Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Chemistry

Minda Nielsen

Occurrence of bisphenols and benzophenones in mussels and fish in Algoa Bay, South Africa

Master's thesis in Natural Science with Teacher Education
Supervisor: Alexandros Asimakopoulos
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Abstract

Endocrine disrupting chemicals (EDCs) are emerging contaminants, and their environmental fate and potential adverse effects on human health and wildlife are not well assessed. Bisphenols (Bps) and benzophenones (BzPs) are ubiquitous EDCs and are used in plastics, in addition to being used in industrial and consumer product applications. Because of their widespread use they have been detected in nearly all matrices and marine ecosystems are often the sink for these organic pollutants. The research on Bps and BzPs in marine organisms are limited, especially on Bps analogues. Therefore, it is necessary to monitor the occurrence and abundance of Bps and BzPs in marine organisms to get insight in the environmental fate and release routes of the pollutants.

A total of 137 mussel samples (*Perna perna*) and 31 fish samples (*Seriola lalandi*, n=11 and *Sardinops sagax*, n=20) sampled in Algoa Bay, South Africa in 2022 were investigated for the occurrence of Bps and BzPs. The analytes were extracted with liquid-liquid extraction and the quantification was performed using UPLC-MS/MS. A total of nine out of the fourteen target analytes were detected. For Bps and BzPs, the highest determined median concentration in mussels at all sites were BpAP and BzP-2 (31.6 and 7.90 ng g^{-1} respectively). For S. lalandi and S. sagax the highest median concentrations for BzPs were BzP-2 (17.4 and 2.76 ng g^{-1} respectively), whereas BpAF (15.0 ng g^{-1}) and BpA (130 ng g^{-1}) respectively. Comparisons of the concentrations and geographical distribution of the species, and the mussel samples from January and September, indicate that environmental and biological factors influence the release sources and exposure of the pollutants.

A risk assessment of the detected pollutants was performed by calculating the estimated daily intake (EDI) for dietary consumption of the fish and mussels. These values were then compared with the tolerable daily intake (TDI) value set by the European Food Safety Authority (EFSA). As a result, the EDI concentrations for Bps exceeded the TDI values, suggesting that the consumption of fish and mussels collected in Algoa Bay, South Africa may pose a risk to human health. The EDIs for BzPs did not exceed the TDI values indicating that no risk regarding BzPs is associated with adverse health effects.

The work conducted in this thesis is a continuation of the research on the occurrence of Bps and BzPs in mussels sampled in the same sites in Algoa Bay, South Africa by Castro et al. (1) in collaboration with the Sustainable Seas Trust (SST), South Africa. SST is the owner of the data used in this thesis.

Sammendrag

Endokrine forstyrrende kjemikaler (EDCs) er framvoksende miljøgifter, og deres skjebne i miljøet og potensielle skadevirkninger på menneskers helse og dyreliv er ikke godt undersøkt. Bisfenoler (Bps) og benzofenoner (BzPs) er EDCs som er til stede overalt og som benyttes i plast, i tillegg til å brukes i industri og forbrukerprodukter. På grunn av deres utbredte anvendelse, har de blitt detektert i nesten alle matriser og marine økosystemer er ofte en mottaker for slik organiske miljøgifter. Forskning på Bps og BzPs i marine organismer er begrenset, spesielt for Bps analoger. Derfor er det nødvendig å overvåke forekomsten og omfanget av Bps og BzPs i marine organismer for å få innsikt i skjebnen til miljøgiftene, samt kunnskap om de ulike utslippsrutene.

Totalt 137 muslingprøver (*Perna perna*, n=137) og 31 fiskeprøver (*Seriola lalandi*, n=11 og *Sardinops sagax*, n=20) ble tatt i Algoa Bay, Sør-Afrika i 2022 for å undersøke forekomsten av Bps og BzPs. Analyttene ble ekstrahert med væske-væske ekstraksjon og kvantifiseringen ble utført ved hjelp av UPLC-MS/MS. Totalt ni av fjorten analytter ble påvist. For muslingprøver tatt i begge måneder, var de høyeste bestemte mediankonsentrasjonene for Bps og BzPs, BpAP og BzP-2 (henholdsvis 31.6 og 7.90 ng g^{-1}). For *S. lalandi* og *S. sagax* var de høyeste mediankonsentrasjonene for BzPs, BzP-2 (henholdsvis 17.4 og 2.76 ng g^{-1}), mens for Bps var det henholdsvis BpAF (15.0 ng g^{-1}) og BpA (130 ng g^{-1}). Sammenligninger av konsentrasjonene og den geografiske fordelingen av muslingprøvene fra januar og september, indikerer at miljømessige og biologiske faktorer påvirker utslippskildene og eksponeringen av miljøgiftene.

En risikovurdering av de påviste forurensningene ble utført ved å beregne estimert daglig inntak (EDI) for kostinntak av fisk og muslinger. Disse verdiene ble deretter sammenlignet med en tolerabelt daglig inntak (TDI) verdi satt av den Europeiske myndighet for næringsmiddeltrygghet (EFSA). Som et resultat oversteg EDI-konsentrasjonene for Bps TDI-verdiene, noe som tyder på at inntak av fisk og muslinger hentet fra Algoa Bay, Sør-Afrika kan utgjøre en risiko for menneskers helsetilstand. EDI-verdiene for BzPs oversteg på sin side ikke TDI-verdiene, noe som indikerer at det ikke foreligger en risiko forbundet med uheldige helseeffekter med hensyn på BzPs.

Arbeidet som ble utført i denne oppgaven er en fortsettelse av forskningen av forekomsten til Bps og BzPs i muslinger hentet fra de samme prøvelokasjonene i Algoa Bay, Sør-Afrika av Castro et al. (1) i samarbeid med Sustainable Seas Trust (SST), Sør-Afrika. SST er eier av all data benyttet i denne masteroppgaven.

Acknowledgements

This thesis concludes my degree in Natural Science with Teacher Education with a specialization in Environmental and Analytical Chemistry at the Norwegian University of Science and Technology (NTNU). The work with this thesis has challenged me and helped me gain knowledge about organic trace level analysis that I will bring with me into my professional career, and I have several people I would like to express my gratitude to for making the thesis possible.

I would like to thank my ever so patient supervisor, Alexandros Asimakopoulos, for letting me be a part of this project and for your positive and easy-going attitude. Your support and quick response to emails have been invaluable during the last few months. Thank you to my co-supervisors Amarein J. Fourie and Tanna Hewett from the Sustainable Seas Trust (SST) in South Africa for executing the sampling and pre-preparation, in addition to answering any questions I had about the sampling and sampling areas. I would also like to express my gratitude to Gabriela Castro and Susana V. Gonzáles for preparing and analyzing the samples. Also, Susana V. Gonzáles, thank you for the never-ending help in the MS-lab and for answering every question enthusiastically no matter how dumb they were. I would also like to thank Anders Lervik for help with constructing the PCA plots and boxplots.

This thesis would not have been completed without the support of Cornelia Horn and Ellen Bull. Thank you for making the five years in Trondheim unforgettable and for sticking with me through all ups and downs, for good conversations, and for every laughter we have shared together. I would also like to thank my family for supporting me and for believing in me no matter what. Thank you to Ingeborg Lysberg, my MS partner-in-crime for every encouragement, help and good advice. At last, I would like to thank my fellow students at my study program, especially Hanne Svergja, for lunch breaks, good conversations and fun times during the last five years.

Contents

Abbreviations

4-OH-BzP	4-hydroxy-benzophenone
API	Atmospheric-pressure ionization
b.w.	Body weight
BpA	Bisphenol A
BpAF	Bisphenol AF
BpAP	Bisphenol AP
BpB	Bisphenol B
BpF	Bisphenol F
BpM	Bisphenol M
BpP	Bisphenol P
BpS	Bisphenol S
Bps	Bisphenols
BpZ	Bisphenol Z
BzP-1	Benzophenone-1
BzP-2	Benzophenone-2
BzP-3	Benzophenone-3
BzP-8	Benzophenone-8
BzPs	Benzophenones
CID	Collision induced dissociation
d.w.	Dry weight
E.U.	European Union
ECHA	European Chemicals Agency
EDCs	Endocrine Disrupting Chemicals
EDI	Estimated daily intake
EFSA	European Food Safety Authorization
ESI	Electrospray ionization
HPLC	High-performance liquid chromatography
IS	Internal standard
LC	Liquid chromatography
m/z	mass-to-charge ratio
MP	Mobile phase
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MW	Molecular weight
PC	Polycarbonate
SCCS	Scientific Committee for Consumer Safety
SML	Specific migration limit

SP	Stationary phase
SRM	Selected reaction monitoring
SST	Sustainable Seas Trust
TA	Target analyte
TDI	Tolerable daily intake
t-TDI	Temporary tolerable daily intake
UPLC	Ultra-performance liquid chromatography
w.w.	Wet weight
WWTPs	Wastewater treatment plants

1 Introduction

Increased anthropogenic activity is putting pressure on the environment and is resulting in the release of contaminants of emerging concerns whose environmental impact and fates are not yet fully understood (2). These emerging contaminants (EC) are often present at very low concentrations and many of them show evidence of being endocrine disrupting chemicals (EDCs) (3). EDCs disrupt the normal functioning of hormones which regulate important biological processes in both humans and wildlife (4). Their long-term effects and fate are uncertain (5) and there are growing concerns about the impact these contaminants may have on ecosystems and human health. Because of this there is an increasing demand to investigate the occurrence, release sources, exposure levels, and the human health effects of different EDCs.

Bisphenols (Bps) and benzophenones (BzPs) are organic chemicals that have demonstrated endocrine disrupting abilities and have a widespread use in industrial and consumer products (6-8). They are ubiquitous, and their release are to a large extent associated with plastic waste and personal care products. Plastic pollution is regarded as an issue of global concern and a lot of plastics end up in marine environments where chemical additives can leach from plastic and have adverse effects on organisms and ecosystems (9).

Bps are heavily used in plastics and the most applied Bps, bisphenol A (BpA), is commonly used as a monomer in polycarbonate (PC) plastics and in epoxy resins. BpA have shown endocrine disrupting abilities at low concentrations (10) and has been proved to leach from plastics (11). This has resulted in regulations constricting the use of BpA in consumer goods and consequently replacing BpA with other Bps analogues (12). These analogues are less investigated than BpA and current research findings indicate that the analogues may have similar endocrine disrupting properties as BpA (13). BzPs are commonly used as UV-filters and UV-stabilizers in personal care products and in plastics (7, 8). The most investigated BzPs, benzophenone-3 (BzP-3) is used in sunscreens and cosmetics, and is frequently detected in aquatic matrices (8). Because of the increased awareness surrounding skin cancers caused by UV-radiation, the use of personal care products containing UV-filters such as BzP-3 has increased the levels of BzP-3 and its metabolites in coastal waters (14).

The investigations of Bps and BzPs analogues are lacking (13) and there exists uncertainties surrounding the environmental effects and fate of the chemicals. Therefore, the aim of this thesis is to assess the occurrence of Bps and BzPs analogues in marine species, mussels (*Perna perna*) and fish (*Seriola lalandi*,

Sardinops sagax), sampled in Algoa Bay, South Africa. This assessment may contribute with knowledge allowing for a better understanding of the environmental fate and potential human exposure risks of the EDCs, as well as providing insight in possible local release sources and their influence. This study is a continuation of the research done by Castro et al. (1) and the non-profit organization the Sustainable Seas Trust (SST) located in Algoa Bay, South Africa.

2 Theoretic Background

2.1 Endocrine Disrupting Chemicals (EDCs)

In all organisms, hormones regulate most of the biological processes such as metabolism, development, and reproduction (4). They are essential for life and may be affected by endocrine disrupting chemicals (EDCs) which are exogenous contaminants that disrupt the normal functioning of the endocrine system in both humans and wildlife (15). These contaminants can be natural or anthropogenic, however human-made EDCs are emerging and the long-term effects and fate of the chemicals are uncertain (5). Also, the potential adverse effects of EDCs may be hard to investigate as some effects may manifest some time after the exposure (5). The group of EDCs are heterogenous and contain a wide variety of compounds such as PCBs, phthalates, bisphenols, and benzophenones (16)

EDCs have several modes of action regarding the effects they manifest in both humans and wildlife, and even small concentrations can lead to adverse effects (4, 16). In humans, research have shown that EDCs have negative effects on reproduction in both males and females, including low sperm count (5, 17) and ovarian insufficiency (5, 18), and have the potential to increase the risk of hormone-sensitive cancer types (4, 5). In wildlife, evidence strongly indicate that the exposure to EDCs is resulting in effects such as eggshell thinning in birds (19-21) and malformations of the reproductive anatomy (imposex) in aquatic invertebrates (5, 22, 23).

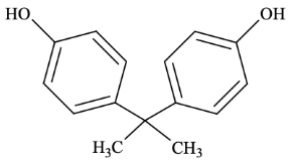
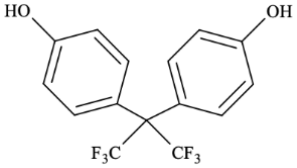
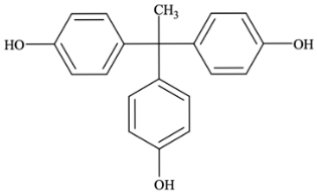
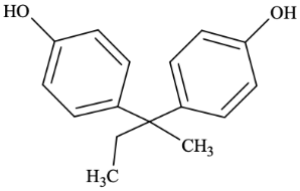
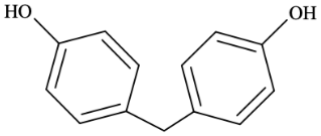
Exposure to EDCs can occur through several potential channels including food, manufactured products, and through water, soil, and air (16). In aquatic matrices, one of the major contamination sources are wastewater treatment plants as the conventional treatments employed are not effectively eliminating EDCs (4, 16). In addition to this, hospital waste disposal, recreational activities, leaching from agriculture and industry are other sources (16).

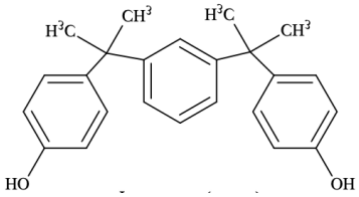
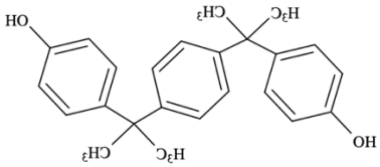
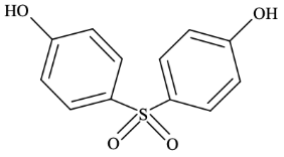
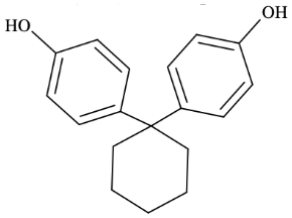
2.1.1 Bisphenols

Bisphenols (Bps) are organic compounds consisting of two hydroxylated benzene rings connected by a carbon bridge (24). They have a wide range of industrial and consumer applications, and different physicochemical properties, depending on the different substituents located on the aromatic rings or on the carbon bridge (6). The most applied bisphenol is bisphenol A (BpA), which has had an estimated volume production of 8 million tons (12, 25), a level that is decreasing (12). BpA is used in epoxy resins and as

monomer in polycarbonate (PC) plastic, and is found in food containers, thermal paper, toys, and clothes made from synthetic fibers (12, 26). BpA has been found to leach from food contact materials either by migration of residual BpA or by hydrolysis of the polymer itself (11). As BpA has shown toxic effects, it is being replaced by analogues that is presumed safer (12), such as the analogues investigated in this thesis. Including BpA these are bisphenol B (BpB), bisphenol AF (BpAF), bisphenol AP (BpAP), bisphenol F (BpF), bisphenol M (BpM), bisphenol P (BpP), bisphenol S (BpS) and bisphenol Z (BpZ). Information such as molecular structure, full name, CAS numbers, molecular weight (MW, g mol^{-1}) and the predicted log K_{ow} is provided in Table 1. The log K_{ow}-values are the same as in Castro et al. (1) and is predicted data from ChemSpider.com.

Table 1: The molecular structure, full name, abbreviation, CAS-number, molecular weight (MW, g mol^{-1}) and partition coefficient (Log K_{ow}) for Bps compounds. The log K_{ow}-values are the same as in Castro et al. (1) and is predicted data from ChemSpider.com

Molecular structure	Compound	Abbreviation	CAS-number	MW (g mol^{-1})	Log K_{ow}
	Bisphenol A	BpA	80-05-7	228.3	3.32
	Bisphenol AF	BpAF	1478-61-1	336.2	4.47
	Bisphenol AP	BpAP	1571-75-1	290.4	4.35
	Bisphenol B	BpB	77-40-7	242.3	4.13
	Bisphenol F	BpF	620-92-8	200.2	2.91

	Bisphenol M	BpM	13595-25-0	346.5	6.37
	Bisphenol P	BpP	2167-51-3	346.5	6.37
	Bisphenol S	BpS	80-09-1	250.3	1.65
	Bisphenol Z	BpZ	843-55-0	268.4	4.46

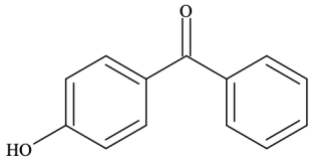
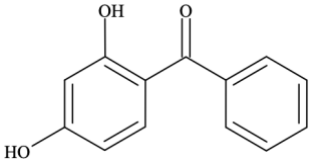
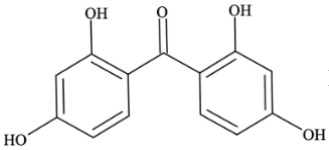
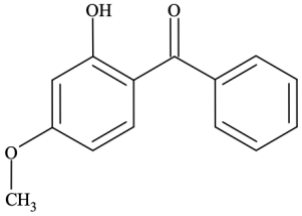
The research on Bps analogues other than BpA is lacking (13) and more studies on their toxic effects and environmental fate is needed. The analogues have many different applications where the main substitute for BpA, Bps, is being becoming more frequently used in thermal paper (25, 27), whereas BpAP is used in plastics, electronics and personal care products such as body wash and hair styling (28). BpA and the other analogues have shown estrogenic effects, and BpAF is suggested to bioaccumulate in rat tissue (29).

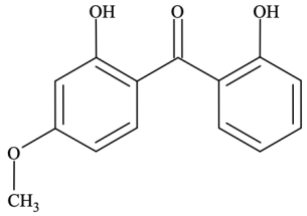
The predominant exposure route of BpA for humans is through dietary intake (30). However, Bps can also be adsorbed through dermal contact by e.g. touching thermal paper (25). In the environment, the major release source of bisphenols are wastewater treatment plants (WWTPs). Traditional wastewater treatments are not efficient enough in removing bisphenols (31, 32) and there are disputes surrounding the optimal treatment (33). Leaching of pollutants from microplastics have been suggested as an exposure route for organisms (34, 35). Significant amounts of plastics are being released into the environment every year, and some of it ends up in the marine environment. Microplastics can be ingested by aquatic organisms and a study conducted by Barboza et al. (35) found evidence of an association between the amount of microplastics in fish muscle and the concentration of bisphenols.

