Distribution and bioaccumulation of current-use herbicides in sediments and biota from Lake St Lucia (South Africa) and adjacent marine environment

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By

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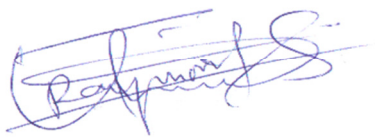
Declaration

The data described in this thesis were collected with approval from the Wits University Animal Ethics Committee (AREC number: 20133201) and iSimangaliso Wetland Park Authority, between January 2018 and April 2021. Experimental work was carried out while registered at the School of Chemistry, University of the Witwatersrand, Johannesburg, under the supervision of Doctor Letitia Pillay and Professor Marc Humphries.

This thesis, submitted for the degree of Doctor of Philosophy in the Faculty of Science, University of the Witwatersrand, Johannesburg, represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others, it is duly acknowledged in the text.



Raymond Lubem Tyohemba
(August 2021)

We certify that the above statement is correct and as the candidate's supervisors we have approved this thesis for submission.

Dr Letitia Pillay
Supervisor
August 2021

Prof. Marc S. Humphries
Supervisor
August 2021

List of publications and contribution of authors

This thesis is based on the following papers

I. **Herbicide residues in sediments from Lake St Lucia (iSimangaliso World Heritage Site, South Africa) and its catchment areas: Occurrence and ecological risk assessment.**

Raymond Lubem Tyohemba*, Letitia Pillay & Marc S. Humphries

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Raymond Tyohemba (RLT) developed the analytical method, analysed the samples, and drafted the original manuscript. Letitia Pillay (LP) collected the samples, reviewed, and edited the write up. Marc S. Humphries (MSH) conceived the study, collected the samples, reviewed, and edited the write up.

II. **Bioaccumulation of current-use herbicides in fish from a global biodiversity hotspot: Lake St Lucia, South Africa**

Raymond Lubem Tyohemba, Letitia Pillay & Marc S. Humphries

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RLT developed the analytical method, analysed the samples, and drafted the original manuscript. LP collected the samples, reviewed, and edited the write up. MSH conceived the study, collected the samples, reviewed, and edited the write up.

III. **Accumulation of commonly used agricultural herbicides in coral reef organisms from iSimangaliso Wetland Park, South Africa**

Raymond Lubem Tyohemba [a], Marc S. Humphries [a], Michael H. Schleyer [b], Sean N. Porter [b]

Sean N. Porter (SNP) conceived of the study and collected samples with Michael H. Schleyer (MHS). RLT validated the analytical method and conducted the laboratory work with MSH. SNP conducted the statistical analyses and interpreted the data with MSH. RLT wrote the first draft which was reviewed and edited by MSH and SNP.

ABSTRACT

Agriculture is one of the leading sources of pollution impacting rivers, lakes and coastal environments worldwide. Chemical herbicides, in particular, are applied in substantial quantities across vast areas of agricultural land to meet growing global food demands. These compounds are generally highly mobile, susceptible to runoff and may impact environments far from their site of application. As a result, the impact of herbicides on aquatic biological communities is of increasing global concern, particularly in regions important for biodiversity conservation.

Lake St Lucia is a large, shallow estuarine system located within iSimangaliso Wetland Park, a UNESCO World Heritage Site on the east coast of South Africa. The system is considered the single most important nursery for estuary-associated fish and invertebrates on the south-east coastline of Africa, and the largest protected estuarine environment for hippos, crocodiles, and aquatic birds on the continent. The offshore marine environment is similarly characterised by high levels of biodiversity and hosts South Africa's only coral reefs. Despite their protected status, these critical estuarine and marine habitats are vulnerable to external pollution pressures. The catchment areas of Lake St Lucia are under intensive commercial and subsistence agriculture and runoff from farmlands enter Lake St Lucia and ultimately the coastal ocean. This study investigated the prevalence and accumulation of several common herbicides currently used in South Africa to evaluate potential impacts on aquatic and marine communities in iSimangaliso Wetland Park. This was assessed by investigating herbicide concentrations in i) river and lake sediments from Lake St Lucia, ii) in tissues from two fish species commonly found at Lake St Lucia, and iii) in several reef coral invertebrates.

Herbicide contaminants including triazines (atrazine, hexazinone, simazine and terbuthylazine), anilides/aniline (acetochlor, alachlor, metolachlor, trifluralin), phenoxy-acids (2,4-D and MCPA) and carbamate (EPTC) were detected in the majority of samples analysed. In sediments, total herbicide concentrations ranged between n.d. – 82.4 ng g^{-1} dw, with acetochlor (3.77 ± 1.3 ng g^{-1}), hexazinone (2.86 ± 1.1 ng g^{-1}) and metolachlor (10.1 ± 8.7 ng g^{-1}) the dominant herbicide residues. The Mkhuze and Mfolozi rivers were identified as important sources of herbicide contamination to Lake St Lucia. A preliminary ecological risk assessment revealed that current herbicide loads could pose a threat to aquatic life, particularly at the algal and aquatic invertebrate community level. Tissue analyses revealed widespread herbicide contamination in fish from Lake St Lucia, with total concentrations in the range of 44.3 – 238 ng g^{-1} and 72.2 – 291 ng g^{-1} dw for *Clarias gariepinus* (African

sharptooth catfish) and *Oreochromis mossambicus* (Mozambique tilapia), respectively. A preliminary human health risk assessment indicated no dietary risk associated with the consumption of both fish species, but that exposure to atrazine and simazine bioaccumulation through life-time consumption presented potential cancer risk to local communities. Herbicide residues were detected in >95% of coral tissue samples, with total average concentrations across sampling sites ranging between 25.2 and 51.3 ng g^{-1} dw. Acetochlor, alachlor and hexazinone were the predominant analytes detected at all sites, but concentrations varied markedly among coral species. On average, highest total herbicide concentrations were measured in soft coral (*Sarcophyton glaucum*; 90.4 ± 60 ng g^{-1} and *Sinularia gravis*; 42.7 ± 25 ng g^{-1}) and sponge (*Theonela swinhoei;* 39.0 ± 40 ng g^{-1}) species, while significantly lower concentrations characterised the two hard coral species sampled (*Echinopora hirsutissima;* 10.5 ± 5.9 ng g^{-1} and *Acropora austera;* 5.20 ± 4.5 ng g^{-1}). Latitudinal variation in herbicide concentrations suggested that runoff originating from St Lucia estuary and Maputo Bay were likely the major sources of contamination to coral reefs.

Results from this study provide first insight into the distribution and bioaccumulation of herbicide contaminants in iSimangaliso Wetland Park. Findings indicate that herbicides accumulate readily within aquatic and marine biota, presenting concerns not only for park management in the region, but for biodiversity conservation globally. Chronic exposure of organisms to herbicides remains largely understudied and potential impacts on ecosystem communities cannot at present be evaluated. Impacts may be subtle and include reproductive suppression or reduced resilience to disease and climate change. Urgent toxicological information is required to help inform monitoring programs and management strategies.

Dedications

I dedicate this work to my beautiful wife Mary for her unwavering support, sacrifice and understanding during my studies at Wits and my lovely children, Civirnen and Ashidoon for bearing with daddy while he was away from home for over three years.

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List of abbreviations and acronyms

CH_3COONa	Sodium acetate
$C_2H_9NaO_5$	Sodium acetate trihydrate
ESI	Electron spray ionization
EPTC	*S*-ethyl dipropylthiocarbamate
EU	European Union
FAO	Food and Agricultural Organisation
GC	Gas chromatography
GBR	Great Barrier Reef
IWP	iSimangaliso Wetland Park
KW-ANOVA	Kruskal-Wallis analysis of variance
KZN	KwaZulu-Natal
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MCPA	Mono-chlorophenoxyacetic acid
MDS	Multi-dimensional scaling
MEC	Measured environmental concentration
MeOH	Methanol
$MgSO_4$	Magnesium sulfate
MRM	Multiple reaction monitoring
MS	Mass spectrometry
OCP	Organochlorine pesticides
PCNB	Pentachloronitrobenzene
PNEC	Predicted no-effect concentration
PSA	Primary secondary amine
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
RQ	Risk quotient
RSD	Relative standard deviation
SD	Standard deviation
TOF	Time-of-flight
US EPA	United State Environmental Protection Agency
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

Herbicides, a group of chemicals which fall under the broader category pesticides, are used to inhibit the growth of weeds and invasive species in residential and agricultural regions (Tadeo et al., 2008). Early chemicals utilized as herbicides, including sulfuric acid, sodium salts, iron, copper sulfate and arsenic compounds, were often non-specific and phytotoxic (Gupta, 2017). The introduction of the selective herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in the 1940s was the turning point in the herbicide industry (Price & Kelton, 2011) and initiated further research on plant growth regulators (Vats, 2015). The introduction of paraquat, diquat and monuron followed in the 1950s, with nitrofen and alachlor appearing in the 1960s. Later entries to market included glyphosate and imidazolinone herbicides in the 1970s, as well as various triazine derivatives that have been in use for over 40 years (Gupta, 2017; Steven & Summer, 1991). Continued efforts to increase food production, improve crop yields, and promote food security have resulted in the increased utilization of pesticides in recent decades (Pimentel, 1996; Hazel, 2002; Mercurio, 2016). Today, global pesticide usage is estimated at approximately 4.1 million tons per annum, with herbicides accounting for around 30% of this (FAO, 2018).

Due to the widespread and intensive use of pesticides, agriculture is one of the main sources of pollution impacting terrestrial aquatic and marine ecosystems worldwide (US EPA, 2000; Shahidul & Tanaka, 2004; Dowd et al., 2008; Parris, 2011). Attention has traditionally focused on impacts associated with organochlorine and organophosphate insecticides, which are known to readily accumulate in the environment (Edwards, 2013; Carvalho, 2017). Several decades of research has revealed the persistent and bioaccumulative nature of these compounds, as well as their toxic effects on humans and biological ecosystems (Readman et al., 1992; Weisskopf et al., 2010; Jayarj et al., 2016; Mamta et al., 2019). As a result, many of the organochlorine and organophosphate compounds originally developed for agricultural use have since been banned or severely restricted on a global basis. However, the potential environmental and health impacts associated with herbicide compounds use have received far less attention. Owing to their high solubility, herbicides are susceptible to runoff and leaching, and thus readily enter groundwater and river systems (Ritter et al., 2002; van Dam et al., 2011). Particularly in relatively water-scarce regions of the world, agricultural activities are often concentrated in river valleys and coastal plains, resulting in the drainage of herbicides into downstream

lakes, wetlands and estuaries (Theron, 2012; Bennett et al., 2018; Lekitan et al 2018; Somboonsuke et al., 2018; Zebire et al., 2019). Herbicide runoff has also been identified as an important source of contamination to coastal areas, particularly coral reefs (Lewis et al., 2009; Packett et al., 2009; Brodie et al., 2012; Kennedy et al., 2012; Davis et al., 2013; Ali et al., 2014; Gallen et al., 2019; Kroon et al., 2020).

Although largely understudied, growing evidence suggests that many herbicide compounds currently in use may be associated with severe toxicological risks even at relatively low doses (Cedergreen & Streibig, 2005). Among the biological effects, herbicide exposure has been linked with various cytotoxic, mutagenic and embryotoxic effects in organisms (Hayes et al., 2002; Nwani et al., 2011; Prado et al., 2012). Moreover, emerging evidence suggests that certain herbicide residues may be susceptible to bioaccumulation (Fernandez & Gardinali, 2016; Fu et al., 2018). The occurrence and potential impact of herbicide residues in aquatic and coastal ecosystems is thus of increasing global concern (Ouyang et al., 2019; Pico et al., 2019; Seibert et al., 2020).

1.2 Transport and fate of herbicides in the aquatic environment

Herbicides are susceptible to transport in the environment, which may occur via leaching, runoff or even volatilization (Queiroz et al., 2009; Mendes et al., 2019). The extent to which herbicides may be transported through various components in the environment is a function of both the stability and physicochemical attributes of individual compounds (Havens et al., 1995; Sondhia, 2014). Important properties influencing the persistence and mobility of herbicide compounds in the environment include its solubility, vapour pressure, octanol-water partition coefficient (Kow), and susceptibility to chemical or microbial degradation (Zabaloy et al., 2011; Curran, 2016).

The runoff and leaching of herbicides is primarily a function of solubility and is considered one of its most critical properties influencing their environmental fate (Reinert and Rodgers, 1987; Mackay, 1980; Curran, 2016). Solubility determines whether the compound is likely to reside exclusively in the water column or be associated with suspended particulate matter. Compounds associated with suspended particulate matter are more likely to accumulate in terrestrial aquatic systems and nearshore environments, while those dissolved in water may move longer distances and impact environments far from their site of application (van Dam et al., 2011).

The adsorption coefficient (Koc) provides an indication for the tendency of herbicide compounds to sorb to sediment particles. The higher the Koc, the greater the role of sorption in removing herbicides from water (Dickson et al., 1981). Due to the partitioning of chemicals into sediment-binding sites, sediment contamination levels can surpass water concentrations by many orders of magnitude (Barron, 2003). The binding of organic contaminants to sediments may be further influenced by organic carbon content, clay content, cation-exchange capacity, pH and sediment particle size (Barron, 2003; Solly et al., 2020).

Herbicide contaminants have the potential to accumulate in aquatic organisms. The octanol-water partition coefficient (Kow) of a herbicide is inversely proportional to its solubility and can be used to predict whether herbicides are likely to accumulate in biological tissues (Mackay, 1980). The larger the Kow the more likely herbicide accumulation within an organism's tissues is (Reinert & Rodgers, 1987). The exposure of organisms to contaminants is usually mediated through dietary pathways, which may include the ingestion of sediment particles and organic matter, but can also occur through non-dietary routes, such as absorption via gills or movement through mucus membranes (Barron, 2003; Kaiser, 2012). Due to diversity in feeding ecology and living patterns, pollutant accumulation in aquatic organisms is often highly species-dependent (Broderius & Kahl, 1985; Hermens et al., 1985; Broderius et al., 1995; McLeod et al., 2014).

Although very little information currently exists, studies have shown that certain herbicides may bioaccumulate in higher-level aquatic organisms. For example, atrazine, simazine, alachlor, acetochlor and metolachlor have been detected in fish from both freshwater and marine environments (Abrantes et al., 2010; Reindl et al., 2015; Ojemaye et al., 2020a). The accumulation of atrazine, trifluralin, simazine, alachlor and metolachlor has also been reported in seagrasses, sea snails and sea cucumbers (Haynes et al., 2000; Salvat et al., 2016).

1.3 Herbicide toxicity and effects on non-target aquatic organisms

While the usefulness of herbicides in enhancing global food production remains undisputable, concerns regarding their effect on non-target organisms have emerged (Jurado et al., 2011; Mercurio, 2016). The effects of certain herbicide compounds on organisms and tissues have been evaluated in several concentration-response and bioassay laboratory studies. These tests have investigated various cytotoxic (Prado et al., 2012), genotoxic and mutagenic (Fernandes et al., 2007; de Campos Ventura et al., 2008; Nwani et al., 2011), embryotoxic and teratogenic (Morgan, 1996; Hayes et al., 2002), and estrogenic

(Xie et al., 2005) effects in aquatic organisms. Chloroacetanilides (including acetochlor, alachlor, butachlor, dimethachlor, metazachlor, metolachlor, pretilachlor and propachlor) have been shown to have both adverse individual and synergistic effects on green algal reproduction (Junghans et al., 2003). Hexazinone has been shown to inhibit the growth of green algae, diatoms and macrophytes (Peterson et al., 1997), while a decrease in photosynthetic efficiency has been reported in autotrophic communities exposed to environmental concentrations of diuron (Ricart et al., 2009; Bhowmick et al., 2021). Bacteria and protozoa have exhibited toxicological responses to alachlor, diuron and glyphosate (Bonnet et al., 2007).

Due to their targeted action mechanism, most herbicides are considered not particularly toxic to fish (Solomon et al., 2013). However, some herbicides, such as oxidative phosphorylation uncouplers are toxic because the target mechanism is common to both plants and animals (Stephenson & Solomon, 2007). Other exceptions include herbicides which interfere with cell division, such as the dinitroaniline based herbicides; trifluralin, benfluralin, ethalfluralin and pretilachlor (McBride & Richards, 1975; BCPC, 2003). Several sub-lethal effects have been shown to manifest through changes in reproduction and increased stress response (USEPA, 2011; Solomon et al., 2013). Atrazine has been shown to induce genotoxic and mutagenic effects in fish species (de Campos Ventura et al., 2008) and cause gonad abnormalities and DNA damage in frogs and tadpoles (Clement et al., 1997; Gammon et al., 2005; Hayes et al., 2002).

Herbicide runoff into coastal areas may have significant toxicological consequences for marine organisms. Although herbicide contamination in subtropical and tropical marine environments is widespread, risks posed to marine species are poorly understood (Thomas et al., 2002; McMahon et al., 2005; Shaw et al., 2010; Kennedy et al., 2012). The Great Barrier Reef off the coast of Queensland in Australia is perhaps the best example of a marine ecosystem threatened by herbicide runoff (and other classes of pesticide). Deteriorating water quality, resulting largely from the use of pesticides within catchment areas, is considered one of the most pressing threats to the ecological integrity and long-term health of the region (Grant et al., 2018; Brodie & Landos, 2019; Gallen et al., 2019). Pesticide residues are found ubiquitously in waterways and waterbodies along the Queensland coast (Brodie et al., 2012; Allen et al., 2017; Warne et al., 2020), although few bioaccumulation (example; Haynes et al., 2000a) or toxicity (example; Magnusson et al., 2013; Flores et al., 2020) studies on marine organisms have been conducted. Potential impacts to coral reefs, which function as important spawning, nursery and feeding areas, are thus of particular concern. Most reef-building corals obtain the majority of their nutritional

requirements via the translocation of metabolites from their photosynthetic endosymbiotic zooxanthellae partners (Lewis et al., 2009). Laboratory studies have indicated that photosystem II (PSII) inhibiting herbicides (e.g., atrazine and diuron) readily penetrate coral tissues and potentially reduce the photosynthetic efficiency of these symbionts (Owen et al., 2003; Negri et al., 2005; Vonk & Kraak, 2020). Several adverse effects have been documented, including photosynthetic suppression, reduced reproductive output, severe bleaching, reduction in tissue lipid content and partial colony mortality (Cantin et al., 2007; Shaw et al., 2008; Lewis et al., 2009; Negri et al., 2011; Richmond et al., 2018).

1.4 Herbicide use and detection in South African aquatic environments

South Africa is currently the largest consumer of agrochemicals in Africa, with over 3000 registered pesticide products (DAFF, 2010; Quinn et al., 2011; Worldometer, 2021). Approximately 27000 tons (2.2 kg ha^{-1}) are used on an annual basis, making the country the 20th highest consumer of pesticides in the world (FAOSTAT, 2020; Worldometer, 2021). Herbicide use in the country is estimated at around 9500 tons per annum (FAO, 2020), of which the most common include glyphosate and triazine derivatives (Dabrowski, 2015). Agricultural pesticides used in South Africa have been ranked based on their environmental mobility and potential human health effects (Dabrowski et al., 2014). This is calculated using a weighted hazard potential (WHP) approach, which allows pesticides to be prioritised according to a combination of three indices viz: quantity of use, toxicity potential and hazard potential (Table 1).

Table 1. List of the top 25 priority pesticides ranked by their weighed hazard potential (WHP) along with estimated annual usage quantities in South Africa. Entries in bold denote compounds analysed in this study.

Rank	Active Ingredient	WHP	Quantity (kg y^{-1})	Common crop applications
1	**Atrazine**	3.626	1 009 920	Maize, sorghum, and sugar cane
2	Mancozeb	3.444	2 765 260	All food stuff
3	**Acetochlor**	1.587	656 545	Cotton, groundnuts, maize, sorghum and sugar cane
4	Ethylene-dibromide	1.221	247 878	Citrus, grains, vegetables
5	**Terbuthylazine**	1.134	674 413	Maize, peas, and sorghum
6	Glyphosate	0.977	3 641 509	Sugar cane, maize, soy beans, wheat, oats, potatoes and peas
7	Sulphur	0.860	90 280	Apples, avocadoes, bananas, beans, citrus, grapes, mangoes, papaya and tomatoes
8	Copper oxychloride	0.837	1 076 206	Tomatoes, potatoes, cucumber, melons, ornamentals
9	Imidacloprid	0.795	214 884	Apples, citrus, maize, sorghum, grapes, sunflower seed, tomatoes and wheat
10	**Metolachlor**	0.653	443 707	Beans, cotton seed, groundnuts, maize, sorghum, soya beans, sugar cane and sunflower
11	**2,4-D**	0.560	331 281	Citrus, maize, potatoes, sorghum, sugar cane and wheat
12	**Alachlor**	0.452	285 892	Groundnuts, maize, pineapples, potatoes, soya beans, sugarcane, and sunflower seed.
13	**MCPA**	0.389	277 000	Maize, potatoes, sorghum, sugarcane, and wheat
14	**Simazine**	0.280	83 253	Apples, grapes, maize, pears, and asparagus
15	Paraquat	0.235	295 866	Cotton seed, maize, and sugar cane
16	Aldicarb	0.209	105 000	Maize, banana, citrus, sweet potatoes, grapes. cotton seed, groundnuts and tomatoes
17	MSM	0.180	245 108	Sugar cane
18	**Trifluralin**	0.168	159 792	Cabbage, cowpeas, groundnuts, kidney beans, soya beans, sunflower seeds, and tomatoes
19	Potassium-phosphite	0.167	236 154	Ornamentals, field nurseries, potatoes
20	Diuron	0.151	96 000	Asparagus and sugar cane
21	Metribuzin	0.133	106 080	Tomatoes, potatoes, maize, carrot
22	**Hexazinone**	0.124	84 607	Pineapples and Sugarcane
23	Cyanamide	0.117	202 860	Wheat, vegetables and fruits
24	Carbofuran	0.105	31 834	Broccoli, cabbage, maize, potatoes, sorghum, sunflower seed, sugar cane and wheat
25	**EPTC**	0.103	178 430	Maize, sorghum, maize, potatoes, sugarcane, sunflower seed, sweet and sweet potatoes

Data sources: Quinn et al. (2011), Dabrowski et al. (2014), Dabrowski (2015)

Relatively few studies have examined the prevalence of herbicide residues in South Africa's aquatic systems despite widespread use in the country (Table 2; Fig. 1). Initial studies conducted in the 1980s through to the early 2000s focused mainly on the monitoring of atrazine in water bodies (Hassett et al., 1987; Pick et al., 1992; Vahrmeijer, 1993; Weaver, 1993; Meintjies et al., 2000). More recently, the presence of other triazine derivatives in surface water systems has been examined (Du Preez et al., 2005; Rimayi et al., 2018). The occurrence of other herbicide classes in South African aquatic systems, including chloroacetanilides, diuron, MCPA, 2,4-D and glyphosate have very recently been reported on (Horn et al., 2019; Curchod et al., 2020).

Less research has focused on the presence of herbicides in sediments and biota. Triazine residues (atrazine, ametryn, propazine and simazine) have been detected in river sediments (Du Preez et al., 2005; Kunene & Mahlambi, 2020), while a recent study by Ojemaye et al. (2020b) reported the presence of triazines (atrazine and simazine) and chloroacetanilides (alachlor, butachlor and metolachlor) in marine sediments. Early studies by Heath & Claassen (1999) examined atrazine bioaccumulation in several indigenous freshwater fish species. Recent assessments have broadened in scope to examine the bioaccumulation of other herbicide residues in aquatic organisms (Rimayi et al., 2018; Barnhoorn & van Dyk, 2020; Ojemaye et al., 2020a; Ojemaye et al., 2020b). Fish from dams in the farming community surrounding Roodeplaat Dam in Gauteng identified terbuthylazine and DCPA in tissues of *Clarias gariepinus* (Barnhoorn & van Dyk, 2020). Low levels of triazine and terbuthylazine metabolites in fish tissue were detected in *Clarias gariepinus* from Hartbeespoort Dam, North West Province (Rimayi et al., 2018). Triazine and anilide herbicides have also been detected in marine biota (fish, mussels, sea urchins and seagrasses) around Cape Town (Ojemaye et al., 2020a; 2020b).

Overall, there remains little information regarding the presence of commonly used herbicides in South African aquatic and marine ecosystems. Bioaccumulation data are particularly scarce, especially for the subtropical eastern parts of the country, which hosts several important conservation parks and the country's only coral reefs.

Table 2: Existing herbicide data for South African freshwater and marine environments Concentrations expressed in ng L^{-1} (water) and ng g^{-1} (sediment and biota). Numbers indicate sampling locations shown in Fig. 1.

	Study area	Concentrations detected	Reference
	Water		
1	Ponds (North West)	Atrazine (390 – 9300) Simazine (1000 – 3200) Terbuthylazine (1040 – 4100)	Du Preez (2005)
2	Rivers (Free State)	Atrazine (130 – 790)	Hassett et al. (1987)
3	Dams (Free State)	Atrazine (<50 – 11500)	Vahrmeijer (1993)
4	Vaal River and dams (Free State)	Atrazine (<250 – 1190)	Meitjies et al. (2000)
5	Rivers (Free State)	2, 4-D (720 – 1080) Glyphosate (420)	Horn et al. (2019)
6	Hartbeespoort Dam , Jukskei River, Crocodile Rivers (North West and Gauteng)	Atrazine (<5 – 1570) Simazine (n.d. – 658) Prometon (n.d. – 344) Propazine (65 – 877)	Rimayi et al (2018)
7	Krom River, Berg River, Hex River (Western Cape)	*S*-metolachlor (1.6 – 15.6) Simazine (6.8 – 67.4) Terbuthylazine (71.8 – 717.0) Terbutryn (4.3 – 43.2) Atrazine (2.3 – 23.2) Diuron (5.9 – 59.0) MCPA (13.4 – 133.4)	Curchod et al. (2020)
8	Camps Bay (Western Cape)	Atrazine (n.d. – 1.9) Metolachlor (n.d. – 2.3) Simazine (<1 – 4.3)	Ojemaye et al. (2020b)
9	Northern Cape	Atrazine (60 – 420)	Weaver (1993)
10	Rivers and dams (Gauteng)	Atrazine (730 – 18000)	Pick et al. (1992)
	Sediment		
1	Ponds (North West)	Atrazine (<0.5)	Du Preez (2005)
11	Msunduzi River, Mbokodweni River and Umgeni River (KZN)	Atrazine (n.d. – 29) Ametryn (n.d. – 7.2) Propazine (n.d. – 28) Simazine (n.d. – 94)	Kunene & Mahlambi (2020)
8	Camps Bay (Western Cape)	Alachlor (n.d. – 15) Metolachlor (32 – 45.3) Simazine (15 – 25)	Ojemaye et al. (2020b)
	Fish tissue		
12	Berg River (Mpumalanga)	Atrazine (0.5)	Health & Claasen (1999)
13	Kalk Bay Harbour (Western Cape)	Alachlor (n.d. – 48) Atrazine (n.d. – 66) Metolachlor (n.d. – 42) Simazine (n.d. – 158)	Ojemaye et al. (2020a)
14	Roodeplaat Dam (Gauteng)	DCPA (<50) Terbuthylazine (<50)	Barnhoorn & Van Dyk (2020)
6	Hartbeespoort Dam, Jukskei River, Crocodile River (North West and Gauteng)	Triazine metabolites (n.d. – 0.5)	Rimayl et al. (2018)
	Marine biota		
8	Camps Bay (Western Cape)	Alachlor (n.d. – 25) Atrazine (5 – 60) Metolachlor (n.d. – 52.9) Simazine (40 – 157.8)	Ojemaye et al. (2020b)

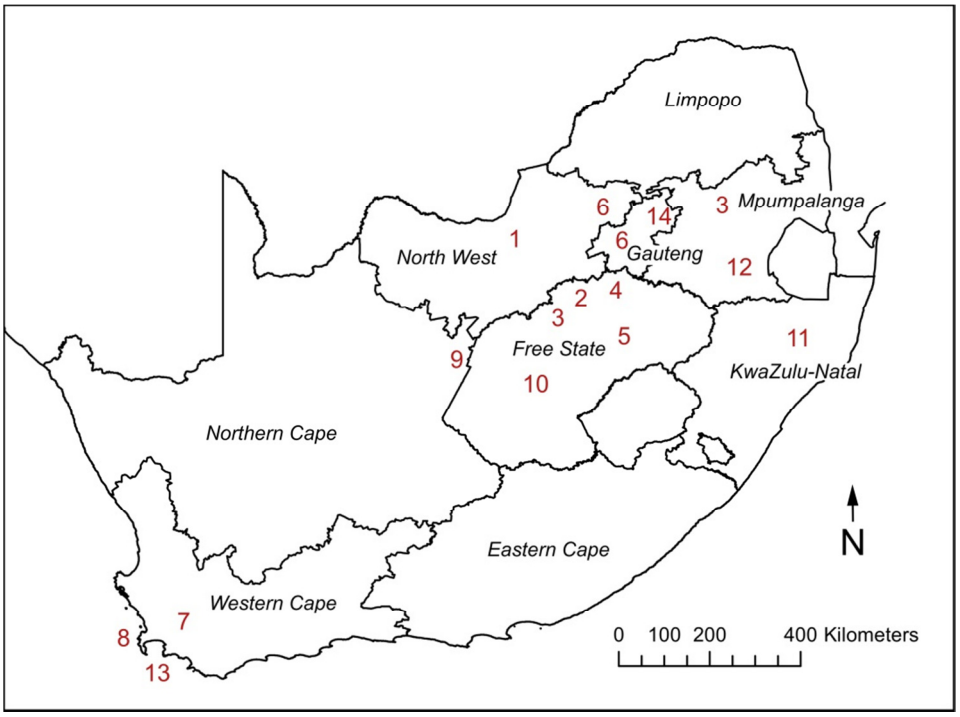

Fig. 1 Location of previous studies conducted in South Africa examining environmental herbicide concentrations. Study details provided in Table 2.

1.5 Study area: Lake St Lucia, iSimangaliso Wetland Park

Lake St Lucia is a large (350 km^2), shallow estuarine system situated on the east coast of South Africa (Fig. 2). The system is considered the largest of its kind in Africa (Porter, 2013) and constitutes the focal point of iSimangaliso Wetland Park (IWP), a UNESCO World Heritage Site. IWP is one of the largest conservation areas in South Africa, second only to Kruger National Park. iSimangaliso stretches across 180 km of coastline from St. Lucia estuary in the south to the border between South Africa and Mozambique in the north. The region forms part of the Maputaland-Pondoland-Albany biodiversity hotspot and is recognised as one of Africa's most important centres of endemism (Van Wyk & Smith, 2001; Smith et al., 2008., Perera et al., 2021), providing critical habitat for a range of species from marine, wetland and savannah environments.

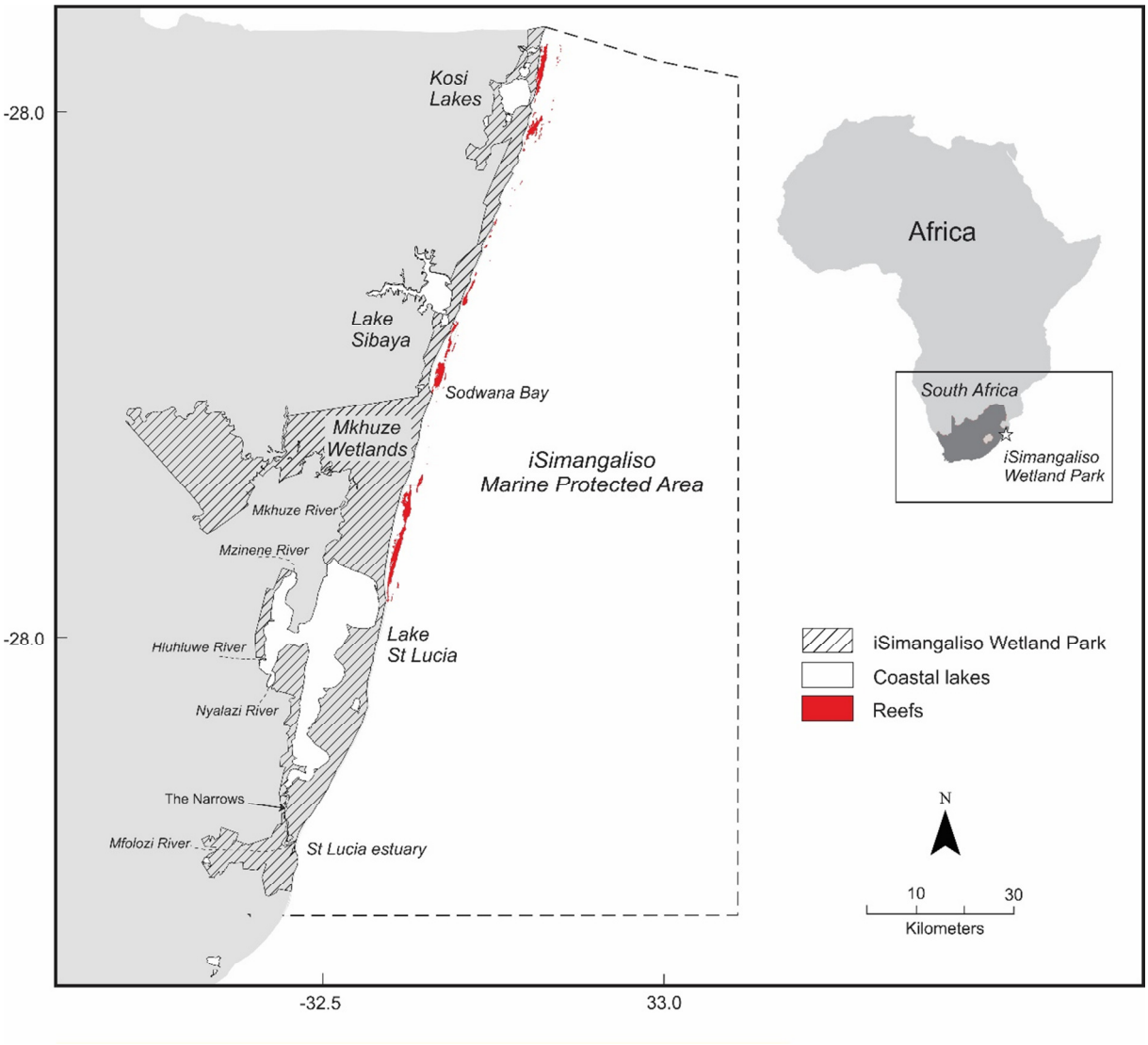

Fig. 2 Major wetlands, coastal lakes and reefs associated with iSimangaliso Wetland Park on the north-east coast of South Africa.

The marine environment lies at the south-western limits of the tropical Western Indian Ocean and is characterised by highly diverse coral reef communities and tropical ichthyofauna (Bolton et al., 2004; Floros et al., 2012; Schleyer et al., 2018). Reefs are dominated by soft coral species, but a variety of scleractinian species, sponges and ascidians are also found (Schleyer & Celliers, 2003; Schleyer & Porter, 2018). Numerous species of marine mammals and sea turtles, as well as deep water pelagic fish also occur throughout the region. The iSimangaliso Marine Protected Area (MPA) covers approximately 10,700 km^2 and constitutes the largest protected marine environment along the South African coastline.

The terrestrial landscape (2,400 km^2) is characterised by a mosaic of interlinked ecosystems and habitat types, including several large coastal lake systems, a diverse variety of

freshwater wetlands, and extensive coastal dune forests (Ellery et al., 2013). Four Ramsar Wetlands of International Importance are located within iSimangaliso, the largest of which is Lake St Lucia (Fig. 2). Lake St Lucia is considered the single most important nursery for estuary-associated fish and invertebrates on the south-east coastline of Africa, and the largest protected estuarine environment for hippos, crocodiles, and aquatic birds on the continent (Porter, 2013). The system comprises three inter-connected lake basins separated from the ocean by a high coastal dune barrier. The only contemporary link to the ocean is via a long sinuous channel known as the Narrows. The mouth of the estuary is susceptible to prolonged periods of closure, with lake water levels driven predominately by fluvial inputs (Cyrus et al., 2011). Fresh water enters Lake St Lucia mainly via inflow from five rivers (Mkhuze, Mzinene, Hluhluwe, Nyalazi and Mfolozi), with minor contributions from groundwater seepage (Whitfield et al., 2006; Jugwanth et al., 2013). The Mkhuze River (catchment area of 6000 km^2) that flows into Lake St Lucia from the north and the Mfolozi River (catchment area of 10 085 km^2) which enters near the estuary mouth are the largest contributors to sediment and water supply.

The catchment areas these rivers drain lie outside the boundary of IWP and are significantly impacted by agriculture. Sugarcane has been cultivated on the lower Mfolozi floodplain since 1911 and now covers an area of approximately 90 km^2 (Searle, 2013). The floodplain also favours subsistence farming of other crops, with sweet potatoes, banana and amadume being the main ones (Dlamini et al., 2021). The Mkhuze floodplain is impacted by small-scale commercial and subsistence farming (Patrick & Ellery, 2006). Many households within the Mnqobokazi Tribal authority have access to fertile land in the Mkhuze swamps for subsistence and small-scale agriculture. Sugarcane cultivation by emerging commercial farmers is increasing on the lower end of the Mkhuze floodplain (Burgoyne et al., 2016) while the growing of maize, cabbage, sugar beets, potatoes, and mangoes along the Mkhuze River is common among local communities (Burgoyne and Kelso, 2014).

1.6 Rationale for the study

Despite their protected status, iSimangaliso's coastal and marine habitats are threatened by pollution originating from sources outside of the park boundary. Land use practices in surrounding catchment areas are identified by management authorities as one of the main threats to IWP (IWPA, 2016). Earlier studies conducted in IWP reported on the presence of DDT (Humphries, 2013) and a range of other legacy organochlorine pesticides (OCPs) in sediments from Lake St Lucia, Lake Sibaya and Kosi Bay (Buah-Kwofie & Humphries, 2017). The presence of OCP residues have also been reported in Mkhuze and Mfolozi river

sediments, suggesting that catchment runoff continues to be an important source of contamination to Lake St Lucia (Buah-Kwofie & Humphries, 2021). Several studies at Lake St Lucia have revealed high levels of OCP bioaccumulation in tissues from fish (Buah-Kwofie and Humphries, 2021) and Nile crocodiles (Buah-Kwofie et al., 2018; Humphries et al., 2021), as well as in cormorant and pelican eggs (Bouwman et al., 2019). Although few contamination studies have been conducted within the iSimangaliso MPA, initial work by Porter et al. (2018) found surprisingly high OCP concentrations in soft coral and sponge species collected from reefs adjacent to Lake St Lucia and Lake Sibaya.

While the occurrence and bioaccumulation of legacy OCPs in IWP is now well documented, no information exists regarding other prominent contaminants, in particular currently used agricultural herbicides. This thesis presents the first investigation into the prevalence and bioaccumulation of current-use herbicides in the aquatic and marine habitats of IWP. The study focuses on Lake St Lucia because of its biological importance, and unlike the groundwater-fed lake systems of Sibaya and Kosi, receives predominantly fluvial input from catchment areas under intensive agriculture. Eleven herbicide residues (Table 3) were targeted for analysis on the basis of them being identified as priority herbicides in South Africa (Table 2) as well as their likely use within the region.

Table 3: Classification and properties of the herbicides analysed in this study

Herbicide	Chemical Family	Solubility (mg L^{-1}) [a-d]	Half-life in soil (d) [a-d]	Soil adsorption coefficient (Koc) [a-d]	Octanol/water partition coefficient (log Kow) [a-d]	Site of action in plants [e-f]
Atrazine	Triazine	33	35 – 50	100	2.50	Inhibitor of photosynthesis at photosystem II
Hexazinone	Triazine	33,000	90	54	1.95	Inhibitor of photosynthesis at photosystem II
Simazine	Triazine	6.2	27 – 102	130	2.10	Inhibitor of photosynthesis at photosystem II
Terbuthylazine	Triazine	8.5	5 – 116	236	3.21	Inhibitor of photosynthesis at photosystem II
Acetochlor	Chloroacetanilide	223	8 – 18	225	4.14	Inhibition of cell division
Alachlor	Chloroacetanilide	170	1 – 30	240	3.09	Inhibition of cell division
Metolachlor	Chloroacetanilide	488	20	200	2.90	Inhibition of cell division
Trifluralin	Dinitroaniline	0.22	57 – 126	8000	4.83	Inhibitor of microtubule assembly
EPTC	Thiocarbamate	375	6 – 30	200	3.20	Inhibitor of lipid synthesis
MCPA	Phenoxy-acid	274	<7	50	-0.71	Mimic the growth hormone auxin
2,4-D	Phenoxy-acid	23,180	<7	20	0.04	Mimic the growth hormone auxin

Data sources: a: Navarro-Ortega et al.(2010), b: Quinn et al. (2011), c: Wauchope et al. (1992), d: Tadeo et al. (2008), e: Vats, (2015), f: Kocher & Manne (2012).

1.7 Thesis objectives and structure

The overarching aim of this study is to evaluate the occurrence and accumulation of selected priority herbicides at Lake St Lucia and adjacent coral reef habitats and assessing potential impacts on terrestrial aquatic and marine ecosystem health.

To address this aim, the following objectives were identified:

- To investigate the prevalence of herbicide residues in sediments from Lake St Lucia and its major catchment areas.

- To investigate the extent to which herbicides may be transferred through the food chain by examining bioaccumulation in two fish species from Lake St Lucia
- To assess the potential influence of herbicide runoff on the marine environment by examining bioaccumulation in five different coral reef invertebrates.

These objectives are addressed in chapters 2, 3 and 4, which are written as journal articles. The focus of each article is distinct, although some overlap and repetition between chapters is unavoidable. The thesis is structured as follows:

Chapter 2: *Herbicide residues in sediments from Lake St Lucia (iSimangaliso World Heritage Site, South Africa) and its catchment areas: Occurrence and ecological risk assessment*

This chapter investigates the occurrence and distribution of herbicide residues in sediments from Lake St Lucia and examines the degree to which the system is affected by contamination arising from agricultural runoff. The chapter has been published in *Environmental Pollution* 267 (2020) 115566: https://doi.org/10.1016/j.envpol.2020.115566

Chapter 3: *Bioaccumulation of current-use herbicides in fish from a global biodiversity hotspot: Lake St Lucia, South Africa*

This chapter reports on the distribution and accumulation of herbicide residues in two common fish species from Lake St Lucia (*Clarias gariepinus*, African sharptooth catfish and *Oreochromis mossambicus,* Mozambique tilapia). The associated ecological and human health risks are discussed. The chapter has been published in *Chemosphere* 284 (2021) 131407: https://doi.org/10.1016/j.chemosphere.2021.131407.

Chapter 4: *Accumulation of commonly used agricultural herbicides in coral reef organisms from iSimangaliso Wetland Park, South Africa*

This chapter examines spatial and inter-species variations in herbicide accumulation in five coral reef organisms found along the Maputaland coast. Included in this assessment are two soft coral species (*Sinularia gravis* and *Sarcophyton glaucum*), two hard coral species (*Acropora austera* and *Echinopora hirsutissima*), and a sponge (*Theonella swinhoei*). The chapter has been submitted to *Science of the Total Environment.*

Chapter 5: The chapter summarises the key findings of the study and offers direction for future work.

