Persistent pharmaceuticals in an urban Western Cape Estuary: effects on microalgal assemblages and sandprawn water filtration

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PLAGIARISM DECLARATION

I know the meaning of plagiarism and declare that all of the work in the dissertation, save for that which is properly acknowledged, is my own.

Signature: Signed by candidate Date: 22 January 2024

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ABSTRACT

Coastal environments comprise distinct and diverse ecosystems of economic and ecological significance that provide invaluable ecosystem functions and services to humans. Despite their significance, coastal environments worldwide are experiencing deterioration due to the growth of the human population and associated activities in coastal areas. Chemicals of emerging concern, including pharmaceuticals and personal care products, are a prominent example of an emerging threat to coastal environments due to their ubiquity and persistence in marine environments globally. However, research on levels of pharmaceutical pollution and associated effects on ecological processes and biota in estuaries is severely lacking in South Africa. Therefore, the consequences for key estuarine biota and the functions they provide are largely unknown, to the best of my knowledge. Therefore, the purpose of this study was to address the above-mentioned knowledge gaps on pharmaceutical pollution in South Africa. This aim was addressed by firstly determining the concentration of pharmaceuticals in the Zandvlei Estuary, which is an urbanized, anthropogenically manipulated system in the Western Cape, South Africa. Thereafter, the interaction between sulfamethoxazole presence and sandprawn density was quantified experimentally to understand the responses of microalgae biomass. An experiment was conducted as the consequences of pharmaceutical pollution on functionally important estuarine biota such as sandprawns and the functions they provide including biofiltration in South African estuaries are, to the best of my knowledge, unknown. Based on field sampling, high concentrations of pharmaceuticals were recorded in the Zandvlei Estuary, particularly near the mouth, where acetaminophen had the highest average concentration in water samples among all sites (4.604 ± 0.453 µg/L) and the highest average concentration in sandprawn samples (11.309 µg/g).

Additionally, pharmaceuticals including sulfamethoxazole, carbamazepine and diclofenac were detected in water samples in all sites and all sandprawn samples. With the use of environmentally relevant sulfamethoxazole concentrations in a laboratory mesocosm experiment, I found that sulfamethoxazole presence negatively affected pelagic microalgae biomass, where micro- and picoplankton declined with sulfamethoxazole concentrations but nanoplankton and benthic microalgae was unaffected. The high levels of pharmaceuticals found in the Zandvlei Estuary are likely a result of increasing sewage spills due to malfunctioning sewage pump stations, which in turn is likely amplified by load shedding (planned electricity outages to manage demand) intensifying over the last seven years. Findings additionally highlight the potential for small, urban temporary-open closed estuaries to be accumulation sites for chemicals of emerging concern, given the order of magnitude greater values recorded in the Zandvlei Estuary compared to False Bay. Moreover, this study has shown that increasing sulfamethoxazole concentrations cause a reduction in phytoplankton biomass and a shift in size classes. These findings need further research to understand ecological repercussions for food web topology and efficiency, for example, given that changes in phytoplankton abundance and traits can generate indirect bottom-up changes to higher trophic levels.

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1. INTRODUCTION

1.1 Background and literature review

Coastal marine environments comprise distinct and diverse ecosystems of economic and ecological significance given the invaluable ecosystem functions and services provided to humans (Barbier, 2011, Barbier, 2017). Coastal ecosystems such as estuaries are critical transition zones that join the sea, freshwater and land and offer services such as coastline protection, nursery grounds for migratory and resident fish, habitat and food for animals and improved water quality (Levin, 2001, Sheaves *et al.*, 2014). Moreover, these critical transition zones play a major role in nutrient cycling and decomposition, which supports bacterial and phytoplankton production that are central to maintaining coastal ecosystem functioning (Levin, 2001, Hopkins, 1998). Coastal environments account for around one-third of all oceanic primary production and are thus some of the most productive ecosystems on Earth (Borges, 2005, Gattuso, 1998). The abundance of recreational activities and unique aesthetic features in coastal areas create tourism hotspots that contribute significantly to local and regional economies (Barbier, 2011, Barbier, 2017).

Despite their importance, coastal environments worldwide are subject to deterioration (Kemp *et al.*, 2005, Worm, 2006, Kotke *et al.*, 2019) due to the growth of the human population and associated activities in coastal areas (Lotze *et al.*, 2006). Although coastal regions make up only 4% of the Earth's landmass, roughly one-third of the human population resides in coastal zones, mainly due to the benefits and services coastal ecosystems provide to humans such as water, food availability and recreation (Barbier, 2017, Barbier, 2011). Coastal development, industrialisation and habitat modification, the exploitation of marine resources, nutrient enrichment, loss of

biodiversity and pollution are some of the anthropogenic threats associated with human populations in and around coastal zones (Crain *et al.*, 2009, Kemp *et al.*, 2005, Culhane *et al.*, 2019, Ojemaye and Petrik, 2021b). Since anthropogenic stressors do not act in isolation, coastal ecosystems often face multiple environmental and anthropogenic stressors concurrently, which can therefore have detrimental impacts on coastal environments through synergistic interactions (Crain *et al.*, 2009, Halpern *et al.*, 2008). The combined effect of multiple coastal stressors significantly impacts ecosystem resilience, which consequently intensifies coastal degradation (Defeo and Elliott, 2021, He and Silliman, 2019, Halpern *et al.*, 2019). Although the causes and consequences of endogenous pressures are potentially manageable by coastal authorities, exogenous pressures including global change stressors and cross-system nutrient inputs threaten ecosystem functioning due to difficulties associated with the management of stress over multiple spatial scales (Defeo and Elliott, 2021). For example, increased frequency of storm surges, sea-level rising and ocean warming threaten coastal ecosystems as these pressures cannot be regulated by local coastal management authorities acting independently (Elliott *et al.*, 2020a, Defeo and Elliott, 2021). Therefore, ecosystem resilience and ecosystem functioning are hindered significantly as the effects of climate change intensify and global change-driven stressors affect the ability of coastal ecosystems to recover after a disruptive event (Defeo and Elliott, 2021).

The nature of human existence is such that societies are constantly progressing and evolving. The implication is therefore that new threats to coastal ecosystems are emerging in parallel, where the consequences to the ecosystem are not fully understood. Chemicals of emerging concern (CECs) including pharmaceutical and personal care products, are an example of an emerging threat to coastal environments (Gaw *et al.*, 2014, Ojemaye and Petrik, 2021b, Daughton, 2016). The fate

and impacts of pharmaceutical and personal care products (PPCPs) in coastal ecosystems are not well understood due to a lack of research, especially in comparison to other anthropogenic threats such as nutrient pollution and eutrophication that have been investigated over decades (Gaw *et al.*, 2014, Ojemaye and Petrik, 2021b). Subsequently, management authorities have a knowledge base for understanding the causes and consequences of these stressors on coastal ecosystems and functioning, whereas the consequences of PPCPs in the environment are largely unknown (Gaw *et al.*, 2014, Ojemaye and Petrik, 2021b, Ojemaye, 2019). More importantly, few studies have quantified how pharmaceuticals can impact key ecological processes in aquatic ecosystems which determine their functionality. PPCPs are termed chemicals of emerging concern as they have become increasingly topical in recent years due to their pervasive use by humans and the increasing frequency of their detection in aquatic ecosystems (Daughton, 2016, Szymańska *et al.*, 2019, Ojemaye, 2019).

Industrial, municipal and household sewage are the primary sources of PPCPs in the marine environment (Gaw *et al.*, 2014, Michael *et al.*, 2013). These chemicals may enter sewage from pharmaceutical manufacturing plants, usage by patients in communities and discharges from hospitals (Daughton, 1999). Other sources of PPCPs in the marine environment include the inappropriate disposal of chemicals into seafills and landfills, runoff from horticulture and animal husbandry practices, and discharges from aquaculture facilities near rivers or in coastal regions (Archer, 2017, Kemper, 2008, Le and Munekage, 2004). The high concentrations and occurrence of pharmaceuticals and their metabolites in coastal marine environments are predominantly due to insufficient removal during wastewater treatment processes. The removal of pharmaceuticals and their metabolites from sewage is largely dependent on the type of pharmaceutical and its

physicochemical properties including solubility and structure (Jones *et al.*, 2005). However, pharmaceuticals in wastewater have a wide variety of removal efficiencies during treatment (Jones *et al.*, 2005). For example, diclofenac, a nonsteroidal anti-inflammatory has removal efficiencies of 69 % and 92 % (Madikizela and Chimuka, 2017b) and the anti-epileptic carbamazepine has removal efficiencies of -40 % and 52 % (Moslah, 2018, Fernández-López *et al.*, 2016), where negative value indicate an increase in pharmaceutical concentration after treatment. Wastewater treatment processes also affect pharmaceutical removal as certain processes are more effective at elimination than others. For example, traditional treatment processes such as sedimentation, flocculation and sand filtration are often ineffective as they are not designed to remove pharmaceuticals (Yang *et al.*, 2017).

The presence of PPCPs in the marine environment is concerning as they can transform, bind to sediment and particulate matter, and bioaccumulate within marine organisms (Gaw *et al.*, 2014, Ojemaye and Petrik, 2021b). Non-target organisms in coastal ecosystems are directly exposed to these contaminants daily (Ojemaye and Petrik, 2021b) and although these pollutants may be found at low concentrations in the environment, they are specifically designed to be biologically active at low concentrations (Fabbri, 2016), with induction of stress and negative impacts on behaviour, metabolism and reproduction reported (Mesquita *et al.*, 2011, Di Poi *et al.*, 2014, Neuparth *et al.*, 2014, Gonzalez-Rey and Bebianno, 2013). Moreover, organisms may be exposed to pharmaceuticals during their entire life cycle due to continuous inputs into aquatic environments (Kovalakova *et al.*, 2020). Significantly, the combined effects of multiple PPCPs in aquatic environments are a serious toxicological concern, due to pharmaceutical mixtures having negative

additive or synergistic impacts on biota (Hoerger *et al.*, 2013, Teixeira and Granek, 2017, Ebele *et al.*, 2017, Stackelberg *et al.*, 2004).

More recently, research on pharmaceutical pollution has focused on antibiotics in the environment, likely due to the rise in antibiotic consumption globally due to human population growth and increased demand for food, specifically animal protein, which necessitates a greater use of antibiotics (Van Boeckel *et al.*, 2015, Zhao *et al.*, 2019). Antibiotics in the environment are notably concerning due to their potential impacts on environmental functions, as they are synthetic, semi-synthetic or natural compounds that can kill or inhibit the metabolic activity or growth of microorganisms. Moreover, high antibiotic concentrations in wastewater treatment plant effluent are associated with an increase in antibiotic-resistant genes (ARGs) and antibiotic-resistant bacteria (ARBs), which can alter microbial community structures and reduce diversity (Harrabi *et al.*, 2019) and threaten marine organisms and human health (Kümmerer, 2009).

Pharmaceuticals including antibiotics, nonsteroidal anti-inflammatories, anticonvulsants, and antibacterials have been detected in estuaries and coastal waters worldwide (Table 1; Kotke *et al.*, 2019, Ojemaye and Petrik, 2021b, Fang *et al.*, 2012). Some of the most commonly detected pharmaceuticals in coastal zones are diclofenac (Bayen *et al.*, 2013, Andreu *et al.*, 2016, Paiga *et al.*, 2017, Ojemaye and Petrik, 2021b), carbamazepine (Bjorlenius *et al.*, 2018, Kotke *et al.*, 2019, Ojemaye and Petrik, 2021b, Ohoro *et al.*, 2021a), acetaminophen (Kotke *et al.*, 2019, Ojemaye and Petrik, 2021b, Koagouw *et al.*, 2021) and sulfamethoxazole (Klosterhaus *et al.*, 2013, Yan *et al.*, 2013, Ohoro *et al.*, 2021a, Ojemaye and Petrik, 2021b).

Table 1: The maximum concentration of selected pharmaceuticals in estuaries globally.

Therapeutic group	Pharmaceutical	Continent	Concentration in water (ng/L)	Study site	Reference
Analgesics and antipyretics	Acetaminophen	Africa	12000*	Blue Lagoon**, South Africa	Agunbiade and Moodley (2014)
			1260*	Blue Lagoon**, South Africa	Matongo *et al.* (2015)
			20000	Gazi Bay, Kenya	Wanjeri *et al.* (2023)
		Asia	2127	Alexander Estuary, Israel	Topaz *et al.* (2020)
			45	Danshuie River Estuary, Taiwan	Fang *et al.* (2019)
			13	Jiulong River Estuary, China	Sun *et al.* (2016)
		Australia	67	Sydney Estuary, Australia	Birch *et al.* (2015)
		Europe	917	Humber Estuary, England	Letsinger *et al.* (2019)
			11	Tejo Estuary, Portugal	Reis-Santos *et al.* (2018)
			440	Bilbao Estuary, Spain	Mijangos *et al.* (2018)
			49	Plentzia Estuary, Spain	Mijangos *et al.* (2018)
			395*	Garonne River Estuary, France	Aminot *et al.* (2016)
			ND	German Bight, Germany	Kotke *et al.* (2019)
		North America	120	Jamaica Bay, USA	Benotti and Brownawell (2007)
			13	Long Island Sound Estuary, USA	Lara-Martin *et al.* (2014)
			110*	Buffalo Estuary, Gulf of Mexico	Scott *et al.* (2016)
Antibiotic	Sulfamethoxazole	Africa	<LOD	Sundays River Estuary, South Africa	Ohoro *et al.* (2021a)
			<LOD	Buffalo River Estuary, South Africa	Ohoro *et al.* (2021a)
			<LOD	Swartkops Estuary, South Africa	Ohoro *et al.* (2021b)
			ND	Blue Lagoon**, South Africa	Matongo *et al.* (2015)
			1500	Blue Lagoon**, South Africa	Agunbiade and Moodley (2014)
			900	Gazi Bay, Kenya	Wanjeri *et al.* (2023)

<LOD: below the limit of detection, ND: not detected, *: maximum average among sites, **: blue lagoon is also known as the Umgeni River Estuary.

Table 1 continued

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Therapeutic group	Pharmaceutical	Continent	Concentration in water (ng/L)	Study site	Reference
Antibiotic	Sulfamethoxazole	Asia	656	Alexander Estuary, Israel	Topaz *et al.* (2020)
			38	Pearl River Estuary, China	Liang *et al.* (2013)
			57	Yangtze Estuary, China	Yan *et al.* (2013)
			30	Danshuie River Estuary, Taiwan	Fang *et al.* (2019)
			1	Jiulong River Estuary, China	Sun *et al.* (2016)
		Europe	<LOD	Tejo Estuary, Portugal	Reis-Santos *et al.* (2018)
			2	Tejo Estuary, Portugal	Fonseca *et al.* (2021)
			12	German Bight, Germany	Kotke *et al.* (2019)
		North America	26	Jamaica Bay, USA	Benotti and Brownawell (2007)
			280*	Buffalo Estuary, Gulf of Mexico	Scott *et al.* (2016)
			67	San Francisco Bay, USA	Klosterhaus *et al.* (2013)
	Trimethoprim	Africa	1560	Sundays River Estuary, South Africa	Ohoro *et al.* (2021a)
			1620	Buffalo River Estuary, South Africa	Ohoro *et al.* (2021a)
			2000*	Swartkops Estuary, South Africa	Ohoro *et al.* (2021b)
			200*	Blue Lagoon**, South Africa	Matongo *et al.* (2015)
			18000	Gazi bay, Kenya	Wanjeri *et al.* (2023)
		Europe	2046	Bilbao Estuary, Spain	Mijangos *et al.* (2018)
			6	Plentzia Estuary, Spain	Mijangos *et al.* (2018)
			247	Humber Estuary, England	Letsinger *et al.* (2019)
			6	Tejo Estuary, Portugal	Fonseca *et al.* (2021)
Anticonvulsant	Carbamazepine	Africa	8750*	Sundays River Estuary, South Africa	Ohoro *et al.* (2021a)
			2690*	Buffalo River Estuary, South Africa	Ohoro *et al.* (2021a)

<LOD: below the limit of detection, ND: not detected, *: maximum average among sites, **: blue lagoon is also known as the Umgeni River Estuary.

Table 1 continued

Therapeutic group	Pharmaceutical	Continent	Concentration in water (ng/L)	Study site	Reference
Anticonvulsant	Carbamazepine	Africa	9500*	Swartkops Estuary, South Africa	Ohoro *et al.* (2021b)
			240*	Blue Lagoon**, South Africa	Matongo *et al.* (2015)
			94	Umgeni River Estuary, South Africa	Rimayi *et al.* (2018)
			6400	Gazi Bay, Kenya	Wanjeri *et al.* (2023)
			18*	Garonne River Estuary, France	Aminot *et al.* (2016)
		Asia	4953	Alexander Estuary, Israel	Topaz *et al.* (2020)
			11	Danshuie River Estuary, Taiwan	Fang *et al.* (2019)
			4	Jiulong River Estuary, China	Sun *et al.* (2016)
		Australia	3	Sydney Estuary, Australia	Birch *et al.* (2015)
		Europe	1256	Tejo Estuary, Portugal	Fonseca *et al.* (2021)
			0.83	Douro Estuary, Portugal	Duarte *et al.* (2023)
			61	Mira Estuary, Portugal	Duarte *et al.* (2023)
			20	German Bight, Germany	Kotke *et al.* (2019)
		North America	34	Jamaica Bay, USA	Benotti and Brownawell (2007)
			140*	Buffalo Estuary, Gulf of Mexico	Scott *et al.* (2016)
			44	San Francisco Bay, USA	Klosterhaus *et al.* (2013)
Antidepressant	Amitriptyline	Europe	0.3	Garonne River Estuary, France	Aminot *et al.* (2016)
			0.8	Douro Estuary, Portugal	Duarte *et al.* (2023)
			44	Mira Estuary, Portugal	Duarte *et al.* (2023)
			1.6	Tejo Estuary, Portugal	Duarte *et al.* (2023)
Fibrates/lipid regulators	Bezafibrate	Africa	5000	Blue Lagoon**, South Africa	Agunbiade and Moodley (2014)
		Asia	1041	Alexander Estuary, Israel	Topaz *et al.* (2020)

<LOD: below the limit of detection, ND: not detected, *: maximum average among sites, **: blue lagoon is also known as the Umgeni River Estuary.

Table 1 continued

Therapeutic group	Pharmaceutical	Continent	Concentration in water (ng/L)	Study site	Reference
Fibrates/lipid regulators	Bezafibrate	Europe	4*	Garonne River Estuary, France	Aminot *et al.* (2016)
			28	Tejo Estuary, Portugal	Fonseca *et al.* (2021)
			0.5	German Bight, Germany	Kotke *et al.* (2019)
		North America	0.2*	Long Island Sound Estuary, USA	Lara-Martin *et al.* (2014)
Non-nucleoside reverse transcriptase inhibitors	Nevirapine	Africa	68	Umgeni River Estuary, South Africa	Rimayi *et al.* (2018)
			1170	Gazi Bay, Kenya	Wanjeri *et al.* (2023)
		Europe	0.5*	Garonne River Estuary, France	Aminot *et al.* (2016)
Non-steroidal anti-inflammatory	Diclofenac	Africa	1260	Warner Beach Estuary, South Africa	Sigonya *et al.* (2022)
			<LOD	Umgeni River Estuary, South Africa	Ngubane *et al.* (2019)
		Asia	730	Alexander Estuary, Israel	Topaz *et al.* (2020)
			11	Jiulong River Estuary, China	Sun *et al.* (2016)
		Europe	251	Humber Estuary, England	Letsinger *et al.* (2019)
			52	Tejo Estuary, Portugal	Reis-Santos *et al.* (2018)
			0.9	German Bight, Germany	Kotke *et al.* (2019)

<LOD: below the limit of detection, ND: not detected, *: maximum average among sites.

Research on pharmaceutical pollution levels and associated effects on ecological processes in estuaries is severely lacking compared to riverine and marine environments. Such knowledge gaps impede understanding of the broader consequences of pharmaceutical pollution in estuarine ecosystems (Reis-Santos *et al.*, 2018, Brausch *et al.*, 2012, Gaw *et al.*, 2014). The complexity, and variability of estuarine ecosystems (due to temporal and spatial changes in salinity, sediment loads and hydrodynamic changes (Chapman and Wang, 2001) along with their productivity and ecological significance as nursery and feeding grounds, necessitate more research concerning pharmaceutical pollution. For example, in terms of temporal variability, benthic sediment that is resuspended during changes in tides or storms can act as a secondary source of pollution, given that pharmaceuticals typically accumulate in sediment. Such variability further complicates our understanding of pharmaceutical pollution (Liang *et al.*, 2013, Ojemaye, 2019). Similarly, salinity changes can influence the bioavailability of contaminants and bioaccumulation in estuarine biota since salinity determines the partitioning of contaminants between the overlying water and sediment (Chapman and Wang, 2001, Li *et al.*, 2021). More specifically, sediment in coastal marine environments acts as a sink and reservoir for pharmaceutical contaminants because their affinity for suspended solids is enhanced with higher salinities (Gilroy *et al.*, 2012).