2.1.2 Benzophenones

Benzophenones (BzPs) are a group of aromatic compounds with the chemical formula Ph_2CO consisting of two benzene rings connected by a carbonyl bridge (36). Their conjugated structure allows them to absorb UV-radiation in the UVA and UVB range and disperse it as lower-energy radiation (8, 37). This property has contributed to a widespread usage of hydroxylated BzPs analogues as UV-filters in consumer products and as UV-stabilizers in polymers such as plastics to avoid photolysis (7, 8). In general, they are lipophilic, relatively photostable and bioaccumulative compounds (38, 39). An overview of the molecular structures, full name, abbreviations, CAS numbers, molecular weight (MW, g mol^{-1}) and the predicted log K_{ow} for the BzPs are provided in Table 2. The log K_{ow}-values are the same as in Castro et al. (1) and is predicted data from ChemSpider.com.

Table 2: The molecular structure, abbreviation, CAS-number, molecular weight (MW, g mol^{-1}) and partition coefficient (Log K_{ow}) for BzPs compounds. The log K_{ow}-values are the same as in Castro et al. (1) and is predicted data from ChemSpider.com.

Molecular structure	Compound	Abbreviation	CAS-number	MW (g mol^{-1})	Log K_{ow}
	4-hydroxybenzophenone	4-OH-BzP	80-05-7	198.2	3.07
	Benzophenone-1	BzP-1	1478-61-1	214.2	2.96
	Benzophenone-2	BzP-2	1571-75-1	246.2	2.78
	Benzophenone-3	BzP-3	77-40-7	228.2	3.79

	Benzophenone-8	BzP-8	620-92-8	244.2	3.82

The water solubility of hydroxylated BzPs is moderate to high (40), which is reflected in their emission routes into the environment. The two major routes of emission are direct emission from recreational activities, e.g. swimming, and indirect emission through the effluent of WWTPs (41, 42). The widespread use of BzPs is resulting in a continuous release of the pollutants into the environment (43). The solubility of BzPs makes them available in aquatic matric and as a result, aquatic matrices are the sink of BzPs (8, 41).

A commonly used UV-filter is benzophenone-3 (BzP-3), often referred to as oxybenzone (44), which is frequently used in sunscreens and cosmetic products. As a result, BzP-3 is often detected in the aquatic environment and there exists more data on its occurrence than of the other derivates investigated in this thesis (8). Some derivates of BzP-3 are benzophenone-1 (BzP-1), benzophenone-2 (BzP-2), benzophenone-8 (BzP-8) and 4-hydroxy-benzophenone (4-OH-BzP) (14, 45), with BzP-1 being the main metabolite (40). These are also five of the target analytes in this thesis. All of the compounds have indicated endocrine disrupting abilities with either estrogenic or anti-androgenic activity (38, 40), and BzP-8 is also linked to genotoxicity (40). BzPs have shown potential for bioaccumulation and have been detected in fish fat (46).

2.2 Study species

Organisms can be used as bioindicators, offering a insight in the presence, impact and extent of pollution (47). They play a crucial role in understanding the fate of pollutants in the environment and assisting in the assessment of the potential health effects they may have on human health and wildlife. The information obtained by using bioindicators is important for policy makers (48) in the progress of protecting the environment. However, not every specie can be used for monitoring and there are some desirable characteristics the organisms should have. The organisms should naturally occur in the research area, have a widespread distribution and display a tolerance for different stressors (48). Different species are used for different classes of pollutants, where fish and molluscs are frequently used as bioindicators for organic pollutants in aquatic ecosystems (48).

2.2.1 *Perna perna*

The brown mussel (*Perna perna*) is a bivalve mollusc belonging to the family Mytilidae. The mussel thrives in tropical and subtropical waters and are naturally occurring in regions of the Western Indian, Mediterranean, and the Atlantic Oceans, native to the coastal areas of Africa, Europe, and South America (47, 49). *P. perna* has a significant commercial and economic value, which is contributed to it being the most consumed shellfish globally (50). In addition to this, the mussel has a crucial ecological value by serving as an engineer species by creating biogenic reefs as well as remediating nutrients (47, 51). Because of its significance, it is important to understand how pollutants affect the species and the potential adverse effects it may have for human health.

Morphologically, *P. perna* consists of two triangular shell valves of a similar size (47), and is characterized by its brown colour, as indicated by its common name. They reach the size of 90-100 mm (52) and are sessile benthic filter feeders with a diet consisting of suspended organic material, phyto- and zooplankton (47, 53). They are sessile meaning that they are fixed in the same place (53). According to a study conducted by Kadokami et al. (54), certain organic pollutants exhibit a higher potential for bioaccumulation in benthic organisms, because the concentration of the pollutants may be higher in sediments. The filter feeding enables *P. perna* to accumulate various types of pollutants with concentrations reflecting the concentration in sediments and in the water column (47). With mussels being important prey for organisms of higher trophic levels such as crabs, whelks and humans (55), consuming them can cause pollutants to move up the trophic levels (47) for contaminants able of biomagnification. Thus, investigating the occurrence of bisphenols and benzophenones in molluscs are important.

2.2.2 *Seriola lalandi*

Seriola lalandi, also called yellowtail kingfish, is a fish species naturally occurring in the subtropical waters of the Indo-Pacific and Atlantic Oceans (56, 57). It is common in the areas of Australia, New Zealand and South Africa, and is a popular recreational sportfish (57). *S. lalandi* is of economic and commercial importance, and due to the species' fast growth rate and high nutritional quality (58), there has been established commercial aquaculture production of the fish in several countries (59). Additionally, in South Africa, the fish was, in 2015, the fourth largest commercial linefishery (58). *S. lalandi* is pelagic and have a somewhat nomadic behaviour (60). The adult fish are typically found in open coastal waters, whereas

juvenile fish gather in schools closer to the surface (57). The fish is long and slender, and as its name implies, has a bright yellow tail.

S. lalandi is a medium sized piscivore (60), meaning that it is a predator feeding on smaller fish such as sardine and mackerel (61). However, they are deemed as opportunistic feeders meaning that their diet may also consist of crustations (60). The maximum size of the fish is debatable, but according to Pepperell the largest official weight was around 50 kg (57). Some pollutants have the ability to bioaccumulate and biomagnify, and as a predator, yellowtail kingfish may have a higher concentration of these pollutants in its muscle tissue than their prey (60). Therefore, it is important to investigate the occurrence of contaminants in species of a higher trophic level to assess the potential effect on the food chain and the bioaccumulation potential of some pollutants.

2.2.3 *Sardinops sagax*

Pilchard (*Sardinops sagax*) is a pelagic fish species in the Clupeidae family which gather in large schools in the coastal areas of the Pacific Ocean and around the southern cape of Africa (57). The species is a small silvery sardine fish with dark spots along the sides of the body, reaching a typical size of 20 cm (57). The pilchard is of both ecological and economic value (62), where the economic value stems from the species essential role as food source for humans, in addition to its usage to feed lifestock and as bait (63). In South Africa, canned pilchard is considered as one of the most important food items (62, 63).

S. sagax has a crucial ecological value because of its function in the marine food web. Due to its role as a foraging fish, and as a filter feeder (64), with a diet consisting of phytoplankton and zooplankton, it makes evergy available as food for organisms higher up in the food web (63). Studies indicate that organic pollutants bioaccumulate in pelagic fish (64, 65) and that this can be a potential risk for organisms of higher trophic levels such as larger fish, seals and seabirds (62). Therefore, it is important to investigate the occurrence of organic pollutants such as bisphenols and benzophenones in organisms of lower trophic levels, in addition to gain knowledge about the potential for direct uptake of contaminants through the human diet.

2.3 Study location – Algoa Bay

The study location in this thesis is the area of Algoa Bay, South Africa, situated in the harbour city of Gqeberha, formerly known as Port Elizabeth (66). The city is situated on the west side of the bay and is the most populated city in the Eastern Cape with around 1 million inhabitants (67). It is located in the southern part of South Africa and is at an important area for the study of global changes as the area is at the interface of three different oceans, the Southern, Indian and Atlantic Oceans (68). Algoa Bay has been a part of a long-term monitoring project since 2007 where the aim is to better understand the complex coastal ecosystem. As a result of this, Algoa Bay has become the best-monitored coastal area in the Southern Hemisphere (67, 68). As Algoa Bay is situated in the Southern Hemisphere, meaning that the summer months are in December to February while winter is in June to August. Precipitation is more heavy in the winter months between May and August, and lowest in summer between December and February (69)

Algoa Bay has a relatively shallow coastal area with depths less than 70 m, and there are five different estuaries that discharge into the bay, including Swartkops and Sunday estuaries (67). Both estuaries have different anthropogenic influence and are surrounded by industry and agriculture (70). Swartkops is situated in the outskirts of Gqeberha, and runs through highly populated and agricultural areas where there are multiple sources contributing to the pollution levels in the river (70). According to Adams et al. (71) effluent from several WWTPs stands for around 50% of the river flow of Swartkops. Other pollution sources include stormwater, and industrial and urban runoff (71). Sunday is situated further away from Gqeberha, to the northeast, and is heavily influenced by agriculture runoff (72).

South Africa is high on the global list of countries being the worst contributors to marine plastic pollution (66), and rivers such a Sunday and Swartkops, are considered to be great sources of plastic pollution (73). The WWTPs of South Africa is contributing to the release of plastics in the environment because of their faulty functionality (66), and a review of the marine plastic pollution in South Africa was executed by Verster and Bouwman (66) and they deducted that up to 40% of all wastewaters in South Africa is left untreated because of detoriated WWTPs.

2.4 Regulations

2.4.1 Bisphenols

Several regulations, assessments, and scientific opinions surrounding the use and exposure of BpA have emerged in the last decades, and the primary focus area of concern has been on the dietary intake of BpA, as the primary route of exposure for humans is ingestion (30). Several of the assessments and scientific opinions have been executed by the European Food Safety Authorization (EFSA) (74-76), and their first full risk assessment report on BpA in food contact material was published in 2006 (77). The impartial scientific opinions and evaluations of EFSA are communicated to the E.U.s member states, policy makers and stakeholders with the purpose of protecting consumers. Numerous countries such as Canada, Australia, China and the European Union (E.U.) member states have all restricted or banned the use of BpA in infant food containers (78). Additionally, the E.U. has restricted the specific migration limit (SML) of BpA to 0.05 mg kg^{-1} from food contact materials into food stuff (79), and restricted the use of BpA in thermal paper (80).

The focus of regulations has been on BpA, and of this author's knowledge at the time of the submission of this thesis, there are no regulations or scientific opinions about the BpA analogues. However, the European Chemicals Agency (ECHA) have proposed that the restriction of several BpA analogues would be included in the REACH Annex XVII (81). The substances proposed for restriction is BpA, BpAF, BpB, BpF, BpS and their salts.

2.4.1.1 TDI

In 2015 EFSA established a temporary tolerable daily intake (t-TDI) for BpA with the threshold being at 4 µg kg^{-1} body weight (b.w.) day^{-1} (76). The TDI provides an estimation of the safe amount of a compound that a person can be exposed to over their lifetime without there being a significant health risk (82). Because of uncertainties and data gaps, the temporary limit was re-evaluated and a new, permanent, TDI was established in December 2022 (83). The new TDI is 20 000 times lower than the previous limit at 0.2 ng kg^{-1} (b.w.) day^{-1} and EFSA concluded that all age groups are exposed to limits above this (83).

2.4.2 Benzophenones

In comparison to bisphenols, there has been less focus on benzophenone compounds, and this is reflected in the regulations and scientific opinions on the chemicals. Nevertheless, with BzP-3 being one of the most

abundantly detected BzP analogues, it has been subjected to regulations. In the E.U., the Scientific Committee for Consumer Safety (SCCS) conducted a safety assessment on BzP-3 in cosmetic products. This resulted in a reduction of the allowed maximum concentration in sunprotective products because of concerns about human health (84). The SCCS concluded that the previous limit of 6% in sunscreen products were too high and lowered the limit to 2.2%, while a limit of 0.5% is allowed if BzP-3 is used to protect the cosmetic formulation (84). The use of the chemical in sunscreens has in later years been banned in Hawaii, and other island nations, because of scientific evidence indicating that BzP-3 is having a negative impact on the health of coral reefs (85-87).

2.4.2.1 TDI

A TDI limit of 30 μg kg^{-1} (b.w.) day^{-1} was in 2009 established for benzophenone after a toxicological evaluation (36). This evaluation was revisited by EFSA in 2017, however the panel concluded that the limit set in 2009 was suitable according to the considered scientific data indicating that the endocrine activity by both benzophenone and 4-OH-BzP is not directly related to any observable toxic effects (88). This conclusion may be subjected to further discussion based on newer research that indicate that BzPs directly interacts with estrogen related receptors (89).

2.5 Estimated Daily Intake

When assessing the potential adverse effects a contaminant can have on human health, it is necessary to estimate the extent of the exposure (82). This can be done through several method, where one of them is by calculating the estimated daily intake (EDI) of the compounds in foodstuff (7). This may be especially important if the main exposure route of the chemical is through dietary intake. The EDI values can be compared to the TDI limits sat by EFSA to evaluate if there is a risk associated with human exposure to the compounds. The EDI is calculated with the following Equation 1 (1, 90, 91).

$$\mathrm{EDI} = \frac{\mathrm{c} \times \mathrm{DC}}{\mathrm{B.W.}} \quad (1)$$

Where c is the concentration of the target analyte in the sample, DC is the estimated daily food consumption and B.W. is the mean body weight of the consumers.

2.6 Previous studies

An overview of the previous studies on the occurrence of Bps and BzPs in mussels from different locations worldwide, up to the release of the preliminary study of this thesis, are presented in Castro et al. (1). As of this author's knowledge, at the time of writing this thesis, there are no new studies on the occurrence in different mussel species.

No studies surrounding the occurrence of the target analytes have been performed on *S. lalandi* and *S. sagax*. Therefore, an overview of the detected concentrations of Bps and BzPs in other fish species from selected locations around the world are presented in Table 3 and Table 4 respectively. The overview is primarily limited to saltwater fish. Out of the target analytes, BpA, BzP-1 and BzP-3 have been more frequently studied. The analysis of the other Bps analogues is lacking, and no studies analyzing BpP or BpM were found. For BzPs, no studies were discovered analyzing BzP-2.

Table 3: Concentrations of Bps (ng g^{-1} d.w.) and reported detection frequencies (DF%) in fish muscles from different locations.

Country	Location	Species	Concentrations ng g^{-1} (DF%)									References
			BpA	BpAF	BpAP	BpB	BpF	BpM	BpP	BpS	BpZ	
Portugal	North East Atlantic Ocean	*Dicentrarchus labrax* (n=50)	9.1[a]	n.d.[b]	n.d.	0.7[a]	n.d.	NA	NA	NA	n.d.	Barboza et al. (35)
		Trachurus trachurus (n=50)	1.4[a]	n.d.	n.d.	1.3[a]	n.d.	NA	NA	NA	n.d.	
		Scomber colias (n=50)	1.0[a]	n.d.	n.d.	10.0[a]	n.d.	NA	NA	NA	n.d.	
Malaysia	Pulau Kupur, Johor	*Trachinotus blochii* (n = 10)	0.322[a]	NA[c]	NA	NA	NA	NA	NA	NA	NA	Ismail et al. (92)
		Lutjanus campechanus (n = 10)	0.084[a]	NA	NA	NA	NA	NA	NA	NA	NA	
		Lates calcarifer (n = 10)	0.078[a]	NA	NA	NA	NA	NA	NA	NA	NA	
Poland	Gulf of Gdansk	*Clupea harengus* (n=11)	19.7-440.1	NA	NA	NA	NA	NA	NA	NA	NA	Staniszewska et al. (93)
		Gadus morhua (n=6)	98.3-755.7	NA	NA	NA	NA	NA	NA	NA	NA	
		Platihthys flesus (n=6)	25.4-798.4	NA	NA	NA	NA	NA	NA	NA	NA	
Spain	Ebro Delta	*Cyprinus carpio, Liza sp., Mugil cephalus, Dicentrarchus labrax, Carassius auratus,* Ictalurus sp., *Silurus glanis* (n=23)	<LOD-1.5 (4%)	<LOD-11.6 (10%)	NA	<LOD	<LOD	NA	NA	<LOD-4.2 (80%)	NA	Gil-Solsona et al. (94)

[a] median concentration

[b] not detected

[c] not analyzed

Table 4: Concentrations of BzPs (ng g^{-1} d.w.) and detection frequencies (DF%) in fish muscles from different locations.

Country	Location	Species	Concentration ng g^{-1} (DF%)					References
			BzP-1	BzP-2	BzP-3	BzP-8	4-OH-BzP	
Taiwan	Miaoli City	Saltwater fish[a] (n=80)	<LOD-0.15 (5%)	NA[b]	<LOD-0.37 (85%)	NA	<LOD-5.73 (86%)	Huang et al. (95)
		Freshwater fish[a] (n=30)	<LOD-0.14 (10%)	NA	<LOD-0.90 (80%)	NA	<LOD-6.23 (53%)	
Portugal	Mediterranean Sea, Atlantic, Atlantic Southwest, North-East Atlantic, Pacific Ocean & Asia Southeast	Mackerel (n=9)	n.d.[b]-5.0 (44%)	NA	n.d.-82.2 (78%)	NA	NA	Cunha et al. (96)
		Monkfish (n=4)	n.d.-36.1 (75%)	NA	5.0-98.7 (100%)	NA	NA	
		Plaice/sole (n=6)	n.d.	NA	n.d.	NA	NA	
		Tuna (n=4)	5.0-34.2 (100%)	NA	n.d.-2.5 (50%)	NA	NA	
Taiwan	Chung-Li	Striped bass (n=1)	1.7	NA	5.7	1.7	NA	Tsai et al. (97)
		Tilapia (n=1)	0.7	NA	5.4	1.5	NA	
		Cod (n=1)	1.0	NA	3.3	0.5	NA	
		Salmon (n=1)	3.6	NA	6.9	2.4	NA	
Norway	Oslo fjord	Cod liver (n=15)	<20-1037 (7%)	NA	NA	NA	NA	Langford et al. (98)
Brazil	Guanabara Bay	*Mugil liza* (n=11)	<LOQ (91%)	NA	3.50-15.4 (100%)	NA	3.02-22.6 (100%)	Molins-Delgado et al. (99)

[a] not specified

[b] not detected

[c] not analyzed

2.7 Analysis of Bisphenols and Benzophenones in Biological Matrices

The analysis of trace organic contaminants, such as Bps and BzPs in biological matrices, needs sufficient sample preparation and extraction steps to be prepared for analysis (100). Furthermore, as the concentration of pollutants in environmental samples may be quite low (100), the analysis instrument must have a sensitivity and selectivity enough to identify and quantify the analytes.

