Sons, New York, NY.

Laverock, B., Smith, C. J., Tait, K., Osborn, A. M., Widdicombe, S., & Gilbert, J. A. (2010). Bioturbating shrimp alter the structure and diversity of bacterial communities in coastal marine sediments. *The ISME journal*, ***4*****(12)**, 1531-1544.

Lee, K. S., Park, S. R., & Kim, Y. K. (2007). Effects of irradiance, temperature, & nutrients on growth dynamics of seagrasses: a review. *Journal of Experimental Marine Biology and Ecology*, ***350*****(1-2)**, 144-175.

Legendre, P., & Anderson, M. J. (1999). Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. *Ecological monographs*, ***69*****(1)**, 1-24.

Lemley, D. A., Adams, J. B., Rishworth, G. M., & Bouland, C. (2019). Phytoplankton responses to adaptive management interventions in eutrophic urban estuaries. *Science of the Total Environment*, ***693***, 133601.

Leung, K. Y., Wang, Q., Zheng, X., Zhuang, M., Yang, Z., Shao, S., Achmon, Y., & Siame, B. A. (2022). Versatile lifestyles of Edwardsiella: Free-living, pathogen, and core bacterium of the aquatic resistome. *Virulence*, ***13*****(1)**, 5-18.

Levermann, A., Clark, P. U., Marzeion, B., Milne, G. A., Pollard, D., Radic, V., & Robinson, A. (2013). The multimillennial sea-level commitment of global warming. *Proceedings of the National Academy of Sciences*, ***110*****(34)**, 13745-13750.

Li, H., Liu, L., Li, M., & Zhang, X. (2013). Effects of pH, temperature, dissolved oxygen, and flow rate on phosphorus release processes at the sediment and water interface in storm sewer. *Journal of analytical methods in chemistry*, *2013*.

Liang, J., Mao, G., Yin, X., Ma, L., Liu, L., Bai, Y., Zhang, T., & Qu, J. (2020). Identification and quantification of bacterial genomes carrying antibiotic resistance genes and virulence factor genes for aquatic microbiological risk assessment. *Water Research*, ***168***, 115160.

Lisa, J. A. (2015). *Linking structure and function of microbial communities responsible for sedimentary nitrogen processes in North Carolina estuaries*. The College of William and Mary.

Lønborg, C., Baltar, F., Carreira, C., & Morán, X. A. G. (2019). Dissolved organic carbon source influences tropical coastal heterotrophic bacterioplankton response to experimental warming. *Frontiers in microbiology*, ***10***, 2807.

Long, Y., Jiang, J., Hu, X., Hu, J., Ren, C., & Zhou, S. (2021). The response of microbial community structure and sediment properties to anthropogenic activities in Caohai wetland sediments. *Ecotoxicology and Environmental Safety*, ***211***, 111936.

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome biology*, ***15*****(12)**, 1-21.

Lu, X. M., & Lu, P. Z. (2014). Characterization of bacterial communities in sediments receiving various wastewater effluents with high-throughput sequencing analysis. *Microbial ecology*, ***67***, 612-623.

Lu, Y., Yuan, J., Lu, X., Su, C., Zhang, Y., Wang, C., Cao, X., Li, Q., Su, J., Ittekkot, V., Garbutt, R. A., Bush, S., Fletcher, S., Wagey, T., Kachur, A., & Sweijd, N. (2018). Major threats of pollution and climate change to global coastal ecosystems and enhanced management for sustainability. *Environmental Pollution*, ***239***, 670-680.

Luna, G. M., Quero, G. M., & Perini, L. (2016). Next generation sequencing reveals distinct fecal pollution signatures in aquatic sediments across gradients of anthropogenic influence. *Advances in Oceanography and Limnology*, ***7*****(2)**.

MacArthur, R. H., & Wilson, E. O. (1967). *The Theory of Island Biogeography (Volume 1)*. Princeton University Press, Princeton, New Jersey, US.

Maire, O., Duchêne, J. C., Grémare, A., Malyuga, V. S., & Meysman, F. J. R. (2007). A comparison of sediment reworking rates by the surface deposit-feeding bivalve *Abra ovata* during summertime and wintertime, with a comparison between two models of sediment reworking. *Journal of Experimental Marine Biology and Ecology*, ***343*****(1)**, 21-36.

Malham, S. K., Rajko-Nenow, P., Howlett, E., Tuson, K. E., Perkins, T. L., Pallett, D. W., Wang, H., Jago, C. F., Jones, D. L., & McDonald, J. E. (2014). The interaction of human microbial pathogens, particulate material and nutrients in estuarine environments and their impacts on recreational and shellfish waters. *Environmental Science: Processes & Impacts*, ***16*****(9)**, 2145-2155.

Malone, T., Malej, A., & Faganeli, J. (2021). *Coastal Ecosystems in Transition*. Hoboken, N. J: John Wiley and Sons.