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CHAPTER 2

Herbicide residues in sediments from Lake St Lucia (iSimangaliso World Heritage Site, South Africa) and its catchment areas: Occurrence and ecological risk assessment

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Abstract

The impact of agricultural pesticides on sensitive aquatic ecosystems is a matter of global concern. Although South Africa is the largest user of pesticides in Africa, few studies have examined the toxicological threats posed by agricultural runoff, particularly to conservation areas of international importance. This study investigated the occurrence of 11 priority listed herbicides in sediments from Lake St Lucia, located on the east coast of South Africa. While characterised by exceptionally high levels of biodiversity, Lake St Lucia is affected by agricultural runoff primarily via inflow from two major rivers; the Mkhuze and Mfolozi. Sediment samples collected from Lake St Lucia and its two major fluvial inputs reveal widespread herbicide contamination of the aquatic environment. Residues were detected in the vast majority of samples analysed, with Mkhuze (27.3 ± 17 ng g^{-1}) and Mfolozi (25.6 ± 20 ng g^{-1}) sediments characterised by similar total herbicide levels, while lower concentrations were typically detected in Lake St Lucia (12.9 ± 12 ng g^{-1}). Overall, the most prominent residues detected included acetochlor (3.77 ± 1.3 ng g^{-1}), hexazinone (2.86 ± 1.4 ng g^{-1}) and metolachlor (10.1 ± 8.7 ng g^{-1}). Ecological assessment using Risk Quotients (RQs) showed that cumulative values for triazines and anilides/aniline herbicide classes presented low to medium risk for algae and aquatic invertebrate communities. Considering the biological importance of Lake St Lucia as a nursery for aquatic organisms, it is recommended that further research on the aquatic health of the system be undertaken. Additional monitoring and investigation into mitigation strategies is suggested, particularly as agricultural activities surrounding Lake St Lucia are likely to expand in the future.

2.1 Introduction

The occurrence and distribution of pesticides in aquatic systems is a matter of global concern and poses significant toxicological threats to both organisms and human health (Dabrowski et al., 2014; Hela et al., 2005; World Health Organization, 2010). Pesticide residues reach surface water mainly through agricultural runoff, with estuarine environments often the ultimate recipients of agricultural nonpoint source pollution (Weber et al., 1995). The rich and diverse ecosystems that typically characterise estuarine and coastal environments are therefore particularly vulnerable to the long-term influences of pesticide usage (Russi et al., 2013). Due to their low solubility in water, many pesticides tend to sorb onto particulate matter and subsequently become incorporated into bed sediments where they may act as long-term sources of contaminants to the aquatic environment (Weber et al., 1995). Estuarine environments may also act as conduits of pollutants to the coastal ocean and pesticide residues have been widely reported in nearshore sediments (Shaw et al., 2010; Xu et al., 2007) and coral reef ecosystems (Bargar et al., 2013; Haynes et al., 2000; Imo et al., 2008). While sediment contamination is often recognised as a major source of ecosystem health stress, little attention has focused on the potential ecotoxicological risks of sediment-associated pesticides, with the vast majority of studies limiting assessment to water quality standards (Carriger and Rand, 2008; Palma et al. 2014; Stamatis et al., 2013; Thurman et al., 1991).

Lake St Lucia (Fig. 1) is the largest estuarine system in Africa and forms an integral component of the UNESCO World Heritage iSimangaliso Wetland Park in South Africa. The lake and its surrounding wetland areas support considerable biodiversity and are host to large hippopotamus and crocodile populations, as well a diverse range of fish and bird species. The adjacent coral reef communities constitute the southern limit of their distribution in the western Indian Ocean and are characterised by exceptionally high levels of biodiversity (Porter and Schleyer, 2019). Although considered the oldest protected estuary in the world (Perissinotto et al., 2013) and afforded Ramsar and World Heritage Site status, Lake St Lucia is impacted by river catchments which lie outside the boundaries of the conservation area. While these catchment areas play a vital role in regulating freshwater and sediment supply to the lake, they have been subjected to significant land-use alterations, most notably in the form of agriculture (Burgoyne and Kelso, 2014; KZN Provincial Government, 2009; Ntombela, 2003; Searle, 2013). The river floodplains are characterised by silt-rich soils which provide fertile agricultural land on an otherwise sandy coastal plain. Commercial sugarcane farming in the region commenced in 1927 and widespread aerial spraying of insecticides to combat tsetse fly and the spread of malaria followed in the 1940s

(Brown, 2008; Maloa, 2001). Many of the applied chemicals were organochlorine pesticides (OCPs) that included DDT, lindane and dieldrin. While these compounds have since been banned for agricultural purposes, several recent studies have shown the extent to which OCPs have accumulated within sediments and biota of Lake St Lucia (Buah-Kwofie et al., 2018a, 2018b; Buah-Kwofie and Humphries, 2017). These compounds have also been shown to enter the marine environment and accumulate within the tissues of coral reef organisms (Porter et al. 2018). Although the occurrence of OCPs remains a major concern in the region, several other pesticide classes have also been used. South Africa is the highest user of pesticides in sub-Saharan Africa (Dalvie et al., 2009), with over 8 000 pesticide formulations having been registered for use. Despite this, little information exists regarding the prevalence and impact of commonly used agricultural herbicides on biologically valuable aquatic ecosystems, such as Lake St Lucia.

The objective of this study was to investigate the occurrence and potential ecotoxicological risks associated with herbicide residues in sediments from Lake St Lucia. The influence of agricultural runoff as a major entry route for pesticide residues into Lake St Lucia was assessed by examining residues in sediments from the two largest fluvial inputs; the Mkhuze and Mfolozi rivers. We focused on 11 herbicide residues, which included triazines (atrazine, hexazinone, simazine and terbuthylazine), anilides/aniline (acetochlor, alachlor, metolachlor and trifluralin), carbamate (EPTC) and phenoxy-acids (2,4-D and MCPA). These compounds were targeted on the basis of them being identified as priority herbicides in South Africa in terms of their potential risk to human health. The analytes included rank among the top 25 priority pesticides identified by Dabrowski et al., (2014) using a weighted hazard potential index based on quantity of use, toxicity potential, environmental exposure potential. This study represents the first investigation into the prevalence of herbicide residues in Lake St Lucia and provides critical baseline data not only for assessing potential ecological risks to the estuarine ecosystem, but also as a potential source to the marine environment.

2. 2 Materials and Methods

2.2.1 *Study area*

Lake St Lucia is large (350 km^2) shallow estuarine system located in KwaZulu-Natal (KZN) on the sub-tropical east coast of South Africa (28°00′26″ S, 32°28′51″ E; Fig. 1). The lake is separated from the ocean by a barrier dune complex, with the only contemporary link to the ocean via a long sinuous channel known as the Narrows. The mouth of the estuary is susceptible to prolonged periods of closure, with lake water levels driven predominately by

fluvial discharge (Cyrus et al., 2011; Cyrus and Vivier, 2006). Lake St Lucia is supplied by five rivers, the catchments of which lie outside the boundaries of the Park. The Mkhuze River that flows into Lake St Lucia from the north and the Mfolozi River which enters near the estuary mouth are the largest contributors to sediment and water supply. The rivers are seasonal, flowing during the wet summer months, but typically reduced to seepage through bed sediments during the drier winter months. Persistent groundwater seepage occurs through shallow coastal plain aquifers, although this contributes only a small component to the overall water balance of the lake (Kelbe et al., 2013).

The Mkhuze (6,000 km^2) and Mfolozi (10,085 km^2) rivers drain extensive catchment areas, which together represent >90% of the watershed area entering Lake St Lucia (Hutchison and Pitman, 1977). Both rivers carry high suspended sediment loads and their lower reaches are characterised by extensive silt-rich floodplains (Grenfell et al., 2009; Humphries et al., 2010). The Mkhuze River drains an extensive natural swamp area (Fig. 1B) that significantly attenuates the discharge of sediment into the northern end of Lake St Lucia. In contrast, flow along the lower reach of the Mfolozi River has been modified by the installation of drainage canals and construction of levees to limit the flooding of adjacent farmlands. For several decades, the Mfolozi River was artificially separated from the St Lucia estuary mouth over concerns that hydrological modifications on the floodplain was causing increased siltation near the St Lucia estuary mouth (Forbes et al., 2020). The management strategy to keep the inlets of the Mfolozi and St Lucia systems separate was eventually abandoned in the early 2000s in response to a prolonged and devastating drought. In 2012, a spillway was established to facilitate the natural relinking of the Mfolozi and St Lucia systems. This connection has been maintained ever since and today the Mfolozi River is a significant contributor to the freshwater and sediment supply of Lake St Lucia.

Approximately 18% of the Mfolozi catchment is under agriculture (Stretch and Maro, 2013), but the majority of this activity is concentrated on the lower reaches of the floodplain (Fig. 1). Agriculture began in the early twentieth century and today commercial sugarcane operations occupy around 90 km^2 of land adjacent to the Mfolozi River. Cultivation on the Mkhuze floodplain is largely confined to the western fringe and limited mainly to community subsistence farming and small-scale commercial agriculture (Dahlberg and Burlando, 2009).

2.2.2 *Sample collection*

A total of 52 grab top-most (~ 5 cm) sediment samples were collected from Lake St. Lucia (n =14) and its two main fluvial inputs; the Mkhuze River (n = 26) and Mfolozi River (n = 12)

using a Van Veen grab sampler and placed in aluminium foil and sealed in zip lock bags. Sampling occurred during 2018/2019 and focused on the lower floodplain reaches of each river where agricultural activities are most concentrated. Samples were frozen on the day of collection and transported to the laboratory where they were then air-dried at room temperature, homogenised using a mortar and pestle, and sieved to <125 µm. The sieved samples were transferred into amber bottles and refrigerated at -18 ^{o}C until analysis.

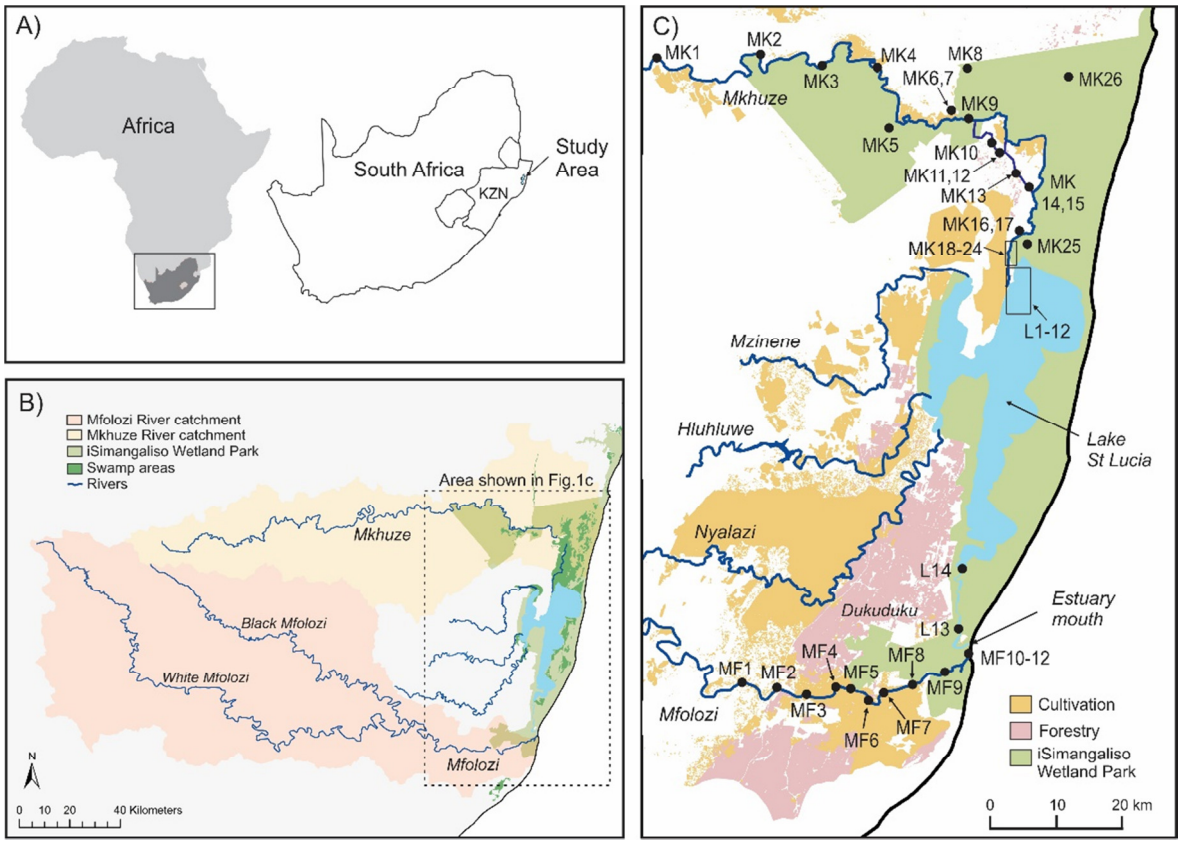

Fig. 1. Study area. A) Map showing the location of the study site situated in the province of KwaZulu-Natal (KZN) on the east coast of South Africa. B) Lake St Lucia and the catchment areas of the Mkhuze and Mfolozi rivers. C) Areas under major cultivation and location of samples collected from Lake St Lucia (L1-14), the Mkhuze River (MK1-26) and Mfolozi River (MF1-12).

2.2.3 *Sample extraction*

The extraction of phenoxy-acid (MCPA and 2,4-D) and multi-residue (acetochlor, alachlor, atrazine, EPTC, hexazinone, metolachlor, simazine, terbuthylazine and trifluralin) herbicides was carried out following modified QUEChERS procedures (Buah-Kwofie and Humphries, 2017; Santilio et al., 2011).

For the extraction of phenoxy-acid herbicides, 5 g of sample was saturated with 10 mL water in a 50 mL centrifuge tube. This was followed by alkaline hydrolysis with 300 µL of 5 N NaOH. After vigorous shaking and allowing the sample to stand for 30 minutes, the mixture was neutralized with 300 µL of 5 N H_2SO_4. The sample was extracted in 10 mL acetonitrile containing 4 g anhydrous $MgSO_4$, 1 g NaCl, 1 g tri-sodium citrate dihydrate, and 0.5 g disodium citrate sesquihydrate. The sample was shaken vigorously by hand, vortexed for 2 minutes and then centrifuged to separate the organic extract. A 5 mL aliquot of the extract was transferred into a tube containing 900 mg $MgSO_4$, 150 mg primary secondary amine (PSA) and 150 mg C18. This mixture was vortexed and then centrifuged to isolate the clean extract. A 3 mL aliquot of the supernatant was concentrated to dryness under vacuum (≤ 40 ^oC) and reconstituted in 50:50 MeOH:H_2O containing 1% formic acid. This was filtered through a 0.45 µm membrane filter and gravimetrically spiked with 20 ng mL^{-1} from a 1000 ng mL^{-1} standard solution containing each analytes reference standard and 50 ng mL^{-1} nicarbazin as internal standard.

Multi-residue herbicides were extracted from samples (10 g) using 20 mL acetonitrile (containing 1% glacial acetic acid) and a mixture of $MgSO_4$, CH_3COONa and $C_2H_9NaO_5$ The mixture was shaken vigorously by hand, vortexed for 2 minutes and then centrifuged to separate the organic extract. The supernatant was transferred into a centrifuge tube containing clean-up sorbents (1.2 g $MgSO_4$, 0.4 g C18, and 0.4 g PSA), vortexed and then centrifuged to separate the clean extract. A 5 mL aliquot of the extract was concentrated to dryness under vacuum (≤ 40 ^oC), reconstituted in hexane. A 20 ng mL^{-1} reference standard mix (from a 1000 ppb solution containing each of the targeted analytes) and 50 ng mL^{-1} pentachloronitrobenzene (PCNB) internal standard spike was gravimetrically added prior to analysis.

2.2.4 *Analysis*

Phenoxy-acids were analysed by LC-MS-MS using a Thermo Scientific Dionex Ultimate 3000 UHPLC system coupled to a Bruker Compact Q-TOF mass spectrometer. Chromatographic separation was performed on a Phenomenex Luna Omega 1.6 µm C18 column (100 Å, 2.1 × 100 mm) using two solvent systems: (A) 0.1% formic acid aqueous solution and (B) MeOH with 1% formic acid. The injection volume was 10 µL, flow rate was set at 0.3 mL min^{-1} and a gradient elution was used with the following conditions: 0-1 min, 5 % B; 2-3 min, 50% B; 4-5 min, 54% B; 6-7 min, 60% B; 8-9 min, 70% B; 11-13 min, 95 B; 14-16 min, 5% B. Multiple reaction monitoring (MRM) parameters were optimized by direct

infusion of a 1000 ppb composite standard solution in the MS and ionized using both positive and negative electron spray ionization (ESI^+ and ESI^-). Analytes were analysed in negative polarity. The parent ions of both target herbicides were observed in a full MS scan and fragmented by varying the collision energy of the ionization to obtain the most abundant product ion when the intensity of parent ion decreased to below 200.

Multi-class analytes using two-dimensional gas chromatography time-of-flight mass spectrometry (GC X GC-TOFMS). Analysis was performed using an Agilent 7890 GC equipped with a Leco Pegasus 4D TOF mass spectrometer. Separation was achieved using a Restek BPx-5 MS column with integra-Guard column coupled to a Rxi-17Sil MS secondary column. Samples of 2 µL were injected in a splitless mode using high purity helium as a carrier gas at a flow rate of 1.4 mL min^{-1}. The GC oven temperature was set to 55 ^oC (held for 1 min), ramped to 200 ^oC at a rate of 8 ^oC min^{-1} and maintained at this temperature for 4 min, and finally ramped to 270 ^oC at a rate of 10 ^oC min^{-1} and maintained at this temperature for 2 min. Data processing and peak identification were performed using the Leco ChromaTOF software and databases. Peaks were identified based on the retention time of specific ions and confirmed by two identifier ions.

Quantification was performed in triplicate against high purity (>98%) reference standards obtained from Sigma-Aldrich. Linear regressions derived from the matrix-matched calibration curves for all multi-residue compounds were $r^2 > 0.99$. Concentration data were tested for normality using a *Shapiro-Wilk* test, which indicated a nonparametric distribution. Statistical relationships between different herbicide classes and mean concentrations in St Lucia, Mkhuze and Mfolozi sediments were examined using *Kruskal-Wallis* analysis of variance (K-W ANOVA) followed by Tukey's post-hoc test. Analytes not detected and <LOD were assigned a value of zero. All statistical analyses were performed in OriginPro 2019b. Significance was set at $p < 0.05$.

2.2.5 *Quality assurance and quality control*

The extraction procedures outlined above were validated by performing spike recovery tests at three different fortification levels. Recoveries (n = 3) ranged between 70 and 81% for phenoxy acids and between 77 and 105% for multi-residue procedures. Analytical limits of detection (LOD) were in the range of 0.05 – 0.29 ng g^{-1} and 0.19 – 0.27 ng g^{-1} for multi-residue and phenoxy acid analytes, respectively. Limits of quantification (LOQ) ranged between 0.17 and 0.97 ng g^{-1} for all analytes. Internal precision (%RSD) was typically <15%. Blanks were run with every sample batch and quality control standards were analysed after

every three samples to monitor and correct for drift in instrument response. HPLC grade solvents (Sigma-Aldrich) were used for all analyses

2.2.6 *Risk assessment*

Aquatic risk was assessed based on the Risk Quotient (RQ) index using the ratio between the Measured Environmental Concentration (MEC) and the Toxic Effect Concentration (TEC) reported in other studies (Hela et al. 2005; Hernando et al., 2006; Papadakis et al., 2015). For MEC, we used the mean and maximum detected concentrations for each pesticide, representing the "general" and "worst case" scenarios, respectively. As benchmark toxicity tests are based on dissolved phase concentrations in pore-water, measured sediment-associated herbicide concentrations were converted to pore-water concentrations using an equilibrium-partitioning approach (Ccanccapa et al., 2016). Pore-water concentrations (C_{pw}) were calculated as: $C_{pw} = \frac{C_s}{K_d}$, where C_s is the sediment concentration of the herbicide and K_d the partitioning coefficient. The partitioning coefficient was estimated as $K_d = K_{oc} \times f_{oc}$ (Hornsby et al., 1996) where K_{oc} , the organic carbon-water partitioning coefficient, is obtained from literature (Hornsby et al., 1996; Navarro-Ortega et al., 2010; Tadeo. 2008), and f_{oc} is the fraction of total organic carbon measured in the sediments. The organic carbon content of sediments was estimated based on loss on ignition at 550 ^{o}C (Kerven et al., 2000; Sleutel et al., 2007). PNEC was based on the acute toxicity values (EC_{50} or LC_{50}) of each herbicide across four taxonomic groups; algae, zooplankton (*Daphnia magna*), benthic invertebrate (*Chironomus riparius)* and fish. These values were obtained from literature and are provided in the Supplementary Information (TS2.2) along with the physicochemical and ecotoxicology parameters used in the calculations.

Since pesticide mixtures are reported to have potential additive, synergistic or antagonistic toxicity effects (ANZECC and ARMCANZ, 2000; Qu et al., 2011), we calculated the RQ for mixtures as: $Total\ RQ = \sum_{1=1}^{n} RQ_i$ at both maximum and mean concentration exposure levels. Risk Quotient levels of concern are placed at RQ = 0.01 to 0.1 (low risk), RQ = 0.1 to <1 (medium risk), RQ = 1 (high risk) and RQ >1 (very high risk).

2.3 Results

2.3.1 *Concentration and distribution of herbicide groups*

Herbicide residues were detected in all samples analysed, with all target herbicide classes present in Lake St Lucia, Mkhuze and Mfolozi sediments (Table 1, Supplementary Information TS2.3). The frequency of detection for the range of compounds analysed was typically higher in samples from the Mkhuze (70 ± 16%) and Mfolozi (77 ± 14%) rivers when compared to Lake St Lucia (53 ± 18%). On average, acetochlor (78%), terbuthylazine (76%), trifluralin (78%) and 2,4-D (75%) were the most frequently detected compounds, while MCPA (51%) and metolachlor (55%) were least prevalent.

On average, total herbicide concentrations in Mkhuze (27.3 ng g^{-1}) and Mfolozi (25.6 ng g^{-1}) sediments were similar, but highly variable, ranging 1.89 – 67.8 ng g^{-1} and 6.65 – 82.4 ng g^{-1}, respectively. Lower average herbicide levels were measured in sediments from Lake St Lucia (12.9 ng g^{-1}), where concentrations ranged between n.d. to 34.7 ng g^{-1}. Triazines and Anilides were the predominant herbicide classes, together contributing >70% of total measured concentrations (Fig. 2). On average, highest concentrations of triazines were detected in sediments from the Mkhuze River (10.1 ± 8.7 ng g^{-1}), followed by the Mfolozi River (6.68 ± 7.7 ng g^{-1}) and Lake St Lucia (4.27 ± 5.0 ng g^{-1}). Similar anilides/aniline concentrations were found in Mkhuze (12.3 ± 10.1 ng g^{-1}) and Mfolozi sediments (11.9 ± 12.6 ng g^{-1}), but were present in noticeably lower concentrations in Lake St Lucia (6.08 ± 6.1 ng g^{-1}). The phenoxy acid and carbamate groups typically contributed <20% and <10% to total concentrations, respectively. Highest phenoxy acid concentrations were measured in Mfolozi sediment (4.81 ± 5.2 ng g^{-1}), while similar carbamate concentrations were found in the Mfolozi (2.17 ± 1.4 ng g^{-1}) and Mkhuze (2.20 ± 1.8 ng g^{-1}) rivers. Lake St Lucia sediments were characterised by lowest carbamate and phenoxy acid concentrations, averaging 1.07 ± 1.7 ng g^{-1} and 1.46 ± 2.3 ng g^{-1}, respectively.

2.3.2 *Variation in herbicide residues*

Herbicide residue concentrations measured in river and lake sediment samples are presented in Fig. 3. Overall, the most prominent residues detected included acetochlor (3.77 ± 1.3 ng g^{-1}), hexazinone (2.86 ± 1.4 ng g^{-1}) and metolachlor (10.1 ± 8.7 ng g^{-1}). Despite considerable variability between individual samples, notable differences in the herbicide signatures of the three sampling areas are evident. Hexazinone (4.40 ± 5.0 ng g^{-1}), acetochlor (3.59 ± 3.2 ng g^{-1}) and trifluralin (3.21 ± 5.2 ng g^{-1}) were prominent in Mkhuze River sediments, while acetochlor (5.12 ± 7.8 ng g^{-1}), metolachlor (3.35 ± 5.2 ng g^{-1}) and 2,4-D (3.70 ± 4.0 ng g^{-1}) were the most notable residues detected in Mfolozi River sediments. Lower concentrations of almost all herbicide residues were found in lake sediments, with hexazinone (2.31 ± 4.0 ng g^{-1}) and acetochlor (2.60 ± 2.4 ng g^{-1}) being the most prominent.

Lake St Lucia was characterised by significantly lower concentrations of simazine and 2,4-D compared to levels measured in Mkhuze and Mfolozi sediments, respectively.

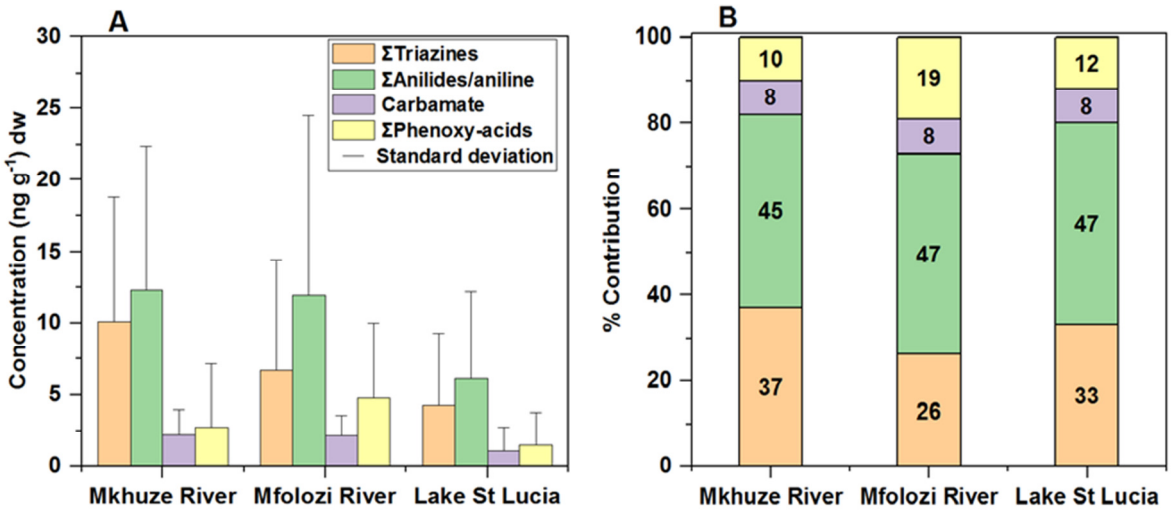

Fig. 2. Comparison between herbicide groups measured in sediments from the Mkhuze, Mfolozi and Lake St Lucia. A) Average total concentration of each herbicide class. B) Relative contribution of each herbicide class to total concentration.

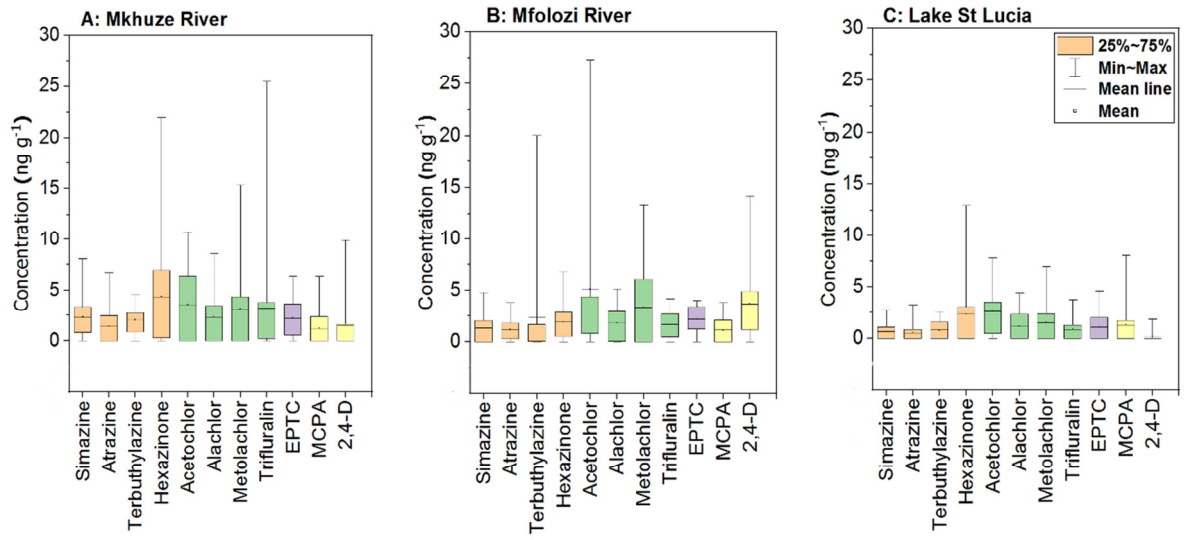

Fig. 3. Box and whisker plots showing distribution in herbicide residue concentrations measured in sediments from (A) Mkhuze River, (B) Mfolozi River, and (C) Lake St Lucia

Table 1. Mean concentration (± SD) of herbicides in surface sediments from St Lucia and its two major catchments

Herbicide	Mkhuze River (n = 26)			Mfolozi River (n = 12)			Lake St Lucia (n = 14)		
	Concentration (ng g^{-1})			Concentration (ng g^{-1})			Concentration (ng g^{-1})		
	Detection (%)	Mean	Range	Detection (%)	Mean	Range	Detection (%)	Mean	Range
Triazines									
Atrazine	69	1.41 ± 1.6	n.d. – 6.71	83	1.14 ± 1.4	n.d. – 3.83	43	0.53 ± 0.9	n.d. – 3.25
Hexazinone	77	4.40 ± 5.0	n.d. – 22.0	83	1.88 ± 2.0	n.d. – 6.87	57	2.31 ± 4.0	n.d. – 13.0
Simazine	88	2.27 ± 1.9	n.d. – 8.11	67	1.30 ± 1.6	n.d. – 4.80	50	0.67 ± 0.9	n.d. – 2.73
Terbuthylazine	88	2.05 ± 1.3	n.d. – 4.57	75	2.37 ± 5.7	n.d. – 20.1	64	0.77 ± 0.9	n.d. – 2.53
Anilides/Aniline									
Acetochlor	73	3.59 ± 3.2	n.d. – 10.8	75	5.12 ± 7.8	n.d. – 12.8	86	2.60 ± 2.4	n.d. – 7.83
Alachlor	69	2.30 ± 2.2	n.d. – 8.6	75	1.81 ± 1.7	n.d. – 5.13	50	1.11 ± 1.5	n.d. – 4.44
Metolachlor	65	3.16 ± 3.8	n.d. – 15.3	50	3.35 ± 5.2	n.d. – 13.3	50	1.52 ± 2.0	n.d. – 6.98
Trifluralin	77	3.21 ± 5.2	n.d. – 25.5	92	1.66 ± 1.3	n.d. – 4.18	57	0.85 ± 1.3	n.d. – 3.77
Carbamate									
EPTC	85	2.20 ± 1.8	n.d. – 6.41	83	2.17 ± 1.4	n.d. – 4.02	43	1.07 ± 1.7	n.d. – 4.62
Phenoxy-acids									
MCPA	46	1.24 ± 1.9	n.d. – 6.5	58	1.11 ± 1.4	n.d. – 3.82	50	1.28 ± 2.3	n.d. – 8.09
2,4-D	65	1.49 ± 2.8	n.d. – 9.94	92	3.70 ± 4.0	n.d. – 14.2	69	0.18 ± 0.5	n.d. – 1.83
Total		27.3 ± 17	1.8 – 67.8		25.6 ± 20	6.65 – 82.4		12.9 ± 12	n.d. – 34.7

n.d. : Not detected

2.4 Discussion

2.4.1 *Prevalence of herbicides*

Sediments from Lake St Lucia and its nearby catchment areas contain measurable concentrations of a range of different herbicide residues. The high frequency of residue detection suggests that herbicide use, and environmental contamination is fairly widespread in the region. While these compounds may enter Lake St Lucia partly via groundwater discharge and atmospheric deposition, runoff from agricultural land is considered to be the likely dominant transport pathway (Buah-Kwofie and Humphries 2017). The Mkhuze and Mfolozi rivers thus act as the main entry points for herbicides and all residues found within river samples were also detected in Lake St Lucia, although typically at lower concentrations and frequencies. Despite differences in agricultural practices (commercial vs subsistence) and cropping intensity, Mkhuze and Mfolozi river sediments were characterised by similar average herbicide concentrations, 27.3 ± 17 ng g^{-1} and 25.6 ± 20 ng g^{-1}, respectively. River sediments contained approximately double the herbicide load measured in Lake St Lucia (12.9 ± 12 ng g^{-1}), reflecting their proximity to agricultural point sources. While the prevalence of herbicides in sediments from the study area is expected to reflect use within the catchment areas, a variety of physicochemical properties (e.g. water solubility, stability, and binding affinity) influence both transport and the accumulation tendency of different residues within the environment.

2.4.2 *Influence of land use and physicochemical parameters*

The Mkhuze and Mfolozi rivers both occupy wide, low-gradient floodplains that experience similar climatic conditions. While several catchment and physicochemical factors may influence the movement of pesticides into watercourses, the quantity and rate of pesticide application is considered the most important indicator for the potential contamination of non-target environments (Dabrowski, 2015). The most recent estimates on pesticide application rates for South Africa are provided by Dabrowski, (2015) who used agricultural land-cover and crop specific pesticide use data to assess the likely spatial distribution of active ingredients across the country. We made use of these data in evaluating the occurrence and distribution of herbicides measured in sediments from our study area.

Over 17,000 kg of 2,4-D and 9,000 kg of 2,4-D amine is reported to be used each year in KZN, largely in sugarcane production (Dabrowski, 2015). It is therefore not surprising that relatively high concentrations of 2,4-D were detected in the majority of Mfolozi River

samples. Lower concentrations of 2,4-D were detected in Mkhuze sediments, where it is likely associated with small-scale subsistence and communal sugarcane growing practices, which have increased significantly in recent years (Burgoyne and Kelso, 2014). 2,4-D is highly soluble and degrades rapidly in soil (half-life <7 days), and its presence in the majority of the sediments analysed here suggests that this compound is applied on a large-scale in the region. MCPA is also often used in sugarcane production, typically in combination with other herbicides (SASRI, 2018), although this compound was detected less frequently and in lower concentrations compared to 2,4-D.

Approximately 97,000 kg of atrazine and 76,000 kg of hexazinone is used on an annual basis in KZN (Dabrowski, 2015). There are 13 hexazinone and 7 atrazine formulations reported to be in use for weed control in sugarcane production in the region (SASRI, 2018). Lower quantities of terbuthylazine and simazine are also used in the cultivation of maize, sugarcane, sorghum, pineapples and a host of other crops (Dabrowski, 2015). Triazines were widely detected in both the Mkhuze and Mfolozi rivers but occurred in higher concentrations in Mkhuze sediments where they are likely associated with community farming that includes popular crops such as maize, cabbage, potatoes and mangoes (Burgoyne and Kelso, 2014). This takes place on communal crop lands situated directly adjacent to the Mkhuze River, which is often the primary source of water for irrigation. Communities that border the Mfolozi River, notably in the vicinity of Dukuduku on the northern side of the river, are also involved in the cultivation of maize, sugarcane, beans, vegetables and fruits (Ntombela, 2003). Despite having a low tendency to adsorb to sediment or suspended matter (Log K_{ow} < 3), atrazine and simazine are considered persistent herbicides (Seybold et al., 1999; Jablonowski et al., 2009) and were detected in most samples, although in relatively low concentrations. Hexazinone was the most prominent triazine residue in both Mkhuze and Lake St Lucia sediments, which was unexpectedly given its high solubility and low tendency to sorb to sediment (K_{ow} = 1.95). Relatively high concentrations of hexazinone (up to 22.0 ng g^{-1}) within Mkhuze sediments suggest widespread use of this compound in communal farming practices.

Anilides/aniline compounds are some of the most widely used in herbicides in the region, with in excess of 20,000 kg of acetochlor, alachlor and metolachlor being used on an annual basis in KZN (Dabrowski, 2015). These herbicides are used in the cultivation of sugarcane, sorghum, maize, sweet potatoes and sunflowers, and were detected in relatively high concentration in sediments from the study area. This likely reflects their widespread use in the region as well as their tendency to absorb to sediment particles (Koc = 200 – 8000). Trifluralin, which is relatively persistent and has a high tendency to bind to soil particles (Koc

= 8000), was particularly prominent in the Mkhuze sediments where it is likely associated with the subsistence farming of soy beans, cabbage, groundnuts, tomatoes and wheat (Quinn et al., 2011).

2.4.3 *Risk assessment and ecological concerns*

Despite being the highest user of pesticides in sub-Saharan Africa, little is known about the environmental occurrence of herbicide residues and associated potential ecological risks in South Africa. Nevertheless, several priority listed pesticides, including atrazine, simazine, terbuthylazine and 2,4-D, have been detected in relatively high concentrations in surface and groundwater from intensive maize production areas in South Africa (Du Preez et al., 2005; George 2014a; Horn et al. 2019; Rimayi et al., 2018). The presence of atrazine and metolachlor has also been reported in river sediments from the eastern Free State (George, 2014b), where maize, wheat and sorghum are widely grown.

We applied a tier-1 ecotoxicological assessment in estimating the risks associated with the herbicide concentrations measured in this study. Cumulative RQ values for each herbicide class were calculated from their corresponding mean and maximum concentrations (Table 2). Triazines and anilides/aniline herbicides were generally detected in samples at levels that presented low (RQ >0.01) and medium (RQ >0.1) risks for algae at mean and maximum concentrations, respectively. Triazines also showed low risk to *D. magna* in Mkhuze River sediment at maximum concentrations. Carbamate and phenoxy acid herbicide groups fell below the low risk threshold for all taxa at all sites. The summed RQ values for each site indicate low and medium risk to algae populations across the study area at mean and maximum total herbicide concentrations, respectively (Fig. 4a). Highest risk was associated with herbicide levels measured in Mkhuze River sediments. Total RQs were overwhelmingly associated with the triazine and anilides/aniline classes across all environmental levels, except for *C. riparius,* where triazines and phenoxy acids tended to be most prominent (Fig. 4b).

Although the individual herbicide concentrations measured are not predicted to pose any environmental risk, cumulative totals suggest potential risk associated with mixtures at the algal and aquatic invertebrate community level. Triazines are known to inhibit photosynthesis in algae (Allinson et al., 2015; Magnusson et al., 2008), leading to potential changes in community structure, food reduction and habitat loss (Bester et al., 1995; Davies et al., 1994; Peterson et al., 1997). Studies have also reported impaired algae production by individual chloroacetanilides (alachlor, acetochlor and metolachlor) and complete algal growth

inhibition when exposed to mixtures of these herbicides (Hostovsky et al., 2014; Junghans et al., 2003).

While there are several limitations to tier-1 risk assessments, the qualitative screening evaluation presented here highlights the potential for the Mkhuze and Mfolozi rivers to act as pathways through which contaminated sediment of biological concern is transported into Lake St Lucia. Although both rivers were characterised by similar total herbicide concentrations, sediment introduced by the Mkhuze River is perhaps of greater ecological concern as this material remains trapped within the system, while Mfolozi-derived deposits would be flushed out to sea during episodic estuary mouth breachings. However, the sediment loads associated with each river differ considerably. In contrast to the extensive swamp system that the Mkhuze River drains upon entering Lake St Lucia, flow on the Mfolozi floodplain is highly modified by drainage canals and levees. This results in the Mfolozi River depositing substantial amounts of silt and clay within the estuary mouth and lower Narrows (Forbes et al., 2020), while sediment inputs associated with the Mkhuze River occur only during exceptionally high flow events. The Mfolozi River is thus expected to be the greatest contributor to the overall contaminant load entering Lake St Lucia.

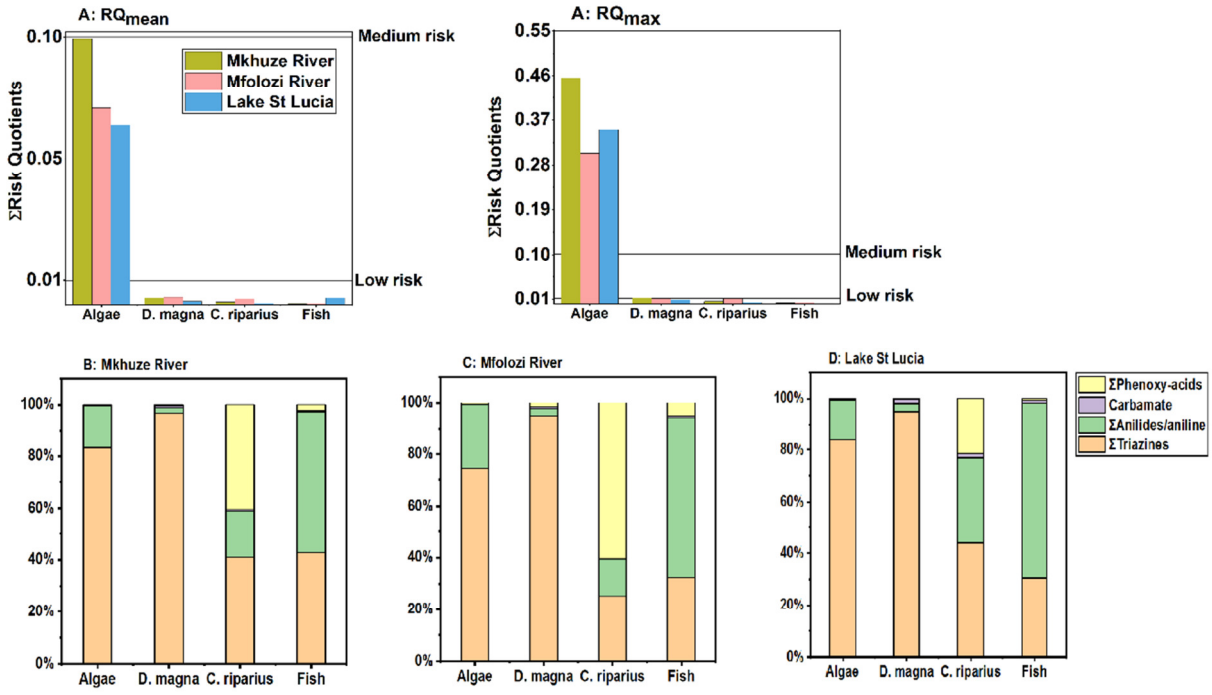

Fig. 4. (A) Summed risk quotients for different taxonomic levels calculated at mean and maximum concentration, (B-D) Relative contribution of each herbicide class to total risk in Mkhuze, Mfolozi and Lake St Lucia sediments at mean concentration.

Table 2. Risk quotients for the different sites and environmental levels based on the mean (**maximum**) concentration of each major herbicide class. Detailed results provided in Table TS2.4.

