

Effects of burrowing sandprawns (*Callichirus kraussi*) on urban estuarine water quality

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ABSTRACT

Eutrophication, one of the leading global water pollution challenges, can be addressed with nature-based solutions (NBSs). NBSs use or mimic natural processes (ecosystem services) to improve water quality, for example microorganisms, plants and filter feeders have already been shown to mitigate eutrophication. However, burrowing endobenthic organisms have not yet been considered as a NBS, despite their potential to greatly impact water quality. Therefore, the purpose of this study was to determine whether these endobenthic organisms could be effective as NBSs, more specifically whether the activities of the common sandprawn (*Callichirus kraussi*) can improve water quality in terms of pelagic nutrient, microalgal and suspended solids concentrations. *C. kraussi* is a benthic ecosystem engineer that, like other endobenthic crustaceans, affects water flow, sediment biogeochemistry and benthic-pelagic fluxes through bioturbation and bioirrigation. Their elaborate burrow systems may act as biological filters for pelagic microalgae, but they may also be a source of nutrient-rich excrement inputs into the water column. To determine the effects of *C. kraussi* on water quality, a laboratory mesocosm experiment and comparative *in situ* analysis were used in this study. In the mesocosm experiment, water quality at 3 increasing *C. kraussi* densities (0 [control], 100 and 200 individuals·m⁻²) were compared. At the end of the experiment, the water column NH₄⁺ concentration in the high density *C. kraussi* treatment was 2.6 times greater compared to the controls. Despite this increase in the NH₄⁺ concentration, the water column chl-a concentrations were 42.8% (50% density treatment) and 44.3% (100% density treatment) lower compared to the controls. Additionally, microalgal biomasses in the sediment were less concentrated on burrow mounds created by *C. kraussi* compared to the adjacent sediment between mounds and within the burrows. However, the overall chl-a concentrations in the sediment did not differ between treatments. This suggests that *C.*

kraussi has the effect of breaking up microalgal mats on the sediment surface with their burrow mounds without changing the overall sediment surface chl-a concentration, thereby maintaining benthic-pelagic coupling. *C. kraussi* did not affect suspended solids concentrations in the water column over the 12-day study period. For the *in situ* component, water quality was compared at sites where *C. kraussi* was present and the adjacent sections where it was absent in the Zandvlei Estuary Nature Reserve. The findings from the mesocosm experiment were reflected *in situ*: Chl-a concentrations in *C. kraussi* habitats were reduced by 43.1% from upstream areas without *C. kraussi*, and again increased by 32.9% from *C. kraussi* habitats to downstream canalised areas without *C. kraussi*. Differences in suspended solids concentrations were also not explained by *C. kraussi* presence *in situ*. The conclusion from this study is that ecosystem engineering by *C. kraussi* has an overall positive effect on water quality in urban estuaries, principally by reducing chl-a concentrations in the water column and maintaining benthic-pelagic coupling. Therefore, *C. kraussi* should be conserved for its water purification ecosystem services. This can be achieved by maintaining soft sediments in estuaries, rather than constructing concrete canals in which *C. kraussi* cannot burrow. Regulation of *C. kraussi* extraction for fishing bait is also necessary to prevent the loss of their filtration function. Furthermore, this study supports the use of *C. kraussi* as a NBS to eutrophication and encourages the consideration of similar endobenthic organisms for this purpose. Endobenthic ecosystem engineering should not be underestimated in addressing the global challenge of eutrophication.

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1 INTRODUCTION

1.1 Background

1.1.1 Water pollution

Due to the expected population growth, economic development and changing consumption patterns, global water demand is expected to continue increasing into the foreseeable future (Vörösmarty et al. 2000; United Nations Water [UN-Water] 2018). However, water availability is becoming unpredictable due to climate change (Oki and Kanae 2006; Intergovernmental Panel on Climate Change 2014). In urban areas, where 68% of the world's population is predicted to live by 2050, water demand may exceed surface water availability by 2050 (Padowski and Gorelick 2014; Flörke et al. 2018; United Nations Department of Economic and Social Affairs 2018). Therefore, we need to ensure that our available water is maintained at the appropriate quality to sustain biodiverse ecosystems and healthy societies.

One of the most prevalent water quality challenges in urban areas is nutrient-loading (Jeppesen et al. 1999; Schindler 2006; United Nations Environment Programme [UNEP] 2016). The main causes of nutrient-loading in water bodies is runoff from agricultural land containing excess nitrates and phosphorus (Jeppesen et al. 1999; Schindler 2006; Organisation for Economic Co-operation and Development 2013). Excess nutrients in a water body may affect water quality directly in terms of odour, taste or health effects, or indirectly by stimulating toxic algal growth in the process of eutrophication (Department of Water Affairs and Forestry 1996; Backer 2002; World Health Organisation 2017).

1.1.2 Eutrophication

Eutrophication is one of the leading global causes of water quality reduction in inland and coastal water bodies (UNEP 2001; Chislock et al. 2013). In South Africa between 2002 and 2012, 78% of the country's 50 largest water bodies were classified as either eutrophic (chlorophyll-a [chl-a] concentration of 20 – 30 $\mu\text{g}\cdot\text{L}^{-1}$) or hypertrophic (chl-a $\geq 30 \mu\text{g}\cdot\text{L}^{-1}$) (Matthews and Bernard 2015). In Europe between 2008 and 2011, 22.6% of rivers, 36.8% of lakes and all reported saline waters were classified as eutrophic or hypertrophic (European Commission 2013).

Eutrophication occurs when excess nutrients (mainly nitrogen [N] and phosphorus [P]) enter a water body and stimulate plant growth. Resultant algal blooms may prevent sunlight from penetrating into the water, causing algae below the surface to die and decompose, during which microorganisms flourish and deplete the water body of oxygen, creating hypoxic or anoxic conditions. Algal blooms may also be toxic, depending on the types of algae that are flourishing. A eutrophic water body can no longer support human activities, such as recreation or consumption, and may lead to biodiversity loss (UNEP 2001; van Ginkel 2011; Chislock et al. 2013).

To prevent or combat eutrophication, different water treatment methods can be used to remove excess nutrients and algae. Methods may be physical, such as filters; chemical, such as substances that precipitate out nutrients; biological, such as natural processes that break down or absorb nutrients; or a combination of these (UN-Water 2018). In general, physical and chemical methods tend to be more expensive, labour-intensive and environmentally disruptive compared to biological methods (Alvarez and Illman 2006; UN-Water 2018).

1.1.3 Nature based solutions

Nature-based solutions (NBS), also known as bioremediation, uses or mimics biological processes to improve water quality and resource management (UN-Water 2018). This water treatment approach is essentially the manipulation of ecosystem services. Ecosystem goods and services are the natural resources and processes that sustain all life on Earth and benefit human societies directly or indirectly (Constanza et al. 1997). For example, some trees provide goods in the form of wood, and services in the form of air or water filtration by absorbing gaseous or dissolved pollutants.

Since social and environmental benefits of conserving ecosystem services are often neglected due to decision-making that primarily focuses on more easily quantifiable financial benefits, Constanza et al. (1994 and 2014) attempted to estimate the global financial value of ecosystem services to humans. The value of ecosystem services (excluding the market value of goods) in 2011 was estimated to be US\$ 125 trillion per year (in 2007 US\$) (Constanza et al. 2014). This equates to approximately US\$ 154 trillion per year in 2019 (accounting for the increased consumer price index of 23.51% and increased average inflation rate of 1.78% since 2007 [Alioth Finance 2019]).

The most recent World Water Development Report (UN-Water 2018) details the importance of NBS for sustainable water management with co-benefits for the economy, society and natural environment. Understanding how biological processes and water quality influence each other may help us predict how changes in water quality will affect ecosystem services; it may also inform us which biological activities improve or degrade water quality.

It has been shown that NBS can be used to address eutrophication: Historically, microorganisms have been the most commonly used to remove excess nutrients from water (Vidali 2001; Alvarez and Illman 2006; Masters and Wendell 2014). Microorganisms, together with specific plants, have been used in natural or artificially constructed wetlands to combat eutrophication (Mitsch and Jørgensen 2004; Vymazal 2007; Wright et al. 2017). In marine environments, the filtering activities of filter feeders such as clams and mussels have also been shown to prevent eutrophication (Cloern 1982; Officer et al. 1982). Conversely, it has been suggested that the decline of the American oysters (*Crassostrea virginica*) in Chesapeake Bay due to overharvesting and disease may have contributed to the increase in phytoplankton and exacerbation of eutrophication in the bay (Newell 1988; Grabowski and Petersen 2007).

The NBSs provided by microorganisms, plants and filter feeders as described above link to the nutrient cycling, waste treatment and cultural ecosystem services as defined by Constanza et al. (1977). Globally, nutrient cycling and waste treatment have been shown to be worth approximately US\$ 27.1 trillion and US\$ 3.7 trillion per year respectively (Constanza et al. 1997, updated to reflect 2019 buying power [Alioth Finance 2019]).

Although there are many benefits to using NBS, they have been neglected in policies and natural resource management, because there are gaps in our understanding of natural ecosystems and their hydrological impacts (UN-Water 2018). Since the hydrological impacts of ecosystems vary with type, location and time, it can be difficult to make generalised assumptions about best water management practices (UN-Water

2018). Therefore, it is important to study the components and processes that affect water quality in different ecosystems to better inform water management decisions.

1.1.4 Benthic-pelagic coupling and its effects on water quality

There remain large gaps in our understanding of coastal and estuarine ecosystems due to their environmental variability, biogeochemical changes and biological interactions (Cloern et al. 2016; Griffiths et al. 2017). These ecosystems provide important ecosystem services, including enhancement of water quality (Agardy et al. 2005; Granek et al. 2010); however, many face challenges such as excessive nutrient-loading, especially in urban settings (Vitousek et al. 1997; Lotze et al. 2006; Cloern et al. 2016). A better understanding of the dynamic exchanges of energy and materials (including nutrients) in these habitats may help to address the issue of eutrophication and inform water management strategies (Griffiths et al. 2017).