Pharmaceuticals have the potential to differentially impact pelagic and benthic estuarine biota, including key estuarine species that drive ecosystem functioning (Costanzo *et al.*, 2005). For example, Fonseca *et al*., (2021) reported that pharmaceutical detection frequencies were higher in benthic than pelagic species in an estuarine environment, suggesting that benthic organisms are more susceptible to pharmaceutical contaminants, likely due to the adsorptive affinity of pharmaceuticals to sediment. This highlights the importance of habitat and diet (e.g. sediment

feeders) in pharmaceutical uptake and bioaccumulation (Fonseca *et al.*, 2021). Nevertheless, pharmaceuticals have been reported to have negatively affected the growth and diversity of pelagic assemblages at environmentally relevant concentrations[1] (Teixeira and Granek, 2017, Shan *et al.*, 2021). However, beyond the effects on benthic and pelagic compartments, pharmaceuticals influence taxa differentially based on their chemical properties (Teixeira and Granek, 2017, Shan *et al.*, 2021, Almeida *et al.*, 2014, Yang *et al.*, 2014)

1.2 Pharmaceutical pollution research in South African estuaries

In South Africa, little research has been conducted on estuarine ecosystems regarding pharmaceutical pollution. However, over 800 million litres of wastewater are discharged into South African estuaries per day (Van Niekerk *et al.*, 2019) containing various persistent organic pollutants and pharmaceuticals, which necessitates additional research regarding pharmaceutical pollution. Riverine input is a major secondary source of pharmaceuticals for estuaries (Aminot *et al.*, 2016). However, pharmaceutical concentrations in estuaries are dependent on riverine flow rate, season, residence times and tidal intrusion because these variables influence pharmaceutical dilution and estuarine flushing (Aminot *et al.*, 2016). In South Africa, estuaries are essential for coastal ecosystem functioning as shown globally (Levin, 2001). Estuaries influence critical and diverse processes such as coastline protection and nutrient cycling (Barbier, 2011, Levin, 2001). As sheltered ecosystems, South African estuaries are particularly important fish and invertebrate nurseries that support commercial fisheries and subsistence harvesting (Whitfield, 1994, Beck *et al.*, 2001). In addition, the functioning of the estuarine ecosystem locally, like all ecosystems, depends on functionally important ecosystem engineers nested therein. One such species is the

[1] pharmaceutical concentrations that are similar to those quantified in the environment.

endobenthic sandprawn *Kraussillichirus kraussi,* but no research has investigated the impacts of pharmaceuticals on ecological functions provided by this species.

Sandprawns have a wide distribution in Southern Africa, extending from Inhambane in Mozambique to Lüderitz Bay in Namibia (Branch *et al.*, 2016). Found in shallow marine ecosystems and estuaries, sandprawns are one of the most dominant benthic organisms in these systems in terms of their high abundance and ecological impacts on benthic ecosystem processes (Pillay *et al.*, 2007a). Sandprawns are influential benthic deposit-feeders that burrow into and rework the sediment, which consequently creates complex bioengineered benthic habitats (Fig. 2; Pillay and Branch, 2011, Pillay, 2019). Moreover, bioturbation activities change biogeochemical characteristics and benthic community composition (Murphy and Kremer, 1992, Meysman *et al.*, 2006, Pillay *et al.*, 2007b). For these reasons, *K. kraussi* is recognised as a significant ecosystem engineer in benthic environments in South Africa (Pillay and Branch, 2011).

Sandprawns construct complex burrow systems that can extend 1m deep into the sediment, in which they spend a large portion of their time budgets actively pumping water through them (Pillay, 2019). Bi-directional pumping by *K. kraussi* facilitates the exchange of water from the pelagic and benthic environments, which oxygenates water within the burrow as well as eliminates faecal matter and potentially toxic chemicals (Pillay and Branch, 2011). As water is exchanged through the burrows, suspended particles including organic matter and phytoplankton may be absorbed onto the sediment in the burrow walls (Pillay and Branch, 2011, Pillay, 2019). The addition of mucous (secreted by sandprawns onto the walls for stability) to burrow walls can also increase particle trapping (Venter *et al.*, 2020). Moreover, prior research has demonstrated that

microbial biofilms line burrow walls which can increase the adsorption of organic matter and phytoplankton (Pillay and Branch, 2011). Additionally, Venter *et al*., (2020) reported a near 50 % decline in pelagic phytoplankton biomass in the presence of sandprawns and an increase in phytoplankton biomass on sandprawn burrow walls compared to the sediment surface. Hence, sandprawn burrows can act as biological filters that can significantly improve water quality, maintain bentho-pelagic coupling and enhance coastal resilience against nutrient pollution and subsequent eutrophication (Venter *et al.*, 2020, Raffaelli *et al.*, 2003). Moreover, Thomas *et al.* (2023), showed that in addition to reducing phytoplankton biomass, sandprawns prevent a shift to nanophytoplankton dominance thereby counteracting eutrophication. This study shows the importance of considering phytoplankton size class responses to sandprawns and other biological processes. Importantly, several other studies have shown the importance of different phytoplankton size classes in terms of mediating food-web interactions and nutrient cycling (Safi and Hayden, 2010, Ward and Shumway, 2004, Jiang *et al.*, 2019).

1.3 Aims and hypotheses

The rise of chemical contamination in estuaries in association with global change highlights the need for holistic ecosystem management (Elliott *et al.*, 2020a, Elliott *et al.*, 2020b). The lack of pharmaceutical baseline data in estuaries is a significant oversight as this data is critical for estuarine management. Therefore, in this study, pharmaceutical concentrations will be determined in the Zandvlei Estuary, located in the Western Cape of South Africa. The estuary is an ideal model urban estuarine ecosystem to understand global change and pollution pressures and can therefore provide valuable insight into stressors and responses of other urban estuaries locally. Also noteworthy is that the system is the only functional fish nursery on the False Bay coastline (Quick

and Harding, 1994). Moreover, the consequences of pharmaceutical pollution on functionally important estuarine biota in South African estuaries are, to the best of my knowledge, unknown. Therefore, following the quantification of levels of pharmaceutical pollution in the Zandvlei Estuary, an experiment will be conducted, in which the effects of specific pharmaceutical pollutants on sandprawn biofiltration and microalgal biomass will be quantified. Despite the limitations of manipulative *ex-situ* experiments approximating natural ecosystems, they are essential to understanding complex ecological processes by limiting confounding factors specifically in the context of estuarine environments (Quinn and Keough, 2002).

There were two main aims in this study. The first was to determine concentrations of pharmaceuticals present in the Zandvlei Estuary water column and in resident sandprawns. The second aim was to quantify the individual and interactive effects of sandprawn density and sulfamethoxazole (SMX) concentrations on pelagic and benthic microalgal biomass and the biofiltration effects of sandprawns. The first project aim was based on a field study, in which samples were collected from the Zandvlei Estuary to identify pharmaceuticals present in the system and their concentrations. The second project aim was achieved using a factorial mesocosm experiment, in which sandprawn density and SMX concentration were manipulated, and ecological responses (mainly pelagic and benthic microalgal assemblages) were determined. SMX was used as the target pharmaceutical in my experiment because firstly SMX is one of the most detected antibiotics in aquatic environments and readily detected in municipal wastewater (Lu *et al.*, 2020, Gobel *et al.*, 2007). Secondly, SMX had the second-highest total average concentration in the Zandvlei Estuary. Thirdly, SMX is a broad-spectrum antibiotic that inhibits bacterial growth and

therefore should negatively affect sandprawn filtration due to the microbial biofilms increasing adsorption of phytoplankton (Baran *et al.*, 2011, Pillay and Branch, 2011, Venter *et al.*, 2020).

The first hypothesis tested in the field study was that pharmaceutical concentrations will be greater in the northern parts of the estuary compared to the mouth, due to riverine input and limited flushing in northern areas. The second hypothesis tested was that pharmaceutical concentrations in sandprawn tissue would be greater than in the estuarine water of the Zandvlei Estuary. This expectation was based on previous studies indicating that pharmaceutical concentrations were greater in biota compared to the surrounding aquatic environment due to certain pharmaceuticals bioaccumulating within biota (Long *et al.*, 2022).

For the experiment, I tested the hypothesis that increasing sandprawn will reduce phytoplankton biomass, based on the findings of Venter *et* al., (2020) that sandprawn presence resulted in a near 50 % decline in phytoplankton biomass. I also hypothesized that the addition of SMX will inhibit sandprawn water biofiltration thus resulting in a reduced rate of pelagic microalgal decline. The second hypothesis is based on the findings of Venter *et al.* (2020), that biofiltration by sandprawns is mediated by overlying waters being pumped through burrows, and subsequent adsorption of phytoplankton cells on burrow walls. However, given that SMX is an antibiotic, the addition of SMX will potentially negatively affect burrow bacteria, thus reducing phytoplankton adhesion potential and hence biofiltration of pelagic microalgae. Additionally, SMX may directly impact the gut microbiome of sandprawns and negatively influence sandprawn activity and biofiltration.

Additionally, it was hypothesised that SMX will directly and indirectly reduce phytoplankton biomass due to the structurally similar organelles and cellular processes of bacteria and some phytoplankton species, particularly cyanobacteria (Guo *et al.*, 2015, Brain *et al.*, 2008) and by inhibiting the resource provisions (e.g. vitamins and nutrients) from bacteria to phytoplankton (Croft, 2005, Cho *et al.*, 2015). This hypothesis was based on research by Teixeira and Granek (2017) indicating that some phytoplankton taxa are negatively affected by SMX, speculating that this was driven by taxa having similar organelle structure to bacteria and a decline in resource provision from bacteria to phytoplankton (Croft *et al*., 2005). Lastly, it is expected that SMX will differently affect pelagic and benthic microalgae. Although no research has been conducted on the differential responses of pelagic and benthic sensitivity to SMX, a contrasting response is expected because of the distinction in species between the pelagic and benthic compartments, specific species sensitivity to antibiotics as well as the affinity of antibiotics to sediment (Hagenbuch and Pinckney, 2012, Kim and Carlson, 2007).

2. METHODS

2.1 Ethics statement

The methodology applied in this study was approved by the Science Faculty Animal Ethics Committee of the University of Cape Town (2022/V6/DP/A). A Cape Nature permit (CN44-87-23853) was obtained for the collection, transportation and use of sandprawns from the Zandvlei Estuary Nature Reserve for research. Permission from the landowners, issued by the Biodiversity Management Branch Ecological Management Committee was obtained to conduct sampling in the Zandvlei Estuary Nature Reserve.

2.2 Field study

2.2.1 Study site

The Zandvlei Estuary is a shallow (mean water depth = 1.4 m), urban system that is situated about 30km from Cape Town, Western Cape, on the northwest shore of False Bay (Fig. 1; Harding, 1994, Venter *et al.*, 2020). The Zandvlei Estuary has been subjected to extensive anthropogenic modification, including canalisation of the lower region, construction of the marina on the east and the addition of a rubble weir at the mouth to control water levels (Harding, 1994). The mouth of the estuary is closed during the winter months when there is high rainfall and mechanically opened for about a week during the summer months when there is low rainfall (Venter *et al.*, 2020).

The Zandvlei Estuary is classified as eutrophic, with the northern region of the system being highly eutrophic (Harding, 1994). Riverine inputs, including drainage from informal settlements with poor sanitation infrastructure, agricultural land and urbanised areas are the main reasons for the Zandvlei Estuary's eutrophic conditions (Venter *et al.*, 2020). In addition, increased nutrient loading from sewage and stormwater input due to the hardening of the Zandvlei Estuary catchment has caused an overgrowth of pondweed (Adams *et al.*, 2020). Frequent sewage spills due to wastewater treatment plant pump (WWTP) failures further threaten the Zandvlei Estuary (Adams *et al.*, 2020). For example, there were ongoing sewage spills in the Zandvlei Estuary during sample collection for both the field study and experiment. Zandvlei is also utilized as a recreational area however, poor water quality and pollution threaten participation in recreational activities (Quick and Harding, 1994). Dense aggregations (176 /m^2 – 240 /m^2) of the sandprawn, *Kraussillichirus kraussi* inhabit a small area (4.9 % of Zandvlei Estuary) in the lower regions of the system (Venter *et al.*, 2020).

Figure 1: Location of the Zandvlei Estuary and marina within (i) Cape Town and (ii) South Africa showing sampling sites (1-5). The arrowed line near site 5 denotes the sandprawn biotope.

2.2.2 Field sampling

Field sampling took place in the Zandvlei Estuary in winter on the 4th of August 2022, during a closed mouth phase. Water samples for pharmaceutical extraction and quantification were collected following Ojemaye and Petrik (2021). Prior to estuarine water collection, glass bottles were washed with liquid soap, rinsed with Millipore water and methanol, and dried overnight at 160 ºC. Before sampling, bottles were rinsed with estuarine water at each site. Triplicate sub-surface (depth 30 cm) water samples (1 L) were collected from 5 sampling sites in the Zandvlei Estuary (Fig. 1). Sub-surface water samples were collected to ensure no floating surface debris

was collected. Physico-chemical variables (water temperature, salinity, dissolved oxygen and pH) and chlorophyll biomass were measured at each site with a YSI 6600 Multiprobe. Water depth per site was measured using a weighted line marked at 30 cm intervals.

Ten sandprawns were collected (for pharmaceutical extraction and quantification) using a stainless steel sandprawn pump (length= 90 cm, diameter= 5 cm) in the sandprawn biotope region of the Zandvlei Estuary (Fig. 1; near site 5). The sandprawns selected were between 4-8 cm in length (from chela to telson) and non-ovigerous. The sandprawns were then individually wrapped in aluminium foil and placed into plastic Ziplock bags. The water and sandprawn samples were stored in a cooler box on ice packs and transported to the Chemical Sciences building at the University of Western Cape for preparation and extraction.

2.2.3 Pharmaceutical preparation and extraction

2.2.3.1 Water samples

Water samples were filtered, and pharmaceutical extractions were carried out according to Ojemaye and Petrik (2021). The water samples were filtered within 24 hours of collection through cellulose filter paper (particle retention 0.45 µm, GF/A 47 mm) to remove any debris or sediment. After filtration, the water samples were stored for 5 days at 4 ºC in a refrigerator until solid-phase extraction (SPE). SPE was done with 500 mg, 6 cc HLB extraction cartridges (Oasis HLB®) connected to a vacuum manifold and pump (VAC ELUT SPS 24 Port Agilent Varian, USA). The extraction cartridges were preconditioned with 7 ml of HPLC-grade methanol and then 7 ml of Milli-Q water. The filtered estuarine water samples were then individually loaded in the preconditioned cartridges, with a flow rate of 5 mL/min.

The cartridges were washed with 5 mL of 40 % v/v methanol. The extraction cartridges containing the analytes were dried under a gentle stream of nitrogen. Subsequently, the analytes were eluted with 2 mL of methanol with a flow rate of 1 mL/min and the eluate was dried under a gentle stream of nitrogen. The dried samples were reconstituted with 100 µl of methanol, placed into individual 200 µm glass inserts within glass vials and stored at -40 ºC until transportation to the Central Analytical Facility (CAF), Stellenbosch for analysis on ice.

2.2.3.2 Sandprawn samples

The 10 sandprawns were individually rinsed with Millipore water before being preserved in a refrigerator at -20 °C, before being prepared for the pharmaceutical extraction processes. Sandprawns were freeze-dried for 5 days to remove moisture (water and haemolymph) to ensure that the solvent could penetrate the sample. The sandprawns were crushed with a pestle and mortar and three samples of approximately 1g of the crushed and dried sandprawns were weighed and put into separate centrifuge tubes. Three dried tissue samples were acquired to assess the variation within the sandprawn population. Extraction was performed with 3 ml of HPLC-grade methanol. The sandprawn samples were placed in an ultrasonic bath (Bransonic MH Ultrasonic bath) for 30 minutes to agitate the sample particles. The samples were centrifuged at 8000 rpm for 10 minutes to separate the suspended particles from the liquid. The supernatant was then combined in a 10 ml volumetric flask and HPLC grade methanol was added to make up a volume of 10 mL. 1 mL of the extracted sample was diluted with 9 mL of Millipore water.

SPE was carried out with 200 mg, 6 cc HLB cartridges (Oasis HLB®) connected to a vacuum manifold and pump (VAC ELUT SPS 24 Port Agilent Varian, USA). SPE for sandprawns was carried out as described for the water sample analysis. The reconstituted sandprawn and water samples were stored at -40 ºC until further analysis.

2.2.4 Pharmaceutical analysis

Pharmaceutical analysis (chemical identification and quantification) of the sandprawns and water samples was done at CAF (Central Analytical Facilities), Stellenbosch, using a Waters Aquity LCMS (Liquid Chromatography-Mass Spectrometer) coupled with a Waters Xevo TQS Mass Spectrophometer. Separation of sample compounds was achieved with a Waters Acquity C18 2.1 x 100 mm, 1.7 µm column. An internal standard solution of 10 ppb was used to spike the water and sandprawn samples. 20 µl was removed from each sample and replaced with an equivalent volume of internal standard solution. The LCMS was also calibrated with a 10 ppb internal standard mixture.

2.2.5 Bioaccumulation factor

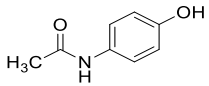
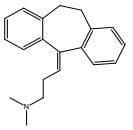
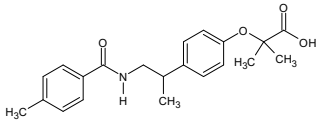
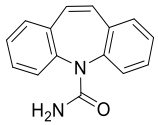
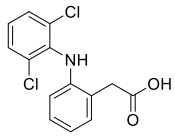
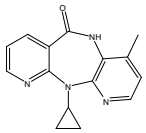
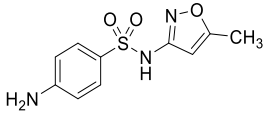
The bioaccumulation factor (BAF) of each detected pharmaceutical in the Zandvlei Estuary was calculated to determine if each pharmaceutical is bioaccumulating within the sandprawn tissue. The BAF was expressed as the ratio of the contaminant concentration in the sandprawn tissue to that of estuarine water. Contaminants were classified as either “bioaccumulative” (BAF > 5000 L/kg) or “potentially bioaccumulative” (2000-5000 L/kg) (Wu *et al.*, 2010, Na *et al.*, 2013)

2.2.6 Quality control and assurance

Quality control and assurance were done according to Ojemaye and Petrik (2021), to circumvent problems associated with blank contamination (Ojemaye and Petrik, 2021b). Blank field samples (MilliQ water) were exposed to the environment and sampling conditions (glass bottles containing MilliQ water were opened and exposed to air and sunlight during sampling) and analysed with the samples as a control for potential contamination. In this study, no contaminants were detected in the analysed blank samples.

The analytes were selectively identified by qualitatively comparing chromatograph spectral peaks of the standard solution and the samples (see Appendix A). The precision of the instrument was assessed by obtaining the relative standard deviation (%) of replicate injections of the standards and samples. The resulting relative standard deviation was <20 %. Linearity was assessed using the obtained calibration curves and estimated using the coefficient of determination (r^2), which ranged from 0.988 to 1.000 for each identified compound (see Appendix A). Accuracy was determined by comparing concentrations (20 ng/L and 100 ng/L) obtained in triplicate pre-spiked and post-spiked samples and expressed in recovery percentages (Table 2). The limit of detection (LOD) and limit of quantification (LOQ) were determined (Table 2) with the sample responses from the slope (S) and the standard deviation of the blank sample responses (α), where LOD = 3.3 α/S and LOQ = 10 α/S.

Table 2: Parameters of liquid chromatography-mass spectrometry, limit of detection (LOD), limit of quantification (LOQ) and the recoveries of target pharmaceuticals.

Pharmaceutical	Therapeutic class	Molecular structure	Molecular weight (g/mol)	Log Kow	RT (min)	Ion transition (m/z)		CE (eV)	LOD (ng/L)	LOQ (ng/L)	Recovery (%)
Acetaminophen	Analgesics and antipyretics		151.16	1.10	3.22	152 > 93	152 > 110	24	0.43	1.30	98.5
Amitriptyline	Tricyclic antidepressants		277.40	4.81	5.45	278 > 117	278 > 233	20	0.07	0.22	98.5
Bezafibrate	Fibrates		361.82	3.81	9.06	360 > 274	360 > 274	20	0.80	2.50	98.9
Carbamazepine	Anticonvulsant		236.27	2.67	6.43	237 > 135 237 > 179	237 > 194	10	0.20	0.50	99.2
Diclofenac	Nonsteroidal anti-inflammatory		296.15	4.06	9.12	296 > 215	296 > 250	15	0.70	2.00	99.1
Nevirapine	non-nucleoside reverse transcriptase inhibitors		266.30	2.5	4.19	267 > 107	267 > 226	25	0.16	0.48	98.2
Sulfamethoxazole	Antibiotic		253.28	1.31	4.50	254 > 147	254 > 156	25	0.30	1.00	98.9

2.3 Experiment

2.3.1 Experimental Design

A twenty-four-day factorial mesocosm experiment was conducted to investigate the individual and interactive effects of sulfamethoxazole (SMX) and sandprawn density on benthic and pelagic microalgal assemblages. The experiment was conducted in the aquarium facility in the John Day Building, University of Cape Town (air temperature = 15 °C; 16-hour day and 8-hour night light cycle). Twenty-seven mesocosms (volume = 0.054 m^3, depth = 50 cm) were filled with sediment (depth = 25 cm) and estuarine water (depth = 25 cm, temperature = 15 °C, salinity = 32 %). Using the randomization function in MS Excel, mesocosms were randomly interspersed in the aquarium to avoid spatial bias (Quinn and Keough, 2002). The experiment was conducted from 5 March 1 April 2023.

Sediment and estuarine water were collected from the sandprawn biotope area (site 5) of Zandvlei Estuary in the Western Cape province of South Africa (Fig. 1). The sediment was added randomly to the mesocosms and large fauna such as gastropods and sandprawns were removed. The estuarine water was homogenised in an 85 L bin, filtered (200 μm) to remove large debris and then added to the mesocosms. Each mesocosm was individually aerated using aquarium facility airlines and airstones (Pillay *et al.*, 2012). Mesocosms were then left undisturbed for at least a day before the sandprawns were added to ensure conditions were relatively uniform and stable in each mesocosm. Sandprawns were collected using a stainless-steel prawn pump (length= 90 cm, Diameter= 5 cm). Sandprawns selected were between 4 and 8 cm in length (chela to telson) and non-ovigerous. Sandprawns were left to acclimatise for at least 2 hours before being placed into the mesocosms according to the treatment designations (Venter *et al.*, 2020).