Herbicide class	Mkhuze River				Mfolozi River				Lake St Lucia			
	Algae	*D. magna*	*C. riparius*	Fish	Algae	*D. magna*	*C. riparius*	Fish	Algae	*D. magna*	*C. riparius*	Fish
Triazines	0.0817	0.0025	0.0004	0.0002	0.0540	0.0027	0.0006	0.0002	0.0554	0.0013	0.0002	0.0001
	(**0.3892**)	(**0.0119**)	(**0.002**)	(**0.0006**)	(**0.2458**)	(**0.0091**)	(**0.004**)	(**0.0008**)	(**0.3041**)	(**0.0077**)	(**0.0010**)	(**0.0004**)
Anilides/aniline	0.0163	0.0001	0.0002	0.0002	0.0182	0.0001	0.0003	0.0003	0.0105	<0.0001	0.0002	0.0002
	(**0.0637**)	(**0.0003**)	(**0.0007**)	(**0.0008**)	(**0.0557**)	(**0.0002**)	(**0.0009**)	(**0.0009**)	(**0.0429**)	(**0.0002**)	(**0.0005**)	(**0.0006**)
Carbamate	0.0001	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	(**0.0001**)	(**0.0001**)	(<**0.0001**)	(**<0.0001**)	(**0.0001**)	(**0.0001**)	(<**0.0001**)	(**<0.0001**)	(**0.0001**)	(**0.0001**)	(<**0.0001**)	(**<0.0001**)
Phenoxy acids	0.0003	<0.0001	0.0004	<0.0001	0.0004	<0.0001	0.0013	<0.0001	0.0003	<0.0001	0.0001	<0.0001
	(**0.0016**)	(**0.0001**)	(**0.0030**)	(**0.0001**)	(**0.0015**)	(**0.0001**)	(**0.0051**)	(**0.0001**)	(**0.0024**)	(**<0.0001**)	(**0.0009**)	(**<0.0001**)

2.5 Conclusions

The results of this study show that herbicides commonly used in commercial and communal farming areas surrounding Lake St Lucia contaminate non-target aquatic environments. Herbicides, particularly those belonging to the triazine and anilides/aniline families, were widely detected in sediments from the Mkhuze and Mfolozi rivers, which are considered the main pathways through which herbicides enter Lake St Lucia. Ecological risk assessment revealed that current herbicide concentrations could pose a threat to sensitive aquatic life, particularly at the algal and aquatic invertebrate community level. However, it should be highlighted that this study represents a preliminary assessment based on a limited number of compounds and did not include some products that are extensively used in South Africa, such as mancozeb and glyphosate. The ecological risk assessment presented here should thus be viewed as conservative and additional monitoring studies are recommended.

Although this study focussed specifically on the occurrence and distribution of herbicides in sediment, it is recommended that further research on aquatic system health be conducted considering the biological importance of Lake St Lucia as nursery for aquatic organisms. Furthermore, the potential exposure of human communities to herbicides and their associated health effects should also be considered. Many rural communities in the region do not have access to treated water and often make use of water collected directly from surface and groundwater resources. The identification of priority areas for management and investigation into mitigation strategies is suggested, particularly as agricultural activities on the Mkhuze floodplain are likely to expand in the future.

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CHAPTER 3

Bioaccumulation of current-use herbicides in fish from a global biodiversity hotspot: Lake St Lucia, South Africa

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Abstract

Agricultural pesticides threaten aquatic systems and biodiversity at a global scale, but limited information is available on the accumulation of current-use herbicides in tissues of aquatic organisms. Here, we examine the potential exposure and accumulation of currently used herbicides in two species of fish from Lake St Lucia, a global biodiversity hotspot located in South Africa. Muscle tissue samples were analysed for 11 widely used multi-residue and phenoxy-acid herbicides. Herbicide residues were detected in all samples analysed, with total concentrations ranging from 44.3 – 238 ng g^{-1} (*Clarias gariepinus*) and 72.2 – 291 ng g^{-1} dw (*Oreochromis mossambicus*). The most prominent herbicides detected included the two phenoxy-acid herbicides, MCPA (17.6 ± 12 ng g^{-1}) and 2,4-D (28.9 ± 16 ng g^{-1}), along with acetochlor (15.4 ± 5.8 ng g^{-1}), atrazine (12.7 ± 7.1 ng g^{-1}) and terbuthylazine (12.4 ± 12 ng g^{-1}). Results indicate that fish at Lake St Lucia accumulate a complex mixture of herbicides, some previously unreported in tissue, highlighting the potential threat that agricultural runoff may pose to conservation areas. However, assessing the impact of herbicide accumulation on wild fish populations is difficult at present and urgent toxicological data are needed to better understand chronic exposure effects on aquatic organisms. A preliminary human health risk assessment indicated minimal risk associated with the consumption of local fish, but potential additive and synergistic effects of contaminant mixtures remain unknown.

3.1 Introduction

Agriculture is one of the leading sources of pollution impacting rivers and lakes globally (Wang, 2006; Mateo-Sagasta et al., 2017; Evans et al., 2019; Sharma et al., 2019). Herbicides are among the most widely used chemicals in the agricultural sector, intensively applied over vast areas as the standard method for weed control (Sharma et al., 2019).

Many commonly used herbicides, including atrazine, acetochlor and 2,4-D, are highly mobile in the environment and their use has resulted in widespread contamination of groundwater (e.g., Sun et al., 2020), streams (e.g., Correia et al., 2020) and estuaries (e.g., Rodrigues et al., 2018). Although herbicide concentrations in surface waters are generally low, even for residues that are relatively mobile and persistent (Solomon et al., 2013), mounting evidence suggests that herbicides can induce sub-lethal physiological and behavioural changes in non-target aquatic organisms (e.g., Stara et al., 2016; Velisek et al., 2016; de Albuquerque et al., 2020). Moreover, certain herbicide residues have been shown to accumulate in organisms, where they may potentially affect different levels of the food chain. Herbicide accumulation has been reported in a variety of fish species (Abrantes et al., 2010; Rimayi et al., 2018; Ojemaye et al., 2020a), as well as in birds and mammals from the Baltic Sea (Reindl et al., 2015). However, the extent to which commonly used herbicides accumulate in organisms and move through the aquatic food chain remains widely underreported and represents a crucial gap in knowledge for environmental decision-making.

South Africa is currently the largest consumer of agrochemicals in Africa, with over 300 different herbicide products registered for use (Quinn et al., 2011; Dabrowski et al., 2014). Despite their widespread use, surprisingly few studies have evaluated the prevalence of herbicide contamination in the region. Nevertheless, herbicide residues, including atrazine, simazine, alachlor, metolachlor, glyphosate and 2,4-D, have recently been reported in South African waters (Horn et al., 2019; Curchod et al., 2020; Ojemaye et al., 2020b) and in a few local fish species (Rimayi et al., 2018; Barnhoorn & van Dyk, 2020; Ojemaye et al., 2020a). The impact of herbicides on non-target organisms and aquatic ecosystem function is of particular concern in areas important for biodiversity conservation. Recently, we reported on the widespread presence of herbicide residues in sediments from Lake St Lucia, South Africa (Tyohemba et al., 2020). Lake St Lucia (Fig. 1) is a large (350 km^2) shallow estuarine system located within the iSimangaliso World Heritage Site and recognised as a global hotspot for biodiversity. The system is considered the single most important nursery for estuary-associated fish and invertebrates on the south-east coastline of Africa, and the largest protected estuarine environment for hippos, crocodiles, and aquatic birds on the continent (Porter, 2013). However, intensive agriculture occurs within the surrounding catchment areas and rivers flowing into Lake St Lucia act as important conduits for the transport of herbicides into the system (Tyohemba et al., 2020). A preliminary ecological risk assessment indicated that herbicides present in Lake St Lucia could have negative effects on algal and macroinvertebrate communities (Tyohemba et al., 2020).

The aim of this study was to evaluate the extent to which commonly used herbicides bioaccumulate in fish at Lake St Lucia. This was assessed by examining herbicide concentrations in muscle tissue samples from two locally abundant fish species; *Oreochromis mossambicus* (Mozambique tilapia) and *Clarias gariepinus* (African sharptooth catfish). Fish occupy a strategic position in the food web as they form the main component in the diet of many higher trophic level species inhabiting Lake St Lucia (e.g., birds and crocodiles) and are also frequently consumed by local communities. The accumulation of contaminants in fish therefore has both potential ecological and human health implications. Potential human health risks associated with the consumption of contaminated fish from Lake St Lucia were assessed using a preliminary quantitative risk assessment.

We report on the presence of 11 herbicide residues, which included triazines (atrazine, hexazinone, simazine and terbuthylazine), anilides/aniline (acetochlor, alachlor, metolachlor and trifluralin), carbamate (EPTC) and phenoxy-acids (2,4-D and MCPA). These herbicides were targeted on the basis of them being identified as priority herbicides in South Africa in terms of their potential risk to human health (Dabrowski et al., 2014), and have also been recently detected in sediments from Lake St Lucia (Tyohemba et al., 2020). To our knowledge, the bioaccumulation of hexazinone, EPTC and MCPA has not previously been assessed in any aquatic vertebrate.

3.2 Materials and Methods

3.2.1 *Study Site*

Lake St Lucia is located on the sub-tropical east coast of South Africa and is considered the largest estuarine lake in Africa (Porter, 2013). The site is a Ramsar listed wetland and forms part of iSimangaliso Wetland Park, a UNESCO World Heritage Site. The lake is a vast shallow-water system (average depth of 1 m) comprising three interconnected basins (North Lake, South Lake and False Bay), which are sustained primarily by fluvial inputs. The only contemporary link to the sea is via an ~21 km long channel known as the Narrows. The estuary mouth, however, is susceptible to prolonged periods of closure and lake water levels are driven primarily by river inflows, with the Mkhuze and Mfolozi rivers being the largest contributors. Combined, these two rivers represent more than 90% of the watershed area entering Lake St Lucia (Hutchison & Pitman, 1977) and capture runoff from land under intensive commercial and subsistence agriculture.

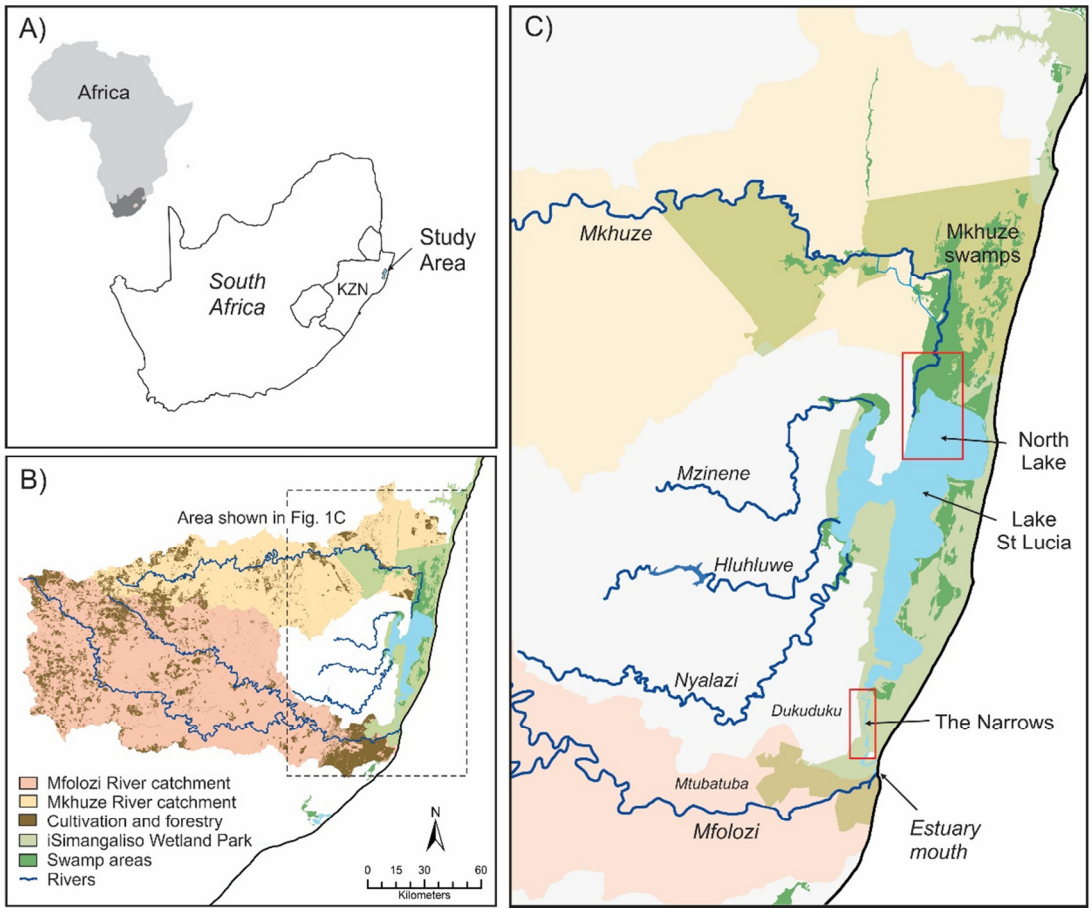

Fig. 1 Study area. a) Location of the study site on the east coast of South Africa. b) Lake St Lucia, its major catchments (Mkhuze and Mfolozi rivers) and areas under significant cultivation. c) The two locations (red boxes) from where fish samples were collected from.

Lake St Lucia is an important nursery ground for juvenile fish and 155 different fish species have been recorded, although their occurrence and distribution within the system varies strongly in response to salinity (Cyrus et al., 2011). *Oreochromis mossambicus* and *C. gariepinus* are two common resident species that typically occur throughout the system. *Clarias gariepinus* is a relatively large, sedentary, bottom-dwelling omnivore, which scavenges on benthic organisms and smaller fish. Adults typically reach an average length of 1 – 1.5 m and can weigh up to 60 kg when fully grown (Skelton, 2002). Growth is very rapid, with males reaching larger sizes than females. *Oreochromis mossambicus* is predominantly detritivorous and feeds primarily on phytoplankton (Skelton, 2002). Adults may reach 39 cm in length and up to 1.1 kg.

3.2.2 *Sample collection*

Sampling focused on two areas of Lake St Lucia (Fig. 1). The northern basin which receives drainage from the Mkhuze River was sampled in November 2018, while the Narrows, which receives inflow primarily from the Mfolozi River, was sampled in March 2019. A total of 57 specimens were collected, which included 40 *C. gariepinus* and 17 *O. mossambicus*. Fish were captured using standard angling techniques and euthanatized by severing the spinal cord with a sharp knife. All specimens were measured for total length and weight. Muscle tissue samples were removed, placed in aluminium foil and immediately frozen. In the laboratory, tissue samples were freeze-dried, homogenized into fine powder and then stored at −18 °C until analysis. Sampling was conducted under permit from Ezemvelo KZN Wildlife (permit number: 4298) and the iSimangaliso Wetland Park Authority. All field methods and laboratory analyses were performed in accordance with procedures approved by the University of the Witwatersrand Animal Ethics Committee (AESC number: 20133201).

3.2.3 *Sample extraction and analysis*

Multi-residue herbicides (acetochlor, alachlor, atrazine, EPTC, hexazinone, metolachlor, simazine, terbuthylazine and trifluralin) and phenoxy-acids (MCPA and 2,4-D) were extracted from 2 g tissue samples following a modified QuECheRS procedure reported by Tyohemba et al. (2020). Multi-residue analytes were extracted from samples using acetonitrile (10 mL) and a mixture of anhydrous magnesium sulfate (6 g), sodium acetate (1.5 g) and sodium acetate trihydrate (1 g). Extract clean-up was performed using a combination of $MgSO_4$ (1.2 g), PSA (0.4 g), C-18 (0.4 g) and florisil (0.4 g). The cleaned extract was concentrated to dryness under vacuum (≤ 40 ^{o}C), gravimetrically spiked with a reference standard and pentachloronitrobenzene (internal standard), and reconstituted in 1 mL hexane for final analysis. Phenoxy-acid herbicides were extracted with acetonitrile (10 mL containing 1% formic acid) following alkaline hydrolysis with NaOH (5 N 300 µL) and H_2SO_4 (5 N 300 µL). Anhydrous $MgSO_4$ (4 g), NaCl (1 g), tri-sodium citrate dihydrate (1 g), and disodium citrate sesquihydrate (0.5 g) were used to aid in the separation of the organic extract. The resulting mixture was frozen at –18 ^{o}C to solidify lipids present in the organic phase and an aliquot of the supernatant was then concentrated to dryness under vacuum (≤ 40 ^{o}C) and reconstituted in 1 mL MeOH:H_2O (50:50) containing 1% formic acid. A reference standard and nicarbazin (internal standard) was gravimetrically added prior to analysis. Tissue lipid content was estimated gravimetrically by extracting wet subsamples (1 g) with 10 mL hexane:acetone (3:1 v/v).

Multi-residue analytes were measured using two-dimensional gas chromatography time-of-flight mass spectrometry (GC X GC-TOFMS). Analysis was carried out using an Agilent 7890 GC equipped with a Leco Pegasus TOFMS. Phenoxy-acids were analysed by LC-MS-MS using a Thermo Scientific Dionex Ultimate 3000 UHPLC system coupled to a Bruker Compact Q-TOF mass spectrometer. All instrument settings were identical to those reported by Tyohemba et al. (2020). Quantification was performed in triplicate against high purity (> 98%) PESTANAL® reference standards purchased from Sigma-Aldrich. Correlation coefficients derived from linear regressions obtained from matrix-matched calibration curves were > 0.99 in all cases.

Concentration data were tested for normality using a *Shapiro-Wilk* test. Where the distribution was parametric, significant differences in herbicide concentration among species and between sampling sites were determined using a student t-test. A Wilcoxon-Mann Whitney U Test was used in cases where the data were not normally distributed. Statistical analyses were performed in Statistica v10. Significance was set at $p < 0.05$ and concentrations below detection were assigned a value of zero. All values are expressed as the arithmetic mean ± SD, unless otherwise stated.

3.2.4 *Quality assurance*

The extraction procedures outlined above were validated by performing spike recovery tests on fish (hake) purchased from a local supermarket. Average recoveries (n = 9) ranged between 72 and 96% for multi-residue analytes, and between 84 and 103% for phenoxy acids (TS 3.1). Analytical limits of detection (LOD) were in the range of 0.14 – 0.31 ng g^{-1} and 0.34 – 0.43 ng g^{-1} for multi-residue and phenoxy-acid analytes, respectively. Limits of quantification (LOQ) ranged between 0.33 and 1.02 ng g^{-1} for all analytes (All data in Table TS 3.1). Samples were analysed in triplicate, with analytical precision (%RSD) typically <15%. Blanks and quality control standards were repeatedly analysed during each sample run to correct for drift in instrument response.

3.2.5 *Human health risk assessment*

Potential risks to human health posed by the consumption of contaminated fish were assessed by calculating the estimated daily intake (EDI) based on average herbicide concentrations measured in each fish species (FAO/WHO, 1987):

$$EDI = \frac{C \times CR}{BW}$$

where *C* is the average concentration of herbicide measured in fish (ng g^{-1} ww), *CR* is the estimated daily fish consumption rate (g d^{-1}), and *BW* is the assumed average body weight set at 60 kg for adults (WHO, 2012). This calculation was performed at both the 50th and 95th percentile of measured concentrations. Although fish is an important component in the diet of communities surrounding Lake St Lucia, limited information on local eating habits and consumption rates exists. To account for this uncertainty, EDI was calculated at two different daily consumption levels; 150.5 g d^{-1} based on national food consumption data (Nel & Steyn, 2002) and a more conservative estimate of 30 g d^{-1} used by Volschenk et al. (2019). These values provide likely maximum and minimum fish consumption rates for communities surrounding Lake St Lucia.

Potential non-cancer health risks were assessed by calculating hazard quotients (HQ) based on acceptable daily intake values (ADI) as:

$HQ = \frac{EDI}{ADI}$ (US EPA, 1991).

ADI values were obtained from the AERU Pesticide Properties Database (Lewis et al., 2020) and the Australian Pesticides Veterinary Medicines Authority (APVMA, 2020). This provides a preliminary quantitative risk assessment and is derived using conservative methods and assumptions to ensure that risks are not underestimated. For preliminary quantitative risk assessment purposes, HQ values ≤0.2 are considered to indicate negligible adverse health effects, while HQ values exceeding this threshold require the implementation of a detailed risk assessment or risk management measures (Health Canada, 2004).

Carcinogenic risk was assessed by deriving lifetime cancer risk (LCR) estimates following US EPA guidelines (US EPA, 1991; Dougherty et al., 2000). LCR was calculated using the equation:

$$LCR = EDI \times CSF$$

where CSF is the cancer slope factor (mg kg^{-1} day^{-1}) obtained from available US government databases (US EPA, 1999; CA-OEHHA, 2001; DEC, 2013). LCR was calculated for 6 of the 11 target analytes for which CSF data were available. A risk level below 10^{-6} is considered acceptable, between 10^{-6} and 10^{-4} is considered to be an area of concern, while greater than

10^{-4} is considered a high cancer risk (US EPA, 1991). Hazard ratios (HR) were calculated as follows (Jiang et al., 2005):

$$HR = \frac{EDI}{BMC}$$

where BMC is the benchmark concentration for cancer effects and calculated as:

$$BMC = \frac{Risk \times BW}{CR \times CSF}$$

The risk is set at one in a million chance (10^{-6}) due to a lifetime of exposure and the amount of fish consumed per kg body weight of an individual per day. A HR >1 indicates potential risk to human health (Dougherty et al., 2000).

3.3 Results and discussion

3.3.1 *Herbicide concentrations*

Fish specimens collected from Lake St Lucia ranged in total weight from 150 to 4450 g for *C. gariepinus* and from 40 to 250 g for *O. mossambicus*. Average tissue lipid content differed significantly ($Z = 3.52$; $p < 0.001$) between species, measuring 7.8 ± 1.7% in *C. gariepinus* and 3.5 ± 1.5% in *O. mossambicus*. Given the large difference in lipid content between species, we compare herbicide concentrations based mainly on a dry weight (dw) basis. All wet weight and lipid-normalised concentration data are provided in supplementary Table S2.

Herbicide residues were detected in all muscle tissue samples analysed. Acetochlor, alachlor, atrazine, simazine and terbuthylazine were detected in every sample, while trifluralin (49%) and hexazinone (66%) were detected least frequently (Table 1). Average total herbicide concentrations were similar between the two species, averaging 129 ± 4.7 ng g^{-1} and 137.7 ± 79 ng g^{-1} in *O. mossambicus* and *C. gariepinus,* respectively. Total triazine, anilides/aniline and carbamate concentrations were similar between species (Fig. 2A), while significantly higher ($p < 0.05$) phenoxy acid concentrations were measured in *C. gariepinus* (43.2 ± 25 ng g^{-1}) compared to *O. mossambicus* (27.8 ± 21 ng g^{-1}). In all cases, total concentrations of the different herbicide groups measured in fish muscle tissues were substantially higher (typically 7 – 30 times) compared to levels previously detected in lake sediments. Although the small sample size makes it difficult to assess spatial variability in

herbicide concentrations within each species, total average herbicide concentrations measured in fish populations from the northern (108.2 ± 33 ng g^{-1}) and southern (153.2 ± 47 ng g^{-1}) parts of Lake St Lucia differed significantly (Z = -3.61; p < 0.001).

The most prominent herbicide residues detected included acetochlor, metolachlor, EPTC, MCPA and 2,4-D (Table 1, Fig. 2B, C). For most herbicides, the average residue concentrations between species were broadly similar, although highest levels were typically detected in *C. gariepinus.* No significant differences in herbicide residue concentrations were detected between species, apart from trifluralin, where average concentrations were significantly higher (Z = 3.45; p < 0.001) in *O. mossambicus* (2.80 ± 2.1 ng g^{-1}) compared to *C. gariepinus* (0.92 ± 1.6 ng g^{-1}).

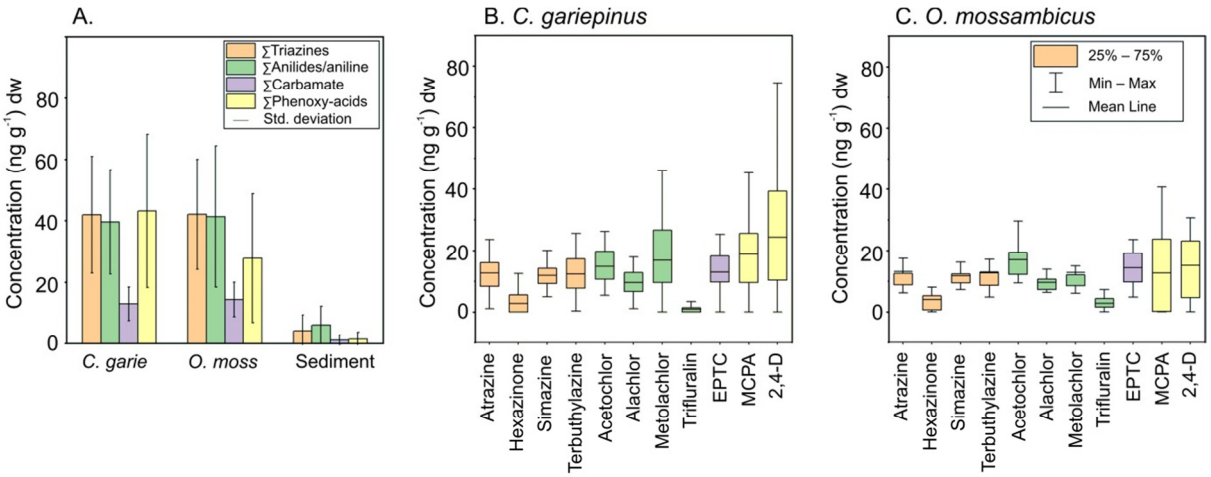

Fig. 2 (A) Comparison between total average (± SD) concentrations of different herbicide groups measured in fish and sediment from Lake St Lucia. Sediment data from Tyohemba et al. (2020). (B, C) Box plots showing variation in individual herbicide concentrations (ng g^{-1} dw) for *C. gariepinus* and *O. mossambicus*, respectively.

Table 1. Mean ± standard deviation and range of herbicide concentrations (ng g^{-1} dw) measured in *Clarias gariepinus* and *Oreochromis mossambicus* tissue samples.

Herbicide			*Clarias gariepinus* (n = 40)			*Oreochromis mossambicus* (n = 17)		
	Solubility (mg L^{-1}) [a-c]	Log Kow [a-c]	Mean	Range	Frequency (%)	Mean	Range	Frequency (%)
Triazines								
Atrazine	33	2.50	12.5 ± 5.3	1.10. – 23.6	100	13.0 ± 10	6.12 – 52.1	100
Hexazinone	33,000	1.95	2.78 ± 3.5	n.d. – 12.4	63	3.95 ± 4.5	n.d. – 18.1	77
Simazine	6.2	2.10	11.9 ± 4.8	n.d. – 23.9	98	11.8 ± 3.5	7.20 – 19.0	100
Terbuthylazine	8.5	3.21	12.4 ± 6.5	0.32 – 25.5	100	12.6 ± 9.3	4.81 – 46.0	100
Anilides/aniline								
Acetochlor	223	4.14	14.8 ± 5.7	5.30 – 26.2	100	17.0 ± 6.0	9.40 – 29.6	100
Alachlor	170	3.09	9.51 ± 4.2	1.00 – 17.8	100	9.56 ± 3.1	6.41 – 16.7	100
Metolachlor	488	2.90	16.8 ± 12	n.d. – 46.0	85	12.8 ± 12	6.00 – 56.6	100
Trifluralin	0.22	4.83	0.92 ± 1.6	n.d. – 6.21	88	2.80 ± 2.1	n.d. – 7.21	33
Carbamate								
EPTC	375	3.20	12.9 ± 5.5	n.d. – 72.8	98	14.3 ± 5.6	25.5 – 109	100
Phenoxy acids								
MCPA	274	-0.71	18.8 ± 12	n.d. – 45.3	95	14.3 ± 14	n.d. – 40.7	77
2,4-D	23180	0.04	24.4 ± 17	n.d. – 74.5	95	17.2 ± 10	n.d. – 30.7	77
Total			138 ± 79	44.3 – 238		129.3 ± 4.7	72.2 – 291	
Lipid content (%)			7.9 ± 1.6	3.5 – 13.3		3.5 ± 1.5	1.9 – 6.3	
Sample Weight (g)			1059 ± 1175	150 – 4550		114 ± 60	40 – 250	

Data sources: a: Tadeo et al., 2008 c: Navarro-Ortega et al. c: 2010 U.S. EPA (2017b)

3.3.2 *Human health risks*

Given that locally caught fish are an important source of protein to neighbouring communities, health risks associated with the consumption of contaminated fish from Lake St Lucia is an important consideration. The preliminary human health risk assessment indicated that EDI and HQ values did not exceed threshold guideline values at both the 50^{th} and 95^{th} percentile of measured concentrations (TS 3.3). However, potential long-term cancer risks (LCR between 10^{-6} and 10^{-4}) were indicated for atrazine (at the 50^{th} and 95^{th} percentile) and simazine (at the 95^{th} percentile) in both fish species at the maximum likely consumption rate. Potential cancer risk was also indicated for acetochlor and alachlor (at the 95^{th} percentile) for both species.

3. 4. Discussion

3.4.1. *Factors affecting bioaccumulation in fish species*

All herbicides previously detected in sediments from Lake St Lucia were found to accumulate in fish muscle tissues. Fish living in contaminated environments are potentially exposed to herbicides through both diet and absorption across their gills (Barron, 2003; Schlenk, 2005; Kaiser, 2012). Bioaccumulation in fish may therefore be influenced by a variety of physico-chemical factors that control the distribution of individual herbicides between different environmental compartments, as well as the specific feeding habits of individual species.

Highly absorptive compounds (log $K_{ow} \geq 2.6$) tend to have greater bioaccumulation potential (Cumming & Rücker, 2017; Hodges et al., 2019). This property likely explains the relatively high concentrations of anilides/aniline herbicides (acetochlor, alachlor, metolachlor) detected in both fish species. These compounds are also known to be relatively persistent (half-lives >100 days) in freshwater (Chen et al., 2014; Elsayed, 2015). Although trifluralin has a high absorption potential (log $K_{ow} = 4.83$), it is characterised by very poor solubility (0.22 mg L^{-1}) and undergoes rapid degradation (Grover et al., 1997). This likely accounts for the relatively low trifluralin concentrations measured in both fish species. Many of the triazine herbicides (atrazine, simazine and terbuthylazine) are similarly characterised by poor solubility, but were nevertheless detected in relatively high concentrations that may be attributed to the reasonably persistent nature of triazine herbicides in aquatic systems (Ferrando et al., 1992; Bester & Hühnerfuss, 1993). Phenoxy acid herbicides (MCPA and 2,4-D) were found in surprisingly high concentrations in both fish species, despite their particularly low absorption

potential (log K_{ow} <0.1). MCPA and 2,4-D are extensively used in commercial sugarcane production in the region (Dabrowski, 2015) and we suspect the elevated concentrations detected reflect the high contaminant loads Lake St Lucia receives from these areas.

Although chemical absorption potential (K_{ow}) is considered a key parameter influencing the bioaccumulation potential of organic contaminants, this can be mitigated by various factors, most significantly by biotransformation (Goutte et al., 2020; Laue et al., 2020). Organic contaminants with high metabolic conversion rates may be easily eliminated by animals, while those that are slowly metabolised are predisposed to bioaccumulation. Moreover, predictive models suggest that chemicals with high metabolic biotransformation rates (log k_M > -0.7) are less likely to biomagnify in higher trophic level organisms even if they have molecular structures conducive to partitioning to lipids (van der Oost et al., 2003; Walters et al., 2016). Herbicides are predicted to undergo relatively rapid metabolic biotransformation (Arnot et al., 2008), although few toxicokinetic studies in fish have been conducted (e.g., Schultz and Hayton, 1999). Thus, while herbicides may accumulate within the muscle tissues of fish, they may be rapidly eliminated and have a low probability of biomagnifying in the food web.

Despite differences in the feeding habits of *C. gariepinus* and *O. mossambicus*, both species exhibited similar herbicide bioaccumulation profiles. This suggests that bioaccumulation may be largely determined by exposure to herbicide-contaminated water, rather than through dietary pathways. Although no water quality monitoring studies have been undertaken to date, it is likely that runoff from large commercial farmlands on the Mfolozi floodplain results in relatively higher herbicide concentrations entering Lake St Lucia via the Mfolozi River compared to the Mkhuze River. This could also explain why total average herbicide concentrations in fish from the southern part of Lake St Lucia were significantly higher compared those sampled from the northern basin. However, it should be noted that sampling was conducted at different periods and differences observed between sites could be due to various temporal fluctuations in environmental conditions (e.g., river discharge, herbicide application/release, photochemical or bacterial degradation) and fish ecophysiology (e.g. metabolism, reproduction, age and sex). Spatial differences in herbicide bioaccumulation should thus be interpreted cautiously.

3.4.2. *Herbicide accumulation and toxicological effects*

Relatively few studies have reported on herbicide accumulation in fish, particularly for the range of residues analysed in this study (Table 2). Atrazine has been the most common

target herbicide, having been detected in fish from Poland (Kaczyriski et al., 2017), South America (Miranda et al., 2008; Ernst et al., 2018), China (Fu et al., 2018) and Africa (Ezemonye et al, 2015; Mohamed & Sabae, 2015). Generally, atrazine concentrations measured in fish from Lake St Lucia were higher than those reported in these studies, but similar to concentrations measured in fish from Kalk Bay harbour, Cape Town (Ojemaye et al., 2020b). Simazine and alachlor tissue concentrations at Lake St Lucia were similar to levels reported in fish from Brazil (Miranda et al., 2008), but acetochlor and metolachlor concentrations were substantially higher compared to those measured in fish from Europe (Belenguer et al., 2014; Pico et al., 2019) and China (Fu et al., 2018). To our knowledge, the bioaccumulation of hexazinone, EPTC and MCPA has not previously been assessed in any aquatic vertebrate.

The acute toxicity of herbicides to fish is reasonably well reported in experimental assays. Triazine herbicides (atrazine, hexazinone, simazine and terbuthylazine) are considered to have relatively low toxicity for fish (Appleby et al., 2005; Hostovsky et al., 2014), although various physiological and biochemical alterations have been reported (Nieves-Puigdoller et al., 2007;; Toughan et al., 2018; Wang et al., 2019). *Teratogenic and genotoxic potentials (Adeyemi et al., 2015), neuroendocrine disruption (Liu et al., 2016) and reduced fertility rates (Bautista et al., 2018) have been reported in zebra fish exposed to atrazine.* Prolonged exposure to simazine has been reported to cause oxidative damage to cell lipids and proteins in common carp (Stara et al., 2012), while exposure to a metabolite of terbuthylazine has been reported to affect the survival, growth and development of carp embryos and larvae (Velisek et al., 2016). Alachlor has been shown to cause delays in hatching, embryogenic malfunctions and skeletal deformations in fish larvae (Lazhar et al., 2012), while low concentrations of trifluralin exposure resulted in reduced growth development in zebra fish (Awkerman et al., 2020). Exposure to ecologically relevant concentrations of 2,4-D has been associated with reduced larvae survival in several freshwater fish species (Dehnert et al., 2021).

In general, acute toxicity tests suggest that fish are most sensitive to herbicide exposure during early developmental stages. However, while laboratory studies provide an indication of the potential risks associated with acute herbicide exposure, chronic effects on fish reproduction are at present difficult to assess in the absence of appropriate toxicological data. The impact of chronic, sub-lethal herbicide exposure on wild fish populations may be subtle and difficult to detect, particularly as effects may combine and interact synergistically with other environmental factors. Evidence that multiple agricultural herbicides currently in

use accumulate in wild fish highlights the critical need for coordinated field and laboratory studies to better understand the effects of long-term herbicide exposure on fish populations.

While the preliminary human health assessment presented here indicates fairly minimal risk associated with the consumption of fish from Lake St Lucia, other possible routes of exposure (other contaminated food and inhalation), as well as potential additive and synergistic effects have not been taken into account. For example, fish from Lake St Lucia are known to accumulate a variety of organochlorine pesticides (Buah-Kwofie and Humphries, 2021; Buah-Kwofie et al., 2018) and the potential additive or synergistic effects associated with multiple contaminants are unknown. Variations in the amount of fish consumed by communities and their predisposition to contaminant exposure risks are additional unknowns. Infants and young children may be particularly vulnerable to exposure.

Table 2. Herbicide residue concentrations (ng g^{-1}) reported in fish muscle tissue from other locations. For comparative purposes, we report our data on a dry weight (dw) and wet weight (ww) basis.

Location		Acetochlor	Alachlor	Metolachlor	Trifluralin	Atrazine	Simazine	Terbuthylazine
Lake St Lucia, South Africa [a]	dw	5.3 – 29.6	1.0 – 17.8	n.d. – 56.6	n.d. – 7.2	1.1 – 52.1	n.d. – 23.9	n.d. – 46.0
	ww	1.3 – 8.5	0.2 – 4.1	n.d. – 16.3	n.d. – 1.2	0.2 – 15.0	n.d. – 5.4	n.d. – 13.3
Africa								
Cape Town, South Africa [b]	dw		n.d.	n.d.		n.d. – 49	n.d. – 158	
Assiut City, Egypt [c]	dw				0.02 – 1.67			
Wada El-Rayan, Egypt [d]	dw					0.01	0.01	
Edo State, Nigeria [e]	dw					90 – 630		
South America								
Porta Grossa, Brazil [f]	dw		n.d. – 32.9			n.d. – 9.57	n.d. – 28.3	n.d. – 2.25
Minas Gerais, Brazil [g]	ww			0.60 – 0.98				
Uruguay rivers [h]	ww			1.2		1.6		
North America								
California, USA [i]	ww				<0.15 – 1.9			
Europe								
Biebrza, Poland [j]	ww			9 – 11 (*S*-isomer)		5		
Jucar, Spain [k]	dw			4.32				
Jucar, Spain [l]	dw	n.d. – 2.6		n.d. – 3.82				
Lake Vela, Portugal [m]	ww		n.d. – 0.19					
Asia								
Northeast China [n]	dw	0.11 – 0.32				0.5 – 2.8		

a: This study
b: Ojemaye et al. (2020)
c: Yahia & Elsharkawy (2014)
d: Mohammed & Sabae (2015)
e: Ezemonye et al. (2015)
f: Miranda et al. (2008)
g: Paulino et al. (2014)
h: Ernst et al. (2018)
i: Sapozhnikiva et al. (2004)
j: Kaczyriski et al. (2017)
k: Belenguer et al. (2014)
l: Pico et al. (2019)
m: Abrantes et al. (2010)
n: Fu et al. (2018)

3.5. Conclusions

The results of this study indicate that various currently used agricultural herbicides accumulate within fish at Lake St Lucia. Although few similar studies have been undertaken to date, these findings add to growing evidence which suggests that wild fish populations are susceptible to chronic herbicide exposure. While herbicides have been associated various acute toxicity responses, evaluating the biological effects of herbicide contaminants measured in fish tissue remains challenging and presents a crucial gap in knowledge for biodiversity conservation management. Various sub-lethal effects may affect fish populations and toxicological data are urgently needed to better understand chronic exposure effects on fish and aquatic ecosystems. Most of our current knowledge regarding the effects of herbicides on fish species comes from laboratory studies and studies on wild fish species are crucial to better understand the impacts of complex chronic exposures to low concentrations of pesticides. The extent to which herbicides move through the aquatic food chain and biomagnify in higher trophic level organisms also remains unknown and warrants further investigation. Finally, our findings draw attention to the potential impact of herbicide transboundary pollution on conservation areas, which to date has been largely overlooked. As with many aquatic ecosystems impacted by agricultural runoff, Lake St Lucia will continue to be vulnerable to herbicide inputs and regular water quality monitoring is essential for evaluating risks and guiding herbicide management in catchment areas.

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CHAPTER 4

Accumulation of commonly used agricultural herbicides in coral reef organisms from iSimangaliso Wetland Park, South Africa

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Abstract

Coral reefs are among the most biodiverse ecosystems on earth but are significantly impacted by agricultural runoff. Despite herbicides being commonly detected in coastal waters, the possibility of herbicide accumulation in coral reef species has largely been overlooked. Here, we investigate the accumulation of several multi-residue herbicides in five species of coral reef invertebrates collected from ten sites along the Maputaland coast, South Africa. Multiple herbicide residues were detected in 95% of the samples analysed, with total average concentrations across sites ranging between 25.2 ng g^{-1} to 51.3 ng g^{-1} dw. Acetochlor, alachlor and hexazinone were the predominant herbicides detected at all sites, with atrazine and simazine being detected less frequently. Significant interactive effects were detected between sites nested in reef complex crossed with species, based on multivariate and total herbicide concentrations. In general, multivariate herbicide concentrations varied significantly between species within and across most sites. Contrastingly, the concentrations of herbicides did not differ between conspecifics at most sites nested in their respective reef complexes. On average, highest total herbicide concentrations were measured in soft coral (*Sarcophyton glaucum;* 90.4 ± 60 ng g^{-1} and *Sinularia gravis*; 42.7 ± 25 ng g^{-1}) and sponge (*Theonela swinhoei;* 39.0 ± 40 ng g^{-1}) species, while significantly lower concentrations were detected in hard corals (*Echinopora hirsutissima;* 10.5 ± 5.9 ng g^{-1} and *Acropora austera;* 5.20 ± 4.5 ng g^{-1}) at most sites. Total herbicide concentrations did not differ however between conspecifics across most sites nested in reef complex. Agricultural runoff entering the ocean via the uMfolozi-St Lucia estuary and Maputo Bay were identified as likely to be the two main sources of herbicide contamination to coral reefs in the region. There is an urgent need to assess the long-term effects of herbicide exposure on coral reef communities.

4.1 Introduction

Deteriorating water quality is considered a major contributor to the decline of coral reefs globally (Burke et al., 2011; Hoegh-Guldberg, 2011; Browne et al., 2015; Brown et al., 2017). Most coral reefs occur in shallow, nearshore waters and are therefore particularly vulnerable to land-based anthropogenic pressures. Yet, despite growing concerns regarding the potential impacts of terrigenous pollutants on coral reef health, our understanding of the long-term cumulative risks remains poor (Nalley et al., 2021). Coral reef organisms are known to have a high capacity for accumulating and concentrating pollutants within their tissues, and several studies have reported on the presence of various metals (Chan et al., 2014; Jafarabadi et al., 2018; van der Schyff et al., 2020) and pesticides (Bargar et al., 2013; Porter et al., 2018; Wang et al., 2008) in coral tissues.

Runoff from agricultural land is considered one of the main contributors to the degradation of water quality and the health of coral reefs (Kennedy et al., 2012; Gallen et al., 2019; Thomas et al., 2020). Agricultural herbicides are used intensively on a worldwide basis, with global consumption estimated at approximately 1.2 million tons per annum (FAO, 2018). Most herbicides currently in use are highly mobile, readily enter the aquatic environment, and thus may potentially be transported far from their site of application (van Dam et al., 2011). As a result, herbicides have been detected in sediment and water from several coral reefs (Haynes et al., 2000; Shaw et al., 2008, 2010; Salvat et al., 2016; Brodie & Landos, 2019; Spilsbury et al., 2020; Warne et al., 2020). Acute toxicity tests suggest that herbicides may readily penetrate coral tissues, potentially reducing zooxanthellar photosynthetic efficiency (Owen et al., 2003; Negri et al., 2005), while longer-term exposure may result in bleaching, reduced reproductive output, and partial or full colony mortality (Cantin et al., 2007). Although high herbicide concentrations continue to enter coral reef ecosystems, the extent to which they accumulate within coral tissues remains unknown.