Benthic-pelagic coupling – the connection between the sediment and water column habitats through mass or energy transfer (Griffiths et al. 2017) – may have profound effects on water quality, but is often overlooked (Stamhuis 1996; Raffaelli et al. 2003). For example, nutrients may be transferred to the water column from the sediment, or vice versa, by benthic activities such as bioturbation or bioirrigation (Koike and Mukai 1983; Raffaelli et al. 2003; Solan et al. 2008).

Robinson et al. (2018) showed that the bioturbation activities of sea cucumbers (*Holothuria scabra*) could influence the eutrophication process by increasing N fixation at elevated carbon concentrations. Results obtained by Pajand et al. (2017) suggest that polychaete *Nereis diversicolor* may affect eutrophication by consuming organic

matter and removing total N and total P from the water column. Kellogg et al. (2014) reviewed 8 field studies to determine the effectiveness of oysters (*Crassostrea virginica*) in mitigating eutrophication; they concluded that oysters can affect the N-cycle, but the effect and its magnitude depends on the ecosystem type (e.g. oyster reefs or aquaculture) and season. Other studies have shown that bioturbating polychaete *Marenzelleria neglecta* (Granberg et al. 2008), zebra mussels (*Dreissena polymorpha*) (McLaughlan and Aldridge 2013) and other benthic filter-feeders (Cloern 1982; Officer et al. 1982) may also significantly affect the eutrophication process. No studies have explicitly investigated the effects of common sandprawns (*Callichirus kraussi*) on eutrophication in urban estuaries.

1.1.5 Common Sandprawns (*Callichirus kraussi*)

Similar to filter feeders, the common sandprawn (*Callichirus kraussi*, formerly known as *Callianassa kraussi*) can significantly influence its environment and is referred to as an “ecosystem engineer” (Pillay and Branch 2011). Ecosystem engineers are organisms that modify, maintain or create habitats and influence the availability of resources to other species by causing physical changes to abiotic or biotic materials around them (Jones et al. 1994). Previous studies have shown that the bioturbation and irrigation activities of burrowing crustaceans such as sandprawns (or ghost-shrimps elsewhere) may influence community structures, water flow patterns, sediment biogeochemistry and nutrient exchanges between burrows and the overlying water column (Waslenchuk et al. 1983; Branch and Pringle 1987; Murphy and Kremer 1992; Siebert and Branch 2005; Pillay et al. 2007; Pillay and Branch 2011; Pillay et al. 2012; Henninger and Froneman 2013).

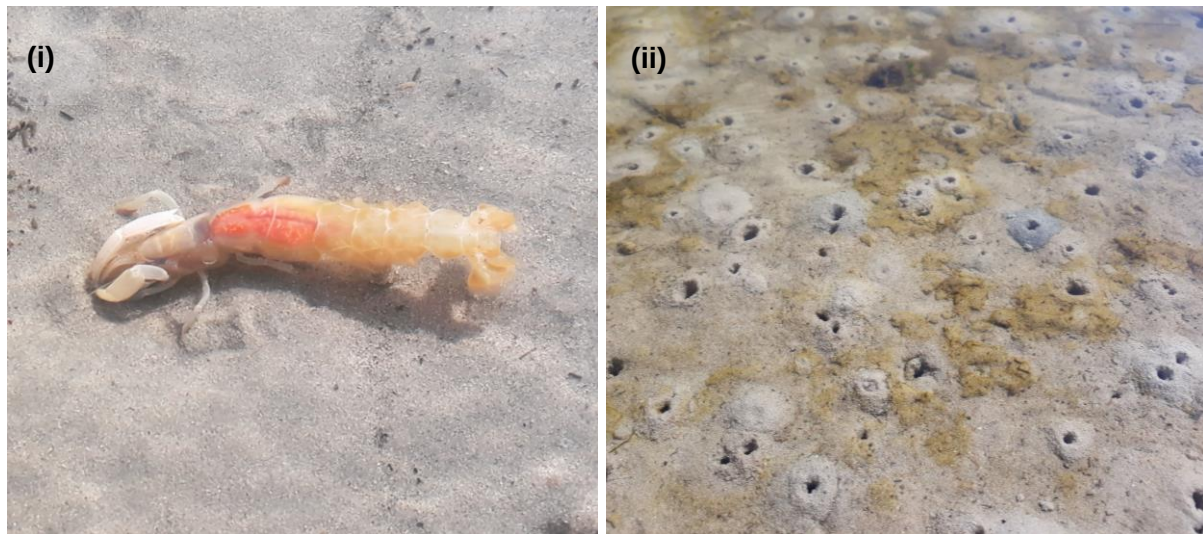


Figure 1. (i) *Callichirus kraussi* (6 cm) creating a new burrow in its estuarine habitat. (ii) Burrow holes and volcano-like mounds created by burrowing activities of *C. kraussi*.

C. kraussi (Figure 1-i) is a dominant thalassinidean crustacean species in marine and estuarine soft sediments along the southern African coast, from Luderitz Bay in Namibia to the Inhambane region in Mozambique (Holthuis 1980; Moyo 2014; Branch et al. 2016). These sandprawns occur in densities of 150-200 individuals·m⁻² (Branch and Pringle 1987; Siebert and Branch 2005) and live in complex, branched burrows (Forbes 1973). They are often used as fishing bait; however, pumping them from the sediment collapses their burrows and may negatively affect sympatric communities (Branch et al. 2016).

Callianassid crustaceans are considered to be some of the most influential bioturbators in terms of the amount and depth of sediment reworked (Cadée 2001). Sandprawns mobilise (e.g. resuspend, erode or shift) sediments through their burrowing activities (Widdows and Brinsley 2002; Moyo 2014), creating volcano-shaped mounds around their burrow openings (Suchanek 1983; Pillay and Branch 2011) (Figure 1-ii).

Pillay et al. (2012) found that sediment turnover from burrows to sediment surface is enhanced at higher sandprawn densities, which leads to the burial of microalgae on the sediment surface (corresponding to results of Suchanek [1983]). Similarly, Branch and Pringle (1987) observed in a field experiment that microalgae accumulated on the sediment surface where sandprawns were absent, but where sandprawns were present, microalgae were relatively more abundant in their burrows. This suggests that sandprawns may combat eutrophication by burying microalgae that colonise in the sediment from the water column or by transporting microalgae into their burrows through active pumping.

Sandprawns irrigate their burrows by beating their pleopods to draw in oxygenated water and flush out anoxic water in which waste has accumulated (Pillay and Branch, 2011). Irrigation allows for the exchange of materials such as nutrients, microalgae and sediment particles between the burrow environment and the overlying water column (Waslenchuk et al. 1983; Murphy and Kremer 1992). Since irrigation events occur intermittently, burrow water composition does not consistently reflect that of the overlying water column (Waslenchuk et al. 1983), i.e. concentration gradients may occur between the benthic and pelagic habitats.

Irrigation may increase nutrient concentrations in the overlying water column if porewater nutrients or sandprawn excrement seep into the burrow water and nutrient-enriched burrow water is flushed out into the overlying water column (Aller 1980; Aller et al. 1983; Koike and Mukai 1983; Waslenchuk et al. 1983; D'Andrea and DeWitt 2009). Alternatively, if concentrations are higher in the overlying water column than in the burrow environment, the concentration gradient may lead to the removal of

nutrients from the overlying water into the burrows through irrigation or diffusion (Aller 1980; Waslenchuk et al. 1983). Therefore, sandprawn irrigation activities may affect eutrophication by influencing nutrient concentrations in the water column.

During irrigation, microalgae can also be transported into the burrows, where they are thought to be adsorbed onto the mucous-lined burrow walls (Griffen et al. 2004; Pillay and Branch 2011). As deposit feeders, sandprawns consume the adsorbed microalgae (Frey and Howard 1975; Coelho 2004). They have also been described as ‘gardeners’, since they accumulate algae on their burrow walls and thereby increase their own food supply (Branch and Pringle 1987; Griffis and Chavez 1988; Pillay and Branch 2011). This adsorption and consumption of algae may effectively reduce pelagic algal concentrations, thereby potentially combating eutrophication.

The combination of bioturbation and irrigation may also affect the concentration of suspended solids in the overlying water column. Previous studies have suggested that sandprawn activities may increase the concentrations of fine suspended solids (Aller and Dodge 1974; Suchanek 1983; Colin et al. 1986). This effect may result in degradation of water quality due to increased turbidity and thus decreased light penetration (Murphy 1985).

1.2 Problem statement

Bioturbation and irrigation by *C. kraussi* are likely to affect the quality of the overlying water column, particularly in terms of eutrophication. However, since this has not explicitly been studied, it is not clear from previous studies whether *C. kraussi* activities are likely to exacerbate or alleviate eutrophication in their environment. For example,

Waslenchuk et al. (1983) and Murphy and Kremer (1992) found callianassid burrow water to be enriched in nutrients (NH_4^+ and PO_4^{3-}), and these nutrients to be flushed into the water column from the burrows, which could aid eutrophication. However, microalgal adsorption onto the burrow walls and subsequent consumption in the presence of *C. kraussi* suggests that they may combat eutrophication. If we do not know how sandprawns affect water quality, it may be unclear what the best estuary management practices would be with regards to the conservation of *C. kraussi* and improvement of water quality.

1.3 Project scope and objectives

The primary objective of this project was to determine how *C. kraussi* affects eutrophication by quantifying its effects on nutrient (N as ammonium [NH_4^+], nitrate [NO_3^-] and nitrite [NO_2^-], and P as phosphate [PO_4^{3-}]) and microalgal (as chl-a) concentrations in the water column. As a secondary objective, the effects of *C. kraussi* on microalgal concentrations at the sediment surface and burrow walls were quantified to form a mechanistic understanding of how microalgal biomass is affected by the presence of sandprawns. The final objective was to quantify the effects of sandprawns on suspended solids concentrations.

To achieve the project objectives, a mesocosm experiment was carried out in a laboratory aquarium. An experimental approach was used because it allowed for the critical evaluation of competing hypotheses (Quinn and Keough 2002) by isolating sandprawns' effects on nutrient and chl-a concentrations from other confounding factors that may affect these concentrations in natural systems. Laboratory

experiments also allowed for replication and generation of results in a short timeframe (Srivastava et al. 2004).