This mesocosm experiment consisted of two treatment groups with three levels, each replicated three times (Fig. 2). The first treatment was sandprawn density (0 %, 50 % and 100 %) and the second was SMX concentration (no SMX, average SMX and high SMX). For sandprawn density, 0 % density was the control and had no sandprawns, but the 50 % density mesocosms received 9 sandprawns and 100 % density received 18 sandprawns. The sandprawn density levels were based on the natural reported range of sandprawn densities (0-200 individuals.m^2) in South African estuaries (Branch, 1987).

The SMX levels used in the experiment were calibrated to natural water column levels recorded in the Zandvlei Estuary (see section 2.2 of the Methods and 3.1.2 of Results). 0.1 µg/L and 0.15 µg/L of SMX were added to the average and maximum SMX mesocosms every 2 days. The SMX used in the experiment was obtained commercially (Sigma-Aldrich) and applied by scaling down the average and maximum SMX concentrations recorded in the Zandvlei Estuary to the volume of water per mesocosm. No SMX mesocosms did not receive SMX for the duration of the experiment.

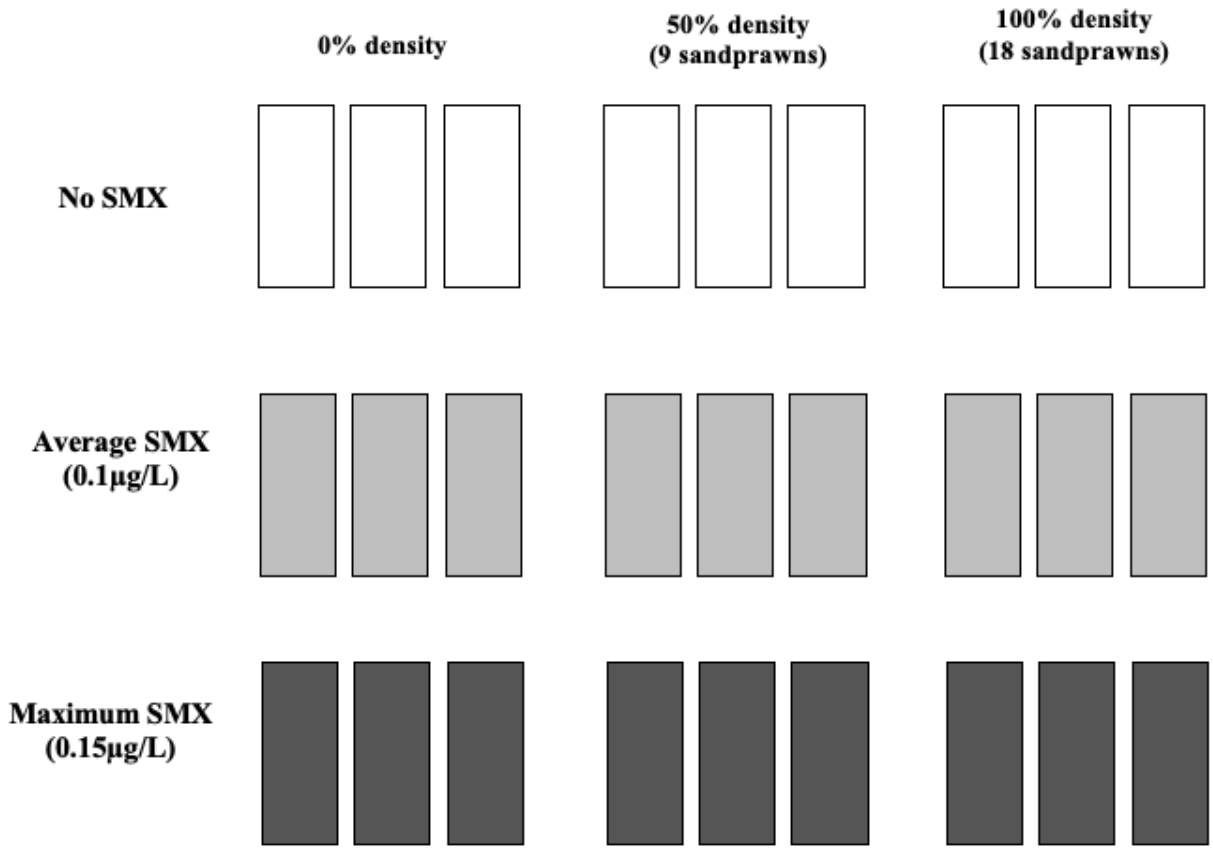


Figure 2: Schematic showing the design of the mesocosm experiment testing the effects of sandprawn density and SMX levels on benthic and pelagic microalgal assemblages (note: mesocosms were randomly interspersed to avoid spatial bias and were not clustered by treatment as illustrated).

2.3.2 Experimental data collection

2.3.2.1 Physico-chemical variables and pelagic inorganic nutrients

Abiotic variables were measured in each mesocosm prior to the start and for the duration of the experiment as conducted by Venter *et al*. (2020). Temperature, pH, dissolved oxygen, and salinity were measured every three days from day 0 for the duration of the experiment using a YSI 6600 mp Multiprobe. To avoid cross-contamination of mesocosms, the multiprobe was rinsed with deionised water after each measurement per mesocosm.

Two 25 mL sub-surface (depth =12 cm) water samples were collected per mesocosm every 4 days using a syringe and pooled together to determine inorganic nutrient concentrations. To avoid cross-contamination between mesocosms separate syringes were utilised for each mesocosm and rinsed following sample collection. From these samples, nitrite (NO^{2-}), nitrate (NO^{3-}), ammonia (NH_4^{+}) and phosphate (PO_4^{3-}) concentrations were measured using a Hanna Instruments Multiparameter Bench Photometer (HI 83203).

2.3.2.2 Suspended solids

On the final day of the experiment, 500 mL water samples were collected from each mesocosm to quantify suspended solid loads. The water samples were vacuum filtered onto previously weighed 0.7 µm micro-glass fibre filters and left to air dry for 15 minutes before being weighed again on an analytical balance (precision = 4 decimal places). Suspended solid loads were determined as the difference in the pre- and post-filtration masses.

2.3.2.3 Sulfamethoxazole concentration

Three 1 L water samples were collected before the start of the experiment from the homogenised estuarine prior to being added to mesocosms. 1 L water samples per mesocosm were also collected on the final day of the experiment to determine the changes in concentration of SMX for the duration of the experiment. The water samples were filtered, and chemical extractions and analysis were performed with an LC-MS following the methods outlined in sections 2.2.3 and 2.2.4.

2.3.2.4 Pelagic and benthic chl-a concentration and sediment reflectance

On the final day of the experiment, 500 mL subsurface (depth = 12 cm) water samples were collected per mesocosm to determine the pelagic chlorophyll (chl-*a*) biomass for micro-, nano- and picoplankton using a separate syringe per mesocosm. Each water sample was filtered within 12 hours of collection sequentially through 20 μm (microplankton), 2.0 μm (nanoplankton) and 0.7 μm (picoplankton) filters (GF/F). Following filtration, the filter papers were separately placed into 10 mL of 90 % acetone and placed into a fridge (< 5 °C) for 48 hours to allow for chl-*a* extraction. The chl-*a* biomass of samples was determined fluorometrically using a Turner Designs Trilogy Fluorometer (Perissinotto *et al.*, 2010).

Following all pelagic sample collection on the final day of the experiment, the remaining water was carefully siphoned out of each mesocosm to ensure the benthos were not disturbed. A benthotorch (bbe Moldaenke) was then used to determine the sediment reflectance and the biomass of diatoms, green algae and cyanobacteria. Sediment reflectance refers to the capacity of sediment to reflect light back to the benthotorch. Sediment reflectance would typically increase when organic matter and microalgal biofilm biomass is low in the sediment. Due to the heterogeneity of

mesocosm sediment surfaces, 9 benthotorch measurements were made per mesocosm, pooled together and averaged.

2.4 Statistical analyses

2.4.1 Field study

PRIMER-E (Version 7) with the PERMANOVA add-on package (Clarke and Gorley, 2006) was used for multivariate analyses of water column pharmaceutical data in the field study. A resemblance matrix was created using the Bray-Curtis method to determine the similarity in pharmaceutical concentrations at each site. A non-metric multidimensional scaling plot (nMDS) was generated from the resemblance matrix to visualize the spatial variation in pharmaceuticals among sampling sites. Vector overlays were added to the nMDS plot to visualize the contributions of individual pharmaceutical compounds that were responsible for spatial differentiation (if any).

2.4.2 Experiment

R Studio (R version 4.2.1, 2022) was used to perform all univariate statistical analyses for the experimental component of this study. The predictor variables included in this study were sulfamethoxazole (SMX) presence (none, average and high), sandprawn density (0 %, 50 % and 100 %) and their interaction. The response variables included physico-chemical (temperature, pH, salinity, dissolved oxygen), inorganic nutrients (NH_4^+, NO_3^-, NO_2^- and PO_4^{3-}), pelagic and benthic chl-*a* concentrations, sediment reflectance and suspended solids data.

2.4.2.1 Physico-chemical variables and pelagic inorganic nutrients

To determine the effect of predictor variables on physico-chemical and inorganic nutrient data, Linear Mixed Effects Models (LMEMs) were fitted by restricted maximum likelihood (REML) with the '*lme4*' package (Bates *et al.*, 2015). Since data collection took place over multiple days, time (days) was included as a random factor for all LMEMs as measurements were expected to change within each treatment over time and were not temporally independent (Venter, 2019). Model assumptions including homogeneity and normality were assessed with the quartile-quartile (Q-Q) plots, histograms, and residuals against predictor variables plots (Zuur *et al.*, 2009). Data were transformed (log or square root) if model assumptions were violated and the model was re-fitted. Analyses of Variances (ANOVAs) were done on the LMEMs using the '*car*' package to determine the significance (p-values) of the explanatory variables and the interaction between the variables. This was because p-values for main and interactive effects were not provided in model outputs.

2.4.2.2 Suspended solids, pelagic and benthic chl-a concentrations and sediment reflectance

To determine the effect predictor variables on benthic and pelagic chl-*a* concentrations, sediment reflectance and suspended solids individual, Linear Models (LMs) were fitted. As with the LMEMs, model assumptions were assessed, and data was transformed accordingly if model assumptions were violated. To determine the significance (p-values) of each explanatory variable, ANOVAs were done on each LM using the '*car*' package. Additionally, AIC (Akaike Information Criterion) values were generated for each response variable using the '*step*' function to determine the best model fit.

3. RESULTS

3.1 Field study

3.1.1 Physico-chemical variables

Temperature, dissolved oxygen and pH varied negligibly between sites and ranged from 13.56 ℃ to 14.60 ℃, 8.24 mg/L to 9.60 mg/L and 8.47 to 8.60 respectively (Table 3). Salinity and depth were more variable among sites, ranging between 15.39 ppt to 16.84 ppt and 1.0 m to 1.7 m, except site 5 where salinity and depth were 18.19 ppt and 0.4 m respectively (Table 3). Chl-*a* concentration generally decreased from site 5 (81.6 µg/L) to site 1 (20.7 3µg/L; Table 3).

Table 3: Pelagic physico-chemical variables measured at sites 1-5 in the Zandvlei Estuary.

Site	Temperature (℃)	Salinity (ppt)	Dissolved oxygen (mg/l)	pH	Depth (m)	Chl-*a* (µg/L)
1	14.35	16.76	9.31	8.52	1.2	81.6
2	14.24	16.84	8.24	8.47	1.4	54.5
3	13.73	15.39	9.6	8.67	1.0	54.4
4	13.56	15.92	9.4	8.70	1.7	51.2
5	14.60	18.19	8.95	8.56	0.4	20.7

3.1.2 Pharmaceutical occurrence

Site 5 was the most contaminated of sampling sites based on total pharmaceutical concentration, followed by site 3, site 2, site 4 and then site 1 (Table 4). Acetaminophen had the greatest concentration in sampling sites (0.121 ± 0.005 to 2.531 ± 0.076 µg/L), while amitriptyline (0.006 ± 0.000 to 0.012 ± 0.001 µg/L) and nevirapine (0.007 ± 0.000 to 0.009 ± 0.001 ug/L) were the least concentrated (Table 4). Sulfamethoxazole and bezafibrate concentrations were the highest at site 5 (Table 4) and along with acetaminophen were the main pharmaceuticals that differentiated this site from the rest (Fig. 3). Carbamazepine and diclofenac were greater at site 3, which differentiated this site from others (Table 4; Fig. 3).

Acetaminophen also had the highest average concentration in the sandprawn samples, (11.309 ± 2.142 µg/g), followed by carbamazepine (3.395 ± 1.169 µg/g), sulfamethoxazole (1.031± 0.532 µg/g) and nevirapine (0.120 ± 0.120 µg/g; Table 5). Diclofenac (0.024 ± 0.008 µg/g), bezafibrate (0.002 ± 0.002 µg/g) and amitriptyline (0.001± 0.001 µg/g) had the lowest average concentrations in the sandprawn samples (Table 5). Variance in pharmaceutical concentrations was evident for the three sandprawn samples measured, suggesting high within-population variability.

Table 4: Average concentration of pharmaceutical contaminants (mean ± SE) in water samples collected from sites 1-5 in the Zandvlei Estuary.

Site	Pharmaceutical concentration (µg/L)							
	Acetaminophen	**Amitriptyline**	**Bezafibrate**	**Carbamazepine**	**Diclofenac**	**Nevirapine**	**Sulfamethoxazole**	**Total**
1	0.12 ± 0.005	0.01 ± 0.0022	0.022 ± 0.0055	0.071 ± 0.0205	0.016 ± 0.0057	0.009 ± 0.0006	0.069 ± 0.0129	0.319 ± 0.0161
2	0.32 ± 0.013	0.01 ± 0.0009	0.027 ± 0.0021	0.067 ± 0.0096	0.037 ± 0.0132	0.009 ± 0.0007	0.094 ± 0.0056	0.562 ± 0.0410
3	1.31 ± 0.058	0.01 ± 0.0003	0.019 ± 0.0031	0.157 ± 0.0586	0.040 ± 0.0060	0.009 ± 0.0006	0.093 ± 0.0163	1.632 ± 0.1801
4	0.33 ± 0.009	0.01 ± 0.0003	0.015 ± 0.0033	0.100 ± 0.0055	0.016 ± 0.0032	0.007 ± 0.0003	0.085 ± 0.0038	0.559 ± 0.0440
5	2.53 ± 0.076	0.01 ± 0.0010	0.028 ± 0.0013	0.082 ± 0.0144	0.021 ± 0.0034	0.008 ± 0.0006	0.138 ± 0.0027	2.817 ± 0.3552
Total	4.604 ± 0.453	0.044 ± 0.001	0.112 ± 0.002	0.478 ± 0.016	0.130 ± 0.005	0.042 ± 0.000	0.480 ± 0.011	

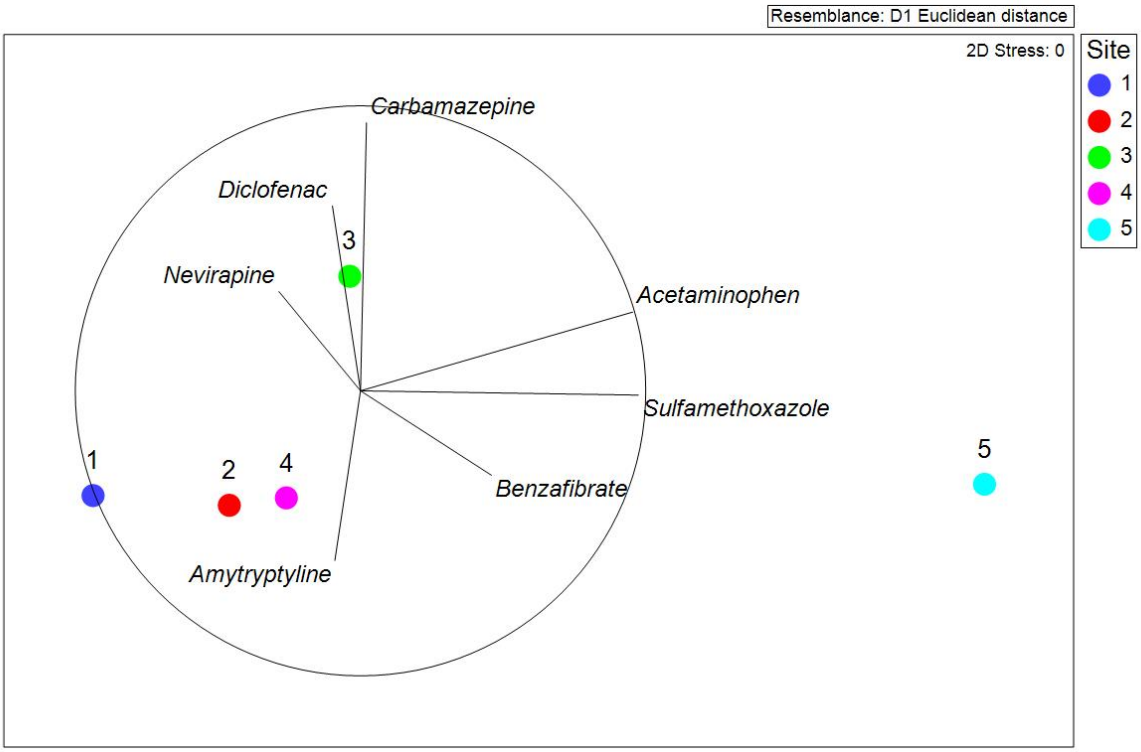


Figure 3: Non-metric multidimensional scaling ordination showing spatial variability in pharmaceutical concentrations among sites 1-5 in the Zandvlei Estuary. Vector overlays show the contribution of individual pharmaceutical compounds that contributed most to spatial differentiation among sites.

Table 5: Concentrations (mean ± SE) of pharmaceutical contaminants in sandprawn tissue samples collected from site 5 in the Zandvlei Estuary. Samples A, B and C represent three samples (1g) collected randomly from pooled dried tissue of 10 sandprawns from site 5.

Sample	Pharmaceutical concentration (µg/g)						
	Acetaminophen	Amitriptyline	Bezafibrate	Carbamazepine	Diclofenac	Nevirapine	Sulfamethoxazole
A	14.398	0.003	0.005	2.612	0.018	0.359	2.094
B	7.194	0	0	5.695	0.040	0	0.520
C	12.334	0	0	1.879	0.013	0	0.480

3.1.3 Bioaccumulation factor

The bioaccumulation factor for nevirapine was the greatest (43775 kg/L), followed by carbamazepine (41257 kg/L), sulfamethoxazole (7450 kg/L) and acetaminophen (4469 kg/L; Table 6). Of these pharmaceuticals, acetaminophen was potentially bioaccumulative in sandprawn samples, while the rest were bioaccumulative compounds. Diclofenac, amitriptyline and bezafibrate had the lowest bioaccumulation factors (Table 6) and were not bioaccumulative.

Table 6: Bioaccumulation factors of detected pharmaceuticals for sandprawn tissue samples in site 5 of the Zandvlei Estuary.

Pharmaceutical compound	**Bioaccumulation factor (L/kg)**
Acetaminophen	4469*
Amitriptyline	92
Bezafibrate	60
Carbamazepine	41257**
Diclofenac	1148
Nevirapine	43775**
Sulfamethoxazole	7450**

Note: ** bioaccumulative compounds, * potentially bioaccumulative

3.2 Experiment

3.2.1 Physico-chemical variables

Variance in dissolved oxygen and pH was minimal over the course of the experiment, ranging from 7.1 ± 0.03 to 7.9 ± 0.04 mg/L and 8.0 ± 0.03 to 8.5 ± 0.05 respectively (Table 7). Despite this minor variation, dissolved oxygen ($p = 0.005$) and pH ($p = 0.009$) levels were significantly affected by the SMX x sandprawn density interaction (Table 8). Temperature varied temporally during the experiment, ranging from 19.4 ± 0.13 to 14.1 ± 0.22 ℃ (Table 7). As with dissolved oxygen and pH, the sandprawn density x SMX interaction significantly explained temperature variation ($p = 0.011$; Table 8), though among-treatment trends were not clear. Salinity increased over time across all treatments from 32.1 ± 0.04 ppt on day 0 to 34.9 ± 0.31 ppt on day 24 (Table 7). Salinity variance was explained by sandprawn density ($p < 0.001$) and the sandprawn density x SMX interaction ($p < 0.001$), but not by SMX presence (Table 8). However, treatment related salinity responses were unclear given the minor variance across treatments (Table 7).

Table 7: Summary of spatio-temporal variation in physico-chemical variables (mean ± SE) across SMX and sandprawn density treatments for days 0, 9, 18 and 24 of the experiment. Sample sizes are shown in square brackets per day of data collection. Results for all days of physico-chemical data collection are shown in Appendix B. (Note: value in italics SE < 0.01).