Coral communities found along the north-eastern coast of South Africa (Fig. 1) constitute the southern limit of their distribution in the western Indian Ocean (Schleyer & Celliers, 2003). The reefs are rich in biodiversity, comprising a mix of tropical and subtropical species (Schleyer & Porter, 2018). Due to their marginal geographical location, these communities have not been notably affected by bleaching (Porter & Schleyer, 2017) and occur along a narrow continental shelf that lacks significant fluvial inputs (Porter et al., 2017). The reefs fall within the iSimangaliso Wetland Park and, although protected by World Heritage status, are subjected to anthropogenic pressures. Porter et al. (2018) recently reported on the widespread accumulation of organochlorine pesticides (OCPs) in reef organisms from the

region. These contaminants are thought to originate largely from agricultural activities occurring within adjacent catchment areas and enter the ocean via groundwater (Porter et al., 2018). Contaminant inputs to the Maputaland coastal environment are unlikely to be limited to legacy OCPs. Numerous agricultural herbicides are currently used in the region and the presence of various residues, including acetochlor, alachlor and hexazinone, have recently been reported in sediments from Lake St Lucia (Tyohemba et al., 2020).

Chronic exposure of reef organisms to herbicides remains largely understudied globally but is essential to assess risks to coral reef communities. This study aimed to assess the accumulation of multi-residue herbicide contaminants in reef organisms from Maputaland, South Africa. Spatial and inter-species variations were investigated by examining herbicide concentrations in a sponge (*Theonella swinhoei*), two soft corals (*Sarcophyton glaucum* and *Sinularia gravis*) and two hard coral species (*Acropora austera* and *Echinopora hirsutissima*) from ten sites along the Maputaland coastline. Nine herbicide residues were targeted, which included triazines (atrazine, hexazinone, simazine and terbuthylazine), anilides/aniline (acetochlor, alachlor, metolachlor and trifluralin) and the carbamate EPTC.

4.2 Materials and Methods

4.2.1 *Sample Collection*

Coral samples were collected from 10 sites along the Maputaland coast in north-east South Africa (Fig. 1). The Maputaland reefs are situated at the south-western limits of the tropical Western Indian Ocean and support a diverse variety of tropical and subtropical coral communities (Schleyer et al., 2018). The south-westerly flowing Agulhas Current is the dominant oceanographic feature along the coastline and brings warm, oligotrophic water to the area. Mean seasonal sea-surface temperatures in the Maputaland region range from 22°C in winter to 26°C in summer (Schleyer et al., 2018). The reefs run parallel to the coastline and are grouped into Northern, Central and Southern complexes, these being respectively found adjacent to Kosi Bay, Lake Sibaya, and north of Lake St Lucia (Fig. 1). The reefs lie offshore of a sandy coastal plain that is characterised by several large coastal waterbodies and a variety of freshwater wetlands (Ellery et al., 2013). These coastal systems are separated from the ocean by a high, vegetated dune barrier and limited sediment exchange results in remarkably clear nearshore waters. The St Lucia estuarine system represents the only significant source of terrestrial sediment in the region (Connell & Porter, 2013; Perrisinotto et al., 2013). Lake St Lucia is supplied by two major rivers in the

region (iMkhuze and uMfolozi), both of which drain large catchment areas that have been significantly impacted by commercial and subsistence agriculture.

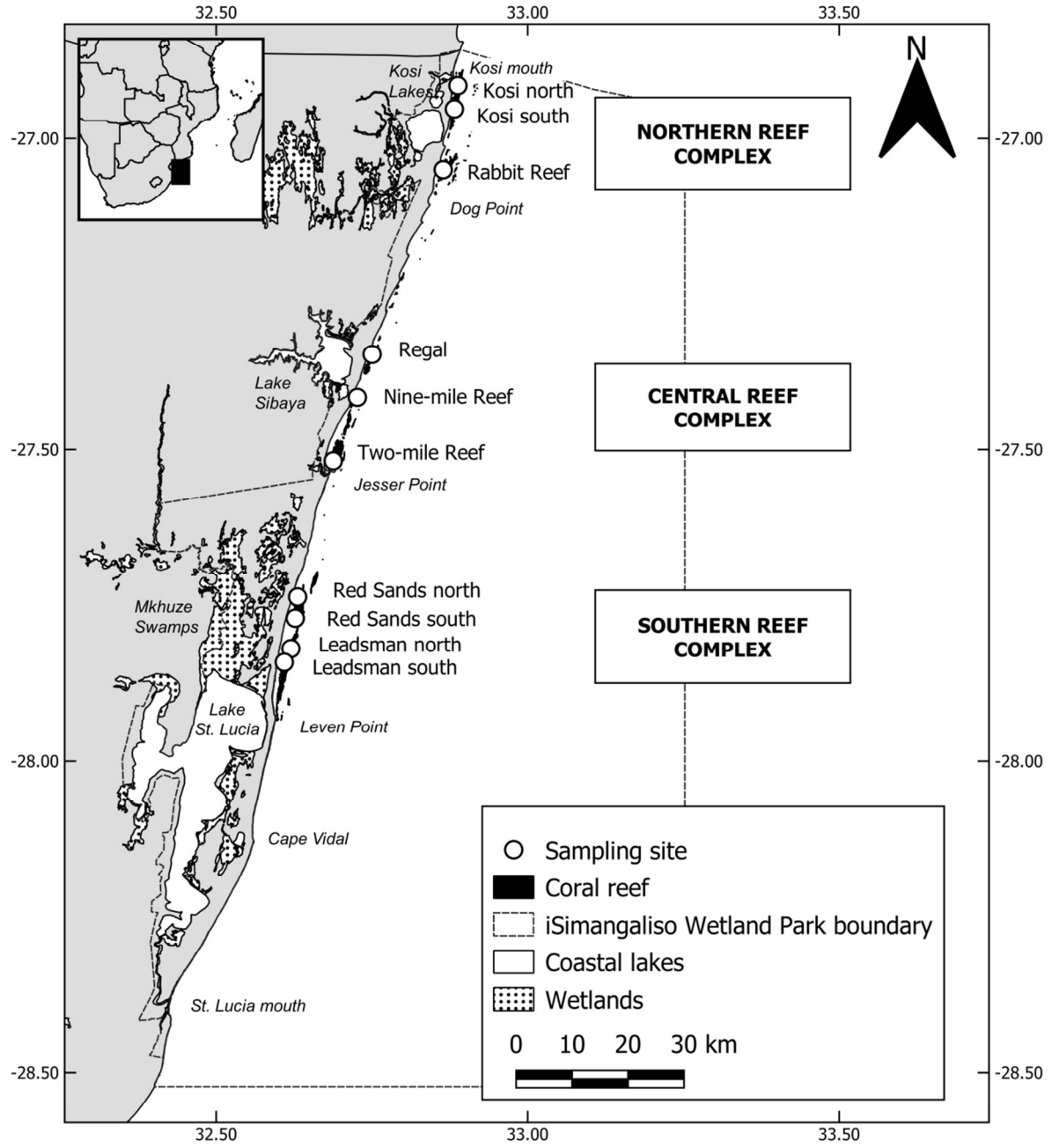

Fig. 1 Location of the ten reef sampling sites within iSimangaliso Wetland Park on the north-east coast of South Africa

Five species of locally abundant sessile coral reef invertebrates were sampled. These included a sponge (*Theonella swinhoei*) and two soft coral species (*Sinularia gravis* and *Sarcophyton glaucum*) which were previously targeted by Porter et al. (2018) who identified them as suitable candidates for assessment of the long-term bioaccumulation of

contaminants in the region. In addition, two hard coral species, *Acropora austera* and *Echinopora hirsutissima,* were collected to expand the range of functional and taxonomic groups investigated. Triplicate tissue samples (~30 g) from independent colonies of each species were collected at ten sites (146 samples in total) during 2019 using SCUBA at depths of 12 – 16 m, except for *A. austera* which could not be found at Regal (Fig. 1). Samples were immediately placed on ice in the field and subsequently stored at -18 ^{o}C prior to laboratory analysis.

4.2.2 *Sample extraction and chemical analysis*

The extraction of multi-residue herbicides from tissue samples was achieved following a modified QuEChERS procedure (Buah-Kwofie & Humphries, 2019). Tissue subsamples were freeze-dried and homogenised into fine powder using an electric blender. Samples of approximately 3 g were initially rehydrated with 5 mL water and then extracted with 6 mL acetonitrile/acetic acid (99:1 v/v). Phase separation of the organic and aqueous phases was achieved using a combination of anhydrous magnesium sulfate (2.5 g), sodium acetate (0.8 g) and sodium acetate trihydrate (0.4 g). The mixture was vortexed and then centrifuged to isolate the organic extract. Sample clean-up was achieved using a combination of magnesium sulfate, C18, florisil, and primary secondary amine (PSA). An aliquot of the cleaned extract was then evaporated to dryness under vacuum (≤ 40 ^{o}C), reconstituted in 1 mL hexane, and spiked with 50 ppb pentachloronitrobenzene (internal standard) for final analysis.

Samples were screened for nine multi-residue herbicides (atrazine, hexazinone, simazine, terbuthylazine, acetochlor, alachlor, metolachlor, trifluralin and EPTC). Analysis was performed by two-dimensional gas chromatography (GC x GC) using an Agilent 7890 GC coupled to a LECO Pegasus Time-of-Flight (TOF) mass spectrometer. Separation was achieved using a Restek BPX-5 primary column coupled to an Rxi-17Sil MS secondary column. Samples of 2 µL were injected in splitless mode using high-purity helium as the carrier gas at a flow rate of 1.4 mL min^{-1}. Temperature was set at 55 ^{o}C (1-minute hold), raised to 200 ^{o}C at a rate of 8 ^{o}C min^{-1} (4-minute hold), and finally increased to 270 ^{o}C at a rate of 10 ^{o}C min^{-1} (2-minute hold). Data processing and peak identification was performed using the Leco ChromaTOF software and databases. Peaks were identified based on the retention time of specific ions and confirmed by two identifier ions. Analyses were performed in triplicate and quantified using high-purity (>98%) PESTANAL® reference standards obtained from Sigma-Aldrich. Coefficients of determination (r^2) derived from linear regression models of the matrix-matched calibration curves for all analytes were >0.99. Analytical

precision (%RSD) across all sample batches was typically <15% (n = 3). Tissue lipid content in each species was estimated gravimetrically by extracting selected sub-samples (n = 10) with hexane:acetone (3:1).

4.2.3 *Quality control*

The herbicide extraction procedure was validated by performing spike recovery tests on three different tissue matrices: soft coral (*S. gravis*), hard coral (*A. austera*) and sponge (*T. swinhoei*). Blank samples were prepared by extracting tissue twice following the procedure described previously. The first extract was discarded, while the second extract was analysed to confirm the absence of target herbicides. This material was then used to test analyte recovery efficiency at three fortification levels (5, 10, and 50 ppb). Recoveries (n = 3) for all herbicide analytes ranged between 68 and 104% (*S. gravis)*, 71 and 95% (*A. austera*) and between 65 and 115% (*T. swinhoei).* Limits of detection (LOD) ranged between 0.10 and 0.60 ng g^{-1}, while limits of quantification (LOQ) ranged between 0.35 and 2.0 ng g^{-1} for all analytes (TS 4.1). Solvent blanks and quality control standards were repeatedly analysed during each run to correct for drift in instrument response.

4.2.4 *Statistical analysis*

Multivariate variation in herbicide concentrations was investigated using a three-factor permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001a, b). Reef complex and species were considered orthogonal fixed effects and site was nested within reef complex and also considered a fixed effect. The analysis was run on a Euclidian distance matrix using 9999 Monte Carlo simulations of the residuals under a reduced model and type III sums of squares. Post hoc pairwise comparisons were undertaken when *a priori* analysis indicated a significant difference ($\alpha = 0.05$). Multivariate patterns were visualised using non-metric multidimensional scaling (nMDS) based on the Euclidian distance matrix with Pearson correlations between each herbicide analyte and the canonical axes displayed as vectors to indicate the strengths and directions of the associations (Kruskal & Wish, 1978). Variation in the concentrations of each of the five herbicides and the total herbicide concentration was investigated using univariate permutational analyses of variance (permANOVA) (Anderson 2001a, b). The identical model and method used to analyse the multivariate data was employed on each analyte and the total herbicide concentrations, except for hexazinone. The preliminary permANOVA on hexazinone indicated that the term Complex resulted in a negative estimate of that term's component of variation. Therefore, we followed the approach suggested by Anderson et al. (2008) and excluded Complex from the

model and maintained site and species thereby ensuring that variation due to space (i.e., sites) and species was still investigated. Both PERMANOVA and permANOVA analyses were supplemented with permutational analyses of dispersion based on distances from group centroids to test for homogeneity of group dispersions (Anderson, 2006). The analyses were undertaken using the software package PERMANOVA+ for PRIMER (Anderson et al., 2008).

4.3 Results

Of the nine herbicide analytes targeted, five (acetochlor, alachlor, hexazinone, atrazine and simazine) were present at quantifiable concentrations (all data provided in supplementary TS 4.2). Herbicide residues were detected in 95% of samples, with total average concentrations across sampling sites ranging between 25.2 and 51.3 ng g^{-1} (Fig. 2A). Highest total concentrations were measured at the two most southerly sites (Leadsman South and Leadsman North). Marked differences in herbicide concentration were observed between species (Fig. 3A). On average (± standard deviation), highest total herbicide concentrations were measured in *Sarcophyton glaucum* (90.4 ± 60 ng g^{-1}). *Sinularia gravis* (42.7 ± 25 ng g^{-1}) and *Theonela swinhoei* (39.0 ± 40 ng g^{-1}) were characterised by relatively lower but similar concentrations, while the two hard coral species exhibited lowest concentrations (*Acropora austera*: 5.8 ± 5.3 ng g^{-1}; *Echinopora hirsutissima*: 10.5 ± 8.4 ng g^{-1}).

Total herbicide concentrations in each sample did not differ significantly among the three reef complexes ($p = 0.1753$) or sites nested within complex ($p = 0.0911$), but did indicate significant variation according to species, the interaction of species and complex and the interaction of sites nested within complex crossed with species ($p = 0.0001$). *Post hoc* tests revealed that except for *E. hirsutissima*, total herbicide concentrations in each species differed from their conspecifics between the Southern and Northern Complexes ($p < 0.05$), whilst *T. swinhoei* ($p = 0.0028$) and *A. austera* ($p = 0.0061$) also showed differences between the Northern and Central Complexes (TS 4.3). Furthermore, most species differed from one another when compared across sites within each complex ($p < 0.05$), the only exceptions were *S. glaucum* and *T. swinhoei,* and *E. hirsutissima* and *A. austera* from the Northern Complex (TS 4.3). Similarly, most species differed from one another at each site ($p < 0.05$), except for most species from Kosi North and Kosi South (TS 4.4). However, total herbicide concentrations for each species generally did not differ from their conspecifics between sites in each of their respective complexes ($p > 0.05$), this was consistently so for *E. hirsutissima* in each of the three complexes (TS 4.4). Dispersions in total herbicide concentrations did not differ among groups of replicates derived from the three-way factor of

site-complex-species (p = 0.5049), nor did they differ for the site-complex interaction (p = 0.9616), but did differ for the complex-species interaction term on occasions usually involving *S. glaucum* from the Southern Complex (p < 0.05).

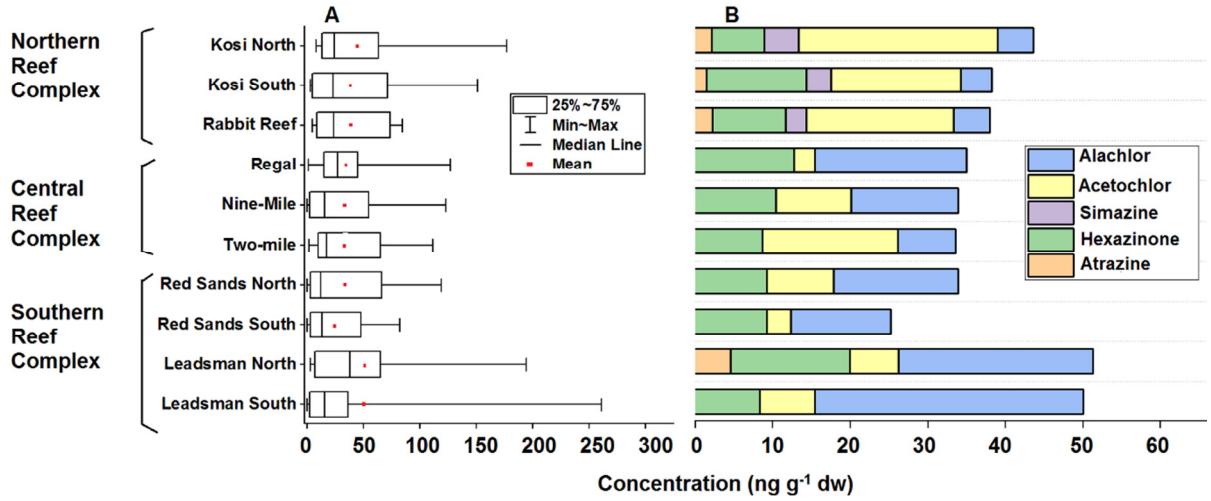

Fig. 2 Variation in herbicide concentrations (ng g^{-1} dw) among sites based on (A) total herbicide concentration and (B) individual herbicide concentrations.

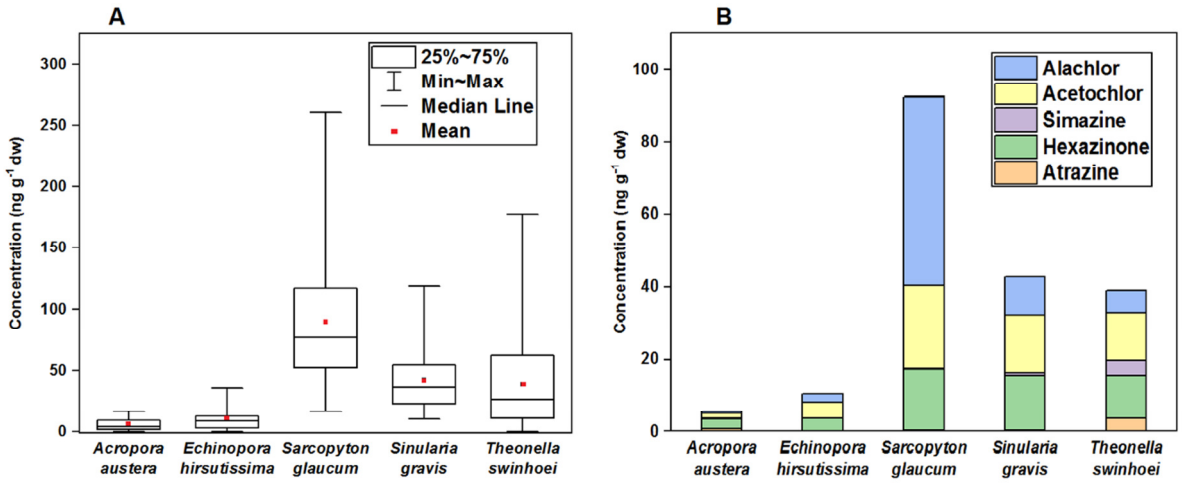

Fig 3. Variation in herbicide concentrations (ng g^{-1} dw) among species based on (A) total herbicide concentration and (B) individual herbicide concentrations.

Acetochlor, alachlor and hexazinone were the predominant analytes detected at all sites and in all reef invertebrates (Fig. 2B, 3B). On average, highest alachlor concentrations were detected at Leadsman North (26.2 ± 56 ng g^{-1}) and Leadsman South (34.6 ± 66 ng g^{-1}), while lowest concentrations were measured at sites from the Northern Reef Complex (4.0 ± 2.7 ng g^{-1}). Alachlor was particularly dominant in *S. glaucum* (51.6 ± 56 ng g^{-1}). Acetochlor concentrations followed an opposite latitudinal trend. Highest average acetochlor

concentrations were measured at sites from the Northern Reef Complex (20.5 ± 17 ng g^{-1}), while lower concentrations characterised sites from the Central (10.5 ± 16.3 ng g^{-1}) and Southern (6.29 ± 9.2 ng g^{-1}) Reef Complexes (Fig. 4).

A priori analyses of alachlor and acetochlor concentrations detected significant interactions between complex and species ($p = 0.0001$) as well as site nested in complex crossed with species ($p < 0.05$). A slight majority (53%) of *post hoc* tests indicated that species generally did not differ in their concentrations of alachlor at most sites, but when they did it was mainly between soft corals and hard corals (*S. gravis* versus *A. austera* and *S. glaucum* versus *E. hirsutissima* (TS 4.5). *Sarcophyton glaucum* differed among complexes ($p < 0.005$), but most other species did not (TS 4.6). Acetochlor exhibited no clear pattern of variation according to the interaction of sites nested in complex crossed with species (TS 4.7), and between complex crossed with species except in *E. hirsutissima* which did not differ between any of the complexes (TS 4.8). For acetochlor, dispersions did not differ among groups of replicates ($p = 0.6052$), but did differ for the complex-species interaction term on occasions usually involving *A. austera* from the Southern Complex ($p < 0.05$). For alachlor, dispersions did not differ among groups of replicates ($p = 0.1088$), nor did they differ for the site-complex interaction term ($p = 0.175$) but did differ for the complex-species interaction term on occasions involving *A. austera* from the Central Complex and *S. glaucum* form the Southern Complex ($p = 0.0002$).

Average hexazinone concentrations were similar across all sites, varying between 6.8 ng g^{-1} and 16.0 ng g^{-1}, and were highest in the soft corals *S. gravis* and *S. glaucum* (Fig. 2B, 3B). Hexazinone also indicated significant interactive effects between sites and species ($p = 0.0003$), with *post hoc* comparisons indicating that concentrations did not differ ($p > 0.05$) between most species at each site and between most sites for each species; exceptions occurred for some site comparisons involving *S. gravis* and *T. swinhoei* (TS 4.9). Dispersions among groups of replicates based on the interaction of site and species did not differ ($p = 0.917$).

Atrazine and simazine were less frequently detected (17% and 9%, respectively) and only in samples from the Northern Reef Complex, apart from 6 samples from Leadsman North that tested positive for atrazine. Average concentrations of simazine (3.46 ± 8.3 ng g^{-1}) and atrazine (1.90 ± 3.3 ng g^{-1}) were similarly low (or absent) across the three reef complexes (Fig. 4). Higher concentrations of simazine were however found in the Northern Complex relative to the Central and Southern complexes where it was absent, but sites in the Northern Complex had similar concentrations. Atrazine and simazine were primarily detected

in samples from *T. swinhoei,* where concentrations averaged 3.83 ± 8.4 ng g^{-1} and 4.18 ± 10 ng g^{-1}, respectively, with only traces of these herbicides found in some of the other species. Similar to the previous analytes, interactive effects between complex and species ($p = 0.0506$) and sites nested in complex crossed with species ($p = 0.0033$) were detected for atrazine, while simazine indicated significant interactive effects between complex and species only ($p = 0.0001$). *Post hoc* tests indicated that species did not differ in their concentrations of atrazine at each site in the majority of pairwise comparisons (94%), nor did each species differ across sites ($p > 0.05$) (TS 4.10). *Post hoc* tests of simazine for pairs of species were restricted to the Northern Complex where *T. swinhoei* differed from *E. hirsutissima* ($p = 0.019$), *A. austera* ($p = 0.0211$) differed from *S. glaucum* ($p = 0.0164$), and *S. gravis* differed from *S. glaucum* ($p = 0.0289$). *Sinularia gravis* and *T. swinhoei* were also found to differ between the Northern and Southern Complexes and the Northern and Central Complexes ($p < 0.05$) (TS 4.11). For simazine, dispersions did not differ among groups of replicates (0.9982) but did differ for the complex-species interaction term ($p = 0.0155$), while dispersions for atrazine differed among groups of replicates ($p = 0.0005$).

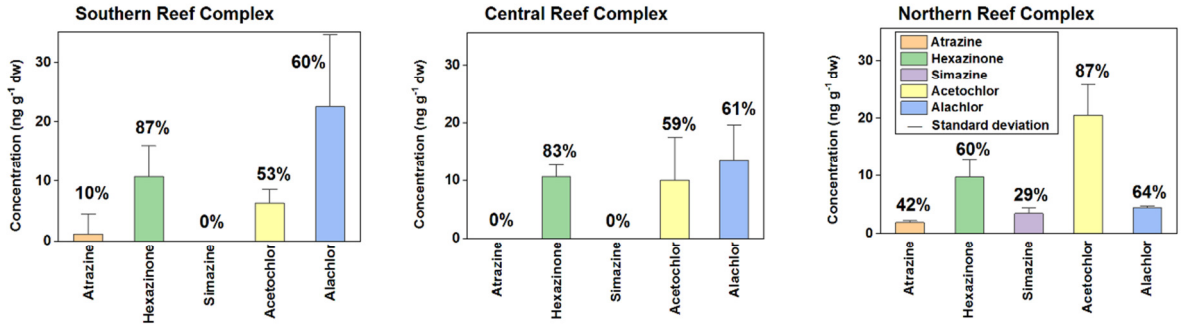

Fig. 4 Average (± SD) herbicide concentrations (ng g^{-1} dw) across the three Maputaland Reef Complexes. Percentages above bars indicate detection frequency.

The non-metric MDS ordination according to sites based on all five analytes revealed a clear separation of samples along a latitudinal gradient (Fig. 5). Samples from the Southern Complex, and to a lesser degree the Central Complex, separated out along an alachlor concentration gradient. In contrast, samples from the Northern Complex generally separated out along an acetochlor gradient. The non-metric MDS ordination according to species revealed clear separation along a gradient driven by the concentration of alachlor and acetochlor (Fig. 6). *A priori* PERMANOVA detected significant differences in the interaction of reef complex and species as well as sites nested within complex crossed with species ($p = 0.0001$). *Post hoc* analyses indicated that each species generally differed from their conspecifics at different reef complexes ($p < 0.05$), except for *E. hirsutissima* (TS 4.12).

Furthermore, species generally differed from each other in each reef complex (TS 4.12). When species were compared among sites within their respective complexes, most species did not differ from their conspecifics, except for *S. glaucum* from the Southern Complex and *S. gravis* from the Central Complex, but species generally did differ from each other at each site, except for Kosi North and Kosi South (TS 4.13).

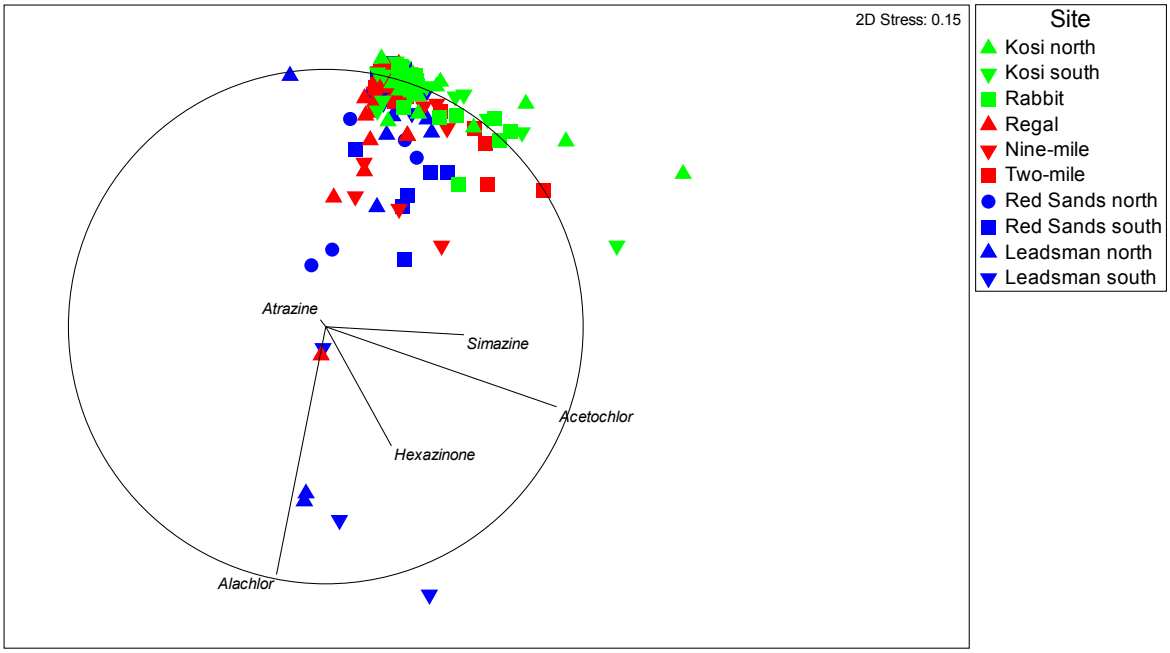

Fig. 5 Non-metric MDS ordination based on the concentration of herbicides according to sites from the Northern (green), Central (red) and Southern (blue) Complexes. Vectors indicate the strengths of the correlations of the five herbicides.

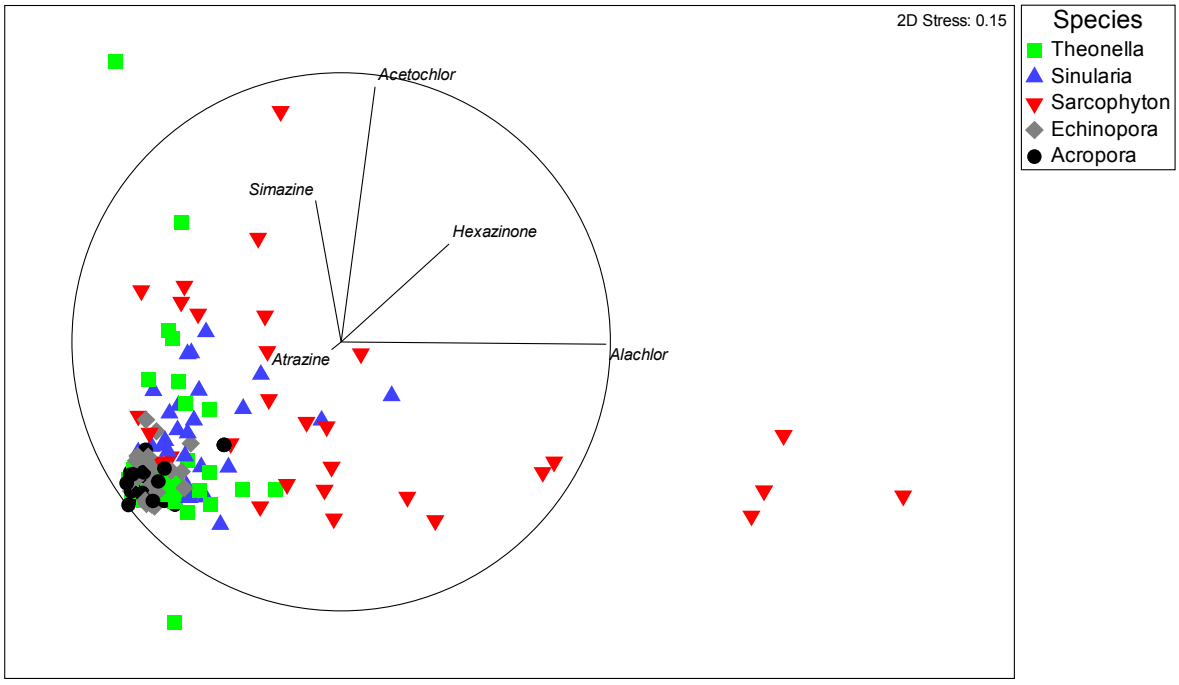

Fig. 6 Non-metric MDS ordination based on the concentration of herbicides according to coral reef species. Vectors indicate the strengths of the correlations of the five herbicides.

Multivariate dispersions did not differ among groups of replicates derived from the three-way factor of site-complex-species ($p = 0.4073$), but did differ for the complex-species interaction term based on the site-complex group centroids ($p = 0.0001$), although most of the *post hoc* comparisons were non-significant.

4.4 Discussion

4.4.1 *Herbicide occurrence in Maputaland coral reef organisms*

Several herbicides were detected in coral reef organisms from Maputaland. Hexazinone, alachlor and acetochlor were the most commonly detected and are known to be widely used within adjacent catchment areas, particularly in sugarcane cultivation (Dabrowski, 2015; Tyohemba et al., 2020). Other herbicides detected included atrazine and simazine, but were found in <20% of the samples analysed, despite being some of the most commonly used in products in the agricultural industry (Dabrowski, 2015). A variety of physico-chemical properties may influence the transport potential and ultimate environmental fate of particular herbicides (Wauchope, 1992; Hargreaves et al., 1999). Herbicides may be transported in dissolved or particulate-bound forms depending on their water solubility and sorption to soil (e.g., Rice et al., 2004). Although water quality sampling was not conducted as part of this study, monitoring data from the Great Barrier Reef suggests that herbicides are transported into nearshore waters largely in dissolved phase (Davis et al., 2012). The delivery of herbicides to Maputaland reefs is thus probably strongly influenced by the solubility of the compounds used in adjacent catchment areas. Hexazinone is highly mobile in the environment (solubility 33,000 mg L^{-1}), while acetochlor (223 mg L^{-1}) and alachlor (170 mg L^{-1}) are moderately soluble (Wauchope et al., 1992). In contrast, atrazine and simazine are very poorly soluble, and tend be retained within sediments (Meakins et al., 1995). Terbuthylazine and Trifluralin are also characterised by very poor solubility (<10 mg L^{-1}), which is probably why they were not found in reef organisms, despite being frequently detected within sediments at Lake St Lucia (Tyohemba et al., 2020).

4.4.2 *Variation in concentrations and potential sources of contamination*

Spatial variations in herbicide accumulation along the Maputaland coastline provide insight into potential contamination sources. The two most prominent herbicides detected

(acetochlor and alachlor) show distinct but opposing latitudinal concentration trends that suggest the presence of at least two major contamination sources in the region. Variation in alachlor concentrations suggests that St Lucia estuary is an important source of herbicide contamination to coastal waters. Lake St Lucia is supplied by several rivers that drain catchment areas heavily impacted by agriculture. Most notably, sugarcane cultivation occurs extensively on the lower uMfolozi River floodplain and runoff from agricultural land probably results in substantial quantities of herbicides being transported offshore, particularly during flood events. The Maputaland coast is characterised by a north-flowing inshore counter current that results in a general northward drift of coastal sediment (Ramsay, 1994; Mitchell et al., 2005). We attribute high alachlor concentrations detected at sites from the Southern Reef Complex to the northward movement of herbicide plumes originating from St Lucia Estuary. uMfolozi-St Lucia flood waters probably also transport substantial quantities of hexazinone to coastal waters.

A general northward increase in acetochlor concentrations suggests that St Lucia Estuary is probably not the only major source of herbicide contamination. Highest acetochlor concentrations were detected at sites from the Northern Reef Complex which, along with the frequent presence of atrazine and simazine, points to a second source of contamination. Kosi Bay represents the most proximal potential source of herbicide contamination to the Northern Reef Complex. The proximity of the Northern Reef Complex to Kosi Bay Estuary could explain why simazine and atrazine, despite being poorly soluble, were detected in samples here. However, the Kosi Bay lakes system is predominantly groundwater-fed and associated with a relatively small catchment area (~600 km^2; Ndlovu, 2015). Although cultivation does occur within the surrounding wetland areas, this is largely subsistence in nature. While contributions from these activities cannot be discounted, we suspect that high acetochlor concentrations measured at sites from the Northern Reef Complex may reflect the influence of river discharge into Maputo Bay (Mozambique), ~90 km north of Kosi Bay. Maputo Bay receives runoff from several sources, the largest being the Maputo River, which drains the Pongola region of South Africa. The lower reach of the Pongola River is characterised by wide alluvial plains, which are under intensive sugarcane cultivation, although the growing of cotton, fruit and maize also occurs in the region (Jaganyi et al., 2009). While no water quality monitoring studies have been undertaken to date, we suspect the Maputo River transports substantial herbicide loads into Maputo Bay, which are then carried southward by the Agulhas Current.

Simulation experiments suggest that herbicides may be relatively persistent in the marine environment (Mercurio et al., 2015). The half-lives of the photosystem-II (PSII) herbicides

(ametryn, atrazine, diuron, hexazinone and tebuthiuron) investigated by Mercurio et al. (2015) were all greater than a year, explaining why herbicides are detected year-round in nearshore waters of the Great Barrier Reef, often far from source rivers (Lewis et al., 2009; Kennedy et al., 2012). It also explains how herbicides originating from St Lucia Estuary and Maputo Bay could reach coral reefs along the Maputaland coastline.

4.4.3 *Potential effects on reef communities*

Globally, few other studies have reported on herbicide accumulation in reef organisms and none of these have included coral species (Table 1). Various herbicides, including alachlor, atrazine and simazine have been detected in fish and invertebrates from reefs in French Polynesia (e.g., Salvat et al., 2016), while low concentrations of atrazine have been measured in several seagrass species off the Queensland coast in Australia (e.g., Haynes et al., 2000). Overall, herbicide concentrations measured in Maputaland reef species are higher compared to these studies, but assessing the risks posed is difficult considering the absence of appropriate toxicological data.

Risks associated with different herbicide contaminants are likely to be dependent on their mode of action. Hexazinone, atrazine and simazine are PSII inhibiting herbicides, which compete with molecules responsible for triggering photosynthetic reactions (Kennedy et al., 2012). Since PSII reactions are common in many photosynthetic organisms, reduced photosynthetic efficiency has been reported in several marine species, including corals (Jones et al., 2003; Cantin et al., 2007; Shaw et al., 2012), microalgae (Magnusson et al., 2008), coralline algae (Negri et al., 2011) and seagrasses (Flores et al., 2013). Photosynthesis inhibition can lead to decreased algal production and may eventually result in coral bleaching (van Dam et al., 2011). Although PSII inhibiting herbicides have the same mode of action, variations in behaviour have been found in a variety of marine phototrophs (Jones & Kerswell, 2003; Magnusson et al., 2010; Negri et al., 2011; Wilkinson et al., 2015). Laboratory-based studies suggest that certain PSII herbicides may bind more effectively, resulting in increased photo-oxidative stress (Krieger-Liszkay, 2005; Krieger-Liszkay & Rutherford, 1998). This may result in varying toxic responses (Negri et al., 2011; Thomas et al., 2020). Moreover, herbicides have been shown to act in an additive manner with regards to PSII inhibition (Magnusson et al., 2010; Wilkinson et al., 2015) and herbicide mixtures are thus likely to be more toxic to reef organisms than their individual concentrations.

Acetochlor and alachlor (chloroacetanilides) are inhibitors of cell division in plants, but their effect in other species is largely unknown (Kim et al., 2020). Aside from inhibiting cell

division, these herbicides are known to interfere with certain enzymes, block plant hormone biosynthesis (Wilkinson, 1982; Molin et al., 1986; Trenkamp et al., 2004; Bach & Faure, 2010) and affect plant respiration and photosynthesis (Sloan & Camper, 1986; Seo et al., 2020). Although studies have rarely reported on the specific toxicity of chloroacetanilide herbicides to reef organisms, experiments on aquatic macrophytes and algae indicate their toxicity to be less than the PSII herbicides (Fairchild et al., 1998). Nevertheless, acute exposure to acetochlor and alachlor has been associated with various physiological and biochemical effects in juvenile fish (Yi et al., 2007; Lazhar et al., 2012; Xu et al., 2016; 2019).

While these studies provide some indication of the potential stresses associated with acute herbicide exposure, the long-term chronic effects on coral reproduction, immune system functionality, and the ability of corals to recover from disturbance are unknown (Lewis et al., 2012). The vulnerability of reef organisms to long-term herbicide exposure is likely to differ. Results from Maputaland show that herbicide accumulation is clearly species-dependent. Fleshy organisms with a high-water throughput have a greater potential to absorb and accumulate herbicides. Samples from *T. swinhoei* (8.0 ± 1.7%), *S. gravis* (9.4 ± 1.1%) and *S. glaucum* (9.7 ± 0.4%) were characterised by significantly higher lipid contents compared to those from *A. austera* (3.8 ± 0.3%) and *E. hirsutissima* (4.0 ± 0.8%) which further suggests the higher accumulation of herbicides in their tissues. Sponges (*T. swinhoei*) are very effective water filters, while soft coral species such as *S. glaucum* are dimporphic, having heterozooids and autozooids, the latter designed to constantly irrigate their fleshy coenenchyme (Fabricius, 1995). The accumulation of high herbicide concentrations in *T. swinhoei* and *S. glaucum* is probably due to the ability of these species to filter large quantities of water through their fleshy tissues. Differences in herbicide accumulation between species may also be attributed to variations in metabolic turnover, differences in excretion pathways, and age of the colony sampled.

The majority of corals have symbiotic algae that supplement their nutrition, and this facility could be impaired by the herbicide residues under consideration. However, corals are also heterotrophic and tentacular feeding could offset the deleterious effects of herbicide residues to an unknown extent. *Theonella swinhoei* is covered by a substantial film of exogenous microbiota, both photosynthetic and heterotrophic (e.g., Keren et al., 2015; Kuo et al., 2019), and these microbes may similarly be affected by, or possibly mitigate the effects of, herbicide accumulation. While the mitigating effects of biotransformation in corals are yet to be studied, predictive kinetic models suggest that herbicides have relatively high metabolic conversion rates ($\log k_M > -0.7$) and thus may be rapidly eliminated by, for example, fish (Arnot et al., 2008). Similarly, biotransformation may be a key process that can mitigate the

bioaccumulation potential of herbicides in coral reef invertebrates and is an important parameter to consider in exposure assessments. Toxicokinetic studies examining bioaccumulation and biotransformation processes in corals thus merit further study.

Although species sensitivity may vary, soft coral and sponge taxa are likely to be most vulnerable to the long-term effects of herbicide exposure. This may cause community compositional shifts, whereby species most sensitive to herbicide exposure are out-competed by less sensitive species. Long-term monitoring at Nine-mile Reef has indicated a steady decline in the cover of soft corals by ~20% over the past two decades (Porter & Schleyer, 2017; Schleyer et al., 2008). Factors contributing to the long-term decline in soft coral cover at this location remain unknown and chronic herbicide exposure could be a contributing factor. Indeed, the impact of long-term herbicide exposure on coral reef communities may be subtle. However, the effects of long-term exposure may combine and interact additively or synergistically with other environmental stressors, potentially reducing the resilience of corals to global climate change (Negri et al., 2011; Nalley et al., 2021).

Table 1: Comparison between herbicide concentrations (ng g^{-1} dw) reported in this study and those measured in reef organisms from around the world.