Comparative field surveys were also undertaken to determine whether results obtained under experimental conditions were evident in the field. This was done because results from laboratory experiments may not always accurately reflect general trends seen in natural systems, since the spatial and temporal scales are artificially constrained (Quinn and Keough 2002; Srivastava et al. 2004). Sandprawns, sediment and water required for both the mesocosm experiments and *in situ* measurements were collected from the Zandvlei Estuary Nature Reserve, Cape Town.

1.4 Hypotheses

The 3 competing hypotheses for this study were that water quality would (a) not be affected, (b) be degraded or (c) be improved with increasing densities of *C. kraussi*. These hypotheses were evaluated in terms of nutrient, chl-a and suspended solids concentrations.

1.5 Project significance

Determining how sandprawns affect eutrophication has important implications for managing urban estuaries and lagoons. If sandprawns combat eutrophication, then managers would be encouraged to create favourable conditions for them, such as soft sediments where the sandprawns can burrow instead of concrete canals. Sandprawns could also be considered as nature-based solutions for eutrophic systems.

If sandprawns aid eutrophication, then this gives estuary managers a focal area to address eutrophication by removing or excluding sandprawns from eutrophic areas. If sandprawns do not affect eutrophication, despite their other extensive environmental effects, then future eutrophication research and models can disregard these organisms under the conditions explored within this study.

2 METHODOLOGICAL APPROACH

2.1 Ethics statement

The methods used in this study were approved by the University of Cape Town (UCT) Science Faculty Animal Ethics Committee (approval number 2018/v10/DP). Sampling was carried out in a nature reserve (Zandvlei Estuary Nature Reserve) with permission from the landowners, issued by the Reserve Manager in the Biodiversity Management Branch of the City of Cape Town. An integrated environmental and fisheries research permit (number RES2018/53) was acquired from the Department of Environmental Affairs and the Department of Agriculture, Forestry and Fisheries for the collection, transportation and housing sandprawns from the Zandvlei Estuary Nature Reserve for research purposes.

2.2 Field study site: Zandvlei Estuary Nature Reserve

The Zandvlei Estuary is fed from the north by 4 main streams and flows south into the ocean at Muizenberg Beach (Hutchings et al. 2016). The estuary mouth and outlet channel have been converted into a concrete canal (Harding 1994), which excludes sandprawns and other benthic organisms from this area.

The mouth is periodically opened (1 week per month in summer, and all through winter). When the estuary is closed, the water level is kept fairly constant (less than 1 m above mean sea level [Hutchings et al. 2016]), to prevent the flooding of houses around the estuary while keeping an adequate depth for recreational activities. During mouth closure, all *C. kraussi* habitats in the estuary are submerged. The water depth above the sediment ranges from approximately 15 cm to 1.4 m.

In the 1990s, Harding (1994) classified the system as eutrophic and reported mean annual concentrations of $1.79 \text{ mg}\cdot\text{L}^{-1}$ total nitrogen, $0.18 \text{ mg}\cdot\text{L}^{-1}$ total phosphorus and $25 \text{ }\mu\text{g}\cdot\text{L}^{-1}$ chl-a. More recently, Haskins (2016) reported median annual concentrations of $0.12 \text{ mg}\cdot\text{L}^{-1}$ total phosphorus and $13 \text{ }\mu\text{g}\cdot\text{L}^{-1}$ chl-a measured in 2015.

Excess nutrients tend to enter the estuary through sewage spills and from surrounding lawns that are enriched with fertiliser and excrement (Haskins 2016). This leads to the nutrient enrichment of the system and stimulates growth of pond weed (*Potamogeton pectinatus*), epiphytic algae (*Cladophora/Enteromorpha* sp.) and phytoplankton (Bacillariophyceae, Chlorophyceae and other minor groups) (Hutchings et al. 2016).

2.3 Experimental design

The experimental design followed Pillay et al. (2012) with modifications. Mesocosms were used to determine the effects of sandprawn densities on nutrient, microalgal and suspended solids concentrations in the water column, as well as microalgal concentrations in the sediment (Figure 2).



Figure 2. Laboratory setup with 9 mesocosms, each half-filled with sediment and half-filled with water from the Zandvlei Estuary Nature Reserve. Values indicate sandprawn density in each mesocosm, with 100% corresponding to the maximum natural density of 200 individuals·m⁻² in South African estuaries.

Nine mesocosms (diameter = 27 cm, volume = 25 L) were half-filled (depth = 18 cm) with intertidal sediment and half-filled (depth = 18 cm above sediment) with water collected from the Zandvlei Estuary Nature Reserve (Figure 3). The sediment was collected at the lower reaches of the estuary (Figure 3-iii), where sandprawns are abundant, and sieved through a 2 mm mesh before being added to each mesocosm. Microorganisms and organic matter were retained in the sediment to maintain near-natural conditions and were randomly distributed in all mesocosms, so that any effects caused by microorganisms should not affect the comparison between mesocosms with and without sandprawns. Estuarine water (salinity = 20‰, temperature = 17°C) was collected approximately 200 m upstream, in the middle of the sandprawn habitat (Figure 3-iii).

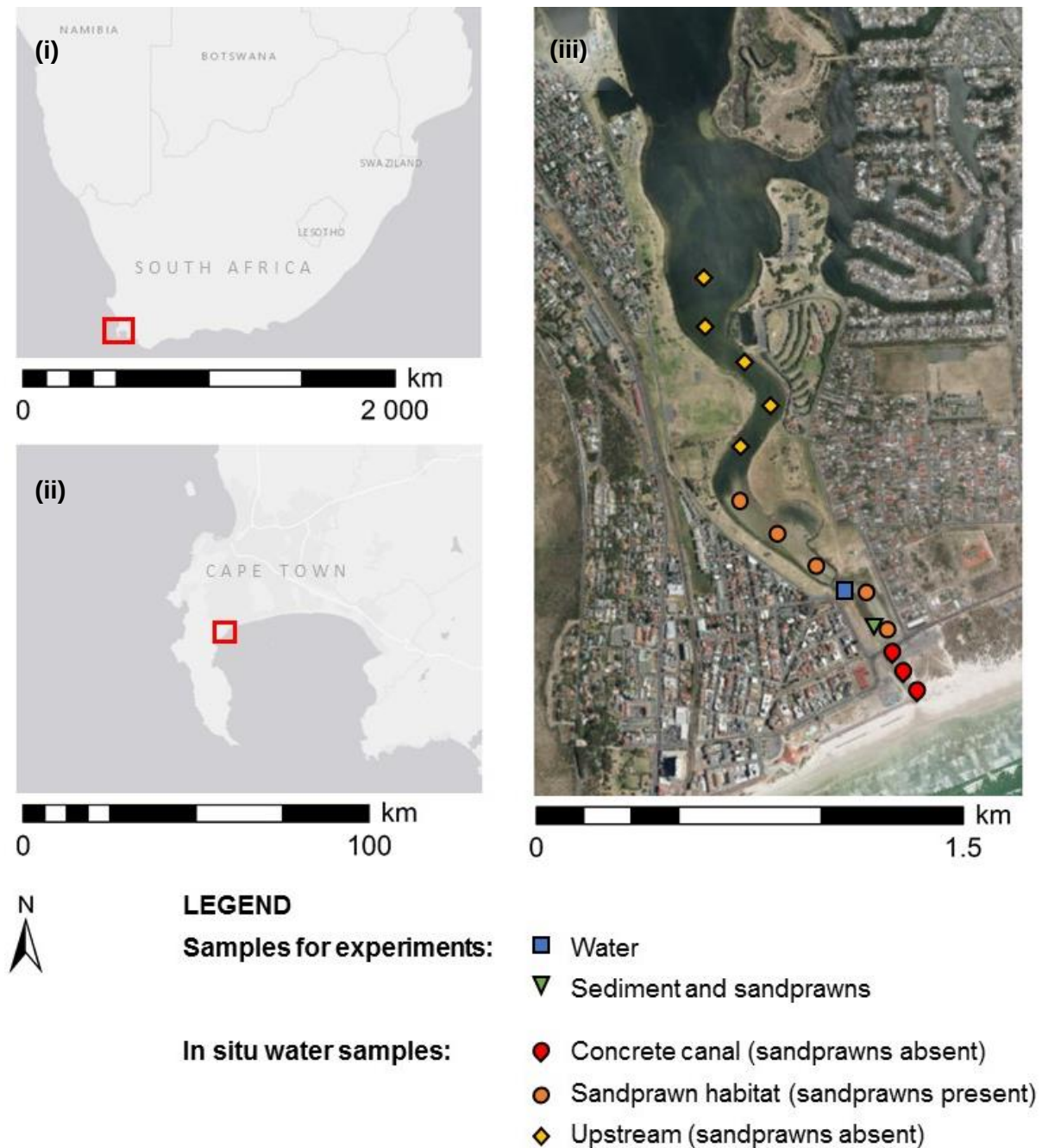


Figure 3. (i) Study site location within South Africa. (ii) Study site location within Cape Town. (iii) Aerial view of the study site, Zandvlei Estuary Nature Reserve. Locations are shown for sites where sandprawns, sediment and water samples were collected for the laboratory experiment and sites where in situ water samples were collected within and adjacent to the sandprawn habitat.

No water flows were induced in the mesocosms, since this mimicked the conditions experienced in the Zandvlei Estuary while the mouth is closed and sandprawns are submerged for 3-week periods. Each individual mesocosm was aerated continuously.

Mesocosms were left to settle for 1 hour before initial (Day 0) water quality measurements were collected and sandprawns added to the designated mesocosms. To ensure uniformity across all mesocosm and to maintain conditions to support sandprawn survival, a multi-meter water sampler (Lovibond ® Water Testing SensoDirect 150 with PT1000) was used to measure conductivity, dissolved oxygen, pH and temperature in each mesocosm on Day 0 and every 2 days thereafter. Sandprawns were not fed following Pillay et al. (2012) given that they are deposit-feeding organisms.

Water was not changed or added during the experiment, in order to keep similar conditions throughout the study such that water column concentrations as affected by sandprawn activities (and not artificial influences) could be compared between different days.