2.7.1 Sample preparation

Sample preparation is a crucial step in trace organic analysis, and the methods used should reflect the physicochemical properties of both the analytes and the matrix (100). The preparation process should include as few steps as possible to avoid contamination in addition to avoid the loss of analyte (101). For biological samples it is often necessary to begin the sample preparation with homogenization to ensure that the sample accurately reflect the composition of the remaining sample. For trace organic analysis it is thereafter necessary to isolate the analytes from the matrix to eliminate components that may interfere with the analysis (102). In addition to this, as the pollutants are present at low quantities it is necessary to carry out an enrichment. Isolation, enrichment, and clean-up can be performed by extraction (101).

2.7.1.1 Liquid-liquid extraction

Liquid-liquid extraction (LLE) is an extraction technique where the analytes in a sample get physically separated from the matrix and transferred to a solvent (103). This is executed by using two immiscible solvents, usually one organic and one aqueous, with the separation based on the principle of partitioning (102). The physicochemical properties decides which solvents are used as “like dissolves like”, and the analytes should be soluble in the extraction solvent (100). The extraction mixture should be stirred to guarantee a sufficient interaction between the two liquid phases, through methods such as ultrasonication or shaking, and the number of extractions must be adequate to extract the analytes from the matrix (104).

2.7.2 UPLC

Ultra-performance liquid chromatography (UPLC) is a liquid chromatography technique that utilize a stationary phase (SP) consisting of sub-2 μm particles, and pressures up to 1300 bar, to separate analytes in a sample (105). The technique allows for both the identification and quantification of analytes in complex matrices and is a valuable analytical tool in environmental monitoring due to its sensitivity that allows analysis at very low levels (106, 107). The separation principle of UPLC is that analytes have distinct

partitioning between the SP and the liquid mobile phase (MP) due to the analytes' physicochemical properties (102, 108).

An UPLC instrument is very similar to the conventional high-performance liquid chromatography (HPLC) instrument. It consists of an injector, pumps, one or more columns, a detector, and a data processing device (102). The main differences are that UPLC utilize shorter columns with a smaller inner diameter as well as a higher pump pressure (102, 105). The smaller column particle size and the applied pressure of UPLC reduce band broadening, resulting in a better separation of the analytes and sharper peaks (105). This increases the signal-to-noise ratio (S/N). Hence, UPLC has a higher sensitivity and selectivity than HPLC and is therefore preferred when analysing complicated matrices with lower quantities. The reduced column size of UPLC also reduce the need of solvent (105). However, one disadvantage with the applied pressure range is that it reduces the lifetime of the column, and it must be replaced more often than columns used in HPLC.

2.7.3 Mass spectrometry

Mass spectrometry (MS) is an analytical technique where the analytes in a sample are ionized and separated based on their mass-to-charge ratio (m/z). This separation can provide qualitative and quantitative information about the analytes (109) such as structural characteristics, chemical properties and determination of the molecular weight (102). MS has high sensitivity and low detection limits, making the technique important in many areas of expertise such as biochemistry and environmental analysis. The MS instrument consists of three major components: the ionization source which ionize the analytes and transition them to the gaseous phase, the mass analyzer where the ions are separated based on their m/z ratio, and the detector that detects the separated ions (109). The signals that are detected are graphically displayed as a mass spectrum.

2.7.3.1 Ion source – Electrospray Ionization

Electrospay Ionization (ESI) is an atmospheric-pressure ionization (API) method where the analytes in a solution are ionized and transferred to the gaseous phase (110). The method effectively removes the solvent without losing analyte and is deemed a soft ionization technique as is causes less fragmentation of the molecules and multiple charged ions (111). This allows for accurate mass measurements since more molecular ions remains intact, however, one limitation is that the reduced fragmentation provides little structural information about the analytes (110). By combining ESI with tandem MS this can be surpassed.

ESI is the preferred ionization method combined with liquid chromatography, and it is used to analyse non-volatile and moderately polar to polar compounds (102, 112). It is useful in analysing macromolecules and depending on the properties of the compounds, can be used in a positive or negative mode (110).

A conventional ESI has a linear trajectory from where the solvent-analyte mixture is injected to where the ions in a gaseous phase enters the mass analyser (110). The ionization happens with the application of an electric filed, 3-5 kV (110), which disperse the solvent-analyte mixture into an aerosol of electrically charged droplets as it exits the inlet source (111). An inert flowing gas, often N_2, is commonly applied to the end of the inlet source to make the dispersal more effective in addition to guiding the ions towards the mass analyser (110, 111). The charged droplets move through the evaporation chamber where a drying gas, N_2, assist in the desolvation process where the surface charge of the droplets increase until it releases the gaseous ions and the solvent is fully evaporated (110, 111). A nozzle and a skimmer are used to separate the analytes from other molecules before it enters the mass analyser (110).

2.7.3.2 Z-spray inlet

There exist various adaptations of ESI, where the utilization of a Z-spray inlet is one of them (110). In comparison with conventional inlets, where the trajectory of the highly charged droplets is linear, the trajectory of Z-spray follows, as the name implies, a Z-shape (110). This shape is more effective in separating the generated ions from the solvent as the electrode attracts the smaller charged droplets while both larger charged droplets and neutrals travels straight forward (110, 112). Also, the shape leads to less build-up on the skimmer resulting in the performance characteristics of the instrument remaining stable (110).

2.7.3.3 Mass analyser – Quadrupole analyser

After ESI, the gaseous ions must be separated based on their m/z ratio before they reach the detector. This separation can be performed by a quadrupole mass analyzer where the separation is based on the ions different trajectories in an oscillating electric field (113). The quadrupole mass analyzer consists of four identical rods arranged parallel to each other where the opposite rods form a pair that is applied the same voltage (113). One of the pairs is applied a certain direct current and radiofrequency, while the other pair is applied the opposite, thus creating an oscillating electrical field (102). The applied voltage is selective,

meaning that only ions with a certain m/z ratio will reach the detector (102). Ions with greater or smaller m/z ratios will either collide with the rods or end up outside the axis to the detector (102, 113).

2.7.4 Tandem mass spectrometry

Tandem mass spectrometry, commonly referred to as MS/MS, is when two or more mass analysers are utilized during analysis. A popular MS/MS instrument is the triple quadrupole where three quadrupoles are linearly combined (113). The three quadrupoles are often denoted as Q1, Q2 and Q3. Both Q1 and Q3 function as mass filters and analysers whereas Q2 is acting as a collision cell (113). With MS/MS a precursor ion is chosen in Q1 and transferred to Q2 where collision induced dissociation (CID) occurs. In the collision cell, an inert collision gas, e.g. Ar or N_2 (114), will fragment the precursor ion into product ions. The product ions will then be filtered, and one or more chosen ions will be analysed. This makes it possible to detect selected fragmentation patterns (113).

Triple quadrupole mass analysers can be utilized in different scan modes, depending on the desired information (113). Selected reaction monitoring (SRM), also known as multiple reaction monitoring (MRM), is the most common mode in quantitative analysis of complex matrices (114). This is because of its heightened sensitivity and selectivity which is due to the absence of scanning (113). In SRM, both Q1 and Q3 filter based on selected m/z ratios, therefore only selected precursor ions that produce fragments of a specific m/z ratio will be detected (113). There may be several product ions where the ion with the highest abundancy and signal is used for quantification. This allows for accurate quantification.

2.8 Quantification

2.8.1 Internal standard

A sample preparation should consists of as few steps as possible to avoid both sample loss and contamination (101). Addition of an internal standard (IS) prior to the sample preparation can compensate for the possible loss of analyte (108). This is due to the known concentration of added IS and the relationship between the analyte and the IS being proportional, meaning that loss of analyte involves the loss of IS with a known concentration. If several analytes are detected, it is recommended to use several internal standards (102, 108).

2.8.1 Calibration curves and linearity

When the concentrations of analytes in a sample is to be determined, a calibration curve is utilized. The calibration curve is constructed by linear regression where the response of known concentrations of the analytes in standard solutions are plotted against the response of the unknown concentrations of analytes in the sample (101). The standard solutions are prepared with relevant concentrations regarding the expected concentration range of the analytes in the sample. The linearity of the constructed calibration curve is important during quantification as the linear dynamic range is used to determine the unknown concentrations (101). To assess if the calibration curve is a good fit for the plotted data points, the regression coefficient (R^2) should be close to $R^2 = 1$ as this indicate that the calibration curve is a perfect fit. A $R^2 = 0$ indicate a bad linearity (101).

2.8.2 Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) can be regarded as a measurement of a method's sensitivity (101), and are important parameters when interpreting the obtained data from an analytical analysis (115). The LOD can be defined as the lowest amount of an analyte in a sample that with confidence can be differentiated from the background noise (101) whereas LOQ is defined as the lowest concentration of the analyte that with an acceptable accuracy and precision can be determined (101). If more than one analyte are analyzed, the LOD and LOQ must be determined separately for each analyte (108).

There exist different approaches for determining the LOD and LOQ (115) where one method is by a visual inspection. In this method, the instrumental LOQ (iLOQ) is visually determined based on the linear dynamic range of the constructed calibration curve. The iLOQ is determined as lowest concentration in the linear range whereas the instrumental LOD (iLOD) can be determined by dividing the iLOQ by 3. The equation for calculating the iLOD is shown in Equation 2.

$$\mathrm{iLOD} = \frac{\mathrm{iLOQ}}{3} \tag{2}$$

The method LOD (mLOD) and method LOQ (mLOQ) are the limits for the entire analytical method (115). The determination of mLOD and mLOQ are based on spiked matrix blanks that went through the entire analytical process (101, 115).

2.8.5 Principal Component Analysis (PCA)

Principal component analysis (PCA) is a statistical technique used to display large quantities of data in a simplified manner. Multivariate data can consist of several dimensions and attributes for each data point, and it can be difficult to find patterns and correlations between variables (116). In a PCA plot the number of dimensions is reduced without losing valuable information, making a visualization of the data easier (117). Instead of discarding any variables, they are compressed into principal components which constitute the axes of the plot (116). In a two-dimensional PCA plot, the data points are presented in a scatterplot and the axes are made up of the two principal components (PC) that accounts for the majority of the variations in the provided data set. A greater variation is accounted for by PC1 than PC2 (117), and they are independent of each other and have different measurement units (118). A type of PCA is a PCA biplot which is a merge of a normal PCA plot and loadings vectors. Loading vectors show the correlation between the variables and the principal components with the angles and direction in regards to the principal components being significant (118).

3 Method

3.1 Chemicals and materials

3.1.1 Chemicals and standard solutions

Analytical standard of both Bps and BzPs of high purity were purchased from Sigma-Aldrich (Steinheim, Germany). Target analyte standards included bisphenol A (BpA, ≥ 99%), bisphenol AF (BpAF, ≥ 99%), bisphenol AP (BpAP, ≥ 99%), bisphenol B (BpB, ≥ 98%), bisphenol F (BpF, ≥ 98%), bisphenol M (BpM, ≥ 99%), bisphenol P (BpP, ≥ 99%), bisphenol S (BpS, ≥ 98%), bisphenol Z (BpZ, ≥ 99%), 4-hydroxybenzophenone (4-OH-BzP, ≥ 98%), benzophenone-1 (BzP-1, ≥ 99%), benzophenone-2 (BzP-2, ≥ 97%), benzophenone-3 (BzP-3, ≥ 98%) and benzophenone-8 (BzP-8, ≥ 98%). Isotopically labelled internal standards (ISs), BpA-$^{13}C_{16}$ (99%), BpAF-$^{13}C_6$ (99%), BpS-$^{13}C_6$ (98%) and BzP-3-$^{13}C_6$ (99%) were obtained from Cambridge Isotope Laboratories in Andover MA, USA. β-Glucuronidase (*Helix pomatia*, type HP-2, aqueous solution, ≥ 100.000 units mL^{-1}) was purchased from Sigma-Aldrich (Steinheim, Germany). Methanol (MeOH, analytical grade) and ammonium hydroxide (25% v/v) was purchased from Merck (Darmstadt, Germany). Ammonium acetate (≥ 98% w/w) and ethyl acetate ($CH_9CO_2CH_2CH_3$, ≥ 99.7% v/v) were obtained from VWR Chemicals (Trondheim, Norway). The water used was purified with a Milli-Q water grade system (Q-option, Elga, Labwater, Veolia Water Systems LTD, U. K.).

3.1.2 Calibration curve

A 9-point calibration curve was constructed with standard solutions of the target analytes and the internal standards (IS, 20 ppb). The analyte concentrations were 0, 0.2, 0.5, 1, 2, 5, 10, 20, 50 ng mL^{-1} with MeOH as a solvent.

3.2 Sample collection

The sampling of the three species, *P. perna*, *S. lalandi* and, *S. sagax*, was executed by the non-profit and charitable organization The Sustainable Seas Trust (SST) which goal is to protect the marine resources of Africa. All the samples were collected in 2022, pre-preparated, and sent to the Norwegian University of Science and Technology (NTNU), around two years after the sample collection of Castro et al. (1). SST is the owner of the data used in this thesis.

3.2.1 Mussels

P. perna (n=137) were collected in Algoa Bay, South Africa in January (n=67) and September (n=70) 2022. The mussels were obtained from 7 sites inside and outside Algoa Bay, the same sites as in Castro et al. (1), with sites 1-3 being inside the Bay area whereas sites 4-7 were located south and southwest outside the Bay, as seen on Figure 1. In Table 5, the sites with associated names and descriptions are provided. Ten mussels were collected at each site except for site 3 during the January sampling where there were harvested 7 mussels because of high ocean swells. All collected mussels were placed in labelled polypropylene (PP) plastic bags and put on ice before transported to the SST lab in Port Elizabeth.

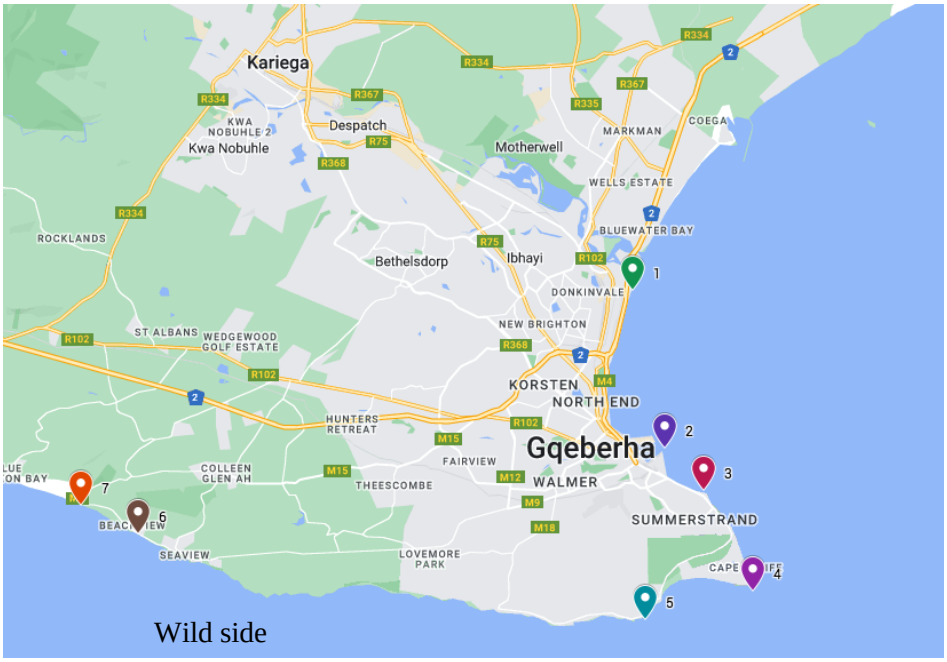


Figure 1: Illustration of the seven mussel sample sites, the same as in Castro et al. (1). Map was created using Google Earth

Table 5: Mussel sites in Algoa Bay, South Africa with associating descriptions gathered from Castro et al. (1) and Fourie et al. (119)

Sites	Description	Details
1	Sewage works	Daily used by fishermen and beachgoers. An outlet from a WWTP is close to the sampling point.
2	Harbour wall	Not frequently used, but open area.
3	Something good	Popular beach used daily by fishermen and beachgoers. Stormwater outlets are near the sampling sites (119)
4	Cape Recife	Permit is needed for access and an outlet from a WWTP is close to the sampling point.
5	Noordhoek	A more rural area, not frequently used.
6	Beachview	Beachgoers and fishermen use the area daily. A small river containing wastewater effluent discharge on the beach.
7	Maitland	Beachgoers and fishermen use the area daily. Perennial river that runs through agricultural land discharge near the sampling site.

3.2.2 Fish

S. lalandi (n=11) were in May 2022 provided by anglers competing in a fishing competition at the Noordhoek Ski Boat Club. The fish were predominantly caught by the anglers at the Wild side of Algoa Bay, as seen in Figure 1. A portion of fillet from the tail-end of the fish was removed and placed in labelled pre-washed PP bags, placed on ice until frozen at −20℃. The pilchard (*S. sagax*, n=20) were received frozen and individually wrapped in July 2022, donated by a local fishery that caught their produce in Algoa Bay. The fish were frozen at −20℃.

3.3 Sample preparation

The sample pre-preparation took place in the laboratory of SST in Port Elizabeth, South Africa. The mussels were removed from their shell and with a stainless-steel spatula scraped into 25 mL PP tubes. The fish were slightly thawed before the preparation. A small portion of the edges of the *S. lalandi* samples were cut off to minimize possible contamination from the cutting that took place in the field. For the *S. sagax*, the entire fillet of one side of the fish were utilized as sample. This was carried out with stainless-steel equipment. The skin of the fish was removed before the samples were placed into 80 mL PP tubes. The wet mass of the samples was recorded before the sample tubes were placed in a freezer at −20℃ for a minimum of 12 hours. The samples were then freeze-dried (1 mbar, −50℃, Beta 1–8 LSCBasic (Martin Christ Gefriertrocknungsanlagen GmbH; Germany)) and the mass recorded, before the samples were

homogenized in their respective PP tubes with the use of a stainless-steel homogenizer. The rest of the analytical process was executed in the laboratory at the Norwegian University of Science and Technology.

3.3.1 Extraction

The extraction procedure of the analytes was executed in accordance with Castro et al. (1) with minor modifications. Approximately 0.1 g of freeze-dried mussel or fish sample was transferred to a labelled PP tube and then spiked with $10\ \mathrm{ng\ mL^{-1}}$ internal standard (IS). Next, the sample was added 1 mL of ammonium acetate (NH_4OAc, 1 M) and ultrasonicated for 45 min, before 1 mL NH_4OAc (1 M) containing 44 units of $\beta-$glucuronidase was added. 44 units of $\beta-$glucuronidase equals to 100 µL of $\beta-$glucuronidase in 100 ml of NH_4OAc (1 M). Then, the sample was incubated for a minimum of 12 hours at 37℃. An LLE was carried out with addition of 4 mL ethyl acetate (AcOEt) before the sample was shaken for 1 hour (150 rpm) and centrifuged for 10 minutes (4000 rpm). The supernatant was collected, and this sequence was repeated a total of three times (3×4 mL AcOEt) where the supernatant was combined in a new PP tube. The combined supernatant was added 2 mL Milli-Q water, shaken for 5 minutes and centrifuged for 10 minutes (4000 rpm). Lastly, under a gentle nitrogen (N_2) stream, the supernatant was concentrated to close to dryness before 1 mL methanol (MeOH) was added.