Location	Reef organism	Species	Atrazine	Simazine	Terbuthylazine	Alachlor	Metolachlor	Trifluralin	Hexazinone	Acetochlor
Maputaland, South Africa[a]	Octocoral	*Sinularia gravis*	n.d. – 4.9	n.d. – 9.2		2.8 – 57.8			3.2 – 4.8	n.d. – 44.6
	Octocoral	*Sarcphyton glaucum*	n.d. – 3.96	n.d.		n.d. – 160			1.3 – 89.0	6.0 – 69.6
	Sponge	*Theonella swinhoei*	n.d. – 43.3	n.d. – 45.1		n.d. – 33.3			n.d. – 26.5	n.d. – 105
	Hard coral	*Acropora austera*	n.d. – 12.8	n.d. – 3.85		n.d. – 6.34			n.d. – 16.2	n.d. – 12.6
	Hard coral	*Echinopora hirsutissima*	n.d. – 2.27	n.d. – 2.31		n.d. – 14.6			n.d.– 12.2	n.d. – 23.0
French Polynesia[b]	Fish	*Chlorurus sordidus*	n.d. – 20	n.d. – 50	n.d. – 90	n.d. – 20	n.d. – 30			
	Fish	*Epinephelus merra*	n.d. – 30	n.d. – 90	n.d. – 130	n.d. – 20	n.d. – 360	n.d. – 10		
	Fish	*Epinephelus hexagonatus*	n.d. – 60				n.d. – 50			
	Shrimp	*Penaeus stylirostris*			n.d. – 10		n.d. – 30	n.d. – 30		
	Sea cucumber	*Halodeima atra*	n.d. – 30	n.d. – 40	n.d. – 90	n.d. – 10	n.d. – 30	n.d. – 10		
	Mud crab	*Scylla serrata*					n.d. – 40	n.d. – 10		
	Sea snail	*Trochus Niloticus*					n.d. – 20			
	Green Macroalgae	*Halimeda*				20 – 40				
	Giant clam	*Tridacna maxima*					n.d. – 10	n.d. – 20		
Queensland Coast, Australia[c]	Seagrass	*Halodule uninervis*	<0.5							
	Seagrass	*Cymodocea serrulata*	<0.5							
	Seagrass	*Zostera capriconi*	<0.5							

n.d. = not detected

a: This study

b: Salvat et al. (2016)

c: Haynes et al. (2000)

4.5 Conclusions

Coral reef organisms exposed to agricultural runoff may accumulate high concentrations of multiple herbicides within their tissues. Reefs in Maputaland, which have been exposed to herbicide contamination for much of the past century, showed varying degrees of herbicide accumulation depending on species and distance from potential sources of contamination. Agricultural runoff entering the ocean via the uMfolozi-St Lucia estuary and Maputo Bay are identified as the two main sources of herbicide contamination to coral reefs in the region. However, herbicide accumulation is probably not unique to Maputaland and our findings have considerable implications for coral reefs globally, especially those receiving regular runoff from adjacent agricultural land. The impacts of long-term herbicide exposure on reef organisms cannot at present be evaluated and requires urgent attention. Assessing risks to coral reefs will require improved knowledge on several fronts, including the persistence and fate of herbicides in coastal waters, interactions of herbicides with environmental parameters (e.g., temperature and turbidity), and the chronic effects of herbicide mixtures.

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CHAPTER 5

CONCLUSIONS

5.1 Summary of the main results

This study investigated the occurrence, bioaccumulation and potential impact of current-use priority herbicides at Lake St Lucia and the nearshore marine environment within iSimangaliso Wetland Park. Accumulation in three environmental components (sediments, estuarine fish, and coral reef invertebrates) was investigated to provide insight into the transport and fate of herbicide residues. It is inferred that runoff from surrounding catchment areas introduces a variety of herbicide contaminants into Lake St Lucia. The Mkhuze and Mfolozi rivers are important conduits for herbicide transport and agricultural activities occurring within these drainage basins thus represent significant sources of contamination to Lake St Lucia. Multiple herbicide residues were found to bioaccumulate in fish from Lake St Lucia. While the accumulation of herbicides in aquatic vertebrates remains largely understudied, this presents potential ecological concerns related to the transfer of contaminants to higher trophic level species . Additionally, fish is a major source of protein for local communities and the regular consumption of fish from Lake St Lucia may be associated with long-term carcinogenic risk. Agricultural runoff also impacts the nearshore marine environment and herbicides were detected in almost all the coral reef invertebrates analysed. While little is currently known about the long-term effects of chronic herbicide exposure on reef organisms, chronic exposure studies suggest potential impacts may include photosynthesis inhibition, reproductive suppression, severe bleaching or partial colony mortality.

5.2 Management implications

The study offers critical baseline data on current-use herbicide contaminants impacting estuarine and marine habitats in iSimangaliso Wetland Park. The results should help guide policy-making and the management of biodiversity resources in the region. Conservation areas are impacted by external pollution pressures and cannot be managed in insolation. Moreover, the last several decades have seen dramatic increases in the number of people living within surrounding catchment areas and land is increasingly being converted to agriculture. This is likely to be accompanied by significant increases in herbicide usage in the area, with higher herbicide loads entering Lake St Lucia and the coastal marine environment.

Conservation management needs to take into account potential future stressors associated with climate change, which may interact additively or antagonistically to magnify the impact of contaminants (Verberk et al., 2016). Complex interactions between contaminants and climate change are possible and particularly concerning for species living on the edge of their physiological tolerance range, rendering them more vulnerable to the dual pressures of climate change and contaminant exposure (Lai et al., 2020; Noyes et al., 2009; Patra et al., 2007; Gordon 2003). The effects of long-term exposure to pollution may also combine and interact synergistically with other environmental factors, potentially reducing the resilience of corals to global climate change (e.g., Negri et al., 2011).

5.3 Limitations of the study and opportunities for future work

It is important to note that this study did not cover all the priority herbicides currently used in South Africa. In particular, it excluded glyphosate, mancozeb and paraquat, which are used in high quantities in South Africa. The study area falls within a region where >90% of South Africa's sugarcane is cultivated. Other agricultural pesticides, such as the insecticides imidacloprid and acetamiprid, are used to control *Fulmekiola serrata* (sugarcane thrips). The variety of agricultural chemicals impacting iSimangaliso is thus likely far greater than just the compounds analysed in this study.

Considerable knowledge gaps exist regarding the transport, persistence and fate of herbicides in the region. No information currently exists on contaminant fluxes or seasonal variations in contaminant inputs. The regular monitoring of herbicide concentrations in river and coastal waters would provide valuable information on herbicide transport and persistence. At present, regular water quality monitoring is not conducted within the park, but is essential for evaluating risks and guiding herbicide management in catchment areas. Monitoring is also essential for identifying herbicides that may need to be discontinued or more tightly regulated.

The toxicological effects of herbicide accumulation are speculative and require further investigation. Ecotoxicological studies are needed to better understand the impacts of long-term herbicide exposure, particularly on coral reef communities. Investigating the bioaccumulation of herbicides in other species as well as possible biomagnification in higher trophic organisms is also necessary for risk assessment.

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APPENDICES

Supplementary Table TS 2.1: Percentage spiked recoveries (n = 3) and % RSD at three fortified levels (ng g^{-1}). LOD limit of detection, LOQ = limit of quantification (ng g^{-1}). Data linked to Chapter 2

	10 ng g^{-1}		100 ng g^{-1}		250 ng g^{-1}			
Analyte	Mean	RSD	Mean	RSD	Mean	RSD	LOD	LOQ
EPTC	79.3	8.7	80.8	6.7	77.4	19.5	10.7	32.56
Trifluralin	78.4	6.0	94.2	6.5	104	12.4	10.7	32.46
Simazine	94.6	0.9	102	7.1	94.1	7.3	18.7	56.59
Atrazine	93.5	5.9	101	6.3	92.9	12.6	11.1	33.63
Terbuthylazine	77.2	5.1	94.3	15.6	102	2.8	12.7	38.62
Acetochlor	89.1	1.1	105	7.4	98.3	10.9	11.7	35.51
Alachlor	98.8	2.8	103	5.2	99.8	4.5	10.9	33.00
Metolachlor	91.3	2.1	92.4	5.4	96.8	12.4	10.2	31.03
Hexazinone	85.3	2.9	82.3	17.7	98.1	5.5	7.2	21.91
	20 ng g^{-1}		**50 ng g^{-1}**		**100 ng g^{-1}**			
MCPA	70.3	2.0	74.5	5.8	81.1	6.2	5.5	16.39
2,4-D	79.3	1.8	74.1	6.6	76.1	8.0	10.6	32.02

Table TS 2.2: Toxicity profile (reference a - f) and physicochemical properties (reference g - j) of studied herbicides Data linked to Chapters 2

Herbicide	Formula	Formula mass (g mol^{-1})	Solubility (mg L^{-1})	Half-life (soil) (d)	Log K_{ow}	Vapour Pressure (mPa)	Soil Sorption (K_{oc})	LC_{50}	EC_{50}		
								C. riparius	Algae	*Daphnia*	Fish
Atrazine	$C_8H_{14}ClN_5$	215.68	33	35-50	2.50	3.8×10^{-2}	100	1000	92.71	72	2000
Simazine	$C_7H_{12}ClN_5$	201.66	6.2	27-102	2.10	2.9×10^{-3}	130	13000	441.86	3600	6400
Terbuthylazine	$C_9H_{16}ClN_5$	229.71	8.5	5-116	3.21	0.15	236	500	16.5	32000	3400
Hexazinone	$C_{12}H_{20}N_4O_2$	252.31	33,000	90	1.95	0.036	54	78000	14	151600	505000
Acetochlor	$C_{14}H_{20}ClNO_2$	269.77	223	8-18	4.14	0.005	225	1600	1430	10600	1600
Alachlor	$C_{14}H_{20}ClNO_2$	269.77	170	1-30	3.09	2.0	240	19500	12.63	9500	6500
Metolachlor	$C_{15}H_{22}ClNO_2$	283.79	488	20	2.9	4.20	200	4089.01	72.67	25000	4900
Trifluralin	$C_{13}H_{16}F_3N_3O_4$	335.28	0.22	57-126	4.83	6.10	8000	1000	2509.25	356	514
EPTC	$C_9H_{19}NOS$	189.32	375	6-30	3.20	0.01	200	23000	3080.25	7500	52700
MCPA	$C_9H_9ClO_3$	200.62	24	<7	-0.71	2.3×10^{-2}	50	10000	1160	190000	748000
2,4-D	$C_8H_6OCl_2O_3$	222.04	23,180	<7	0.04	1.86×10^{-2}	20	2400	33800	100000	120000

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Supplementary Table TS2.3: Average (n = 3) herbicide concentrations (ng g^{-1} dw) ±SD in sediment samples collected from Lake St Lucia (samples L1 – 14), Mkhuze River (samples MK1 – 26) and Mfolozi River (samples MF1 – 12). Data linked to Chapter 2

Sample ID	EPTC	Trifluralin	Simazine	Atrazine	Terbuthylazine	Acetochlor	Alachlor	Metolachlor	Hexazinone	MCPA	2,4-D	Total
MK1	0.59 ± 0.6	0.23 ± 0.1	0.07 ± 0.4	n.d	0.35 ± 0.2	n.d	n.d	0.35 ± 0.1	0.32 ± 0.0	n.d	n.d	1.91
MK2	0.17 ± 0.0	n.d	n.d	n.d	n.d	1.07 ± 0.4	n.d	1.01 ± 0.2	1.29 ± 0.4	n.d	n.d	3.54
MK3	2.70 ± 0.2	1.54 ± 0.5	2.26 ± 0.3	0.22 ± 0.1	2.73 ± 0.2	n.d	n.d	n.d	0.89 ± 0.5	n.d	n.d	10.3
MK4	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	3.48 ± 0.1	n.d	n.d	3.48
MK5	0.31 ± 0.1	1.97 ± 0.1	1.88 ± 0.2	1.38 ± 0.4	2.63 ± 0.2	6.73 ± 0.7	3.80 ± 0.3	2.78 ± 0.1	9.15 ± 0.3	1.02 ± 0.3	2.77 ± 0.5	34.4
MK6	n.d	n.d	0.59 ± 0.0	n.d	0.67 ± 0.2	n.d	n.d	n.d	2.12 ± 0.7	2.36 ± 0.6	9.94 ± 0.4	15.7
MK7	1.75 ± 0.2	3.80 ± 0.3	5.35 ± 0.6	0.52 ± 0.3	2.81 ± 0.1	3.86 ± 0.7	4.52 ± 0.6	2.41 ± 0.3	n.d	n.d	n.d	25.0
MK8	1.03 ± 0.3	4.88 ± 0.5	3.94 ± 0.6	3.08 ± 0.2	4.14 ± 0.9	10.8 ± 0.4	8.61 ± 0.8	11.7 ± 0.1	11.0 ± 0.2	n.d	0.67 ± 0.3	59.8
MK9	n.d	n.d	n.d	1.89 ± 0.0	n.d	n.d	n.d	n.d	n.d	n.d	n.d	1.89
MK10	1.98 ± 1.1	1.15 ± 0.2	0.96 ± 0.4	1.81 ± 0.1	1.58 ± 0.3	4.21 ± 0.4	1.97 ± 0.2	4.34 ± 0.3	n.d	5.87 ± 0.3	5.76 ± 0.3	29.6
MK11	3.04 ± 0.0	11.99 ± 0.1	1.29 ± 0.2	n.d	2.20 ± 0.1	n.d	n.d	n.d	0.66 ± 0.1	n.d	n.d	19.2
MK12	3.02 ± 0.9	0.53 ± 0.0	0.82 ± 0.3	1.23 ± 0.2	0.88 ± 0.2	4.34 ± 0.7	2.04 ± 1.0	3.08 ± 0.1	n.d	0.52 ± 0.1	n.d	16.5
MK13	1.20 ± 0.3	3.40 ± 0.2	2.70 ± 0.5	2.00 ± 0.3	1.40 ± 0.3	6.64 ± 0.7	2.75 ± 0.2	4.17 ± 0.3	3.55 ± 0.2	3.16 ± 0.2	n.d	31.0
MK14	2.59 ± 0.5	5.36 ± 0.4	3.71 ± 0.6	3.11 ± 0.7	3.83 ± 0.2	9.29 ± 0.9	5.55 ± 0.1	7.21 ± 0.9	8.85 ± 0.2	n.d	0.25 ± 0.2	49.8
MK15	4.58 ± 0.5	n.d	3.58 ± 0.3	3.00 ± 0.6	2.49 ± 0.2	5.3 ± 0.4	4.04 ± 0.6	4.93 ± 0.5	n.d	1.37 ± 0.5	n.d	29.3
MK16	1.69 ± 0.2	n.d	0.81 ± 0.1	n.d	1.64 ± 0.6	1.56 ± 0.3	n.d	n.d	2.75 ± 0.3	4.12 ± 0.7	4.61 ± 0.4	17.2
MK17	3.98 ± 0.2	1.10 ± 0.3	1.44 ± 0.1	1.24 ± 0.3	0.94 ± 0.4	5.46 ± 1.2	1.31 ± 0.8	2.72 ± 0.9	3.25 ± 0.3	6.37 ± 0.2	9.02 ± 0.2	36.8

Sample ID	EPTC	Trifluralin	Simazine	Atrazine	Terbuthylazine	Acetochlor	Alachlor	Metolachlor	Hexazinone	MCPA	2,4-D	Total
MK18	1.37 ± 0.9	3.25 ± 0.4	4.81 ± 0.3	2.44 ± 0.3	2.71 ± 0.9	3.34 ± 1.0	2.91 ± 0.7	3.85 ± 0.9	6.09 ± 0.3	2.89 ± 0.3	1.65 ± 0.5	35.3
MK19	4.01 ± 0.2	25.5 ± 0.1	2.95 ± 0.7	1.15 ± 0.2	3.87 ± 0.5	1.46 ± 0.5	2.54 ± 0.6	n.d	9.02 ± 0.7	n.d	n.d	50.5
MK20	5.19 ± 0.5	4.00 ± 0.3	1.84 ± 0.2	2.57 ± 0.2	2.39 ± 0.3	5.14 ± 0.3	3.44 ± 0.5	5.14 ± 0.2	5.43 ± 0.9	0.60 ± 0.1	n.d	35.7
MK21	2.27 ± 0.7	3.64 ± 0.2	2.76 ± 0.6	3.17 ± 0.5	2.76 ± 0.4	2.91 ± 0.5	3.18 ± 0.3	3.60 ± 0.8	8.57 ± 0.7	2.59 ± 0.1	2.89 ± 0.6	38.3
MK22	6.41 ± 0.8	2.97 ± 0.9	3.38 ± 0.4	0.98 ± 0.3	4.57 ± 0.5	0.75 ± 0.1	2.13 ± 0.5	n.d	6.97 ± 0.4	n.d	n.d	28.2
MK23	3.9 ± 0.1	1.52 ± 0.3	1.98 ± 0.2	n.d	2.25 ± 0.4	n.d	0.56 ± 0.2	n.d	3.1 ± 0.2	n.d	1.23 ± 0.5	14.5
MK24	3.63 ± 0.6	4.72 ± 1.0	8.11 ± 0.0	6.71 ± 0.2	3.31 ± 0.3	7.49 ± 0.7	4.73 ± 0.7	7.13 ± 0.2	22.0 ± 0.8	n.d	n.d	67.8
MK25	n.d	0.69 ± 0.3	2.09 ± 0.1	n.d	0.87 ± 0.3	6.65 ± 0.5	2.95 ± 0.3	2.33 ± 0.2	5.89 ± 0.2	1.35 ± 0.2	n.d	22.8
MK26	1.75 ± 0.3	1.29 ± 0.8	1.77 ± 0.7	0.08 ± 0.0	2.24 ± 0.2	6.4 ± 0.6	2.7 ± 0.3	15.3 ± 0.4	n.d	n.d	n.d	31.6
MF1	3.69 ± 0.2	0.61 ± 0.7	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.47 ± 0.2	1.76 ± 0.3	6.53
MF2	2.29 ± 0.5	0.17 ± 0.0	0.02 ± 0.0	0.55 ± 0.2	0.94 ± 0.3	2.87 ± 0.3	0.48 ± 0.4	n.d	0.81 ± 0.2	1.61 ± 0.2	5.48 ± 0.1	15.2
MF3	1.33 ± 0.5	0.36 ± 0.2	0.75 ± 0.2	0.77 ± 0.3	0.28 ± 0.0	3.16 ± 0.5	1.36 ± 0.3	n.d	3.81 ± 0.6	n.d	3.32 ± 0.2	15.1
MF4	1.14 ± 0.7	1.36 ± 0.2	n.d	0.16 ± 0.0	0.56 ± 0.2	1.62 ± 0.7	0.15 ± 0.1	7.24 ± 1.6	n.d	n.d	0.28 ± 0.2	12.5
MF5	3.12 ± 1.1	4.18 ± 0.9	4.80 ± 0.3	3.48 ± 0.63	2.39 ± 0.3	n.d	n.d	n.d	1.55 ± 0.3	n.d	n.d	19.5
MF6	2.66 ± 1.1	2.6 ± 0.5	3.79 ± 0.7	3.83 ± 0.6	3.23 ± 0.1	n.d	n.d	n.d	6.87 ± 0.2	n.d	0.78 ± 0.4	23.8
MF7	4.02 ± 0.6	n.d	n.d	2.48 ± 0.7	20.1 ± 1.0	27.3 ± 1.8	5.13 ± 0.1	13.1 ± 0.4	2.52 ± 0.1	3.50 ± 0.3	4.32 ± 0.5	82.4
MF8	2.51 ± 0.6	1.34 ± 0.8	2.29 ± 0.2	1.06 ± 0.1	0.73 ± 0.1	12.9 ± 0.3	2.49 ± 0.2	4.92 ± 0.1	0.64 ± 0.2	n.d	1.62 ± 0.2	30.5
MF9	n.d	2.45 ± 0.2	1.33 ± 0.2	0.5 ± 0.2	0.14 ± 0.1	4.75 ± 0.1	3.76 ± 0.1	1.16 ± 0.1	1.92 ± 0.5	0.12 ± 0.1	1.56 ± 0.4	17.7
MF10	3.9 ± 0.6	1.06 ± 0.2	1.75 ± 0.2	0.43 ± 0.4	n.d	4.10 ± 0.9	2.83 ± 0.3	13.34 ± 0.7	0.87 ± 0.4	2.48 ± 0.3	4.03 ± 0.5	34.8

Sample ID	EPTC	Trifluralin	Simazine	Atrazine	Terbuthylazine	Acetochlor	Alachlor	Metolachlor	Hexazinone	MCPA	2,4-D	Total
MF11	1.38 ± 0.2	2.76 ± 0.1	0.88 ± 0.2	0.39 ± 0.14	0.08 ± 0.1	2.32 ± 0.1	2.53 ± 0.1	0.43 ± 0.1	3.12 ± 0.2	1.31 ± 0.1	7.12 ± 0.3	22.3
MF12	n.d	3.07 ± 0.4	n.d	n.d	n.d	2.46 ± 0.6	2.95 ± 0.7	n.d	0.42 ± 0.2	3.82 ± 0.1	14.17 ± 0.8	26.9
L1	n.d.	n.d	n.d.	n.d.	0.11 ± 0.3	2.16 ± 1.5	n.d	n.d.	n.d.	0.72 ± 0.6	n.d	3.00
L2	4.12 ± 1.0	0.22 ± 0.1	2.19 ± 0.7	3.25 ± 0.5	2.19 ± 1.0	2.02 ± 0.6	0.74 ± 0.8	6.98 ± 1.5	n.d.	n.d	n.d	21.7
L3	n.d.	n.d	n.d.	n.d.	n.d	n.d	n.d	n.d.	n.d.	n.d	n.d	n.d.
L4	0.97 ± 0.5	1.25 ± 0.3	1.48 ± 0.2	n.d.	0.52 ± 0.2	7.83 ± 0.4	3.10 ± 0.1	2.36 ± 0.3	3.07 ± 0.4	0.55 ± 0.1	n.d	21.1
L5	1.99 ± 0.9	2.18 ± 0.3	2.73 ± 0.6	0.82 ± 0.5	0.6 ± 0.5	6.15 ± 0.38	2.32 ± 0.3	3.01 ± 0.3	5.29 ± 0.1	n.d.	n.d	25.1
L6	2.95 ± 0.2	0.53 ± 0.2	n.d.	0.59 ± 0.1	1.36 ± 0.3	2.66 ± 1.0	1.44 ± 0.3	2.31 ± 0.5	13.0 ± 0.2	1.87 ± 0.1	1.83 ± 0.1	28.5
L7	n.d.	n.d.	0.83 ± 0.2	1.03 ± 0.2	1.56 ± 0.5	2.44 ± 0.3	0.68 ± 0.5	1.09 ± 0.2	1.56 ± 0.1	n.d.	n.d	9.19
L8	n.d.	0.07 ± 0.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.11 ± 0.2	1.68 ± 1.0	n.d	1.86
L9	n.d.	0.67 ± 0.5	n.d.	n.d.	0.14 ± 0.2	0.05 ± 0.0	n.d.	n.d.	0.51 ± 0.1	3.84 ± 2.5	n.d	5.21
L10	n.d.	n.d.	n.d.	n.d.	n.d.	0.45 ± 0.6	n.d.	n.d.	n.d.	8.09 ± 2.1	n.d	8.54
L11	n.d.	n.d.	0.04 ± 0.1	n.d.	n.d.	3.00 ± 0.6	n.d.	n.d.	0.49 ± 0.5	n.d.	n.d	3.53
L12	n.d.	n.d.	n.d.	n.d.	n.d.	0.78 ± 0.2	n.d.	n.d.	n.d.	n.d.	n.d	0.78
L13	0.34 ± 0.2	3.24 ± 0.2	1.05 ± 0.1	0.94 ± 0.4	2.53 ± 0.4	3.52 ± 0.2	2.77 ± 0.4	2.12 ± 0.4	n.d.	n.d.	0.67 ± 0.1	17.18
L14	4.62 ± 0.8	3.77 ± 0.2	1.0 ± 0.1	0.74 ± 0.0	1.75 ± 0.7	5.39 ± 0.8	4.44 ± 0.7	3.39 ± 0.5	8.3 ± 0.3	1.23 ± 0.1	n.d	34.7

n.d. : not detected

Supplementary Table TS2.4: Calculated risk quotients for the different sites and trophic levels based on mean (**maximum**) herbicide concentrations. Data linked to Chapter 2

	Mkhuze River				Mfolozi River				Lake St Lucia			
	Algae	*D. magna*	*C. riparius*	Fish	Algae	*D. magna*	*C. riparius*	Fish	Algae	*D. magna*	*C. riparius*	Fish
Atrazine	0.0019 (**0.0090**)	0.0025 (**0.0116**)	0.0002 (**0.0008**)	0.0001 (**0.0004**)	0.0021 (**0.0069**)	0.0026 (**0.0089**)	0.0002 (**0.0006**)	<0.0001 (**0.0003**)	0.0010 (**0.0058**)	0.0012 (**0.0075**)	<0.0001 (**<0.0001**)	<0.0001 (**0.0003**)
Simazine	0.00049 (**0.0018**)	0.0001 (**0.0002**)	<0.0001 (**0.0001**)	<0.0001 (**0.0001**)	0.0004 (**0.0014**)	0.0001 (**0.0002**)	<0.0001 (**0.0001**)	<0.0001 (**0.0001**)	0.0002 (**0.0008**)	<0.0001 (**0.0001**)	<0.0001 (**<0.0001**)	<0.0001 (**0.0001**)
Terbuthylazine	0.0066 (**0.0147**)	<0.0001 (**<0.0001**)	0.0002 (**0.0005**)	<0.0001 (**0.0001**)	0.0101 (**0.0860**)	<0.0001 (**<0.0001**)	0.0003 (**0.002**)	<0.0001 (**0.0004**)	0.0033 (**0.0108**)	<0.0001 (<**0.0001**)	0.0001 (**0.0004**)	<0.0001 (**0.0001**)
Hexazinone	0.0728 (**0.3638**)	<0.0001 (**<0.0001**)	<0.0001 (**0.0001**)	<0.0001 (**<0.0001**)	0.0415 (**0.1515**)	<0.0001 (**<0.0001**)	<0.0001 (**<0.0001**)	<0.0001 (**<0.0001**)	0.0509 (**0.2866**)	<0.0001 (<**0.0001**)	<0.0001 (**<0.0001**)	<0.0001 (**<0.0001**)
Acetochlor	0.0001 (**0.0004**)	<0.0001 (**0.0001**)	0.0001 (**0.0004**)	0.0001 (**0.0004**)	0.0003 (**0.0007**)	<0.0001 (**0.0001**)	0.0002 (**0.0005**)	0.0002 (**0.0006**)	0.0001 (**0.0004**)	<0.0001 (**0.0001**)	0.0001 (**0.0004**)	0.0001 (**0.0004**)
Alachlor	0.0134 (**0.0501**)	<0.0001 (**0.0001**)	<0.0001 (<**0.0001**)	<0.0001 (**0.0001**)	0.0141 (**0.0398**)	<0.0001 (**0.0001**)	<0.0001 (**<0.0001**)	<0.0001 (**0.0001**)	0.0086 (**0.0345**)	<0.0001 (**0.0001**)	<0.0001 (**<0.0001**)	<0.0001 (**0.0001**)
Metolachlor	0.0027 (**0.0132**)	<0.0001 (**<0.0001**)	<0.0001 (**0.0002**)	<0.0001 (**0.0002**)	0.0038 (**0.0153**)	<0.0001 (**<0.0001**)	0.0001 (**0.0003**)	<0.0001 (**0.0002**)	0.0017 (**0.0080**)	<0.0001 (<**0.0001**)	<0.0001 (**0.0001**)	<0.0001 (**0.0001**)
Trifluralin	<0.0001 (**<0.0001**)	<0.0001 (**0.0001**)	<0.0001 (**<0.0001**)	<0.0001 (**0.0001**)	<0.0001 (**<0.0001**)	<0.0001 (**<0.0001**)	<0.0001 (**<0.0001**)	<0.0001 (**<0.0001**)	<0.0001 (**<0.0001**)	<0.0001 (<**0.0001**)	<0.0001 (**<0.0001**)	<0.0001 (<**0.0001**)
EPTC	0.0001 (**0.0001**)	<0.0001 (**0.0001**)	<0.0001 (<**0.0001**)	<0.0001 (**<0.0001**)	0.0001 (**0.0001**)	<0.0001 (**<0.0001**)	<0.0001 (<**0.0001**)	<0.0001 (**<0.0001**)	<0.0001 (**<0.0001**)	<0.0001 (**0.0001**)	<0.0001 (**<0.0001**)	<0.0001 (<**0.0001**)
MCPA	0.0003 (**0.0014**)	<0.0001 (**<0.0001**)	<0.0001 (**0.0002**)	<0.0001 (**<0.0001**)	0.0003 (**0.0011**)	<0.0001 (**<0.0001**)	<0.0001 (**0.0001**)	<0.0001 (**<0.0001**)	0.0004 (**0.0023**)	<0.0001 (<**0.0001**)	<0.0001 (**0.0003**)	<0.0001 (<**0.0001**)
2,4-D	<0.0001 (**0.0002**)	<0.0001 (**0.0001**)	0.0004 (**0.0026**)	<0.0001 (**0.0001**)	0.0001 (**0.0004**)	<0.0001 (**<0.0001**)	0.0013 (**0.0049**)	<0.0001 (**0.0001**)	<0.0001 (**0.0001**)	<0.0001 (<**0.0001**)	0.0001 (**0.0006**)	<0.0001 (<**0.0001**)
ΣRQ	0.0983 (**0.4546**)	0.0026 (**0.0123**)	0.0010 (**0.0049**)	0.0004 (**0.0014**)	0.0726 (**0.3031**)	0.0028 (**0.0095**)	0.0022 (**0.0095**)	0.0005 (**0.0019**)	0.0663 (**0.3494**)	0.0013 (**0.0079**)	0.0005 (**0.0024**)	0.0002 (**0.0010**)

Supplementary Table TS3.1: Percentage spiked recoveries (n = 3) and % RSD at three fortified levels (ng g^{-1}). LOD limit of detection, LOQ = limit of quantification (ng g^{-1}). Data linked to Chapter 3

	10 ng g^{-1}		**100 ng g^{-1}**		**250 ng g^{-1}**			
Analyte	Mean	RSD	Mean	RSD	Mean	RSD	LOD	LOQ
EPTC	72.5	5.9	89.7	10.7	91.9	4.4	0.09	0.31
Trifluralin	86.3	4.1	76.2	3.4	95.0	14.6	0.15	0.51
Simazine	72.2	6.9	80.0	11.4	79.3	13.6	0.29	0.98
Atrazine	76.8	8.0	73.5	2.7	90.5	12.6	0.12	0.39
Terbuthylazine	87.5	2.1	80.7	11.4	90.6	6.7	0.18	0.61
Acetochlor	76.6	2.6	81.8	3.2	95.5	9.9	0.11	0.35
Alachlor	86.3	11.3	78.1	8.3	88.3	2.8	0.22	0.73
Metolachlor	89.6	3.8	80.4	3.7	92.6	8.6	0.08	0.27
Hexazinone	79.1	9.1	72.0	10.2	87.4	7.1	0.15	0.49
	20 ng g^{-1}		**50 ng g^{-1}**		**100 ng g^{-1}**			
MCPA	89.6	1.5	100.9	4.6	102.9	9.1	0.36	1.2
2,4-D	84.4	5.7	87.4	4.1	96.1	7.7	0.40	1.3

Supplementary Table TS3.2: Average (n = 3) dry weight (dw), wet weight (ww) and lipid weight (lw) herbicide concentrations (ng g^{-1})in fish tissues samples collected from Lake St Lucia. Sample TF 1-14: Tilapia in Northern Lake, TNAR 1-3 : Tilapia in the Narrows, CF 2-11 : Catfish in Northern Lake, NAR 1-30 : Catfish in the Narrows. Data linked to Chapter 3

Sample ID		Atrazine	Hexazinone	Simazine	Terbuthylazine	Acetochlor	Alachlor	Metolachlor	Trifluralin	EPTC	MCPA	2,4-D	TOTAL	±SD
TF 1	dw	8.50	5.20	8.60	4.80	12.0	7.60	9.70	1.40	6.70	24.8	15.3	104.6	6.3
	ww	1.67	1.02	1.69	0.94	2.35	1.49	1.90	0.27	1.31	4.86	3.00	20.5	1.2
	lw	83.3	51.0	84.3	47.0	117.6	74.5	95.1	13.7	65.7	243.0	149.9	1025.1	61.5
TF 2	dw	6.10	n.d	8.60	5.40	13.3	6.90	7.90	0.60	8.30	0.00	15.1	72.2	5.0
	ww	1.11	n.d	1.57	0.98	2.42	1.26	1.44	0.11	1.51	n.d	2.75	13.2	0.9
	lw	28.5	n.d	40.13	25.2	62.07	32.2	36.87	2.8	38.73	n.d	70.5	336.9	23.4
TF 3	dw	10.7	0.60	11.9	8.60	17.6	8.70	12.0	3.30	4.8	29.2	20.8	128.2	8.3
	ww	1.58	0.09	1.76	1.27	2.6	1.29	1.78	0.49	0.71	4.32	3.08	19.0	1.2
	lw	66.0	3.70	73.38	53.03	108.53	53.65	74.0	20.35	29.6	180.1	128.3	790.6	51.0
TF 4	dw	9.50	5.2	12.4	10.3	18.2	10.2	11.7	4.4	15.6	17.4	11	125.9	4.4
	ww	1.41	0.77	1.84	1.52	2.69	1.51	1.73	0.65	2.31	2.58	1.63	18.6	0.7
	lw	63.9	35.0	83.4	69.3	122.4	68.6	78.7	29.6	105.0	117.1	74.0	847.0	29.7
TF 5	dw	12.5	7.3	10.3	15	14.7	10.6	9.2	2.5	12.6	13.8	4.7	113.2	4.1
	ww	2.23	1.3	1.83	2.67	2.62	1.89	1.64	0.45	2.24	2.46	0.84	20.2	0.7
	lw	74.2	43.3	61.1	89.0	87.2	62.9	54.6	14.8	74.8	81.9	27.9	671.7	24.2
TF 6	dw	11.9	n.d	11.1	11.4	17.0	12.5	11.3	5.10	19.1	23.8	23.1	146.3	7.2
	ww	2.27	n.d	2.12	2.18	3.25	2.39	2.16	0.97	3.65	4.55	4.41	28.0	1.4
	lw	90.9	n.d	84.8	87.1	129.9	95.5	86.3	39.0	145.9	181.8	176.5	1117.7	54.7
TF 7	dw	10.1	3.8	8.3	7.5	12.0	7.1	8.4	1.9	16.1	n.d	n.d	75.2	5.1
	ww	2.07	0.78	1.70	1.54	2.46	1.46	1.72	0.39	3.30	n.d	n.d	15.4	1.0
	lw	50.5	19	41.5	37.5	60	35.5	42.0	9.50	80.5	n.d	n.d	376.0	25.3
TF 8	dw	13.4	0.80	16.2	16.6	19.5	13.6	14.00	5.30	21.1	n.d	n.d	120.5	6.5
	ww	1.93	0.12	2.33	2.39	2.81	1.96	2.02	0.76	3.04	n.d	n.d	17.4	1.1

Sample ID		Atrazine	Hexazinone	Simazine	Terbuthylazine	Acetochlor	Alachlor	Metolachlor	Trifluralin	EPTC	MCPA	2,4-D	TOTAL	±SD
	lw	52.2	3.11	63.05	64.61	75.89	52.93	54.49	20.63	82.12	n.d	n.d	469.0	31.0
TF 9	dw	10.4	8.00	11.5	11.5	13.5	10.4	10.4	5.40	16.1	17.8	26	141.0	5.6
	ww	1.99	1.53	2.20	2.20	2.58	1.99	1.99	1.03	3.08	3.4	4.97	27.0	1.1
	lw	94.6	72.8	104.6	104.6	122.8	94.6	94.6	49.1	146.4	161.9	236.5	1282.4	50.6
TF 10	dw	9.60	3.80	11.5	9.10	11.9	7.30	9.20	1.50	16.5	n.d	30.7	111.1	8.3
	ww	1.27	0.50	1.52	1.20	1.57	0.96	1.21	0.20	2.18	n.d	4.05	14.7	1.1
	lw	66.7	26.4	79.9	63.2	82.7	50.7	63.9	10.4	114.6	n.d	213.3	771.8	58.0
TF 11	dw	7.90	3.90	10.0	8.00	9.40	7.20	6.00	3.00	9.70	0.50	24.6	90.2	6.2
	ww	1.67	0.82	2.11	1.69	1.98	1.52	1.27	0.63	2.05	0.11	5.19	19.0	1.3
	lw	43.9	21.7	55.5	44.4	52.2	40.0	33.3	16.7	53.9	2.78	136.6	500.9	34.5
TF 12	dw	8.80	n.d	10.3	10.5	13.7	7.30	8.90	1.70	13.0	1.50	n.d	75.7	5.2
	ww	1.39	n.d	1.63	1.66	2.16	1.15	1.41	0.27	2.05	0.24	n.d	12.0	0.8
	lw	63.2	n.d	74.0	75.4	98.4	52.4	63.9	12.2	93.4	10.8	n.d	543.7	37.1
TF 13	dw	14.0	18.1	19.0	17.1	20.1	16.7	14.9	7.20	23.6	3.60	20.4	174.7	5.9
	ww	1.85	2.39	2.51	2.26	2.65	2.20	1.97	0.95	3.12	0.48	2.69	23.1	0.8
	lw	77.0	99.6	104.5	94.1	110.6	91.9	82.0	39.6	129.8	19.8	112.2	960.9	32.3
TF 14	dw	8.10	n.d	9.40	10.4	12.1	6.50	8.10	1.80	14.5	8.40	20.3	99.6	5.6
	ww	1.56	n.d	1.80	2.00	2.32	1.25	1.56	0.35	2.78	1.61	3.90	19.1	1.1
	lw	28.8	n.d	33.42	36.98	43.02	23.11	28.8	6.4	51.56	29.9	72.2	354.1	19.8
TNAR1	dw	17.5	0.90	7.20	9.30	28.6	6.40	7.00	n.d	6.00	0.20	n.d	83.1	8.7
	ww	4.68	0.24	1.92	2.49	7.64	1.71	1.87	n.d	1.6	0.05	n.d	22.2	2.3
	lw	74.4	3.82	30.6	39.5	121.5	27.2	29.8	n.d	25.5	0.85	n.d	353.2	37.1
TNAR2	dw	52.1	3.7	18.8	46	29.6	13.9	56.6	n.d	20.5	33	17.1	291.3	19
	ww	15.03	1.07	5.42	13.27	8.54	4.01	16.33	n.d	5.91	9.52	4.93	84.0	5.4
	lw	277.8	19.7	100.3	245.3	157.9	74.1	301.8	n.d	109.3	176.0	91.2	1553.4	101
TNAR3	dw	10.6	5.9	15.2	13	25.0	9.7	12.6	2.5	19.2	40.7	28.2	182.6	11.0
	ww	2.77	1.54	3.97	3.39	6.52	2.53	3.29	0.65	5.01	10.62	7.36	47.7	2.9

Sample ID		Atrazine	Hexazinone	Simazine	Terbuthylazine	Acetochlor	Alachlor	Metolachlor	Trifluralin	EPTC	MCPA	2,4-D	TOTAL	±SD
	lw	50.3	28.0	72.1	61.7	118.6	46.0	59.8	11.9	91.1	193.1	133.8	866.2	52
CF2	dw	3.60	n.d	5.10	0.90	12.0	4.10	n.d	n.d	4.80	12.5	1.30	44.3	4.5
	ww	0.64	n.d	0.91	0.16	2.15	0.73	n.d	n.d	0.86	2.24	0.23	7.92	0.8
	lw	10.4	n.d	14.7	2.60	34.7	11.8	n.d	n.d	13.9	36.1	3.75	127.9	13
CF3	dw	1.10	n.d	n.d	0.300	20.9	1.00	n.d	n.d	10.3	30.2	19.5	83.3	11
	ww	0.20	n.d	n.d	0.06	3.89	0.19	n.d	n.d	1.92	5.62	3.63	15.5	2.0
	lw	2.11	n.d	n.d	0.58	40.1	1.92	n.d	n.d	19.8	57.9	37.4	159.7	21
CF4	dw	8.20	n.d	9.40	7.80	10.0	7.50	9.20	3.30	14.8	8.10	14.1	92.4	4.2
	ww	1.05	n.d	1.2	1.00	1.28	0.96	1.18	0.42	1.89	1.04	1.8	11.8	0.5
	lw	13.81	n.d	15.8	13.1	16.8	12.6	15.5	5.56	24.9	13.6	23.8	155.6	7.1
CF5	dw	7.7	n.d	8.8	7.7	13.8	8.1	n.d	n.d	13.5	26.1	10.6	96.3	7.6
	ww	1.56	n.d	1.79	1.56	2.8	1.64	n.d	n.d	2.74	5.3	2.15	19.5	1.6
	lw	22.7	n.d	25.9	22.7	40.6	23.8	n.d	n.d	39.7	76.8	31.2	283.3	23
CF6	dw	16.1	0.3	13.1	12.9	26.2	11.3	13.1	2.5	18.2	19.9	44.8	178.4	12
	ww	3.41	0.06	2.78	2.73	5.55	2.4	2.78	0.53	3.86	4.22	9.50	37.8	2.5
	lw	56.0	1.04	45.5	44.8	91.2	39.3	45.5	8.69	63.3	69.2	155.7	620.0	42
CF7	dw	10.8	1.20	9.70	7.80	13.9	9.00	n.d	n.d	10.3	7.2	n.d	69.9	5.1
	ww	2.12	0.24	1.90	1.53	2.72	1.76	n.d	n.d	2.02	1.41	n.d	13.7	1.0
	lw	24.6	2.73	22.1	17.8	31.7	20.5	n.d	n.d	23.5	16.4	n.d	159.3	12
CF8	dw	11.0	6.80	12.0	10.0	10.1	9.10	14.8	n.d	11.5	17.9	6.50	109.7	4.6
	ww	2.16	1.33	2.35	1.96	1.98	1.78	2.90	n.d	2.25	3.51	1.27	21.5	0.9
	lw	52.6	32.5	57.4	47.8	48.3	43.5	70.8	n.d	55.0	85.6	31.1	524.4	22
CF9	dw	10.0	6.00	10.8	7.90	11.8	7.50	n.d	n.d	10.9	7.30	7.80	80.0	4.0
	ww	1.82	1.09	1.97	1.44	2.15	1.37	n.d	n.d	1.98	1.33	1.42	14.6	0.7
	lw	25.6	15.4	27.7	20.3	30.3	19.2	n.d	n.d	27.9	18.7	20.0	205.1	10
CF10	dw	12.1	n.d	13.2	10.1	18.6	10.0	12.2	n.d	7.30	12.5	74.5	170.5	20
	ww	2.26	n.d	2.47	1.89	3.48	1.87	2.28	n.d	1.37	2.34	13.9	31.9	3.8

Sample ID		Atrazine	Hexazinone	Simazine	Terbuthylazine	Acetochlor	Alachlor	Metolachlor	Trifluralin	EPTC	MCPA	2,4-D	TOTAL	±SD
	lw	64.7	n.d	70.5	54.0	99.4	53.4	65.2	n.d	39.0	66.8	398.0	911.0	109
CF11	dw	6.20	n.d	6.40	5.80	7.40	6.30	3.90	n.d	7.00	26.0	15.3	84.3	7.3
	ww	1.09	n.d	1.13	1.02	1.30	1.11	0.69	n.d	1.23	4.58	2.69	14.8	1.3
	lw	12.8	n.d	13.3	12.0	15.3	13.0	8.08	n.d	14.5	53.8	31.7	174.6	15
NAR1	dw	5.80	n.d	4.90	4.30	5.30	2.80	5.20	n.d	3.30	17.8	40.2	89.6	12
	ww	1.52	n.d	1.28	1.13	1.39	0.73	1.36	n.d	0.86	4.66	10.52	23.5	3.0
	lw	20.7	n.d	17.5	15.4	18.9	10.0	18.6	n.d	11.8	63.6	143.6	320.0	42
NAR2	dw	12.2	n.d	13.1	12.7	10.4	8.80	14.1	n.d	9.90	23.3	30.8	135.3	8.9
	ww	2.85	n.d	3.06	2.97	2.43	2.06	3.30	n.d	2.31	5.45	7.20	31.6	2.1
	lw	30.5	n.d	32.8	31.8	26.0	22.0	35.3	n.d	24.8	58.3	77.0	338.3	22
NAR3	dw	7.90	0.20	11.0	8.70	16.9	8.00	11.3	n.d	n.d	35.5	39.9	139.4	13
	ww	2.12	0.05	2.95	2.34	4.54	2.15	3.03	n.d	n.d	9.53	10.71	37.4	3.6
	lw	22.6	0.57	31.5	24.9	48.4	22.9	32.4	n.d	n.d	101.7	114.3	399.3	39
NAR4	dw	12.6	n.d	11.5	13.1	13.5	11.0	11.6	n.d	15.2	41.4	58.4	188.3	17
	ww	2.81	n.d	2.57	2.93	3.02	2.46	2.59	n.d	3.40	9.25	13.05	42.1	3.9
	lw	35.6	n.d	32.5	37.0	38.1	31.1	32.8	n.d	42.9	116.9	164.9	531.8	49
NAR5	dw	11.4	n.d	7.70	6.70	14.0	6.50	14.9	n.d	8.10	22.8	24.5	116.6	8.0
	ww	2.55	n.d	1.72	1.5	3.13	1.45	3.33	n.d	1.81	5.09	5.47	26.1	1.8
	lw	34.5	n.d	23.3	20.3	42.4	19.7	45.1	n.d	24.5	69.1	74.2	353.3	24
NAR6	dw	17.1	10.3	19.2	19.1	16.8	13.5	30.8	n.d	20.1	18.5	22.3	187.7	7.7
	ww	4.15	2.5	4.65	4.63	4.07	3.27	7.47	n.d	4.87	4.48	5.41	45.5	1.9
	lw	52.5	31.6	58.9	58.6	51.6	41.4	94.5	n.d	61.7	56.8	68.4	575.9	23.5
NAR7	dw	11.1	5.00	11.1	11.6	10.8	6.10	17.9	n.d	9.90	19.9	20.6	124.0	6.3
	ww	2.49	1.12	2.49	2.60	2.42	1.37	4.02	n.d	2.22	4.47	4.62	27.8	1.4
	lw	28.4	12.8	28.4	29.6	27.6	15.6	45.7	n.d	25.3	50.8	52.6	316.7	16
NAR8	dw	11.8	1.10	13.8	15.8	20.8	8.90	22.4	n.d	10.4	n.d	39.3	144.3	12
	ww	2.90	0.27	3.39	3.88	5.10	2.18	5.50	n.d	2.55	n.d	9.64	35.4	2.9