Mangiafico, S. S. (2016). Summary and Analysis of Extension. *Program Evaluation in R, version, **1*****(1)**. [Version 1.18.7.]. rcompanion.org/handbook

Mann, B. C., Bezuidenhout, J. J., Swanevelder, Z. H., & Grobler, A. F. (2021). MinION 16S datasets of a commercially available microbial community enables the evaluation of DNA extractions and data analyses. *Data in Brief*, ***36***, 107036.

Matcher, G. F., Froneman, P. W., Meiklejohn, I., & Dorrington, R. A. (2018). Distinct responses of bacterial communities to agricultural and urban impacts in temperate southern African estuaries. *Estuarine, Coastal and Shelf Science*, ***200***, 224-233.

Matcher, G., Froneman, P. W., & Dorrington, R. A. (2015). *Aquatic microbial diversity: a sensitive and robust toll for assessing ecosystem health and functioning* (No. 2038/1, p. 14). WRC report.

Mateo-Sagasta, J., Zadeh, S. M., Turral, H., & Burke, J. (2017). Water pollution from agriculture: a global review. Executive summary.

Matthews, M. W., & Bernard, S. (2015). Eutrophication and cyanobacteria in South Africa's standing water bodies: A view from space. *South African journal of science, **111*****(5)**, 1-8.

McArdle, B. H., & Anderson, M. J. (2001). Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology*, ***82*****(1)**, 290-297.

McLellan, S. L., & Eren, A. M. (2014). Discovering new indicators of fecal pollution. *Trends in microbiology*, ***22*****(12)**, 697-706.

McMurdie, P. J., & Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PloS one*, ***8*****(4)**, e61217.

Millennium Ecosystem Assessment. (MEA; 2005). Coastal systems. In: Hassan R, Scholes R, Ash N, editors. Ecosystems and human well-being: current state and trends. Vol. 1. Washington (DC): Island Press; p. 513–549.

Morán, X. A. G., López-Urrutia, Á. N. G. E. L., Calvo-Díaz, A. L. E. J. A. N. D. R. A., & Li, W. K. (2010). Increasing importance of small phytoplankton in a warmer ocean. *Global Change Biology*, ***16*****(3)**, 1137-1144.

Moyo, R. K. (2014). *Ecology and behaviour of burrowing prawns and their burrow symbionts* (Master's thesis, University of Cape Town).

Moyo, R., Pillay, D., & Baeza, J. A. (2017). Symbiont-mediated shifts in sandprawn behaviour: Implications for ecosystem functioning in marine soft-sediment ecosystems. *Journal of Experimental Marine Biology and Ecology*, ***486***, 296-304.

Mvungi, E. F., & Pillay, D. (2019). Eutrophication overrides warming as a stressor for a temperate African seagrass (Zostera capensis). *Plos one*, ***14*****(4)**, e0215129.

Naidoo, S., & Olaniran, A. O. (2014). Treated wastewater effluent as a source of microbial pollution of surface water resources. *International journal of environmental research and public health*, ***11*****(1)**, 249-270.

National Research Council. (2011). Climate stabilization targets: emissions, concentrations, & impacts over decades to millennia. *National Academies Press*.

Neumann, B., Vafeidis, A. T., Zimmermann, J., & Nicholls, R. J. (2015). Future coastal population growth and exposure to sea-level rise and coastal flooding-a global assessment. *PloS one*, ***10*****(3)**, e0118571.

Nixon, S. W. (1995). Coastal marine eutrophication: a definition, social causes, and future concerns. *Ophelia*, ***41*****(1)**, 199-219.

Numberger, D., Ganzert, L., Zoccarato, L., Mühldorfer, K., Sauer, S., Grossart, H. P., & Greenwood, A. D. (2019). Characterization of bacterial communities in wastewater with enhanced taxonomic resolution by full-length 16S rRNA sequencing. *Scientific reports*, ***9*****(1)**, 9673.

Ohore, O. E., Ifon, B. E., Wang, Y., Kazmi, S. S. U. H., Zhang, J., Sanganyado, E., Jiao, X., Liu, W., & Wang, Z. (2023). Vertical changes in water depth and environmental variables drove the antibiotics and antibiotic resistomes distribution, and microbial food web structures in the estuary and marine ecosystems. *Environment International*, 108118.

Ohore, O. E., Zhang, S., Guo, S., Addo, F. G., Manirakiza, B., & Zhang, W. (2021a). Ciprofloxacin increased abundance of antibiotic resistance genes and shaped microbial community in epiphytic biofilm on Vallisneria spiralis in mesocosmic wetland. *Bioresource Technology*, ***323***, 124574.

Ohore, O. E., Zhang, S., Guo, S., Manirakiza, B., Addo, F. G., & Zhang, W. (2021b). The fate of tetracycline in vegetated mesocosmic wetlands and its impact on the water quality and epiphytic microbes. *Journal of Hazardous Materials*, ***417***, 126148.

Oksanen, J., Simpson, G. L., Blanchet, G. F., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, H. M. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M. De., Durand, S., Evangelista, H. B. A., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M. O., Lahti, L., McGlinn, D., Ouellette, M., Cunha, E. R., Smith, T., Stier, A., Braak, C.

