IMPACTS OF ANTIMICROBIAL COMPOUNDS IN URBAN WATERWAYS RECEIVING SEWER OVERFLOWS

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Abstract

Personal care products (PCPs) are a subset of emerging contaminants detected in waterways globally and include antimicrobial compounds, preservatives, organic UV-filters and industrial chemicals. These compounds are washed down household drains every day and can enter waterways via stormwater, sewer overflows and wastewater treatment plant effluents. There is growing concern about the effects of PCPs on aquatic ecosystems as many of these compounds impact microorganisms and are endocrine disruptors. Though research into the presence of PCPs is increasing, the majority of studies originate from Europe and North America and there is limited data on the presence and effects of PCPs in New Zealand waterways. The results from the work undertaken in this thesis will contribute to the development of any future regulations for PCPs in New Zealand.

The occurrence of PCPs and effects on bacterial community structure were investigated over six months in two Christchurch urban streams previously impacted by sewer overflows: Dudley Creek Diversion and Cross Stream. The UV-filters benzophenone-3 and octyl-methoxycinnamate, and bisphenol A were frequently detected. Concentrations of UV-filters were lower during the winter months. Other detected compounds included methyl paraben, octylphenol, o-phenylphenol and triclosan. Compounds were detected in the low ng/L range in stream water and low ng/g range in sediment. As no sewer overflows occurred over the course of the study there were limited differences observed between upstream and downstream concentrations. Previous sewer overflows were likely to be the source of benzophenone-3 at Dudley Creek Diversion as downstream sediment concentrations were significantly higher than upstream. Triclosan was also detected in two sediment samples downstream at Dudley Creek Diversion in March and April but was not detected in any upstream samples. The sediment bacterial communities at Dudley Creek Diversion were significantly different at upstream and downstream sites of the overflow outfall indicating that contaminants derived from sewerage inputs may alter sediment bacterial community composition. Though concentrations were lower than those reported to have toxic effects in waterways, several compounds were identified using multivariate multiple regression with distance-based linear modelling as having an effect on the structure of bacterial communities including benzophenone-3, octyl-methoxycinnamate, triclosan and bisphenol A.

The effect of the antimicrobial triclosan on the photosynthetic activity of the green alga *Stigeoclonium* sp. and cyanobacteria *Phormidium autumnale* was investigated. *Stigeoclonium* sp. was more sensitive to triclosan than *Phormidium autumnale*, the 96 hr EC_{50} values were 1.23 and 3.17 mg/L, respectively. Both organisms examined are commonly found in New Zealand rivers and these results provide new information on the effect of PCPs in New Zealand waterways.

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Abbreviations and Acronyms

3PBOH	3-phenoxybenzyl alcohol
4MBC	4-methylbenzylidene camphor
ACN	Acetonitrile
APEO	Alkylphenol ethoxylates
ASE	Accelerated solvent extraction
BP1	Benzophenone-1
BP3	Benzophenoe-3
BPA	Bisphenol A
BPA-DA	Bisphenol A diacetate
bParaben	Butyl paraben
bzParaben	Benzyl paraben
DCM	Dichloromethane
DO	Dissolved oxygen
E1	Estrone
E2	17β-estradiol
E3	Estriol
ECs	Emerging contaminants
EE2	17α-ethinyl estradiol
eParaben	Ethyl paraben
GCMS	Gas chromatography mass spectrometry
GPC	Gel permeation chromatography
HPLC	High performance liquid chromatography
HTS	High through-put sequencing
IPA	Isopropyl alcohol
LOD	Limit of detection
mParaben	Methyl paraben
MQ	MilliQ
mTCS	Methyl triclosan
NP	Nonylphenol
OMC	Ocyl-methoxycinnamate
OP	Octylphenol
OPP	O-phenylphenol
OTU	Operational taxonomic unit
PCPs	Personal care products
PCR	Polymerase chain reaction
pParaben	Propylparaben
SPE	Solid phase extraction
TCS	Triclosan
TOC	Total organic carbon

TSS	Total suspended solids
WWTP	Wastewater treatment plant

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1 Introduction

Stream ecosystems in urbanised areas are impacted by multiple stressors and as a result are vulnerable to severe degradation and modification.[1] An increase in impervious surface cover leading to augmented surface runoff has been identified as the major effect of urbanisation on urban streams.[2] Streams that drain urban land are regularly found to be in an ecologically degraded state.[3] This observation has led to the term "urban stream syndrome" being used to describe the often degraded state of these ecosystems.[3] Urban stormwater has been demonstrated to contribute to the deteriorating quality of receiving waters, carrying nutrient pollution and conventional contaminants such as heavy metals.[4] In recent years stormwater contamination by a wide range of organic compounds has been highlighted with over 650 compounds identified as being present at trace concentrations.[4] Though the primary source of contaminants is urban stormwater runoff, sewer overflows and wastewater treatment plant (WWTP) effluents can also be major contributors.[3] Personal care products (PCPs) are a class of organic contaminants for which there is little data for urban streams in New Zealand.

It has been acknowledged that the lack of regulations in place for PCPs is an issue for managing these compounds.[5] There is very little monitoring of these unregulated compounds to determine their presence in the aquatic environment.[5] The European Union has developed strategies to deal with some PCPs however globally limits and regulations for PCPs have not been specifically set for water bodies.[5] Knowledge of the presence and effects of PCPs in New Zealand waterways is critical to inform the development of any future regulations. This study investigates the occurrence and impact of PCPs in two Christchurch urban streams: Dudley Creek Diversion and Cross Stream.

1.1 Significance of personal care products as emerging contaminants

In most cases, the term 'emerging contaminants' (ECs) is used to describe unmonitored or unregulated contaminants. Research on their presence, and impacts on human health and the ecosystem could mean more ECs become candidates for regulation in future.[6] Emerging contaminants have likely been present in the environment since their introduction, however as analytical instruments have become more sensitive in recent times and ECs are now detected, concern about their effects on ecosystems is growing.[6]

Personal care products (PCPs) are used every day in households resulting in the release of chemical ingredients, along with degradation products, into the environment.[7] Personal care products are a subset of emerging contaminants and are recognised as trace pollutants which have recently been identified in freshwater systems. These compounds may pose a threat to human and ecosystem health.[8] Compounds classed as PCPs include antimicrobial compounds, preservatives, organic UV-filters and industrial

chemicals such as BPA.[9] Sewer overflows and stormwater runoff have been recognised worldwide as a source of PCPs entering urban waterways.[10]

Personal care products are often studied alongside pharmaceuticals which are used to treat disease and are also released into the aquatic environment via domestic wastewater.[11] There have been more than 150 studies investigating the presence of pharmaceuticals and personal care products (PPCPs) in receiving waters.[12] However, often only a few PCPs are focused on in an individual study and the suite of compounds analysed between studies are not consistent, making it difficult to compare levels of PCP pollution.[12] Further, 80% of studies on the occurrence of PPCPs come from Europe and North America.[12] Of these studies, considerably more research has been conducted into investigating the occurrence of pharmaceuticals in the environment than personal care products and there is a large focus on water bodies receiving WWTP effluent.[7] To gain a more comprehensive understanding of PCP contamination in waterways on a global scale, studies in other regions are needed, along with expanding the range of compounds analysed, with a larger focus on personal care products.[12]

1.2 Key target analytes and effects on physiological processes

Triclosan

Triclosan is a broad spectrum synthetic antimicrobial compound primarily found in many personal care products including toothpaste, hand soap and deodorant. Other uses include incorporation into plastic materials and kitchen utensils.[13] Triclosan is classed as a halogenated aromatic hydrocarbon with phenolic, diphenyl ether and polychlorinated biphenyl functionalities (Figure 1.2a).[14] Triclosan's mode of action for bacteria is the inhibition of the enzyme enoyl acyl carrier protein reductase leading to blocked lipid biosynthesis.[15] In PCPs, triclosan is included at around 0.1% to 0.3% (w/w) and the formulations are applied externally.[14] Consequently triclosan is washed off into domestic wastewater with no metabolic changes.[14] Release from PCPs into domestic wastewater is the main source of triclosan in the environment.[14] A recent review calls for triclosan to be considered on the priority list for emerging contaminants due to its widespread use and toxic effects on aquatic organisms.[14] Lack of available monitoring data could be a key reason for the absence of triclosan on lists prioritising contaminants of concern.[14]

Triclosan can readily photodegrade[13] and has a low aqueous solubility and with a high octanol-water partitioning coefficient (log K_{OW} = 4.8), has the potential to bioaccumulate.[14] The degradation product of triclosan, methyl triclosan (mTriclosan) is more lipophilic than the parent compound and is resistant to photodegradation causing further environmental concern.[14, 16]

Both triclosan and mTriclosan have been found to bioaccumulate in algae, with concentrations in algae detected at an order of magnitude higher than water samples from the same stream.[17] Accumulation has also been observed in higher trophic level species such as snails,[18] fish[18] and dolphins[19]. Several studies have indicated triclosan toxicity for a number of species including invertebrates, fish, macrophytes and algae.[13, 20] Algae show the greatest sensitivity to triclosan with adverse effects on growth displayed below 1 µg/L.[7]

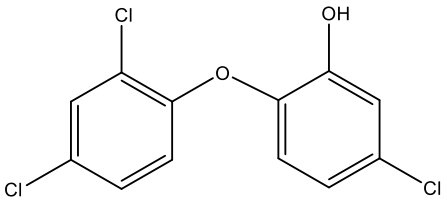


Figure 1.2a. Triclosan

Parabens

Parabens are esters of para-hydroxybenzoic acid and have either an alkyl or benzyl group.[21] Parabens are typically found in a wide range of foodstuffs, pharmaceuticals and cosmetics as preservatives due to their broad spectrum antimicrobial and antifungal properties and have been used for approximately 100 years.[21] Increased alkyl chain length gives increased antimicrobial function and decreased water solubility, often resulting in the use of multiple parabens in products to achieve optimal antimicrobial activity.[22] The European Union regulates the use of parabens in cosmetics. However, for the environment, particularly water, there are no guidelines for acceptable concentrations.[21] The structures of methyl and propyl paraben are presented in Figure 1.2b.