Sample ID		Atrazine	Hexazinone	Simazine	Terbuthylazine	Acetochlor	Alachlor	Metolachlor	Trifluralin	EPTC	MCPA	2,4-D	TOTAL	±SD
	lw	47.9	4.46	56.0	64.1	84.4	36.1	90.9	n.d	42.2	n.d	159.4	585.3	47
NAR9	dw	8.30	6.40	9.10	8.50	11.8	5.00	12.9	n.d	7.60	23.6	24.4	117.6	7.4
	ww	1.84	1.42	2.01	1.88	2.61	1.11	2.85	n.d	1.68	5.22	5.39	26.0	1.6
	lw	13.9	10.7	15.2	14.2	19.7	8.34	21.5	n.d	12.7	39.4	40.7	196.2	12
NAR10	dw	10.1	7.50	10.9	9.20	9.60	8.20	17.3	n.d	12.3	10.8	30.7	126.6	7.6
	ww	2.28	1.69	2.46	2.08	2.17	1.85	3.9	n.d	2.78	2.44	6.93	28.6	1.7
	lw	26.4	19.6	28.5	24.0	25.1	21.4	45.2	n.d	32.1	28.2	80.2	330.7	20
NAR11	dw	10.4	6.40	11.6	9.90	11.1	8.60	14.6	0.90	13.1	23.9	13.7	124.2	5.7
	ww	2.63	1.62	2.93	2.50	2.81	2.17	3.69	0.23	3.31	6.04	3.46	31.4	1.4
	lw	34.6	21.3	38.6	33.0	37.0	28.6	48.6	3.0	43.6	79.6	45.6	413.7	19
NAR12	dw	8.00	3.10	9.20	5.70	6.60	6.40	13.3	n.d	9.50	1.50	2.60	65.9	4.0
	ww	1.92	0.74	2.2	1.37	1.58	1.53	3.19	n.d	2.28	0.36	0.62	15.8	0.9
	lw	20.3	7.88	23.4	14.5	16.8	16.3	33.8	n.d	24.2	3.81	6.61	167.5	10
NAR13	dw	21.7	1.80	11.1	24.9	14.4	7.20	41.5	n.d	9.10	7.60	5.50	144.8	12
	ww	5.76	0.48	2.94	6.61	3.82	1.91	11.0	n.d	2.41	2.02	1.46	38.4	3.2
	lw	65.2	5.41	33.4	74.8	43.3	21.6	124.7	n.d	27.3	22.8	16.5	435.1	36
NAR14	dw	21.5	3.10	9.90	20.2	15.4	8.50	46.0	n.d	13.2	36.7	22.9	197.4	14
	ww	5.23	0.75	2.41	4.92	3.75	2.07	11.2	n.d	3.21	8.93	5.57	48.0	3.4
	lw	71.1	10.3	32.7	66.8	50.9	28.1	152.1	n.d	43.7	121.4	75.7	652.8	46
NAR15	dw	22.3	3.10	12.7	25.5	15.0	9.40	30.8	n.d	12.8	16.1	17.5	165.2	9.1
	ww	4.84	0.67	2.76	5.53	3.26	2.04	6.68	n.d	2.78	3.49	3.80	35.9	2.0
	lw	66.9	9.30	38.1	76.5	45.0	28.2	92.4	n.d	38.4	48.3	52.5	495.8	27
NAR16	dw	15.7	n.d	9.10	18.3	8.10	4.60	16.9	n.d	8.50	43.3	45.3	169.8	16
	ww	3.45	n.d	2.00	4.02	1.78	1.01	3.71	n.d	1.87	9.52	9.96	37.3	3.4
	lw	35.1	n.d	20.3	40.9	18.1	10.3	37.8	n.d	19.0	96.7	101.2	379.3	35
NAR17	dw	8.1	2.5	11.2	6.7	11.1	6.6	10	1.4	13.4	19.9	29.2	120.1	7.9
	ww	1.83	0.57	2.53	1.52	2.51	1.49	2.26	0.32	3.03	4.5	6.61	27.2	1.8

Sample ID		Atrazine	Hexazinone	Simazine	Terbuthylazine	Acetochlor	Alachlor	Metolachlor	Trifluralin	EPTC	MCPA	2,4-D	TOTAL	±SD
	lw	23.6	7.28	32.6	19.5	32.3	19.2	29.1	4.08	39.0	58.0	85.0	349.8	23
NAR18	dw	5.40	1.70	5.60	3.30	7.60	4.30	9.90	n.d	10.5	28.6	39.3	116.2	12
	ww	1.41	0.44	1.46	0.86	1.98	1.12	2.58	n.d	2.74	7.46	10.3	30.3	3.2
	lw	19.7	6.22	20.5	12.1	27.8	15.7	36.2	n.d	38.4	104.6	143.7	424.9	45
NAR19	dw	15.9	8.00	18.5	15.5	18.2	15.5	25.8	n.d	18.5	13.9	18.1	167.9	6.6
	ww	4.24	2.13	4.93	4.13	4.85	4.13	6.88	n.d	4.93	3.71	4.83	44.8	1.8
	lw	57.3	28.8	66.7	55.8	65.6	55.8	93.0	n.d	66.7	50.1	65.2	604.9	24
NAR20	dw	15.6	12.4	18.9	14.2	21.2	16.0	27.6	1.40	19.2	11.1	1.80	159.4	7.8
	ww	2.89	2.30	3.50	2.63	3.92	2.96	5.11	0.26	3.55	2.05	0.33	29.5	1.4
	lw	48.4	38.5	58.6	44.0	65.7	49.6	85.6	4.34	59.5	34.4	5.58	494.2	24
NAR21	dw	15.0	2.60	17.0	14.1	23.6	15.3	28.8	n.d	18.2	2.30	41.8	178.7	12
	ww	3.53	0.61	4.01	3.32	5.56	3.60	6.79	n.d	4.29	0.54	9.85	42.1	2.9
	lw	37.2	6.45	42.2	35.0	58.5	37.9	71.4	n.d	45.1	5.7	103.7	443.2	31
NAR22	dw	18.2	n.d	19.8	17.4	22.1	15.6	36.2	0.90	18.1	13.4	42.9	204.6	13
	ww	4.76	n.d	5.18	4.55	5.78	4.08	9.46	0.24	4.73	3.5	11.2	53.5	3.3
	lw	73.2	n.d	79.6	70.0	88.9	62.7	145.6	3.62	72.8	53.9	172.5	822.8	51
NAR23	dw	11.1	1.40	10.5	7.40	6.20	6.90	8.60	2.60	12.1	7.90	9.40	84.1	3.3
	ww	2.53	0.32	2.40	1.69	1.42	1.58	1.96	0.59	2.76	1.80	2.15	19.2	0.8
	lw	33.1	4.18	31.3	22.1	18.5	20.6	25.7	7.76	36.1	23.6	28.1	251.0	9.9
NAR24	dw	12.5	1.5	14.5	15.3	20.8	12.8	18.6	n.d	12.4	n.d	23.0	131.4	8.1
	ww	3.35	0.40	3.88	4.10	5.57	3.43	4.98	n.d	3.32	n.d	6.16	35.2	2.2
	lw	40.1	4.82	46.6	49.1	66.8	41.1	59.7	n.d	39.8	n.d	73.9	421.9	26
NAR25	dw	14.6	n.d	9.6	17	12.4	12.7	13.6	3.00	19.2	11.1	10.0	123.2	5.6
	ww	4.09	n.d	2.69	4.76	3.47	3.56	3.81	0.84	5.38	3.11	2.8	34.5	1.6
	lw	44.0	n.d	28.9	51.2	37.4	38.3	41.0	9.04	57.9	33.5	30.1	371.3	17
NAR26	dw	12.2	n.d	10.1	11.1	13.4	12.7	13.8	4.40	19.0	38.8	42.8	178.3	13
	ww	2.78	n.d	2.30	2.53	3.06	2.90	3.15	1.00	4.33	8.85	9.76	40.7	3.0

Sample ID		Atrazine	Hexazinone	Simazine	Terbuthylazine	Acetochlor	Alachlor	Metolachlor	Trifluralin	EPTC	MCPA	2,4-D	TOTAL	±SD
	lw	32.8	n.d	27.2	29.9	36.1	34.2	37.2	11.8	51.2	104.4	115.2	480.0	35
NAR27	dw	23.6	9.20	23.9	24.6	23.4	17.2	36.3	6.20	25.2	16.8	n.d	206.4	10
	ww	4.62	1.80	4.68	4.81	4.58	3.37	7.10	1.21	4.93	3.29	n.d	40.4	2.0
	lw	53.1	20.7	53.8	55.3	52.6	38.7	81.7	14.0	56.7	37.8	n.d	464.3	23
NAR28	dw	18.3	1.50	15.6	18.9	17.9	14.9	31.2	3.70	13.7	8.40	11.2	155.3	8.1
	ww	4.68	0.38	3.99	4.84	4.58	3.81	7.99	0.95	3.51	2.15	2.87	39.8	2.1
	lw	59.9	4.91	51.1	61.9	58.6	48.8	102.1	12.1	44.9	27.5	36.7	508.4	27
NAR29	dw	16.9	n.d	20.0	19.4	25.5	14.7	n.d	2.30	20.6	45.3	33.1	197.8	14
	ww	3.79	n.d	4.48	4.35	5.71	3.29	n.d	0.52	4.62	10.15	7.42	44.3	3.1
	lw	49.7	n.d	58.8	57.1	75.0	43.2	n.d	6.76	60.6	133.2	97.4	581.7	41
NAR30	dw	19.5	8.20	16.5	23.6	23.6	17.8	36.9	4.00	23.9	25.1	39.1	238.2	10
	ww	4.34	1.83	3.67	5.25	5.25	3.96	8.21	0.89	5.32	5.59	8.70	53.0	2.3
	lw	46.5	19.6	39.4	56.3	56.3	42.5	88.0	9.54	57.0	59.9	93.3	568.3	25

n.d. : not detected

Supplementary Table TS 3.3 Estimated daily intake (EDI) (ng kg^{-1} bw d^{-1}), hazard quotient (HQ), hazard ratio (HR) and lifetime cancer risk (LCR) calculated for 50th percentile and 95th percentile (in parentheses) concentrations (ng g^{-1} wet weight). Estimate ranges are provided based on likely maximum (MAX) and minimum (MIN) fish consumption rates for communities near Lake St Lucia. Acceptable daily intake (ADI) values from AERU Pesticide Properties Database (Lewis et al., 2020) and the Australian Pesticides Veterinary Medicines Authority (Australian Pesticides and Veterinary Medicines Authority, 2020). LC50 values in fish based on 96 hr exposure in rainbow trout and sheepshead minnow from US EPA pesticide ecotoxicity database (USEPA 2021). Values in bold indicate risk.

		Acetochlor	**Alachlor**	**Metolachlor**	**Trifluralin**	**Atrazine**	**Hexazinone**	**Simazine**	**Terbuthylazine**	**MCPA**	**2,4-D**	**EPTC**
ADI (ng kg^{-1} d^{-1})		3600	10000	100000	15000	20000	50000	5000	4000	50000	50000	9000
Cancer Slope Factor (CSF)		0.0327	0.056	0.0092	0.0077	0.23	–	0.092	–	–	–	–
LC50 (ppb)		380 – 1200[a]	1400 – 2800[a]	3900[a]	22[a]	5300[a]	<420000[a]	>10000[a]	–	91000[a]	143000[b]	19960[a]
Clarias gariepinus												
EDI	MAX	7.8 (14)	5.0 (10)	8.3 (24.1)	– (2.3)	6.8 (12)	1.0 (5.3)	6.3 (12)	6.5 (13)	10.0 (24)	13.5 (28)	7.0 (12)
	MIN	1.6 (2.8)	1.0 (2.0)	1.7 (4.8)	– (0.5)	1.4 (2.4)	0.2 (1.1)	1.3 (2.4)	1.3 (2.7)	2.0 (4.8)	2.7 (5.7)	1.4 (2.5)
HQ (10^{-4})	MAX	21.9 (39.0)	5.0 (10.0)	0.8 (2.4)	– (1.5)	3.4 (6.0)	0.2 (1.1)	13 (24)	16.3 (33)	2.0 (4.8)	2.7 (5.7)	7.8 (14)
	MIN	4.3 (7.8)	1.0 (2.0)	0.2 (0.5)	– (0.3)	0.7 (1.2)	<0.01 (0.2)	2.5 (4.7)	3.3 (6.6)	0.4 (1.0)	0.5 (1.1)	1.6 (2.7)
HR	MAX	0.6 (**1.2**)	0.7 (**1.4**)	0.2 (0.6)	– (0.04)	**3.9** (**6.9**)	–	**1.4** (**2.7**)	–	–	–	–
	MIN	0.03 (0.05)	0.03 (0.06)	0.01 (0.02)	– (<0.01)	0.16 (0.28)	–	0.06 (0.11)	–	–	–	–
LCR (10^{-6})	MAX	0.25 (0.46)	0.28 (0.56)	0.08 (0.22)	– (0.02)	**1.6** (**2.8**)	–	0.58 (**1.1**)	–	–	–	–
	MIN	0.05 (0.09)	0.06 (0.11)	0.02 (0.04)	– (<0.01)	0.31 (0.55)	–	0.12 (0.22)	–	–	–	–
Oreochromis mossambicus												
EDI	MAX	6.5 (19.6)	3.8 (7.0)	4.5 (14.8)	1.0 (2.5)	4.5 (17.1)	2.0 (4.3)	4.5 (10.8)	5.0 (13.5)	4.0 (24.3)	7.5 (14.0)	5.8 (13.0)
	MIN	1.3 (3.9)	0.8 (1.4)	0.9 (3.0)	0.2 (0.5)	0.9 (3.4)	0.4 (0.9)	0.9 (2.2)	1.0 (2.7)	0.8 (4.9)	1.5 (2.8)	1.2 (2.6)
HQ (10^{-4})	MAX	18.1 (54.3)	3.8 (7.0)	0.5 (1.5)	0.7 (1.7)	2.3 (8.5)	0.4 (0.9)	9.0 (21.6)	12.5 (33.9)	0.8 (4.9)	1.5 (2.8)	6.4 (15)
	MIN	3.6 (10.8)	0.8 (1.4)	0.1 (0.3)	0.1 (0.3)	0.5 (1.7)	0.1 (0.2)	1.8 (4.3)	2.5 (6.8)	0.2 (1.0)	0.3 (0.6)	1.3 (2.9)
HR	MAX	0.5 (**1.6**)	0.5 (**1.0**)	0.1 (0.3)	0.02 (0.05)	**2.6** (**9.8**)	–	**1.0** (**2.5**)	–	–	–	–
	MIN	0.02 (0.06)	0.02 (0.04)	<0.01 (0.01)	<0.01 (<0.01)	0.1 (0.39)	–	0.04 (0.1)	–	–	–	–

		Acetochlor	Alachlor	Metolachlor	Trifluralin	Atrazine	Hexazinone	Simazine	Terbuthylazine	MCPA	2,4-D	EPTC
LCR (10^{-6})	MAX	0.21 (0.64)	0.21 (0.39)	0.04 (0.14)	0.01 (0.02)	**1.0** (**3.9**)	–	0.42 (**1.0**)	–	–	–	–
	MIN	0.04 (0.13)	0.04 (0.08)	0.01 (0.03)	<0.01 (<0.01)	0.21 (0.78)	–	0.08 (0.20)	–	–	–	–

[a:] LC50 of herbicides for rainbow trout

[b:] LC50 of 2,4-D (as dimethylamine salt) for sheepshead minnow

Supplementary Table TS4.1: Percentage spiked recoveries (n = 3) and % Relative Standard Deviation (RSD) at three levels (ng g^{-1}). LOD limit of detection, LOQ =limit of quantification (ng g^{-1}). Data linked to Chapter 4

	SPIKING LEVEL (ppb)	ACR	%RSD	SIN	%RSD	THE	%RSD	LOD	LOQ
EPTC	50	84.7	2.5	74.8	2.1	82.3	3	0.16	0.55
	10	76.8	4.9	94.6	1.1	83	7.2		
	5	85.2	15.2	87.9	8.3	84.1	3.4		
Trifluralin								0.24	0.82
	50	88.0	9.1	102.6	5.1	87.7	6.7		
	10	78.6	1.5	104.4	6.6	94.5	5.8		
	5	74.0	9.5	70.5	14.3	85	4		
Simazine								0.55	1.88
	50	75.7	10.3	79.1	4.3	114.5	4.3		
	10	76.7	5.1	86.4	6.8	93.1	7.4		
	5	72.2	3.1	72.7	9.1	95.5	2.9		
Atrazine								0.26	0.91
	50	76.6	3	72.5	2.2	81.7	0.6		
	10	72.3	4.5	76.6	4.6	70.5	3.9		
	5	70.6	5.7	67.3	6.6	63.8	1.9		
Terbuthylazine								0.60	1.99
	50	84.0	2.3	74.7	2.1	94.1	4.6		
	10	92.1	1.8	79.8	6.9	79.3	3.8		
	5	83.0	6	88.3	6.6	83.8	10.2		
Acetochlor								0.41	1.45
	50	81.4	3.5	82.5	6.3	84.8	1.3		
	10	74.2	5.2	72.7	3.3	88.6	3.4		
	5	72.1	3.6	68.3	2.3	89	4		
Alachlor								0.56	1.80
	50	73.7	5.7	83.5	4.5	97.4	10.6		
	10	80.1	4.7	96.5	5.9	93.7	5.5		
	5	76.1	9.9	74.3	13.4	70.9	3.2		
Metolachlor								0.27	0.91
	50	95.0	6.9	82.0	3.3	104.7	0.4		
	10	83.3	10.5	84.6	2.2	88.9	6.7		
	5	72.2	6.9	75.1	6.7	65.4	2.9		
Hexazinone								0.10	0.35
	50	91.1	4.9	75.1	5.6	89.3	3.5		
	10	76.3	2	86.1	8.3	88	0.6		
	5	72.6	6.4	68.2	5	88.4	1.2		

ACR: *Acropora austera* (Hard coral)
SIN: *Sinularia gravis* (Soft coral)
THE: *Theonella swinhoei* (Sponge).

Supplementary Table TS4.2: Average (n = 3), (Range) herbicide concentrations (ng g^{-1}) dry weight in Reef organisms from Maputaland. Data linked to Chapter 4

Site	*Species*	Atrazine	Hexazinone	Simazine	Acetochlor	Alachlor
Kosi North	*S. gravis*	n.d.	14.8 (n.d. – 22.9)	n.d.	22.6 (14.5 – 38.3)	5.1 (n.d. – 9.3)
	S. glaucum	n.d.	5.3 (n.d. – 9.6)	n.d.	29.6 (7.3 – 57.2)	n.d.
	T. swinhoei	5.3 (n.d. – 9.9)	12.4 (3.0 – 16.3)	22.2 (n.d. – 45.1)	59.7 (5.1 – 105.0)	13.0 (9.1 – 15.8)
	A. austera	4.3 (n.d. – 12.8)	0.9 (n.d. – 2.6)	n.d.	2.4 (n.d. – 5.5)	3.8 (n.d. – 6.3)
	E. hirsutissima	0.8 (n.d. – 2.3)	1.0 (n.d. – 2.9)	n.d.	14.3 (9.7 – 22.9)	1.0 (n.d. – 3.0)
Kosi South	*S. gravis*	1.0 (n.d. – 3.1)	3.2 (n.d. – 9.6)	4.7(n.d. – 9.2)	16.0 (6.2 – 30.8)	5.7 (3.7 – 5.7)
	S. glaucum	n.d.	41.7 (n.d. – 89.0)	n.d.	41.8 (18.8 – 57.6)	3.1 (n.d. – 4.9)
	T. swinhoei	5.4 (2.4 – 9.0)	14.1 (9.0 – 18.9)	10.1 (n.d. – 19.9)	22.8 (n.d. – 43.3)	7.0 (3.2 – 9.7)
	A. austera	0.9 (n.d. – 1.6)	1.2 (n.d. – 3.7)	1.3 (n.d. – 3.8)	0.9 (n.d. – 2.6)	n.d.
	E. hirsutissima	n.d.	4.4 (2.7 – 5.6)	n.d.	2.2 (n.d. – 6.5)	4.1 (n.d. – 12.2)
Rabbit Reef	*S. gravis*	1.4 (n.d. – 3.3)	4.2 (n.d. – 12.5)	2.5 (n.d. – 4.1)	11.8 (10.2 – 13.3)	4.8 (n.d. – 9.4)
	S. glaucum	0.6 (n.d. – 1.7)	29.9 (10.3 – 50.6)	n.d.	39.5 (18.1 – 54.1)	7.6 (6.3 – 9.5)
	T. swinhoei	7.0 (1.7 – 10.7)	11.3 (7.8 – 15.2)	9.5 (n.d. – 16.5)	32.4 (21.0 – 44.3)	8.4 (7.8 – 9.5)
	A. austera	1.7 (n.d. – 2.6)	n.d.	0.7 (n.d. – 2.2)	6.3 (2.3 – 12.6)	1.5 (n.d. – 2.4)
	E. hirsutissima	0.2 (n.d. – 0.7)	1.6 (n.d. – 4.8)	0.8 (n.d. – 2.3)	4.9 (2.0 – 7.1)	1.1 (n.d. – 2.3)
Regal	*S. gravis*	n.d.	21.7 (19.7 – 25.5)	n.d.	1.5 (0.8 – 2.6)	2.8 (2.7 – 2.8)
	S. glaucum	n.d.	1.8 (n.d. – 4.9)	n.d.	7.7 (n.d. – 16.5)	65.6 (38.8 – 106.0)
	T. swinhoei	n.d.	23.3 (20.9 – 26.5)	n.d.	n.d.	10.1 (n.d. – 23.8)
	A. austera					
	E. hirsutissima	n.d.	3.9 (n.d. – 7.5)	n.d.	1.7 (n.d. – 3.9	n.d.

Site	*Species*	Atrazine	Hexazinone	Simazine	Acetochlor	Alachlor
Nine Mile	*S. gravis*	n.d.	9.3 (7.8 – 10.6)	n.d.	25.0 (19.8 – 30.3)	9.6 (6.2 – 15.1)
	S. glaucum	n.d.	24.8 (14.9 – 43.4)	n.d.	18.3 (2.5 – 31.6)	48.2 (48.0 – 48.3)
	T. swinhoei	n.d.	16.2 (11.9 – 21.0)	n.d.	n.d.	11.1 (n.d. – 33.3)
	A. austera	n.d.	n.d.	n.d.	n.d.	n.d.
	E. hirsutissima	n.d.	1.4 (n.d. – 2.7)	n.d.	5.1 (n.d. – 8.0)	n.d.
Two-Mile	*S. gravis*	n.d.	16.7 (12.1 – 21.7)	n.d.	37.1 (26.4 – 44.6)	9.3 (7.3 – 12.1)
	S. glaucum	n.d.	7.9 (n.d. – 14.2)	n.d.	42.9 (10.9 – 69.6)	21.5 (3.7 – 32.7)
	T. swinhoei	n.d.	4.9 (1.0 – 9.1)	n.d.	2.0 (n.d. – 6.0)	0.9 (0.8 – 1.0)
	A. austera	n.d.	8.7 (2.7 – 13.4)	n.d.	n.d.	n.d.
	E. hirsutissima	n.d.	4.5 (1.9 – 9.2)	n.d.	5.3 (4.4 – 7.0)	5.5 (n.d. – 12.8)
Red Sands	*S. gravis*	n.d.	30.5 (23.2 – 40.8)	n.d.	23.7 (17.9 – 33.0)	43.5 (28.6 – 57.8)
	S. glaucum	n.d.	1.3 (n.d. – 3.6)	n.d.	17.1 (n.d. – 28.4)	36.3 (32.5 – 43.0)
	T. swinhoei	n.d.	5.6 (n.d. – 10.1)	n.d.	2.4 (0.7 – 4.0)	0.9 (n.d. – 1.8)
	A. austera	n.d.	0.4 (n.d. – 1.2)	n.d.	n.d.	n.d.
	E. hirsutissima	n.d.	8.0 (3.0 – 12.2)	n.d.	n.d.	n.d.
New Red Sands	*S. gravis*	n.d.	24.8 (2.7 – 39.1)	n.d.	9.4 (n.d. – 14.8)	4.9 (4.0 – 5.7)
	S. glaucum	n.d.	5.8 (n.d. – 9.3)	n.d.	6.0 (n.d. – 14.6)	57.0 (25.0 76.4)
	T. swinhoei	n.d.	4.1 (n.d. – 8.5)	n.d.	n.d.	1.7 (n.d. – 5.1)
	A. austera	n.d.	9.8 (2.7 – 16.2)	n.d.	n.d.	n.d.
	E. hirsutissima	n.d.	1.5 (n.d. – 4.5)	n.d.	n.d.	0.9 (n.d. – 2.8)
Leadsman	*S. gravis*	n.d.	21.5 (15.2 – 28.3)	n.d.	11.8 (6.6 – 21.8)	10.3 (8.3 – 12.1)
	S. glaucum	2.0 (0.8 – 4.0)	28.0 (13.1 – 46.0)	n.d.	6.9 (5.3 – 8.0)	159.6 (158.7 – 160.4)

Site	*Species*	Atrazine	Hexazinone	Simazine	Acetochlor	Alachlor
	T. swinhoei	20.6 (8.5 – 43.3)	19.4 (17.8 – 22.0)	n.d.	10.5 (n.d. – 22.8)	5.5 (n.d. – 13.7)
	A. austera	n.d.	3.8 (n.d. – 6.9)	n.d.	0.9 (n.d. – 2.7)	n.d.
	E. hirsutissima	n.d.	7.4 (3.1 – 11.2)	n.d.	1.5 (n.d. – 2.5)	n.d.
New Leadsman	*S. gravis*	n.d.	7.1 (1.5 – 10.1)	n.d.	n.d.	8.4 (5.7 – 10.1)
	S. glaucum	n.d.	24.2 (n.d. – 37.9)	n.d.	22.5 (15.6 – 28.0)	153.1 (101.3 – 195.0)
	T. swinhoei	n.d.	6.0 (n.d. – 10.0)	n.d.	1.0 (n.d. – 2.0)	3.5 (n.d. – 5.3)
	A. austera	n.d.	0.7 (n.d. – 2.2)	n.d.	0.5 (n.d. – 1.6)	1.4 (n.d. – 4.2)
	E. hirsutissima	n.d.	3.6 (1.4 – 6.7)	n.d.	11.5 (n.d. – 19.6)	6.6 (1.1 – 14.6)

n.d. : not detected

Table TS 4.3

***Post hoc* pair-wise tests for Reef Complex crossed with Species for total herbicide concentration data.**

Term 'Reef Complex x Species' for pairs of levels of factor 'Reef Complex'

Within level '*Sinularia gravis*' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	0.85095	0.4167	9832	0.4035
Southern, Northern	2.6344	0.0184	9819	**0.0226**
Central, Northern	1.8632	0.0915	9861	0.0885

Within level '*Sarcophyton glaucum*' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	1.7023	0.1103	9848	0.1102
Southern, Northern	2.3157	0.0333	9837	**0.0336**
Central, Northern	0.69939	0.4931	9839	0.4998

Within level '*Theonella swinhoei*' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	0.44828	0.6644	9834	0.6678
Southern, Northern	4.7544	0.0008	9823	**0.0006**
Central, Northern	3.8434	0.0026	9838	**0.0028**

Within level '*Echinopora hirsutissima*' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	0.33034	0.749	9846	0.7545
Southern, Northern	0.48796	0.6424	9837	0.6303
Central, Northern	1.0973	0.2965	9838	0.2956

Within level '*Acropora austera*' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	0.94995	0.3661	9822	0.3506
Southern, Northern	2.4538	0.0243	9853	**0.0292**
Central, Northern	3.3737	0.0039	9782	**0.0061**

Term 'Complex x Species' for pairs of levels of factor 'Species'

Within level 'Southern' of factor 'Complex'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	4.1217	0.0017	9815	**0.001**
Sinularia, Theonella	6.0079	0.0001	9855	**0.0001**
Sinularia, Echinopora	8.24	0.0001	9815	**0.0001**
Sinularia, Acropora	11.2	0.0001	9836	**0.0001**
Sarcophyton, Theonella	5.9366	0.0001	9824	**0.0001**
Sarcophyton, Echinopora	6.5649	0.0002	9828	**0.0001**
Sarcophyton, Acropora	7.0117	0.0001	9804	**0.0001**
Theonella, Echinopora	2.5538	0.0203	9838	**0.0197**
Theonella, Acropora	5.225	0.0005	9824	**0.0003**
Echinopora, Acropora	2.0014	0.0608	9847	0.0641

Within level 'Central' of factor 'Complex'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	2.5295	0.028	9840	**0.0277**
Sinularia, Theonella	3.3996	0.0073	9834	**0.0047**

Sinularia, Echinopora	8.7289	0.0003	9829	**0.0001**
Sinularia, Acropora	10.658	0.0001	9823	**0.0001**
Sarcophyton, Theonella	3.9521	0.0033	9858	**0.0029**
Sarcophyton, Echinopora	5.2129	0.0008	9813	**0.0003**
Sarcophyton, Acropora	5.694	0.0003	9831	**0.0003**
Theonella, Echinopora	2.5697	0.0176	9856	**0.0257**
Theonella, Acropora	3.8247	0.0016	9853	**0.0023**
Echinopora, Acropora	3.4289	0.0053	9839	**0.006**

Within level 'Northern' of factor 'Complex'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	2.3805	0.0302	9826	**0.0356**
Sinularia, Theonella	3.1909	0.0059	9851	**0.0073**
Sinularia, Echinopora	3.733	0.0028	9828	**0.0027**
Sinularia, Acropora	4.6028	0.0008	9838	**0.0009**
Sarcophyton, Theonella	0.71626	0.493	9855	0.49
Sarcophyton, Echinopora	4.0414	0.0015	9840	**0.0014**
Sarcophyton, Acropora	4.3361	0.0011	9854	**0.0009**
Theonella, Echinopora	4.7955	0.0008	9843	**0.0003**
Theonella, Acropora	5.078	0.0009	9859	**0.0003**
Echinopora, Acropora	1.3434	0.2019	9838	0.1997

Table TS 4.4
***Post hoc* pair-wise comparisons for Site nested in Reef Complex crossed with Species for total herbicide concentration data.**

Term 'Site(Reef Complex) x Species' for pairs of levels of factor 'Species'

Within level 'Southern' of factor 'Reef Complex'
Within level 'Leadsman north' of factor 'Site'

Groups		t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton		2.2175	0.0983	10	0.0897
Sinularia, Theonella		1.2764	0.2991	10	0.2668
Sinularia, Echinopora		7.3124	0.0989	10	**0.0013**
Sinularia, Acropora		9.9763	0.1034	10	**0.0006**
Sarcophyton, Theonella		1.908	0.2995	10	0.13
Sarcophyton, Echinopora		2.9964	0.102	10	**0.0412**
Sarcophyton, Acropora		3.0945	0.0996	10	**0.0359**
Theonella, Echinopora		4.9671	0.102	10	**0.0098**
Theonella, Acropora		5.6383	0.0998	10	**0.0056**
Echinopora, Acropora	1.2843	0.2984	10	0.2738	

Within level 'Southern' of factor 'Reef Complex'
Within level 'Leadsman south' of factor 'Site'

Groups		t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton		4.2817	0.0959	10	**0.0124**
Sinularia, Theonella		0.88078	0.5007	10	0.43
Sinularia, Echinopora		0.60288	0.6102	10	0.5876
Sinularia, Acropora		4.0073	0.1012	10	**0.0148**
Sarcophyton, Theonella		4.3768	0.0996	10	**0.013**
Sarcophyton, Echinopora		4.0352	0.1003	10	**0.0166**
Sarcophyton, Acropora		4.5849	0.0985	10	**0.0104**
Theonella, Echinopora		1.003	0.4005	10	0.3673
Theonella, Acropora		1.5132	0.3028	10	0.199
Echinopora, Acropora	1.8731	0.1914	10	0.1265	

Within level 'Southern' of factor 'Reef Complex'
Within level 'Red Sands south' of factor 'Site'

Groups		t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton		2.8069	0.0989	10	**0.0498**
Sinularia, Theonella		8.0738	0.0917	10	**0.0013**
Sinularia, Echinopora		8.1427	0.1009	10	**0.0011**
Sinularia, Acropora		9.1089	0.0999	7	**0.001**
Sarcophyton, Theonella		4.0658	0.1002	10	**0.0142**
Sarcophyton, Echinopora		4.1345	0.1042	10	**0.0141**
Sarcophyton, Acropora		4.9517	0.1014	7	**0.0077**
Theonella, Echinopora		0.20801	0.7039	10	0.8534
Theonella, Acropora		3.109	0.1014	7	**0.0383**
Echinopora, Acropora	2.8001	0.1004	7	0.0524	

Within level 'Southern' of factor 'Reef Complex'
Within level 'Red Sands north' of factor 'Site'

Groups		t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton		2.0418	0.2008	10	0.1145
Sinularia, Theonella		3.094	0.103	10	**0.0343**
Sinularia, Echinopora		3.6512	0.0956	10	**0.019**
Sinularia, Acropora		2.7446	0.0996	10	0.0504
Sarcophyton, Theonella		5.5069	0.1035	10	**0.0055**
Sarcophyton, Echinopora		6.1589	0.103	10	**0.0041**
Sarcophyton, Acropora		5.1899	0.096	10	**0.008**

Theonella, Echinopora		0.79871	0.6957	7	0.4708
Theonella, Acropora		0.69577	0.6	10	0.5192
Echinopora, Acropora	1.7785	0.3035	10	0.158	

Within level 'Central' of factor 'Reef Complex'
Within level 'Two-mile' of factor 'Site'

Groups		t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton		0.33572	0.7978	10	0.7568
Sinularia, Theonella		5.5644	0.0994	10	**0.0056**
Sinularia, Echinopora		4.7481	0.0999	10	**0.01**
Sinularia, Acropora		5.446	0.0994	10	**0.0055**
Sarcophyton, Theonella		2.4986	0.102	10	0.0672
Sarcophyton, Echinopora		2.2014	0.0993	10	0.0927
Sarcophyton, Acropora		2.4626	0.1002	10	0.0716
Theonella, Echinopora		1.6935	0.299	10	0.1674
Theonella, Acropora		0.20173	0.8091	10	0.8471
Echinopora, Acropora	1.4581	0.3084	10	0.2185	

Within level 'Central' of factor 'Reef Complex'
Within level 'Nine-mile' of factor 'Site'

Groups		t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton		2.7065	0.1018	10	**0.0492**
Sinularia, Theonella		1.1342	0.3026	10	0.3248
Sinularia, Echinopora		6.4153	0.1018	10	**0.0025**
Sinularia, Acropora		7.9892	0.0987	4	**0.0015**
Sarcophyton, Theonella		2.9825	0.0964	10	**0.0422**
Sarcophyton, Echinopora		5.0634	0.0988	10	**0.008**
Sarcophyton, Acropora		5.4892	0.0951	4	**0.0063**
Theonella, Echinopora		1.518	0.1079	10	0.2018
Theonella, Acropora		2.0137	0.097	4	0.1141
Echinopora, Acropora	3.3525	0.0965	4	**0.0268**	

Within level 'Central' of factor 'Reef Complex'
Within level 'Regal' of factor 'Site'

Groups		t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton		1.8668	0.0997	10	0.1375
Sinularia, Theonella		1.0735	0.4003	10	0.344
Sinularia, Echinopora		6.1423	0.0994	10	**0.0037**
Sinularia, Acropora		10.726	0.1002	4	**0.0005**
Sarcophyton, Theonella		1.5471	0.0983	10	0.1967
Sarcophyton, Echinopora		2.6414	0.1032	10	0.0559
Sarcophyton, Acropora		2.8639	0.0996	4	**0.0432**
Theonella, Echinopora		4.0631	0.1032	10	**0.0145**
Theonella, Acropora		5.1732	0.0957	4	**0.0083**
Echinopora, Acropora	2.4601	0.1	4	0.0755	

Within level 'Northern' of factor 'Reef Complex'
Within level 'Rabbit' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	6.562	0.1035	10	**0.0026**
Sinularia, Theonella	3.9725	0.0979	10	**0.0163**
Sinularia, Echinopora	2.0233	0.2013	10	0.1122
Sinularia, Acropora	1.679	0.2063	10	0.1665
Sarcophyton, Theonella	1.0813	0.3997	10	0.3362
Sarcophyton, Echinopora	24.738	0.0979	10	**0.0001**
Sarcophyton, Acropora	15.943	0.0985	10	**0.0002**
Theonella, Echinopora	7.4217	0.1003	10	**0.0023**

Theonella, Acropora		6.7147	0.1013	10	**0.0029**
Echinopora, Acropora	0.42867	0.7958	10	0.6898	

Within level 'Northern' of factor 'Reef Complex'
Within level 'Kosi south' of factor 'Site'

Groups		t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton		1.5034	0.3022	10	0.2051
Sinularia, Theonella		1.9278	0.2996	10	0.1285
Sinularia, Echinopora		3.2226	0.0944	10	**0.0327**
Sinularia, Acropora		5.9654	0.0996	10	**0.0054**
Sarcophyton, Theonella		0.69023	0.4909	10	0.5238
Sarcophyton, Echinopora		2.0405	0.1042	10	0.1129
Sarcophyton, Acropora		2.228	0.0983	10	0.0923
Theonella, Echinopora		3.2763	0.1005	10	**0.0312**
Theonella, Acropora		3.8783	0.1013	10	**0.0161**
Echinopora, Acropora	1.4671	0.3999	10	0.2158	

Within level 'Northern' of factor 'Reef Complex'
Within level 'Kosi north' of factor 'Site'

Groups		t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton		0.4042	0.5026	10	0.7033
Sinularia, Theonella		1.7209	0.1885	10	0.1581
Sinularia, Echinopora		1.9543	0.2026	10	0.1213
Sinularia, Acropora		2.5336	0.1009	10	0.0655
Sarcophyton, Theonella		1.8754	0.2041	10	0.1361
Sarcophyton, Echinopora		1.181	0.2989	10	0.3081
Sarcophyton, Acropora		1.6241	0.1022	10	0.1802
Theonella, Echinopora		2.4439	0.1	10	0.0717
Theonella, Acropora		2.6053	0.1012	10	0.0614
Echinopora, Acropora	1.1483	0.3945	10	0.3157	

Term 'Site(Reef Complex) x Species' for pairs of levels of factor 'Site'

Within level 'Southern' of factor 'Reef Complex'
Within level 'Sinularia' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	6.1286	0.0999	10	**0.0034**
Leadsman north, Red Sands south	4.7812	0.1013	10	**0.0075**
Leadsman north, Red Sands north	0.43196	0.7025	10	0.6845
Leadsman south, Red Sands south	7.4634	0.1064	10	**0.0018**
Leadsman south, Red Sands north	2.2866	0.0998	10	0.0761
Red Sands south, Red Sands north	4.0167	0.0999	10	**0.0165**

Within level 'Southern' of factor 'Reef Complex'
Within level 'Sarcophyton' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	0.91235	0.3992	10	0.4223
Leadsman north, Red Sands south	1.9211	0.2957	10	0.1284
Leadsman north, Red Sands north	1.615	0.2969	10	0.1845
Leadsman south, Red Sands south	3.2726	0.1019	10	**0.033**
Leadsman south, Red Sands north	2.9565	0.0962	10	**0.0399**
Red Sands south, Red Sands north	0.92626	0.4984	10	0.4072

Within level 'Southern' of factor 'Reef Complex'
Within level 'Theonella' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	4.4292	0.0968	10	**0.0114**
Leadsman north, Red Sands south	5.0152	0.1002	10	**0.0071**
Leadsman north, Red Sands north	5.0764	0.0934	10	**0.008**
Leadsman south, Red Sands south	0.30512	0.7057	10	0.7775
Leadsman south, Red Sands north	0.73891	0.51	10	0.5034
Red Sands south, Red Sands north	0.61721	0.6986	10	0.5678

Within level 'Southern' of factor 'Reef Complex'
Within level 'Echinopora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	1.2333	0.3916	10	0.2905
Leadsman north, Red Sands south	0.19663	0.6986	10	0.8565
Leadsman north, Red Sands north	1.9615	0.1959	10	0.1197
Leadsman south, Red Sands south	1.3187	0.3968	10	0.259
Leadsman south, Red Sands north	1.9073	0.3111	10	0.1268
Red Sands south, Red Sands north	1.8688	0.2059	10	0.1372

Within level 'Southern' of factor 'Reef Complex'
Within level 'Acropora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	0.96275	0.4058	10	0.3844
Leadsman north, Red Sands south	3.3136	0.0993	7	**0.03**
Leadsman north, Red Sands north	1.2385	0.3996	10	0.2885
Leadsman south, Red Sands south	1.3064	0.3986	4	0.2564
Leadsman south, Red Sands north	1.6648	0.2031	10	0.1677
Red Sands south, Red Sands north	2.3856	0.1011	7	0.0754

Within level 'Central' of factor 'Reef Complex'
Within level 'Sinularia' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	1.7543	0.1972	10	0.1557
Two-mile, Regal	3.7969	0.0983	10	**0.0218**
Nine-mile, Regal	2.9884	0.0988	10	**0.0451**

Within level 'Central' of factor 'Reef Complex'
Within level 'Sarcophyton' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	0.62163	0.5938	10	0.5657
Two-mile, Regal	0.077387	1	10	0.9421
Nine-mile, Regal	0.52023	0.5957	10	0.6342

Within level 'Central' of factor 'Reef Complex'
Within level 'Theonella' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	1.405	0.1038	10	0.2222
Two-mile, Regal	3.5978	0.1005	10	**0.0219**
Nine-mile, Regal	0.40249	0.6958	10	0.7102