J. F. T., & Weedon, J. (2022). Vegan: community ecology package. http://CRAN. R-project. org/package= vegan.

Orel, N., Fadeev, E., Klun, K., Ličer, M., Tinta, T., & Turk, V. (2022). Bacterial indicators are ubiquitous members of pelagic microbiome in anthropogenically impacted coastal ecosystem. *Frontiers in Microbiology*, ***12***, 765091.

Orth, R. J., Carruthers, T. J., Dennison, W. C., Duarte, C. M., Fourqurean, J. W., Heck, K. L., Hughes, A. R., Kendrick, G. A., Kenworthy, W. J., Olyarnik, S., & Short, F.T. (2006). A global crisis for seagrass ecosystems. *Bioscience*, ***56*****(12)**, 987-996.

Papaspyrou, S., Gregersen, T., Cox, R. P., Thessalou-Legaki, M., & Kristensen, E. (2005). Sediment properties and bacterial community in burrows of the ghost shrimp *Pestarella tyrrhena* (Decapoda: Thalassinidea). *Aquatic Microbial Ecology*, ***38*****(2)**, 181-190.

Perkins, T. L., Clements, K., Baas, J. H., Jago, C. F., Jones, D. L., Malham, S. K., & McDonald, J. E. (2014). Sediment composition influences spatial variation in the abundance of human pathogen indicator bacteria within an estuarine environment. *PloS one*, ***9*****(11)**, e112951.

Pillay, D. (2019). Ecosystem engineering by thalassinidean crustaceans: response variability, contextual dependencies and perspectives on future research. *Diversity*, ***11*****(4)**, 64.

Pillay, D. (2019). Ecosystem engineering by thalassinidean crustaceans: response variability, contextual dependencies and perspectives on future research. *Diversity*, ***11*****(4)**, 64.

Pillay, D., & Branch, G. M. (2011). Bioengineering effects of burrowing thalassinidean shrimps on marine soft-bottom ecosystems. *Oceanography and Marine Biology: an Annual Review* 49: 137–192.

Pillay, D., Branch, G. M., & Forbes, A. T. (2007). Effects of *Callianassa kraussi* on microbial biofilms and recruitment of macrofauna: a novel hypothesis for adult–juvenile interactions. *Marine Ecology Progress Series*, ***347***, 1-14.

Pillay, D., Branch, G. M., & Forbes, A. T. (2007c). Effects of *Callianassa kraussi* on microbial biofilms and recruitment of macrofauna: a novel hypothesis for adult–juvenile interactions. *Marine Ecology Progress Series*, ***347***, 1-14.

Pillay, D., Branch, G. M., Griffiths, C. L., Williams, C., & Prinsloo, A. (2010). Ecosystem change in a South African marine reserve (1960–2009): role of seagrass loss and anthropogenic disturbance. *Marine Ecology Progress Series*, ***415***, 35-48.

Pillay, D., Williams, C., & Whitfield, A. K. (2012). Indirect effects of bioturbation by the burrowing sandprawn *Callichirus kraussi* on a benthic foraging fish, *Liza richardsonii*. *Marine Ecology Progress Series*, ***453***, 151-158.

Pomeroy, L. R., Hanson, R. B., McGillivary, P. A., Sherr, B. F., Kirchman, D., & Deibel, D. (1984). Microbiology and chemistry of faecal products of pelagic tunicates: rates and fates. *Bulletin of Marine Science*, ***35*****(3)**, 426-439.

Poth, M., & Focht, D. D. (1985). 15N kinetic analysis of N2O production by Nitrosomonas europaea: an examination of nitrifier denitrification. *Applied and environmental microbiology*, ***49*****(5)**, 1134-1141.

Psych: procedures for psychological, psychometric, and personality research. https://CRAN.R-project.org/package=psych (2021).

Pusceddu, A., Fiordelmondo, C., & Danovaro, R. (2005). Sediment resuspension effects on the benthic microbial loop in experimental microcosms. *Microbial ecology*, ***50***, 602-613.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids research*, ***41*****(D1)**, D590-D596.

Qwabe, W. (2019). *Interactions between ecosystem engineering by burrowing sandprawns (Callichirus kraussi) and nutrients: consequences for benthic community structure & ecosystem functioning*.

Raymond, P. A., & Spencer, R. G. (2015). riverine DOM. In *Biogeochemistry of marine dissolved organic matter* (pp. 509-533). Academic Press.

Reichardt, W. (1988). Impact of bioturbation by Arenicola marina on microbiological parameters in intertidal sediments. *Marine ecology progress series. Oldendorf*, ***44*****(2)**, 149-158.

Reise, K. (2002). Sediment mediated species interactions in coastal waters. *Journal of Sea Research*, ***48*****(2)**, 127-141.

Richardson, K., & Jørgensen, B. B. (1996). Eutrophication: definition, history and effects. *Eutrophication in coastal marine ecosystems*, ***52***, 1-19.