Three sandprawn treatments were used in the experiment, namely controls, 50% natural density and 100% natural density, with 3 replicates of each. In the control mesocosms, sandprawns were absent; in the 50% and 100% treatment mesocosms, 6 and 11 sandprawns respectively were added to each mesocosm, corresponding to 50% and 100% of the maximum natural sandprawn density of 200 individuals·m⁻² in South African estuaries (Branch and Pringle 1987).

The sandprawns were collected from the Zandvlei Estuary Nature Reserve using standard stainless-steel pumps (length = 90 cm, diameter = 5 cm). Only sandprawns

that were not carrying eggs and were between approximately 4 cm and 8 cm in length (chela to telson) were selected. After extraction from the sediment, the sandprawns were placed in loose, moistened layers of newspaper and transported to the aquarium facility at UCT, where they were distributed amongst the buckets according to the designated treatments.

Sandprawns of different sizes were equally distributed among the mesocosms as far as possible, such that each treatment with sandprawns had roughly equal percentages of smaller (closer to 4 cm) and larger (closer to 8 cm) sandprawns present. Sandprawns were not measured at this stage, since the sizes were generally similar and taking measurements may have caused stress and therefore may have affected sandprawn behaviour and the validity of the experimental results.

During the experiment, visible burrow holes in each mesocosm were counted as an approximation of the number of sandprawns living in each mesocosm. It has been shown in past studies that the number of burrow openings closely corresponds to the number of sandprawns present (Wynberg and Branch 1994; Nel and Branch 2013; Moyo et al. 2018), such that burrow opening counts can be used to estimate the abundance of sandprawns within an area. Sandprawns were routinely added to each mesocosm in the event of burrow holes being reduced, such that at least 5 holes were present in the 50% mesocosms and 10 holes were present in the 100% mesocosms.

2.4 Experimental data collection

2.4.1 *Chl-a concentrations in the water column*

On Day 0 (before sandprawns were added), and every 2 days thereafter until Day 11, 3 mL water samples were collected from each mesocosm to measure chl-a concentrations. Two water samples were taken from each mesocosm: at depths of 6 cm (low level) and 12 cm (high level) above the sediment surface. This approach was followed to determine if potential sandprawn effects on chl-a were depth-specific. Chl-a concentrations ($n = 18$ for each day) were determined fluorometrically using a Turner Designs Trilogy Fluorometer. Chl-a was measured in Relative Fluorescence Units (RFUs) and the values were converted to concentration units of $\mu\text{g}\cdot\text{L}^{-1}$ with a calibration curve.

2.4.2 *Nutrient concentrations in the water column*

On Day 1, 7 and 9, 40 mL water samples were collected from the high and low depth levels of each mesocosm to measure nutrient concentrations. N was measured as ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-), which are regarded as the most important forms of N for algal growth (Department of Water Affairs and Forestry 1996; Howitt and Udvardi 2000). P was measured as orthophosphate (PO_4^{3-}).

The NH_4^+ , NO_3^- , NO_2^- and PO_4^{3-} concentrations of each sample was determined with a Hanna Instruments Multiparameter Bench Photometer for Aquaculture (HI 83203). Appropriate reagents and methods (Table 1) were used according to the specifications in the instruction manual.

Table 1. Reagents and methods used to determine concentrations of nutrient parameters in water samples from mesocosms.

Parameters	Reagent code	Range (mg·L ⁻¹)	Method
Ammonia (NH ₃ -N, NH ₃ , NH ₄ ⁺)	HI 93715-01	0.00 – 10.00	Nessler
Nitrate (NO ₃ ⁻ -N, NO ₃ ⁻)	HI 93728-01	0.0 – 30.0	Cadmium reduction
Nitrite (NO ₂ ⁻ -N, NO ₂ ⁻ , Na·NO ₂)	HI 93707-01	0.00 – 1.15	Diazotization
Phosphate (PO ₄ ³⁻ , P, P ₂ O ₅)	HI 93713-01	0.00 – 2.50	Ascorbic acid

2.4.3 Suspended solids in the water column

After the final measurements of chl-a (Day 11) and nutrient concentrations (Day 9), a 500 mL water sample was collected from each mesocosm to determine the suspended solids concentration. A watch glass and 0.7 µm micro-glass fibre paper were labelled and weighed on a Mettler Toledo AE100 Analytical Balance (± 0.00005) for each sample. The water samples were individually filtered through their corresponding filter papers using a Büchner funnel and flask, before being dried on their respective watch glasses at 70 °C overnight. The mass of suspended solids per sediment sample was determined by weighing the watch glass, dried filter and suspended solids on the same balance and deducting the masses of the watch glass and filter.

2.4.4 Chl-a concentrations in the sediment

At the termination of the experiment (2 weeks), each mesocosm was drained of water by drilling 4 holes (diameter = 3 mm) equally spaced around the bucket, 5 cm above

the base. Once the water was drained, 6 surface sediment cores (diameter = 1.5 cm, depth = 1 cm) were collected from each mesocosm. The points at which the samples were taken were randomised by placing a 1 cm x 1 cm grid across each mesocosm and generating 6 random coordinates within the grid area using Microsoft Excel.

For the 50% and 100% sandprawn treatments, 3 of the samples were taken from the burrow mounds, while the remaining 3 samples were taken from the areas between the burrow mounds. Additionally, sediment samples were collected from the burrow walls by sectioning the burrow to a depth of 1 cm and carefully scraping the sand away, as per the method employed by Moyo et al. (2018). Three different burrow lining samples were collected per mesocosm.

All sediment chl-a samples (surface and burrow wall) were placed in 30 mL of 90% acetone and refrigerated for 46 hours so that the chl-a could leach into the supernatant liquid. Subsequently, chl-a concentrations of the supernatant liquid for each sample was determined fluorometrically as outlined for water column chl-a determinations. Each sediment sample was then filtered, dried and weighed as per the method for determining suspended solids in the water column. Chl-a concentrations were standardised by expressing values relative to mass of sediment sample collected, since it was not possible to collect the exact same volume of sediment for each sample.

At the end of the 2-week experiment, the sandprawns were returned to the Zandvlei Estuary Nature Reserve.

2.5 Field data collection

Field data were collected from the Zandvlei Estuary Nature Reserve to determine if trends in mesocosm water column chl-a and suspended solids in relation to sandprawns were also evident *in situ*. Surface (10 – 20 cm below the surface) and bottom (1.5 m below the surface) water samples for chl-a and suspended solids were collected on the 29th of October 2018, when the estuary mouth had been closed for 18 days and 3 days before it was due to be opened again. A comparative approach was adopted for this component: water column chl-a and suspended solids concentrations recorded in a continuous, discrete sandprawn habitat were compared with upstream and downstream samples in which sandprawns were absent.

Five samples were collected in the sandprawns habitat, while another 5 samples were collected upstream where sandprawns were absent (Figure 3-iii). Additionally, 3 samples were collected downstream of the sandprawn habitat which was canalised (Figure 3), thus effectively excluding sandprawns due to there being no access to burrowing medium. All water samples were collected roughly 180 m apart across the upstream biotopes sampled, and roughly 80 m apart across the downstream canal.

Samples were processed at UCT, where chl-a and suspended solids were measured as previously described for the experimental component.

2.6 Statistical analyses

R statistical software (R Core Team 2019) was used to perform all statistical analyses. The response variables that were considered in this study were the concentrations of chl-a, suspended solids and the nutrients PO_4^{3-} , NH_4^+ , NO_3^- and NO_2^- .

The explanatory variables that were considered were the density of sandprawns present (0%, 50% and 100% for mesocosms; 'Absent' and 'Present' for field samples), the sediment section (on volcano-like mound, between mounds and on burrow walls), and the water depth where applicable. If water depth did not explain significant variation in the response variables, then only the upper level was used, to avoid pseudo-replication and since effects observed further away from the sandprawns would be more meaningful from ecological and management perspectives.

2.6.1 Experimental conditions

Environmental factors measured with the multi-meter water sampler were compared between mesocosms for the 10 days that data were collected. Analyses of Variance (ANOVAs) were applied using the 'aov' function in the 'stats' package (Chambers 1992; R Core Team 2019) to determine if significant variations in water conductivity, dissolved oxygen, pH and temperature were explained by the mesocosm number.

If the p-value was significant ($p < 0.05$), indicating significant differences between mesocosms, Linear Models (LMs) were fitted to the data using the 'lm' function in the 'stats' package (Chambers 1992; Wilkinson and Rogers 1973; R Core Team 2019). The significances of the explanatory variables (treatment and day) were determined with ANOVAs of the LMs using the 'car' package (Fox and Weisberg, 2011), which calculated F- and p-values. In the cases where p-values were significant ($p < 0.05$), the Multiple Comparisons of Means (Tukey Contrasts) in the 'lsmeans' package (Lenth 2016) were applied to the LMs to identify between which groups the significant differences occurred.

2.6.2 *Water nutrients and chl-a concentrations in mesocosms*

A Kruskal-Wallis Rank Sum Test was performed on each treatment group individually (control, 50% treatment and 100% treatment) for the entire study period, to determine whether different mesocosms within the same treatment group explained significant variation in the response variables. The treatments could only be meaningfully compared to each other if there was no significant variation within each treatment. The 'kruskal.test' package (Hollander and Wolfe 1973) was used to perform the test.

To determine which explanatory variables significantly explained the variation in chl-a and nutrient concentrations over 11 days, Linear Mixed-Effects Models (LMMs) were fitted by Restricted Maximum Likelihood (REML) estimation using the 'lme4' package (Bates et al. 2015). Since measurements were taken over multiple days, the days were considered as a random effect, as concentrations were expected to change over time within each treatment, and measurements from the same mesocosms could not be considered as independent. Diagnostic plots of the models were also generated to check for non-linearity, unequal error variances and outliers.

ANOVAs were applied to the LMMs, as per the LMs of the environmental conditions, to determine the significance of the main fixed effects, and p-values were generated with Wald Chi-square tests using the default sums of squares type II (Mangiafico 2015). Multiple Comparisons of Means (Tukey Contrasts) were also applied to the LMMs to identify between which groups the significant differences occurred.

A posterior predictive simulation (Gelman and Hill 2006) was used to explore the model fit. The final LMM was evaluated using the interquartile range of the chl-a

concentrations as the summary statistic. The model was deemed to represent the data adequately if the (one-tailed) posterior predictive p-value was >0.78 , indicating that at least 78% of observed data falls within the simulated distribution (Bates et al. 2015).