Day	SMX presence	Sandprawn density (%)	Temperature (°C) [3]	Salinity (ppt) [3]	Dissolved oxygen (mg/l) [3]	pH [3]
0	None	0	19.2 ± 0.07	32.1 ± 0.05	7.2 ± 0.02	8.0 ± 0.03
		50	19.2 ± 0.11	32.2 ± 0.02	7.1 ± 0.05	8.0 ± 0.03
		100	19.4 ± 0.08	32.1 ± 0.06	7.1 ± 0.02	8.1 ± 0.01
	Average	0	19.2 ± 0.13	32.1 ± 0.01	7.2 ± 0.03	8.1 ± 0.04
		50	19.3 ± 0.07	32.1 ± 0.01	7.1 ± 0.02	8.0 ± 0.02
		100	19.3 ± 0.09	32.2 ± 0.08	7.1 ± 0.02	8.0 ± 0.04
	High	0	19.2 ± 0.10	32.1 ± 0.06	7.2 ± 0.03	8.1 ± 0.02
		50	19.2 ± 0.08	32.1 ± 0.09	7.1 ± 0.03	8.0 ± 0.02
		100	19.2 ± 0.02	32.1 ± 0.05	7.1 ± 0.04	8.0 ± 0.01
9	None	0	15.3 ± 0.21	33.2 ± 0.20	7.7 ± 0.03	8.2 ± 0.03
		50	15.3 ± 0.07	32.9 ± 0.08	7.7 ± 0.01	8.2 ± 0.01
		100	15.2 ± 0.15	33.0 ± 0.42	7.9 ± 0.23	8.2 ± 0.04
	Average	0	15.3 ± 0.05	33.2 ± 0.27	7.7 ± 0.02	8.2 ± 0.04
		50	15.4 ± 0.10	32.9 ± 0.17	7.6 ± 0.01	8.1 ± 0.07
		100	15.3 ± 0.10	33.1 ± 0.24	7.6 ± 0.01	8.2 ± 0.07
	High	0	15.1 ± 0.09	33.4 ± 0.02	7.8 ± 0.01	8.2 ± 0.02
		50	15.1 ± 0.13	32.9 ± 0.36	7.7 ± 0.04	8.2 ± 0.02
		100	15.5 ± 0.15	32.7 ± 0.16	7.7 ± 0.05	8.2 ± 0.02
18	None	0	18.5 ± 0.14	33.6 ± 0.15	7.3 ± 0.03	8.1 ± 0.07
		50	18.2 ± 0.06	33.3 ± 0.08	7.4 ± *0.01*	8.1 ± 0.05
		100	18.4 ± 0.08	33.5 ± 0.54	7.4 ± 0.04	8.2 ± 0.07
	Average	0	18.3 ± 0.02	33.8 ± 0.15	7.4 ± 0.03	8.2 ± 0.05
		50	18.3 ± 0.13	33.2 ± 0.27	7.4 ± 0.04	8.1 ± 0.07
		100	18.4 ± 0.15	33.6 ± 0.30	7.4 ± 0.04	8.2 ± 0.04
	High	0	18.3 ± 0.06	34.1 ± 0.16	7.4 ± 0.01	8.2 ± 0.06
		50	18.2 ± 0.32	33.3 ± 0.64	7.4 ± 0.02	8.1 ± 0.07
		100	18.7 ± 0.39	32.9 ± 0.13	7.3 ± 0.07	8.0 ± 0.11
24	None	0	14.5 ± 0.20	34.4 ± 0.12	7.8 ± 0.09	8.4 ± 0.10
		50	14.3 ± 0.14	34.3 ± 0.17	7.9 ± 0.03	8.3 ± 0.05
		100	14.1 ± 0.12	34.9 ± 0.28	7.9 ± 0.03	8.5 ± 0.03
	Average	0	14.4 ± 0.29	34.8 ± 0.46	7.8 ± 0.06	8.4 ± 0.10
		50	14.8 ± 0.59	34.0 ± 0.47	7.7 ± 0.15	8.3 ± 0.10
		100	14.4 ± 0.36	34.4 ± 0.56	7.9 ± 0.07	8.3 ± 0.10
	High	0	14.3 ± 0.23	34.8 ± 0.30	7.8 ± 0.05	8.4 ± 0.02
		50	14.2 ± 0.10	34.3 ± 0.42	7.9 ± 0.02	8.4 ± 0.02
		100	14.5 ± 0.22	34.1 ± 0.21	7.9 ± 0.07	8.4 ± 0.08

Table 8: Results of type II Wald Chi-Square test showing main and interactive effects of predictor variables (SMX and sandprawn density) on environmental response variables. Significant p-values are shown in bold, and levels of significance are shown as $p < 0.05$*, $p < 0.01$**, $p < 0.001$***.

Response variable	Explanatory variable	df	$\chi 2$	Significance level
Temperature	SMX	2	2.60	0.273
	Sandprawn density	2	1.05	0.592
	SMX x sandprawn density	4	13.01	**0.011***
Salinity	SMX	2	2.58	0.275
	Sandprawn density	2	34.13	**< 0.001*****
	SMX x sandprawn density	4	19.09	**< 0.001*****
Dissolved oxygen	SMX	2	0.90	0.639
	Sandprawn density	2	0.03	0.984
	SMX x sandprawn density	4	14.67	**0.005****
pH	SMX	2	1.65	0.439
	Sandprawn density	2	5.40	0.067
	SMX x sandprawn density	4	13.57	**0.009****

3.2.2 Pelagic inorganic nutrients

Among the pelagic inorganic nutrients, the concentration of ammonia had the greatest range (0.4 to 11.7 mg/L) followed by nitrite (0.3 to 10.3 mg/L) and phosphate (0.3 to 8.5 mg/L, Table 9). Variations in phosphate concentrations throughout the experiment were significantly explained by sandprawn density ($p = 0.035$; Table 10), with levels generally increasing from control to 100 % sandprawn density. Nitrite concentrations were significantly affected by SMX presence ($p = 0.001$; Table 10), with levels generally increasing from control to high SMX (Table 9). Variation in ammonia concentration throughout the experiment was not explained significantly by SMX presence ($p = 0.248$), sandprawn density ($p = 0.461$) or the sandprawn density x SMX interaction ($p = 0.906$; Table 10). Nitrate concentrations were below the detection limit (0.0 mg/L) and were thus excluded from the results and statistical analyses.

Table 9: Summary of the spatio-temporal variation in the concentration (mg/L) of pelagic inorganic nutrients (mean ± SE) across SMX and sandprawn density treatments for days 0, 8, 16 and 24 of the experiment. Sample sizes are shown in square brackets per day of data collection. Results for all days of organic nutrient data collection are shown in Appendix B.

Day	SMX presence	Sandprawn density (%)	Nitrite (NO^{2-}) [3]	Phosphate (PO_4^3) [3]	Ammonium (NH_4^+) [3]
0	None	0	7.7 ± 1.20	0.3 ± 0.18	10.4 ± 1.48
		50	7.0 ± 1.73	0.5 ± 0.33	7.3 ± 2.71
		100	10.3 ± 1.67	1.6 ± 0.30	10.8 ± 0.52
	Average	0	7.7 ± 0.67	0.9 ± 0.47	1.7 ± 0.97
		50	8.0 ± 0.58	1.3 ± 0.20	7.5 ± 3.72
		100	8.0 ± 0.58	1.4 ± 0.19	9.4 ± 1.31
	High	0	7.0 ± 0.58	0.3 ± 0.30	5.8 ± 3.20
		50	6.3 ± 1.20	1.4 ± 0.17	4.2 ± 2.61
		100	6.0 ± 2.00	1.7 ± 0.12	5.3 ± 1.75
8	None	0	5.7 ± 1.67	1.5 ± 0.09	3.9 ± 3.40
		50	5.7 ± 0.88	0.8 ± 0.44	1.1 ± 0.07
		100	7.3 ± 0.88	1.5 ± 0.33	1.6 ± 0.46
	Average	0	7.3 ± 0.33	0.9 ± 0.38	0.7 ± 0.05
		50	6.7 ± 0.67	1.0 ± 0.07	1.4 ± 0.30
		100	9.7 ± 2.40	1.8 ± 1.13	2.2 ± 0.61
	High	0	10.3 ± 1.86	1.2 ± 0.54	0.4 ± 0.10
		50	7.3 ± 2.67	0.6 ± 0.63	4.7 ± 3.52
		100	7.3 ± 0.33	1.6 ± 0.19	2.0 ± 0.60
16	None	0	4.3 ± 1.33	1.6 ± 0.12	4.2 ± 3.54
		50	3.3 ± 1.20	1.6 ± 0.44	3.4 ± 2.25
		100	1.0 ± 1.00	2.4 ± 0.23	5.8 ± 3.33
	Average	0	0.3 ± 0.33	1.9 ± 0.96	10.5 ± 1.16
		50	6.0 ± 3.79	2.4 ± 0.99	8.6 ± 3.06
		100	4.3 ± 0.67	3.7 ± 0.78	8.1 ± 2.39
	High	0	4.3 ± 1.45	1.5 ± 0.49	6.0 ± 2.71
		50	5.7 ± 0.67	2.6 ± 1.75	5.6 ± 2.07
		100	3.0 ± 0.58	2.6 ± 0.68	6.4 ± 1.76
24	None	0	7.0 ± 1.67	2.1 ± 0.77	2.2 ± 1.75
		50	7.7 ± 0.88	2.7 ± 0.09	10.2 ± 1.41
		100	10.0 ± 0.58	3.2 ± 0.12	10.7 ± 0.53
	Average	0	6.7 ± 3.71	2.6 ± 0.69	11.2 ± 0.21
		50	2.0 ± 2.00	1.9 ± 0.27	8.4 ± 2.00
		100	2.0 ± 1.15	3.8 ± 1.54	9.0 ± 2.85
	High	0	4.3 ± 0.88	0.4 ± 0.38	11.7 ± 0.68
		50	5.7 ± 1.33	1.9 ± 0.64	11.6 ± 0.07
		100	6.7 ± 0.33	1.2 ± 0.36	5.9 ± 2.77

Table 10: Results of type II Wald Chi-Square test showing main and interactive effects of the predictor variables (SMX and sandprawn density) on inorganic nutrient response variables. Significant p-values are shown in bold, and levels of significance are shown as $p < 0.05$*, $p < 0.01$**, $p < 0.001$***.

Response variable	Explanatory variable	df	$\chi 2$	Significance level
Nitrite	SMX	2	13.19	**0.001****
	Sandprawn density	2	1.32	0.518
	SMX x sandprawn density	4	2.13	0.712
Phosphate	SMX	2	0.20	0.905
	Sandprawn density	2	6.72	**0.035***
	SMX x sandprawn density	4	1.02	0.907
Ammonia	SMX	2	2.79	0.248
	Sandprawn density	2	1.55	0.461
	SMX x sandprawn density	4	1.03	0.906

3.2.3 Suspended solids

Suspended solid loads varied minimally across all treatments, ranging from 0.21 g to 0.35 g (Fig. 4). SMX presence ($p = 0.167$) and the sandprawn density x SMX interaction ($p = 0.084$) did not explain any variation in suspended solid loads in the mesocosms (Table 11). Although SMX presence was not significant, it generally improved model fit (AIC = -77.04; see appendix B). Variation in suspended solid loads was explained by sandprawn density ($p < 0.001$; Table 11), with suspended sediment generally being greater in the presence of sandprawns (Fig. 4).

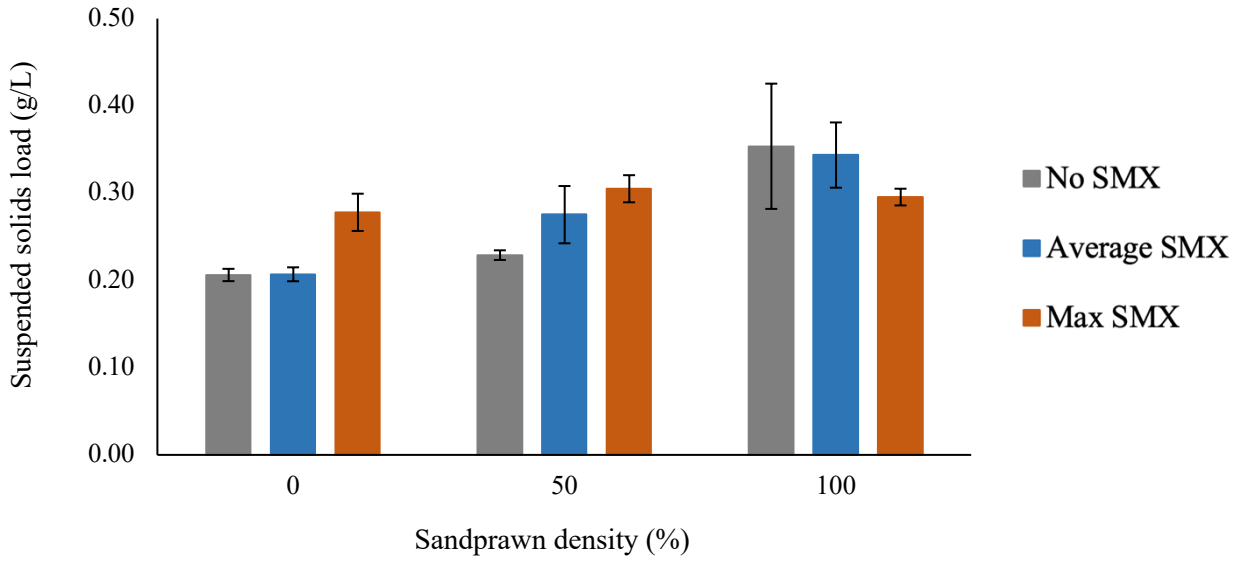


Figure 4: The effect of SMX (none, average and maximum) and sandprawn density (0 %, 50 % and 100 %) on the suspended solid loads (mean ± SE) at the end of the experiment.

Table 11: Results of an ANOVA (type II) showing the main and interactive effects of the predictor variables (SMX and sandprawn density) on suspended solid loads. Significant p- p-values are shown in bold, and levels of significance are shown as $p < 0.05$*, $p < 0.01$**, $p < 0.001$***.

Response variable	Explanatory variable	df	Sum of squares	F-value	Significance level
Suspended solids	SMX	2	0.097	1.98	0.167
	Sandprawn density	2	0.570	11.69	**<0.001****
	SMX x sandprawn density	4	0.238	2.45	0.084
	Residuals	18	0.439		

3.2.4 Pelagic chl-a concentration

Total chlorophyll biomass varied significantly with SMX presence ($p = 0.008$; Table 12) and generally decreased with increasing SMX concentration. Similarly, variation in microplankton ($p = 0.009$) and picoplankton ($p = 0.019$) biomass was also explained by SMX concentration (Table 12), with declining trends evident for both response variables. (Fig. 5). Sandprawn density did not explain variation in total chlorophyll, microplankton, and picoplankton biomass, however, sandprawn density did improve model fit for all three models (AIC = -67.16, AIC = -50.69, and AIC = -53.52 respectively; see appendix B). Variation in nanoplankton biomass was not explained by SMX level ($p = 0.072$), sandprawn density ($p = 0.394$), or the interaction between these predictor variables ($p = 0.511$; Table 12).

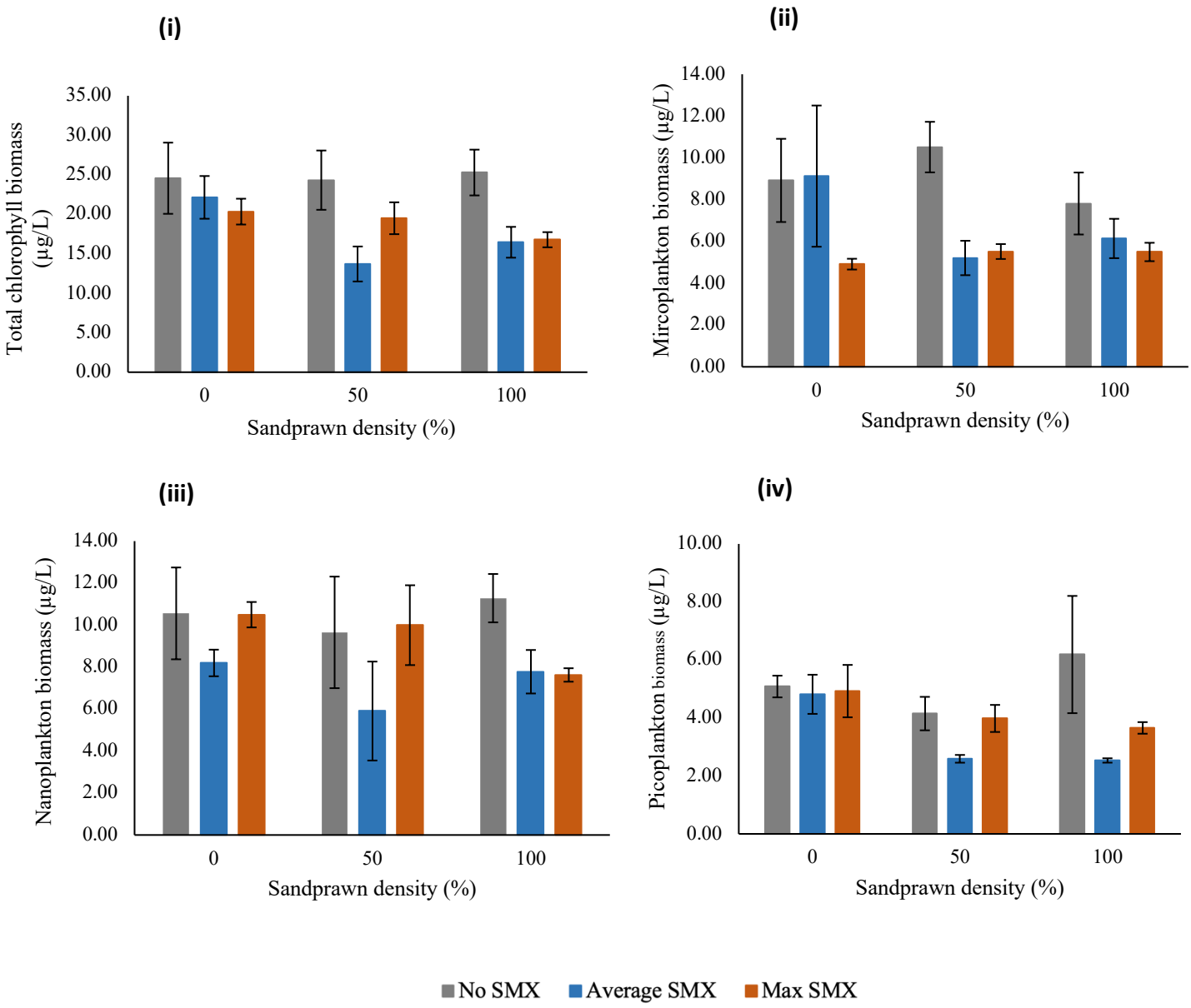


Figure 5: The effect of SMX (none, average and maximum) and sandprawn density (0 %, 50 % and 100 %) on the biomass (mean ± SE) of (i) total chlorophyll (ii) microplankton (iii) nanoplankton and (iv) picoplankton at the end of the experiment.

Table 12: Results of an ANOVA (type II) showing the main and interactive effects of the predictor variables (SMX and sandprawn density) on total chlorophyll biomass, microplankton biomass, nanoplankton biomass and picoplankton biomass. Significant p-values are shown in bold, and levels of significance are shown as p< 0.05*, p < 0.01**, p < 0.001***.

Response variable	Explanatory variable	df	Sum of squares	F-value	p-value
Total chlorophyll biomass	SMX	2	0.630	6.33	**0.008****
	Sandprawn density	2	0.163	1.63	0.223
	SMX x sandprawn density	4	0.272	1.37	0.285
	Residuals	18	0.896		
Microplankton biomass	SMX	2	1.170	6.28	**0.009****
	Sandprawn density	2	0.044	0.24	0.791
	SMX x sandprawn density	4	0.460	1.23	0.332
	Residuals	18	1.678		
Nanoplankton biomass	SMX	2	0.778	3.06	0.072
	Sandprawn density	2	0.249	0.98	0.394
	SMX x sandprawn density	4	0.432	0.85	0.511
	Residuals	18	2.284		
Picoplankton biomass	SMX	2	0.087	4.95	**0.019***
	Sandprawn density	2	0.054	3.05	0.721
	SMX x sandprawn density	4	0.516	1.46	0.255
	Residuals	18	0.087		

3.2.5 Benthic chl-a concentration

Diatom biomass was not significantly affected by SMX presence (p = 0.240), sandprawn density (p = 0.066), or the SMX x sandprawn density interaction (p = 0.671; Table 13). Benthic cyanobacteria biomass was significantly affected by sandprawn density (p = 0.040; Table 13) but trends were unclear (Fig. 6). Although SMX presence did not significantly affect benthic cyanobacteria biomass, it did improve model fit (AIC = -43.96; see appendix B). Benthic green algae concentrations were mostly 0 chl-a/cm² and thus were excluded from the results and not statistically analysed.

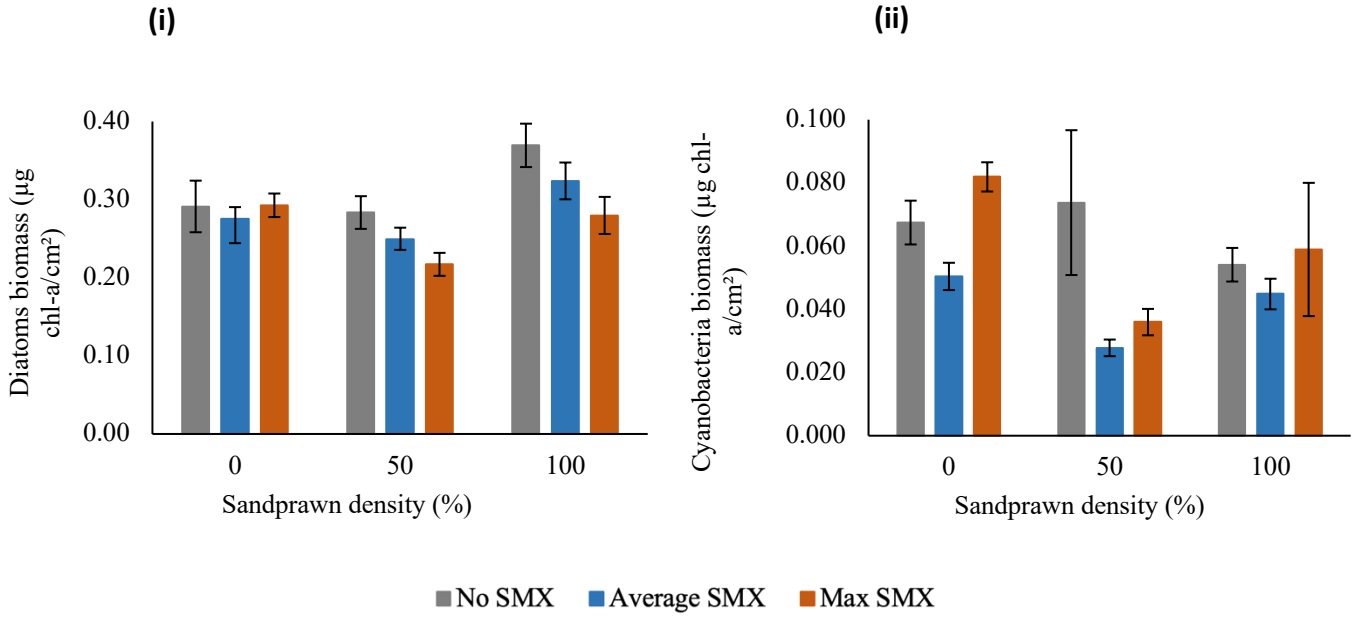


Figure 6: The effect of SMX (none, average and maximum) and sandprawn density (0 %, 50 % and 100 %) on the concentration (mean ± SE) of benthic (i) diatoms and (ii) cyanobacteria at the end of the experiment.