Parabens have displayed estrogenic activity, a cause of human health and environmental concern however the potency of the effect is still debated.[23] In rainbow trout ethyl-,propyl- and butyl paraben all showed estrogenic activity between 100 and 300 mg/kg body weight however the level of exposure required to reach these concentrations would not be typical in the environment. [22a] Reduced sperm counts in animals have also been reported, but not at environmentally relevant concentrations.[22b]

Parabens have been shown to biodegrade under aerobic conditions and have an estimated low to moderate potential for bioaccumulation with log K_{OW} values in the range of 1.96 and 3.57.[22a]

i. ii

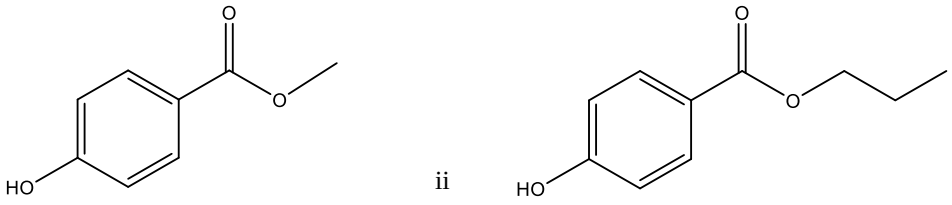


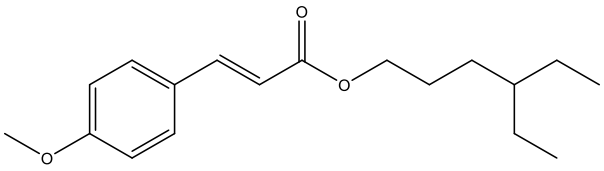
Figure 1.2b. Methyl paraben (i) and Propyl paraben (ii).

UV filters

Sunscreen and many products contain UV filters including skin creams, cosmetics and hair dyes.[24] UV-filters are also used in building materials and paints.[25] Organic UV-filters absorb UV light to protect the skin or hair. Like other PCPs UV filters are washed off and enter domestic waste water or can be directly input into rivers/lakes during swimming or bathing.[24, 26] With growing public awareness of the harmful damage of UV radiation there has been an increase in the use of sunscreen, causing concern regarding the environmental impact of UV filters.[27] As a result, research on the occurrence of UV filters in PCPs and the environment is increasing.[26]

Due to the high lipophilicity of some UV-filters, there is potential for bioaccumulation in aquatic organisms.[24] The log K_{OW} values for compounds in this study have been reported as 3.8 for benzophenone-3 (BP-3; Figure 1.2c), 5.1 for 4-Methylbenzylidene camphor 5.1 (4-MBC) and 6.0 for octyl methoxycinnamate (OMC; Figure 1.2c).[24] UV-filters have been measured in fish and 4-MBC has been reported in fish at substantially higher concentrations than in respective surface water samples.[24, 28] Effects on various organisms have also been examined with 4-MBC and OMC showing estrogenic effects in fish and BP3 adversely effecting reproduction.[29] Microalgae have been shown to be more sensitive to UV-filters than organisms of higher trophic levels.[26] An EC_{50} value is defined as the concentration of a toxicant that induces a response halfway between the baseline and maximum response. For BP3 exposure the EC_{50} for the microalgae *Isochrisis galbana* 13.87 µg/L compared with 3118.19 µg/L for sea urchin larvae.[30]

i.



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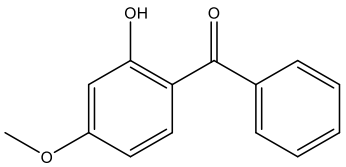
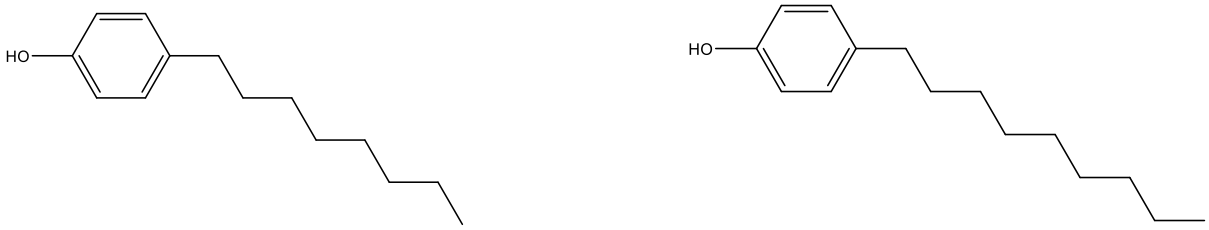


Figure 1.2c. Octyl methoxycinnamate (i) and benzophenone-3 (ii).

Surfactant degradation products

Nonylphenol (NP) and octylphenol (OP) ethoxylates are the most commonly used alkylphenol ethoxylates (APEOs) non-ionic surfacants.[31] Alkylphenol ethoxylates are used in formulations for many products including detergents[32], pesticides[32], textiles[33] and paints[33] and are ubiquitous in wastewater due to their widespread use.[32] There are two main degradation pathways for APEOs, the non-oxidative

pathway which occurs mostly in WWTPs and the oxidative pathway which is dominant in natural waters.[31] Nonylphenol and OP are degradation products of their corresponding ethoxylates and are of concern due their endocrine system toxicity which is greater than the parent compounds.[31] Nonylphenol and OP are relatively stable in the environment.[34] Both OP and NP are confirmed estrogen mimics.[35] Estrogenic responses have been caused in fish by both NP and OP including vitellogenin production induced by both alkylphenols in male fish at 5 µg/L.[34]



i. ii

Figure 1.2d. Octylphenol (i) and nonylphenol (i).

Bisphenol A

Bisphenol A (BPA) is a widely used chemical, important industrially in the production of epoxy resins and polycarbonate plastics.[36] Many everyday products contain these plastics including DVDs, sports equipment, food cans and reusable bottles.[36] Bisphenol A is frequently detected in surface waters globally.[37] The primary route for BPA into the environment is sewage effluent and landfill sites with some contribution from leaching of BPA from products into the environment.[37] The half-life for BPA in freshwater is less than 5 days with bacteria being the primary influence on its degradation. [38]

Exposure of aquatic organisms to BPA has increased as usage of products containing the chemical has risen.[37, 39] Endocrine disruption is of particular concern and has been observed in aquatic organisms as BPA is classified as a xenoestrogen.[38-39] Delayed emergence in the midge *Chhironomus riparus* has been reported at exposure of just 0.078 µg/L and at an exposure of 1.75-2.4 µg/L a reduction in sperm density was measured in the brown trout, *Salmo trutta f. fario*.[37] Significant inhibition of growth was observed for the algae *Chlorella pyrenoidosa* and *Scenedesmus obliquus* in acute toxicity tests with EC_{50} values of 63.53 and 26.72 mg/L respectively.[40] Another study has also reported an EC_{50} value for growth inhibition of the alga *Cyclotella caspia* in the mg/L range (7.96 mg/L).[41]

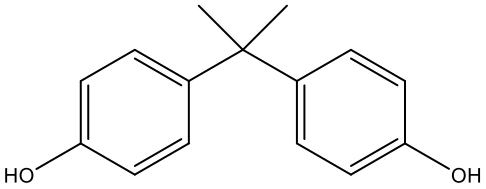


Figure 1.2e. Bisphenol A

1.3 Fate of emerging contaminants in the environment

Organic chemicals including emerging contaminants can undergo a range of physical, chemical and biological processes once released into the environment. These processes may leave the chemical structure unchanged or result in transformation of the chemical.[42] Chemical, photochemical, and biological, particularly microbial transformations generate new compounds.[42] Transformation products are often also environmentally relevant due to increased toxicity or persistence. For example BP3 can be degraded to BP1 which has been shown to be weakly estrogenic[7, 43] while methyl triclosan, the microbial degradation product of triclosan, is of concern due to greater environmental stability and lipophilicity compared to its parent compound.[13]

Sorption can prevent degradation of the chemical by other processes such as biodegradation[44] and photodegradation,[45] while reducing the bioavailability of the compound.[42] The chemical structure of the pollutant and the mineral and organic matter content of the sediments or suspended particulates govern the extent to which sorption processes in the aquatic environment occur.[42] Neutral chemicals, such as those studied in this thesis, will adsorb onto sediments and suspended particulates with neutral hydrophobic surfaces which becomes more favourable with increasing amounts of organic carbon.[42] The octanol/water partitioning coefficient K_{OW} describes the ability of organic contaminants to sorb to particulates and sediment.[46] Compounds with high log K_{OW} values (>5) are likely to easily absorb to sediments.[46]

Accumulation of emerging contaminants may also occur in biota as described for specific chemicals in section 1.3. For aquatic biota direct partitioning between the water, sediments, and organisms is one of the key bioaccumulation processes as well as the more complicated partitioning between consumed food and the internal transport of the chemical within the organism.[42] Metabolism of the chemical may or may not occur before excretion, or the parent compound and/or any degradation products may accumulate in the organism within different tissues.[42]

Photochemical transformation due to sunlight is considered the most important abiotic degradation process for organic chemicals in surface waters.[47] Direct photodegradation may occur if compounds are able to absorb sunlight in the UV wavelength region.[48]. Indirect photodegradation can occur in the presence of photoreactive chemicals, such as nitrate and humic substances, which can generate strong oxidants after reactions under sunlight. The oxidants can then degrade contaminants present in the water.[48]