3.4 UPLC-MS/MS

The instrumental analysis is identical to the one applied in Castro et al. (1). The analysis of the 14 target analytes were carried out with the use of an UPLC-MS/MS instrument with a triple quadrupole MS (Xevo TQ-S) using a Z-spray ESI source being connected to an Acquity UPLC I-Class system. All were obtained from Waters (Milford, MA, U.S). The utilized mobile phase consisted of a gradient with a mixture of Milli-Q grade water containing 0.1 % v/v ammonium acetate (A) and MeOH (B) and had a constant flow of 300 µL min^{-1} with an injection volume of 4 µL. The programmed mobile phase gradient is given in Table 6.

Table 6: The programmed mobile phase gradient consisting of a mixture of Milli-Q grade water with 0.1 % v/v ammonium hydroxide (A) and MeOH (B). The flow rate was constant at 300 µL min^{-1}.

Time (min)	A (%)	B (%)
0	75	25
0.1	25	75
3.7	1	99
4.6	75	25

The utilized SP was a Kinex C18 EVO column (50×2.1 mm, 1.7 µm), coupled with a C18 security guard (2 2.1 mm i. d.), purchased from Phenomenex (Torrance, CA, U. S.). The temperature of the UPLC precolumn and column were kept constant at 30 ℃. The ionization source was ESI with a negative ionization mode (ESI-) with applied N_2 (350 ℃, 650 Lh^{-1}) gas. The capillary voltage was -1.5 kV with a cone voltage of 50 B and source offset voltage at 30 V. The mass spectrometer was run in SRM mode, and the MS parameters are provided in Castro et al. (1) and information about the target analytes and associated IS is provided in Appendix D in Table D1. The added internal standard (IS) solutions were 100 ppm and 50 µL of each were added to the samples.

3.5 Method Performance Characteristics

Method performance characteristics for the method was validated in Castro et al. (1).

Field and laboratory blanks were made, both in the SST lab in Port Elizabeth and in the lab at NTNU. The response of the blanks was subtracted from the sample set to account for any contamination occurring during the analytical process.

3.6 Data processing and treatment

The data presented in this thesis was processed using different softwares. The UPLC-MS/MS data was obtained with MassLynx (v4.1), and the integrations were performed with the use of TargetLynx (Waters, Milford, U.S.). The data was processed, and descriptive statistics were carried out with Excel (Microsoft, 2019), and the PCA biplot were constructed with the use of Past4. The map of the sample sites for *P. perna* was created using Google Earth. All concentrations were reported as ng g^{-1} dry weight (d.w.), except the EDI values that were reported as ng kg^{-1} body weight (b.w.) day^{-1}.

4 Results and Discussion

4.1 Method Performance Characteristics and Quantification

The method performance was validated by Castro et al. (1).

4.1.1 Linearity

The constructed calibration curves that were utilized to estimate the concentrations of the 14 target analytes all had a regression coefficient $R^2 > 0.994$, suggesting that the curves were a good fit given that the value were close to $R^2 = 1$. The R^2 for all analytes are presented in Appendix D, Table D1.

4.1.2 Limit of detection and limit of quantification

The instrumental limit of quantification (iLOQ) was visually decided from the constructed calibration curves. The limits for all analytes were determined to be the concentration at the lowest end of the linear range. From the iLOQ, the instrumental limit of detection (iLOD) was calculated with Equation 2 presented in chapter 2.8.2. The iLODs ranged from 0.2 to 1.0 ng mL^{-1}, whereas the iLOQ spanned from 0.6 to 3.0 ng mL^{-1}. The iLODs and ILOQs for the target analytes are presented in Table B1, provided in Appendix B. All of the concentrations that fell below the iLOD were excluded from the data set in further calculations.

At a nominal mass of 0.1 g sample, the method limit of detection (mLOD) and the method limit of quantification (mLOQ) were calculated. The mLODs and mLOQs for the Bps and BzPs are presented in Table 7. The mLOD for the target analytes ranged from 2 to 10 ng g^{-1}, while mLOQ spanned from 6 to 30 ng g^{-1}.

Table 7: Method limit of detection (mLOD) and method limit of quantification (mLOQ) for the target analytes.

Analyte	mLOD (ng g^{-1})	mLOQ (ng g^{-1})
BpA	2	6
BpB	2	6
BpAF	5	15
BpAP	2	6
BpF	5	15
BpM	2	6
BpP	2	6
BpS	2	6
BpZ	10	30
4-OH-BzP	5	15
BzP-1	2	6
BzP-2	2	6
BzP-3	5	15
BzP-8	2	6

4.2 Descriptive statistics and concentrations

The concentrations of Bps (n=9) and BzPs (n=5) were quantified in the three species *P. perna* (n=137), *S. lalandi* (n=11) and *S. sagax* (n=20) collected from Algoa Bay, South Africa. The concentrations were estimated through constructed calibration curves with the relative response as a function of the concentration of the target analytes and IS. Out of the 14 target analytes, five Bps (BpA, BpAF, BpAP, BpF, BpS) and 4 BzPs (BzP-1, BzP-2, BzP-3, BzP-8) were detected and had concentrations above iLOD. Compounds (BpB, BpM, BpP, BpZ, 4-OH-BzP) with concentrations below iLOD is excluded from further investigation.

4.2.1 *P. perna*

Among the 14 target analytes there were four Bps analogues (BpA, BpAF, BpAP, BpS) and four BzPs analogues (BzP-1, BzP-2, BzP-3, BzP-4) that were detected in the mussel samples collected in both months. For these compounds the concentrations were detected and descriptive statistics such as detection frequency (DF), median, mean, minimum (Min) and maximum (Max) values were calculated. The data is presented in Table 8.

Table 8: Concentrations and descriptive statistics of the detected target analytes in *P. perna* sampled in January and September 2022 in Algoa Bay, South Africa

Site	Descriptive statistics	Concentrations (ng g^{-1})									
		BpA	BpAF	BpAP	BpS	BzP-1	BzP-2	BzP-3	BzP-8	$\Sigma_{(4)}$Bps	$\Sigma_{(4)}$BzPs
1	DF (n= 20) %	20	55	60	10	5	90	10	25	70	90
	Median	9.27	8.74	34.1	7.70	4.31	11	5.79	3.37	37.4	12.2
	Average	10.2	8.39	64.7	7.70	4.31	12.0	5.79	3.85	66.0	14.0
	Min	2.53	4.70	2.36	4.11	4.31	3.15	5.05	2.18	3.60	3.15
	Max	19.6	9.82	180	11.3	4.31	26.3	6.52	7.23	189	32.8
2	DF (n=20) %	25	50	50	55	n. d.	70	15	10	95	80
	Median	20.9	9.68	20.2	34.7	n. d.	5.06	5.69	5.74	34.8	5.38
	Average	26.2	9.82	30.3	31.6	n. d.	7.77	9.01	5.74	46.3	9.21
	Min	12.2	8.45	1.87	13.0	n. d.	1.58	5.38	5.19	19.2	1.58
	Max	49.7	11.9	78.2	45.1	n. d.	23.9	16.0	6.28	138	23.9
3	DF (n= 17) %	18	47	59	53	6	77	18	12	94	88
	Median	6.04	7.38	40.2	49.5	6.79	6.80	6.44	2.40	52.8	6.80
	Average	6.81	7.76	53.6	51.3	6.79	8.65	6.49	2.40	67.5	9.56
	Min	1.97	5.38	2.87	42.7	6.79	2.01	6.09	1.97	42.7	2.01
	Max	12.4	10.6	204	61.0	6.79	20.6	6.94	2.82	211	27.1
4	DF (n=20) %	n. d.[a]	50	60	50	5	80	15	15	95	90
	Median	n. d.	10.3	48.7	10.4	3.78	5.93	7.50	2.38	43.0	7.21
	Average	n. d.	9.60	64.4	11.0	3.78	7.26	7.65	2.43	51.5	8.34
	Min	n. d.	7.31	6.51	4.01	3.78	2.28	5.69	2.16	9.65	2.16
	Max	n. d.	11.0	189	16.2	3.78	16.8	9.76	2.73	200	16.8
5	DF (n =20) %	5	50	65	40	5	75	15	5	90	80
	Median	3.23	7.56	21.7	11.8	6.97	5.25	4.94	4.31	17.7	8.05
	Average	3.23	7.16	36.2	12.0	6.97	7.21	3.96	4.31	35.6	8.21
	Min	3.23	0.90	2.8	4.39	6.97	0.99	0.87	4.31	5.97	1.85
	Max	3.23	9.93	136	16.6	6.97	16.9	6.09	4.31	161	16.9
6	DF (n= 20) %	20	50	70	25	5	90	20	5	95	90
	Median	10.3	7.89	22.3	14.9	3.95	9.11	5.60	5.89	19.3	10.2
	Average	10.8	8.08	20.7	12.3	3.95	11.8	5.51	5.89	25.0	13.6
	Min	6.46	7.07	1.23	1.66	3.95	0.57	4.48	5.89	2.41	0.57
	Max	16.3	9.13	57.8	19.1	3.95	31.5	6.35	5.89	81.6	36.0
7	DF (n= 20) %	40	45	70	55	5	75	5	20	85	85
	Median	15.5	10.5	29.2	8.99	4.18	10.1	5.08	5.11	47.0	10.1
	Average	17.3	12.6	51.6	9.99	4.18	9.22	5.08	4.61	63.8	9.77
	Min	12.8	8.38	21.8	5.04	4.18	2.83	5.08	2.75	12.4	3.62
	Max	25.0	24.2	105	16.0	4.18	14.4	5.08	5.46	147	17.1

All sites	DF (n= 137) %	18	50	62	41	4	80	14	13	89	86
	Median	13.7	8.73	31.6	15.1	4.25	7.90	6.09	3.69	35.1	8.68
	Average	15.1	9.03	45.5	21.5	5.00	9.30	6.32	3.97	49.7	10.5
	Min	1.97	0.90	1.23	1.66	3.78	0.57	0.87	1.97	2.41	0.57
	Max	49.7	24.2	204	61.0	6.97	31.5	16.0	7.23	211	36.0

BpB, BpF, BpM, BpP, BpZ and 4-OH-BzP is not included as their concentration <iLOD and had a DF=0

[a] not detected

The mussel samples (n=137) were collected in January and September 2022. The total DF for $\Sigma_{(4)}$Bps and $\Sigma_{(4)}$BzPs for all sites were 89 and 86% respectively. The highest DFs were for BzP-2 (80%) and BpAP (62%). In Castro et al. (1) the four highest DFs had an order of magnitude BpA (100%) > BzP-2 (99%) > BpAP = BpS (91%). In this thesis, the DF for BpA was determined to be 18% which is five times lower than in the preliminary study. The order of magnitude for pollutants, based on the median concentrations of all sites, was BpAP (31.6) > BpS (15.1) > BpA (13.7) > BzP-2 (7.90) > BpAF (8.73) > BzP-3 (6.09) > BzP-1 (4.25) > BzP-8 (3.69 ng g^{-1}). The median concentration of $\Sigma_{(4)}$Bps and $\Sigma_{(4)}$BzPs for the seven sites were 35.1 and 8.68 ng g^{-1} respectively. This indicates that the estimated concentrations of BzPs were generally lower than that of Bps.

In this project the estimated BpA concentrations varied from 1.97 to 49.7 ng g^{-1}. These concentrations differ from other studies where higher concentrations have been detected. In Gatidou et al. (120), the estimated BpA concentrations in mussels (*Mytilus galloprovincialis, Madiola barbatus, Venus gallina*) collected in the coastal areas of Greece had a range of 209 to 626 ng g^{-1} d.w. The sampling in Gatidou et al. (120) took place in 2006, before restrictions on the use of BpA in food materials were implemented in the E.U. (121). Another study that also assessed the concentration of BpA in mussels sampled before the regulations were enforced also had a higher estimated concentration range of 3.30 to 714 ng g^{-1} d.w. (122). These concentration ranges are larger than the concentrations detected in *P. perna* in this thesis, and in Castro et al. (1) where the concentrations varied from 7.70 to 129 ng g^{-1} d.w..

Newer research examining the occurrence of BpA in *Mytilus galloprovincialis* in the Slovenian coastal waters estimated a significantly smaller concentration range of 0.03 to 0.22 ng g^{-1} w.w (123). This decrease in concentrations in mussels sampled in Europe may reflect the regulations, indicating that the application of BpA is decreasing. The usage and production of PC plastics containing BpA in food contact materials are unregulated in African countries with South Africa being the only country to have banned the use of

BpA in baby bottles (124). This may a contributing factor to why the estimated concentrations in this thesis and in Castro et al. (1) were higher than in the European samples. However, other factors may also influence this.

The occurrence of the other Bps analogues in mussels are less investigated (13). Out of the three other Bps analogues detected (BpAF, BpAP, BpS), only BpS has been detected in other studies, except for in Castro et al. (1). In Liao and Kannan (7), mussels (n=186) collected in the Chinese Bohai Sea in the period of 2006-2015 had an estimated concentration range of BpS of n.d. to 4.68 ng g^{-1} d.w. with a DF = 1.08%. In contrast, the range of BpS in this thesis was 1.66 to 61.0 ng g^{-1} with a DF = 41%. As BpS is considered as the main replacement of BpA (25, 27), the elevated concentrations in the samples from Aloga Bay may indicate that the production of BpS is increasing.

Similarly to the concentrations estimated in this thesis, the concentration of BpAP was the highest of the Bps analogues in Castro et al. (1) with a median concentration of all seven sites at 150 ng g^{-1} d.w.. In addition to BpS, BpAP is another major substitute for BpA with a variety of applications from electronics to personal care products (28). The detected concentrations of BpAP may indicate that BpAP is used to substitute BpA in different applications.

The median concentration of $\Sigma_{(4)}$BzPs for the seven sites was 8.68 ng g^{-1}, and the estimated concentrations were generally lower than that of Bps. This is the same trend as in the preliminary study were the ΣBzPs was an eightfold lower than ΣBps (1). BzP-2 was the benzophenone analogue with highest DF (80%) and highest median concentration (7.90 ng g^{-1}). As with the Bps, there are limited studies conducted on the occurrence of BzPs in mussels, and except for Castro et al. none have detected BzP-2 in mussels (1).

The other BzPs analogues have been detected in various studies, which are reporting higher concentration ranges than reported in this thesis (1). The occurrence of BzPs in mussels was investigated in Liao and Kannan (7). In this study the concentration range of BzP-3 was n.d. to 58.6 ng g^{-1}, while the ranges for BzP-1 and BzP-8 was n.d.-1.31 and n.d.-1.59 ng g^{-1} d.w. respectively. In contrast to this, a study by Cunha et al. reported higher concentrations for BzP-1 (n.d.-94.2 ng g^{-1} d.w.) than for BzP-3 (n.d.-85.5 ng g^{-1} d.w.) (96). In this thesis BzP-3 had the second highest median concentration at 6.09 ng g^{-1} and a concentration range

of 0.87 to 16.0 ng g^{-1}, while BzP-1 had a range of 3.78 to 6.97 ng g^{-1} and a median estimated concentration of 4.25 ng g^{-1}.

There can be several factors influencing the occurrence of BzPs in mussels. Even though BzP-3 is most applied, BzP-1, BzP-2 and BzP-8 also have applications as UV-filters and additives in plastics and personal care products (7). Although, the use of BzP-1 and BzP-8 have been prohibited for use in cosmetics in the E.U. (45), suggesting that the detected levels may be lower because of this. In contrast to this, the higher concentration of BzP-1 of BzP-8 may be due to them being major metabolites in the degradation of BzP-3 (45), especially BzP-1. BzP-2 are also a metabolite of BzP-3 (14).

4.2.1.1 Comparison of the two sampling periods

This project analyzed *P. perna* samples that were collected in January and September 2022. This allows for a seasonal comparison of the occurrence and abundance of Bps and BzPs in mussels. Descriptive statistics including the median, mean, min and max concentrations of the detected analytes are presented in Table C1and Table C2 provided in Appendix C for January and September samples respectively.

The P. Perna samples (n=67) collected in January had a $\Sigma_{(4)}$Bps DF = 100% meaning that one or more of the bisphenol analogues were detected in every mussel sample. For $\Sigma_{(4)}$BzPs, the frequency was lower at DF = 82%. The Bps analogues generally showed higher DFs than the BzPs, except that of BsP-2 that had a DF = 75%. For all sites, the median concentrations for The $\Sigma_{(4)}$Bps and $\Sigma_{(4)}$BzPs were determined at 48.2 and 13.7 ng g^{-1} respectively with the major contributors being BpAP (39.9 ng g^{-1}) and BzP-2 (12.4 ng g^{-1}). BpAP was the Bps with the overall highest median concentrations at all seven sites with a range spanning 21.1 (site 2) to 62.6 ng g^{-1} (site 7), whereas BzP-2 had the highest median concentrations at every site from 5.50 (site 7) to 17.9 ng g^{-1} (site 1).

The *P. perna* samples (n=70) collected in September 2022 had $\Sigma_{(4)}$Bps DF = 79% whereas the $\Sigma_{(4)}$BzPs DF = 90% for all seven sites. Four of the detected analytes (BzP-1, BzP-1, BpA, BpAF) were only detected in two out of the 70 mussel samples. The median concentrations of all sites for Bps varied from 5.04 (BpAF) to 16.0 ng g^{-1} (BpA), with the $\Sigma_{(4)}$Bps being 19.3 ng g^{-1}. For the BzPs analogues, the $\Sigma_{(4)}$BzPs for the sites is 5.97 ng g^{-1} where the main contributor is BzP-3 (6.22 ng g^{-1}).

When comparing the determined concentrations in the mussel samples, it is evident that less Bps and BzPs were detected in the samples from September than those from January. In September, only three of the target analytes had DFs above 20%. These were BpAP (37%), BpS (56%) and BzP-2 (84%). For the January samples, all analytes had DF above 20% except for BzP-1 (6%) and BzP-3 (13). The median concentration for the January samples were generally higher than for September samples except for BpS (15.5), BpA (16.0) and BzP-3 (6.22 ng g^{-1}).

4.2.2 Concentrations and statistics for Bps and BzPs in *S. lalandi* and *S. sagax*

The fish samples were captured at two different areas in the Bay area and consisted of samples of *S. lalandi* (n=11) and *S. sagax* (n=20). The *S. lalandi* and the *S. sagax* samples were collected in May and July respectively. The target analytes detected in the fish samples differed and where five Bps (BpA, BpAF, BpAP, BpF, BpS) and three BzPs (BzP-1, BzP-2, BzP-8) were detected in *S. sagax*, the detected analytes in *S. lalandi* excludes BpF, BpAP and BzP-1 as showed in Table. For both species the DF = 100 and DF = 87% for ΣBps and ΣBzPs respectively, with the major contributors being BpAF (100%) and BzP-8 (65%). The median concentrations were 63.4 ng g^{-1} for ΣBps and 5.58 ng g^{-1} for ΣBzPs with BpA (127 ng g^{-1}) and BzP-2 (15.2 ng g^{-1}). BpF was only detected in one *S. sagax* sample.