Within level 'Central' of factor 'Reef Complex'
Within level 'Echinopora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)

Two-mile, Nine-mile	2.2954	0.0964	10	0.0832
Two-mile, Regal	2.4267	0.0998	10	0.0674
Nine-mile, Regal	0.31132	0.5974	10	0.7693

Within level 'Central' of factor 'Reef Complex'
Within level 'Acropora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	2.7467	0.0998	4	0.0536
Two-mile, Regal	2.7467	0.0973	4	**0.0495**
Nine-mile, Regal	Denominator is 0			

Within level 'Northern' of factor 'Reef Complex'
Within level 'Sinularia' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	0.67116	0.6933	10	0.5374
Rabbit, Kosi north	1.2359	0.3965	10	0.277
Kosi south, Kosi north	0.9157	0.3955	10	0.4055

Within level 'Northern' of factor 'Reef Complex'
Within level 'Sarcophyton' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	0.24543	0.6935	10	0.8217
Rabbit, Kosi north	2.9246	0.1009	10	**0.0438**
Kosi south, Kosi north	1.3036	0.2932	10	0.2599

Within level 'Northern' of factor 'Reef Complex'
Within level 'Theonella' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	0.57709	0.802	10	0.5985
Rabbit, Kosi north	1.1078	0.3975	10	0.3259
Kosi south, Kosi north	1.2887	0.296	10	0.2619

Within level 'Northern' of factor 'Reef Complex'
Within level 'Echinopora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	0.44571	0.6979	10	0.6723
Rabbit, Kosi north	1.6965	0.1958	10	0.1608
Kosi south, Kosi north	0.99432	0.5023	10	0.378

Within level 'Northern' of factor 'Reef Complex'
Within level 'Acropora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	1.6651	0.0978	10	0.17
Rabbit, Kosi north	0.26079	1	10	0.8105
Kosi south, Kosi north	4.1671	0.0973	10	**0.0144**

Table TS 4.5

***Post hoc* pair-wise tests for Site nested in Reef Complex crossed with Species for alachlor concentration data.**

Term 'Site(Reef Complex) x Species' for pairs of levels of factor 'Species'

Within level 'Southern' of factor 'Reef Complex'
Within level 'Leadsman north' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	103.28	0.1037	10	**0.0002**
Sinularia, Theonella	1.121	0.402	10	0.3163
Sinularia, Echinopora	9.275	0.0988	4	**0.0007**
Sinularia, Acropora	9.275	0.1011	4	**0.0011**
Sarcophyton, Theonella	30.137	0.1015	10	**0.0002**
Sarcophyton, Echinopora	336.95	0.1046	4	**0.0001**
Sarcophyton, Acropora	336.95	0.1052	4	**0.0001**
Theonella, Echinopora	1.3278	0.3995	2	0.2597
Theonella, Acropora	1.3278	0.4021	2	0.2617
Echinopora, Acropora	Denominator is 0			

Within level 'Southern' of factor 'Reef Complex'
Within level 'Leadsman south' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	5.2557	0.0936	10	**0.0059**
Sinularia, Theonella	2.1732	0.1	10	0.0964
Sinularia, Echinopora	0.40419	0.6995	10	0.7012
Sinularia, Acropora	3.5555	0.1046	7	**0.0249**
Sarcophyton, Theonella	5.427	0.1038	10	**0.0065**
Sarcophyton, Echinopora	5.2673	0.1044	10	**0.0054**
Sarcophyton, Acropora	5.5079	0.1028	7	**0.0055**
Theonella, Echinopora	0.69176	0.8023	10	0.5356
Theonella, Acropora	0.94095	0.398	4	0.3967
Echinopora, Acropora	1.2002	0.2959	7	0.3006

Within level 'Southern' of factor 'Reef Complex'
Within level 'Red Sands south' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	0.7915	0.6049	10	0.4672
Sinularia, Theonella	5.0438	0.0975	10	**0.0079**
Sinularia, Echinopora	5.1612	0.1012	4	**0.0068**
Sinularia, Acropora	5.1612	0.1009	4	**0.0061**
Sarcophyton, Theonella	10.334	0.1024	10	**0.0003**
Sarcophyton, Echinopora	10.727	0.0999	4	**0.0005**
Sarcophyton, Acropora	10.727	0.1035	4	**0.0005**
Theonella, Echinopora	1.7054	0.3943	2	0.1631
Theonella, Acropora	1.7054	0.3971	2	0.1616
Echinopora, Acropora	Denominator is 0			

Within level 'Southern' of factor 'Reef Complex'
Within level 'Red Sands north' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	3.2284	0.1028	10	**0.0329**
Sinularia, Theonella	1.7965	0.2933	7	0.1492
Sinularia, Echinopora	3.7114	0.1036	7	**0.0212**
Sinularia, Acropora	9.6094	0.0971	4	**0.0013**
Sarcophyton, Theonella	3.409	0.1032	7	**0.0287**
Sarcophyton, Echinopora	3.4696	0.0976	7	**0.0263**

Sarcophyton, Acropora	3.5335	0.1034	4	**0.023**
Theonella, Echinopora	0.39395	1	2	0.7106
Theonella, Acropora	1	1	1	0.3841
Echinopora, Acropora	1	1	1	0.3801

Within level 'Central' of factor 'Reef Complex'
Within level 'Two-mile' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	1.3425	0.3985	10	0.2497
Sinularia, Theonella	5.8658	0.0943	10	**0.0041**
Sinularia, Echinopora	0.93186	0.492	10	0.405
Sinularia, Acropora	6.4811	0.095	4	**0.003**
Sarcophyton, Theonella	2.2959	0.1032	10	0.0873
Sarcophyton, Echinopora	1.6403	0.208	10	0.1736
Sarcophyton, Acropora	2.3935	0.1008	4	0.0739
Theonella, Echinopora	1.2128	0.3999	10	0.2941
Theonella, Acropora	16.799	0.1025	4	**0.0003**
Echinopora, Acropora	1.4429	0.3989	2	0.2266

Within level 'Central' of factor 'Reef Complex'
Within level 'Nine-mile' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	13.963	0.0945	10	**0.0002**
Sinularia, Theonella	0.13193	1	7	0.9041
Sinularia, Echinopora	3.469	0.0954	4	**0.0239**
Sinularia, Acropora	3.469	0.1018	4	**0.0254**
Sarcophyton, Theonella	3.3433	0.0994	7	**0.0297**
Sarcophyton, Echinopora	563.36	0.0659	6	**0.0001**
Sarcophyton, Acropora	563.36	0.0682	6	**0.0001**
Theonella, Echinopora	1	1	1	0.3571
Theonella, Acropora	1	1	1	0.3771
Echinopora, Acropora	Denominator is 0			

Within level 'Central' of factor 'Reef Complex'
Within level 'Regal' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	3.0544	0.1003	10	**0.0394**
Sinularia, Theonella	1.0271	0.3972	10	0.3638
Sinularia, Echinopora	64.026	0.1036	4	**0.0001**
Sinularia, Acropora	Negative			
Sarcophyton, Theonella	2.5511	0.1046	10	0.0661
Sarcophyton, Echinopora	3.1886	0.0989	4	**0.0321**
Sarcophyton, Acropora	Negative			
Theonella, Echinopora	1.4151	0.4032	2	0.2345
Theonella, Acropora	Negative			
Echinopora, Acropora	Denominator is 0			

Within level 'Northern' of factor 'Reef Complex'
Within level 'Rabbit' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	0.95254	0.3983	10	0.3931
Sinularia, Theonella	1.3005	0.3024	10	0.2616
Sinularia, Echinopora	1.3379	0.4039	7	0.2599
Sinularia, Acropora	1.182	0.3945	7	0.3036
Sarcophyton, Theonella	0.75772	0.5011	10	0.4969
Sarcophyton, Echinopora	5.4796	0.0984	10	**0.0057**
Sarcophyton, Acropora	4.9145	0.1037	10	**0.0068**
Theonella, Echinopora	8.4685	0.1027	10	**0.0016**

Theonella, Acropora	7.391	0.1014	10	**0.0016**
Echinopora, Acropora	0.4066	0.8004	7	0.7009

Within level 'Northern' of factor 'Reef Complex'
Within level 'Kosi south' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	1.3284	0.3012	10	0.257
Sinularia, Theonella	0.58405	0.7083	10	0.5982
Sinularia, Echinopora	0.39263	0.8051	7	0.7112
Sinularia, Acropora	4.975	0.1034	4	**0.0078**
Sarcophyton, Theonella	1.5616	0.3037	10	0.1906
Sarcophyton, Echinopora	0.2131	1	4	0.8458
Sarcophyton, Acropora	1.9953	0.4033	2	0.1219
Theonella, Echinopora	0.66113	0.5944	7	0.5504
Theonella, Acropora	3.6163	0.1001	4	**0.0252**
Echinopora, Acropora	1	1	1	0.3764

Within level 'Northern' of factor 'Reef Complex'
Within level 'Kosi north' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	1.874	0.3986	2	0.1353
Sinularia, Theonella	2.3394	0.2055	10	0.0829
Sinularia, Echinopora	1.4215	0.3945	4	0.2278
Sinularia, Acropora	0.39374	0.8013	7	0.7138
Sarcophyton, Theonella	6.4622	0.1042	4	**0.0028**
Sarcophyton, Echinopora	1	1	1	0.3677
Sarcophyton, Acropora	1.962	0.399	2	0.115
Theonella, Echinopora	5.3656	0.0993	7	**0.006**
Theonella, Acropora	3.31	0.1007	10	**0.0312**
Echinopora, Acropora	1.2925	0.3939	4	0.2597

Term 'Site(Reef Complex) X Species' for pairs of levels of factor 'Site'

Within level 'Southern' of factor 'Reef Complex'
Within level 'Sinularia' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	1.1201	0.3976	10	0.3362
Leadsman north, Red Sands south	3.8997	0.107	10	**0.0168**
Leadsman north, Red Sands north	4.4372	0.0993	10	**0.0112**
Leadsman south, Red Sands south	4.1144	0.1078	10	**0.0144**
Leadsman south, Red Sands north	2.3772	0.1958	10	0.0811
Red Sands south, Red Sands north	4.5711	0.1013	10	**0.0108**

Within level 'Southern' of factor 'Reef Complex'
Within level 'Sarcophyton' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	Negative			
Leadsman north, Red Sands south	29.56	0.1041	10	**0.0009**
Leadsman north, Red Sands north	5.1184	0.0971	10	**0.0174**
Leadsman south, Red Sands south	4.2156	0.0994	10	**0.0131**
Leadsman south, Red Sands north	3.0122	0.0963	10	**0.0401**
Red Sands south, Red Sands north	1.259	0.4024	10	0.2767

Within level 'Southern' of factor 'Reef Complex'
Within level 'Theonella' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	0.44203	0.9008	7	0.6789
Leadsman north, Red Sands south	1.1012	0.3937	7	0.3341
Leadsman north, Red Sands north	0.84896	0.6985	4	0.4386
Leadsman south, Red Sands south	1.4232	0.3968	7	0.2317
Leadsman south, Red Sands north	0.74176	0.395	4	0.4953
Red Sands south, Red Sands north	0.4481	1	4	0.68

Within level 'Southern' of factor 'Reef Complex'
Within level 'Echinopora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	1.6097	0.0997	4	0.1888
Leadsman north, Red Sands south	Denominator is 0			
Leadsman north, Red Sands north	1	1	1	0.3667
Leadsman south, Red Sands south	1.6097	0.1043	4	0.1802
Leadsman south, Red Sands north	1.3468	0.1985	7	0.2506
Red Sands south, Red Sands north	1	1	1	0.3809

Within level 'Southern' of factor 'Reef Complex'
Within level 'Acropora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	1	1	1	0.3786
Leadsman north, Red Sands south	Denominator is 0			
Leadsman north, Red Sands north	Denominator is 0			
Leadsman south, Red Sands south	1	1	1	0.3712
Leadsman south, Red Sands north	1	1	1	0.3715
Red Sands south, Red Sands north	Denominator is 0			

Within level 'Central' of factor 'Reef Complex'
Within level 'Sinularia' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	0.096583	0.8982	10	0.9266
Two-mile, Regal	4.5509	0.1022	10	**0.0103**
Nine-mile, Regal	2.469	0.1019	10	0.0668

Within level 'Central' of factor 'Reef Complex'
Within level 'Sarcophyton' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	2.9726	0.1013	10	**0.0398**
Two-mile, Regal	1.966	0.1015	10	0.1212
Nine-mile, Regal	0.84833	0.7052	10	0.4419

Within level 'Central' of factor 'Reef Complex'
Within level 'Theonella' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	0.92103	1	7	0.3989
Two-mile, Regal	1.292	0.4069	10	0.2669
Nine-mile, Regal	0.077327	1	4	0.9445

Within level 'Central' of factor 'Reef Complex'
Within level 'Echinopora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	1.4429	0.4033	2	0.2207
Two-mile, Regal	1.4429	0.3936	2	0.2165
Nine-mile, Regal	Denominator is 0			

Within level 'Central' of factor 'Reef Complex'

Within level 'Acropora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	Denominator is 0			
Two-mile, Regal	Denominator is 0			
Nine-mile, Regal	Denominator is 0			

Within level 'Northern' of factor 'Reef Complex'
Within level 'Sinularia' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	0.30368	0.7982	10	0.7763
Rabbit, Kosi north	0.075112	1	7	0.9443
Kosi south, Kosi north	0.20448	0.8983	10	0.849

Within level 'Northern' of factor 'Reef Complex'
Within level 'Sarcophyton' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	2.3974	0.0961	10	0.0712
Rabbit, Kosi north	7.7305	0.1008	4	**0.0013**
Kosi south, Kosi north	1.9953	0.3947	2	0.1181

Within level 'Northern' of factor 'Reef Complex'
Within level 'Theonella' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	0.68411	0.9005	10	0.5345
Rabbit, Kosi north	2.21	0.2059	10	0.0894
Kosi south, Kosi north	2.1446	0.1975	10	0.1024

Within level 'Northern' of factor 'Reef Complex'
Within level 'Echinopora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	0.72207	1	4	0.5037
Rabbit, Kosi north	0.085522	1	4	0.9361
Kosi south, Kosi north	0.73524	1	2	0.5043

Within level 'Northern' of factor 'Reef Complex'
Within level 'Acropora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	1.9882	0.3965	2	0.1188
Rabbit, Kosi north	1.1068	0.402	7	0.319
Kosi south, Kosi north	1.962	0.3991	2	0.115

Table TS 4.6

***Post hoc* pair-wise tests for Reef Complex crossed with Species for alachlor concentration data.**

Term 'Reef Complex x Species' for pairs of levels of factor 'Reef Complex'

Within level 'Sinularia' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	3.6142	0.0018	9854	**0.0034**
Southern, Northern	4.2102	0.0005	9848	**0.001**
Central, Northern	1.1784	0.2612	9815	0.2672

Within level 'Sarcophyton' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	4.5251	0.0014	9967	**0.0018**
Southern, Northern	9.7281	0.0001	9956	**0.0003**
Central, Northern	5.5297	0.0003	9854	**0.0003**

Within level 'Theonella' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	1.0936	0.3094	9852	0.2939
Southern, Northern	4.0503	0.0021	9844	**0.0019**
Central, Northern	0.47893	0.6545	9851	0.6408

Within level 'Echinopora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	0.035228	0.9594	9698	0.9751
Southern, Northern	0.090683	0.9285	9828	0.9304
Central, Northern	0.11288	0.9097	9788	0.9162

Within level 'Acropora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	Negative			
Southern, Northern	1.9533	0.0694	9788	0.0695
Central, Northern	0.79877	0.4514	7409	0.4304

Term 'Reef Complex x Species' for pairs of levels of factor 'Species'

Within level 'Southern' of factor 'Reef Complex'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	9.3568	0.0001	9952	**0.0001**
Sinularia, Theonella	5.6031	0.0001	9837	**0.0001**
Sinularia, Echinopora	6.2032	0.0001	9871	**0.0001**
Sinularia, Acropora	7.5193	0.0001	9615	**0.0001**
Sarcophyton, Theonella	11.201	0.0001	9956	**0.0001**
Sarcophyton, Echinopora	11.351	0.0001	9947	**0.0001**
Sarcophyton, Acropora	11.618	0.0001	9950	**0.0001**
Theonella, Echinopora	0.63708	0.5343	9825	0.5324
Theonella, Acropora	2.0279	0.0537	9860	0.0626
Echinopora, Acropora	1.3843	0.1906	9823	0.1837

Within level 'Central' of factor 'Reef Complex'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	5.0137	0.0005	9840	**0.0004**
Sinularia, Theonella	0.030378	0.9733	9816	0.9758
Sinularia, Echinopora	3.2816	0.0063	9780	**0.0063**
Sinularia, Acropora	5.2087	0.0001	9299	**0.0001**
Sarcophyton, Theonella	4.3496	0.0009	9853	**0.0012**
Sarcophyton, Echinopora	5.6988	0.0004	9831	**0.0002**
Sarcophyton, Acropora	4.4986	0.0002	9284	**0.0005**
Theonella, Echinopora	1.2062	0.2813	9706	0.2551
Theonella, Acropora	Negative			
Echinopora, Acropora	Negative			

Within level 'Northern' of factor 'Reef Complex'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	1.1203	0.2888	9841	0.2896
Sinularia, Theonella	2.6093	0.0234	9843	**0.0258**
Sinularia, Echinopora	1.6314	0.1316	9807	0.1261
Sinularia, Acropora	2.2925	0.0439	9834	**0.0394**
Sarcophyton, Theonella	5.2293	0.0007	9821	**0.0002**
Sarcophyton, Echinopora	0.98812	0.3537	9832	0.3409
Sarcophyton, Acropora	1.9448	0.0728	9836	0.08
Theonella, Echinopora	4.3812	0.0018	9831	**0.0006**
Theonella, Acropora	6.5709	0.0004	9821	**0.0001**
Echinopora, Acropora	0.1793	0.8724	9868	0.8639

Table TS 4.7
***Post hoc* pair-wise tests for Site nested in Reef Complex crossed with Species for acetochlor concentration data.**

Term 'Site(Reef Complex) x Species' for pairs of levels of factor 'Site'

Within level 'Southern' of factor 'Reef Complex'
Within level 'Sinularia' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	2.3514	0.1035	4	0.0788
Leadsman north, Red Sands south	1.736	0.2922	10	0.1689
Leadsman north, Red Sands north	0.35464	0.7048	10	0.747
Leadsman south, Red Sands south	5.0625	0.0975	4	**0.0063**
Leadsman south, Red Sands north	1.9912	0.3937	2	0.1167
Red Sands south, Red Sands north	2.1631	0.0986	10	0.0956

Within level 'Southern' of factor 'Reef Complex'
Within level 'Sarcophyton' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	4.2065	0.1011	10	0.0143
Leadsman north, Red Sands south	1.1721	0.391	10	0.3093
Leadsman north, Red Sands north	0.19275	0.9007	10	0.8538
Leadsman south, Red Sands south	0.57077	0.7012	10	0.5998
Leadsman south, Red Sands north	2.8924	0.1006	10	**0.0462**
Red Sands south, Red Sands north	1.139	0.401	7	0.3134

Within level 'Southern' of factor 'Reef Complex'
Within level 'Theonella' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	1.4361	0.4	7	0.2228
Leadsman north, Red Sands south	1.2211	0.3974	10	0.2793
Leadsman north, Red Sands north	1.5905	0.4015	2	0.1844
Leadsman south, Red Sands south	1.2354	0.3071	10	0.2805
Leadsman south, Red Sands north	1.7171	0.3992	2	0.162
Red Sands south, Red Sands north	2.4759	0.0977	4	0.07

Within level 'Southern' of factor 'Reef Complex'
Within level 'Echinopora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	1.6889	0.4075	7	0.162
Leadsman north, Red Sands south	1.9479	0.3994	2	0.1229
Leadsman north, Red Sands north	1.9479	0.4053	2	0.1211
Leadsman south, Red Sands south	1.9507	0.4069	2	0.1217
Leadsman south, Red Sands north	1.9507	0.3915	2	0.1211
Red Sands south, Red Sands north	Denominator is 0			

Within level 'Southern' of factor 'Reef Complex'
Within level 'Acropora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	0.35513	1	2	0.7341
Leadsman north, Red Sands south	1	1	1	0.3654
Leadsman north, Red Sands north	1	1	1	0.3733
Leadsman south, Red Sands south	1	1	1	0.3778
Leadsman south, Red Sands north	1	1	1	0.37
Red Sands south, Red Sands north	Denominator is 0			

Within level 'Central' of factor 'Reef Complex'
Within level 'Sinularia' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	1.9244	0.2015	10	0.1276
Two-mile, Regal	6.4416	0.1021	10	**0.0026**
Nine-mile, Regal	7.6752	0.0972	10	**0.0021**

Within level 'Central' of factor 'Reef Complex'
Within level 'Sarcophyton' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	1.2847	0.2996	10	0.265
Two-mile, Regal	1.9759	0.2011	10	0.1217
Nine-mile, Regal	1.0858	0.3004	10	0.3386

Within level 'Central' of factor 'Reef Complex'
Within level 'Theonella' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	1	1	1	0.3727
Two-mile, Regal	1	1	1	0.3694
Nine-mile, Regal	Denominator is 0			

Within level 'Central' of factor 'Reef Complex'
Within level 'Echinopora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	0.096603	1	10	0.9232
Two-mile, Regal	2.5632	0.1021	10	0.0611
Nine-mile, Regal	1.2184	0.3979	7	0.2955

Within level 'Central' of factor 'Reef Complex'
Within level 'Acropora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	Denominator is 0			
Two-mile, Regal	Denominator is 0			
Nine-mile, Regal	Denominator is 0			

Within level 'Northern' of factor 'Reef Complex'
Within level 'Sinularia' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	0.55501	0.8982	10	0.6083
Rabbit, Kosi north	1.383	0.1006	10	0.2392
Kosi south, Kosi north	0.61476	0.4952	10	0.5665

Within level 'Northern' of factor 'Reef Complex'
Within level 'Sarcophyton' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	0.14097	0.7026	10	0.8982
Rabbit, Kosi north	0.54195	0.7997	10	0.6119
Kosi south, Kosi north	0.64828	0.6021	10	0.5484

Within level 'Northern' of factor 'Reef Complex'

Within level 'Theonella' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	0.67943	0.6016	10	0.5433
Rabbit, Kosi north	0.90964	0.5082	10	0.4048
Kosi south, Kosi north	1.1617	0.2978	10	0.3102

Within level 'Northern' of factor 'Reef Complex'
Within level 'Echinopora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	1.0138	0.2975	7	0.3756
Rabbit, Kosi north	2.0544	0.1008	10	0.1076
Kosi south, Kosi north	2.492	0.098	7	0.0647

Within level 'Northern' of factor 'Reef Complex'
Within level 'Acropora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	1.6572	0.1983	7	0.1691
Rabbit, Kosi north	1.107	0.4022	10	0.3329
Kosi south, Kosi north	0.81309	0.6985	4	0.4595

Term 'Site(Reef Complex) x Species' for pairs of levels of factor 'Species'

Within level 'Southern' of factor 'Reef Complex'
Within level 'Leadsman north' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	0.96551	0.6054	10	0.3888
Sinularia, Theonella	0.15086	1	10	0.8868
Sinularia, Echinopora	2.0358	0.1046	10	0.1139
Sinularia, Acropora	2.1357	0.0963	7	0.0984
Sarcophyton, Theonella	0.54698	0.7008	10	0.6133
Sarcophyton, Echinopora	4.8954	0.1008	10	**0.0089**
Sarcophyton, Acropora	4.9136	0.1024	7	**0.0066**
Theonella, Echinopora	1.3602	0.4047	7	0.2397
Theonella, Acropora	1.4401	0.3888	4	0.218
Echinopora, Acropora	0.47514	1	4	0.6654

Within level 'Southern' of factor 'Reef Complex'
Within level 'Leadsman south' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	6.2089	0.1008	4	**0.0038**
Sinularia, Theonella	1.7171	0.3975	2	0.1615
Sinularia, Echinopora	1.9507	0.3992	2	0.1247
Sinularia, Acropora	1	1	1	0.3732
Sarcophyton, Theonella	5.8636	0.097	10	**0.0036**
Sarcophyton, Echinopora	1.5828	0.1992	10	0.1877
Sarcophyton, Acropora	5.9972	0.0995	7	**0.0041**
Theonella, Echinopora	1.7755	0.4	7	0.147
Theonella, Acropora	0.5782	0.7049	4	0.5889
Echinopora, Acropora	1.853	0.3945	4	0.1388

Within level 'Southern' of factor 'Reef Complex'
Within level 'Red Sands south' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	0.66389	0.6929	10	0.5502

Sinularia, Theonella	4.4657	0.1021	10	**0.0113**
Sinularia, Echinopora	5.0625	0.1011	4	**0.0066**
Sinularia, Acropora	5.0625	0.1016	4	**0.0065**
Sarcophyton, Theonella	1.6864	0.4006	10	0.1649
Sarcophyton, Echinopora	1.968	0.3951	2	0.121
Sarcophyton, Acropora	1.968	0.3964	2	0.1165
Theonella, Echinopora	2.4759	0.0986	4	0.0672
Theonella, Acropora	2.4759	0.1043	4	0.0684
Echinopora, Acropora	Denominator is 0			

Within level 'Southern' of factor 'Reef Complex'
Within level 'Red Sands north' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	0.51733	0.8016	7	0.6329
Sinularia, Theonella	1.9912	0.4071	2	0.1155
Sinularia, Echinopora	1.9912	0.4047	2	0.1166
Sinularia, Acropora	1.9912	0.3966	2	0.1167
Sarcophyton, Theonella	1.3686	0.4035	2	0.2461
Sarcophyton, Echinopora	1.3686	0.3997	2	0.2452
Sarcophyton, Acropora	1.3686	0.4032	2	0.2386
Theonella, Echinopora	Denominator is 0			
Theonella, Acropora	Denominator is 0			
Echinopora, Acropora	Denominator is 0			

Within level 'Central' of factor 'Reef Complex'
Within level 'Two-mile' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	0.32051	0.8061	10	0.766
Sinularia, Theonella	6.0007	0.1005	7	**0.004**
Sinularia, Echinopora	5.7069	0.0988	10	**0.0051**
Sinularia, Acropora	6.7407	0.1032	4	**0.0025**
Sarcophyton, Theonella	2.3676	0.099	7	0.0787
Sarcophyton, Echinopora	2.1856	0.1082	10	0.0899
Sarcophyton, Acropora	2.4993	0.1028	4	0.0645
Theonella, Echinopora	1.5568	0.3068	7	0.1906
Theonella, Acropora	1	1	1	0.368
Echinopora, Acropora	6.4532	0.1005	4	**0.0034**

Within level 'Central' of factor 'Reef Complex'
Within level 'Nine-mile' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	0.74735	0.7143	10	0.4892
Sinularia, Theonella	8.2912	0.0956	4	**0.0012**
Sinularia, Echinopora	5.0489	0.1023	10	**0.0072**
Sinularia, Acropora	8.2912	0.1024	4	**0.0014**
Sarcophyton, Theonella	2.1497	0.0999	4	0.1007
Sarcophyton, Echinopora	1.4871	0.3008	10	0.2108
Sarcophyton, Acropora	2.1497	0.1086	4	0.1006
Theonella, Echinopora	1.9927	0.3935	2	0.121
Theonella, Acropora	Denominator is 0			
Echinopora, Acropora	1.9927	0.3981	2	0.1188

Within level 'Central' of factor 'Reef Complex'
Within level 'Regal' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	1.2893	0.4031	10	0.276
Sinularia, Theonella	2.6044	0.102	4	0.0574
Sinularia, Echinopora	0.15277	0.7926	10	0.8881
Sinularia, Acropora	Negative			
Sarcophyton, Theonella	1.6033	0.3976	2	0.1827
Sarcophyton, Echinopora	1.2207	0.4081	7	0.2915

Sarcophyton, Acropora	Negative			
Theonella, Echinopora	1.4168	0.4077	2	0.2304
Theonella, Acropora	Denominator is 0			
Echinopora, Acropora	Negative			

Within level 'Northern' of factor 'Reef Complex'
Within level 'Rabbit' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	2.5299	0.0991	10	0.0692
Sinularia, Theonella	3.0496	0.0976	10	**0.0381**
Sinularia, Echinopora	4.0026	0.1002	10	**0.0175**
Sinularia, Acropora	1.6519	0.2979	10	0.17
Sarcophyton, Theonella	0.55297	0.707	10	0.6065
Sarcophyton, Echinopora	3.1406	0.097	10	**0.0367**
Sarcophyton, Acropora	2.9145	0.0969	10	**0.0462**
Theonella, Echinopora	4.0063	0.0988	10	**0.0148**
Theonella, Acropora	3.5124	0.1015	10	**0.0242**
Echinopora, Acropora	0.42236	0.8027	10	0.696

Within level 'Northern' of factor 'Reef Complex'
Within level 'Kosi south' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	1.851	0.1972	10	0.1328
Sinularia, Theonella	0.46358	0.6973	10	0.666
Sinularia, Echinopora	1.7596	0.1987	7	0.156
Sinularia, Acropora	1.9906	0.1016	7	0.1262
Sarcophyton, Theonella	1.1074	0.3975	10	0.3371
Sarcophyton, Echinopora	3.3161	0.1004	7	**0.0289**
Sarcophyton, Acropora	3.4734	0.1022	7	**0.026**
Theonella, Echinopora	1.615	0.4087	4	0.185
Theonella, Acropora	1.7382	0.4011	4	0.1576
Echinopora, Acropora	0.55186	1	2	0.6105

Within level 'Northern' of factor 'Reef Complex'
Within level 'Kosi north' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	0.42083	0.7026	10	0.6969
Sinularia, Theonella	1.2252	0.4064	10	0.2887
Sinularia, Echinopora	0.93585	0.4966	10	0.3964
Sinularia, Acropora	2.5364	0.0985	10	0.0664
Sarcophyton, Theonella	0.92035	0.497	10	0.4022
Sarcophyton, Echinopora	1.0054	0.5958	10	0.3791
Sarcophyton, Acropora	1.8492	0.0996	10	0.1382
Theonella, Echinopora	1.5376	0.4118	10	0.1985
Theonella, Acropora	1.9581	0.1927	10	0.1219
Echinopora, Acropora	2.5654	0.0991	10	0.0581

Table TS 4.8

***Post hoc* pair-wise tests for Reef Complex crossed with Species for acetochlor concentration data.**

Term 'Reef Complex x Species' for pairs of levels of factor 'Reef Complex'

Within level 'Sinularia' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	3.3124	0.0076	9833	**0.0052**
Southern, Northern	1.4119	0.1818	9841	0.1859
Central, Northern	1.0469	0.3024	9821	0.3197

Within level 'Sarcophyton' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	1.5199	0.1514	9838	0.1426
Southern, Northern	3.427	0.0055	9788	**0.0046**
Central, Northern	1.4368	0.1735	9846	0.1674

Within level 'Theonella' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	1.3895	0.2048	9850	0.1891
Southern, Northern	3.6339	0.0033	9886	**0.0019**
Central, Northern	3.4663	0.0058	9773	**0.0037**

Within level 'Echinopora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	0.40278	0.7189	9859	0.7015
Southern, Northern	1.7042	0.1101	9830	0.1155
Central, Northern	1.5774	0.1414	9863	0.1371

Within level 'Acropora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	Negative			
Southern, Northern	2.577	0.0166	9880	**0.0214**
Central, Northern	1.1773	0.2888	8787	0.2711

Term 'Reef Complex x Species' for pairs of levels of factor 'Species'

Within level 'Southern' of factor 'Reef Complex'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	0.57859	0.5697	9832	0.5691
Sinularia, Theonella	2.896	0.01	9827	**0.0098**
Sinularia, Echinopora	3.1119	0.0062	9854	**0.0062**
Sinularia, Acropora	5.179	0.0002	9822	**0.0001**
Sarcophyton, Theonella	3.1154	0.0076	9812	**0.0078**
Sarcophyton, Echinopora	3.2904	0.0042	9813	**0.0049**
Sarcophyton, Acropora	4.8721	0.0009	9829	**0.0002**
Theonella, Echinopora	0.098234	0.9066	9794	0.9235
Theonella, Acropora	1.8304	0.0656	9833	0.0868
Echinopora, Acropora	1.9101	0.0701	9840	0.0735

Within level 'Central' of factor 'Reef Complex'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	0.25342	0.811	9841	0.801
Sinularia, Theonella	9.3218	0.0003	9811	**0.0001**
Sinularia, Echinopora	7.4138	0.0001	9818	**0.0002**
Sinularia, Acropora	7.0228	0.0001	9221	**0.0001**
Sarcophyton, Theonella	3.3699	0.0058	9801	**0.0066**
Sarcophyton, Echinopora	2.8447	0.015	9816	**0.0151**
Sarcophyton, Acropora	2.106	0.0474	9271	0.0581
Theonella, Echinopora	2.8536	0.0174	9815	**0.0163**
Theonella, Acropora	Negative			
Echinopora, Acropora	2.8953	0.0145	9295	**0.0125**

Within level 'Northern' of factor 'Reef Complex'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	2.4927	0.0293	9843	**0.0303**
Sinularia, Theonella	1.8818	0.0846	9843	0.0871
Sinularia, Echinopora	2.4195	0.0339	9836	**0.0315**
Sinularia, Acropora	3.5485	0.0034	9811	**0.0042**
Sarcophyton, Theonella	0.10102	0.9238	9837	0.9272
Sarcophyton, Echinopora	4.018	0.0022	9851	**0.0014**
Sarcophyton, Acropora	4.5998	0.0007	9831	**0.0007**
Theonella, Echinopora	2.844	0.0143	9846	**0.0138**
Theonella, Acropora	3.218	0.0087	9832	**0.0083**
Echinopora, Acropora	1.8683	0.0833	9854	**0.089**

Table TS 4.9

***Post hoc* pair-wise tests for Site crossed with Species for hexazinone concentration data.**

Term 'Site x Species' for pairs of levels of factor 'Species'

Within level 'Leadsman north' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	0.62443	0.7075	10	0.5656
Sinularia, Theonella	0.52276	0.8031	10	0.6375
Sinularia, Echinopora	3.1707	0.1016	10	**0.034**
Sinularia, Acropora	4.1231	0.0979	10	**0.0148**
Sarcophyton, Theonella	0.88065	0.6043	10	0.4302
Sarcophyton, Echinopora	2.0793	0.1003	10	0.1065
Sarcophyton, Acropora	2.4588	0.1017	10	0.0704
Theonella, Echinopora	4.5065	0.0993	10	**0.011**
Theonella, Acropora	6.5254	0.0947	10	**0.0035**
Echinopora, Acropora	1.1546	0.396	10	0.3212

Within level 'Leadsman south' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	1.3688	0.4005	10	0.243
Sinularia, Theonella	0.26208	0.6048	10	0.8058
Sinularia, Echinopora	1.0845	0.2947	10	0.3378
Sinularia, Acropora	2.1944	0.1968	7	0.0987
Sarcophyton, Theonella	1.4495	0.3954	7	0.2178
Sarcophyton, Echinopora	1.6803	0.4027	10	0.1674
Sarcophyton, Acropora	1.9285	0.3999	4	0.1245
Theonella, Echinopora	0.69894	0.6031	10	0.5184
Theonella, Acropora	1.6766	0.4029	4	0.1717
Echinopora, Acropora	1.6273	0.2031	7	0.1752

Within level 'Red Sands south' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	5.3861	0.1002	10	**0.0067**
Sinularia, Theonella	4.1104	0.1074	10	**0.0142**
Sinularia, Echinopora	3.7778	0.1049	10	**0.0195**
Sinularia, Acropora	5.6689	0.0991	7	**0.0054**
Sarcophyton, Theonella	1.3434	0.3999	7	0.2452
Sarcophyton, Echinopora	2.294	0.2013	10	0.0823
Sarcophyton, Acropora	0.71544	0.6991	4	0.5132
Theonella, Echinopora	0.61867	0.6099	10	0.5736
Theonella, Acropora	1.7302	0.4004	4	0.1569
Echinopora, Acropora	2.8001	0.0983	7	0.0522

Within level 'Red Sands north' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	1.6357	0.2981	10	0.1761
Sinularia, Theonella	1.7993	0.3072	10	0.1547
Sinularia, Echinopora	2.0585	0.1972	7	0.1095
Sinularia, Acropora	1.2677	0.4116	10	0.276
Sarcophyton, Theonella	0.44343	0.8036	7	0.679
Sarcophyton, Echinopora	1.314	0.4008	4	0.2637
Sarcophyton, Acropora	0.80265	0.393	10	0.4696
Theonella, Echinopora	0.91419	0.6942	4	0.4124
Theonella, Acropora	1.2185	0.3032	10	0.293
Echinopora, Acropora	1.9758	0.201	7	0.1189

Within level 'Two-mile' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	1.7472	0.2071	10	0.1529
Sinularia, Theonella	3.2226	0.0961	10	**0.032**
Sinularia, Echinopora	3.3513	0.099	10	**0.0279**
Sinularia, Acropora	1.9125	0.2012	10	0.1284
Sarcophyton, Theonella	0.62582	0.6015	10	0.5653
Sarcophyton, Echinopora	0.70972	0.5981	10	0.5203
Sarcophyton, Acropora	0.13895	0.8978	10	0.8956
Theonella, Echinopora	0.11762	1	10	0.9111
Theonella, Acropora	0.94735	0.4052	10	0.4045
Echinopora, Acropora	1.0523	0.1969	10	0.3572

Within level 'Nine-mile' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	1.6673	0.1003	10	0.1686
Sinularia, Theonella	2.482	0.0999	10	0.0692
Sinularia, Echinopora	6.9541	0.098	10	**0.0024**
Sinularia, Acropora	11.291	0.1036	4	**0.0003**
Sarcophyton, Theonella	0.89367	0.6	10	0.4217
Sarcophyton, Echinopora	2.5102	0.1024	10	0.0672
Sarcophyton, Acropora	2.6729	0.1034	4	0.0586
Theonella, Echinopora	5.327	0.0966	10	**0.0063**
Theonella, Acropora	6.0852	0.0993	4	**0.0039**
Echinopora, Acropora	1.8489	0.3937	2	0.1401

Within level 'Regal' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	8.1323	0.0954	10	**0.0012**
Sinularia, Theonella	0.61128	0.4933	10	0.5692
Sinularia, Echinopora	6.152	0.0973	10	**0.0046**
Sinularia, Acropora	Negative			
Sarcophyton, Theonella	9.4964	0.1006	10	**0.0008**
Sarcophyton, Echinopora	0.78443	0.7033	7	0.4878
Sarcophyton, Acropora	Negative			
Theonella, Echinopora	7.0708	0.0985	10	**0.0021**
Theonella, Acropora	Negative			
Echinopora, Acropora	Negative			

Within level 'Rabbit' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	2.0842	0.1954	7	0.1066
Sinularia, Theonella	1.5198	0.2937	7	0.2085
Sinularia, Echinopora	0.57385	1	2	0.5996
Sinularia, Acropora	1	1	1	0.3769
Sarcophyton, Theonella	1.5742	0.2929	10	0.1855
Sarcophyton, Echinopora	2.4105	0.1019	7	0.0726
Sarcophyton, Acropora	2.5709	0.0902	4	0.0596
Theonella, Echinopora	3.5999	0.1021	7	**0.0233**
Theonella, Acropora	5.2229	0.1028	4	**0.0059**
Echinopora, Acropora	1	1	1	0.3762

Within level 'Kosi south' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	1.479	0.3914	4	0.2129

Sinularia, Theonella	2.5228	0.1959	7	0.0616
Sinularia, Echinopora	0.36425	0.8003	7	0.731
Sinularia, Acropora	0.58023	1	2	0.595
Sarcophyton, Theonella	1.064	0.3929	10	0.3422
Sarcophyton, Echinopora	1.4428	0.4028	10	0.2246
Sarcophyton, Acropora	1.5658	0.3983	4	0.1933
Theonella, Echinopora	3.2234	0.0994	10	**0.0316**
Theonella, Acropora	4.1277	0.0992	7	**0.0154**
Echinopora, Acropora	2.1448	0.1961	7	0.1003

Within level 'Kosi north' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	1.2021	0.4017	7	0.2995
Sinularia, Theonella	0.27573	0.5941	10	0.7983
Sinularia, Echinopora	1.8492	0.4043	4	0.143
Sinularia, Acropora	1.8659	0.4054	4	0.137
Sarcophyton, Theonella	1.2919	0.3014	10	0.2656
Sarcophyton, Echinopora	1.4422	0.3988	4	0.2189
Sarcophyton, Acropora	1.492	0.4013	4	0.2064
Theonella, Echinopora	2.3621	0.0985	7	0.0825
Theonella, Acropora	2.3927	0.0969	7	0.0749
Echinopora, Acropora	0.077053	1	2	0.9388

Term 'Site x Species' for pairs of levels of factor 'Site'

Within level 'Sinularia' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	3.0472	0.101	10	**0.0399**
Leadsman north, Red Sands south	1.3763	0.3039	10	0.2408
Leadsman north, Red Sands north	0.27774	0.8953	10	0.8015
Leadsman north, Two-mile	1.0156	0.4999	10	0.3618
Leadsman north, Nine-mile	3.1528	0.1003	10	**0.0325**
Leadsman north, Regal	0.046485	1	10	0.9632
Leadsman north, Rabbit	3.0859	0.1044	7	**0.0347**
Leadsman north, Kosi south	3.6864	0.1019	7	**0.0213**
Leadsman north, Kosi north	0.8112	0.6883	10	0.4573
Leadsman south, Red Sands south	3.8972	0.0975	10	**0.018**
Leadsman south, Red Sands north	1.528	0.3027	10	0.1971
Leadsman south, Two-mile	2.4208	0.1003	10	0.071
Leadsman south, Nine-mile	0.73494	0.6992	10	0.5051
Leadsman south, Regal	4.2934	0.1035	10	**0.0133**
Leadsman south, Rabbit	0.59266	0.7997	7	0.5965
Leadsman south, Kosi south	0.91941	0.203	7	0.4044
Leadsman south, Kosi north	0.96572	0.3958	10	0.3902
Red Sands south, Red Sands north	0.45673	0.8004	10	0.6697
Red Sands south, Two-mile	2.2971	0.1016	10	0.0887
Red Sands south, Nine-mile	3.9594	0.1058	10	**0.0166**
Red Sands south, Regal	1.5583	0.2058	10	0.1847
Red Sands south, Rabbit	3.9128	0.102	7	**0.0164**
Red Sands south, Kosi south	4.4084	0.0966	7	**0.0111**
Red Sands south, Kosi north	1.7264	0.0986	10	0.1596
Red Sands north, Two-mile	0.69825	0.4984	10	0.5191
Red Sands north, Nine-mile	1.3796	0.3931	10	0.2463
Red Sands north, Regal	0.27171	0.8994	10	0.8027
Red Sands north, Rabbit	1.7258	0.1971	7	0.1581
Red Sands north, Kosi south	1.8507	0.2013	7	0.134
Red Sands north, Kosi north	0.7465	0.4874	10	0.489
Two-mile, Nine-mile	2.5554	0.0981	10	0.062
Two-mile, Regal	1.4728	0.2991	10	0.217
Two-mile, Rabbit	2.5106	0.1946	7	0.0623
Two-mile, Kosi south	3.1781	0.0981	7	**0.0347**
Two-mile, Kosi north	0.24763	0.9076	10	0.8159