Table 13: Results of an ANOVA (type II) showing the main and interactive effects of the predictor variables (SMX and sandprawn density) on benthic diatom biomass and cyanobacteria biomass. Significant p-values are shown in bold, and levels of significance are shown as p < 0.05*, p < 0.01**, p < 0.001***.

Response variable	Explanatory variable	df	ss	F-value	p-value
Diatoms	SMX	2	0.142	1.55	0.240
	Sandprawn density	2	0.292	3.17	0.066
	SMX x sandprawn density	4	0.110	0.60	0.671
	Residuals	18	0.829		
Cyanobacteria	SMX	2	0.904	3.45	0.054
	Sandprawn density	2	1.015	3.87	**0.040***
	SMX x sandprawn density	4	0.870	1.66	0.203
	Residuals	18	2.359		

3.2.6 Sediment reflectance

Sediment reflectance was significantly affected by sandprawn density ($p = 0.002$), but not by SMX level ($p = 0.519$) or the interaction between sandprawn density and SMX level ($p = 0.880$; Table 14). However, SMX presence did improve model fit for sediment reflectance (AIC = -85.53; see Appendix B). Sediment reflectance increased from 21 % in the 0 % sandprawn density mesocosms to 26 % in the 50 % sandprawn density mesocosms and 27 % in the 100 % sandprawn density mesocosms (Fig. 7).

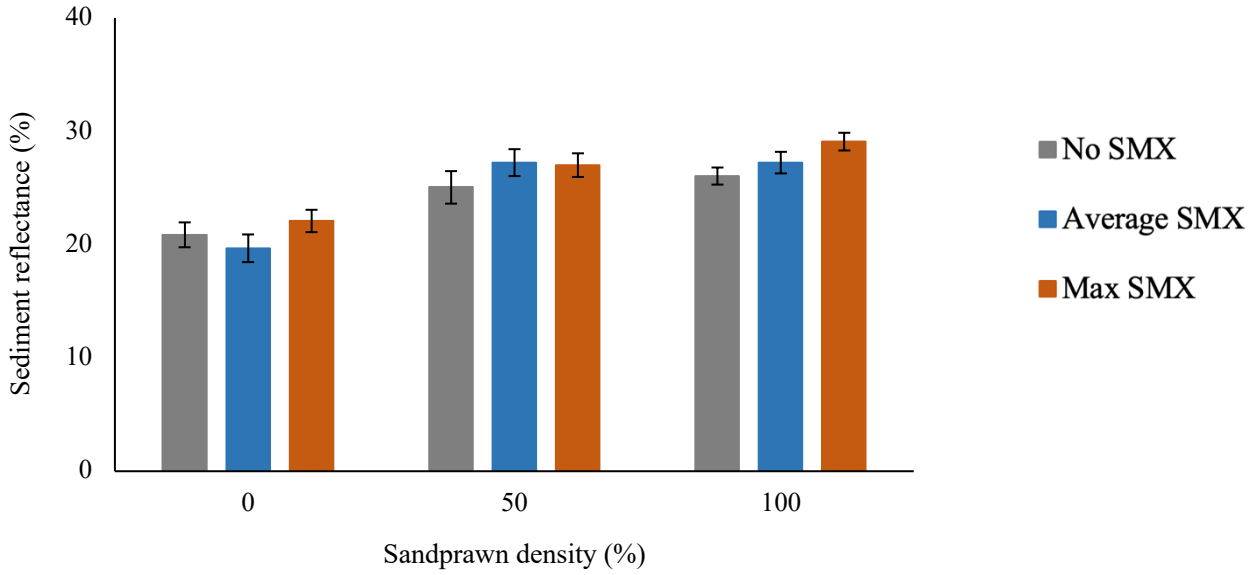


Figure 7: The effect of SMX (none, average and maximum) and sandprawn density (0 %, 50 % and 100 %) on the sediment reflectance (mean ± SE) at the end of the experiment.

Table 14: Results of an ANOVA (type II) showing the main and interactive effects of the predictor variables (SMX and sandprawn density) on sediment reflectance. Significant p-values are shown in bold, and levels of significance are shown as p<0.05*, p<0.01**, p<0.001***.

Response variable	Explanatory variable	df	ss	F-value	p-value
Sediment reflectance	SMX	2	0.034	0.68	0.519
	Sandprawn density	2	0.437	8.87	**0.002****
	SMX x sandprawn density	4	0.029	0.29	0.880
	Residuals	18	0.444		

4. DISCUSSION

Coastal ecosystems are susceptible to emerging anthropogenic stressors including pharmaceutical pollution, given the proximity of coastal communities to the environment and humans' dependence on pharmaceuticals (Crain *et al.*, 2009, Griffiths *et al.*, 2017, Bernhardt *et al.*, 2017, Gaw *et al.*, 2014). As a significant driver of global change, chemical pollutants threaten ecosystem functioning, species diversity and ultimately ecosystem health (Gaw *et al.*, 2014). Pharmaceutical pollution is attributed to the pervasive use of pharmaceuticals by humans, in addition to municipal, industrial and household sewage discharge (Gaw *et al.*, 2014). Urbanization, along with the growth of informal settlements, poor sanitation and improper disposal, also contribute to increased pharmaceutical pollution in South Africa and a decline in water quality (Gqomfa *et al.*, 2021, Archer, 2017). Furthermore, poorly maintained, malfunctioning and overloaded wastewater treatment plants, along with regular power outages, often exacerbate the problem (Wall, 2021, Potgieter *et al.*, 2019).

In South Africa, research on chemicals of emerging concern in the marine environment is insufficient, particularly in estuarine ecosystems. Moreover, research has centred on the

identification and quantification of chemicals in the environment, but the effects of these chemicals on key marine biota and essential ecosystem processes are neglected. Pharmaceutical concentrations and bioaccumulation in sessile invertebrate species have often been quantified because the limited mobility of these indicator organisms allows scientists to better understand local patterns in contamination and causal drivers (Ruhi *et al.*, 2016). However, to advance the understanding of emerging pollutant threats at the ecosystem level, research needs to focus on quantifying consequences for ecosystem functions performed by key species so that potential changes to ecosystem functioning can be assessed. To better understand the quantities and consequences of pharmaceuticals in an estuarine environment in the Western Cape of South Africa, a field study and an *ex-situ* experiment were conducted. Firstly, the field study was used to determine the concentration of pharmaceuticals in representative sites in the Zandvlei Estuary. Secondly, the experiment was done to determine the impact of a persistent antibiotic, sulfamethoxazole on microalgal assemblages and the water filtration function performed by *Kraussillichirus kraussi*, commonly known as sandprawns.

4.1 Pharmaceuticals in the Zandvlei Estuary

The field study revealed that relatively high concentrations (0.01 ± 0.0022 to 2.53 ± 0.076 µg/L) of pharmaceuticals were present in the Zandvlei Estuary. In contrast to the hypothesis, pharmaceutical concentrations were especially greater in site 5, near the sandprawn biotope. Acetaminophen (analgesic and antipyretic) was the most dominant pharmaceutical across all sites and in the sandprawn tissue samples. This is not surprising considering that acetaminophen is one of the most widely prescribed drugs in both the public and private health sectors in South Africa and can be purchased over the counter without a prescription (Osunmakinde *et al.*, 2013). Other

dominant pharmaceuticals detected in the Zandvlei Estuary were sulfamethoxazole (antibiotic), carbamazepine (anticonvulsant) and diclofenac (non-steroidal anti-inflammatory). These pharmaceuticals along with acetaminophen are also often detected in water bodies worldwide, as they are commonly prescribed (Osunmakinde *et al.*, 2013, Madikizela and Chimuka, 2017a, Moslah, 2018). The pharmaceuticals examined were detected in all sites and at high levels, which raises concern about the pervasive use of these pharmaceuticals and their discharge into the estuary, likely through untreated or partially treated sewage from wastewater treatment plants (WWTPs) (Swartz *et al.*, 2018b, Swartz *et al.*, 2018a).

Particular pharmaceutical concentrations detected in the Zandvlei Estuary water samples were greater compared to other sampled South African estuaries (Table 1) including the Sundays Estuary and the Buffalo Estuary (Ohoro *et al.*, 2021a). For example, sulfamethoxazole was below the limit of detection (LOD) in the Sundays and Buffalo Estuaries in all seasons sampled (Ohoro *et al.*, 2021a) and carbamazepine was below LOD in all seasons but winter. During this season, carbamazepine concentrations were around 50 and 17 times higher in the Sundays and Buffalo estuaries respectively compared to the Zandvlei Estuary (Ohoro *et al.*, 2021a). Similarly, sulfamethoxazole, bezafibrate, diclofenac and acetaminophen concentrations were at least 5 times higher in the Umgeni Estuary than in the Zandvlei Estuary (Agunbiade and Moodley, 2014, Sigonya *et al.*, 2022). Furthermore, in winter, the Warner Beach Estuary had levels of diclofenac that were around 30 times greater than values recorded in the Zandvlei Estuary (Sigonya *et al.*, 2022). In contrast, pharmaceutical levels detected in the Zandvlei Estuary were greater than in the Tejo Estuary, Portugal (Reis-Santos *et al.*, 2018), Jiulong River Estuary, China (Sun *et al.*, 2016), the Long Island Sound Estuary, USA (Lara-Martin *et al.*, 2014) and the Sydney Estuary, Australia

(Birch *et al.*, 2015). For example, the concentrations of acetaminophen were over 100 times higher and the concentrations of sulfamethoxazole, bezafibrate and carbamazepine were at least 5 times higher in the Zandvlei Estuary compared to the above mentioned international estuaries (Reis-Santos *et al.*, 2018, Sun *et al.*, 2016, Lara-Martin *et al.*, 2014, Birch *et al.*, 2015). However, the concentrations of nevirapine were at least 100 times lower in the Zandvlei Estuary than in Gazi Bay (coastal lagoon), Kenya (Wanjeri *et al.*, 2023).

Importantly, pharmaceutical concentrations in the Zandvlei Estuary were considerably higher compared to False Bay (Ojemaye and Petrik, 2021a), into which the estuary discharges under open-mouth states. In False Bay across all sampling sites, the highest average concentrations of diclofenac, acetaminophen, sulfamethoxazole and carbamazepine were 3.70 ng/L, 4.79 ng/L, 1.88 ng/L and 1.56 ng/L respectively (Ojemaye and Petrik, 2021a), whereas the highest average concentrations in the Zandvlei Estuary of the diclofenac, acetaminophen, sulfamethoxazole and carbamazepine were 40 ng/L, 2530 ng/L, 137 ng/L and 157 ng/L respectively[2]. The composition of pharmaceuticals was however similar between the Zandvlei Estuary and False Bay, as the previously stated pharmaceuticals were detected in both systems (Ojemaye and Petrik, 2021a). Multiple factors can influence the concentration of pharmaceuticals in aquatic environments, including the sizes of the human population and urban area (Zhou *et al.*, 2011, Jiang *et al.*, 2014), dilution, mixing and residence times in receiving water bodies (Comeau *et al.*, 2008) and the distance from sewage outfalls (Gulkowska *et al.*, 2007). Given that the ocean plays a considerable role in the dilution of persistent chemicals (Ojemaye *et al.*, 2023), the near 100-fold increase in pharmaceutical concentrations of carbamazepine, sulfamethoxazole and acetaminophen in the

[2] values for the Zandvlei Estuary have been converted from μg/L to ng/L to facilitate comparisons with False Bay

Zandvlei Estuary relative to False Bay can be attributed to dilution in False Bay and the higher residence time in the estuary (Fonseca *et al*., 2020).

After sample collection, I learnt through residents and the reserve manager that a sewage spill had occurred before and during sampling. Thus, the high levels of pharmaceuticals recorded within the estuary during my sampling may be a consequence of the above-mentioned spill. Sewage spills due to power outages affecting infrastructure and equipment at the sewage pump station near the mouth of the Zandvlei Estuary are a likely explanation for the high levels of pharmaceuticals detected in this study, especially at site 5. In August 2021, the City of Cape Town measured and reported high levels of *Escherichia coli* in the Zandvlei Estuary, with values of 430000 and 106000 cfu/100ml in the north and main body of the vlei respectively, which further suggests a link between sewage spills and high pharmaceutical concentrations recorded in the estuary (Human, 2021, CCT, 2019, Government, 2018). It is important to mention that during a sewage spill, the Zandvlei Estuary mouth may be opened to allow for flushing into False Bay, but mouth opening may not be immediate and only occur up to a week after the spill has been identified.

In this present study, pharmaceutical concentrations were quantified in sandprawn tissue to understand bioaccumulation in these organisms. More broadly, sandprawns are widely distributed in southern Africa, from Mozambique on the east coast to Namibia on the West, where they are abundant in estuaries and sheltered bays (Branch *et al.*, 2017). The ubiquity and wide distribution of sandprawns are traits that potentially make them suitable indicator species with regard to understanding patterns of pharmaceutical pollution within southern African estuaries and coastal environments. Moreover, sandprawns are key benthic ecosystem engineers, but their potential to

mitigate eutrophication through their newly discovered biofiltration ability and thereby mediate bentho-pelagic coupling (Venter *et al.*, 2020), implies a need to understand bioaccumulation of pharmaceuticals in these engineers and consequences for functions they provide. In comparison to the water samples, pharmaceutical concentrations detected in sandprawn tissue samples were substantially greater than expected. For example, the average concentration of acetaminophen in the sandprawns from site 5 was around 4.5 times greater than that in the estuarine water from site 5, indicating that pharmaceuticals were accumulating in the sandprawns.

In the present study, field-derived estimates indicated that nevirapine (non-nucleoside reverse transcriptase inhibitors), carbamazepine and sulfamethoxazole were bioaccumulative compounds in sandprawns (BAF > 5000L/kg), whereas acetaminophen was potentially bioaccumulative (BAF = 2000-5000 L/kg). The bioaccumulation of compounds is influenced by their chemical properties including water solubility and the octanol/water partition coefficient (log K_{ow}). Regarding the latter, compounds with a log K_{ow} > 1 are more likely to accumulate in organisms (Kümmerer, 2009). In my study, all the detected pharmaceuticals have a log K_{ow} >1, which supports the findings that nevirapine, carbamazepine, sulfamethoxazole and acetaminophen were accumulating in sandprawns. Average field-derived bioaccumulation factors (BAFs) of 208 and 0.6 were reported for carbamazepine in the ribbed mussel (*Geukensia demissa*; (Klosterhaus *et al.*, 2013) and in freshwater snails (*Planorbid* spp.) respectively (Du *et al.*, 2015). Wilkinson *et al*., (2018) reported BAF values for acetaminophen of 37.04 in the freshwater snail, *Bithynia tentaculata* and 26.4 in the amphipod, *Gammarus pulex*. In contrast, Ojemaye and Petrik (2021) reported BAF ranges[3] for carbamazepine of 18029 to 43479, sulfamethoxazole of 28817 to 110525 and acetaminophen of

[3] Range reported in Ojemaye and Petrik (2021) and not averages.

23327 to 117 451 in the sea snail, *Oxystele* spp. Thus, apart from Ojemaye and Petrik (2021), the BAFs for acetaminophen and carbamazepine reported in the present study for sandprawns (Table X) are around 100 and 200 times greater respectively than the other previously mentioned studies.

Similar to the invertebrates investigated in False Bay, sandprawns in the Zandvlei Estuary were bioaccumulating pharmaceuticals, however, the BAF values recorded in False Bay were greater than in the Zandvlei Estuary. The difference in BAFs between the bay and the estuary is likely a product of multiple interacting processes. Firstly, pharmaceutical uptake can be influenced by species-specific traits such as lifestyle and trophic positions (Meredith-Williams *et al.*, 2012, Grabicová *et al.*, 2022). The invertebrates used by Ojemaye and Petrik (2021) to assess BAFs were sedentary and epibenthic rock or sediment dwellers, whereas the sandprawns used in my study are comparatively more mobile and burrow into the sediment, which may provide a buffer from high levels of pharmaceuticals in the water (Pillay *et al.*, 2007a). Secondly, the physico-chemical properties of the water, including pH and temperature can influence the pharmaceutical uptake and bioaccumulation in aquatic organisms (Chen *et al.*, 2017a, Chen *et al.*, 2017b). For example, the bioaccumulation of acidic pharmaceuticals (such as sulfamethoxazole) in aquatic organisms increased with a decrease in pH as metabolism slows with acidification in the aquatic environments (Serra-Compte *et al.*, 2018, Kroeker *et al.*, 2014, Kroeker *et al.*, 2010, Bethke *et al.*, 2023). Additionally, Serra-Compte *et al*., (2018) reported a decrease in bioaccumulation with an increase in temperature. This outcome was due to temperature increasing metabolism and resulted in lower bioaccumulation (Bethke *et al.*, 2023). In the case of sulfamethoxazole conversely, an increase in metabolism in aquatic organisms can stimulate pharmaceutical adsorption and bioaccumulation (Heugens *et al.*, 2003, Serra-Compte *et al.*, 2018). High bioaccumulation of pharmaceuticals by

marine invertebrates in False Bay is noteworthy given the relatively lower concentration of pharmaceuticals in the seawater compared to the invertebrates. Therefore, it must be recognized that high levels of pharmaceuticals can be present and accumulate in biota even when levels in the surrounding estuarine or marine environment (water) are low, as seen in False Bay, which is likely due to the dilution of contaminants (Ojemaye and Petrik, 2021a, Ojemaye *et al.*, 2023).

In the Zandvlei Estuary, apart from chl-*a* biomass, pelagic physico-chemical variables did not differ meaningfully between sampling sites, though depth and salinity did display a degree of inter-site variability. Chl-*a* biomass was considerably greater at site 1 (81.6 µg/L) compared to site 5 (20.7 µg/L), which is consistent with the pattern described by Venter *et al*., (2020), with sites in the upper reaches being eutrophic relative to sites closer to the mouth of the estuary. Noteworthy though, is that although the spatial chl-*a* pattern I recorded is consistent with that of Venter *et al*., (2019), chl-*a* values measured in site 5 (near the sandprawn biotope close to the mouth) in my study are almost double that reported by Venter *et al*., (2020), who reported values from 2016 to 2019. Additionally, Harding (1994) reported mean chl-*a* biomass from 1978 to 1991 of 11 to 16 ug/L near the sandprawn biotope, which is also lower than recorded in my study. Overall, the greater chl-*a* levels in my study for site 5 relative to those of Venter *et al*., (2020) and Harding, (1994) suggest an increase in phytoplankton biomass in the lower reaches in recent years, though this suggestion needs to be interpreted with caution since I collected a single temporal replicate. Nevertheless, The the higher chl-*a* biomass in site 5 of the Zandvlei Estuary is worth flagging from a management perspective and may be explained by various changes that have occurred in the system. Zandvlei Estuary was subject to extensive dredging periodically from the 1940s to the 1970s and continued until 2001 when dredging was paused (Harding, 1994, Goverment, 2018,

Lemley *et al.*, 2019). However, dredging was re-introduced due to the high sedimentation rates and the formation of sandflats near the mouth, which is in close proximity to the sandprawn biotope (Goverment, 2018, Lemley *et al.*, 2019). Thus, dredging was re-instituted to increase the flow and flushing of the system into False Bay (Goverment, 2018). Importantly, dredging of shallow estuaries has a severe impact on benthic microalgae and fauna, as the physical removal of sediment can negatively impact benthic fauna establishment and recruitment (Ray, 2005). Periodic dredging near the mouth region and sandprawn biotope may partially explain the increase in chl-*a* biomass near the sandprawn biotope (site 5) in the Zandvlei Estuary during this study. Sediment removal via dredging inevitably leads to sandprawn removal and/or sediment disturbance. This is very likely to reduce sandprawn population sizes, density and/or physiology. (Venter *et al.*, 2020) showed experimentally that increasing sandprawn density was associated with a near 50 % reduction in phytoplankton biomass, likely due to phytoplankton cells being adsorbed onto burrow walls and consumed during bi-directional water pumping. Thus, dredging-induced reductions in sandprawn population sizes, density or physiological performance may have resulted in compromised water filtration, and hence phytoplankton proliferation. This is an issue that needs management attention, given that sandprawn water filtration can be an important nature-based process that can limit phytoplankton proliferation (Venter *et al.*, 2020).