For *S. lalandi* the DF values varied from 9 to 100% for det detected compounds with the order of magnitude BpAF (100%) > BpS (73%) > BzP-8 (64) > BpA = BzP-2 (9%). For $\Sigma_{(3)}$Bps the median concentration was 16.9 ng g^{-1}. The *S. sagax* samples had $\Sigma_{(5)}$Bps DF = 100% with DF values for all the detected analytes of BpAF (100%) > BpA (100%) > BzP-8 (65%) > BzP-1 (45%) > BpAP (40%) > BpS = BzP-2 (30%) > BpF (5%). The median $\Sigma_{(3)}$Bps for S. sagax was 138 ng g^{-1}.

Out of all *S. lalandi* samples, BpA was only detected in one sample (2.79 ng g^{-1}) a level that is on line with studies performed by Ismail et al. (0.078-0.322 ng g^{-1} d.w.) (92), Gil-Solsona et al. (<LOD-1.5 ng g^{-1} d.w.) (94) and Barboza et al. (1.0-9.1 ng g^{-1} d.w.) (35). These studies were conducted in Spain, Malaysia, and Portugal respectively. In contrast of this, the median concentration for BpA in *S. sagax* was 130 ng g^{-1} with a concentration range of 43.5 to 189 ng g^{-1}. This is much higher than the levels detected in the aforementioned studies. A study performed in the Gulf of Gdansk, Poland, reported levels from 19.7 to 798.4 ng g^{-1} d.w. in three different fish species (93). The reported levels may reflect that the fish samples

used in the study was sampled before the restrictions on BpA in Europe were implemented. Also, the concentrations of the different fish species in the mentioned studies varied, indicating that comparing the concentrations in *S. lalandi* and *S. sagax* with other species may not cover all aspects.

The detected concentrations for BpAF in *S. lalandi* varied from 10.7 to 25.4 ng g^{-1}, whereas in *S. sagax* this range was 7.25-15.2 ng g^{-1}. Both species had DF = 100% for BpAF. Lower levels than this was reported in Gil-Solsana et al. at <LOD to 11.6 ng g^{-1} d.w. in various saltwater species samples in Ebro Delta in Spain (94). BpAF was not detected in Barboza et al. (35). Literature suggests that BpAF are more persistent than the other Bps analogues (29, 125), which may be a reason for the detected concentrations.

BpS was detected in concentrations ranging from <LOD to 4.2 ng g^{-1} d.w. in Gil-Solsana et al., with a DF =80% for 23 samples (94). In comparison, *S. lalandi* had concentrations of 2.42 to 13.7 ng g^{-1} while the concentrations for *S. sagax* varied from 4.37 to 25.3 ng g^{-1}. The levels detected in this project are higher than those reported in literature. BpAP was detected in *S. sagax* with a median concentration of 9.96 ng g^{-1}, however, the pollutant was not detected in the Barboza et al. This may reflect the type of pollution released in Algoa Bay, as well as indicating that BpS and BpAP have an increased production as replacements of BpA.

4.3 Species differences

A visual presentation of the determined concentrations for all the three species are presented in boxplots provided in Figure 2. The plots are based on the ΣBps and ΣBzPs concentrations detected in the three species at the different locations. Outliers are marked as an *x*. The variance of the concentrations is displayed as whiskers and show that the largest variance was for ΣBps concentration detected in *S. sagax*, and that ΣBps for *S. lalandi* had little variation in comparison.

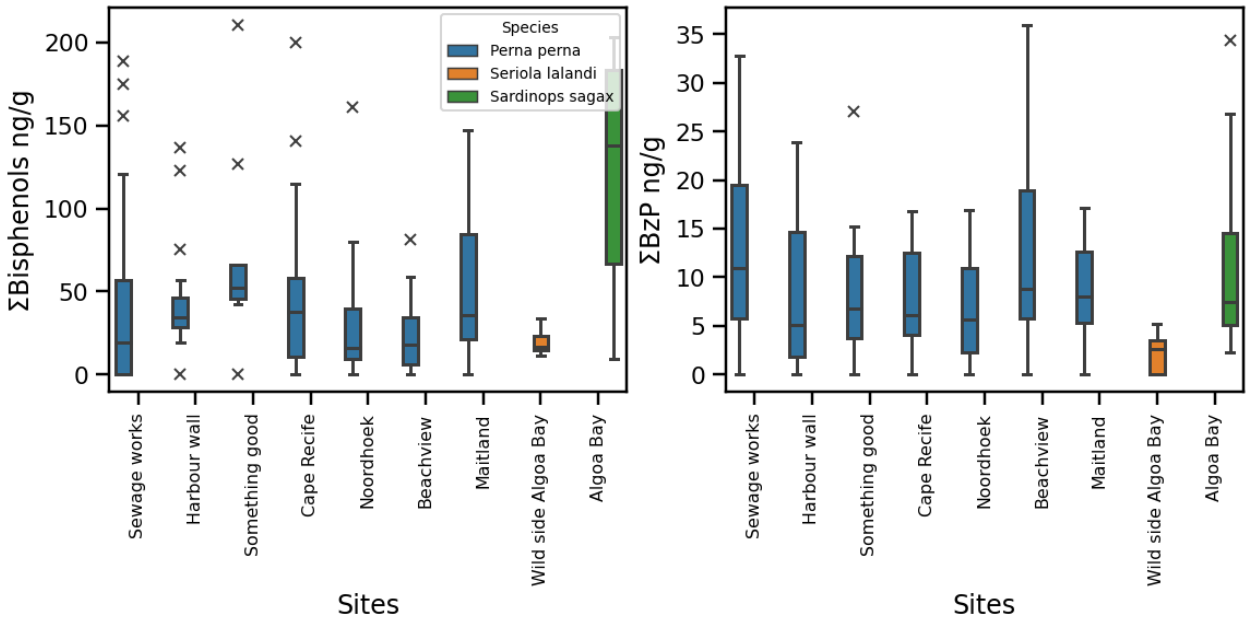


Figure 2: Box-plots of detected ΣBps (left) and ΣBzPs (right) concentrations in *P.perna* (blue), *S. lalandi* (Orange) and *S. sagax* (green)

When comparing the DFs and determined concentrations for *P. perna*, *S. lalandi* and *S. sagax* it is evident that the Bps and BzPs analogues have different occurrence rates and abundance. For instance, BpA had a DF = 90% and a median concentration of 130 ng g^{-1} for *S. sagax*, and a DF = 9% and a median concentration of 2.79 ng g^{-1} for *S. lalandi*. In *P. perna*, the median concentration for all sites were 13.7 ng g^{-1} with a DF = 18%. The species have different lifestyles, dietary intake, trophic levels and size, and these factors may all influence the occurrence of the pollutants (126). Factors such as rates of biodegradation and excretion of the pollutants may also contribute. In addition to this, the sampling occurred at different times of the year suggesting that environmental factors such as seasonal differences may play a role. Therefore, a comparison between the species must be carried out with caution, and more research investigating these environmental and biological factors are needed.

About 10% of all plastic waste ends up in the marine environment (126) and there is a concern surrounding the leaching of pollutants such as Bps and BzPs from the plastics. South Africa has a large problem with marine plastic pollution (66) and effluent from WWTPs can be an important source of microplastics containing organic pollutants (34, 126). Microplastics can be ingested by marine organisms, especially filter-feeders, and a study by Barboza et al. (35) found evidence of an association between the amount of

microplastics in fish tissue and the concentration of Bps. *P. perna* and *S. sagax* are both filter feeders and may have a higher concentration of microplastics as the filtering does not differentiate between food and plastics. This may explain why the concentrations detected in *P. perna* and *S. sagax* is higher than for *S. lalandi* which preys on other fish. However, this can also be caused by other factors as the occurrence of microplastics in the species was not investigated in this project. One factor can be that the fish were caught just outside of Algoa Bay, while the other species were collected in the Bay area or in coastal waters.

The habitat of the species in relation to the pollution release sources may also be a factor. The mussels are benthic and was located closer to the coast where are more anthropogenic activity. Hydrophobic pollutants, such as Bps and BzPs, have shown tendencies to adsorb onto particles and accumulate in the marine sediments (127) this may indicate that the compounds are not carried far away from the release point in the seawater. This may explain why the mussels generally had higher detection frequencies than e.g. *S. lalandi*. However, *S. sagax* had much higher concentrations of BpA. In summary, more research is needed to get insight in the differences in abundance and occurrence rate in the different species.

4.4 Geographical distribution of Bps and BzPs

To get a better insight in the potential local release sources of Bps and BzPs analogues in *P. perna*, an overview of the geographical distribution of the pollutants were constructed and are presented in Figure 3 and Figure 4 for Bps and BzPs respectively. A geographical distribution of the pollutants was also addressed by Castro et al. at the same locations; therefore, this part will contain a brief comparison. The median concentrations for mussels sampled in both sampling periods were used and $\Sigma_{(4)}$Bps had a range of 44.3 to 103.2 ng g^{-1} with an order of magnitude for $\Sigma_{(4)}$Bps being site 3 (103) > site 2 (85.5) > site 4 (69.5) > site 1 (59.8) > site 7 (64.2) > site 6 (55.3) > site 5 (44.3 ng g^{-1}). The median concentration range of $\Sigma_{(4)}$BzPs was 16.9 to 24.6 ng g^{-1} with a distribution order of site 6 (24.6) > site 1 (24.5) > site 7 (24.4) > site 3 (22.4) > site 5 (21.5) > site 4 (19.6) > site 2 (16.5 ng g^{-1}).

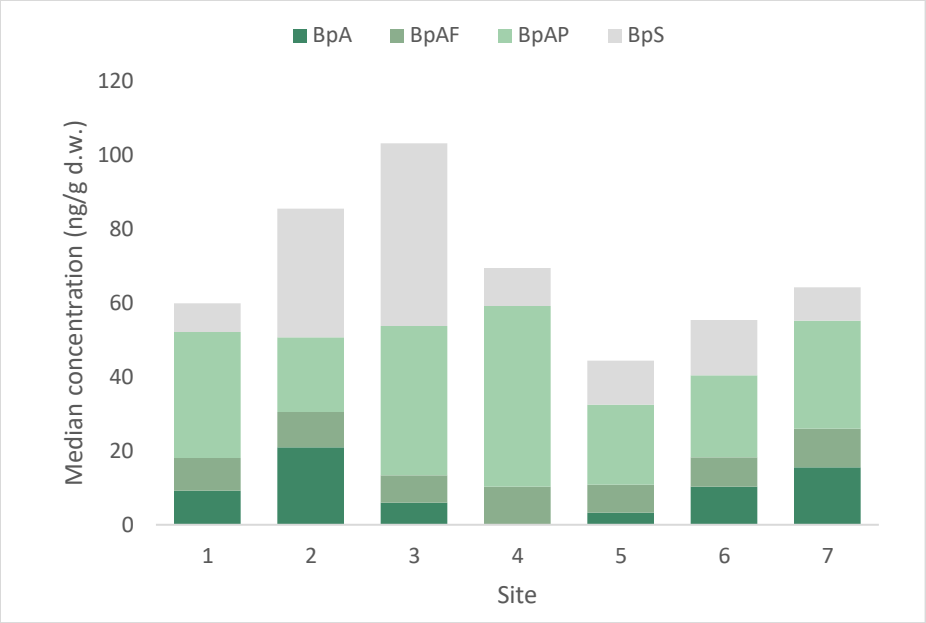


Figure 3: Illustration of the geographical distribution of Bps based on the $\Sigma_{(4)}$Bps concentration in *P. perna* samples from both months

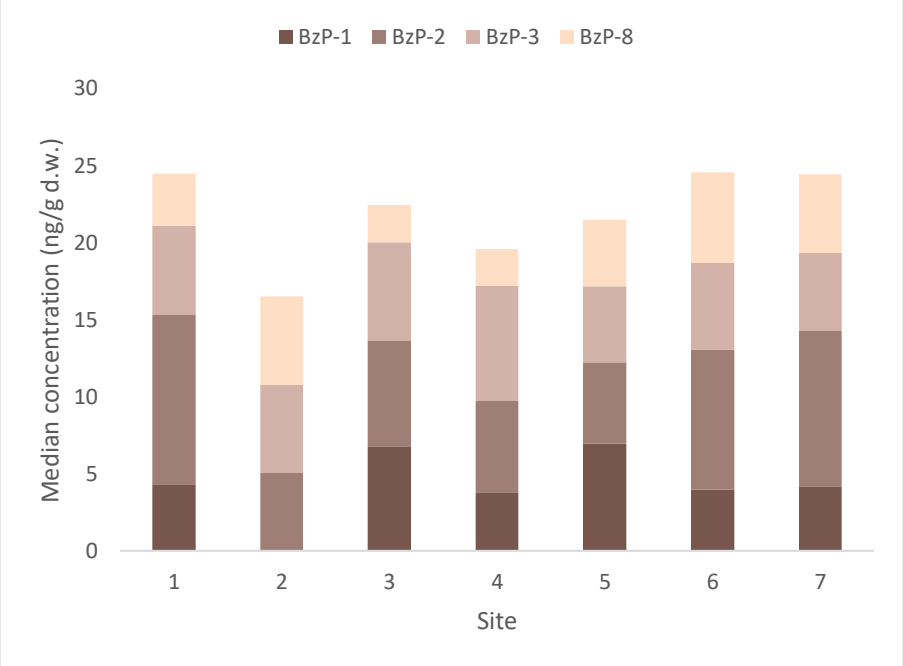


Figure 4: Illustration of the geographical distribution of BzPs based on the $\Sigma_{(4)}$BzPs concentration in *P. perna* samples from both months

The different sites are, as illustrated in Figure 1, located inside and outside of the Bay area. Site 1-3 are located inside Algoa Bay whereas site 4-7 are located west and southwest of the Bay. There are variations in the anthropogenic influence in the sampling areas as some areas are located more remotely than others and are used less frequently by boaters, fishermen and beachgoers. This may influence the concentrations of Bps and BzPs in these areas. The geographical distribution of Bps and BzP in this project does not follow the same pattern as in Castro et al. (1) and there exist a great variation in the geographical distribution of the January and September samples. Illustrations of the geographical patterns in the samples from January and September are provided respectively as Figure E1 in Appendix E. The entirety of this suggests that the release and uptake of the contaminants are influenced by many factors.

Like in Castro et al. there are greater variations in the concentrations of ΣBps at the different sites, than for the ΣBzPs where three of the sites (1, 6, and 7) had nearly similar concentrations at 24.6, 24.5 and 24.4 ng g^{-1} respectively. In this project, the lowest detected concentrations of ΣBps were at site 5 (44.3 ng g^{-1}) which corresponds with the reported geographical pattern in Castro et al. where site 5 also had the lowest detected median concentrations (1). The median concentration of ΣBzPs was 21.5 ng g^{-1}. Site 5 is in an area which is harder to access by the public and it is further away from residential areas. However, there is boat traffic in the area.

The site with the highest concentration of ΣBps in this project is site 3 (median 103 ng g^{-1}), which is daily used by both beachgoers and fishermen. Nearby the sampling site, there are stormwater outlets that can carry pollution from nearby residential areas into the coastal waters. For ΣBzPs the median concentration was 22.4 ng g^{-1}. Compared to the other sites, this site may by more influenced by direct emission from beachgoers. BzPs are commonly used in personal care products such as sunscreens, hair products and cosmetics (44) which may be washed off the skin and hair during swimming. With the site being inside the Bay area, the ocean currents may carry pollution from estuaries such as Swartkops that may include agricultural runoff, wastewater effluent and microplastics (70, 71).

Sampling site 1 and 4 were situated close to WWTPs. The very outlet of a WWTP were situated at site 1 and there was a smell of sewage during sampling. The location is a popular spot for fishermen and is closest to the mouth of the Swartkops estuary. This suggests that the levels of pollutants may be high at this location. The concentrations of ΣBps and ΣBzPs were 59.8 and 24.5 ng g^{-1} respectively. Site 4 has restricted

access and is located on the tip of the Bay area, a location that may contribute to the ΣBps and ΣBzPs concentrations of 69.5 and 19.6 ng g^{-1} (median) by boat traffic and effluent from nearby WWTPs.

Sampling site 6 is located outside of the Bay near an urban area and is used daily by both beachgoers and fishermen. There are several stormwater outlets and smaller river outlets that discharge close to the site, and Castro et al. and Fourie et al. have suggested that these rivers contain domestic wastewater from the residential area close by (1, 119). The median concentrations of ΣBps and ΣBzPs at site 6 were 55.3 and 24.6 ng g^{-1} respectively. In Castro et al. the major contributor at site 6 was BpAP with concentration 686 ng g^{-1} (median). BpAP are used in body-wash and hair-products (28) and may therefore contribute to the high concentrations.

Site 2 had the lowest median concentration for ΣBzPs at 16.5 ng g^{-1} but second highest concentration for ΣBps (85.5 ng g^{-1}). The sampling site is in the harbour and the mussels were attached to the harbour wall. The area is affected by boat traffic going in and out of the harbour, and there is a non-perennial river nearby that runs through urban settlement (119). The area is not frequently used for recreational activities, however just as with site 1 and 3, because of their position, effluent from nearby WWTPs, microplastics and polluted river water may have been carried to the site by ocean currents.

The median concentrations of ΣBps and ΣBzPs were 64.2 and 24.4 ng g^{-1} for site 7 which is located at a popular beach near the outlet av an estuary (119). The estuary runs through agricultural areas and may carry and deposit pollution into the marine coastal areas. It is reported by Castro et al. and Fourie et al. to open-closed (1, 119), however the status of the estuary for the samples in this project is not known.

4.4.1 Principal Component Analysis (PCA)

A PCA biplot for the determined ΣBps and ΣBzPs concentrations, and the three species, was constructed to examine the differences between the sites. The PCA biplot is provided in Figure 5. Every species was assigned a value and the minimum concentration was set as 0.01 ng g^{-1} to give every data point a value. The two first PCs accounted for 75% of the variance, where PC1 and PC2 accounted for 43 and 32 % respectively.

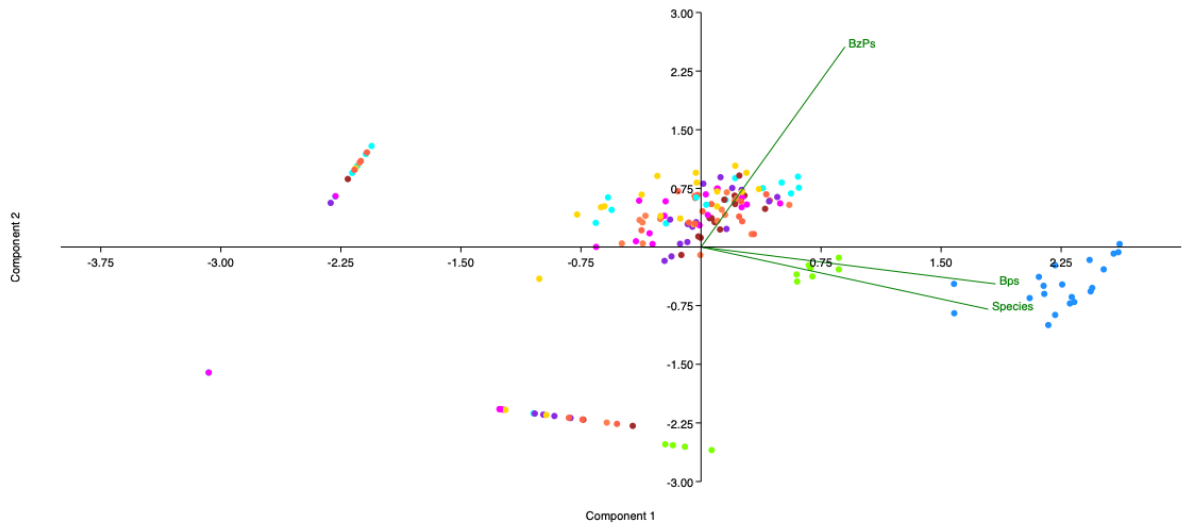


Figure 5: PCA plot with variables ΣBps concentrations, ΣBzPs concentrations, and species as nominal values. Component 1 (PC1) and Component 2 (PC2) accounts for 43 and 32% of the variance respectively.