Though many personal care products may undergo degradation as described above, parent compounds may continuously be released into the environment due to their frequent use and introduction to domestic wastewater.[7] Thus the term “pseudo-persistent” is commonly used to describe such contaminants.[49]

1.4 Photosynthetic microorganisms in urban streams

The impacts on streams in urban areas have been studied in recent years using the change of structure and function of aquatic biota as a focus.[50] The greatest abundance of plant biomass in aquatic environments is comprised of algae, making an appropriate choice for aquatic toxicological studies. However the effect of urbanisation on algal biomass has rarely been studied.[3] Studies have investigated the impacts of urbanisation on algae through correlating community composition with particular water quality parameters such as salinity, pH and nutrient concentrations.[50] However, algae have been underutilised in the field of antimicrobial ecotoxicity. This is despite their unique role as primary producers and crucial links in aquatic food webs.[17] Further studies are required to improve knowledge on the effects of antimicrobial compounds on benthic microorganisms, including algae and bacteria. This will assist in improved predictions of the implications for aquatic ecosystems exposed to these antimicrobial compounds.[17]

Typically soaps, toothpastes and other personal care products (PCPs) contain antimicrobial compounds which are designed to kill or prevent the growth of potentially harmful microorganisms.[8] Sewer overflows release domestic wastewater into streams and non-target microorganisms can be inadvertently exposed to antimicrobial compounds.[8] Species diversity and abundance can be affected, potentially altering the nutrient processing capacity and natural food web structure of affected streams.[8] Microorganisms are particularly sensitive indicators to environmental change and reflect the health of an aquatic ecosystem.[8]

1.5 Effects of triclosan on freshwater microorganisms

There is growing concern about the effect of TCS on aquatic ecosystems, largely due to the compound's antimicrobial properties.[51] Uncertainties remain on the effects of TCS on benthic organisms.[7] A recent study conducted in the USA used an artificial stream experiment to expose microbial communities to environmentally relevant concentrations of TCS. Bioassays were conducted on exposed samples.[51] Triclosan exposure had a significant effect on the proportion of TCS resistant bacteria within sediments.[51] There was a 6-fold increase in the relative number of cyanobacterial sequences, increasing from <1% of the total community to just over 5%.[51] In turn, there was a clear die-off of algae in the community. This suggests that cyanobacteria are more resistant to TCS than algae which could have serious consequences for freshwater ecosystems as cyanobacteria are a public health concern.[51] Previous studies have also shown that exposure to TCS decreases the biomass of some green algae genera relative to algal communities in non-impacted sites, potentially altering community structure.[8]

1.6 Cyanobacteria and risk to human health

Cyanobacteria are microorganisms with both bacterial and algal characteristics. When environmental conditions are favorable some species can multiply rapidly.[52] Proliferations may form as blooms, benthic mats and crusts.[52] Some cyanobacteria produce toxins (cyanotoxins) which can be harmful and potentially fatal when animals including humans are exposed to them.[52] *Phormidium*, a genera of cyanobacteria in which toxin production has been confirmed, is common in lakes and rivers in New Zealand.[52-53] Anatoxin-a and homoanatoxin-a are two neurotoxins produced by *Phormidium.* There have been confirmed dog fatalities from these toxins in New Zealand.[53]

Every summer there is a risk of exposure to cyanotoxins as people take part in recreational freshwater activities. Sewer overflows may cause concentrations of antimicrobial compounds such as triclosan to increase in streams, which may suppress algal growth and enable cyanobacteria to proliferate in the absence of competition from other organisms if cyanobacteria are more resistant to the compound.[51] Little is known about the triggers of cyanobacteria proliferations and toxin production. When benthic mats detach from a substrate, it is thought that cyanotoxins may be released into the water as the mats degrade.[52] Therefore, a risk is also posed to drinking water supplies.[52]

1.7 Project Objectives

Currently there are no regulations regarding acceptable concentrations of chemicals derived from PCPs in New Zealand waterways and there is limited data available regarding their occurrence and toxicity.[54] As these compounds could have ecological impacts at environmental concentrations it is important for more research on their existence and toxicity to be conducted.[54] Little is known about the triggers of cyanobacteria proliferations and more understanding is needed to determine if antimicrobials such as TCS create favourable conditions for growth of cyanobacteria in New Zealand waterways.

The overall aims of this thesis are to:

- Determine if sewer overflows are a significant source of emerging contaminants and in particular anti-microbial compounds in Canterbury urban waterways.
- Characterise the composition and relative abundance of benthic microbial communities upstream and downstream of sewer overflow sites.
- Measure the effects of triclosan on two common species of photosynthetic organisms (a cyanobacterium and green alga) found in urban waterways.

1.8 Thesis Structure

The following two chapters present the experimental work carried out as part of this thesis. The first component was a field study which investigated the occurrence of emerging contaminants and their impact on microbial community structure in two urban streams (Chapter two). In Chapter Three the results of a study measuring the effects of the antimicrobial compound triclosan on the cyanobacterium *Phormidium autumnale* and green alga *Stigeoclonium sp.* are presented. Key conclusions and recommendations are summarised in Chapter Four.

2 Temporal variation of emerging organic contaminants and bacterial communities in urban streams

2.1 Introduction

Chemical contamination of urban streams can have significant impacts on their ecology.[2] The amount of contamination and class of contaminants present in streams is dependent on the extent and type of urbanisation and consequently stormwater drainage, as well as the presence of sewer overflows and wastewater treatment plant (WWTP) effluent.[2] Altered physio-chemical parameters and an increase in contaminants in urban streams is generally observed including oxygen demand, conductivity, suspended solids, nutrients and metals.[2] There is limited data on concentrations of personal care products (a subset of emerging organic contaminants) in urban streams. Sources of PCPs include hygiene products such as hand soap, cleaning products and industrial chemicals as described in section 1.1.[9] These are washed down the drain everyday thus entering domestic wastewater, and potentially the environment.[7] Advancements in WWTP technologies have resulted in reductions in chemical contaminants entering receiving waterways. However, sewer overflows are still common word wide, and untreated sewage can enter streams during storms when the sewer system reaches capacity.[2]

Dudley Creek Diversion and Cross Stream are two urban streams in Christchurch, New Zealand which have sewer overflow outfalls entering them. The two streams were selected for this study and were investigated over a six month period from March – September 2015. Water and sediment samples were collected monthly and analysed for a suite of phenolic antimicrobial compounds and other compounds commonly found in PCPs, as well as for trace elements, nutrients, water quality parameters and bacterial community composition.

2.1.1 Benthic microorganisms and molecular taxonomy

In aquatic environments, microorganisms are often found in complex, surface attached communities called biofilms.[55] As biofilms are attached to a surface, the residence time of microorganisms is longer relative to the transport of water and thus microorganisms' community structure may undergo change over time.[55] Benthic biofilms generally dominate microbial life in streams and their biodiversity can determine the metabolic health of their microbial communities and consequently the functioning of the aquatic ecosystem they reside in.[55] Proteobacteria, Bacteroidetes and cyanobacteria are the most prominent bacterial groups in freshwater biofilms.[55] Sediment bacterial communities are also integral to ecosystem function.[56] Environmental factors such as pH, organic carbon and nitrogen have often been able to explain bacterial community variation in streams. .[55] Urban streams are known to receive

increased inputs of contaminants such as nutrients and metals, and bacterial community structure in sediments has been shown to be significantly different between urban and nonurban stream sites.[57]

There is limited data for the impacts of emerging contaminants on microbial communities in urban streams. The antimicrobial compound triclosan has been shown to impact sediment bacterial community structure and algae. Increased relative abundance of cyanobacteria has been observed in a bacterial sediment community after exposure with a decrease in abundance of green algae,[51] green algae have also been visually damaged in biofilm communities after triclosan exposure while diatoms in the same community recovered.[58] Triclosan can also inhibit biofilm development and decrease biofilm bacterial diversity.[59]

High through-put sequencing (HTS) has advanced the study of microbial communities and numerous studies have used HTS to characterise bacterial communities in a range of environments.[60] High through-put sequencing allows multiple samples to be sequenced at once and tens of thousands of amplicon sequences may be generated from a single sample.[60] High through-put sequencing was used to characterise bacterial community compositions at Dudley Creek Diversion and Cross stream upstream and downstream of known sewerage overflow sites. Thereby the influence of PCPs, among other water quality variables, on structuring bacterial community composition was examined.

2.1.2 Study Objectives

The objectives of the field study were to:

- Determine the occurrence of PCPs and steroid hormones at Dudley Creek Diversion and Cross Stream in sediment and water samples at sites upstream and downstream of sewer overflow outfalls.
- Characterise bacterial community composition in biofilm and sediment samples from the study sites and determine whether there is variation between sites.
- Determine which environmental variables explain variation in bacterial community composition.

2.2 Methods

2.2.1 Sampling

The two urban streams, Cross Stream and Dudley Creek Diversion, were selected after consultation with the Christchurch City Council (CCC). Analysis of data provided by the CCC showed that of the water bodies receiving sewer overflows in Christchurch which could be classified as streams and were safe to sample water, sediment and biofilms, these two had received the most overflows in the last three years. Both Dudley Creek and Cross Stream (Figure 2.2.1) are in the Avon River catchment and drain

a number of impervious surface including roofs, road surfaces, pavements and parking areas.[61] Cross Stream is located in the western area of the catchment. Dudley Creek Diversion is a timber lined drain designed to aid in the prevention of flooding of Dudley Creek, a major tributary of the Avon River that drains a large area of Christchurch between Elizabeth II Drive and Bealey Avenue.[62] Sampling took place as close to the 30th of each month as possible, beginning on the 30th of March 2015 and ending on the 15th of September 2015 (referred to as the sampling taken place for August as sampling was not possible that month). At each overflow site three sampling points were selected using a gradient approach: 10 m upstream of the overflow outfall (control), 5 m downstream of the outfall, and 50 m downstream of the outfall. Sampling began at the point furthest downstream to ensure substrate and flows were not disturbed upstream of any point before sampling. Water samples were collected before sediment and biofilm samples. Samples were collected for analysis of PCPs and steroid hormones, heavy metals, nutrients, suspended solids, total organic carbon (TOC) and benthic bacteria community composition (sediment and biofilms). Only samples collected upstream and 5 m downstream of the two outfalls were eventually analysed due to financial and time constraints.