Confidence intervals (CIs) were used to compare changes in chl-a and nutrient concentrations between the treatments over the relevant period (11 days for chl-a and 9 days for nutrients). The 'Hmisc' package (Harrell 2018) was used to calculate and plot 95% CI ribbons with non-parametric bootstrapping of 1000 replicates. Sample means were compared visually, with an overlap of $\leq 50\%$ between 95% CI ribbons indicating a p-value ≤ 0.05 ; and an overlap of $\leq 5\%$ between 95% CI ribbons indicating a p-value ≤ 0.001 (Cumming and Finch 2005).

2.6.3 *Water chl-a in situ and all suspended solids*

Since these data were collected on a single day, no random effects were required in analysing the responses in chl-a and suspended solids concentrations in field water samples and suspended solids concentrations in the mesocosm water columns. Therefore, LMs were fitted to the data as for experimental conditions. Further analyses to determine the significances of the explanatory variables and to identify where differences occurred were the same as for the LMMs, using ANOVAs and Multiple Comparisons of Means (Tukey Contrasts).

2.6.4 *Sediment chl-a concentrations in mesocosms*

Since sediment chl-a analyses included random effects, LMMs and the statistical approach as per water column chl-a concentrations were used to analyse the data.

Mesocosm number was included as a random effect for each LMM, since multiple samples from each mesocosm could not be considered as independent (Winter 2013).

Overall sediment surface and burrow chl-a concentrations were compared between treatments. Sandprawn density and sediment area (between mounds, on mound or in burrows) were used as explanatory variables to determine whether they explained significant variation in chl-a concentrations.

3 RESULTS

3.1 Environmental variables in mesocosm experiment

There was no significant variation in dissolved oxygen ($F_{8,36} = 0.06$, $p = 1.000$), pH ($F_{8,36} = 0.22$, $p = 0.986$) and temperature ($F_{8,36} = 0.15$, $p = 0.996$) across all mesocosms for the study period; however, there was significant variation in conductivity ($F_{8,36} = 5.94$, $p < 0.0001$) (Table 2; detailed results in Appendix A).

Variability in conductivity was explained by sandprawn density ($F_{2,38} = 11.23$, $p = 0.0001$) and days ($F_{4,38} = 9.86$, $p < 0.0001$), but not by their interaction ($F_{8,30} = 0.18$, $p = 0.991$). Conductivity increased over time and in the presence of sandprawns. In all mesocosms, conductivity on Day 0 was significantly lower compared to the subsequent days (Day 2: $p = 0.026$; Day 4: $p = 0.001$; Day 6: $p = 0.001$; Day 8: $p < 0.0001$). Over the study period, conductivity in the control was 4% lower than in the 50% density treatment ($p = 0.002$) and 5% lower than in the 100% density treatment ($p = 0.001$) (Table 2). There was no significant difference in conductivity between the 50% and 100% density treatments ($p = 0.664$).

Table 2. Mean and standard error of environmental variables from Day 0 to Day 8 in laboratory mesocosms with different *Callichirus kraussi* densities.

Density (individuals·m ⁻²)	Temperature (°C)	pH	Dissolved oxygen (mg·L ⁻¹)	Conductivity (mS·cm ⁻¹)
0	14.3 ± 0.42	8.3 ± 0.02	9.8 ± 0.12	27.8 ± 0.41
100	14.2 ± 0.43	8.3 ± 0.02	9.8 ± 0.12	29.1 ± 0.28
200	14.2 ± 0.42	8.3 ± 0.02	9.8 ± 0.14	29.4 ± 0.30

3.2 Nutrient and chl-a concentrations in the water column

Water depth did not significantly explain variation in nutrient concentrations (Table 3), chl-a concentrations in the mesocosms ($\chi^2 = 0.01$, df = 1, p = 0.928) or chl-a concentrations *in situ* ($F_{1,19} = 0.39$, p = 0.540). Therefore, only measurements from the upper water levels were considered for further analyses.

3.2.1 Experimental nutrient concentrations

Sandprawn density significantly explained variation in NH_4^+ concentration (Table 3). After 9 days, the NH_4^+ concentration was 2.6 times greater in the high density sandprawn treatment (100% natural density) compared to the control (Figure 4-i).

Sandprawn density did not significantly explain variation in PO_4^{3-} or NO_2^- concentrations (Table 3). NO_3^- concentrations were below the 0.0 mg·L⁻¹ detection limit.

Table 3. Effects of water depth (lower and upper thirds of water column) and sandprawn density (0%, 50% and 100% of maximum natural density) on nutrient concentration in a laboratory mesocosm experiment. Significances were determined using Wald Chi-square tests (type II) generated with analyses of variance of linear mixed-effects models fitted by restricted maximum likelihood estimation (Bates et al. 2015). Where water depth was not significant, the model for sandprawn density only included data for the upper water level. Values in bold denote statistical significance.

Nutrient	Explanatory variable	χ^2	df	p-value
Phosphate (PO_4^{3-})	Water depth	0.17	1	0.684
	Sandprawn density	0.56	2	0.754
Nitrite (NO_2^-)	Water depth	0.00	1	0.988
	Sandprawn density	2.28	2	0.320
Ammonium (NH_4^+)	Water depth	0.01	1	0.931
	Sandprawn density	20.85	2	< 0.0001

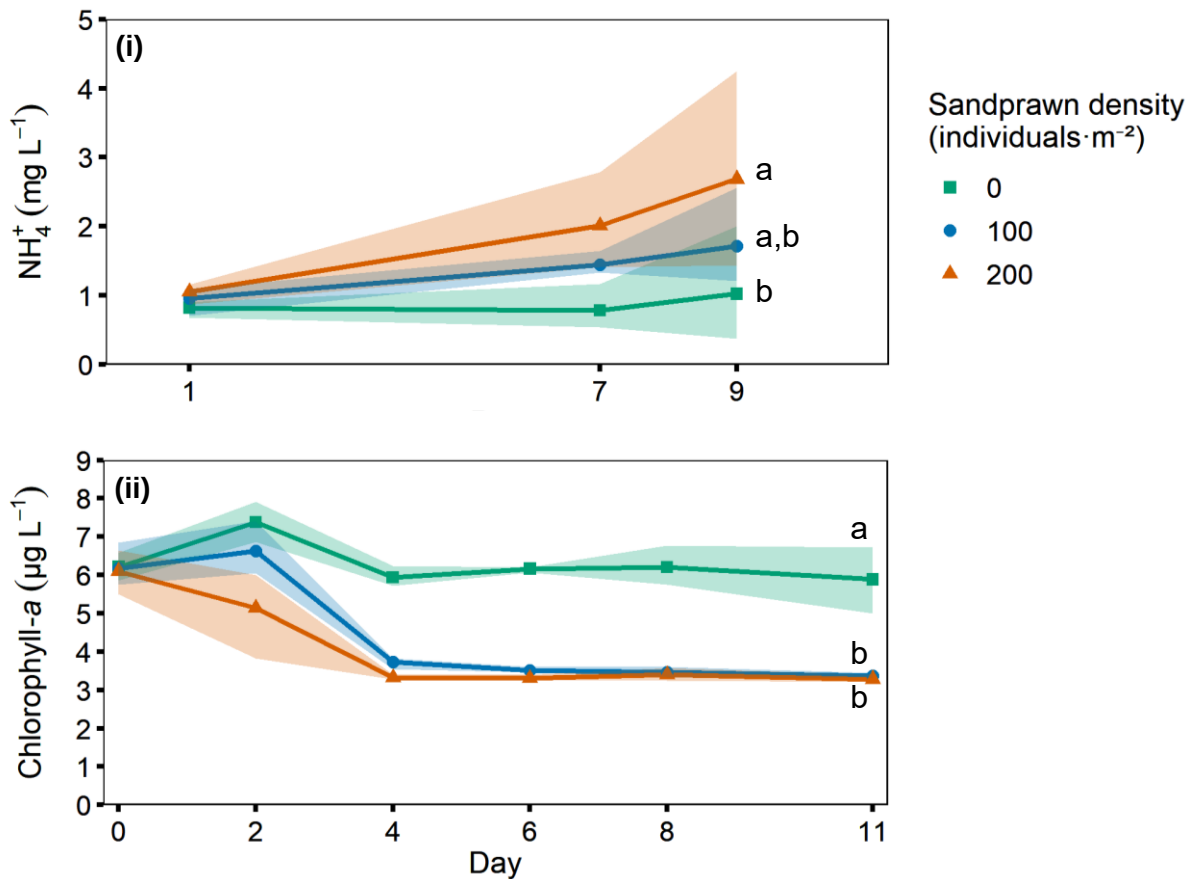


Figure 4. Change in (i) ammonium and (ii) chlorophyll-a concentrations in the water column of mesocosms during a laboratory experiment, where 3 sandprawn density treatments were considered. Each point represents the mean of the 3 measurements from replicate mesocosms for each sandprawn density. Shading represents the 95% confidence interval. Different letters (a, b), generated with Tukey Contrasts, indicate significant differences ($p < 0.05$) between concentrations for each sandprawn density over the entire study period.

3.2.2 Experimental chl-a concentrations

Sandprawn density treatment explained significant variation in chl-a concentrations over the entire study period ($\chi^2 = 105.41$, $df = 2$, $p < 0.0001$) (Figure 4-ii). From start to end of the experiment, chl-a concentrations were reduced by 45-46% in mesocosms containing sandprawns: from 6.2 ± 0.34 SE $\mu\text{g}\cdot\text{L}^{-1}$ to 3.4 ± 0.05 SE $\mu\text{g}\cdot\text{L}^{-1}$ in the 50% density treatment, and from 6.1 ± 0.33 SE $\mu\text{g}\cdot\text{L}^{-1}$ to 3.3 ± 0.06 SE $\mu\text{g}\cdot\text{L}^{-1}$ in the 100%

density treatment. In the controls, the minimum (and final) concentration over the entire study period was 5.9 ± 0.16 SE $\mu\text{g}\cdot\text{L}^{-1}$, with a starting concentration of 6.2 ± 0.21 SE $\mu\text{g}\cdot\text{L}^{-1}$. Compared to the controls, the final chl-a concentrations for the 50% and 100% density treatments were 42.8% and 44.3% lower respectively at the end of the study (both $p < 0.0001$) (Figure 4-ii). The final LMM had a posterior predictive p-value of 0.975 and was considered to be adequately representative of the data.