Three separate clusters can be observed in the PCA biplot. The largest cluster located in the middle of the plot are the data points for the mussel samples. These points are in proximity to each other and have no obvious pattern, not allowing for a separation of the values. This indicates that the concentrations of ΣBps and ΣBzPs in the mussel samples at the different locations are relatively similar. The two other clusters are located on the right side of the biplot and consists of scattered data points in lime green and blue, whereas the blue cluster is located at the rightmost area of the plot. The lime green points are the values for *S. lalandi* while the blue points are the values of *S. sagax*. As with the concentrations provided in Table 8, the PCA plot display the values of *S. sagax* as having higher concentrations of ΣBps than of ΣBzPs. This is indicated by the location of the *S. sagax* data points being at the rightmost area of the plot in the same direction as the ΣBps loadings vector.

The loading vectors of a biplot give information about the correlation between the variables and the principal components (118). In this biplot, the angle between the loading vectors for ΣBps and ΣBzPs are relatively large which indicate that there is a low correlation between the contaminants at these sampling sites. This suggests that it is not possible to extract information about one of the chemical groups from

analyzing the other, and it is necessary to investigate the occurrence and abundance of both Bps and BzPs analogues when carrying out analysis.

To investigate this further, another PCA plot with a larger number of variables were constructed and is provided in Figure 6. The same minimum concentration of 0.01 ng g^{-1} were used and the plotted variables were ΣBps concentrations, ΣBzPs concentrations, sites and the three species. The PC1 and PC2 accounted for 16 and 12% of the variance respectively, however the plot displays the same patterns as depicted in Figure 2 where the plots are gathered in three clusters. The grey crosses are for the *S. lalandi* samples collected in Wild Side Algoa Bay, while the yellow squares are *S. sagax* that were collected in Algoa Bay. The largest cluster are the mussel samples.

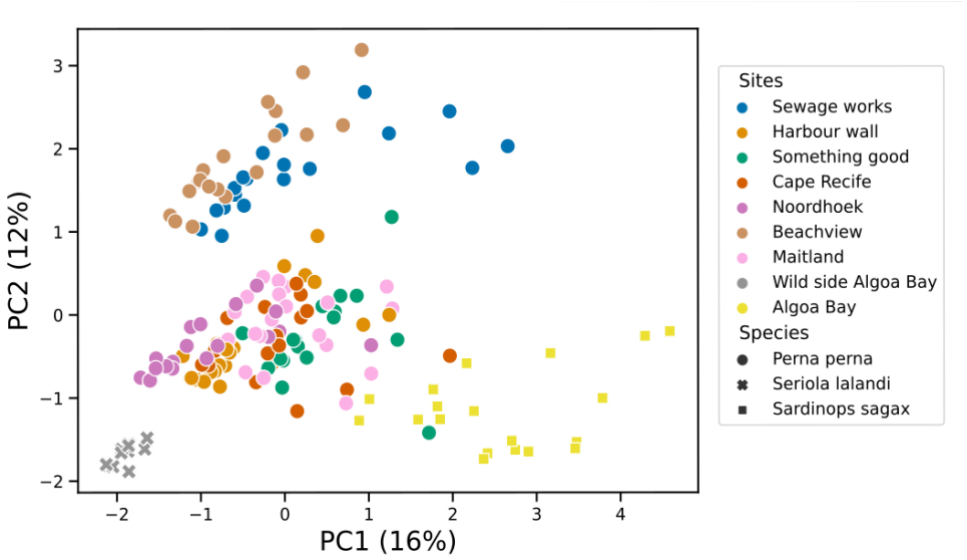


Figure 6: PCA plot with the variables: ΣBps concentrations, ΣBzPs concentrations, sites and the three species. PC1 and PC2 accounted for 16 and 12% respectively.

4.5 Potential exposure and health risk assessment

The contaminants examined in this thesis are all classified as pollutants with potential endocrine disrupting abilities that may have damaging effects such as reprotoxicity (82). Therefore, it is important to assess the risk these compounds may have on human health by dietary intake of mussels and fish. This was executed

by the calculation of an estimated daily intake (EDI) with Equation 1, which will be compared to the TDI limits sat by EFSA. The EDIs were calculated separately for the fish and the mussels, and for the compounds that had concentrations above the iLOD.

For comparison reasons, the average body weight of South Africans was set to the same value as in Castro et al. (1) at 70 kg. The same nominal value was also used in Nekhoroshkov et al. (128) and is the recommended weight for adults to use in risk assessments proposed by EFSA (129). The concentrations of the Bps and BzPs analogues used to calculate the EDIs are the median and maximum concentrations to account for median and high exposure scenarios, in the same way as in Castro et al. (1). As the concentrations were estimated based on the dry weight of the mussel and fish samples, the concentrations were converted to wet weight. For the mussels, the average moisture content is estimated to be 80% (1, 7).

Mussels are an important part of the diet of South Africans (1, 130), especially for people living in coastal areas. The average consumption of mussels varies between individuals, due to several factors such as age, dietary preferences, and geographic location (128). For instance, the average mussel consumption in Europe is higher than in South Africa (128). The assumed average consumption of mussels used in this thesis is the same as in Castro et al. (1) and Nekhoroshkov et al. (128) at 200 g w.w. per week which is 26.6 g w.w. per day. The calculated EDIs for the Bps and BzPs analogues detected in *P. perna* are presented in Table 9 and Table 10 respectively.

Table 9: Estimated daily intake (ng kg^{-1} b.w. day^{-1}) for Bps analogues in *P. perna*.

	Sites	BpA		BpAF		BpAP		BpS		$\sum_{(4)}$Bps	
		Median	Max	Median	Max	Median	Max	Median	Max	Median	Max
Both months	1	0.76	1.60	0.71	0.80	2.79	14.67	0.63	0.92	3.05	15.43
	2	1.71	4.06	0.79	0.98	1.65	6.39	2.84	3.69	2.84	11.17
	3	0.49	1.02	0.60	0.87	3.29	16.63	4.04	4.98	4.31	17.22
	4	n.d.[a]	n.d.	0.85	0.90	3.98	15.45	0.85	1.33	3.51	16.33
	5	0.26	0.26	0.62	0.81	1.77	11.09	0.97	1.35	1.45	13.17
	6	0.84	1.33	0.64	0.75	1.82	4.73	1.22	1.56	1.58	6.66
	7	1.27	2.05	0.86	1.97	2.38	8.57	0.73	1.31	3.84	12.02
	All sites	1.12	4.06	0.71	1.97	2.58	16.63	1.23	4.98	2.87	17.22

BpB, BpF, BpM, BpP and BpZ was excluded from calculations because of concentrations <iLOD

[a] not detected

Table 10: Estimated daily intake (ng kg^{-1} b.w. day^{-1}) for BzPs analogues in *P. perna*.

	Sites	BzP-1		BzP-2		BzP-3		BzP-8		$\sum_{(4)}$BzP	
		Median	Max	Median	Max	Median	Max	Median	Max	Median	Max
Both months	1	0.35	0.35	0.90	2.15	0.47	0.53	0.28	0.59	1.00	2.68
	2	n.d.[a]	n.d.	0.41	1.96	0.46	1.31	0.47	0.51	0.44	1.96
	3	0.55	0.55	0.56	1.68	0.53	0.57	0.20	0.23	0.56	2.21
	4	0.31	0.31	0.48	1.38	0.61	0.80	0.19	0.22	0.59	1.38
	5	0.57	0.57	0.43	1.38	0.40	0.50	0.35	0.35	0.66	1.38
	6	0.32	0.32	0.74	2.57	0.46	0.52	0.48	0.48	0.83	2.94
	7	0.34	0.34	0.82	1.18	0.41	0.41	0.42	0.45	0.82	1.40
	All sites	0.35	0.57	0.65	2.57	0.50	1.31	0.30	0.59	0.71	2.94

4-OH-BzP was excluded from calculations because of concentrations <iLOD

[a] not detected

The calculated EDIs for the $\Sigma_{(4)}$Bps have median concentrations spanning 1.45 to 4.31 ng kg^{-1} b.w. day^{-1} with the median concentration for all sites being 2.87 ng kg^{-1} b.w. day^{-1}. For the median $\Sigma_{(4)}$Bps the major contributor was BpAP 2.58 ng kg^{-1} b.w. day^{-1}. The median concentrations for $\sum_{(4)}$BzP was 0.7 ng kg^{-1} b.w. day^{-1} with values for all sites ranging from 0.44 to 1.00 ng kg^{-1} b.w. day^{-1}. For the benzophenone analogues, the largest contributors were BzP-2 at 0.65 ng kg^{-1} b.w. day^{-1} and BzP-3 with a concentration of 0.50 ng kg^{-1} b.w. day^{-1}. Both median EDIs for Bps and BzPs are significantly lower than the values reported in Castro et al. (1), where the median EDI for all sites for ∑Bps and ∑BzPs were 13.2 and 1.64 ng kg^{-1} b.w. day^{-1} respectively. Also, the concentrations for a maximum exposure scenario for both pollutants were lower than in the preliminary study (1). The maximum EDIs for $\Sigma_{(4)}$Bps and $\sum_{(4)}$BzP were estimated to be 17.22 and 2.94 ng kg^{-1} b.w. day^{-1} respectively.

Both fish species investigated in this thesis are important food sources, especially the pilchard that is commonly canned (63). According to the Food and Agriculture Organization of the United Nations (FAO), the global fish consumption increased from 9.0 to 20.3 kg per capita per year in the span of 1990 to 2018 (131). This increase would indicate that the recommended weekly fish consumption for adults of about 300 g per week (132) are being met. However, the fish consumption of South Africans is reported as being significantly lower than this at 7.4 kg per capita per year (133) equivalently to a daily intake of 20.3 g. Consequently, this daily consumption rate was used in the EDI calculations to make the estimate more realistic. As the reported moisture content in fish usually falls within the range of 60-80 % (134), the moisture content used to convert d.w. to w.w. for both fish species was the median value of 70%. The EDI values for *S. lalandi* and *S. sagax* for Bps and BzPs are shown in Table 11 and Table 12 respectively.

Table 11: Estimated daily intake (ng kg^{-1} b.w. day^{-1}) for Bps analogues in *S. lalandi* and *S. sagax*.

Species	BpA		BpAF		BpAP		BpF		BpS		$\sum_{(5)}$Bps	
	Median	Max	Median	Max	Median	Max	Median	Max	Median	Max	Median	Max
S. lalandi	0.24	0.24	1.31	2.21	n.d.[a]	n.d.	n.d.	n.d.	n.d.	n.d.	1.47	2.93
S. sagax	11.33	16.46	0.90	1.32	0.48	1.06	1.68	1.68	0.80	0.80	11.98	17.70
Both species	11.06	16.46	0.95	2.21	0.48	1.06	1.68	1.68	0.80	0.80	5.51	17.70

BpB, BpM, BpP, BpZ was excluded from calculations because of concentrations <iLOD

[a] not detected

Table 12: Estimated daily intake (ng kg^{-1} b.w. day^{-1}) for BzPs analogues in *S. lalandi* and *S. sagax*.

Species	BzP-1		Bzp-2		BzP-3		BpZ-8		$\sum_{(4)}$BzPs	
	Median	Max	Median	Max	Median	Max	Median	Max	Median	Max
S. lalandi	0.29	1.19	n.d.[a]	n.d.	0.24	0.24	0.23	0.32	0.30	0.45
S. sagax	0.87	2.20	0.28	0.44	1.51	2.33	0.49	0.92	0.65	2.99
Both species	0.50	2.20	0.28	0.44	1.32	2.33	0.33	0.92	0.49	2.99

4-OH-BzP was excluded from calculations because of concentrations <iLOD

[a]not detected

As previously mentioned, only BpA and BpAF was detected in *S. lalandi* out of the nine target Bps compounds, whereas BzP-1, BzP-3 and BzP-8 were detected out of the BzPs. For both *S. lalandi* and *S. sagax* the median $\sum_{(5)}$Bps and $\sum_{(4)}$BzPs EDIs were estimated to be 5.51 and 0.49 ng kg^{-1} b.w. day^{-1} respectively. For *S. lalandi* the main contributors were BpAF (1.31 ng kg^{-1} b.w. $day^{-1)}$ for Bps and BzP-1 (0.29 ng kg^{-1} b.w. day^{-1}) for BzPs. BpAF has been suggested as being a xenoestrogen with higher activity than BpA (135). For *S. sagax* the major contributors were BpA (11.33 ng kg^{-1} b.w. day^{-1}) and BzP-3 (1.51 ng kg^{-1} b.w. day^{-1}). BpA has showed adverse effects on both humans and wildlife at small concentrations (136). The EDIs calculated for a high exposure scenario in both species are 17.70 and 2.99 ng kg^{-1} b.w. day^{-1} for $\sum_{(5)}$Bps and $\sum_{(4)}$BzPs respectively.

The TDI limits set by EFSA for BpA and BzP were at 0.2 ng kg^{-1} b.w. day^{-1} and 30 µg kg^{-1} b.w. day^{-1} respectively (36, 83). Exceeding these limits may pose a significant health risk for humans (82). The EDIs for both the median and maximum exposure scenarios for ∑Bps in fish and mussels exceed the limit of 0.2 ng kg^{-1} b.w. day^{-1}. In mussels, the median ∑Bps for all sites are higher by almost a sixfold, whereas the maximum risk exposure is higher by a twentyfold. For the fish species, the total median ∑Bps EDI is higher than the TDI by a twofold, and the maximum is 15 orders of magnitude higher. This suggests that the consumption of mussels and fish pose a threat to human health, and that the exposure of the pollutants are concerning. For BzPs, none of the calculated EDIs presented are above the TDI of 30 µg kg^{-1} b.w. day^{-1} which is 20 000 times larger than the limit for BpA. The highest maximum EDI of 2.99 µg kg^{-1} b.w. day^{-1} (fish, $\sum_{(4)}$BzPs) is 10 000 orders of magnitude smaller than the TDI, indicating that the occurrence of BzPs in fish and mussel pose no threat to the human population.

The daily dietary intake limit of 20.3 g for fish may be a bit conservative, and by calculating an EDI with the recommended dietary intake of 300 g per week, the EDIs for Bps would exceeds the TDI limit of 0.2 ng kg^{-1} b.w. day^{-1}. Also, as the population in coastal areas generally have a higher consumption of seafood than people living inland (128), the adposed risk of adverse health effects may be higher for them. As the potential adverse effects in other Bps analogues are less studies, the high concentration of BpAF in a popular fish for consumption. *S. sagax*, may pose a higher risk for humans as the compound has shown evidence of bioaccumulating in the tissue in rats (29, 125).

Since the study conducted by Castro et al. (1) were published, the t-TDI was replaced with a new limit (83). As a result of this, the Bps EDI concentrations estimated in the research have exceeded the TDI sat by EFSA. The new, lower, limit ensured that all the EDIs in the study are above the tolerable threshold and therefore poses a risk for human health. However, the limits for BzPs have not changed and the EDIs in that study were lower than the TDI.

To conduct a more in-depth risk assessment of the exposure of Bps and BzPs it is not sufficient to solely look at the EDI for fish and mussels. This is because the compounds are ubiquitous, and humans can be exposed through other food stuff and through nondietary exposure routes such as dermal exposure (25, 30, 137). This may especially be the case for BzPs analogues which are used more in personal care products that are applied directly on the skin (10). Huang et al. (138) compared data from literature on the occurrence

of BpA in urine samples, where the world estimated daily exposure level of BpA was above 30 ng kg^{-1} b.w. day^{-1}. For children, this estimate was two orders of magnitude higher at 60.08 ng kg^{-1} b.w. day^{-1} (138). This may suggest that the consumption of mussels or fish is a smaller part of a larger issue when discussing exposure routes and the potential adverse effects of Bps and BzPs. Also, this indicates that more research on characterizing the different exposure routes is needed. However, this is beyond the scope of this thesis.

5 Conclusion

The aim of this project was to assess the occurrence of Bps (n=9) and BzPs (n=5) in biological samples of *P. perna* (n=137), *S. lalandi* (n=11) and *S. sagax* (n=20) sampled in Algoa Bay, South Africa. Research on the occurrence of Bps analogues, other than BpA, in aquatic organisms is limited, and restrictions on the use of BpA are resulting in the replacement of BpA with other Bps analogues that may have potential adverse effects on marine ecosystems and human health. The research is also limited on the occurrence of BzPs UV-filters and their environmental fate. In total, five of the Bps (BpA, BpAF, BpAP, BpF, BpS) and four of the BzPs (BzP-1, BzP-2, BzP-3, BzP-8) were detected with concentrations above iLOD.

In mussels, the highest detection occurrence rate and abundance for Bps analogues was BpAP with DF = 62%, a median concentration of 31.6 ng g^{-1} and a range of 31.6-204 ng g^{-1}. For BzPs, BzP-2 had a DF = 80% with a median concentration of 7.90 ng g^{-1}. The concentration range of BzP-2 in mussels were 0.57-31.5 ng g^{-1}. All the detected target analytes, except for BpF were detected in *P. perna*. The two fish species had differences in abundance and detection frequency with *S. sagax* displaying a high median concentration and concentration range for BpA at 130 ng g^{-1} and 43.5-189 ng g^{-1} respectively. Also, BpF was detected in one *S. sagax* sample. On the other hand, less analytes were detected in *S. lalandi (*BpA, BpAF, BpS, BzP-2, BzP-8) where BpAF had a concentration range of 10.7-25.4 ng g^{-1} with a median concentration of 15.0 ng g^{-1}. For BzPs, the highest median concentration for *S. sagax* and *S. lalandi* was for BzP-2 (17.4 and 2.76 ng g^{-1} respectively). Out of the detected analytes, no BzP-3 was detected in the fish species. The decreased concentrations of BpA in comparison with concentrations of other studies and the preliminary study for this thesis indicate that it is being replaced by other Bps analogues.

The occurrence and abundance of Bps and BzPs varied with species and with sample site for the mussels. There were clear differences in the mussels sampled in January and September 2022, and factors such as seasonal variations, rainfall, trophic level, and habitat have been suggested to influence the release sources as well as the uptake of pollutants in the organisms. An assessment of the geographical distribution of the mussel samples suggested that anthropogenic activities such as recreational activities and effluent from WWTPs may be important sources of Bps and BzPs into the marine environment.