Additionally, sewage spills have become more frequent in the Zandvlei Estuary due to a notable rise in load shedding in the last seven years (Fig. 8) and malfunctioning sewage pump stations in the Zandvlei Estuary catchment area (CCT, 2023). Loadshedding in South Africa refers to scheduled and strategic power blackouts by Eskom, the national electricity provider. Load shedding is implemented to prevent grid collapse caused by electricity shortages. Although sewage

is not directly discharged into the Zandvlei Estuary catchment from WWTPs, during load shedding, sewage pump stations may trip or not have enough power to operate, resulting in an overflow of sewage into stormwater drains and into the estuary (CCT, 2023). The indirect discharge of sewage into the Zandvlei Estuary can elevate nutrient loading and consequently induce eutrophication and phytoplankton blooms (Adams *et al.*, 2020, Khangaonkar and Yun, 2023). Moreover, both untreated and treated sewage often contain emerging containments including pharmaceuticals that can impact the diversity, behaviour and activity of functionally important biota, as pharmaceutical compounds are biologically active and designed to work at low concentrations (Shan *et al.*, 2021, Santos *et al.*, 2010). The high levels of pharmaceuticals recorded in this study, especially site 5, which was the most polluted, may reflect an increasing trend of sewage spills and load shedding over the last seven years. Additionally, this high pharmaceutical load I recorded may compromise sandprawn biofiltration, leading to greater phytoplankton biomass than was recorded by Venter *et al*., (2020), when national load shedding was significantly reduced[4] (Fig. 8). The data required to conclusively validate the processes described are limited but these processes are worth flagging for management consideration. Reduced sandprawn filtration and increased dredging, nutrient loading and pharmaceutical inputs into the Zandvlei Estuary are likely part of a complex, multi-stressor environment that can facilitate phytoplankton proliferation in the long term.

[4] The experiment of Venter *et al*., (2020) was conducted in 2018.

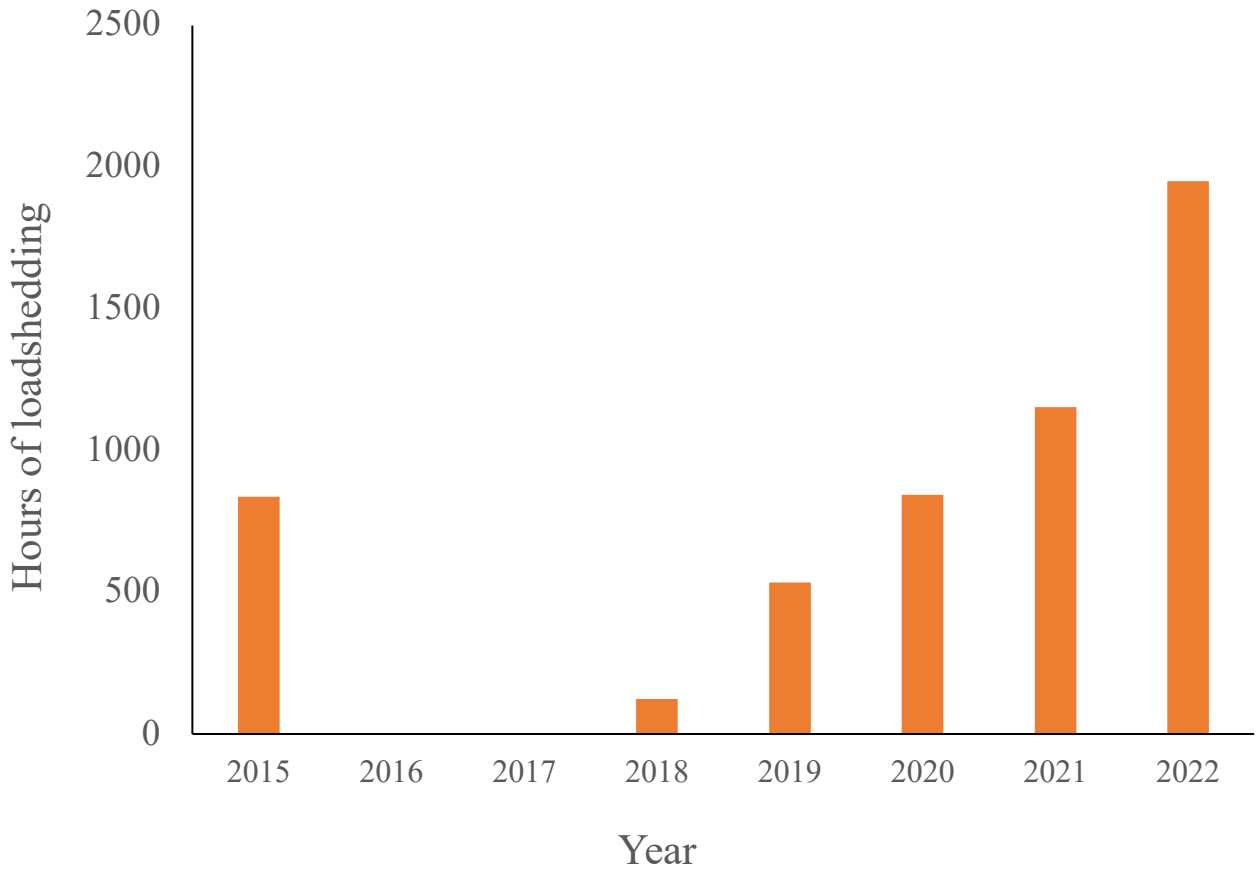


Figure 8: Total hours of national loadshedding experienced in South Africa per year from 2015 to 2022 (BusinessTech, 2022).

4.2 The effect of sandprawn density and sulfamethoxazole on phytoplankton

The main finding emanating from the 24-day *ex-situ* experiment was that total pelagic chl-*a* biomass declined with increasing concentrations of sulfamethoxazole (SMX) in mesocosms which was consistent with the hypothesis. This is a novel and important outcome of this research since few studies have linked SMX to microalgal productivity, whereas much more is known regarding its effects on bacteria. This finding is consistent with that reported by Teixeira and Granek (2017) and Ferrari (2004), where marine microalgal growth rates declined over time when exposed to environmentally relevant concentrations[5] of SMX. In my study is difficult to elucidate the mechanisms by which increasing SMX levels drove chl-*a* biomass declines. However, this result is likely underpinned by the complex relationship between marine microalgae and bacteria that is essential for their survival (Guo *et al.*, 2015, Liu *et al.*, 2012).

More specifically, studies have suggested that a decrease in phytoplankton biomass may be due to antibiotics indirectly affecting vitamin availability and the nutrient supply from bacteria to marine algae through alteration/inhibition of bacterial structure and activity (Croft, 2005, Guo *et al.*, 2015, Teixeira and Granek, 2017). For example, Croft *et al*., (2005) demonstrated that auxotrophic phytoplankton such as diatoms acquire B12 from bacteria in return for fixed carbon. Therefore, antibiotics in the environment likely negatively impact bacteria and thereby secondarily affect certain microalgal species that are reliant on them. Moreover, it was demonstrated that certain mutualistic bacteria supply low molecular organic carbon and dissolved inorganic carbon to microalgae in exchange for fixed organic carbon (Cho *et al.*, 2015). Additionally, antibiotics such as SMX can affect some algal organelles, mainly chloroplasts, since they have a similar structure

[5] pharmaceutical concentrations that are similar to those quantified in the environment.

and cellular processes to bacteria in terms of ribosomal proteins and translation (Guo *et al.*, 2015, Brain *et al.*, 2008). Thus, while microalgae are not the target organisms of antibiotics such as SMX, microalgae may be affected by their presence through indirect effects on bacteria (*via* resource provision) or direct effects on organelles that are structurally similar to those of bacteria.

Another important finding arising from this study was that the phytoplankton assemblage was differentially affected by SMX concentration. Specifically, microplankton and picoplankton were both significantly and negatively impacted by SMX concentration whereas, nanoplankton was not. These results are similar to those of Shan *et al*., (2021), who reported that increasing SMX concentration led to a change in dominant phytoplankton species, with cyanobacteria increasing dominance, resulting subsequently in a phytoplankton community structure shift. Specific cyanobacterial species are among the nanoplankton size class and SMX is an antibiotic that is known to have less cytotoxicity to specific cyanobacterial species (Hu and Wei, 2006, Reynolds, 2006, Shan *et al.*, 2021). For instance, when concentrations of SMX were not fatal to *Microcystis aeruginosa* (a common harmful cyanobacteria species present in South African estuaries), SMX concentrations of 50-125 ug/L enhanced photosynthesis and metabolic activity, which subsequently increased overall phytoplankton biomass (Zhang *et al.*, 2020, Harding and Paxton, 2001). In a study by Xu *et al.*, (2021), SMX indirectly facilitated the growth of certain cyanobacteria species including various *Microcystis* species, likely due to the SMX enhancing the availability of nutrients to these cyanobacteria species. Additionally, the dominance and growth promotion of *Microcystis* spp. was also due to the inhibition of other cyanobacterial taxa by SMX (Xu *et al.*, 2021). However, the responses of nanophytoplankton to SMX in my experiment could also reflect other taxonomic or biological trait differences (Gama *et al.*, 2005).

Historically Cyanophyceae have been shown to form harmful algal blooms (HABs) in the South African estuaries, with *Microcystis* and *Anabaena* spp. being common cyanobacterial components in algal blooms, which in turn adversely affects ecological functioning (Harding, 2000, Harding and Paxton, 2001, Lemley *et al.*, 2019). Importantly, a notable increase in Cyanophyceae (*Microcystis aeruginosa*) was recently documented in the Zandvlei Estuary (Lemley *et al.*, 2019). Microalgal species identification was not carried out in my study due to time limitations, but if cyanobacteria within the nanoplankton size class were not negatively affected by SMX concentrations, potential shifts to cyanobacterial dominance are cause for concern, necessitating further research/quantification in future. This is additionally relevant given previous research showing that increasing SMX concentrations can accelerate cyanobacterial blooms (Xu *et al.*, 2021).

Phytoplankton are essential components of marine ecosystems because they provide the trophic resources on which almost all ocean life depends (Benoiston *et al.*, 2017, Bowler *et al.*, 2010). Phytoplankton generate more than 45% of Earth's annual net primary production, yet only constitute around 1% of photosynthetic biomass (Field *et al.*, 1998, Katz *et al.*, 2004). Phytoplankton make up the base of the food web and therefore play a critical role in trophic interactions, specifically mediated by phytoplankton size and community structure (Froneman, 2006). Phytoplankton cell size influences nutrient uptake, phytoplankton diversity, metabolism, and critical biogeochemical processes and directly relates to food web structure and dynamics (Jiang *et al.*, 2019, Litchmean *et al.*, 2007, Finkel *et al.*, 2010, Marañón, 2015). For example, the dominance of larger phytoplankton (microplankton) is often associated with shorter and simpler

herbivore food webs (Guenther *et al.*, 2008, Jiang *et al.*, 2019). Similarly, the dominance of smaller phytoplankton (nanoplankton and picoplankton) is associated with more complex microbial food webs (Guenther *et al.*, 2008). Thus, phytoplankton size profoundly influences local food web structure and carbon flow through aquatic ecosystems (Jiang *et al.*, 2019). In my experiment, declines in micro- and picoplankton biomass with increasing SMX concentrations together with neutral changes to nanoplankton may generate shifts in the estuarine food web structure (White, 1978) that we do not yet understand.

Although pelagic chlorophyll biomass was significantly and negatively influenced by increasing SMX concentration in my mesocosm experiment, benthic diatom and cyanobacteria biomass were not. This differential response between pelagic and benthic chl-*a* in response to SMX could be due to different traits among benthic and pelagic microalgae. For example, benthic microalgae are known to secrete extrapolymeric substances (EPSs) that provide protection to the algal cell walls from the external environment (Pan, 2010, Christopher *et al.*, 2012). More specifically, Christopher *et al*., (2020) demonstrated that EPSs reduce cell damage in benthic microalgae when exposed to harmful substances such as heavy metals. The protection of benthic microalgae by EPS in my study is a possible reason that SMX did not negatively influence benthic microalgae to the same extent as pelagic microalgae. It is also possible that with greater exposure time during the experiment, SMX effects on benthic diatoms at least may have become evident. This is because visual trends indicated an average decline in diatom biomass with increasing SMX concentration and increasing sandprawn density, which if this experiment was prolonged would have been amplified. Nevertheless, the data are suggestive of greater diatom tolerance to SMX than pelagic phytoplankton. It is important to note that sandprawn density statistically impacted benthic

cyanobacteria biomass. However, this outcome was marginal, with trends for cyanobacteria biomass with increasing sandprawn density being obscure.

During my experiment, pelagic nitrite concentration was statistically influenced by SMX levels, but patterns were obscure, making it difficult to discern whether variance in nitrite concentrations was causally linked to SMX or other stochastic events. Previous research has however suggested that increasing SMX concentration can inhibit denitrification, thus leading to an accumulation of nitrite in the pelagic environment (Xu *et al.*, 2020, Chen *et al.*, 2021). This outcome was attributed to SMX diminishing the nitrite reducing ability of microbes and altering the microbial community composition (Underwood et al., 2011). Moreover, pelagic phosphate concentration variability was linked statistically to sandprawn density during the experiment. Although previous research has suggested that the density of certain callianassid species significantly increases pelagic phosphate concentrations (Waslenchuk *et al.*, 1983), variability in phosphate levels in my experiment across sandprawn treatments was observed, making it difficult to mechanistically link sandprawn density to phosphate levels. Similarly, variations in pelagic temperature, salinity, dissolved oxygen and pH were significantly affected by either sandprawn density, SMX presence or their interaction. However, variation for these variables throughout the experiment was minimal, suggesting that changes that occurred were unlikely to be of biological significance.

In contrast to recent studies conducted by Venter *et al*., (2020) and De Cerff (2022), sandprawn density in this study did not affect total chlorophyll biomass in the water column during my study. Venter *et al*., (2020) reported almost a 50% decline in chl-*a* biomass over an 11-day period and similarly, over 18 days, De Cerff (2022) recorded a decline in total phytoplankton where

abundance in the control treatments was 1.9 times higher than maximum density treatments. This decline in phytoplankton biomass was attributed to sandprawn biofiltration, in which pelagic phytoplankton cells are adsorbed onto burrow walls during bi-directional water pumping by sandprawns. This mechanism was supported by chl-*a* levels being greater along burrow walls than surface sediment, and that sandprawns are not classified as filter-feeders (Venter *et al.*, 2020, De Cerff, 2022, Branch *et al.*, 2017). In contrast to the hypothesis and past studies, biofiltration in the form of phytoplankton biomass reduction by sandprawns was absent in my study. Interestingly, sandprawn bioturbation activity was seemingly unhindered since sediment reflectance (based on BenthoTorch data) generally increased with sandprawn density, indicating density-dependent increases in turned-over sediment. Similarly, suspended solids in the water column increased with sandprawn density, suggesting that sandprawns were mobilizing sediment from the benthos into the pelagic environment. Lastly, observations through the glass mesocosms during the experiment indicated that bioturbation by sandprawns was occurring based on increased sediment rugosity with sandprawn density.

Early research on the diet of sandprawns using dietary markers indicated that fine suspended particulate organic matter was the main trophic resource of sandprawns in the Gamtoos Estuary (Schlacher and Wooldridge, 1996). Subsequent work using a coupled stable-isotope/fatty acid approach indicated that sandprawns utilize marine particulate organic matter as a primary source of food throughout the year and may switch to benthic microalgae during winter months when pelagic phytoplankton production is low (Antonio and Richoux, 2014). The findings of Antonio and Richoux (2014) are consistent with the optimal foraging model, whereby deposit feeders ingest the smallest particles available first as it is more beneficial in terms of energy expenditure (Taghon

et al., 1978, Stamhuis *et al.*, 1998). During material collection in the experiment, high sediment organic matter content was observed *in situ*, presumably due to the frequent and ongoing sewage spills recorded in the Zandvlei Estuary; sewage spills in aquatic environments are often associated with an increase in organic matter content (Bhat and Qayoom, 2021). During this experiment, it is probable that sandprawns were not filtering phytoplankton due to the high observed organic matter content in the water, thus leading to reduced biofiltration. However, it is also important to note that the water used during this experiment was collected from site 5, which based on my field sampling, was the most polluted, containing the highest levels of pharmaceuticals in the estuary. Thus, it is possible that high concentrations of pharmaceuticals (other than SMX used in my experiment) may have impaired biofiltration by sandprawns.

4.3 Synthesis and conclusion

This study has provided novel research on the quantities and potential impacts of pharmaceutical pollution in an urban estuarine environment. However, findings that have emanated need to be interpreted in the context of study limitations so that liberal overextrapolation is avoided. For example, sampling for the field component entailing pharmaceutical concentration quantification in the Zandvlei Estuary was a once off event and therefore, does not consider seasonal/temporal variation. Hence, seasonal sampling is required to better approximate *in situ* variability in pharmaceutical levels. It is important to note that during this study, seasonal sampling was not feasible due to the high costs of chemical analysis. This in turn highlights the need for more funding to be invested in research on pharmaceutical pollution, specifically due to the high concentrations detected in this study and the lack of research on this topic in South Africa. Regarding the experimental component of my study, it is important to recognize that temporal and

spatial limitations are part of manipulative *ex-situ* experiments, in addition to the limited ability to extrapolate to natural conditions. Nonetheless, to quantify causal interactive and individual effects of sulfamethoxazole and sandprawn density on microalgal biomass, a mesocosm experiment was essential to eliminate confounding factors that are highly prominent in heterogeneous ecosystems such as estuaries. Lastly, this study focused on a local estuarine ecosystem and therefore organisms, sediment and water used for the experiment were collected from the Zandvlei Estuary. However, the implication is that the organisms used in the experiment may have been exposed to high concentrations of pharmaceuticals over the last few years and may have adopted compensatory behaviours in response. Therefore, extrapolation of the findings of this study across marine ecosystems needs to be done with caution, but findings are nevertheless of value in providing insight into how small, polluted urban estuaries may be affected by pharmaceutical pollution and how key biotic components may respond.

The action of multiple stressors, particularly in coastal environments, can generate strong synergistic effects on ecological processes, and it is therefore critical to understand interactions between chemical pollution and other stressors such as nutrient enrichment on ecosystem functioning. Unfortunately, research on the impacts of pharmaceuticals on marine biota is severely lacking, specifically in the context of key species that influence ecosystem functionality and resilience. In this study, results demonstrated that sandprawns in the Zandvlei Estuary were bioaccumulating pharmaceuticals, however, further research is necessary to quantify the effects of bioaccumulated pharmaceuticals on sandprawn physiological functioning, while additional studies can shed light on pharmaceutical effects on sandprawn burrow microenvironments and burrow microbial community structure, given that these structures are key determinants of benthic

ecosystem functioning. This is especially relevant given that sandprawns are key ecosystem engineers and have a wide distribution in South Africa and past research on their biofiltration function (Pillay *et al.*, 2007b, Branch *et al.*, 2017, Venter *et al.*, 2020).

The presence of antibiotics, antibiotic resistant genes (ARGs) and antibiotic resistant bacteria (ARBs) in the environment is of great concern with regard to microbial communities, marine organisms and human health (Taylor *et al.*, 2011, Zheng *et al.*, 2021). However, there is limited research available on the prevalence and consequences of ARGs and ARBs on marine and estuarine ecosystems, especially in southern Africa. ARGs are commonly detected in coastal environments and can negatively impact microbial diversity and biogeochemical cycles by altering bacterial community structure (Zheng *et al.*, 2021). ARGs can affect humans through direct or indirect contact including swimming in the environment and ingesting seafood potentially reducing the therapeutic potential of medication to humans (Jiang *et al.*, 2014, Harris *et al.*, 2012). Thus, more research is imperative to further the understanding of antibiotic resistance in the environment and its effects on biota, transfer through trophic levels and on human health.

One of the most prominent findings in this study was the decline in total phytoplankton biomass, particularly that of microplankton and picoplankton, with increasing concentrations of SMX. Shifts in phytoplankton size classes can have important ramifications for food web topology and efficiency in the environment (Guenther *et al.*, 2008, Jiang *et al.*, 2019). Such ramifications are worth further investigation in the context of emerging contaminants including pharmaceuticals. Moreover, to supplement the results obtained in this study, the identification of phytoplankton

species can shed more light on changes in phytoplankton community structure induced by pharmaceuticals.

These ideas are particularly relevant in expanding knowledge of emerging mechanisms by which global change effects manifest in coastal ecosystems. This research has highlighted the role of SMX in reducing microalgal biomass but with differential size-based effects. This study has also demonstrated the potential for small temporary-open closed urban estuaries such as the Zandvlei Estuary, to be collection sites for chemicals of emerging concern, which may consequently impact resident biota. Overall, these findings demonstrate a need for integrative ecosystem management plans to mitigate sewage spills by the municipalities and estuarine managers. Moreover, early warning systems need to be developed to minimize sewage and pharmaceutical inputs into the Zandvlei Estuary and similar systems. Lastly, public education is necessary to inform residents about the threats of pharmaceutical and sewage pollution but to also motivate authorities to reduce pollution in ecosystems on which people depend.