Figure 2.2.1. Dudley Creek Diversion and Cross Stream.

2.2.1.1 Samples for chemical analysis

Personal care product samples

Water samples were collected in 4 L amber glass bottles. A 4 L duplicate sample and sample for spiking were also collected each day. Samples were filtered in the laboratory on the same day as collection through Whatman GF/C filters before acidification to pH 2 using sulphuric acid.[63] Filters were frozen at -20 ˚C for analysis of suspended solids for emerging contaminants.

Sediment samples were collected in 250 mL polystyrene containers with a stainless steel spatula cleaned with ethanol between sites. In the laboratory sediments were sub-sampled (approximately 200 g) for emerging contaminant analysis and frozen at -20 ˚C until extraction.

Trace elements

Samples for total and dissolved metal analysis were collected at each sampling point in 50 mL polyethylene centrifuge tubes. A duplicate sample was taken at one sampling point each day. Water samples were processed in the clean room at the University of Canterbury. Samples for dissolved metal analysis were filtered with mixed cellulose filters (0.45 µm Millex). All water samples were acidified to pH<2 with ultrapure quartz distilled nitric acid. Samples were refrigerated until analysis by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS).[64] Sediment samples for metal analysis were subsampled from the same collection container as the emerging contaminants and stored in the same manner.

Nutrients

Nutrient samples were collected in 50mL polyethylene centrifuge tubes. One sample was collected at each upstream and downstream site for total nitrogen (TN) and total phosphorous (TP), a second sample for dissolved reactive phosphorous (DRP), nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+) analysis. The TN and TP samples were not filtered and approximately 40-45 mL was collected. The samples for DRP, NO_3-N , NO_2-N, and NH_4-N were filtered with a 0.45 µm filter and approximately 40-45 mL was collected. All nutrient samples were stored frozen (-20 ˚C) until analysis.

Total suspended solids and total organic carbon

Suspended solid samples and TOC samples were collected from each sampling point in 1 L Schott bottles and 1 L amber glass bottles, respectively. A duplicate sample was collected each day for each analyte. All samples were stored refrigerated until analysis.

2.2.1.2 Samples for molecular analysis

Triplicate biofilm samples were taken using Whirl-pak™ Speci-sponges™ at each sampling point. The entire top and side surfaces of three rocks was swabbed at each site.[65] Triplicate sediment samples were also taken by filling three 1.7 mL DNA free Eppendorf tubes with sediment to three quarters full with a sterilised stainless steel spatula. Gloves were changed at each site and ethanol was used to wash down bags and spatulas to ensure cleanliness. Each sample was stored in a separate plastic bag. All molecular samples were stored frozen at -20 ˚C until analysis.

2.2.2 Materials

Materials for emerging organic contaminants analysis

Chemicals

Individual 1000 µg/L natives standard solutions were prepared in acetonitrile (ACN) from solid standards of *p*-hydroxybenzoic acid butyl ester (mParaben), *p*-hydroxybenzoic acid ethyl ester (eParaben), *p*-hydroxybenzoic acid propyl ester (pParaben), *p*-hydroxybenzoic acid butyl ester (bParaben), benzyl 4-hydroxybenzoate (benzyl paraben), 4-octylphenol (OP), 4-methyl-benzylidene camphor (4-MBC), (2-hydroxy-4-methoxyphenyl)-phenylmethanone (BP-3) and octyl-methoxycinnamate (OMC), all purchased from Accustandard, 4-chloro-3,5-dimethylphenol (chloroxylenol), 2-phenylphenol (*o*-phenylphenol), 2-chlorobuta-1,3-diene (chlorophene), 4,4-isopropylidenediphenol diacetate (BPA diacetate), 2,4-dihydroxybenzophenone (BP-1), 3-phenoxybenzyl alcohol (3-PBOH), 2,2 -bis(4-hydroxy-phenyl)propane (BPA), 2,4,4'-trichloro-2'-methoxydiphenyl ether (mTriclosan), estrone (E1), 17β-estradiol (E2), estriol (E3), and 17α-ethinylestradiol (EE2), all purchased from Sigma-Aldrich, 4-*n*-nonylphenol (NP) purchased from Fluka, and 5-chloro-2-(2,4-dichlorophenoxy)phenol (triclosan) purchased from Dr Ehrenstorfer GmbH. The individual standards were then diluted to prepare a 1 µg/L mixed natives standard.

The internal standard 2,2-bis(4-hydroxy-3-methylphenyl)propane (BPC) was purchased from Sigma-Aldrich. Three additional internal standards were used for the sediment samples; ^{13}C2-mono-2ethylhexyl phthalate (^{13}C2-mEHP), ^{13}C2-monoethyl phthalate (^{13}C2-mEP), and ^{13}C6-3-phenoxybenzoic acid (^{13}C6-3PBA,) and were purchased from Cambridge Isotope Laboratories Inc.

Carbon-13 labelled surrogates including $^{13}C_6$-mParaben, $^{13}C_6$-bParaben, $^{13}C_6$-NP, $^{13}C_{12}$-triclosan and $^{13}C_6$-BPA, purchased from Cambridge Isotope Laboratories Inc, were prepared in acetonitrile to make a 1 µg/mL combined standard.

The solvents used at the University of Canterbury were all HPLC grade including acetonitrile (ACN), methanol (MeOH), dichloromethane (DCM), and acetone. A purification system at the University of Canterbury was used to obtain MilliQ (MQ) water (Millipore, USA). Sodium sulfate (puriss. P.a. ACS, anhydrous, granulated), purchased from Sigma Aldrich, and concentrated sulphuric acid (ACS grade) were also used throughout the project.

For derivatisation of standards and samples, *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) for GC derivatisation, ≥98.5% was purchased from Fluka. 2-mercaptoethanol and ammonium iodide were purchased from Aldrich.

For work carried out at Plant and Food Research, HPLC grade MeOH (Optima), DCM (submicron filtered), acetone (submicron filtered), isopropanol (IPA, submicron filtered) and pentane (pesticide

grade) were purchased from Fisher Scientific. MQ water was obtained from the in-house purification system (Integral 5 Millipore Instrument, USA). Dipotassium phosphate (powder, A.C.S reagent) was purchased from J.T Baker. Potassium dihydrogen phosphate (monobasic, molecular biology grade) was purchased from Sigma. Sodium sulphate (anhydrous, AR grade) was purchased from Mallinckrodt. Celite was purchased from Sigma. Diatomaceous earth and Ottawa sand were purchased from Restek.

Materials

Strata-X 33u Polymeric Reversed Phase solid phase extraction (SPE) cartridges (500 mg/6 mL), Strata Florisil clean-up cartridges (1000 mg/6 mL) and GF/C Whatman filter papers were used throughout for solid phase extractions.

For Accelerated Solvent Extractions (ASE) undertaken at Plant and Food Research, GF/C filter papers and ASE filter papers were purchased from Dionex. Strata-X 33u SPE cartridges (1g/20 mL giga tube) and Strata Florisil clean-up cartridges (2g/12 mL) were used for SPE and florisil clean up steps.

2.2.3 Personal care products and steroid hormones analysis

2.2.3.1 Water samples

Sample Extraction

The filtered water samples were extracted the day following sampling using the method of Emnet et al (2015).[63, 66] Solid phase extraction (SPE) cartridges were rinsed with 4 × 5 mL DCM/MeOH (95:5). The solvent was allowed to soak into the cartridge for 2 minutes after each addition before flowing through the cartridges under gravity. Cartridges were then dried under vacuum before elution of 2 × 5 mL MeOH followed by 2 × 5 mL MQ. Again the solvent soaked into the cartridge for 2 minutes on each addition. Samples were connected to the SPE cartridges with transfer tubes secured with plugs and extracted at a flow rate of 15 – 20 mL/min. Sample bottles were rinsed with 30 mL of MQ water after sample extraction which was also passed through the cartridge. Cartridges were dried under vacuum before the florisil clean-up step. Florisil cartridges were filled to the top of the label with granular sodium sulfate and rinsed with 2 × 5 mL acetone then dried under vacuum. The SPE cartridges were connected to the top of the florisil/sodium sulphate cartridges and eluted into 40 mL amber glass vials with 6 × 5 mL DCM/MeOH (95:5). The solvent was allowed to soak into the cartridge for 2 minutes each elution. Samples were then split in two by drying each sample down under nitrogen and gentle heating (~ 40 °C) before a quantitative transfer (1 × 500 µL, 2 × 250 µL) to a 10 mL volumetric flask and being made up to volume. A 5 mL glass bulb pipette was then used to transfer half of the sample to an amber glass vial. The remaining half of the sample was transferred to a new amber glass vial. Extracts were stored at 4˚c until analysis.

Quality assurance/Quality control

All glassware was rinsed three times with MeOH, DCM and ACN before use. The sodium sulfate and GF/C filter papers were both rinsed with MeOH and ACN. The sodium sulphate had previously been baked at 500°C to remove organic carbon and moisture.