The EDIs of the pollutants were calculated and compared with the TDI limits sat by EFSA. The EDIs for ΣBps in *P. perna*, *S. lalandi* and *S. sagax*, at both median and maximum exposure scenarios, exceeded the

TDI, indicating that dietary intake of mussels and fish with Bps pose a health threat to humans. The EDIs for ΣBzPs were much lower than the TDI, suggesting that a dietary intake of mussels and fish do not pose a threat to humans regarding BzPs at the current limit.

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Appendices

Appendix A – Samples

Table A1: Determined concentrations (ng g^{-1} d.w.) of the detected Bps and BzPs in fish samples. Y = Yellowtail (*S. lalandi*, n = 11) and PP = Pilchard (*S. sagax*, n = 20)

	Concentrations (ng g^{-1} d. w.)									
Samples	BpA	BpAF	BpAP	BpF	BpS	BzP-1	BzP-2	BzP-8	$\Sigma_{(5)}$Bps	$\Sigma_{(3)}$BzP
Y1	2.79	15.42	n. d.	n. d.	13.67	n. d.	n. d.	3.63	31.88	3.63
Y2	n. d.[a]	23.32	n. d.	n. d.	3.37	n. d.	2.76	2.42	26.69	5.18
Y3	n. d.	15.69	n. d.	n. d.	n. d.	n. d.	n. d.	3.66	15.69	3.66
Y4	n. d.	15.04	n. d.	n. d.	5.03	n. d.	n. d.	2.67	20.06	2.67
Y5	n. d.	10.68	n. d.	n. d.	3.21	n. d.	n. d.	2.66	13.89	2.66
Y6	n. d.	13.12	n. d.	n. d.	2.56	n. d.	n. d.	2.16	15.68	2.16
Y7	n. d.	13.61	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	13.61	n. d.
Y8	n. d.	25.42	n. d.	n. d.	8.29	n. d.	n. d.	n. d.	33.71	n. d.
Y9	n. d.	14.05	n. d.	n. d.	2.82	n. d.	n. d.	3.42	16.87	3.42
Y10	n. d.	15.59	n. d.	n. d.	2.42	n. d.	n. d.	n. d.	18.01	n. d.
Y11	n. d.	11.36	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	11.36	n. d.
PP1	n. d.	9.57	n. d.	n. d.	n. d.	n. d.	n. d.	5.58	9.57	5.58
PP2	84.12	11.37	4.23	n. d.	n. d.	n. d.	n. d.	8.26	99.72	8.26
PP3	47.98	13.05	n. d.	n. d.	n. d.	n. d.	n. d.	4.86	61.03	4.86
PP4	183.11	10.68	6.66	n. d.	n. d.	4.37	n. d.	3.98	200.46	8.35
PP5	189.20	7.88	6.33	n. d.	n. d.	n. d.	n. d.	7.45	203.41	7.45
PP6	149.72	10.87	7.96	n. d.	n. d.	5.11	n. d.	n. d.	168.54	5.11
PP7	126.93	10.46	n. d.	n. d.	n. d.	3.22	n. d.	n. d.	137.39	3.22
PP8	68.92	9.31	n. d.	n. d.	n. d.	2.77	n. d.	3.03	78.22	5.80
PP9	127.11	10.80	n. d.	n. d.	n. d.	2.31	n. d.	n. d.	137.92	2.31
PP10	43.47	10.31	n. d.	n. d.	n. d.	2.14	n. d.	7.32	53.78	9.46
PP11	111.46	7.25	n. d.	n. d.	4.37	n. d.	12.10	6.96	123.08	19.05
PP12	n. d.	15.17	n. d.	n. d.	n. d.	n. d.	n. d.	2.32	15.17	2.32
PP13	171.86	9.35	12.18	n. d.	9.85	n. d.	26.82	n. d.	203.24	26.82
PP14	50.67	12.71	n. d.	n. d.	n. d.	3.76	n. d.	10.53	63.39	14.28
PP15	138.03	9.91	n. d.	9.15	n. d.	4.80	n. d.	n. d.	157.09	4.80
PP16	133.39	10.54	4.59	n. d.	n. d.	3.16	n. d.	2.70	148.52	5.87
PP17	154.93	8.92	3.70	n. d.	25.28	n. d.	15.23	n. d.	192.82	15.23
PP18	163.30	8.98	n. d.	n. d.	10.07	n. d.	24.53	9.79	182.35	34.33
PP19	159.72	8.30	4.76	n. d.	13.87	n. d.	19.59	5.60	186.65	25.19
PP20	47.03	14.37	n. d.	n. d.	6.52	n. d.	7.38	n. d.	67.92	7.38

[a] not detected, below LOD

Table A2: Determined concentrations (ng g^{-1} d.w.) of the detected Bps and BzPs in mussel (P. perna, n=137) samples from January (n=67) and September (n=70)

Sampling month	Samples	Concentrations (ng g^{-1} d. w.)									
		BpA	BpAF	BpAP	BpS	BzP-1	BzP-2	BzP-3	BzP-8	$\Sigma_{(4)}$Bps	$\Sigma_{(4)}$BzP
September	Site 1.1	n. d.[a]	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
	Site 1.2	n. d.	4.70	n. d.	n. d.	n. d.	6.00	n. d.	n. d.	4.70	6.00
	Site 1.3	n. d.	n. d.	n. d.	4.11	n. d.	3.82	n. d.	n. d.	4.11	3.82
	Site 1.4	n. d.	n. d.	n. d.	n. d.	4.31	8.33	n. d.	n. d.	n. d.	12.64
	Site 1.5	n. d.	n. d.	3.60	n. d.	n. d.	6.40	n. d.	2.18	3.60	8.58
	Site 1.6	n. d.	n. d.	n. d.	n. d.	n. d.	6.85	n. d.	n. d.	n. d.	6.85
	Site 1.7	n. d.	n. d.	n. d.	n. d.	n. d.	3.15	n. d.	n. d.	n. d.	3.15
	Site 1.8	n. d.	n. d.	n. d.	n. d.	n. d.	8.62	n. d.	n. d.	n. d.	8.62
	Site 1.9	n. d.	n. d.	n. d.	n. d.	n. d.	12.42	n. d.	4.00	n. d.	16.42
	Site 1.10	n. d.	n. d.	18.67	n. d.	n. d.	4.91	n. d.	n. d.	18.67	4.91
	Site 2.1	n. d.	n. d.	n. d.	39.67	n. d.	3.02	n. d.	n. d.	39.67	3.02
	Site 2.2	n. d.	n. d.	n. d.	45.11	n. d.	2.26	15.98	n. d.	45.11	18.24
	Site 2.3	n. d.	n. d.	31.93	43.53	n. d.	5.20	n. d.	n. d.	75.46	5.20
	Site 2.4	n. d.	n. d.	19.24	n. d.	n. d.	5.57	n. d.	n. d.	19.24	5.57
	Site 2.5	n. d.	n. d.	n. d.	n. d.	n. d.	2.56	n. d.	n. d.	n. d.	2.56
	Site 2.6	n. d.	n. d.	n. d.	35.02	n. d.	n. d.	5.69	n. d.	35.02	5.69
	Site 2.7	n. d.	n. d.	n. d.	34.74	n. d.	2.81	n. d.	n. d.	34.74	2.81
	Site 2.8	n. d.	n. d.	1.87	33.29	n. d.	1.85	n. d.	n. d.	35.16	1.85
	Site 2.9	n. d.	n. d.	n. d.	32.58	n. d.	1.58	n. d.	n. d.	32.58	1.58
	Site 2.10	n. d.	n. d.	n. d.	34.77	n. d.	4.92	n. d.	n. d.	34.77	4.92
	Site 3.1	n. d.	n. d.	n. d.	49.49	n. d.	3.62	n. d.	n. d.	49.49	3.62
	Site 3.2	n. d.	n. d.	n. d.	46.18	n. d.	6.80	n. d.	n. d.	46.18	6.80
	Site 3.3	n. d.	5.38	n. d.	60.62	n. d.	n. d.	n. d.	n. d.	66.00	n. d.
	Site 3.4	n. d.	n. d.	n. d.	42.66	n. d.	2.01	n. d.	n. d.	42.66	2.01
	Site 3.5	n. d.	n. d.	79.09	47.31	n. d.	11.03	n. d.	n. d.	126.41	11.03
	Site 3.6	n. d.	n. d.	n. d.	52.21	n. d.	n. d.	6.09	n. d.	52.21	6.09
	Site 3.7	n. d.	n. d.	5.17	61.00	n. d.	4.98	n. d.	n. d.	66.17	4.98
	Site 3.8	n. d.	n. d.	n. d.	46.19	n. d.	3.76	n. d.	n. d.	46.19	3.76
	Site 3.9	n. d.	n. d.	n. d.	n. d.	n. d.	5.59	n. d.	n. d.	n. d.	5.59
	Site 3.10	n. d.	n. d.	2.87	56.09	n. d.	5.28	6.94	n. d.	58.96	12.22
	Site 4.1	n. d.	n. d.	n. d.	n. d.	n. d.	9.49	n. d.	n. d.	n. d.	9.49
	Site 4.2	n. d.	n. d.	n. d.	9.65	n. d.	n. d.	n. d.	n. d.	9.65	n. d.
	Site 4.3	n. d.	n. d.	n. d.	15.47	n. d.	2.45	n. d.	n. d.	15.47	2.45
	Site 4.4	n. d.	n. d.	n. d.	10.07	n. d.	4.89	n. d.	n. d.	10.07	4.89
	Site 4.5	n. d.	n. d.	n. d.	9.88	n. d.	2.28	n. d.	n. d.	9.88	2.28
	Site 4.6	n. d.	n. d.	n. d.	11.11	n. d.	4.64	n. d.	n. d.	11.11	4.64

	Site 4.7	n. d.	n. d.	n. d.	10.72	n. d.	n. d.	5.69	n. d.	10.72	5.69
	Site 4.8	n. d.	n. d.	n. d.	15.53	n. d.	5.47	n. d.	n. d.	15.53	5.47
	Site 4.9	n. d.	n. d.	57.91	n. d.	n. d.	5.08	7.50	n. d.	57.91	12.58
	Site 4.10	n. d.	n. d.	6.51	16.24	n. d.	2.72	9.76	n. d.	22.75	12.48
	Site 5.1	n. d.	n. d.	80.15	n. d.	n. d.	11.76	n. d.	n. d.	80.15	11.76
	Site 5.2	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
	Site 5.3	n. d.	n. d.	n. d.	15.31	n. d.	3.42	n. d.	n. d.	15.31	3.42
	Site 5.4	n. d.	n. d.	n. d.	10.12	n. d.	n. d.	n. d.	n. d.	10.12	n. d.
	Site 5.5	n. d.	n. d.	7.37	n. d.	n. d.	3.81	4.94	n. d.	7.37	8.75
	Site 5.6	n. d.	n. d.	2.84	16.48	n. d.	2.50	n. d.	n. d.	19.32	2.50
	Site 5.7	n. d.	n. d.	n. d.	9.21	n. d.	n. d.	n. d.	n. d.	9.21	n. d.
	Site 5.8	n. d.	n. d.	5.43	10.69	n. d.	n. d.	6.09	n. d.	16.12	6.09
	Site 5.9	n. d.	n. d.	n. d.	13.00	n. d.	2.56	n. d.	n. d.	13.00	2.56
	Site 5.10	n. d.	n. d.	n. d.	n. d.	n. d.	3.18	n. d.	n. d.	n. d.	3.18
	Site 6.1	n. d.	n. d.	19.34	n. d.	3.95	8.40	6.35	n. d.	19.34	18.70
	Site 6.2	n. d.	n. d.	1.23	1.66	n. d.	0.57	n. d.	n. d.	2.90	0.57
	Site 6.3	n. d.	n. d.	34.42	n. d.	n. d.	5.33	n. d.	n. d.	34.42	5.33
	Site 6.4	n. d.	n. d.	n. d.	n. d.	n. d.	7.90	n. d.	n. d.	n. d.	7.90
	Site 6.5	n. d.	n. d.	n. d.	10.61	n. d.	n. d.	n. d.	n. d.	10.61	n. d.
	Site 6.6	n. d.	n. d.	8.22	15.41	n. d.	5.97	n. d.	n. d.	23.63	5.97
	Site 6.7	n. d.	n. d.	3.58	n. d.	n. d.	6.25	n. d.	n. d.	3.58	6.25
	Site 6.8	n. d.	n. d.	2.41	n. d.	n. d.	4.59	n. d.	n. d.	2.41	4.59
	Site 6.9	n. d.	n. d.	3.82	n. d.	n. d.	6.49	n. d.	n. d.	3.82	6.49
	Site 6.10	n. d.	n. d.	n. d.	14.89	n. d.	6.00	n. d.	n. d.	14.89	6.00
	Site 7.1	n. d.	n. d.	23.91	n. d.	n. d.	12.94	n. d.	n. d.	23.91	12.94
	Site 7.2	n. d.	n. d.	24.97	8.99	n. d.	8.08	n. d.	n. d.	33.96	8.08
	Site 7.3	n. d.	n. d.	n. d.	n. d.	n. d.	7.55	n. d.	n. d.	n. d.	7.55
	Site 7.4	n. d.	n. d.	n. d.	n. d.	n. d.	13.35	n. d.	n. d.	n. d.	13.35
	Site 7.5	14.71	n. d.	n. d.	n. d.	n. d.	13.47	n. d.	n. d.	14.71	13.47
	Site 7.6	n. d.	n. d.	n. d.	12.35	n. d.	3.62	n. d.	n. d.	12.35	3.62
	Site 7.7	n. d.	n. d.	n. d.	n. d.	n. d.	14.44	n. d.	n. d.	122.18	14.44
	Site 7.8	n. d.	n. d.	n. d.	n. d.	n. d.	11.70	n. d.	n. d.	23.69	11.70
	Site 7.9	n. d.	n. d.	n. d.	n. d.	n. d.	10.39	n. d.	n. d.	36.99	10.39
	Site 7.10	n. d.	n. d.	n. d.	n. d.	n. d.	10.07	n. d.	n. d.	n. d.	10.07
January	Site 1.1	n. d.	9.28	179.57	n. d.	n. d.	20.15	n. d.	3.37	188.85	23.53
	Site 1.2	n. d.	9.82	31.61	n. d.	n. d.	12.57	5.05	7.23	41.43	24.86
	Site 1.3	n. d.	8.67	24.63	n. d.	n. d.	10.02	n. d.	n. d.	33.29	10.02
	Site 1.4	2.53	7.98	36.60	n. d.	n. d.	12.47	n. d.	n. d.	47.10	12.47
	Site 1.5	19.55	9.38	58.00	n. d.	n. d.	17.90	n. d.	2.48	86.92	20.38
	Site 1.6	10.82	8.71	101.12	n. d.	n. d.	25.76	n. d.	n. d.	120.65	25.76
	Site 1.7	n. d.	8.93	15.19	n. d.	n. d.	11.95	n. d.	n. d.	24.12	11.95

Site 1.8	n. d.	9.29	146.31	n. d.	n. d.	26.28	6.52	n. d.	155.60	32.80
Site 1.9	7.72	8.74	158.34	n. d.	n. d.	19.21	n. d.	n. d.	174.80	19.21
Site 1.10	n. d.	6.76	2.36	11.30	n. d.	n. d.	n. d.	n. d.	20.42	n. d.
Site 2.1	49.68	9.46	77.60	n. d.	n. d.	16.41	n. d.	n. d.	136.74	16.41
Site 2.2	n. d.	11.48	5.86	13.06	n. d.	n. d.	n. d.	5.19	30.39	5.19
Site 2.3	12.18	10.55	n. d.	n. d.	n. d.	12.30	n. d.	n. d.	22.73	18.58
Site 2.4	n. d.	8.62	21.06	n. d.	n. d.	23.94	n. d.	n. d.	29.68	23.94
Site 2.5	34.30	9.97	78.24	n. d.	n. d.	14.05	n. d.	n. d.	122.51	14.05
Site 2.6	n. d.	11.94	44.89	n. d.	n. d.	12.35	5.38	n. d.	56.83	17.73
Site 2.7	20.88	8.45	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	49.01	n. d.
Site 2.8	n. d.	9.91	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	25.78	n. d.
Site 2.9	13.97	9.27	10.04	n. d.	n. d.	n. d.	n. d.	n. d.	33.28	n. d.
Site 2.10	n. d.	8.51	12.54	n. d.	n. d.	n. d.	n. d.	n. d.	21.05	n. d.
Site 3.1	n. d.	7.76	36.57	n. d.	n. d.	n. d.	n. d.	n. d.	44.33	6.79
Site 3.2	n. d.	10.13	33.36	n. d.	n. d.	20.61	6.44	n. d.	43.49	27.05
Site 3.3	n. d.	7.24	203.47	n. d.	n. d.	n. d.	n. d.	n. d.	210.72	n. d.
Site 3.4	6.04	7.28	52.91	n. d.	n. d.	12.37	n. d.	2.82	66.24	15.20
Site 3.5	1.97	7.47	43.91	n. d.	n. d.	12.55	n. d.	1.97	53.35	14.53
Site 3.6	n. d.	10.63	51.23	n. d.	n. d.	11.40	n. d.	n. d.	61.85	11.40
Site 3.7	12.43	6.20	27.15	n. d.	n. d.	12.42	n. d.	n. d.	45.78	12.42
Site 4.1	n. d.	11.04	96.16	7.49	n. d.	n. d.	n. d.	n. d.	114.69	n. d.
Site 4.2	n. d.	11.02	32.00	n. d.	3.78	9.43	n. d.	2.38	43.01	15.59
Site 4.3	n. d.	7.31	25.32	n. d.	n. d.	16.84	n. d.	n. d.	32.64	16.84
Site 4.4	n. d.	10.70	189.13	n. d.	n. d.	13.56	n. d.	n. d.	199.83	13.56
Site 4.5	n. d.	8.07	46.43	n. d.	n. d.	6.45	n. d.	n. d.	54.50	6.45
Site 4.6	n. d.	10.58	49.17	4.01	n. d.	n. d.	n. d.	2.16	63.76	2.16
Site 4.7	n. d.	8.74	131.53	n. d.	n. d.	4.88	n. d.	n. d.	140.27	4.88
Site 4.8	n. d.	10.42	39.87	n. d.	n. d.	6.39	n. d.	2.73	50.29	9.12
Site 4.9	n. d.	7.89	50.59	n. d.	n. d.	7.96	n. d.	n. d.	58.49	7.96
Site 4.10	n. d.	10.27	48.25	n. d.	n. d.	13.66	n. d.	n. d.	58.51	13.66
Site 5.1	n. d.	6.02	58.50	n. d.	n. d.	14.27	n. d.	n. d.	64.52	14.27
Site 5.2	n. d.	8.95	135.71	16.56	6.97	2.40	n. d.	4.31	161.22	13.69
Site 5.3	n. d.	9.32	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	9.32	n. d.
Site 5.4	n. d.	6.36	34.06	n. d.	n. d.	7.36	n. d.	n. d.	40.42	7.36
Site 5.5	3.23	8.06	21.67	n. d.	n. d.	16.92	n. d.	n. d.	32.97	16.92
Site 5.6	n. d.	9.93	25.44	4.39	n. d.	5.25	n. d.	n. d.	39.76	5.25
Site 5.7	n. d.	7.90	20.41	n. d.	n. d.	13.62	n. d.	n. d.	28.32	13.62
Site 5.8	n. d.	0.90	5.07	n. d.	n. d.	0.99	0.87	n. d.	5.97	1.85
Site 5.9	n. d.	7.21	67.50	n. d.	n. d.	10.61	n. d.	n. d.	74.71	10.61
Site 5.10	n. d.	6.96	6.18	n. d.	n. d.	9.51	n. d.	n. d.	13.14	9.51
Site 6.1	n. d.	7.50	n. d.	n. d.	n. d.	14.68	4.91	n. d.	7.50	19.59