Robins, P. E., Lewis, M. J., Simpson, J. H., Howlett, E. R., & Malham, S. K. (2014). Future variability of solute transport in a macrotidal estuary. *Estuarine, Coastal and Shelf Science*, ***151***, 88-99.

Rocke, E., Cheung, S., Gebe, Z., Dames, N. R., Liu, H., & Moloney, C. L. (2020). Marine Microbial Community Composition During the Upwelling Season in the Southern Benguela. *Frontiers in Marine Science*, 7, 255.

Rodríguez, J., Gallampois, C. M., Timonen, S., Andersson, A., Sinkko, H., Haglund, P., Berglund, Å. M., Ripszam, M., Figueroa, D., Tysklind, M., & Rowe, O. (2018). Effects

of organic pollutants on bacterial communities under future climate change scenarios. *Frontiers in microbiology*, ***9***, 2926.

Rogers, L., Power, K., Gaora, P. Ó., & Fanning, S. (2016). Escherichia coli and other Enterobacteriaceae: occurrence and detection. In *Encyclopedia of food and health* (pp. 545-551). Elsevier Inc..

Romero, J., Lee, K. S., Pérez, M., Mateo, M. A., & Alcoverro, T. (2006). Nutrient dynamics in seagrass ecosystems. *Seagrasses: biology, ecology, and conservation*, 227-254.

Rosenqvist, T., Danielsson, M., Schleich, C., Ahlinder, J., Brindefalk, B., Pullerits, K., Dacklin, I., Salomonsson, E. N., Sundell, D., Forsman, M., Keucken, A., Radstrom, P., & Paul, C. J. (2023). Succession of bacterial biofilm communities following removal of chloramine from a full-scale drinking water distribution system. *npj Clean Water*, ***6*****(1)**, 41.

Rowden, A. A., Jones, M. B., & Morris, A. W. (1998). The role of *Callianassa subterranea* (Montagu)(Thalassinidea) in sediment resuspension in the North Sea. *Continental Shelf Research*, ***18*****(11)**, 1365-1380.

Santoro, A. E., & Boehm, A. B. (2007). Frequent occurrence of the human-specific Bacteroides faecal marker at an open coast marine beach: relationship to waves, tides and traditional indicators. *Environmental Microbiology*, ***9*****(8)**, 2038-2049.

Scott, T. M., Rose, J. B., Jenkins, T. M., Farrah, S. R., & Lukasik, J. (2002). Microbial source tracking: current methodology and future directions. *Applied and environmental microbiology*, ***68*****(12)**, 5796-5803.

Seidel, L., Broman, E., Nilsson, E., Ståhle, M., Ketzer, M., Pérez-Martínez, C., Turner, S., Hylander, S., Pinhassi, J., Forsman, A., & Dopson, M. (2023). Climate change-related

warming reduces thermal sensitivity and modifies metabolic activity of coastal benthic bacterial communities. *The ISME Journal*, ***17*****(6)**, 855-869.

Selje, N., & Simon, M. (2003). Composition and dynamics of particle-associated and free-living bacterial communities in the Weser estuary, Germany. *Aquatic Microbial Ecology*, ***30*****(3)**, 221-237.

Short, F. T., & Wyllie-Echeverria, S. (1996). Natural and human-induced disturbance of seagrasses. *Environmental conservation*, ***23*****(1)**, 17-27.

Shuval, H. (2003). Estimating the global burden of thalassogenic diseases: human infectious diseases caused by wastewater pollution of the marine environment. *Journal of water and health*, ***1*****(2)**, 53-64.

Siebert, T., & Branch, G. M. (2005a). Interactions between *Zostera capensis*, *Callianassa kraussi* and *Upogebia africana*: deductions from field surveys in Langebaan Lagoon, South Africa. *African Journal of Marine Science*, ***27*****(2)**, 345-356.

Siebert, T., & Branch, G. M. (2005b). Interactions between *Zostera capensis*, *Callianassa kraussi* and *Upogebia africana*: deductions from field surveys in Langebaan Lagoon, South Africa. *African Journal of Marine Science*, ***27*****(2)**, 345-356.

Song, H. K., Song, W., Kim, M., Tripathi, B. M., Kim, H., Jablonski, P., & Adams, J. M. (2017). Bacterial strategies along nutrient and time gradients, revealed by metagenomic analysis of laboratory microcosms. *FEMS Microbiology Ecology*, ***93*****(10)**, fix114.

Spellerberg, I. F., & Fedor, P. J. (2003). A tribute to Claude Shannon (1916–2001) and a plea for more rigorous use of species richness, species diversity and the 'Shannon–Wiener'Index. *Global ecology and biogeography*, ***12*****(3)**, 177-179.

Stackebrandt, E., and Liesack. W. (1993). Nucleic acids and classification, p. 152-189. In M. Goodfellow and A. G. O'Donnell (ed.), Handbook of new bacterial systematics. Academic Press, London, England.

Statham, P. J. (2012). Nutrients in estuaries—An overview and the potential impacts of climate change. *Science of the total environment*, ***434***, 213-227.