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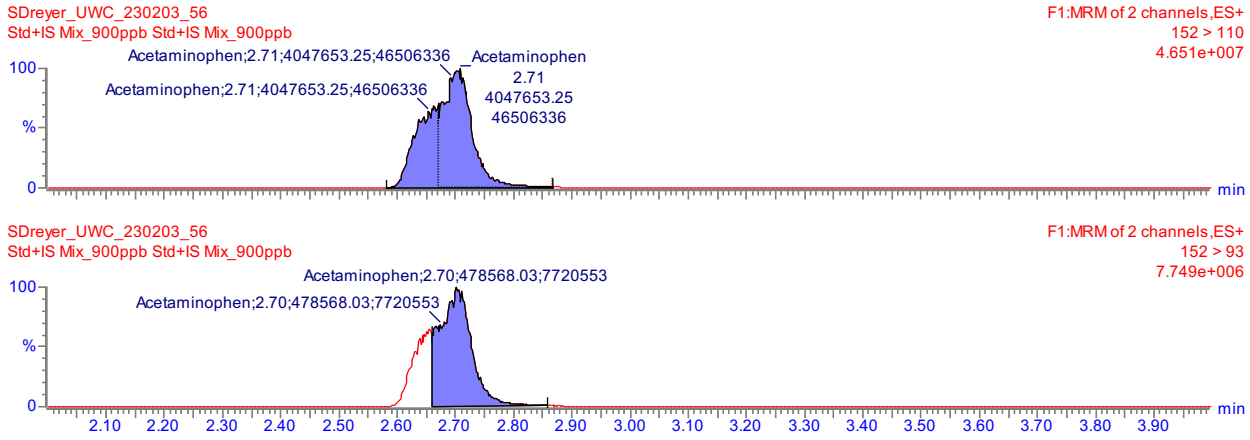
6. APPENDIX

6.1 Appendix A: Field study pharmaceutical results

(a)



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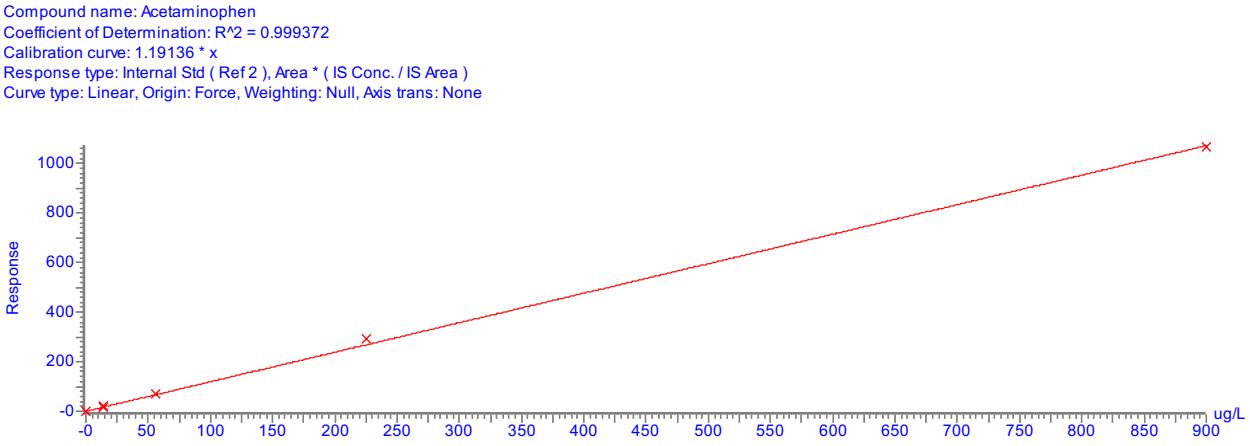
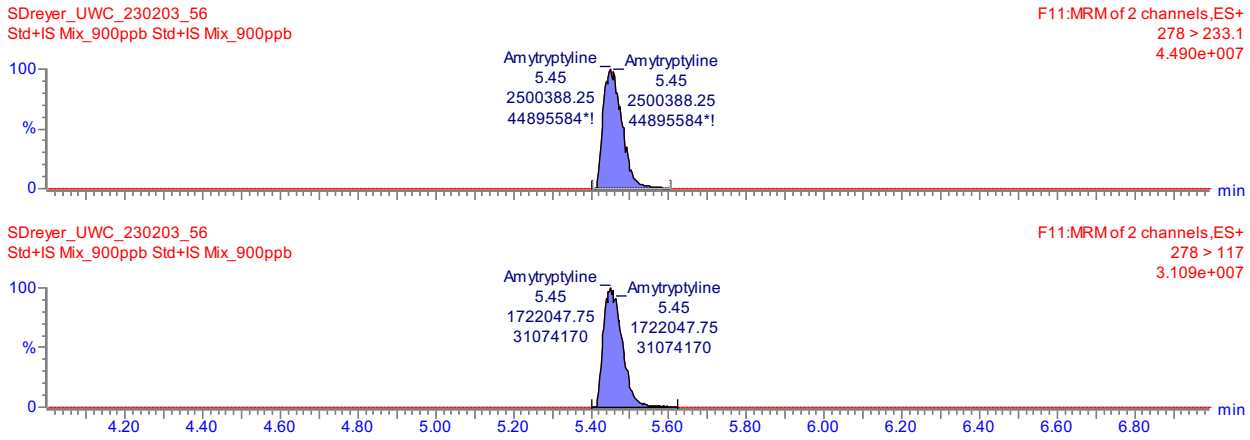


Figure 9: (a) Chromatographs and (b) calibration curve for the compound acetaminophen quantified from the Zandvlei Estuary samples.

(a)



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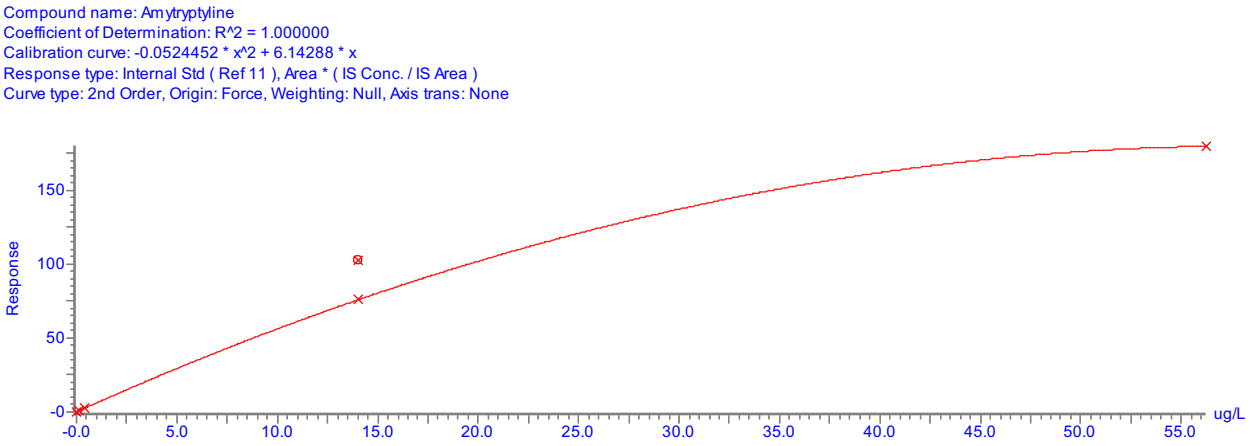
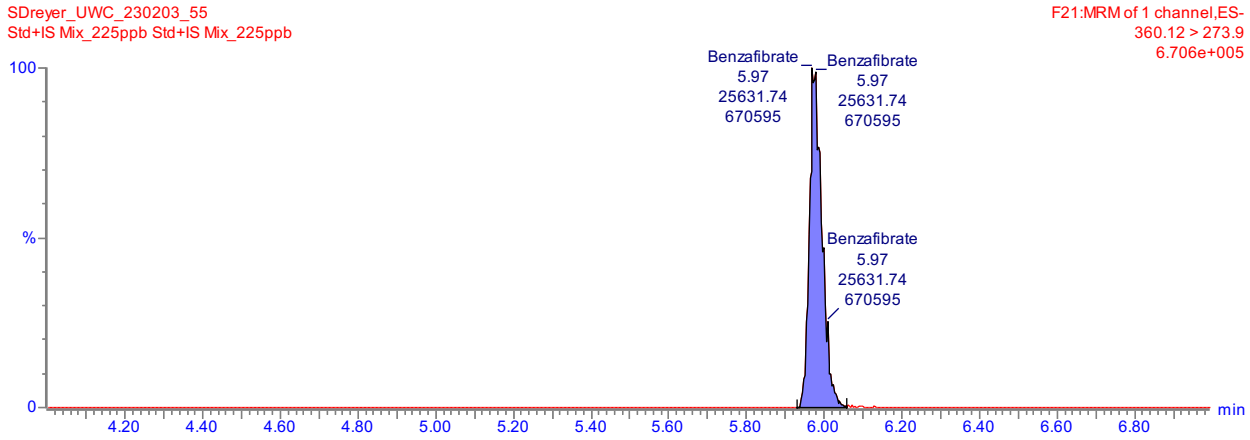


Figure 10: (a) Chromatographs and (b) calibration curve for the compound amytryptyline quantified from the Zandvlei Estuary samples.

(a)



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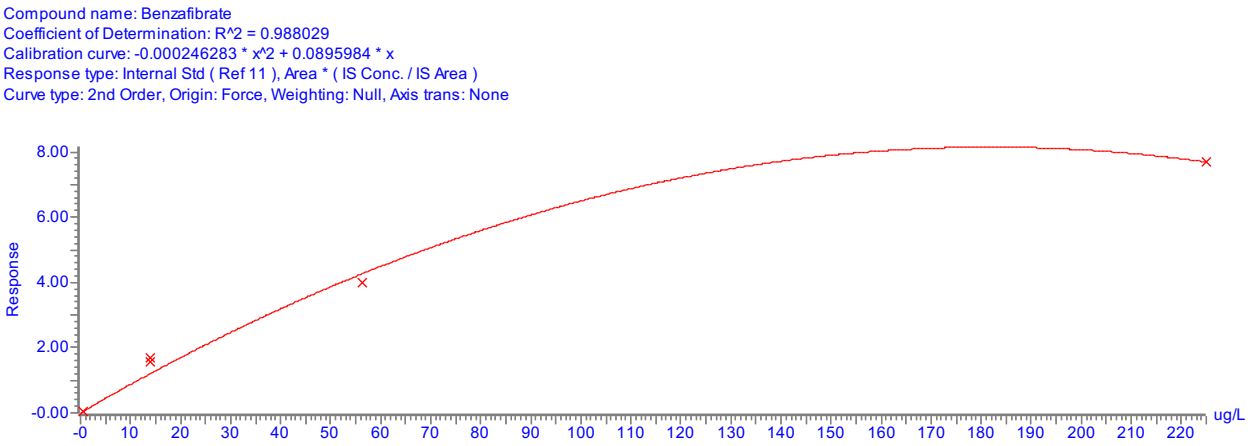
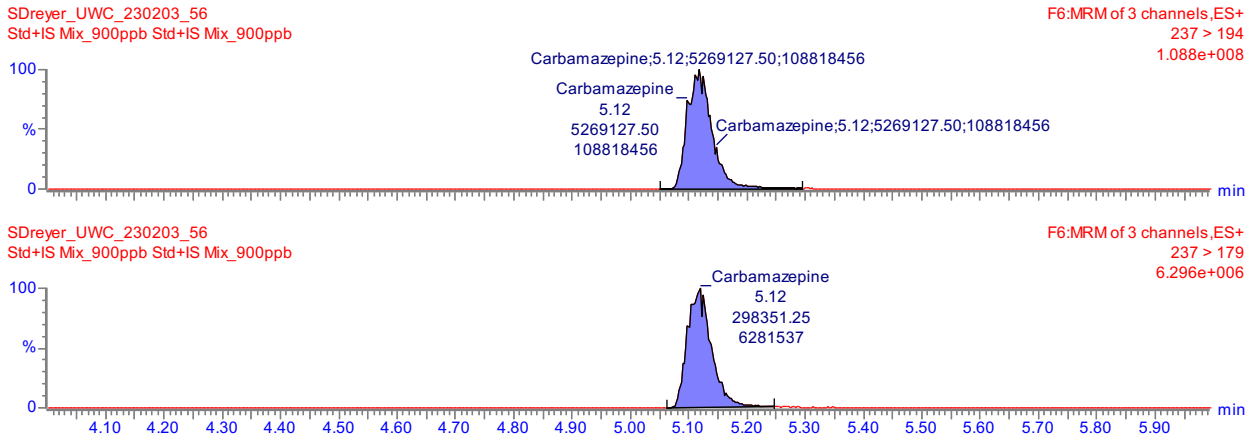


Figure 11: (a) Chromatograph and (b) calibration curve for the compound bezafibrate quantified from the Zandvlei Estuary samples.

(a)



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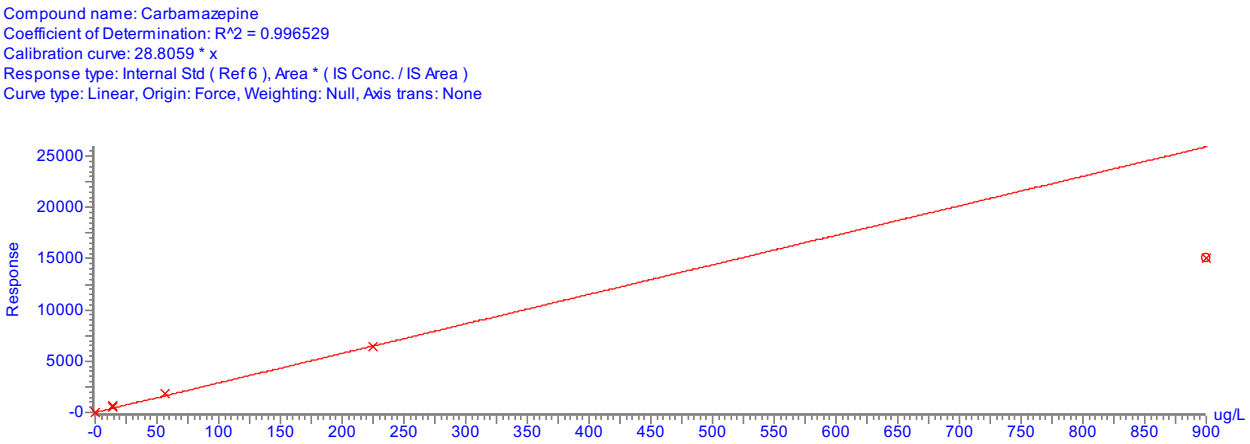
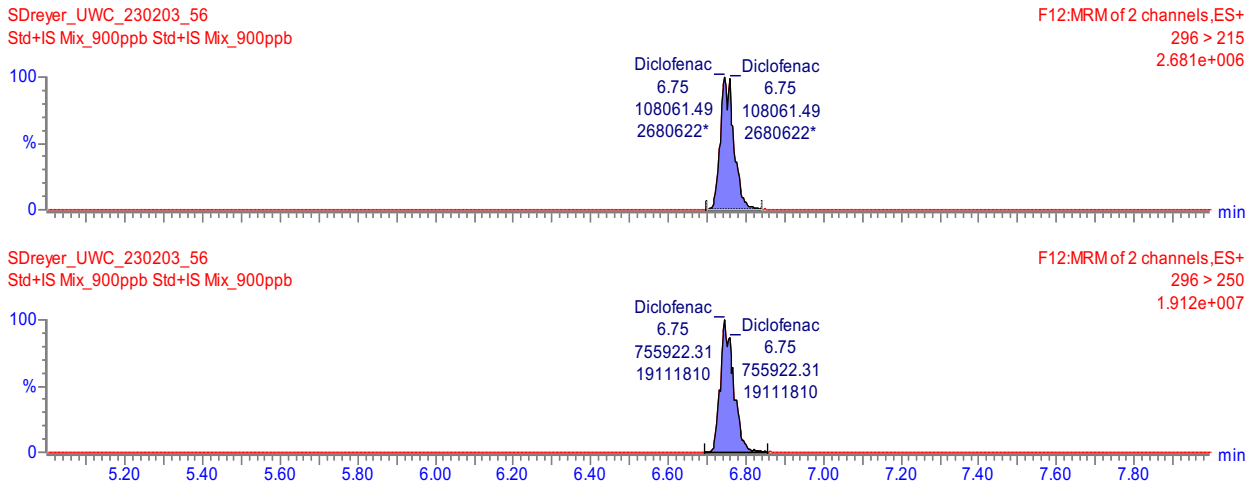


Figure 12: (a) Chromatographs and (b) calibration curve for the compound carbamazepine quantified from the Zandvlei Estuary samples.

(a)



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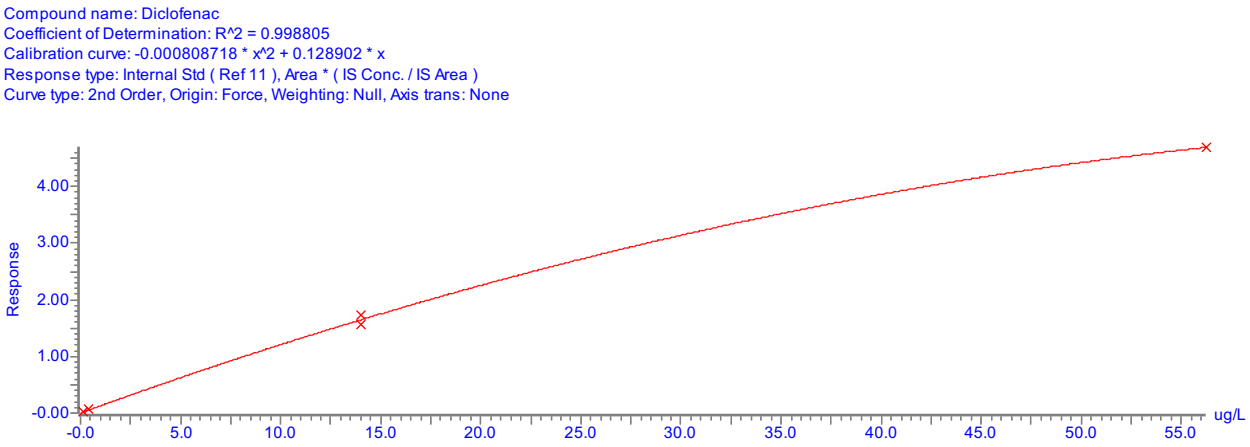
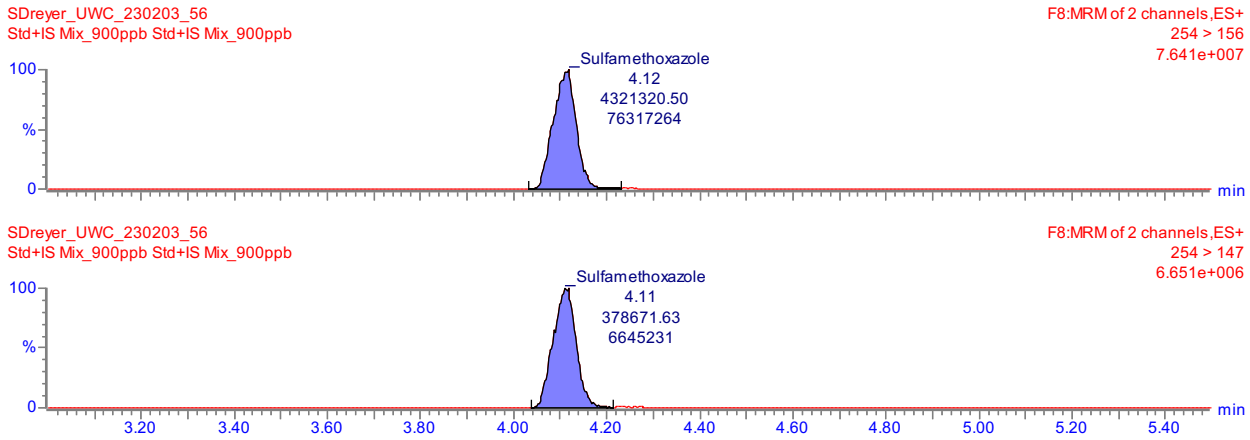


Figure 13: (a) Chromatographs and (b) calibration curve for the compound diclofenac quantified from the Zandvlei Estuary samples.

(a)



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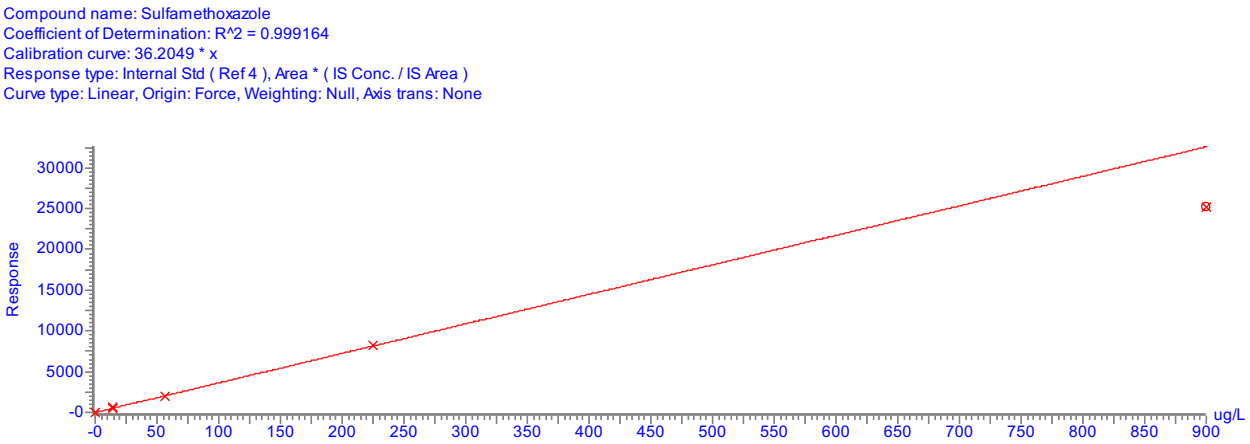
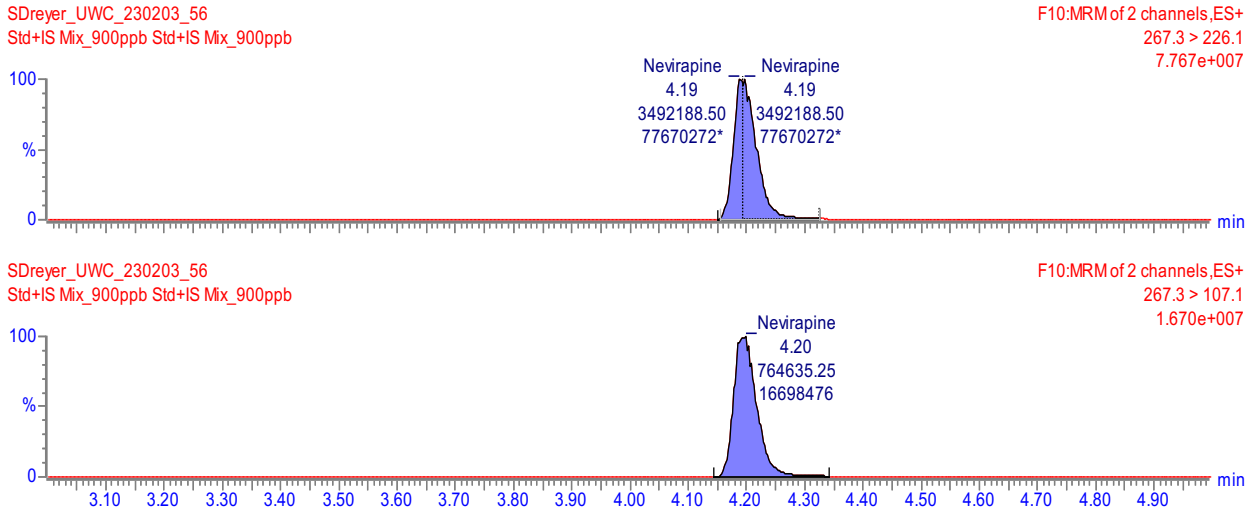


Figure 14: (a) Chromatographs and (b) calibration curve for the compound sulfamethoxazole quantified from the Zandvlei Estuary samples.