Quality control samples were included in every extraction batch, prepared along with the field samples. As described above for the water samples, a duplicate and spike were collected at one sampling point each day. The duplicate was prepared in the same manner as other the other samples. The spiked sample was spiked with 100 µL of a 1 µg/L mixed natives standard. A 4 L MQ sample and an SPE cartridge were also spiked with 100 µL of the same mixed natives standard. Every extraction batch also included a MQ blank and cartridge blank to account for any contribution of analytes to the samples during extraction. All stream samples, blanks and spikes were spiked with 50 µL of the 1 µg/mL ^{13}C labelled surrogate standard in order to determine the analyte recovery for each sample extraction. A comparative standard was also prepared prior to extraction by dispensing the same amount of the natives and surrogate standards used for the spiking above into a pre-weighed amber glass vial. The vial was weighed again after addition of the standards. Percentage spike recoveries relative to the comparative standard for natives and ^{13}C labelled surrogates are presented in Tables 2.2.3a – f.

Due to the fast flowing water at the two streams, the duplicate sample taken on each sampling occasion was not truly a duplicate. To confirm the repeatability of the extraction, duplicate samples (4 L) were subsequently collected from both Dudley Creek Diversion and Cross Stream. The duplicates were mixed together and measured out to 4 L each (2 L from each original duplicate) prior to extraction and subsequent analysis. Duplicate % differences are displayed in Table 2.2.3g.

Table 2.2.3a. ^{13}C labelled surrogate percentage recoveries for stream water (4L), n = 22 (RSD = relative standard deviation, C.I = confidence interval).

Compound	Average	%RSD	Lower 95% C.I	Upper 95% C.I
BPA (ring $^{13}C_6$)	104.9	26.3	91.8	118.0
bParaben (ring $^{13}C_6$)	113.6	24.3	102.3	124.8
E2 (ring $^{13}C_6$)	91.6	15.3	85.9	97.4
mParaben (ring $^{13}C_6$)	122.1	34.2	105.0	139.1
NP (ring $^{13}C_6$)	113.3	16.9	105.5	121.1
TCS (ring $^{13}C_6$)	104.6	26.2	93.4	115.8

Table 2.2.3b. ^{13}C labelled surrogate percentage recoveries for MQ water (4L), n = 14 (RSD = relative standard deviation, C.I = confidence interval).

Compound	Average	%RSD	Lower 95% C.I	Upper 95% C.I
BPA (ring $^{13}C_6$)	117.3	31.9	94.1	140.5
bParaben (ring $^{13}C_6$)	105.4	24.5	91.9	119.0
E2 (ring $^{13}C_6$)	89.6	18.6	80.9	98.4
mParaben (ring $^{13}C_6$)	105.5	28.0	90.0	121.0
NP (ring $^{13}C_6$)	118.3	21.0	105.3	131.3
TCS (ring $^{13}C_6$)	104.2	21.4	92.5	115.9

Table 2.2.3c. ^{13}C labelled surrogate percentage recoveries for SPE cartridges, n = 14 (RSD = relative standard deviation, C.I = confidence interval).

Compound	Average	%RSD	Lower 95% C.I	Upper 95% C.I
BPA (ring $^{13}C_6$)	105.3	26.1	88.2	122.3
bParaben (ring $^{13}C_6$)	103.6	15.8	95.0	112.2
E2 (ring $^{13}C_6$)	81.5	12.1	76.4	86.7
mParaben (ring $^{13}C_6$)	103.4	20.7	92.2	114.7
NP (ring $^{13}C_6$)	111.2	19.1	100.0	122.3
TCS (ring $^{13}C_6$)	103.0	13.5	95.7	110.3

Table 2.2.3d. Percentage spike recoveries for stream water (4 L), n = 7 (RSD = relative standard deviation, C.I = confidence interval).

Compound	Average	%RSD	Lower 95% C.I	Upper 95% C.I
3PBOH	118.1	14.4	105.5	130.7
4MBC	133.1	17.2	116.2	150.0
BP-1	134.3	14.3	120.0	148.5
BP3	136.3	16.5	119.6	152.9
BPA	126.4	8.6	118.4	134.4
BPA-DA	122.7	15.0	109.0	136.3
bParaben	132.1	14.7	117.8	146.4
bzParaben	127.0	16.8	111.2	142.8
Chlorophene	113.2	14.0	101.5	124.9
Chloroxylenol	131.4	17.2	114.6	148.1
E1	94.8	57.5	54.4	135.1
E2	102.3	21.4	86.1	118.5
E3	105.0	12.3	93.7	116.3
EE2	111.1	12.1	101.1	121.0
eParaben	115.8	17.3	101.0	130.7
mParaben	124.8	18.2	108.0	141.7
mTCS	112.8	13.3	101.7	123.8
NP	105.8	13.7	95.0	116.6
OMC	125.2	15.4	110.9	139.4
OP	136.1	16.5	119.4	152.8
OPP	212.4	31.4	163.0	261.8
pParaben	113.2	18.6	97.6	128.8
TCS	115.1	14.2	103.0	127.3

Table 2.2.3e. Percentage spike recoveries for MQ water (4 L), n = 7 (RSD = relative standard deviation, C.I = confidence interval).

Compound	Average	%RSD	Lower 95% C.I	Upper 95% C.I
3PBOH	111.3	12.7	100.8	121.8
4MBC	123.5	12.5	112.1	135.0
BP-1	117.5	18.3	101.6	133.5
BP3	111.9	34.8	83.0	140.8
BPA	103.2	11.0	94.8	111.6
BPA-DA	116.6	9.6	108.4	124.9
bParaben	112.9	13.4	101.7	124.1
bzParaben	116.9	12.6	106.0	127.8
Chlorophene	108.6	9.3	101.1	116.0
Chloroxylenol	169.8	34.6	126.3	213.3
E1	80.0	47.8	51.7	108.4
E2	101.6	13.0	91.8	111.4
E3	79.2	51.3	43.6	114.8
EE2	104.8	6.3	99.9	109.7
eParaben	107.0	16.7	93.8	120.3
mParaben	112.3	19.2	96.4	128.3
mTCS	113.5	12.6	102.9	124.1
NP	101.1	10.3	93.4	108.8
OMC	116.0	15.3	102.9	129.2
OP	124.3	19.0	106.8	141.8
OPP	177.0	29.4	138.4	215.5
pParaben	107.3	14.0	96.2	118.4
TCS	108.9	9.3	101.4	116.4

Table 2.2.3f. Percentage spike recoveries for SPE cartridge (4 L), n = 7 (RSD = relative standard deviation, C.I = confidence interval).

Compound	Average	%RSD	Lower 95% C.I	Upper 95% C.I
3PBOH	99.4	9.6	92.3	106.5
4MBC	108.9	9.1	101.5	116.2
BP-1	102.3	10.2	94.6	110.0
BP3	95.1	32.5	72.2	118.0
BPA	94.2	10.1	87.2	101.3
BPA-DA	105.8	7.1	100.3	111.4
bParaben	100.1	9.2	93.3	106.9
bzParaben	104.3	9.0	97.4	111.3
Chlorophene	97.5	8.0	91.7	103.3
Chloroxylenol	130.7	34.0	97.8	163.6
E1	93.5	48.9	59.6	127.3
E2	84.2	14.7	75.0	93.3
E3	88.1	12.6	78.4	97.8
EE2	92.1	10.7	84.8	99.4
eParaben	94.3	11.8	86.1	102.6
mParaben	96.7	13.0	87.4	106.0
mTCS	95.0	8.9	88.7	101.3
NP	92.6	11.0	85.1	100.1
OMC	98.5	16.0	86.9	110.1
OP	98.7	16.0	87.0	110.4
OPP	132.8	33.4	99.9	165.6
pParaben	94.7	9.6	88.0	101.4
TCS	97.4	8.0	91.6	103.1

Table 2.2.3g. Percentage difference of ^{13}C surrogates and detected compounds between duplicates from Dudley Creek Diversion (DC) and Cross Stream (CS).

Compound	DC % Difference	CS % Difference
BP3	37.6	29.6
BPA	5.4	29.5
BPA (ring $^{13}C_6$)	0.6	4.2
bParaben (ring $^{13}C_6$)	7.3	6.1
E2 (ring $^{13}C_6$)	2.4	4.9
mParaben	10.7	ND
mParaben (ring $^{13}C_6$)	7.7	6.7
NP (ring $^{13}C_6$)	19.6	5.6
OMC	61.6	51.7
OP	5.3	ND
TCS (ring $^{13}C_6$)	3.0	6.7

2.2.3.2 Sediment and particulate phase samples

Sample Preparation

For particulate phase samples, a cellulose filter and GFC grade filter paper were inserted into a capped 22 mL stainless steel ASE cell, pushed firmly to the base. A small amount of diatomaceous earth was compacted atop of the filters. The filter paper containing the sample was folded in half and carefully cut into small strips as to not remove any particulates. The sample was added to four grams of diatomaceous earth and the mixture was blended for 5 sec in an IKA high speed laboratory mill. The homogenized mixture was compacted into the ASE cell by bumping the cell on the bench and using a cell packing tool to apply pressure. Another two grams of diatomaceous earth was blended for 5 sec in the laboratory mill to "rinse" any residual sample on the mill base and blade and compacted into the ASE cell as before.

Thawed sediments (ca. 30 g, wet weight) were packed into pre-weighed ASE cells with a cellulose and GFC grade filter paper as above. The cell was weighed again and the weight of packaged sediment recorded.

The remaining space in the ASE cell was filled with solvent cleaned Ottawa sand for both particulate phase and sediments before spiking with 50 µL of the 1 µg/L 13C-labelled surrogate standard. A cellulose filter paper was placed in the top of the cell with the packing tool and the cell was sealed with a stainless steel cap.