3.2.3 In-situ chl-a concentrations

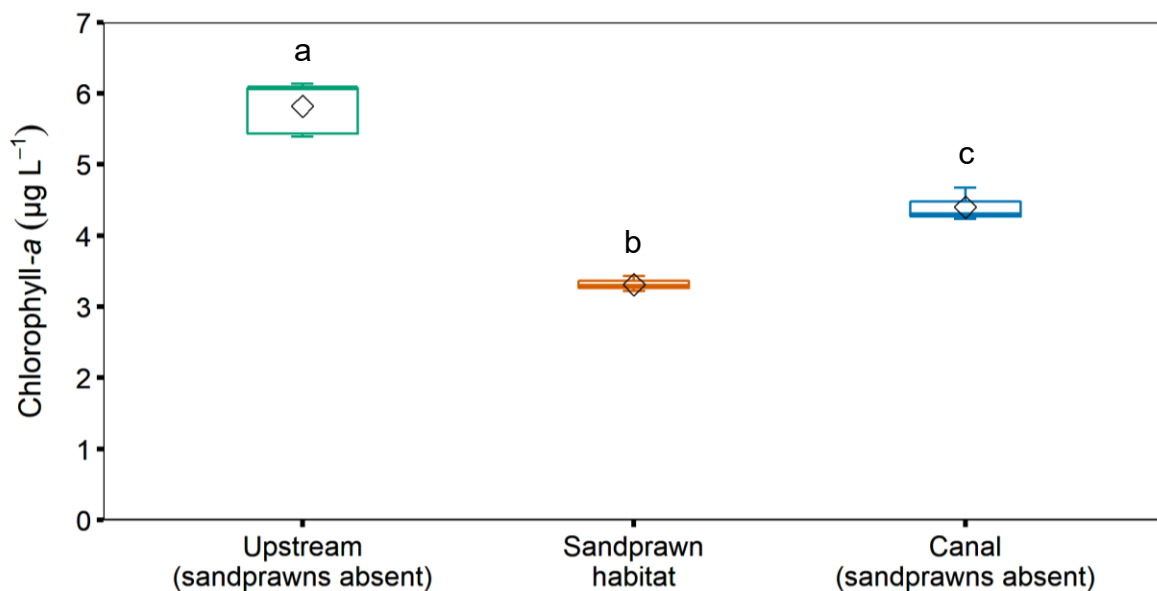


Figure 5. Comparison of chlorophyll-a concentrations in the water column at different sections of the Zandvlei Estuary Nature Reserve: 5 sites upstream from and 5 sites within the sandprawn habitat, as well as 3 sites between the sandprawn habitat and estuary mouth where a concrete canal excludes sandprawn from the area. The boxplot hinges represent the first and third quartiles; upper and lower whiskers end in horizontal bars at ± 1.5 times the inter-quartile range; thickened lines represent the median; and diamonds indicates the mean for each area. Different letters (a, b, c), generated with Tukey Contrasts, indicate significant differences ($p < 0.05$) between chlorophyll-a concentrations for each area.

Sandprawn presence explained significant variation in chl-a concentrations at sampling sites *in situ* ($F_{2,10} = 112.00$, $p < 0.0001$). All 3 sites were significantly different, with chl-a concentrations being the highest upstream (5.8 ± 0.38 SE $\mu\text{g}\cdot\text{L}^{-1}$), the lowest in the sandprawn habitat (3.3 ± 0.08 SE $\mu\text{g}\cdot\text{L}^{-1}$), and intermediate in the concrete canal downstream from the sandprawn habitat (4.4 ± 0.24 SE $\mu\text{g}\cdot\text{L}^{-1}$) (Figure 5).

3.3 Suspended solids in the water column

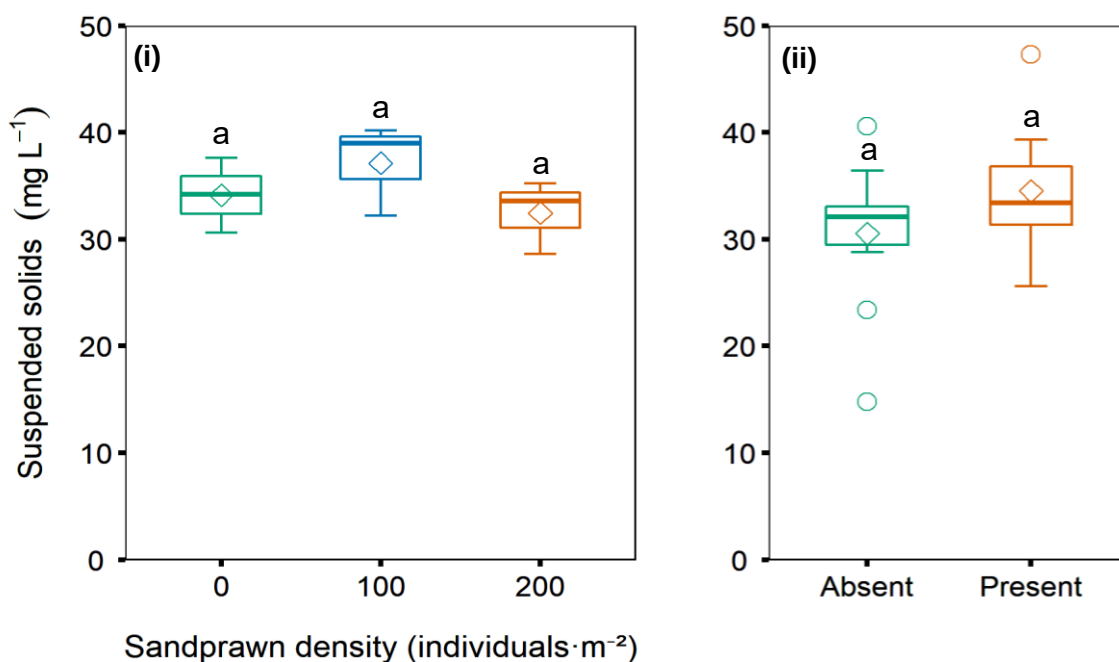


Figure 6. Variation in suspended solids concentrations in the water column among (i) different sandprawn densities in laboratory mesocosms and (ii) sandprawn presence and absence in the Zandvlei Estuary Nature Reserve. Boxplot hinges represent the first and third quartiles; upper and lower whiskers end in horizontal bars at ± 1.5 times the inter-quartile range; circles represent outliers; thickened lines represents medians; and diamonds indicate means. Letters (a) generated by Tukey Contrasts indicate that concentrations were not significantly different ($p > 0.05$).

Sandprawn density did not explain any significant variation in the suspended solids concentration in the water column of either the mesocosms ($F_{2,6} = 1.18$, $p = 0.370$) (Figure 6-i) or the estuary ($F_{1,17} = 2.31$, $p = 0.147$) (Figure 6-ii).

However, water depth in the estuary explained significant variation in suspended solids concentration ($F_{1,17} = 4.98$, $p = 0.039$). Suspended solids concentration was significantly greater in deep samples ($35.5 \pm 2.03 \text{ mg}\cdot\text{L}^{-1}$), compared to the surface samples ($29.6 \pm 1.82 \text{ mg}\cdot\text{L}^{-1}$).

3.4 Chl-a concentrations in the sediment

There was no significant difference in overall sediment chl-a concentrations among the sandprawn densities ($\chi^2 = 3.84$, $df = 2$, $p = 0.147$) (Figure 7, denoted by asterisks). Therefore, sediment chl-a concentrations were pooled for all 3 treatments to compare differences between the types of sediment area overall. Variation in sediment chl-a concentrations was significantly explained by the type of sediment area where samples were collected, namely on mounds, between mounds and within burrows ($\chi^2 = 14.73$, $df = 2$, $p = 0.001$) (Figure 7, denoted by letters a and b).