(a)



(b)

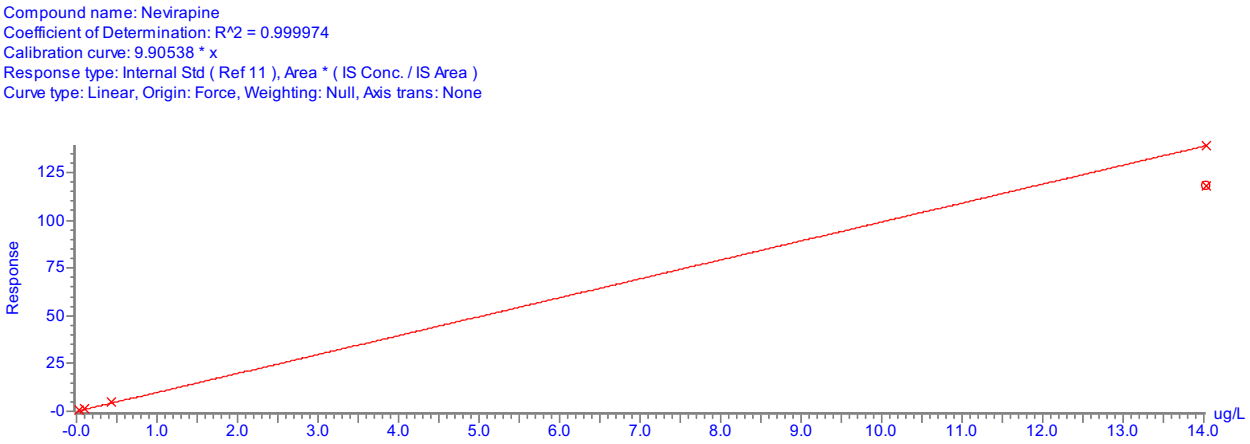


Figure 15: (a) Chromatographs and (b) calibration curve for the compound nevirapine quantified from the Zandvlei Estuary samples.

6.2 Appendix B: Experimental results

Table 15: Spatio-temporal variation in physico-chemical variables (mean ± SE) across SMX and sandprawn density treatments over the 24-day experiment. Sample sizes are shown in square brackets per day of data collection. (Note: value in italics SE < 0.01).

Day	SMX presence	Sandprawn density (%)	Temperature (°C) [3]	Salinity (ppt) [3]	Dissolved oxygen (mg/l) [3]	pH [3]
0	None	0	19.2 ± 0.07	32.1 ± 0.05	7.2 ± 0.02	8.0 ± 0.03
		50	19.2 ± 0.11	32.2 ± 0.02	7.1 ± 0.05	8.0 ± 0.03
		100	19.4 ± 0.08	32.1 ± 0.06	7.1 ± 0.02	8.1 ± 0.01
	Average	0	19.2 ± 0.13	32.1 ± 0.01	7.2 ± 0.03	8.1 ± 0.04
		50	19.3 ± 0.07	32.1 ± 0.01	7.1 ± 0.02	8.0 ± 0.02
		100	19.3 ± 0.09	32.2 ± 0.08	7.1 ± 0.02	8.0 ± 0.04
	High	0	19.2 ± 0.10	32.1 ± 0.06	7.2 ± 0.03	8.1 ± 0.02
		50	19.2 ± 0.08	32.1 ± 0.09	7.1 ± 0.03	8.0 ± 0.02
		100	19.2 ± 0.02	32.1 ± 0.05	7.1 ± 0.04	8.0 ± 0.01
3	None	0	19.7 ± 0.11	31.9 ± 0.06	7.1 ± 0.02	8.2 ± 0.01
		50	19.9 ± 0.08	32.0 ± 0.22	6.9 ± 0.02	8.2 ± 0.04
		100	19.9 ± 0.15	32.0 ± 0.13	7.0 ± 0.04	8.1 ± 0.05
	Average	0	19.8 ± 0.14	32.0 ± 0.15	7.0 ± 0.05	8.2 ± 0.02
		50	19.9 ± 0.03	32.1 ± 0.05	6.9 ± 0.05	8.1 ± 0.05
		100	19.8 ± 0.13	32.4 ± 0.16	7.0 ± 0.03	8.2 ± 0.04
	High	0	19.7 ± 0.01	32.1 ± 0.08	7.0 ± 0.04	8.2 ± 0.02
		50	19.8 ± 0.12	32.0 ± 0.08	6.9 ± 0.03	8.2 ± 0.05
		100	19.9 ± 0.11	31.9 ± 0.06	6.9 ± 0.03	8.1 ± 0.03
6	None	0	15.8 ± 0.14	32.5 ± 0.09	7.6 ± 0.06	8.1 ± 0.06
		50	15.7 ± 0.13	32.4 ± 0.11	7.7 ± 0.04	8.2 ± 0.03
		100	15.6 ± 0.09	32.6 ± 0.16	7.7 ± 0.03	8.1 ± 0.06
	Average	0	15.7 ± 0.10	32.7 ± 0.19	7.7 ± 0.02	8.2 ± 0.03
		50	15.8 ± 0.19	32.5 ± 0.07	7.6 ± 0.04	8.1 ± 0.01
		100	15.8 ± 0.22	32.7 ± 0.12	7.6 ± 0.05	8.1 ± 0.06
	High	0	15.6 ± 0.11	32.7 ± 0.15	7.6 ± 0.04	8.2 ± 0.01
		50	15.6 ± 0.10	32.3 ± 0.24	7.7 ± 0.03	8.1 ± 0.05
		100	15.9 ± 0.12	32.4 ± 0.09	7.7 ± 0.16	8.0 ± 0.13
9	None	0	15.3 ± 0.21	33.2 ± 0.20	7.7 ± 0.03	8.2 ± 0.03
		50	15.3 ± 0.07	32.9 ± 0.08	7.7 ± 0.01	8.2 ± 0.01
		100	15.2 ± 0.15	33.0 ± 0.42	7.9 ± 0.23	8.2 ± 0.04
	Average	0	15.3 ± 0.05	33.2 ± 0.27	7.7 ± 0.02	8.2 ± 0.04
		50	15.4 ± 0.10	32.9 ± 0.17	7.6 ± 0.01	8.1 ± 0.07
		100	15.3 ± 0.10	33.1 ± 0.24	7.6 ± 0.01	8.2 ± 0.07
	High	0	15.1 ± 0.09	33.4 ± 0.02	7.8 ± 0.01	8.2 ± 0.02
		50	15.1 ± 0.13	32.9 ± 0.36	7.7 ± 0.04	8.2 ± 0.02
		100	15.5 ± 0.15	32.7 ± 0.16	7.7 ± 0.05	8.2 ± 0.02

Table 15 continued

Day	SMX presence	Sandprawn density (%)	Temperature (°C) [3]	Salinity (ppt) [3]	Dissolved oxygen (mg/l) [3]	pH [3]
12	None	0	15.0 ± 0.17	33.1 ± 0.29	7.8 ± 0.01	8.2 ± 0.19
		50	15.0 ± 0.14	32.8 ± 0.22	7.8 ± 0.01	8.1 ± 0.03
		100	14.7 ± 0.07	32.9 ± 0.42	7.8 ± 0.01	8.1 ± 0.07
	Average	0	14.9 ± 0.15	33.2 ± 0.09	7.8 ± 0.03	8.2 ± 0.04
		50	15.0 ± 0.17	32.8 ± 0.22	7.7 ± 0.02	8.1 ± 0.07
		100	14.8 ± 0.20	32.9 ± 0.39	7.8 ± 0.03	8.1 ± 0.04
	High	0	14.8 ± 0.12	33.5 ± 0.09	7.8 ± 0.02	8.2 ± 0.04
		50	14.7 ± 0.10	32.5 ± 0.35	7.8 ± 0.01	8.1 ± 0.07
		100	15.0 ± 0.12	32.7 ± 0.30	7.7 ± 0.03	8.0 ± 0.12
15	None	0	13.6 ± 0.10	33.9 ± 0.21	7.5 ± 0.01	8.1 ± 0.11
		50	13.6 ± 0.10	32.6 ± 0.10	7.7 ± 0.13	8.1 ± 0.06
		100	13.5 ± 0.22	33.7 ± 0.57	7.8 ± 0.13	8.3 ± 0.04
	Average	0	13.6 ± 0.18	34.2 ± 0.40	7.8 ± 0.11	8.1 ± 0.10
		50	13.7 ± 0.27	33.9 ± 0.19	7.9 ± 0.07	8.1 ± 0.08
		100	13.6 ± 0.28	33.9 ± 0.33	7.8 ± 0.12	8.1 ± 0.12
	High	0	13.5 ± 0.15	34.4 ± 0.11	7.8 ± 0.12	8.2 ± 0.07
		50	13.8 ± 0.08	33.3 ± 0.40	7.8 ± 0.07	8.2 ± 0.03
		100	13.7 ± 0.10	33.5 ± 0.12	7.7 ± 0.15	7.9 ± 0.20
18	None	0	18.5 ± 0.14	33.6 ± 0.15	7.3 ± 0.03	8.1 ± 0.07
		50	18.2 ± 0.06	33.3 ± 0.08	7.4 ± *0.01*	8.1 ± 0.05
		100	18.4 ± 0.08	33.5 ± 0.54	7.4 ± 0.04	8.2 ± 0.07
	Average	0	18.3 ± 0.02	33.8 ± 0.15	7.4 ± 0.03	8.2 ± 0.05
		50	18.3 ± 0.13	33.2 ± 0.27	7.4 ± 0.04	8.1 ± 0.07
		100	18.4 ± 0.15	33.6 ± 0.30	7.4 ± 0.04	8.2 ± 0.04
	High	0	18.3 ± 0.06	34.1 ± 0.16	7.4 ± 0.01	8.2 ± 0.06
		50	18.2 ± 0.32	33.3 ± 0.64	7.4 ± 0.02	8.1 ± 0.07
		100	18.7 ± 0.39	32.9 ± 0.13	7.3 ± 0.07	8.0 ± 0.11
21	None	0	21.1 ± 0.05	34.0 ± 0.10	7.1 ± 0.02	8.4 ± 0.02
		50	21.1 ± 0.08	33.8 ± 0.10	7.1 ± 0.05	8.4 ± 0.02
		100	21.2 ± 0.11	33.8 ± 0.46	7.1 ± 0.03	8.4 ± 0.02
	Average	0	21.2 ± 0.16	34.4 ± 0.40	7.0 ± 0.04	8.3 ± 0.04
		50	21.2 ± 0.04	33.8 ± 0.16	7.1 ± 0.05	8.3 ± 0.04
		100	21.3 ± 0.11	33.9 ± 0.28	7.1 ± 0.05	8.3 ± 0.05
	High	0	21.1 ± 0.10	34.7 ± 0.05	7.1 ± 0.02	8.3 ± 0.03
		50	21.2 ± 0.16	33.8 ± 0.47	7.1 ± 0.06	8.3 ± 0.04
		100	21.0 ± 0.03	33.6 ± 0.19	7.1 ± 0.05	8.3 ± 0.03

Table 15 continued

Day	SMX presence	Sandprawn density (%)	Temperature (°C) [3]	Salinity (ppt) [3]	Dissolved oxygen (mg/l) [3]	pH [3]
24	None	0	14.5 ± 0.20	34.4 ± 0.12	7.8 ± 0.09	8.4 ± 0.10
		50	14.3 ± 0.14	34.3 ± 0.17	7.9 ± 0.03	8.3 ± 0.05
		100	14.1 ± 0.12	34.9 ± 0.28	7.9 ± 0.03	8.5 ± 0.03
	Average	0	14.4 ± 0.29	34.8 ± 0.46	7.8 ± 0.06	8.4 ± 0.10
		50	14.8 ± 0.59	34.0 ± 0.47	7.7 ± 0.15	8.3 ± 0.10
		100	14.4 ± 0.36	34.4 ± 0.56	7.9 ± 0.07	8.3 ± 0.10
	High	0	14.3 ± 0.23	34.8 ± 0.30	7.8 ± 0.05	8.4 ± 0.02
		50	14.2 ± 0.10	34.3 ± 0.42	7.9 ± 0.02	8.4 ± 0.02
		100	14.5 ± 0.22	34.1 ± 0.21	7.9 ± 0.07	8.4 ± 0.08

Table 16: Summary of the spatio-temporal variation in the concentration (mg/L) of pelagic inorganic nutrients (mean ± SE) across SMX and sandprawn density treatments over the 24-day experiment. Sample sizes are shown in square brackets per day of data collection.

Day	SMX presence	Sandprawn density (%)	Nitrite (NO^{2-}) [3]	Phosphate (PO_4^3) [3]	Ammonium (NH_4^+) [3]
0	None	0	7.7 ± 1.20	0.3 ± 0.18	10.4 ± 1.48
		50	7.0 ± 1.73	0.5 ± 0.33	7.3 ± 2.71
		100	10.3 ± 1.67	1.6 ± 0.30	10.8 ± 0.52
	Average	0	7.7 ± 0.67	0.9 ± 0.47	1.7 ± 0.97
		50	8.0 ± 0.58	1.3 ± 0.20	7.5 ± 3.72
		100	8.0 ± 0.58	1.4 ± 0.19	9.4 ± 1.31
	High	0	7.0 ± 0.58	0.3 ± 0.30	5.8 ± 3.20
		50	6.3 ± 1.20	1.4 ± 0.17	4.2 ± 2.61
		100	6.0 ± 2.00	1.7 ± 0.12	5.3 ± 1.75
4	None	0	8.7 ± 0.33	2.2 ± 0.41	12.0 ± 0.24
		50	8.7 ± 0.67	1.5 ± 0.63	11.1 ± 0.32
		100	7.7 ± 0.67	1.4 ± 0.20	11.6 ± 0.35
	Average	0	8.7 ± 0.67	1.3 ± 0.48	7.6 ± 3.57
		50	7.0 ± 2.08	1.5 ± 0.36	5.5 ± 2.50
		100	5.7 ± 0.88	1.1 ± 0.32	0.9 ± 0.18
	High	0	7.7 ± 0.67	1.4 ± 0.44	0.3 ± 0.09
		50	7.0 ± 0.58	1.1 ± 0.33	0.7 ± 0.17
		100	6.7 ± 2.33	1.0 ± 0.48	1.0 ± 0.09
8	None	0	5.7 ± 1.67	1.5 ± 0.09	3.9 ± 3.40
		50	5.7 ± 0.88	0.8 ± 0.44	1.1 ± 0.07
		100	7.3 ± 0.88	1.5 ± 0.33	1.6 ± 0.46
	Average	0	7.3 ± 0.33	0.9 ± 0.38	0.7 ± 0.05
		50	6.7 ± 0.67	1.0 ± 0.07	1.4 ± 0.30
		100	9.7 ± 2.40	1.8 ± 1.13	2.2 ± 0.61
	High	0	10.3 ± 1.86	1.2 ± 0.54	0.4 ± 0.10
		50	7.3 ± 2.67	0.6 ± 0.63	4.7 ± 3.52
		100	7.3 ± 0.33	1.6 ± 0.19	2.0 ± 0.60
12	None	0	7.7 ± 0.33	2.3 ± 1.63	0.7 ± 0.15
		50	9.7 ± 0.88	2.1 ± 0.38	3.8 ± 2.33
		100	8.3 ± 0.88	1.6 ± 0.32	2.1 ± 0.49
	Average	0	2.0 ± 0.00	1.3 ± 0.67	0.9 ± 0.21
		50	1.0 ± 0.58	1.8 ± 0.52	2.3 ± 0.63
		100	0.7 ± 0.67	1.5 ± 0.20	3.4 ± 1.52
	High	0	0.0 ± 0.00	1.9 ± 0.66	0.5 ± 0.07
		50	0.0 ± 0.00	2.4 ± 0.64	1.3 ± 0.36
		100	2.0 ± 0.00	2.8 ± 0.37	2.0 ± 0.16

Table 16 continued.

Day	SMX presence	Sandprawn density (%)	Nitrite (NO^{2-}) [3]	Phosphate (PO_4^3) [3]	Ammonium (NH_4^+) [3]
16	None	0	4.3 ± 1.33	1.6 ± 0.12	4.2 ± 3.54
		50	3.3 ± 1.20	1.6 ± 0.44	3.4 ± 2.25
		100	1.0 ± 1.00	2.4 ± 0.23	5.8 ± 3.33
	Average	0	0.3 ± 0.33	1.9 ± 0.96	10.5 ± 1.16
		50	6.0 ± 3.79	2.4 ± 0.99	8.6 ± 3.06
		100	4.3 ± 0.67	3.7 ± 0.78	8.1 ± 2.39
	High	0	4.3 ± 1.45	1.5 ± 0.49	6.0 ± 2.71
		50	5.7 ± 0.67	2.6 ± 1.75	5.6 ± 2.07
		100	3.0 ± 0.58	2.6 ± 0.68	6.4 ± 1.76
20	None	0	5.0 ± 2.65	1.3 ± 0.15	6.1 ± 1.19
		50	3.0 ± 0.58	2.9 ± 1.19	1.6 ± 0.22
		100	5.0 ± 0.58	0.8 ± 0.33	3.1 ± 0.32
	Average	0	2.3 ± 1.45	1.6 ± 0.25	3.4 ± 2.26
		50	0.0 ± 0.00	1.5 ± 0.29	8.2 ± 2.27
		100	4.0 ± 2.00	1.2 ± 0.18	5.5 ± 3.46
	High	0	2.3 ± 1.45	3.4 ± 0.07	9.6 ± 1.66
		50	4.0 ± 1.00	2.5 ± 0.65	10.1 ± 0.62
		100	3.3 ± 0.88	3.1 ± 0.20	8.3 ± 2.10
24	None	0	7.0 ± 1.67	2.1 ± 0.77	2.2 ± 1.75
		50	7.7 ± 0.88	2.7 ± 0.09	10.2 ± 1.41
		100	10.0 ± 0.58	3.2 ± 0.12	10.7 ± 0.53
	Average	0	6.7 ± 3.71	2.6 ± 0.69	11.2 ± 0.21
		50	2.0 ± 2.00	1.9 ± 0.27	8.4 ± 2.00
		100	2.0 ± 1.15	3.8 ± 1.54	9.0 ± 2.85
	High	0	4.3 ± 0.88	0.4 ± 0.38	11.7 ± 0.68
		50	5.7 ± 1.33	1.9 ± 0.64	11.6 ± 0.07
		100	6.7 ± 0.33	1.2 ± 0.36	5.9 ± 2.77

Table 17: Akaike Information Criterion (AIC) values for the experimental response variables with each of the predictor variable and the interactions between the predictor variables.

Response variable	Predictor variable	AIC
Suspended solids	SMX, sandprawn density, interaction	-93.24
	SMX, interaction	-93.24
	Sandprawns, interaction	-93.24
	SMX, Sandprawns	-89.53
	SMX	-77.04
	Sandprawns	-89.93
	Interaction	-93.24
Total chlorophyll	SMX x sandprawn density x interaction	-73.96
	SMX x interaction	-73.96
	Sandprawns, interaction	-73.96
	SMX, sandprawns	-74.81
	SMX	-75.29
	Sandprawns	-67.16
	Interaction	-73.96
Microplankton	SMX x sandprawn density x interaction	-57.02
	SMX x interaction	-57.02
	Sandprawns, interaction	-57.02
	SMX, sandprawns	-58.48
	SMX	-61.92
	Sandprawns	-50.69
	Interaction	-57.02
Nanoplankton	SMX x sandprawn density x interaction	-48.69
	SMX x interaction	-48.69
	Sandprawns, interaction	-48.69
	SMX, sandprawns	- 52.01
	SMX	-53.64
	Sandprawns	-49.21
	Interaction	-48.69
Picoplankton	SMX x sandprawn density x interaction	-58.49
	SMX x interaction	-58.49
	Sandprawns, interaction	-58.49
	SMX, sandprawns	-58.90
	SMX	-56.74
	Sandprawns	-53.52
	Interaction	-58.49

Table 17 continued

Response variable	Predictor variable	AIC
Diatoms	SMX x sandprawn density x interaction	-76.05
	SMX x interaction	-76.05
	Sandprawns, interaction	-76.05
	SMX, sandprawns	-80.69
	SMX	-77.38
	Sandprawns	-80.88
	Interaction	-76.05
Cyanobacteria	SMX x sandprawn density x interaction	-47.81
	SMX x interaction	-47.81
	Sandprawns, interaction	-47.81
	SMX, sandprawns	-47.34
	SMX	-43.96
	Sandprawns	-44.67
	Interaction	-47.81
Sediment reflectance	SMX x sandprawn density x interaction	-92.91
	SMX x interaction	-92.91
	Sandprawns, interaction	-92.91
	SMX, sandprawns	-99.22
	SMX	-85.53
	Sandprawns	-101.37
	Interaction	-92.91