Site 6.2	10.93	8.13	29.17	n. d.	n. d.	30.29	n. d.	n. d.	48.23	30.29
Site 6.3	9.58	8.72	40.73	n. d.	n. d.	16.84	n. d.	n. d.	59.02	16.84
Site 6.4	n. d.	7.64	n. d.	n. d.	n. d.	9.83	n. d.	n. d.	43.50	9.83
Site 6.5	n. d.	7.07	n. d.	n. d.	n. d.	10.60	n. d.	n. d.	7.07	10.60
Site 6.6	n. d.	8.58	n. d.	19.10	n. d.	n. d.	n. d.	n. d.	27.67	n. d.
Site 6.7	n. d.	8.98	25.25	n. d.	n. d.	31.50	4.48	n. d.	34.23	35.98
Site 6.8	n. d.	9.13	25.35	n. d.	n. d.	15.42	n. d.	n. d.	34.48	15.42
Site 6.9	16.27	7.46	57.83	n. d.	n. d.	13.26	6.30	n. d.	81.55	19.56
Site 6.10	6.46	7.60	2.20	n. d.	n. d.	19.04	n. d.	5.89	16.26	24.94
Site 7.1	n. d.	10.05	n. d.	n. d.	n. d.	5.40	n. d.	n. d.	35.09	5.40
Site 7.2	n. d.	8.38	21.83	n. d.	n. d.	5.50	n. d.	n. d.	30.20	5.50
Site 7.3	n. d.	8.53	22.41	16.04	n. d.	n. d.	n. d.	n. d.	46.98	n. d.
Site 7.4	n. d.	10.50	77.90	5.04	4.18	n. d.	n. d.	2.75	93.44	6.93
Site 7.5	n. d.	11.63	62.57	7.36	n. d.	2.83	5.08	n. d.	81.56	7.91
Site 7.6	24.71	9.06	102.60	9.76	n. d.	n. d.	n. d.	n. d.	146.13	n. d.
Site 7.7	16.38	24.16	97.53	8.99	n. d.	n. d.	n. d.	4.96	147.06	4.96
Site 7.8	13.94	n. d.	78.34	11.44	n. d.	11.86	n. d.	5.25	103.72	17.11
Site 7.9	13.66	16.73	29.06	7.73	n. d.	7.13	n. d.	5.46	67.18	12.60
Site 7.10	12.81	14.22	29.27	8.65	n. d.	n. d.	n. d.	n. d.	64.94	n. d.

[a] not detected, below LOD

Appendix B – iLOD and iLOQ

Table B1: Instrumental limit of detection (iLOD) and instrumental limit of quantification (iLOQ) determined with the use of the estimated calibration curves for each target analyte.

Analyte	iLOD (ng mL^{-1})	iLOQ (ng mL^{-1})
BpA	0.2	0.6
BpB	0.2	0.6
BpAF	0.5	1.5
BpAP	0.2	0.6
BpF	0.5	1.5
BpM	0.2	0.6
BpP	0.2	0.6
BpS	0.2	0.6
BpZ	1.0	3.0
4-OH-BzP	0.5	1.5
BzP-1	0.2	0.6
BzP-2	0.2	0.6
BzP-3	0.5	1.5
BzP-8	0.2	0.6

Appendic C – Decriptive statistics mussels sampled January and September 2022

Table C1: Descriptive statistics and concentration (ng g^{-1} d.w.) of detected analytes in *P. perna* sampled in January in Algoa Bay, South Africa.

Site	Descriptive statistics	Concentrations (ng g^{-1} d. w.)									
		BpA	BpAF	BpAP	BpS	BzP-1	BzP-2	BzP-3	BzP-8	$\sum_{(4)}$Bps	$\sum_{(4)}$BzP
1	DF (n= 10) %	40	100	100	10	n. d.[a]	90	20	30	100	90
	Median	9.27	8.83	47.3	11.3	n. d.	17.9	5.79	3.37	67.0	20.4
	Mean	10.2	8.75	75.4	11.3	n. d.	17.4	5.79	4.36	89.3	20.1
	Min	2.53	6.76	2.36	11.3	n. d.	10.0	5.05	2.48	20.4	10.0
	Max	19.6	9.82	180	11.3	n. d.	26.3	6.52	7.23	189	32.8
2	DF (n=10) %	50	100	70	30	n. d.	50	10	20	100	60
	Median	20.9	9.68	21.1	15.9	n. d.	14.1	5.38	5.74	31.8	17.1
	Mean	26.2	9.82	35.8	16.2	n. d.	15.8	5.38	5.74	52.8	16.0
	Min	12.2	8.45	5.86	13.1	n. d.	12.3	5.38	5.19	21.1	5.19
	Max	49.7	11.9	78.2	19.7	n. d.	23.9	5.38	6.28	137	23.9
3	DF (n= 7) %	43	100	100	n. d.	14	71	14	29	100	86
	Median	6.04	7.47	43.9	n. d.	6.79	12.4	6.44	2.40	53.4	13.5
	Mean	6.81	8.10	64.1	n. d.	6.79	13.9	6.44	2.40	75.1	14.6
	Min	1.97	6.20	27.2	n. d.	6.79	11.4	6.44	1.97	43.5	6.79
	Max	12.4	10.6	204	n. d.	6.79	20.6	6.44	2.82	211	27.0
4	DF (n=10) %	n. d.	100	100	20	10	80	n. d.	30	100	90
	Median	n. d.	10.3	48.7	5.75	3.78	8.70	n. d.	2.38	58.5	9.12
	Mean	n. d.	9.60	70.8	5.75	3.78	9.90	n. d.	2.43	81.6	10.0
	Min	n. d.	7.31	25.3	4.01	3.78	4.88	n. d.	2.16	32.6	2.16
	Max	n. d.	11.0	189	7.49	3.78	16.8	n. d.	2.73	200	16.8
5	DF (n =10) %	10	100	90	20	10	90	10	10	100	90
	Median	3.23	7.56	25.4	10.5	6.97	9.51	0.87	4.31	36.4	10.6
	Mean	3.23	7.16	41.6	10.5	6.97	8.99	0.87	4.31	47.0	10.3
	Min	6.46	6.96	2.20	19.1	0.00	5.40	4.48	5.89	7.07	5.40
	Max	3.23	9.93	136	16.6	6.97	16.9	0.87	4.31	161	16.9
6	DF (n= 10) %	40	100	70	10	n. d.	90	30	10	100	90
	Median	10.3	7.89	29.2	19.1	n. d.	15.4	4.91	5.89	34.4	19.6
	Mean	10.8	8.08	30.9	19.1	n. d.	17.9	5.23	5.89	36.0	20.3
	Min	6.46	7.07	2.20	19.1	n. d.	9.83	4.48	5.89	7.07	9.83
	Max	16.3	9.13	57.8	19.1	n. d.	31.5	6.30	5.89	81.6	36.0
7	DF (n= 10) %	60	90	90	80	10	50	10	40	100	70
	Median	15.2	10.5	62.6	8.82	4.18	5.50	5.08	5.11	74.4	6.93
	Mean	17.8	12.6	57.9	9.37	4.18	6.54	5.08	4.61	81.6	8.63
	Min	12.8	8.38	21.8	5.04	4.18	2.83	5.08	2.75	30.2	4.96
	Max	25.0	24.2	103	16.0	4.18	11.9	5.08	5.46	147	17.1

All sites	DF (n= 67) %	34	99	88	25	6	75	13	24	100	82
	Median	12.8	8.74	39.9	9.76	5.48	12.4	5.08	3.84	48.2	13.7
	Mean	15.0	9.15	55.5	11.0	5.43	13.2	5.00	4.08	65.8	14.4
	Min	1.97	0.90	2.20	4.01	3.78	0.99	0.87	1.97	5.97	1.85
	Max	49.7	24.2	204	19.7	6.97	31.5	6.52	7.23	211	36.0

BpB, BpF, BpM, BpP, BpZ and 4-OH-BzP is not included as their concentration <iLOD and they had a DF=0

[a] not detected

Table C2: Descriptive statistics and concentrations (ng g^{-1} d.w.) of detected analytes in *P. perna* sampled in September in Algoa Bay, South Africa.

Site	Descriptive statistics	Concentrations (ng g^{-1} d. w.)									
		BpA	BpAF	BpAP	BpS	BzP-1	BzP-2	BzP-3	BzP-8	$\sum_{(4)}$Bps	$\sum_{(4)}$BzP
1	DF (n= 10) %	n. d.[a]	10	20	10	10	90	n. d.	20	40	90
	Median	n. d.	4.70	11.1	4.11	4.31	6.40	n. d.	3.09	4.41	6.85
	Mean	n. d.	4.70	11.1	4.11	4.31	6.72	n. d.	3.09	7.77	7.89
	Min	n. d.	4.70	3.60	4.11	4.31	3.15	0.00	2.18	3.60	3.15
	Max	n. d.	4.70	18.7	4.11	4.31	12.4	0.00	4.00	18.7	16.4
2	DF (n=10) %	n. d.	n. d.	30	80	n. d.	90	20	n. d.	90	100
	Median	n. d.	n. d.	19.2	34.9	n. d.	2.81	10.8	n. d.	35.0	3.97
	Mean	n. d.	n. d.	17.7	37.3	n. d.	3.31	10.8	n. d.	39.1	5.15
	Min	n. d.	n. d.	1.87	32.6	n. d.	1.58	5.69	n. d.	19.2	1.58
	Max	n. d.	n. d.	31.9	45.1	n. d.	5.57	16.0	n. d.	75.5	18.2
3	DF (n= 10) %	n. d.	10	30	90	n. d.	80	20	n. d.	90	90
	Median	n. d.	5.38	5.17	49.5	n. d.	5.13	6.51	n. d.	52.2	5.59
	Mean	n. d.	5.38	29.0	51.3	n. d.	5.38	6.51	n. d.	61.6	6.23
	Min	n. d.	5.38	2.87	42.7	n. d.	2.01	6.09	n. d.	42.7	2.01
	Max	n. d.	5.38	79.1	61.0	n. d.	11.0	6.94	n. d.	126	12.2
4	DF (n=10) %	n. d.	n. d.	20	80	n. d.	80	30	n. d.	90	90
	Median	n. d.	n. d.	32.2	10.9	n. d.	4.77	7.50	n. d.	11.1	5.47
	Mean	n. d.	n. d.	32.2	12.3	n. d.	4.63	7.65	n. d.	18.1	6.66
	Min	n. d.	n. d.	6.51	9.65	n. d.	2.28	5.69	n. d.	9.65	2.28
	Max	n. d.	n. d.	57.9	16.2	n. d.	9.49	9.76	n. d.	57.9	12.6
5	DF (n =10) %	n. d.	n. d.	40	60	n. d.	60	20	n. d.	80	70
	Median	n. d.	n. d.	6.40	11.8	n. d.	3.30	5.51	n. d.	14.2	3.42
	Mean	n. d.	n. d.	24.0	12.5	n. d.	4.54	5.51	n. d.	21.3	5.46
	Min	n. d.	n. d.	2.84	9.21	n. d.	2.50	4.94	n. d.	7.37	2.50
	Max	n. d.	n. d.	80.2	16.5	n. d.	11.8	6.09	n. d.	80.2	11.8
6	DF (n= 10) %	n. d.	n. d.	70	40	10	90	10	n. d.	90	90
	Median	n. d.	n. d.	3.82	12.8	3.95	6.00	6.35	n. d.	10.6	6.00
	Mean	n. d.	n. d.	10.4	10.7	3.95	5.72	6.35	n. d.	12.9	6.87
	Min	n. d.	n. d.	1.23	1.66	3.95	0.57	6.35	n. d.	2.41	0.57
	Max	n. d.	n. d.	34.4	15.4	3.95	8.40	6.35	n. d.	34.4	18.7
7	DF (n= 10) %	20	n. d.	50	30	n. d.	100	n. d.	n. d.	70	100
	Median	16.0	n. d.	23.9	12.4	n. d.	11.1	n. d.	n. d.	23.9	11.1
	Mean	16.0	n. d.	40.3	11.6	n. d.	10.6	n. d.	n. d.	38.3	10.6
	Min	14.7	n. d.	23.5	8.99	n. d.	3.62	n. d.	n. d.	12.4	3.62
	Max	17.3	n. d.	105	13.5	n. d.	14.4	n. d.	n. d.	122	14.4

All sites	DF (n= 70) %	3	3	37	56	3	84	14	3	79	90
	Median	16.0	5.04	13.4	15.5	4.13	5.28	6.22	3.09	19.3	5.97
	Mean	16.0	5.04	23.0	26.0	4.13	6.01	7.50	3.09	30.1	7.05
	Min	14.7	4.70	1.23	1.66	3.95	0.57	4.94	2.18	2.41	0.57
	Max	17.3	5.38	105	61.0	4.31	14.4	16.0	4.00	126	18.7

BpB, BpF, BpM, BpP, BpZ and 4-OH-BzP is not included as their concentration <LOD and they had a DF=0

[a] not detected

Appendix D – Cromatographic and mass spectrometric parameters

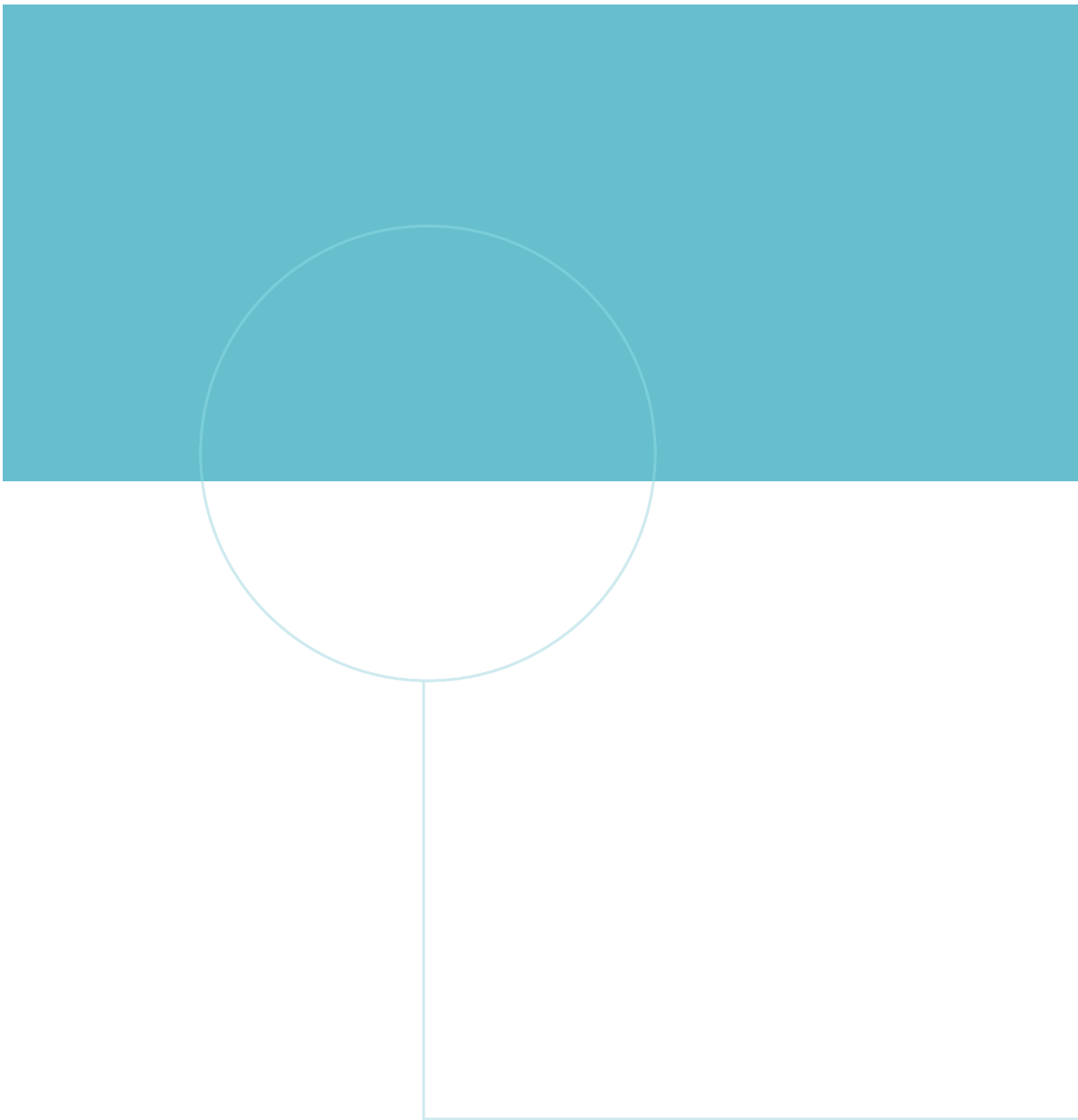
The chromatographic and mass spectrometric parameters were the same as in Castro et al. with exception of the internal standards associated with the target analytes. Therefore, the standards are presented in Table E1.The regression coefficient R^2 for the calibration curves are also provided.

Table D1: Overview of target analyte and associated internal standards, and R^2

Analyte	Internal standard	Linearity, R^2
BpA	BpA-$^{13}C_6$	0.999
BpAF	BpAF-$^{13}C_6$	0.999
BpAP	BpA-$^{13}C_6$	0.999
BpB	BpA-$^{13}C_6$	0.998
BpF	BpAF-$^{13}C_6$	0.994
BpM	BzP-3-$^{13}C_6$	0.999
BpP	BzP-3-$^{13}C_6$	0.999
BpS	BpS-$^{13}C_6$	0.998
BpZ	BzP-3-$^{13}C_6$	0.994
BzP-1	BpS-$^{13}C_6$	0.997
BzP-2	BpS-$^{13}C_6$	0.997
BzP-3	BzP-3-$^{13}C_6$	0.998
BzP-8	BpAF-$^{13}C_6$	0.998
4-OH-BzP	BpS-$^{13}C_6$	0.999

Appendix E – Geographical distribution January and September

Figure E1: Illustration of the geographical distribution of Bps (green) and BzPs (brown) in *P. perna.* Median concentration of ΣBps and ΣBzPs were used.



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