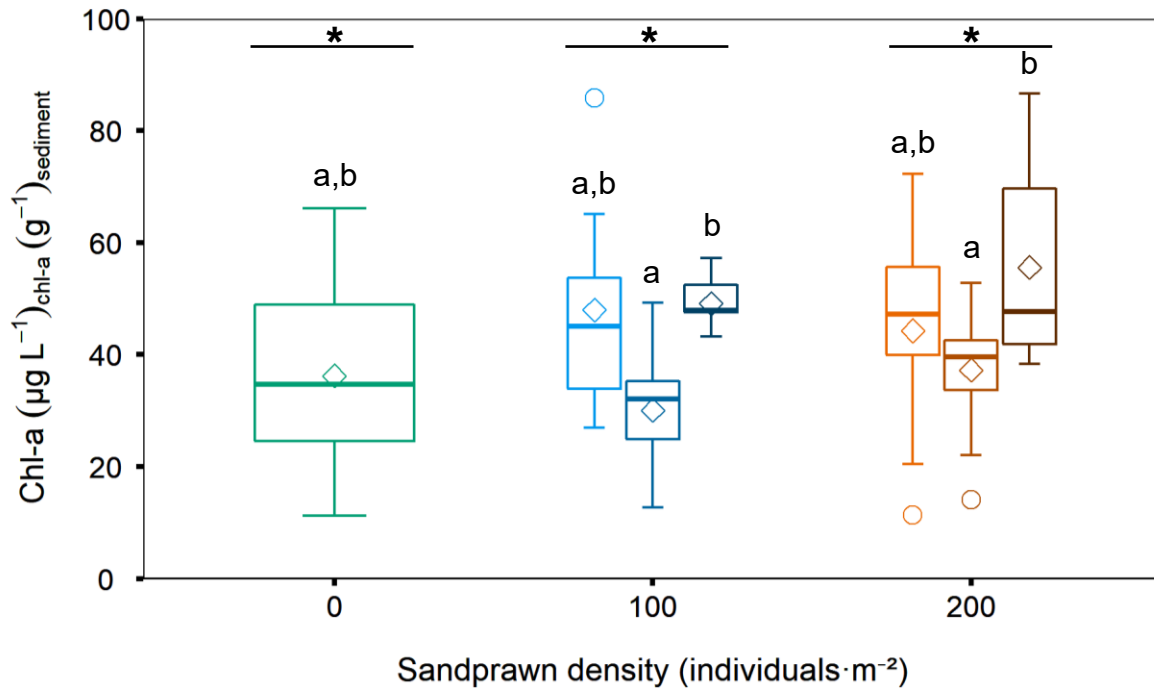
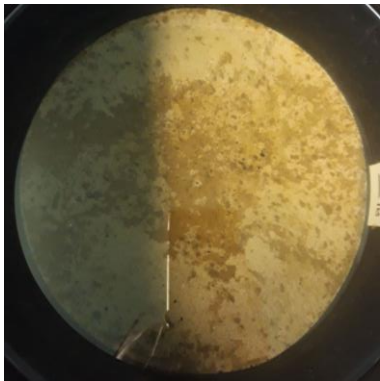
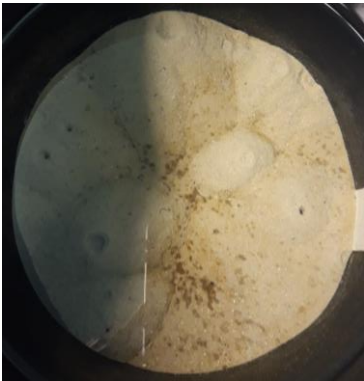
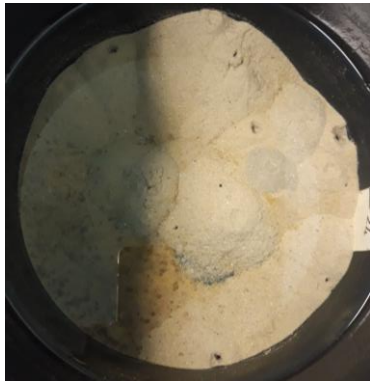
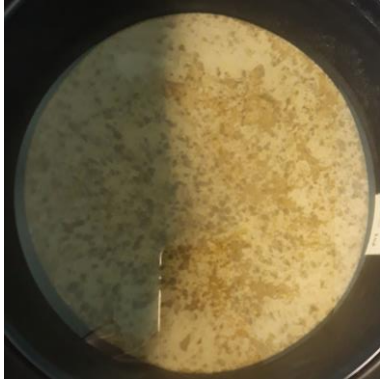
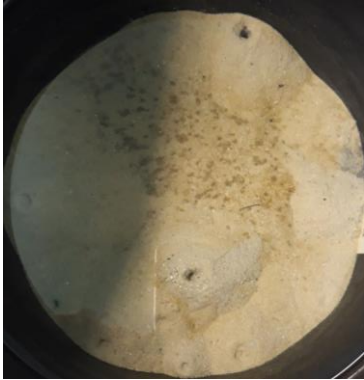
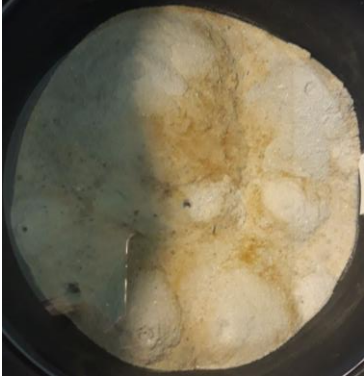
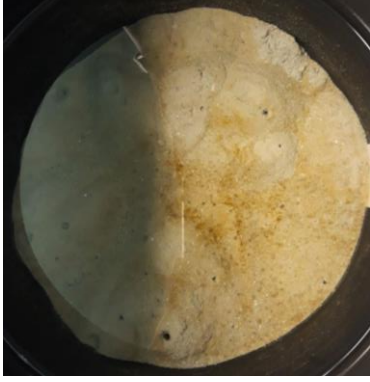


Figure 7. Variation in chlorophyll-a concentrations among different sandprawn densities at 3 sediment areas within the laboratory mesocosms. The lightest/only boxplot at each density denotes sediment from areas between burrow mounds; the intermediate-shade boxplots denote sediment from the burrow mounds; and the darkest boxplots denote sediment from the burrow walls. Boxplot hinges represent the first and third quartiles; upper and lower whiskers end in horizontal bars at ± 1.5 times the inter-quartile range; circles represent outliers; thickened lines represent the median; and diamonds indicate the mean. Across all 3 density treatments, total sediment chlorophyll-a concentrations were not significantly different (indicated by *). Therefore, sediment area concentrations were pooled and compared across all 3 treatments, with different letters (a, b) indicating significant differences ($p < 0.05$) in concentrations between sediment areas (generated with Tukey Contrasts).

The chl-a concentration in the non-mound sediment in all mesocosms (41.2 ± 2.95 [$\mu\text{g}\cdot\text{L}^{-1}$]_{chl-a}·[g⁻¹]_{sediment}) was similar to the chl-a concentration within the burrows in the 50% and 100% density mesocosms (52.4 ± 3.08 [$\mu\text{g}\cdot\text{L}^{-1}$]_{chl-a}·[g⁻¹]_{sediment}). These chl-a concentrations in sediment adjacent to the mounds were significantly higher than chl-a concentrations on the mounds (33.7 ± 2.86 [$\mu\text{g}\cdot\text{L}^{-1}$]_{chl-a}·[g⁻¹]_{sediment}). Microalgal

masses around, and not on top of, the mounds can be seen in photos of the sediment surfaces taken just before sediment cores were collected (Table 4).

Table 4. Sediment surfaces after 12 days of an experiment with 3 replicate mesocosms of each sandprawn density treatment. Dark areas on the left of each photo were shadows cast by the sides of the mesocosm-buckets.

Sandprawn density (individuals·m ⁻²)		
0	100	200
		
		
		

4 DISCUSSION

The overall effect of *C. kraussi* on water quality is mixed (Table 5). On the one hand, high densities of sandprawns seem to increase concentrations of NH_4^+ , which should stimulate algal growth in N-limited marine environments and thereby exacerbate eutrophication. However, despite the nutrient increase, sandprawns seem to reduce algal concentrations in the water column and break up algal mats in the sediment, which suggests that they can combat some of the biological end-effects of eutrophication. Furthermore, sandprawns do not seem to have a significant effect on overall suspended solids concentrations in the water column, despite their vigorous bioturbation activities.

Table 5. Summary of effects of *Callichirus kraussi* on water quality parameters in terms of concentration in the water column, based on the result from the present study.

Parameter	Decreased	Unaffected	Increased
Conductivity			X
Ammonium (NH_4^+)			X
Nitrite (NO_2^-)		X	
Phosphate (PO_4^{3-})		X	
Chlorophyll-a	X		
Suspended solids		X	

4.1 Effects of sandprawns on eutrophication

The present study's results suggest that sandprawns at maximum natural density increase NH_4^+ concentrations in the water column. As part of the NH_4^+ measurements, NH_3 and $\text{NH}_3\text{-N}$ were also measured and showed virtually the same trends as NH_4^+ (Appendix B), which allows for the comparison with studies that considered NH_3 and $\text{NH}_3\text{-N}$. Results of this study are consistent with those found by Koike and Mukai (1983) and D'Andrea and DeWitt (2009), but interestingly seem to contradict those of Pillay et al. (2012), who found no significant variations in NH_3 concentrations among different *C. kraussi* densities. Bridging this gap, Murphy (1985) reported an initial increase in NH_3 concentrations in aquaria with callianassids (*Callinassa californiensis*) compared to controls without callianassids, but found that these concentrations decreased and became similar after 5 days. Therefore, NH_4^+ concentration differences may be time-dependent.

An increase in NH_4^+ concentrations with increasing *C. kraussi* densities could be due to excess NH_4^+ produced by the excretion of benthic fauna and microorganisms, which is flushed out into the overlying water through irrigation (Koike and Mukai 1983; D'Andrea and DeWitt 2009). This increase in NH_4^+ -ions is also reflected by the observation of increased conductivity in the treatments where sandprawns were present compared to treatments without sandprawns (Shcherbakov et al. 2009).

If the sandprawn-induced increase in NH_4^+ is not merely temporary, it could degrade water quality. The World Health Organisation (2017) provides drinking-water quality guidelines for NH_3 as it pertains to odour ($< 1.5 \text{ mg}\cdot\text{L}^{-1}$) and taste ($< 35 \text{ mg}\cdot\text{L}^{-1}$), but

states that NH_3 has no direct human health consequences at these concentrations. However, $\text{NH}_3\text{-N}$ may cause chronic effects in aquatic species at $> 15 \mu\text{g} \cdot \text{L}^{-1}$ and be toxic at $> 100 \mu\text{g} \cdot \text{L}^{-1}$ (Department of Water Affairs and Forestry [DWAF] 1996). According to the mesocosm results in the current study, the elevated ammonia concentrations as quantified in the presence of sandprawns may decrease water quality in terms of its odour, but is unlikely to affect its toxicity for humans or aquatic biota. However, *in situ* nutrient concentrations were not measured and may differ from those measured in the mesocosms.

An increase in nutrients such as NH_4^+ could also be indirectly detrimental to water quality by stimulating harmful algal growth and thereby turning a water body eutrophic (DWAF 1996). In coastal marine environments, which are N-limited (Ryther and Dunstan 1971; Eppley et al. 1979; Smith 1984), the increase in NH_4^+ is expected to cause an increase in chl-a concentrations. However, this does not seem to be the case when sandprawns are present.

According to the results of the mesocosm experiment, despite the increase in NH_4^+ concentrations, microalgal concentrations in the water decreased in the presence of *C. kraussi*, suggesting that they at least partially combat eutrophication. Note that high nutrient concentrations may still be present *in situ* despite the decrease in microalgal concentrations (these were not measured in this study). Experimental effects on pelagic microalgae were evident throughout the water column, and is not only localised close to the sediment layer.