Sample Extraction

Samples were extracted using a Dionex ASE 200 accelerated solvent extraction (ASE) system. Samples were consecutively extracted with two methods. The extraction was first carried out with a 50:50 mixture of water/IPA at a temperature of 120 °C and 2000 psi for 10 min under static extraction conditions. The extraction was repeated under the same conditions and the solvent extract collected in a silanized 60 mL glass vial. The same sample cell was then twice extracted with a 20:80 water/IPA mixture at 180 °C and 2000 psi for 10 min and the extract collected in a second silanized 60 mL glass vial containing 3 mL of pentane to help reduce the loss of analytes by volatilisation. The sample was then rinsed with a 60% flush volume of the 20:80 water/IPA mixture and the solvent collected in the same glass vial. "Rinse" ASE cells were employed between sample cells to prevent any carry over of samples.

Solid Phase Extraction Clean-Up

The two vials containing the solvent extracts for each sample were combined in a single 1 L glass Schott bottle. Vials were rinsed and added to the same Schott bottle with 4 × 25 mL aliquots of phosphate buffer to ensure recovery of the solvent mix. A final volume of 650 mL was attained by adding

additional phosphate buffer. Organic contaminants were extracted from the phosphate buffer-IPA solution onto Strata-X SPE cartridges (1 g, 20 mL) at a flow rate of approximately 15 mL/minute. Once all solution had passed through the SPE cartridge the glass Schott bottle was rinsed three times with separate 20 mL aliquots of Milli-Q water which were also passed through the SPE cartridge. The cartridges were dried together under full vacuum for three minutes, then individually until the majority of residual water was removed.

Solid Phase Extraction and florisil clean-up

Contaminants were eluted from the Strata-X SPE cartridge and passed through a florisil SPE cartridge (Strata, 1 g, 6 mL) as a clean-up stage. A mixture of DCM-MeOH (95:5, 6 × 5 mL) was used to elute the contaminants into previously silanized 40 mL amber glass vials. In the third batch of the extracted samples the florisil cartridges became blocked due to cementing of the sodium sulfate from potential residual water eluted from the SPE cartridges. To complete the extraction for batch three, the SPE cartridges were connected to new florisil cartridges and elution was resumed. The sodium sulfate and florisil was removed from the blocked florisil cartridges and ground up with a cleaned pestle and mortar. The ground florisil and sodium sulfate were transferred into fresh SPE cartridges and extracted separately with 30 mL of the DCM-MeOH mixture. The extracts were then dried under nitrogen gas and combined.

All extracts were reduced to near-dryness under nitrogen gas and gentle heating (40 °C) before being quantitatively transferred with 1 × 0.5 mL and 2 × 0.25 mL DCM/MeOH (95:5) into GC vials. All extracts were stored refrigerated until further clean up.

Gel Permeation Chromatography Clean-Up

The 1 mL extracts were transferred into champagne glass shaped GPC vials followed by a quantitative transfer of 2 × 0.25 mL DCM/MeOH (95:5) via Pasteur pipettes packed with a small amount of solvent washed cotton wool and approximately 10 mg of Celite Hyflo Supercell filter agent. Gel permeation chromatography (GPC) was carried out using a Shimadzu Class VP GPC system controlled by a SCL-10A VP System Controller and fitted with a LC-10AT VP Liquid Chromatograph connected to a SIL-10AP Auto Injector, a SPD-10A UV-Vis Detector, and a FRC-10A Fraction Collector. Samples were injected (1500 µL) and eluted with DCM/MeoH (95:5) as the mobile phase at a flow rate of 2.5 mL/min. The desired fraction of GPC eluent corresponding to the band of target compounds was collected in 2 × 22 mL glass vials (previously silanized).

The collected fraction was reduced to near-dryness under nitrogen gas and gentle heating before a quantitative transfer with 4 × 0.25 mL DCM/MeOH (95:5) into 10 mL volumetric flasks. The extract was made up to 10 mL and split into two 15 mL amber glass vials with a 5 mL glass bulb pipette. The samples were stored refrigerated (4 °C) until GC-MS analysis.

Dry Weight Determination

Approximately 1 g of wet sediment was weighed into dry, labelled pre-weighed aluminium dishes. The dry weights of samples in the first batch of sediments were determined in triplicate. As the relative standard deviation was less than 5% for all samples, duplicates were used there on. The samples were dried in an oven at 105 ℃ for 24 hrs followed by cooling in a desiccator for at least 10 minutes prior to weighing.

Quality assurance/Quality control

Every two batches of samples extracted by ASE (n = 7) alternately included a blank and spike ASE cell (the spike was spiked with 50 µL of the 1 µg/L standard solution and ^{13}C-labelled surrogate standard). The blank and spiked cells contained diatomaceous earth and Ottawa sand but no sample. A comparative standard was prepared at the same time as the samples were spiked. Spike recoveries for samples and diatomaceous earth are displayed in Tables 2.2.3h – k. The diatomaceous earth, Ottawa sand, and cellulose filter papers were solvent extracted prior to use. The sodium sulfate was baked overnight at 500 °C before use. During the SPE clean-up a cartridge blank was included.

Low recoveries for all ^{13}C surrogates except NP were observed in sediment samples. This was likely due to matrix effects arising from the presence of co-extracted matrix components originating from the sediment. However recoveries for the diatomaceous earth were acceptable with the exception of OP.

Table 2.2.3h. ^{13}C labelled surrogate percentage recoveries for sediment samples, n=17 (RSD = relative standard deviation, C.I = confidence interval).

Compound	Average	%RSD	Lower 95% C.I	Upper 95% C.I
BPA (ring $^{13}C_6$)	31.3	36.8	25.8	36.7
bParaben (ring $^{13}C_6$)	2.0	400.0	0.0	5.7
E2 (ring $^{13}C_6$)	26.4	76.0	16.9	35.9
mParaben (ring $^{13}C_6$)	6.2	249.6	0.0	13.6
NP (ring $^{13}C_6$)	81.6	32.4	69.0	94.1
TCS (ring $^{13}C_6$)	0.0	0.0	0.0	0.0

Table 2.2.3i. ^{13}C labelled surrogate percentage recoveries for particulate phase samples, n=22 (RSD = relative standard deviation, C.I = confidence interval).

Compound	Average	%RSD	Upper 95% C.I	Lower 95% C.I
BPA (ring $^{13}C_6$)	61.4	19.1	56.4	66.4
bParaben (ring $^{13}C_6$)	89.5	22.0	81.0	97.9
E2 (ring $^{13}C_6$)	67.2	23.3	60.5	73.9
mParaben (ring $^{13}C_6$)	125.7	27.7	110.8	140.6
NP (ring $^{13}C_6$)	76.7	27.9	67.6	85.8
TCS (ring $^{13}C_6$)	74.8	23.7	67.2	82.3

Table 2.2.3j. ^{13}C labelled surrogate percentage recoveries for diatomaceous earth spike, n=5 (RSD = relative standard deviation, C.I = confidence interval).

Compound	Average	%RSD	Lower 95% C.I	Upper 95% C.I
BPA (ring $^{13}C_6$)	79.5	34.9	55.2	103.8
bParaben (ring $^{13}C_6$)	89.8	15.6	77.6	102.1
E2 (ring $^{13}C_6$)	83.5	10.6	75.8	91.2
mParaben (ring $^{13}C_6$)	129.9	3.5	125.9	133.9
NP (ring $^{13}C_6$)	89.9	6.6	84.7	95.0
TCS (ring $^{13}C_6$)	83.6	12.7	74.2	92.9

Table 2.2.3k. Percentage spike recoveries for diatomaceous earth spike, n=5 (RSD = relative standard deviation, C.I = confidence interval).

Compound	Average	%RSD	Lower 95% C.I	Upper 95% C.I
3PBOH	138.7	7.9	129.0	148.3
4MBC	147.2	13.3	130.1	164.3
BP-1	96.1	33.4	67.9	124.2
BP3	134.3	11.9	120.2	148.3
BPA	114.8	22.6	92.0	137.5
BPA-DA	28.9	56.7	14.5	43.3
bParaben	96.8	12.0	86.6	107.0
bzParaben	91.1	23.9	72.0	110.2
Chlorophene	100.0	13.3	88.4	111.6
E1	66.6	57.6	33.0	100.2
E2	89.2	13.9	78.3	100.0
E3	86.2	23.0	68.8	103.6
EE2	91.0	20.1	75.0	107.0
eParaben	151.7	7.2	142.2	161.3
mParaben	169.3	25.1	132.0	206.7
mTCS	89.4	5.0	85.5	93.3
NP	78.4	33.2	55.6	101.3
OMC	120.2	21.0	98.1	142.4
OP	312.3	49.8	176.0	448.7
pParaben	120.2	14.1	105.4	135.0
TCS	101.1	12.9	89.7	112.6

2.2.4 GC-MS analysis

Sample Derivatisation

Preparation of MSTFA reaction mixture

In a 5 mL glass reaction vial 11.4 mg of ammonium iodide, 17 µL of 2-mercaptoethanol and 285 µL of MSTFA were mixed by vortex then incubated at 65 °C until the ammonium iodide was fully dissolved. The contents were vortexed periodically to aid in the dissolution of the ammonium iodide. The vial was then cooled to room temperature before the addition of 2715 µL of MSTFA. The mixture was homogenised by vortex and the vial was purged with nitrogen gas and sealed tightly to exclude air and moisture. The reaction mix was stored at 4 °C for up to 10 days before it was necessary to prepare a fresh mixture.

Derivatisation of sample extracts

Samples were dried under nitrogen gas at 40°C before being quantitatively transferred to derivatisation vials (previously silanized) with 3 × 0.5 mL DCM/MeOH (95:5). Samples were again evaporated to dryness under nitrogen gas before adding 100 µL of the internal standard (1 µg/L BPC) and 30 µL of MSTFA reaction mix. Vials were vortexed and incubated for 45 minutes at 65°C. After incubation the vials were allowed to cool to room temperature before being made up to 1 mL with iso-octane and transferred to GC vials. Samples were stored at 4 °C before analysis. A 10 and 100 µg/L derivatisation check and a derivatisation blank were included in each batch of 24 samples.