Steward, C. C., Nold, S. C., Ringelberg, D. B., White, D. C., & Lovell, C. R. (1996). Microbial biomass and community structures in the burrows of bromophenol producing and non-producing marine worms and surrounding sediments. *Marine Ecology Progress Series*, ***133***, 149-165.

Stewart, J. R., Gast, R. J., Fujioka, R. S., Solo-Gabriele, H. M., Meschke, J. S., Amaral-Zettler, L. A., Del Castillo, E., Polz, M. F., Collier, T. K., Strom, M. S., Sinigalliano, C. D., Moeller, P. DR., & Holland, A. F. (2008). The coastal environment and human health: microbial indicators, pathogens, sentinels and reservoirs. *Environmental health*, ***7***, 1-14.

Su, Z., Li, A., Chen, J., Huang, B., Mu, Q., Chen, L., & Wen, D. (2020). Wastewater discharge drives ARGs spread in the coastal area: a case study in Hangzhou Bay, China. *Marine pollution bulletin*, ***151***, 110856.

Suchanek, T. H., & Colin, P. L. (1986). Rates and effects of bioturbation by invertebrates and fishes at Enewetak and Bikini Atolls. *Bulletin of Marine Science*, *38*(1), 25-34.

Sutherland, K. P., Porter, J. W., Turner, J. W., Thomas, B. J., Looney, E. E., Luna, T. P., Meyers, M. K., Futch, J. C., & Lipp, E. K. (2010). Human sewage identified as likely source of white pox disease of the threatened Caribbean elkhorn coral, Acropora palmata. *Environmental microbiology*, ***12*****(5)**, 1122-1131.

Thornton, D. C. (2014). Dissolved organic matter (DOM) release by phytoplankton in the contemporary and future ocean. *European Journal of Phycology*, ***49*****(1)**, 20-46.

Thornton, J. A., McMillan, P. H., & Romanovsky, P. (1989). Perceptions of water pollution in South Africa: Case studies from two water bodies (Hartbeespoort Dam and Zandvlei). *South African Journal of Psychology*, ***19*****(4)**, 199-204.

tidyr: tidy messy data. https://tidyr.tidyverse.org (2022).

Tools for microbiome analysis in R. http://microbiome.github.com/microbiome (2017).

Townsend, E. C., & Fonseca, M. S. (1998). Bioturbation as a potential mechanism influencing spatial heterogeneity of North Carolina seagrass beds. *Marine Ecology Progress Series*, ***169***, 123-132.

Traving, S. J., Rowe, O., Jakobsen, N. M., Sørensen, H., Dinasquet, J., Stedmon, C. A., Andersson, A., & Riemann, L. (2017). The effect of increased loads of dissolved organic matter on estuarine microbial community composition and function. *Frontiers in microbiology*, ***8***, 351.

United Nations Environment Programme (UNEP). 2001. Water quality: The impact of eutrophication. *Lakes and Reservoirs*, 3:4-25.

United Nations (UN). (2017). The sustainable development goals report 2017. New York: (publisher unknown).

US EPA. (2012). Recreational water quality criteria. *Health and Ecological Criteria Division OoSaT*, 42.

Van Ginkel, C. E. (2011). Eutrophication: Present reality & future challenges for South Africa. *Water SA*, ***37*****(5)**, 693-702.

Venter, O. (2019). *Effects of burrowing sandprawns (Callichirus kraussi) on urban estuarine water quality* (Master's thesis, Faculty of Science).

Venter, O., Pillay, D., & Prayag, K. (2020). Water filtration by burrowing sandprawns provides novel insights on endobenthic engineering and solutions for eutrophication. *Scientific Reports*, ***10*****(1)**, 1913.

Venter, O., Pillay, D., & Prayag, K. (2020). Water filtration by burrowing sandprawns provides novel insights on endobenthic engineering and solutions for eutrophication. *Scientific Reports*, ***10*****(1)**, 1913.

Volkenborn, N., Polerecky, L., Wethey, D. S., & Woodin, S. A. (2010). Oscillatory porewater bioadvection in marine sediments induced by hydraulic activities of Arenicola marina. *Limnology and oceanography*, ***55*****(3)**, 1231-1247.

Von Sperling, M. (2007). *Wastewater characteristics, treatment and disposal*. IWA publishing.

Waltham, N. J., Elliott, M., Lee, S. Y., Lovelock, C., Duarte, C. M., Buelow, C., Simenstad, C., Nagelkerken, I., Claassens, L., Wen, C. K., Barletta, M., Connolly, R. M., Gillies, C., Mitsch, W. J., Ogburn, M. B., Purandare, J., Possingham, H., & Sheaves, M. (2020). UN decade on ecosystem restoration 2021–2030—what chance for success in restoring coastal ecosystems?. *Frontiers in Marine Science*, ***7***, 71.

Wang, M., & Chen, Y. (2018). Generation and characterization of DOM in wastewater treatment processes. *Chemosphere*, ***201***, 96-109.