It is likely that water column microalgal biomass was reduced in the presence of sandprawns by being transported into the burrows through irrigation and subsequently

consumed by the sandprawns, preventing the build-up of microalgae in the burrows. Since overall sediment chl-a concentrations were similar in the presence and absence of sandprawns, it can be concluded that the microalgae removed from the water column were not merely concentrated in the top sediment layer. Rather, the reduced microalgal concentrations in the water, similar microalgal concentrations in the sediment surface and increased NH_4^+ concentrations in the water in the presence of sandprawns suggests that microalgae were consumed by sandprawns. Furthermore, burrow environments were enriched with microalgae, relative to the surrounding mounds, which is in accordance with the findings of Branch and Pringle (1987). The microalgae were most likely transported into the burrows through irrigation and not water current, since there was little water flow in the mesocosms (the only water flow that occurred was caused by aeration and sandprawn activity).

In addition to their desirable effect on microalgae in the water column, sandprawns also seem to prevent a homogeneous algal mat from covering the sediment surface, which agrees with the findings of Neumann et al. (1970). In the absence of sandprawns, the sediment layer was observed to be fairly uniformly covered in benthic algae; whereas in the presence of sandprawns, the volcano-like mounds provided breaks in algal mat formation and had lower concentrations of microalgae. These island-like breaks in the algae may be enough to maintain benthic-pelagic coupling and thereby prevent benthic habitats from becoming anoxic and potentially uninhabitable by benthic species (McGlatherly et al. 2012; Griffiths et al. 2017).

4.2 Effect of sandprawns on suspended solids

According to the results in the current study, sandprawns do not seem to affect water quality in terms of suspended solids. Even in the mesocosms, which were shallow water environments (water depth = 18 cm), bioturbation did not significantly increase suspended solids in the water column over the study periods (although concentrations may have been intermittently increased during bioirrigation events).

In situ, a greater variation was found between suspended solids concentrations at different water depths than between areas with and without sandprawns. This suggests that water movement, rather than bioturbation, may have a greater impact on suspended solids concentrations (Miller and Stenberg 1988; de Jonge and van Beusekom 1995).

These findings seemingly contradict those reported by Aller and Dodge (1974), Suchanek (1983), Murphy (1985) and Colin et al. (1986). However, all of these studies, except Murphy (1985), used sediment traps to show that callianassids cause resuspension and reworking of sediment, but did not measure and compare suspended solids concentrations in the water column at habitats with and without callianassids. It is possible that solids, especially larger particles, settle again soon after being resuspended through bioturbation (Aller and Dodge 1974). In other words, suspended solids concentrations could be temporarily increased during bioturbation or irrigation events, but may not necessarily be permanently increased in the water column. In such a case, the findings in the present study do not truly contradict the other studies mentioned, since the present study was focused on determining effects

on suspended solids concentrations over the entire study period and not specifically during intermittent bioturbation or irrigation events.

Similarly to the present study, Murphy (1985) compared concentrations of suspended particulate matter in the water column between areas with and without callianassids (*Callianassa californiensis*) using field surveys and laboratory experiments. In field surveys, he reported increased suspended solids concentrations in the area with *Callianassa*; however, the 2 areas sampled were heterogeneous with tidal currents suggested to have been stronger in the area with *Callianassa*. In laboratory mesocosm experiments, he showed that water turbidity, as measured with a nephelometer and calibrated with suspended solids concentration measurements, increased in the presence of *C. californiensis*.

It is possible that these results differ from the present study's results because of the different methods employed and environmental conditions experienced in the experiments. For example, finer sediment may have been more susceptible to resuspension and slower to settle in mesocosms, increasing suspended solids concentrations. Murphy (1985) also replaced 90% of water with lagoon water every other day, which means that his measurement of suspended solids was short-term (after 48 hours maximum), while the present study measured suspended solids after 12 days, giving finer suspended solids more time to settle after being added to the mesocosms. Furthermore, Murphy (1985) used smaller aquaria (19 L divided into 2 sections: experiment and control), so burrowing effects may have been amplified.

Differences in biological traits, for example variability in sediment turnover behaviour of Callianassid burrowers between Murphy's (1985) *C. californiensis* and the present

study may also explain variability in outcomes (Pillay 2019). Murphy (1985) also included clams (*Mercenaria mercenaria*) in the aquaria and added diatoms (*Thalassiosira pseudonana*) daily to feed the *M. mercenaria*, which may have influenced the burrowing activity and therefore the suspended solids concentrations.

4.3 Observations *in situ* compared to mesocosm experiment

The concentrations measured and trends observed in the laboratory mesocosms generally reflected those found *in situ*. This supports the use of laboratory mesocosm experiments to gain quick, precise and replicated results that represent processes in larger scale ecological systems, while still providing important information on potential causal drivers (Srivastava et al. 2004; Benton et al. 2007). This also shows that controlled laboratory experiments may be scaled up to the ecosystem level. Measurements from the natural ecosystem also offered valuable information that supplemented experimental findings, showing that causal drivers identified in small-scale experiments were also potentially present under larger scale, real-world conditions.

Therefore, findings of this study suggest that mesocosm experiments can be used to gain insights into ecological processes, but should be supplemented with *in situ* studies to form a more holistic understanding of phenomena occurring in larger scale ecosystems.

5 CONCLUSION

This study has shown that *C. kraussi* can improve water quality by decreasing microalgal biomass in water; can maintain benthic-pelagic coupling by creating breaks

in sediment microalgae; does not affect suspended solids concentrations; and does not increase ammonia concentrations to concentrations that may be toxic to humans or aquatic species. However, longer term data and more extensive analyses would be required to determine how sandprawns affect other water quality parameters, for example metal and hydrocarbon concentrations.

Based on the findings of this study, the persistence of *C. kraussi* in urban estuaries should ideally be encouraged. This can be achieved by maintaining soft sediment habitats in which *C. kraussi* can burrow, instead of creating canalised or hard-bottomed estuaries. Additionally, the expansion of sandprawn habitats should not automatically be discouraged without a clear understanding of the effects on the ecosystem – for example, if the system is eutrophic, an increase in numbers of sandprawns could improve the water quality. Furthermore, the removal of sandprawns for bait should be regulated or prohibited to avoid the destruction of sandprawn burrows, which disrupts the ecosystem services that these organisms provide.

With their positive effects on water quality, sandprawns can be considered for nature-based solutions. If their effects are to be mimicked by artificial processes, a more thorough understanding of the mechanism behind their removal of microalgae should be acquired. But sandprawns can also be directly used in artificial systems (similarly to organisms in artificial wetlands) to remove microalgae, or encouraged to persist in natural systems to prevent eutrophication.

Recognising that *C. kraussi* provides an important ecosystem service by improving water quality will hopefully lead to a more serious considerations of benthic organisms during water management decision-making. According to the findings of this study, it

is clear that that benthic-pelagic coupling is an important consideration in water quality management. Even though endobenthic organisms are not as readily observed as pelagic species, their extensive effects on water quality should not be underestimated. We therefore need to understand benthic-pelagic coupling in more detail and work with these natural processes to manage our limited water supply sustainably, particularly in the context of deep-dwelling endobenthic bioturbators.

6 REFERENCES

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7 APPENDICES

7.1 Appendix A: Experimental conditions results

Table A. Mean and standard error of environmental variables measured over time in laboratory mesocosms with different sandprawn densities.

Day	Density (sandprawns·m ⁻²)	Temperature (°C)	pH	Dissolved oxygen (mg·L ⁻¹)	Conductivity (mS·cm ⁻¹)
0	0	17.4 ± 0.14	8.2 ± 0.00	9.4 ± 0.01	26.7 ± 0.41
	50	17.4 ± 0.22	8.2 ± 0.01	9.4 ± 0.01	27.2 ± 0.13
	100	17.2 ± 0.15	8.2 ± 0.00	9.2 ± 0.10	27.6 ± 0.3
2	0	13.6 ± 0.05	8.3 ± 0.00	9.4 ± 0.03	27.4 ± 0.36
	50	13.5 ± 0.05	8.3 ± 0.00	9.3 ± 0.03	29 ± 0.05
	100	13.5 ± 0.08	8.2 ± 0.01	9.2 ± 0.03	29.3 ± 0.10
4	0	13.2 ± 0.07	8.3 ± 0.00	10.2 ± 0.04	28.2 ± 0.42
	50	13.0 ± 0.08	8.3 ± 0.00	10.2 ± 0.04	29.6 ± 0.07
	100	13.0 ± 0.09	8.3 ± 0.01	10.2 ± 0.05	29.8 ± 0.13
6	0	13.6 ± 0.04	8.3 ± 0.00	10.4 ± 0.01	28.1 ± 0.47
	50	13.5 ± 0.06	8.4 ± 0.00	10.5 ± 0.01	29.7 ± 0.05
	100	13.4 ± 0.08	8.3 ± 0.01	10.4 ± 0.04	30 ± 0.10
8	0	13.8 ± 0.05	8.4 ± 0.00	9.7 ± 0.04	28.8 ± 0.47
	50	13.8 ± 0.07	8.4 ± 0.00	9.6 ± 0.04	30 ± 0.06
	100	13.7 ± 0.08	8.4 ± 0.00	9.8 ± 0.09	30.3 ± 0.10

7.2 Appendix B: Comparison of ammonia results

Table B. Mean and standard error of ammonia and ammonium variables measured over time in laboratory mesocosms with different sandprawn densities.

Day	Density (sandprawns·m ⁻²)	NH ₄ ⁺ (mg·L ⁻¹)	NH ₃ (mg·L ⁻¹)	NH ₃ -N (mg·L ⁻¹)
1	0	0.79 ± 0.085	0.75 ± 0.080	0.62 ± 0.065
	50	1.02 ± 0.078	0.97 ± 0.074	0.80 ± 0.061
	100	0.94 ± 0.08	0.88 ± 0.075	0.73 ± 0.063
7	0	0.72 ± 0.103	0.68 ± 0.096	0.56 ± 0.080
	50	1.45 ± 0.053	1.37 ± 0.050	1.13 ± 0.040
	100	2.00 ± 0.243	1.88 ± 0.229	1.54 ± 0.189
9	0	0.82 ± 0.241	0.78 ± 0.227	0.64 ± 0.186
	50	1.69 ± 0.266	1.60 ± 0.251	1.31 ± 0.207
	100	3.00 ± 0.742	2.82 ± 0.700	2.32 ± 0.575