Nine-mile, Regal	5.9935	0.103	10	**0.0027**
Nine-mile, Rabbit	1.2104	0.402	7	0.2964
Nine-mile, Kosi south	1.835	0.3106	7	0.1426
Nine-mile, Kosi north	0.73749	0.3984	10	0.5119
Regal, Rabbit	3.8408	0.0988	7	**0.0175**
Regal, Kosi south	4.9611	0.1035	7	**0.0087**
Regal, Kosi north	0.9086	0.6051	10	0.4132
Rabbit, Kosi south	0.18063	1	2	0.8599
Rabbit, Kosi north	1.2513	0.4048	4	0.2713
Kosi south, Kosi north	1.4344	0.4045	4	0.2215

Within level 'Sarcophyton' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	0.2469	0.8012	10	0.8181
Leadsman north, Red Sands south	2.7508	0.1003	10	0.0514
Leadsman north, Red Sands north	2.1999	0.0985	10	0.0908
Leadsman north, Two-mile	1.9089	0.2054	10	0.1229
Leadsman north, Nine-mile	0.23467	0.7948	10	0.8307
Leadsman north, Regal	2.6824	0.1017	10	0.0538
Leadsman north, Rabbit	0.12638	0.8026	10	0.9025
Leadsman north, Kosi south	0.49776	0.7941	10	0.645
Leadsman north, Kosi north	2.2651	0.1009	10	0.0865
Leadsman south, Red Sands south	1.8778	0.3921	7	0.1314
Leadsman south, Red Sands north	1.4696	0.4029	7	0.2153
Leadsman south, Two-mile	1.2651	0.4069	7	0.2679
Leadsman south, Nine-mile	0.044596	1	10	0.9677
Leadsman south, Regal	1.8282	0.3989	7	0.1389
Leadsman south, Rabbit	0.34116	0.6956	10	0.7508
Leadsman south, Kosi south	0.61487	0.6996	7	0.5769
Leadsman south, Kosi north	1.5191	0.4059	7	0.1966
Red Sands south, Red Sands north	1.4351	0.3942	7	0.2278
Red Sands south, Two-mile	1.5285	0.4067	7	0.1957
Red Sands south, Nine-mile	2.5133	0.104	10	0.0611
Red Sands south, Regal	0.27412	0.6993	7	0.7986
Red Sands south, Rabbit	2.4468	0.095	10	0.0699
Red Sands south, Kosi south	1.5628	0.3938	7	0.2
Red Sands south, Kosi north	1.3025	0.3975	7	0.2586
Red Sands north, Two-mile	0.41179	0.5989	7	0.6942
Red Sands north, Nine-mile	1.95	0.1003	10	0.1263
Red Sands north, Regal	1.2099	0.403	7	0.2893
Red Sands north, Rabbit	2.006	0.1001	10	0.1153
Red Sands north, Kosi south	1.3798	0.3996	7	0.2419
Red Sands north, Kosi north	0.1411	1	7	0.8923
Two-mile, Nine-mile	1.6582	0.1015	10	0.1683
Two-mile, Regal	1.3717	0.4037	7	0.2404
Two-mile, Rabbit	1.7764	0.2021	10	0.1502
Two-mile, Kosi south	1.2903	0.4005	7	0.2654
Two-mile, Kosi north	0.53146	0.6037	7	0.6233
Nine-mile, Regal	2.4432	0.1057	10	0.0683
Nine-mile, Rabbit	0.33917	0.6998	10	0.7523
Nine-mile, Kosi south	0.61426	0.6971	10	0.5796
Nine-mile, Kosi north	2.0166	0.1	10	0.1145
Regal, Rabbit	2.393	0.1013	10	0.0754
Regal, Kosi south	1.5411	0.4026	7	0.197
Regal, Kosi north	1.0734	0.3983	7	0.3439
Rabbit, Kosi south	0.41706	0.8028	10	0.6948
Rabbit, Kosi north	2.059	0.1001	10	0.1055
Kosi south, Kosi north	1.4026	0.3991	7	0.2426

Within level 'Theonella' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	4.0278	0.1021	10	**0.0152**

Leadsman north, Red Sands south	4.3028	0.1026	10	**0.0115**
Leadsman north, Red Sands north	5.5174	0.0981	10	**0.0064**
Leadsman north, Two-mile	5.3953	0.1045	10	**0.0065**
Leadsman north, Nine-mile	1.0928	0.3931	10	0.3272
Leadsman north, Regal	1.8335	0.2017	10	0.1429
Leadsman north, Rabbit	3.251	0.1019	10	**0.0319**
Leadsman north, Kosi south	1.7151	0.3009	10	0.1604
Leadsman north, Kosi north	1.4469	0.0972	10	0.2261
Leadsman south, Red Sands south	0.10988	1	7	0.9164
Leadsman south, Red Sands north	0.4837	0.7912	7	0.6511
Leadsman south, Two-mile	0.28571	0.8992	10	0.7913
Leadsman south, Nine-mile	2.501	0.1051	10	0.0683
Leadsman south, Regal	4.9384	0.0976	10	**0.0089**
Leadsman south, Rabbit	1.3952	0.3025	10	0.2416
Leadsman south, Kosi south	1.9097	0.2015	10	0.1277
Leadsman south, Kosi north	1.1221	0.3059	10	0.3223
Red Sands south, Red Sands north	0.37291	0.798	7	0.7312
Red Sands south, Two-mile	0.16869	0.9021	10	0.8696
Red Sands south, Nine-mile	2.6716	0.0969	10	0.0587
Red Sands south, Regal	5.2203	0.0973	10	**0.0063**
Red Sands south, Rabbit	1.5579	0.202	10	0.1897
Red Sands south, Kosi south	2.0614	0.199	10	0.1084
Red Sands south, Kosi north	1.2184	0.3004	10	0.2952
Red Sands north, Two-mile	0.23321	0.6983	10	0.8284
Red Sands north, Nine-mile	3.328	0.0966	10	**0.0306**
Red Sands north, Regal	6.4498	0.1022	10	**0.0034**
Red Sands north, Rabbit	2.1808	0.197	10	0.0926
Red Sands north, Kosi south	2.6277	0.0983	10	0.0593
Red Sands north, Kosi north	1.5445	0.3018	10	0.1949
Two-mile, Nine-mile	3.1647	0.0987	10	**0.0343**
Two-mile, Regal	6.3507	0.1014	10	**0.0038**
Two-mile, Rabbit	1.9809	0.208	10	0.1181
Two-mile, Kosi south	2.4571	0.1997	10	0.0696
Two-mile, Kosi north	1.4065	0.3088	10	0.2339
Nine-mile, Regal	2.2514	0.1976	10	0.0843
Nine-mile, Rabbit	1.4372	0.1965	10	0.2205
Nine-mile, Kosi south	0.54946	0.7037	10	0.6106
Nine-mile, Kosi north	0.70986	0.7029	10	0.5257
Regal, Rabbit	4.4059	0.1003	10	**0.0111**
Regal, Kosi south	2.7846	0.1036	10	**0.0476**
Regal, Kosi north	2.1806	0.0986	10	0.0976
Rabbit, Kosi south	0.77512	0.5	10	0.4778
Rabbit, Kosi north	0.20826	0.7994	10	0.8403
Kosi south, Kosi north	0.30771	0.8043	10	0.7694

Within level 'Echinopora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	1.3186	0.1956	10	0.2546
Leadsman north, Red Sands south	0.18895	0.801	10	0.8559
Leadsman north, Red Sands north	2.1036	0.1988	7	0.1011
Leadsman north, Two-mile	0.85523	0.5038	10	0.4464
Leadsman north, Nine-mile	2.3998	0.0948	10	0.0769
Leadsman north, Regal	1.0776	0.4051	10	0.3478
Leadsman north, Rabbit	2.029	0.1969	7	0.1165
Leadsman north, Kosi south	1.1791	0.2979	10	0.3013
Leadsman north, Kosi north	2.5127	0.1012	7	0.0659
Leadsman south, Red Sands south	1.4111	0.2063	10	0.2274
Leadsman south, Red Sands north	0.96428	0.5009	7	0.3937
Leadsman south, Two-mile	0.3267	0.7003	10	0.7658
Leadsman south, Nine-mile	1.2302	0.3972	10	0.2911
Leadsman south, Regal	0.11004	0.8997	10	0.9224
Leadsman south, Rabbit	0.89181	0.4973	7	0.4214
Leadsman south, Kosi south	0.43847	0.6969	10	0.6814
Leadsman south, Kosi north	1.41	0.3049	7	0.2294
Red Sands south, Red Sands north	2.1197	0.2058	7	0.1016
Red Sands south, Two-mile	0.98389	0.3019	10	0.3824

Red Sands south, Nine-mile	2.3574	0.0969	10	0.0811
Red Sands south, Regal	1.1911	0.304	10	0.2955
Red Sands south, Rabbit	2.0553	0.2044	7	0.1119
Red Sands south, Kosi south	1.2814	0.2958	10	0.2768
Red Sands south, Kosi north	2.4653	0.0997	7	0.07
Red Sands north, Two-mile	1.098	0.504	7	0.3377
Red Sands north, Nine-mile	0.042027	1	4	0.9706
Red Sands north, Regal	0.91285	0.7076	4	0.4127
Red Sands north, Rabbit	0.044747	1	2	0.9664
Red Sands north, Kosi south	1.6827	0.2003	7	0.1674
Red Sands north, Kosi north	0.29414	1	2	0.7839
Two-mile, Nine-mile	1.2692	0.2988	10	0.2679
Two-mile, Regal	0.19619	0.9032	10	0.8527
Two-mile, Rabbit	1.042	0.4919	7	0.355
Two-mile, Kosi south	0.050274	1	10	0.9626
Two-mile, Kosi north	1.4143	0.3007	7	0.24
Nine-mile, Regal	1.0756	0.5001	7	0.3424
Nine-mile, Rabbit	0.095198	1	4	0.9261
Nine-mile, Kosi south	2.5754	0.0973	10	0.064
Nine-mile, Kosi north	0.36605	1	4	0.7341
Regal, Rabbit	0.85726	0.7001	4	0.4421
Regal, Kosi south	0.2136	0.806	10	0.8427
Regal, Kosi north	1.2328	0.4029	4	0.2859
Rabbit, Kosi south	1.5493	0.1939	7	0.1972
Rabbit, Kosi north	0.3335	1	2	0.7563
Kosi south, Kosi north	2.6425	0.2031	7	0.059

Within level 'Acropora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	1.4068	0.397	4	0.2327
Leadsman north, Red Sands south	1.6321	0.4013	4	0.1748
Leadsman north, Red Sands north	1.3582	0.3034	10	0.2505
Leadsman north, Two-mile	1.3014	0.3009	10	0.266
Leadsman north, Nine-mile	1.8658	0.3898	2	0.1324
Leadsman north, Regal	Negative			
Leadsman north, Rabbit	1.8658	0.3932	2	0.1363
Leadsman north, Kosi south	1.0856	0.4022	4	0.3402
Leadsman north, Kosi north	1.3176	0.3962	4	0.2574
Leadsman south, Red Sands south	0.39685	1	2	0.7106
Leadsman south, Red Sands north	2.271	0.0999	7	0.0859
Leadsman south, Two-mile	2.4435	0.1013	7	0.0714
Leadsman south, Nine-mile	1	1	1	0.3749
Leadsman south, Regal	Negative			
Leadsman south, Rabbit	1	1	1	0.3649
Leadsman south, Kosi south	0.33087	1	2	0.7574
Leadsman south, Kosi north	0.11274	1	2	0.9192
Red Sands south, Red Sands north	2.3856	0.1023	7	0.0771
Red Sands south, Two-mile	2.5958	0.1053	7	0.0626
Red Sands south, Nine-mile	1	1	1	0.3751
Red Sands south, Regal	Negative			
Red Sands south, Rabbit	1	1	1	0.3718
Red Sands south, Kosi south	0.6304	1	2	0.566
Red Sands south, Kosi north	0.48352	1	2	0.6434
Red Sands north, Two-mile	0.21582	0.7008	10	0.8373
Red Sands north, Nine-mile	2.5034	0.1003	4	0.069
Red Sands north, Regal	Negative			
Red Sands north, Rabbit	2.5034	0.0982	4	0.066
Red Sands north, Kosi south	2.0912	0.1993	7	0.1017
Red Sands north, Kosi north	2.2235	0.1033	7	0.0907
Two-mile, Nine-mile	2.7467	0.0973	4	**0.0486**
Two-mile, Regal	Negative			
Two-mile, Rabbit	2.7467	0.1022	4	**0.0499**
Two-mile, Kosi south	2.2029	0.2037	7	0.0877

Two-mile, Kosi north	2.3799	0.1012	7	0.0747
Nine-mile, Regal	Denominator is 0			
Nine-mile, Rabbit	Denominator is 0			
Nine-mile, Kosi south	1	1	1	0.3723
Nine-mile, Kosi north	1	1	1	0.3691
Regal, Rabbit	Denominator is 0			
Regal, Kosi south	Negative			
Regal, Kosi north	Negative			
Rabbit, Kosi south	1	1	1	0.3791
Rabbit, Kosi north	1	1	1	0.3775
Kosi south, Kosi north	0.22863	1	2	0.8305

Table TS 4.10

***Post hoc* pair-wise tests for Site nested in Reef Complex crossed with Species for atrazine concentration data.**

Term 'Site(Reef Complex) x Species' for pairs of levels of factor 'Species'

Within level 'Southern' of factor 'Reef Complex'
Within level 'Leadsman north' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	1.9663	0.1025	4	0.1174
Sinularia, Theonella	1.8162	0.0929	4	0.1451
Sinularia, Echinopora	Denominator is 0			
Sinularia, Acropora	Denominator is 0			
Sarcophyton, Theonella	1.6363	0.0981	10	0.1798
Sarcophyton, Echinopora	1.9663	0.1024	4	0.1215
Sarcophyton, Acropora	1.9663	0.1	4	0.1213
Theonella, Echinopora	1.8162	0.0989	4	0.144
Theonella, Acropora	1.8162	0.0961	4	0.1524
Echinopora, Acropora	Denominator is 0			

Within level 'Southern' of factor 'Reef Complex'
Within level 'Leadsman south' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	Denominator is 0			
Sinularia, Theonella	Denominator is 0			
Sinularia, Echinopora	Denominator is 0			
Sinularia, Acropora	Denominator is 0			
Sarcophyton, Theonella	Denominator is 0			
Sarcophyton, Echinopora	Denominator is 0			
Sarcophyton, Acropora	Denominator is 0			
Theonella, Echinopora	Denominator is 0			
Theonella, Acropora	Denominator is 0			
Echinopora, Acropora	Denominator is 0			

Within level 'Southern' of factor 'Reef Complex'
Within level 'Red Sands south' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	Denominator is 0			
Sinularia, Theonella	Denominator is 0			
Sinularia, Echinopora	Denominator is 0			
Sinularia, Acropora	Denominator is 0			
Sarcophyton, Theonella	Denominator is 0			
Sarcophyton, Echinopora	Denominator is 0			
Sarcophyton, Acropora	Denominator is 0			
Theonella, Echinopora	Denominator is 0			
Theonella, Acropora	Denominator is 0			
Echinopora, Acropora	Denominator is 0			

Within level 'Southern' of factor 'Reef Complex'
Within level 'Red Sands north' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	Denominator is 0			
Sinularia, Theonella	Denominator is 0			
Sinularia, Echinopora	Denominator is 0			
Sinularia, Acropora	Denominator is 0			
Sarcophyton, Theonella	Denominator is 0			
Sarcophyton, Echinopora	Denominator is 0			
Sarcophyton, Acropora	Denominator is 0			
Theonella, Echinopora	Denominator is 0			
Theonella, Acropora	Denominator is 0			

Echinopora, Acropora	Denominator is 0			

Within level 'Central' of factor 'Reef Complex'
Within level 'Two-mile' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	Denominator is 0			
Sinularia, Theonella	Denominator is 0			
Sinularia, Echinopora	Denominator is 0			
Sinularia, Acropora	Denominator is 0			
Sarcophyton, Theonella	Denominator is 0			
Sarcophyton, Echinopora	Denominator is 0			
Sarcophyton, Acropora	Denominator is 0			
Theonella, Echinopora	Denominator is 0			
Theonella, Acropora	Denominator is 0			
Echinopora, Acropora	Denominator is 0			

Within level 'Central' of factor 'Reef Complex'
Within level 'Nine-mile' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	Denominator is 0			
Sinularia, Theonella	Denominator is 0			
Sinularia, Echinopora	Denominator is 0			
Sinularia, Acropora	Denominator is 0			
Sarcophyton, Theonella	Denominator is 0			
Sarcophyton, Echinopora	Denominator is 0			
Sarcophyton, Acropora	Denominator is 0			
Theonella, Echinopora	Denominator is 0			
Theonella, Acropora	Denominator is 0			
Echinopora, Acropora	Denominator is 0			

Within level 'Central' of factor 'Reef Complex'
Within level 'Regal' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	Denominator is 0			
Sinularia, Theonella	Denominator is 0			
Sinularia, Echinopora	Denominator is 0			
Sinularia, Acropora	Denominator is 0			
Sarcophyton, Theonella	Denominator is 0			
Sarcophyton, Echinopora	Denominator is 0			
Sarcophyton, Acropora	Denominator is 0			
Theonella, Echinopora	Denominator is 0			
Theonella, Acropora	Denominator is 0			
Echinopora, Acropora	Denominator is 0			

Within level 'Northern' of factor 'Reef Complex'
Within level 'Rabbit' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	0.80053	0.6843	4	0.4622
Sinularia, Theonella	1.9751	0.2041	10	0.1184
Sinularia, Echinopora	1.2469	0.3956	4	0.2832
Sinularia, Acropora	0.27782	1	7	0.7892
Sarcophyton, Theonella	2.3339	0.0963	7	0.0809
Sarcophyton, Echinopora	0.50639	1	2	0.6406
Sarcophyton, Acropora	1.1444	0.3967	4	0.3208
Theonella, Echinopora	2.4845	0.1003	7	0.0647
Theonella, Acropora	1.8583	0.2932	10	0.1359
Echinopora, Acropora	1.6486	0.402	4	0.1783

Within level 'Northern' of factor 'Reef Complex'
Within level 'Kosi south' of factor 'Site'

			Unique	

Groups	t	P(perm)	perms	P(MC)
Sinularia, Sarcophyton	1	1	1	0.3761
Sinularia, Theonella	1.9878	0.1903	7	0.1152
Sinularia, Echinopora	1	1	1	0.3702
Sinularia, Acropora	0.15574	1	4	0.8871
Sarcophyton, Theonella	2.7991	0.1015	4	**0.0487**
Sarcophyton, Echinopora	Denominator is 0			
Sarcophyton, Acropora	1.8643	0.3984	2	0.1351
Theonella, Echinopora	2.7991	0.0997	4	**0.0478**
Theonella, Acropora	2.2861	0.1031	10	0.085
Echinopora, Acropora	1.8643	0.4049	2	0.1359

Within level 'Northern' of factor 'Reef Complex'
Within level 'Kosi north' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	Denominator is 0			
Sinularia, Theonella	1.8262	0.391	2	0.1385
Sinularia, Echinopora	1	1	1	0.3716
Sinularia, Acropora	1	1	1	0.3696
Sarcophyton, Theonella	1.8262	0.4091	2	0.1485
Sarcophyton, Echinopora	1	1	1	0.3792
Sarcophyton, Acropora	1	1	1	0.3723
Theonella, Echinopora	1.5116	0.3925	4	0.1982
Theonella, Acropora	0.19353	1	4	0.8595
Echinopora, Acropora	0.80953	1	2	0.4536

Term 'Site(Reef Complex) X Species' for pairs of levels of factor 'Site'

Within level 'Southern' of factor 'Reef Complex'
Within level 'Sinularia' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	Denominator is 0			
Leadsman north, Red Sands south	Denominator is 0			
Leadsman north, Red Sands north	Denominator is 0			
Leadsman south, Red Sands south	Denominator is 0			
Leadsman south, Red Sands north	Denominator is 0			
Red Sands south, Red Sands north	Denominator is 0			

Within level 'Southern' of factor 'Reef Complex'
Within level 'Sarcophyton' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	1.9663	0.1057	4	0.1234
Leadsman north, Red Sands south	1.9663	0.1017	4	0.1168
Leadsman north, Red Sands north	1.9663	0.0957	4	0.1182
Leadsman south, Red Sands south	Denominator is 0			
Leadsman south, Red Sands north	Denominator is 0			
Red Sands south, Red Sands north	Denominator is 0			

Within level 'Southern' of factor 'Reef Complex'
Within level 'Theonella' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	1.8162	0.104	4	0.1444
Leadsman north, Red Sands south	1.8162	0.1044	4	0.1434
Leadsman north, Red Sands north	1.8162	0.0968	4	0.1491
Leadsman south, Red Sands south	Denominator is 0			
Leadsman south, Red Sands north	Denominator is 0			
Red Sands south, Red Sands north	Denominator is 0			

Within level 'Southern' of factor 'Reef Complex'
Within level 'Echinopora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	Denominator is 0			
Leadsman north, Red Sands south	Denominator is 0			
Leadsman north, Red Sands north	Denominator is 0			
Leadsman south, Red Sands south	Denominator is 0			
Leadsman south, Red Sands north	Denominator is 0			
Red Sands south, Red Sands north	Denominator is 0			

Within level 'Southern' of factor 'Reef Complex'
Within level 'Acropora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	Denominator is 0			
Leadsman north, Red Sands south	Denominator is 0			
Leadsman north, Red Sands north	Denominator is 0			
Leadsman south, Red Sands south	Denominator is 0			
Leadsman south, Red Sands north	Denominator is 0			
Red Sands south, Red Sands north	Denominator is 0			

Within level 'Central' of factor 'Reef Complex'
Within level 'Sinularia' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	Denominator is 0			
Two-mile, Regal	Denominator is 0			
Nine-mile, Regal	Denominator is 0			

Within level 'Central' of factor 'Reef Complex'
Within level 'Sarcophyton' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	Denominator is 0			
Two-mile, Regal	Denominator is 0			
Nine-mile, Regal	Denominator is 0			

Within level 'Central' of factor 'Reef Complex'
Within level 'Theonella' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	Denominator is 0			
Two-mile, Regal	Denominator is 0			
Nine-mile, Regal	Denominator is 0			

Within level 'Central' of factor 'Reef Complex'
Within level 'Echinopora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	Denominator is 0			
Two-mile, Regal	Denominator is 0			
Nine-mile, Regal	Denominator is 0			

Within level 'Central' of factor 'Reef Complex'
Within level 'Acropora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	Denominator is 0			
Two-mile, Regal	Denominator is 0			
Nine-mile, Regal	Denominator is 0			

Within level 'Northern' of factor 'Reef Complex'
Within level 'Sinularia' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	0.26076	1	4	0.8121
Rabbit, Kosi north	1.5756	0.3997	2	0.1862
Kosi south, Kosi north	1	1	1	0.3755

Within level 'Northern' of factor 'Reef Complex'
Within level 'Sarcophyton' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	1	1	1	0.3751
Rabbit, Kosi north	1	1	1	0.3805
Kosi south, Kosi north	Denominator is 0			

Within level 'Northern' of factor 'Reef Complex'
Within level 'Theonella' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	0.50572	0.7982	10	0.6435
Rabbit, Kosi north	0.44838	0.593	10	0.6842
Kosi south, Kosi north	0.02731	1	10	0.9813

Within level 'Northern' of factor 'Reef Complex'
Within level 'Echinopora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	1	1	1	0.3665
Rabbit, Kosi north	0.64106	1	2	0.5528
Kosi south, Kosi north	1	1	1	0.3675

Within level 'Northern' of factor 'Reef Complex'
Within level 'Acropora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	0.89114	0.3965	7	0.4266
Rabbit, Kosi north	0.58249	1	4	0.5867
Kosi south, Kosi north	0.79444	1	4	0.4647

Table TS 4.11
***Post hoc* pair-wise tests for Reef Complex crossed with Species for simazine concentration data.**

Term 'Reef Complex x Species' for pairs of levels of factor 'Species'

Within level 'Southern' of factor 'Reef Complex'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	Denominator is 0			
Sinularia, Theonella	Denominator is 0			
Sinularia, Echinopora	Denominator is 0			
Sinularia, Acropora	Denominator is 0			
Sarcophyton, Theonella	Denominator is 0			
Sarcophyton, Echinopora	Denominator is 0			
Sarcophyton, Acropora	Denominator is 0			
Theonella, Echinopora	Denominator is 0			
Theonella, Acropora	Denominator is 0			
Echinopora, Acropora	Denominator is 0			

Within level 'Central' of factor 'Reef Complex'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	Denominator is 0			
Sinularia, Theonella	Denominator is 0			
Sinularia, Echinopora	Denominator is 0			
Sinularia, Acropora	Denominator is 0			
Sarcophyton, Theonella	Denominator is 0			
Sarcophyton, Echinopora	Denominator is 0			
Sarcophyton, Acropora	Denominator is 0			
Theonella, Echinopora	Denominator is 0			
Theonella, Acropora	Denominator is 0			
Echinopora, Acropora	Denominator is 0			

Within level 'Northern' of factor 'Reef Complex'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	2.454	0.0312	7299	**0.0289**
Sinularia, Theonella	2.2494	0.044	9825	**0.0472**
Sinularia, Echinopora	2.1214	0.051	9736	0.0597
Sinularia, Acropora	1.5883	0.1412	9858	0.1387
Sarcophyton, Theonella	2.7726	0.0159	9123	**0.0164**
Sarcophyton, Echinopora	1	0.3925	257	0.3346
Sarcophyton, Acropora	1.3618	0.2562	4426	0.1961
Theonella, Echinopora	2.7179	0.0164	9847	**0.019**
Theonella, Acropora	2.6271	0.0205	9860	**0.0211**
Echinopora, Acropora	0.74213	0.4987	9527	0.4754

Term 'Reef Complex x Species' for pairs of levels of factor 'Reef Complex'

Within level 'Sinularia' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	Denominator is 0			
Southern, Northern	2.8336	0.0095	8477	**0.0151**
Central, Northern	2.454	0.0267	7152	**0.0287**

Within level 'Sarcophyton' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	Denominator is 0			
Southern, Northern	Denominator is 0			

Central, Northern	Denominator is 0			

Within level 'Theonella' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	Denominator is 0			
Southern, Northern	3.2015	0.0057	9511	**0.006**
Central, Northern	2.7726	0.0189	9166	**0.0157**

Within level 'Echinopora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	Denominator is 0			
Southern, Northern	1.1547	0.2899	516	0.274
Central, Northern	1	0.3981	256	0.3342

Within level 'Acropora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	Denominator is 0			
Southern, Northern	1.5725	0.156	6131	0.136
Central, Northern	Negative			

Table TS 4.12

***Post hoc* pair-wise tests for Reef Complex crossed with Species for multivariate herbicide data.**

Term 'Reef Complex x Species' for pairs of levels of factor 'Reef Complex'

Within level 'Sinularia' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	2.6115	0.0046	9967	**0.0042**
Southern, Northern	2.7951	0.0018	9957	**0.0022**
Central, Northern	1.7869	0.0473	9966	0.0538

Within level 'Sarcophyton' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	3.6924	0.0017	9958	**0.0014**
Southern, Northern	6.276	0.0002	9956	**0.0001**
Central, Northern	2.8889	0.0006	9961	**0.0013**

Within level 'Theonella' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	1.6121	0.0448	9948	0.0703
Southern, Northern	3.3458	0.0022	9940	**0.0022**
Central, Northern	3.1101	0.0056	9942	**0.0052**

Within level 'Echinopora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	0.67529	0.748	9954	0.7079
Southern, Northern	1.5131	0.0945	9953	0.102
Central, Northern	1.075	0.3429	9954	0.3324

Within level 'Acropora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Southern, Central	Negative			
Southern, Northern	2.1397	0.0043	9953	**0.0084**
Central, Northern	1.6362	0.051	9956	0.0712

Term 'Reef Complex x Species' for pairs of levels of factor 'Species'

Within level 'Southern' of factor 'Reef Complex'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	7.4931	0.0001	9954	**0.0001**
Sinularia, Theonella	3.5466	0.0001	9955	**0.0002**
Sinularia, Echinopora	4.6855	0.0001	9948	**0.0001**
Sinularia, Acropora	5.6629	0.0001	9925	**0.0001**
Sarcophyton, Theonella	9.0761	0.0001	9947	**0.0001**
Sarcophyton, Echinopora	9.6551	0.0001	9960	**0.0001**
Sarcophyton, Acropora	10.022	0.0001	9951	**0.0001**
Theonella, Echinopora	1.4942	0.0742	9951	0.1071
Theonella, Acropora	2.1168	0.0018	9952	**0.0123**
Echinopora, Acropora	1.4793	0.1028	9942	0.1109

Within level 'Central' of factor 'Reef Complex'

Unique

Groups	t	P(perm)	perms	P(MC)
Sinularia, Sarcophyton	3.5153	0.0012	9948	**0.0006**
Sinularia, Theonella	3.8661	0.0002	9951	**0.0001**
Sinularia, Echinopora	6.7523	0.0002	9945	**0.0001**
Sinularia, Acropora	6.4571	0.0001	9931	**0.0002**
Sarcophyton, Theonella	3.8177	0.001	9946	**0.0004**
Sarcophyton, Echinopora	4.4723	0.0004	9936	**0.0001**
Sarcophyton, Acropora	3.4186	0.0001	9932	**0.0011**
Theonella, Echinopora	2.6284	0.0033	9948	**0.0067**
Theonella, Acropora	1.4358	0.1628	9956	0.1591
Echinopora, Acropora	1.3498	0.1531	9942	0.1709

Within level 'Northern' of factor 'Reef Complex'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	2.1109	0.0207	9956	**0.027**
Sinularia, Theonella	1.9514	0.0561	9942	0.0544
Sinularia, Echinopora	2.0902	0.0159	9950	**0.0189**
Sinularia, Acropora	2.8728	0.0017	9950	**0.0022**
Sarcophyton, Theonella	1.218	0.2382	9964	0.2289
Sarcophyton, Echinopora	3.1131	0.0021	9944	**0.0028**
Sarcophyton, Acropora	3.465	0.0012	9936	**0.0003**
Theonella, Echinopora	2.9513	0.0066	9922	**0.0073**
Theonella, Acropora	3.2419	0.0055	9942	**0.0042**
Echinopora, Acropora	1.4774	0.0983	9944	0.1109

Table TS 4.13

***Post hoc* pair-wise tests for Sites nested in Reef Complex crossed with Species for multivariate herbicide data.**

Term 'Site nested in Reef Complex crossed with Species' for pairs of levels of factor 'Species'

Within level 'Southern' of factor 'Reef Complex'
Within level 'Leadsman north' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	9.7592	0.0996	10	**0.0031**
Sinularia, Theonella	1.3985	0.0998	10	0.2052
Sinularia, Echinopora	2.9708	0.0961	10	**0.0148**
Sinularia, Acropora	3.4366	0.1031	10	**0.0095**
Sarcophyton, Theonella	7.2816	0.0971	10	**0.0053**
Sarcophyton, Echinopora	11.978	0.0999	10	**0.0019**
Sarcophyton, Acropora	12.101	0.1044	10	**0.0022**
Theonella, Echinopora	1.8586	0.1046	10	0.09
Theonella, Acropora	2.0082	0.0994	10	0.0705
Echinopora, Acropora	1.0925	0.4011	10	0.3341

Within level 'Southern' of factor 'Reef Complex'
Within level 'Leadsman south' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	4.8452	0.1005	10	**0.0088**
Sinularia, Theonella	1.0637	0.4978	10	0.3655
Sinularia, Echinopora	1.5208	0.294	10	0.1492
Sinularia, Acropora	2.6667	0.1037	10	**0.0247**
Sarcophyton, Theonella	4.9925	0.0969	10	**0.0053**
Sarcophyton, Echinopora	4.7602	0.1026	10	**0.0069**
Sarcophyton, Acropora	5.1139	0.0956	10	**0.007**
Theonella, Echinopora	1.3724	0.3975	10	0.2111
Theonella, Acropora	1.4459	0.2991	10	0.1983
Echinopora, Acropora	1.6536	0.2018	10	0.1502

Within level 'Southern' of factor 'Reef Complex'
Within level 'Red Sands south' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	2.1256	0.107	10	**0.0454**
Sinularia, Theonella	4.699	0.1037	10	**0.0039**
Sinularia, Echinopora	4.8022	0.0984	10	**0.0029**
Sinularia, Acropora	5.2663	0.0949	7	**0.0026**
Sarcophyton, Theonella	3.8854	0.1025	10	**0.0064**
Sarcophyton, Echinopora	4.1547	0.1052	10	**0.0045**
Sarcophyton, Acropora	4.2591	0.0997	7	**0.005**
Theonella, Echinopora	0.85336	0.5008	10	0.46
Theonella, Acropora	1.8094	0.2048	7	0.1186
Echinopora, Acropora	2.8001	0.099	7	0.0517

Within level 'Southern' of factor 'Reef Complex'
Within level 'Red Sands north' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	2.6596	0.1003	10	**0.0347**
Sinularia, Theonella	1.8274	0.203	10	0.1118
Sinularia, Echinopora	2.0663	0.098	10	0.0786
Sinularia, Acropora	1.4386	0.3052	10	0.1929
Sarcophyton, Theonella	3.2285	0.0959	10	**0.024**
Sarcophyton, Echinopora	3.3134	0.0944	10	**0.0214**
Sarcophyton, Acropora	3.2987	0.0974	10	**0.0222**
Theonella, Echinopora	0.78924	0.6991	7	0.5207
Theonella, Acropora	1.1943	0.3018	10	0.2873

Echinopora, Acropora	1.9402	0.1972	7	0.1186

Within level 'Central' of factor 'Reef Complex'
Within level 'Two-mile' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	0.77483	0.4989	10	0.5325
Sinularia, Theonella	5.3873	0.1015	10	**0.0034**
Sinularia, Echinopora	4.3918	0.0978	10	**0.0061**
Sinularia, Acropora	5.5194	0.1015	10	**0.0032**
Sarcophyton, Theonella	2.2888	0.1001	10	0.0681
Sarcophyton, Echinopora	2.0147	0.0995	10	0.1019
Sarcophyton, Acropora	2.391	0.0959	10	0.0704
Theonella, Echinopora	1.0415	0.4951	10	0.3878
Theonella, Acropora	0.97845	0.3995	10	0.399
Echinopora, Acropora	1.5737	0.1994	10	0.1291

Within level 'Central' of factor 'Reef Complex'
Within level 'Nine-mile' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	3.1757	0.0933	10	**0.0111**
Sinularia, Theonella	2.1409	0.101	10	**0.0454**
Sinularia, Echinopora	4.7427	0.1036	10	**0.0041**
Sinularia, Acropora	6.7954	0.1062	4	**0.0004**
Sarcophyton, Theonella	2.485	0.1035	10	**0.0339**
Sarcophyton, Echinopora	4.2836	0.0995	10	**0.0032**
Sarcophyton, Acropora	4.5399	0.1014	4	**0.0031**
Theonella, Echinopora	1.636	0.1023	10	0.1424
Theonella, Acropora	1.722	0.1005	4	0.1344
Echinopora, Acropora	1.9809	0.1016	4	0.1025

Within level 'Central' of factor 'Reef Complex'
Within level 'Regal' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	3.1124	0.0995	10	**0.0305**
Sinularia, Theonella	1.0053	0.5049	10	0.3809
Sinularia, Echinopora	5.6795	0.1014	10	**0.0035**
Sinularia, Acropora	Negative			
Sarcophyton, Theonella	2.6791	0.0972	10	**0.0456**
Sarcophyton, Echinopora	3.091	0.0982	10	**0.0354**
Sarcophyton, Acropora	Negative			
Theonella, Echinopora	2.8359	0.1005	10	**0.0178**
Theonella, Acropora	Negative			
Echinopora, Acropora	Negative			

Within level 'Northern' of factor 'Reef Complex'
Within level 'Rabbit' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	2.2583	0.1015	10	**0.0358**
Sinularia, Theonella	2.2834	0.1023	10	**0.0384**
Sinularia, Echinopora	1.4686	0.1011	10	0.1673
Sinularia, Acropora	1.2422	0.3042	10	0.2731
Sarcophyton, Theonella	1.2524	0.3021	10	0.2641
Sarcophyton, Echinopora	2.794	0.1032	10	**0.0153**
Sarcophyton, Acropora	2.755	0.0994	10	**0.0157**
Theonella, Echinopora	3.4273	0.1022	10	**0.0107**
Theonella, Acropora	3.2032	0.1029	10	**0.0131**
Echinopora, Acropora	0.63313	0.9037	10	0.6963

Within level 'Northern' of factor 'Reef Complex'
Within level 'Kosi south' of factor 'Site'

			Unique	

Groups	t	P(perm)	perms	P(MC)
Sinularia, Sarcophyton	1.5699	0.2937	10	0.1809
Sinularia, Theonella	0.86786	0.5888	10	0.4892
Sinularia, Echinopora	1.4876	0.1023	10	0.1592
Sinularia, Acropora	1.8512	0.0962	10	0.0973
Sarcophyton, Theonella	1.1204	0.3992	10	0.3253
Sarcophyton, Echinopora	1.8892	0.099	10	0.1184
Sarcophyton, Acropora	2.024	0.1007	10	0.1044
Theonella, Echinopora	1.6947	0.2035	10	0.1273
Theonella, Acropora	1.9381	0.0972	10	0.0808
Echinopora, Acropora	1.0864	0.2991	10	0.3413

Within level 'Northern' of factor 'Reef Complex'
Within level 'Kosi north' of factor 'Site'

Groups	t	P(perm)	Unique perms	P(MC)
Sinularia, Sarcophyton	0.69182	0.7025	10	0.6246
Sinularia, Theonella	1.2887	0.3956	10	0.2649
Sinularia, Echinopora	1.3866	0.2035	10	0.2009
Sinularia, Acropora	2.0481	0.0967	10	0.0685
Sarcophyton, Theonella	1.1334	0.3078	10	0.3149
Sarcophyton, Echinopora	1.0245	0.5983	10	0.3714
Sarcophyton, Acropora	1.7907	0.0969	10	0.1238
Theonella, Echinopora	1.6249	0.4056	10	0.1719
Theonella, Acropora	1.92	0.0959	10	0.115
Echinopora, Acropora	1.8606	0.1009	10	0.0872

Term 'Site(Reef Complex) x Species' for pairs of levels of factor 'Site'

Within level 'Southern' of factor 'Reef Complex'
Within level 'Sinularia' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	2.6313	0.1004	10	**0.0209**
Leadsman north, Red Sands south	2.8576	0.0984	10	**0.02**
Leadsman north, Red Sands north	0.49506	0.8	10	0.7825
Leadsman south, Red Sands south	4.232	0.1033	10	**0.0055**
Leadsman south, Red Sands north	1.6146	0.3037	10	0.1561
Red Sands south, Red Sands north	2.5319	0.1059	10	**0.0232**

Within level 'Southern' of factor 'Reef Complex'
Within level 'Sarcophyton' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	Negative			
Leadsman north, Red Sands south	7.3622	0.1013	10	**0.0045**
Leadsman north, Red Sands north	4.2834	0.095	10	**0.0187**
Leadsman south, Red Sands south	3.7587	0.0973	10	**0.0151**
Leadsman south, Red Sands north	2.857	0.102	10	**0.0403**
Red Sands south, Red Sands north	1.2349	0.406	10	0.2768

Within level 'Southern' of factor 'Reef Complex'
Within level 'Theonella' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	1.8495	0.0967	10	0.0865
Leadsman north, Red Sands south	1.8703	0.0999	10	0.0889
Leadsman north, Red Sands north	1.9774	0.1028	10	0.065
Leadsman south, Red Sands south	0.62716	0.4976	10	0.6441
Leadsman south, Red Sands north	0.60178	0.5947	10	0.6083
Red Sands south, Red Sands north	0.66216	0.6964	10	0.6307

Within level 'Southern' of factor 'Reef Complex'
Within level 'Echinopora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	1.6219	0.3001	10	0.1385
Leadsman north, Red Sands south	0.44181	0.7961	10	0.7448
Leadsman north, Red Sands north	2.015	0.1927	7	0.0975
Leadsman south, Red Sands south	1.7843	0.0983	10	0.1021
Leadsman south, Red Sands north	1.7176	0.2972	10	0.1335
Red Sands south, Red Sands north	2.049	0.2029	7	0.1008

Within level 'Southern' of factor 'Reef Complex'
Within level 'Acropora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Leadsman north, Leadsman south	1.2106	0.3925	7	0.2814
Leadsman north, Red Sands south	1.5478	0.3999	4	0.1811
Leadsman north, Red Sands north	1.3454	0.2982	10	0.2497
Leadsman south, Red Sands south	0.89196	1	2	0.4531
Leadsman south, Red Sands north	2.1533	0.0992	7	0.084
Red Sands south, Red Sands north	2.3856	0.1004	7	0.0745

Within level 'Central' of factor 'Reef Complex'
Within level 'Sinularia' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	1.8708	0.2007	10	0.109
Two-mile, Regal	5.5057	0.1048	10	**0.0019**
Nine-mile, Regal	5.95	0.1042	10	**0.0025**

Within level 'Central' of factor 'Reef Complex'
Within level 'Sarcophyton' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	1.7054	0.1031	10	0.0962
Two-mile, Regal	1.9578	0.0962	10	0.0708
Nine-mile, Regal	1.2486	0.1975	10	0.2647

Within level 'Central' of factor 'Reef Complex'
Within level 'Theonella' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	1.2984	0.1006	10	0.2431
Two-mile, Regal	2.5977	0.1035	10	**0.0201**
Nine-mile, Regal	0.52667	0.5936	10	0.6963

Within level 'Central' of factor 'Reef Complex'
Within level 'Echinopora' of factor 'Species'

Unique

Groups	t	P(perm)	perms	P(MC)
Two-mile, Nine-mile	1.2009	0.1947	10	0.2802
Two-mile, Regal	1.2845	0.2021	10	0.2442
Nine-mile, Regal	1.1627	0.2977	10	0.3096

Within level 'Central' of factor 'Reef Complex'
Within level 'Acropora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Two-mile, Nine-mile	2.7467	0.1009	4	0.0529
Two-mile, Regal	Negative			
Nine-mile, Regal	Denominator is 0			

Within level 'Northern' of factor 'Reef Complex'
Within level 'Sinularia' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	0.48315	1	10	0.8427
Rabbit, Kosi north	1.2592	0.2048	10	0.2535
Kosi south, Kosi north	1.0057	0.4943	10	0.4098

Within level 'Northern' of factor 'Reef Complex'
Within level 'Sarcophyton' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	0.39339	0.8034	10	0.8464
Rabbit, Kosi north	1.2622	0.2991	10	0.2523
Kosi south, Kosi north	1.2012	0.4026	10	0.2931

Within level 'Northern' of factor 'Reef Complex'
Within level 'Theonella' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	0.60764	0.8001	10	0.6834
Rabbit, Kosi north	0.90337	0.4979	10	0.4245
Kosi south, Kosi north	1.1072	0.305	10	0.3299

Within level 'Northern' of factor 'Reef Complex'
Within level 'Echinopora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	0.93962	0.4962	10	0.4437
Rabbit, Kosi north	1.8196	0.0999	10	0.1181
Kosi south, Kosi north	1.9717	0.0911	10	0.0776

Within level 'Northern' of factor 'Reef Complex'
Within level 'Acropora' of factor 'Species'

Groups	t	P(perm)	Unique perms	P(MC)
Rabbit, Kosi south	1.469	0.2	10	0.1751
Rabbit, Kosi north	0.87574	0.7058	10	0.4942
Kosi south, Kosi north	1.0095	0.4006	10	0.3909