Wang, X. H., Pinardi, N., & Malacic, V. (2007). Sediment transport and resuspension due to combined motion of wave and current in the northern Adriatic Sea during a Bora event

in January 2001: A numerical modelling study. *Continental Shelf Research*, ***27*****(5)**, 613-633.

Warrior, H., & Carder, K. (2005). Production of hypersaline pools in shallow basins by evaporation. *Geophysical research letters*, ***32*****(18)**.

Water, U. N. (2018). 2018 un world water development report, nature-based solutions for water.

Waycott, M., Duarte, C. M., Carruthers, T. J., Orth, R. J., Dennison, W. C., Olyarnik, S., Calladine, A., Fourqurean, J. W., Heck Jr, K. L., Hughes, A. R., Kendrick, G. A., Kenworthy, W. J., Short, F. T., & Williams, S. L. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the national academy of sciences*, ***106*****(30)**, 12377-12381.

Webster, N. S., Wagner, M., & Negri, A. P. (2018). Microbial conservation in the Anthropocene. *Environmental Microbiology*, ***20*****(6)**, 1925-1928.

Wickham, H. (2007). Reshaping data with the reshape package. *Journal of statistical software*, *21*, 1-20.

Wickham, H., Chang, W., & Wickham, M. H. (2016). Package 'ggplot2'. *Create elegant data visualisations using the grammar of graphics. Vsersion*, ***2*****(1)**, 1-189.

Widdows, J., Brinsley, M. D., Salkeld, P. N., & Lucas, C. H. (2000). Influence of biota on spatial and temporal variation in sediment erodability and material flux on a tidal flat (Westerschelde, The Netherlands). *Marine Ecology Progress Series*, ***194***, 23-37.

Withers, P. C. (1992). Comparative animal physiology (pp. 542-545). Philadelphia: *Saunders College Pub.*.

Woodruff, J. D., Irish, J. L., & Camargo, S. J. (2013). Coastal flooding by tropical cyclones and sea-level rise. *Nature*, ***504*****(7478)**, 44-52.

World Health Organization, (WHO: 1998). Guidelines for safe recreational water environments: coastal and fresh waters draft for consultation ref. EOS/Draft/98.14, Geneva, October 1998, 207.

World Health Organization, (WHO: 2003). Looking back: Looking ahead: Five decades of challenges and achievements in environmental sanitation and health. *(No Title)*.

World Health Organization, (WHO: 2018). Waterborne disease related to unsafe water and sanitation. WHO Housing and health guidelines, 172 pages. ISBN 978-92-4-155037-6http://www.who.int/sustainable-development/housing/health-risks/waterborne-disease/en/ Accessed 21 January 2020.

Wu, Q. L., Zwart, G., Schauer, M., Kamst-van Agterveld, M. P., & Hahn, M. W. (2006). Bacterioplankton community composition along a salinity gradient of sixteen high-mountain lakes located on the Tibetan Plateau, China. *Applied and Environmental Microbiology*, ***72*****(8)**, 5478-5485.

Wu, J., Yunus, M., Islam, M. S., & Emch, M. (2016). Influence of climate extremes and land use on fecal contamination of shallow tubewells in Bangladesh. *Environmental science & technology*, ***50*****(5)**, 2669-2676.

Wu, Y., Lin, H., Yin, W., Shao, S., Lv, S., & Hu, Y. (2019). Water quality and microbial community changes in an urban river after micro-nano bubble technology in situ treatment. *Water*, ***11*****(1)**, 66.

Wyda, J. C., Deegan, L. A., Hughes, J. E., & Weaver, M. J. (2002). The response of fishes to submerged aquatic vegetation complexity in two ecoregions of the Mid-Atlantic Bight: Buzzards Bay and Chesapeake Bay. *Estuaries*, ***25***, 86-100.

Xiao, K. Q., Beulig, F., Kjeldsen, K. U., Jørgensen, B. B., & Risgaard-Petersen, N. (2017). Concurrent methane production and oxidation in surface sediment from Aarhus Bay, Denmark. *Frontiers in microbiology*, ***8***, 1198.

Xu, F. Y., Liu, Y. J., Ma, H. X., Zhang, Y., Su, S. B., Shen, C. J., & Lu, C. P. (2012). Characterization of a novel podoviridae-phage infecting *Serratia marcescens* isolated in China. *Bing du xue bao= Chinese Journal of Virology*, ***28*****(4)**, 439-443.

Xu, H., Chen, Z., Huang, R., Cui, Y., Li, Q., Zhao, Y., Wang, X., Mao, D., Luo, Y., & Ren, H. (2021). Antibiotic resistance gene-carrying plasmid spreads into the plant endophytic bacteria using soil bacteria as carriers. *Environmental Science & Technology*, ***55*****(15)**, 10462-10470.

Zhang, X. H., Liu, J., Liu, J., Yang, G., Xue, C. X., Curson, A. R., & Todd, J. D. (2019). Biogenic production of DMSP and its degradation to DMS—their roles in the global sulfur cycle. *Science China Life Sciences*, ***62***, 1296-1319.