Due to the low recovery of the ^{13}C surrogate compounds in the sediment samples, small aliquots of the second half of the sediment samples were re-analysed, trialling different dilutions of the sample. The final selected method of preparation was to dry 0.5 mL of sample under nitrogen and make the sample up to a final volume of 200 µL.

Instrumental Analysis

Gas Chromatography/Mass spectrometry (GC-MS) using a Shimadzu GC-2010 Gas Chromatograph, interfaced with a Shimadzu AOC-20i Auto Injector and a Shimadzu GCMS-QP2010Plus detector was used to analyse derivatised sample extracts and calibration standards. The Shimadzu GCMS Solution software (Version 2.70) was used for instrumental control, data acquisition and data processing. An Rxi-5Sil column (5% diphenyl/95% dimethyl polysiloxane) 30 m x 0.25 mm ID, 0.25 µm film thickness, with an integrated guard column (10 m, Integra-Guard) (Restek, Belleftone USA) was used for compound separation. Derivatised samples and calibration standards were injected into the injection port (1 µL) at 280 °C in splitless mode. The splitless time was 1 min and the split flow rate was 50 – 100 mL/min. The initial oven temperature (100 °C) was held for 5 min, before being increased at a rate of 10 °C/min to 300 °C where the temperature was finally held for 15 min, for a total run time of 30 min. The carrier gas was helium at a flow rate of 1 mL/min.

Electron ionisation (EI) was used in selected ion mode (SIM) to obtain spectra at 70 eV with the ion source held at 200 °C and the GC-MS interface at 250 °C. Calibration of the MS against perflurotributylamine (PFTBA) was carried out before each run using the autotune function.

Retention times and m/z ratios used for individual compounds are presented in Table 2.2.4a. Retention times and m/z ratios for the ^{13}C labelled surrogates are presented in Table 2.2.4b. A nine point calibration curve (0, 1, 2.5, 5, 10, 25, 50, 100 and 250 ng/L) was used to quantify target analytes against the relative response of the internal standard.

Table 2.2.4a. Retention times and SIM mode detection parameters of analytes.

Compound	Rt (min)	Quantifier ion (*m/z*)	Qualifier ions (*m/z*)
$^{13}C_6$ 3-PBA*	17.86	277	233, 392, 203
$^{13}C_6$ mEP*	14.67	255	227, 181, 166
3PBOH	17.05	183	227,272, 257, 211
4MBC	19.38	254	128, 155, 239
BP-1	20.60	343	344, 164, 271
BP3	19.48	285	286, 242, 223, 180
BPA	20.654	357	358, 372, 171
BPA-DA	21.92	213	228, 270
bparaben	16.17	195	193, 210, 266, 251
BPC*	21.27	385	386, 400
bzParaben	20.348	193	300, 85
Chlorophene	17.778	275	290, 165
Chloroxylenol	11.62	213	228, 215
E1	24.35	342	218, 244, 327
E2	24.58	416	285, 129, 326
E3	26.526	504	414, 345, 386
EE2	25.26	425	285, 232, 440
eParaben	13.75	238	193, 223, 210
mEHP $^{13}C_6$*	19.65	225	302, 254, 232
mParaben	12.79	224	209, 177, 193
mTCS	19.91	252	302, 254, 232
NP	17.84	292	180, 165
OMC	21.92	178	161, 133, 290
OP	14.71	207	151,208, 191
OPP	14.19	211	227, 242
pParaben	14.97	237	210, 193, 252
TCS	19.91	360	345, 362, 310

*Internal Standard

Table 2.2.4b. Retention times and SIM mode detection parameters of isotopically labelled surrogates.

Compound	Rt (min)	Quantifier ion (*m/z*)	Qualifier ions (*m/z*)
BPA (ring $^{13}C_{12}$)	20.65	369	370, 384
nparaben (ring $^{13}C_6$)	16.17	216	201, 199, 272
E2l (ring $^{13}C_6$)	24.59	288	422, 332
mparaben (ring $^{13}C_6$)	12.79	215	230, 199
NP (ring $^{13}C_{12}$)	17.84	186	298, 171
TCS (ring $^{13}C_{12}$)	19.94	372	359, 374, 322

Calibration Curve

Calibration standards were prepared by adding 50 µL of the 1 µg/mL stock internal standard to each derivatisation vial and the appropriate amounts of native and ^{13}C surrogate standards to create nine calibration standards (0, 1, 2.5, 5, 10, 25, 50, 100 and 250 ng/mL). Standards were reduced to dryness under a gentle flow of N_2 and heating (~40 ºC). The MSTFA derivatisation mix was then added to each vial (30 µL) and derivatised in the same way as for samples. Standards were made up to 500 µL with isooctane. A new set of calibration standards were prepared every 48 hrs, and analysed after the first 10 samples in a batch. The intensity of the chromatogram analyte peaks were referenced against the internal standard to form a calibration curve with nine points in units of ng/mL. The concentration of each compound in each standard was entered into the calibration software, with a consistent concentration of 100 ng/mL used for the internal standard for each sample. The ratio of calibrated concentrations was recorded against the ratio of the target analyte peak area and internal standard peak area. For quantification of a compound in actual samples the ratio of the compound peak area and the internal standard of the sample's chromatogram was calculated and reported via the calibration curve by the software.

The internal standard BPC was used for all stream water and particulate phase samples. For sediment samples, three additional internal standards were added for quantification to overcome potential matrix enhancements in certain parts of the chromatograph. The internal standard mEP-$^{13}C_6$ was used to quantify the analytes chloroxylenol – bParaben. 3-PBA-$^{13}C_6$ was used to quantify the analytes 3-PBOH – NP. mEHP $^{13}C_6$ was used to quantify the analytes 4-MBC – BP1. BPC was used for analytes BPA – E3.

Instrumental and analysis Quality assurance/Quality control

Before analysis of each batch the syringe needle was cleaned with toluene and DCM by sonication. The injection needle was also programmed to rinse three times each with isooctane, toluene and DCM before and after each sample injection.

An isooctane blank was injected at the beginning of a new sample run to flush the system of any volatiles which may have accumulated in the system since the previous analysis. The injection of the first 10 samples in a batch followed this with a repeat injection of the tenth sample. The full set of calibration standards, or alternatively, 0, 10 and 100 ng/L standards (every second batch) was then injected with a repeat injection of a sample and a standard to confirm the calibration, followed by the injection of a new isooctane blank to ensure there was no sample carry over. The remaining samples in the batch of 24 were then injected followed by another repeat sample and standard injection and finally another isooctane blank.

Limits of detection (LOD) were calculated using EPA Method 8280A.[67] The following equation was used to calculate the LOD:

$$LOD = \frac{2.5 \times Cis \times Hn \times D}{His \times RF}$$

Where:

Cis = The concentration of the internal standard in the sample.

Hn = The peak height of the noise for the quantitation ion at the target analyte's retention time if the target analyte is absent from the sample or near the target analyte's retention time if the target analyte is present in the sample.

D = The dilution factor.

His = The peak height of the internal standard.

RF = The response factor, or the ratio of the area of the target analyte to that of the internal standard multiplied by the ratio of the concentration of the internal standard to that of the target analyte in the lowest concentration calibration standard in which the target analyte is still detected.

The limit of detection for each compound was calculated for three samples and averaged. Limits of detection are displayed in Table 2.2.4c.

Table 2.2.4c. Limits of detection (LOD) of analytes in stream water and sediment, with lower and upper limits of 95% confidence, n = 3 (C.I = confidence interval).

Compound	LOD (Stream water, ng/L)	Lower 95% C.I	Upper 95% C.I	LOD (Sediment, ng/g)	Lower 95% C.I	Upper 95% C.I
3PBOH	1.15	0.43	1.87	0.29	0.11	0.47
4MBC	6.97	1.38	12.56	1.74	0.34	3.14
BP-1	0.60	0.17	1.04	0.15	0.04	0.26
BP3	1.22	0.35	2.10	0.31	0.09	0.52
BPA	0.27	0.09	0.45	0.07	0.02	0.11
BPA-DA	1.46	0.57	2.35	0.34	0.11	0.57
bParaben	0.77	0.43	1.11	0.19	0.11	0.28
bzParaben	1.72	1.09	2.35	0.43	0.27	0.59
Chlorophene	1.39	0.35	2.44	0.35	0.09	0.61
Chloroxylenol	0.68	0.32	1.04	0.17	0.08	0.26
E1	8.15	2.10	14.20	0.97	0.28	1.65
E2	2.62	0.94	4.29	0.40	0.18	0.62
E3	3.14	1.07	5.20	0.97	0.27	1.67
EE2	3.16	0.34	5.99	0.65	0.19	1.11
eParaben	1.01	0.13	1.89	0.25	0.03	0.47
mParaben	1.31	1.07	1.56	0.33	0.27	0.39
mTric	3.30	0.37	6.23	0.82	0.09	1.56
NP	3.94	1.12	6.75	0.98	0.28	1.69
OMC	1.09	0.44	1.74	4.95	0.00	14.11
OP	0.13	0.11	0.16	0.03	0.03	0.04
OPP	1.11	0.03	2.18	0.28	0.01	0.55
pParaben	1.72	0.68	2.76	0.51	0.10	0.92
Tric	1.14	0.24	2.03	0.28	0.06	0.51

2.2.5 Trace element analysis

Sediment Preparation

Sediment samples were dried at 70°C to ensure any bacteria were destroyed. Once samples were completely dry they were sieved (< 2mm) and sediments weighed (1 g ± 0.05g) into individual 50 mL polycarbonate centrifuge tubes for each sample. To each sample, 4 mL of 50% nitric acid and 10 mL of 20% hydrochloric acid was added and samples were left to stand overnight. Samples were refluxed on a hotplate for 40 min at 80 °C. Samples were cooled to room temperature before making the samples up to 20 mL with MQ water. Samples were again left overnight to allow particulates to settle out. Samples were diluted in the University of Canterbury clean room by adding 0.5 mL of sample to 10 mL of 2% nitric acid to give a 21 × dilution.[68] QA/QC samples included a duplicate, two blanks and certified reference material (CRM; Standard Reference Material® 2702, National Institute of Standards and Technology, USA) which were prepared in the same manner as samples.