Ziegler, M., Roik, A., Porter, A., Zubier, K., Mudarris, M. S., Ormond, R., & Voolstra, C. R. (2016). Coral microbial community dynamics in response to anthropogenic impacts near a major city in the central Red Sea. *Marine pollution bulletin*, ***105*****(2)**, 629-640.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009), Mixed Effects Models and Extensions in Ecology with R, 580 pp., Springer, New York, NY.

6. APPENDICES

6.1 Appendix A: Experimental conditions results

Table A: Mean and standard error of abiotic environmental variables measured over the course of the mesocosm experiment across different sandprawn densities

Treatment	Day	Sandprawn density	Temp (°C)	Cond (mS/cm)	Sal (ppt)	pH	Turbidity (NTU)	DO (%)
Low temp, mesotrophic	0	0	13.7±0.1	46.3±0.3	30.1±0.2	8±0.1	9±2.9	93.4±1.3
		50	13.6±0	45.7±0.2	29.6±0.2	8±0	8.7±2.1	94.9±0.1
		100	13.7±0.1	45.7±0.1	29.6±0.1	7.9±0	8.7±1.5	94.6±0
	3	0	14.7±0.3	45.7±0.3	29.7±0.2	8.1±0.1	7.3±1.1	93±2
		50	14.3±0.4	44.1±0.3	28.5±0.2	8±0.1	6.1±0.3	94.4±0.6
		100	14.4±0.2	44.2±0	28.5±0	8.1±0.1	6.1±0.3	93.5±1.9
	6	0	15.1±0.3	45.7±0.4	29.6±0.3	8.1±0	8.3±2.5	93.5±0.2
		50	14.3±0.1	44.8±0.3	28.9±0.3	8.1±0	5.9±0.1	93.5±0.8
		100	14.8±0.3	44.6±0.1	28.8±0	8.1±0.1	6.7±1	94.2±0.6
	9	0	15.3±0.4	45.7±0.6	29.7±0.4	8.1±0	7.1±0.9	93.8±1.2
		50	14.7±0.1	44.7±0	28.9±0	8.1±0	6.1±0.5	94.3±0.6
		100	15.3±0.4	44.5±0.1	28.5±0.4	8.1±0	6.7±0.9	94±0.7
	12	0	15.4±0.3	46±0.3	29.8±0.3	8.2±0.1	5.8±0	93.6±1.9
		50	14.6±0.1	45.1±0	29.2±0	8.1±0.1	5.8±0.1	93.4±1.6
		100	16.5±1.2	44.9±0.2	29.1±0.1	8.2±0.1	5.9±0.2	93.1±1.2
	15	0	15.1±0.3	46.5±0.3	30.2±0.2	8.1±0	6±0.3	94.5±0.2
		50	14.4±0.1	45.6±0.1	29.5±0.1	8.2±0.1	5.7±0	94.1±0.7
		100	14.9±0.3	45.4±0	29.5±0	8.4±0.3	8.3±1.4	94.5±0.9
High temp, mesotrophic	0	0	13.9±0.3	46.7±0.2	30.3±0.1	8±0.1	8.1±1.3	95.1±0.5
		50	13.8±0.2	46.7±0.3	30.3±0.2	8±0	10±1.6	94.4±0.3
		100	13.8±0.2	46.3±0.6	30±0.4	8±0.1	8.8±2	95.3±0.7
	3	0	30±1	49.3±0.2	32.1±0.2	8.3±0.1	7.8±1.5	97±0.6
		50	28.2±0.8	47.4±0.7	30.8±0.5	8.3±0	6.1±0	95.4±0.3
		100	27.8±0.4	46.1±0.9	29.8±0.6	8.3±0	6.7±0.3	96.8±0.5
	6	0	29.7±0.5	51.5±0.8	33.8±0.6	8.3±0.1	6.7±0.3	96.9±0.6
		50	28.7±0.4	49.5±0.9	32.3±0.7	8.3±0	8.7±1.9	96.6±0.3
		100	28.6±0.2	47.5±1.7	30.8±1.2	8.2±0.1	6.5±0	96±0.8

Table A (contd.)

Treatment	Day	Sandprawn density	Temp (°C)	Cond (mS/cm)	Sal (ppt)	pH	Turbidity (NTU)	DO (%)
	9	0	29±0.5	53.1±1.3	34.9±0.9	8.4±0	6.6±0.4	96.9±0.3
		50	29.1±0.8	51.3±1.1	33.6±0.8	8.4±0	6.5±0.4	96.7±0.9
		100	28.7±0.3	48.5±2	31.6±1.5	8.3±0.1	6.6±0.1	98.5±1
	12	0	29±0.3	54.6±1.5	35.8±1.2	8.4±0	6.2±0.1	101.5±1.3
		50	29.5±0.7	52.1±1.1	34.2±0.8	8.3±0	6.4±0.2	98.9±0.5
		100	28.6±0.3	49.8±1.9	32.5±1.4	8.3±0	6.2±0.1	97.5±0.5
	15	0	29.4±0.3	55.4±1.5	36.6±1.1	8.4±0	6.9±0.7	97.3±0.5
		50	29.4±0.7	53.8±1.2	35.4±0.9	8.3±0	6.4±0.2	96.9±0.1
		100	28.8±0.1	51.3±2.2	33.6±1.6	8.3±0	6.7±0.6	97.2±0.4
Low temp, eutrophic	0	0	13.7±0.2	45.6±0.1	29.5±0.1	8±0	6.2±0.1	95.3±0.1
		50	13.6±0.1	45.3±0.2	29.3±0.2	8±0	8.7±1.2	94.9±0.2
		100	13.6±0.1	45.6±0.3	29.5±0.2	8±0.1	8.9±2.1	93.9±1
	3	0	14±0.1	44.6±0.2	28.8±0.2	8±0	6±0.2	95.8±0.5
		50	14±0.3	43.3±0.2	27.9±0.2	8.1±0.1	5.9±0.1	93.6±1.1
		100	14.9±0.3	43.4±0.2	28.3±0.4	8.2±0.1	5.9±0.1	92.8±0.7
	6	0	14.5±0.1	44.4±0.4	28.7±0.3	8.1±0.1	6.9±0.4	93.8±1.2
		50	14.3±0.4	43.7±0.2	28.2±0.2	8.1±0.1	5.9±0.2	93.4±0.8
		100	15.1±0.4	43.8±0.2	28.3±0.1	8.1±0	6.5±0.5	94.4±0.1
	9	0	14.9±0.1	44.2±0.2	28.6±0.1	8.1±0	6±0.1	94.3±1
		50	14.5±0.3	43.9±0.4	28.3±0.3	8.1±0	5.8±0	94.5±0.1
		100	15.5±0.4	44.2±0.2	28.6±0.2	8.1±0	5.8±0.1	94.6±0
	12	0	14.8±0.1	44.5±0.2	28.8±0.1	8.1±0	5.8±0	95.2±0.1
		50	14.5±0.3	44.4±0.3	28.7±0.2	8.1±0.1	5.7±0	92.2±2
		100	15.5±0.6	44.4±0.3	28.7±0.2	8.2±0.1	5.8±0.1	94.1±0.8
	15	0	14.8±0.2	45±0.2	29.1±0.2	8.1±0	6.9±1	94.3±0.6
		50	14.3±0.3	44.8±0.4	29±0.3	8.1±0	5.8±0.1	94.5±0.4
		100	15.1±0.4	45±0.3	29.1±0.2	8.1±0	6.8±1	94.4±0.8

Table A (contd.)

Treatment	Day	Sandprawn density	Temp (°C)	Cond (mS/cm)	Sal (ppt)	pH	Turbidity (NTU)	DO (%)
High temp, eutrophic	0	0	13.6±0	45.1±0.2	29.1±0.1	8±0	10.7±0	95±0.3
		50	13.5±0	45.4±0	29.4±0	8±0	8.7±1.6	94.9±0.3
		100	13.5±0.1	45.6±0.3	29.5±0.2	8±0	9.4±1.8	94.9±0.4
	3	0	28.8±0.5	46.9±1.1	30.3±0.7	8.3±0	6.6±0.3	95.7±0.6
		50	27.8±0.6	45.7±0.3	29.5±0.2	8.3±0.1	6.5±0.1	97.8±0.9
		100	26.8±0.4	44.9±0.1	29±0.1	8.3±0.1	6.2±0	96.1±0.4
	6	0	29.6±0.7	46.5±1	31.1±0.4	8.4±0.1	7.6±0.7	95.9±0.6
		50	29±0.5	46.6±1.4	36.8±5.5	8.4±0.1	6.6±0.1	96.2±0.5
		100	27.4±0.2	46.9±0.2	30.4±0.1	8.3±0.1	6.3±0	96.1±0.8
	9	0	29.3±0.8	48.8±0.6	31.6±0.5	8.4±0.1	6.3±0.1	96.6±0.4
		50	29±0.2	49.7±0.2	32.4±0.1	8.4±0	6.3±0.1	96.5±0.2
		100	28±0.2	48.5±0.3	31.6±0.2	8.3±0	6.2±0.1	97.8±0.4
	12	0	28.9±0.4	50.1±1	32.7±0.7	8.4±0	6.5±0.1	99±1.4
		50	28.8±0.4	51.8±0.3	33.9±0.3	8.4±0	6.5±0.3	97.1±0.5
		100	27.8±0.2	50.4±0.5	32.9±0.4	8.3±0	6.4±0.2	100.2±2.2
	15	0	28.8±0.1	51.5±1	33.8±0.8	8.4±0	7±0.7	97.7±0
		50	29.4±0.3	52.9±0.6	34.8±0.4	8.3±0	6.2±0.1	96.1±0.3
		100	28.1±0.2	52±0.5	34.1±0.4	8.2±0	6.2±0.1	97.8±0.9