Analysis

Sediment and water samples were analysed by Agilent 7500 series ICP-MS fitted with a collision cell (He gas) to remove polyatomic interference. Metals analysed for included aluminium (^{27}Al), antimony (^{121}Sb) arsenic (^{75}As), cadmium (^{111}Cd), chromium (^{53}Cr), copper (^{63}Cu), lead (^{208}Pb), iron (^{57}Fe), manganese (^{55}Mn), nickel (^{60}Ni) and zinc (^{66}Zn). An internal standard, Rhodium (^{108}Rh), was added on-line. A water CRM was included in each batch (Synthetic 1643 CRM, Inorganic Ventures). A duplicate sub-sample was analysed after every 10 samples, followed by a duplicate and 3 mL triplicate sub-sample spiked with 30 µL of a 100 ppb standard after every 20th sample. Before each run, the instrument was calibrated with a blank and standards ranging from 0.1-1000 ppb. Percentage differences were calculated for duplicates, and spike and CRM recoveries were recorded for quality control (Table 2.2.5a – e). Detection limits for water and sediment are displayed in Table 2.2.5f.

Table 2.2.5a. Percentage differences for field duplicates, n=7 (C.I = confidence interval).

Element	Average % Difference	Std. Dev.	Lower 95% C.I	Upper 95% C.I
Al	10.9	4.3	7.5	14.4
As	5.8	5.4	1.5	10.1
Cu	18.9	20.5	2.5	35.3
Fe	11.0	18.5	-3.8	25.8
Mn	8.7	12.9	-1.6	19.1
Ni	18.2	11.8	8.7	27.6
Pb	11.6	9.2	4.2	18.9
Sb	20.3	6.8	14.8	25.7
Zn	14.0	13.3	3.3	24.6

Table 2.2.5b. Percentage spike recoveries for trace metal analysis of water samples, n=4 (C.I = confidence interval).

Element	Average	Std. Dev.	Lower 95% C.I	Upper 95% C.I
Al	108.2	9.8	98.6	117.8
As	104.3	9.5	95.0	113.6
Cd	105.7	10.1	95.9	115.6
Cr	100.8	17.1	84.1	117.6
Cu	101.3	12.7	88.9	113.7
Fe	153.8	63.8	91.3	216.3
Mn	109.1	13.8	95.5	122.6
Ni	100.4	9.6	91.0	109.8
Pb	104.3	6.8	97.6	111.0
Sb	93.5	10.7	83.1	104.0
V	105.0	10.4	94.9	115.1
Zn	134.0	29.5	105.1	162.9

Table 2.2.5b. Percentage spike recoveries for trace metal analysis of sediment samples, n=2 (C.I = confidence interval).

Element	Average	Std. Dev.	Lower 95% C.I	Upper 95% C.I
Al	189.6*	80.1	78.6	300.5
As	104.1	17.2	80.2	128.0
Cd	103.9	3.3	99.4	108.5
Co	108.5	17.0	84.9	132.1
Cr	102.6	16.0	80.4	124.8
Cu	100.6	2.9	96.5	104.6
Fe	224.9*	150.2	16.7	433.0
Mn	106.4	13.2	88.1	124.7
Ni	100.8	12.0	84.2	117.4
Pb	118.3	18.9	92.0	144.5
Sb	117.2	6.4	108.3	126.0
V	102.2	20.7	73.5	130.8
Zn	125.2	0.6	124.4	126.1

*****Al and Fe recoveries are high due to relatively low spike concentration compared to sample concentration.

Table 2.2.5d. Sediment CRM % recoveries, n=2 (C.I = confidence interval).

Element	Average	Std. Dev.	Lower 95% C.I	Upper 95% C.I
As	87.7	12.6	70.3	105.1
Cd	97.3	0.1	97.1	97.5
Co	78.1	6.3	69.4	86.7
Cr	63.9	4.8	57.2	70.6
Mn	80.1	8.8	68.0	92.3
Ni	63.7	11.8	47.3	80.1
Pb	84.4	21.5	54.5	114.2
Sb	17.6	2.2	14.7	20.6
V	68.3	7.5	57.8	78.7

Table 2.2.5e. Water CRM % recoveries, n=4 (C.I = confidence interval).

Element	Average	Std. Dev.	Lower 95% C.I.	Upper 95% C.I.
Al	118.4	13.9	104.9	132.0
As	104.3	8.5	96.0	112.7
Cd	103.2	1.2	102.0	104.4
Co	108.7	6.9	101.9	115.5
Cr	101.0	19.3	82.1	119.9
Cu	104.1	4.6	99.6	108.6
Fe	137.1	8.4	128.9	145.3
Mn	114.5	7.8	106.9	122.2
Ni	104.6	7.1	97.6	111.6
Pb	103.6	4.5	99.1	108.1
Sb	113.5	5.2	108.4	118.6
V	111.6	8.6	103.2	120.0
Zn	101.6	5.3	96.4	106.8

Table 2.2.5f. Limits of detection (LOD) for water trace element analysis of water (µg/L) and sediment.

Element	LOD (ug/L)	LOD (ug/g)
Al	10	4.2
V	1	0.42
Cr	1	0.42
Mn	0.1	0.04
Fe	1	0.42
Co	0.1	0.04
Ni	1	0.42
Cu	0.1	0.04
Zn	1	0.42
As	0.1	0.04
Cd	0.1	0.04
Sb	0.1	0.04
Pb	0.1	0.04

2.2.6 Nutrients

Filtered water samples collected from sampling points 10 m upstream of the sewer overflow and 5 m downstream of the sewer overflow point at each stream were analysed by Hill Laboratories. Total NH_4-N (LOD = 0.010 mg/L), NO_2-N (0.002 mg/L), NO_3-N + NO_2-N (0.002 mg/L), and DRP (LOD = 0.004 mg/L) were tested for using the following methods, respectively: Phenol/hypochlorite colorimetry; Automated Azo dye colorimetry, automated cadmium reduction and molybdenum blue colorimetry.[69]

2.2.7 Total organic carbon analysis

Calibration Standard Preparation

A 1000 µg/L standard stock solution was prepared for total carbon (TC) and inorganic carbon (IC) analyses. For the TC stock standard reagent grade Potassium hydrogen phthalate (KHP) was dried for an hour at 110 °C. After cooling in a desiccator, 2.125 g of KHP was made up to 1000 mL in a volumetric flask with MQ water and stirred. The 1000 µg/L IC standard was prepared by drying reagent grade sodium bicarbonate in a desiccator overnight. Reagent grade sodium carbonate was dried for an hour at 250 °C then cooled in a desiccator. After cooling, 3.497 g of sodium bicarbonate and 4.412 g of sodium carbonate were made up to 1000 mL in a volumetric flask with MQ water and stirred. The 1000 µg/L TC and IC standards were then serially diluted to obtain two sets of calibration standards at concentrations of 100 µg/L, 50 µg/L, 25 µg/L, 10 µg/L, and 5 µg/L.

Analysis

Samples were analysed for TC and IC on the Shimadzu ASI-L TOC analyser in the Department of Chemical and Process Engineering, University of Canterbury. Samples, including a duplicate and blank were then analysed for TC and IC in acid washed glass tubes. The average percentage difference for duplicates was 7.58%. Total organic carbon (TOC) was calculated by subtracting IC from TC values obtained.

2.2.8 Total suspended solids analysis

For each total suspended solids (TSS) sample a Whatman GF/C 47mm filter paper was dried at 105˚C for at least one hour. Filter papers were cooled in a desiccator for 30 mins then weighed. One litre of sample was filtered through one dried and pre-weighed filter paper under vacuum. The graduated cylinder and filtration equipment was rinsed with MQ water so all solids were collected on the filter paper. Vacuum was applied until all liquid was removed from the filter paper. Filter papers were dried at 105˚C for an hour before being cooled for 30 mins in the desiccator and reweighed to determine the mass of the filter paper plus solids. A duplicate sample was included in each batch. The average percentage difference for duplicates was 18.7%.

2.2.9 Molecular analysis

Biofilm samples were prepared for DNA extraction by first adding 40 mL of DNA free water (Life Technologies, USA) to the bags containing the Whirl-pak™ Speci-sponges™ (Nasco, USA) under laminar flow. The biofilm was then squeezed out of the sponges using a stomacher (Colworth 400; AJ Seward, UK) for 2 mins. Water and biofilm was then transferred to 50 mL centrifuge tubes under laminar flow and samples were centrifuged (3000 × g for 10 mins). The supernatant was decanted off and the pellets had DNA extracted from them with the PowerSoil® DNA Isolation Kit (MoBio Laboratories, USA) following the provided instructions.

Sediment samples (ca. 0.25 g) were added to tubes provided in the PowerSoil® DNA Isolation Kit (MoBio Laboratories, USA) and were extracted following the same provided instructions.

The bacterial specific primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′)[70], modified to include Ilumina™ adapters, were used to amplify a region of the 16S rRNA gene approximately 400 bp in length. PCR reactions were carried out in 50 μL volumes made up with 25 μL of AmpliTaq Gold® 360 master mix (Life Technologies), 5 μL CG inhibitor (Life Technologies), 1 μL of each 10 μM primer, 16 μL of DNA free water and about 20 ng of template DNA. PCR cycling conditions were: 95 °C 10 mins, followed by 27 cycles of 95 °C 30 secs, 50 °C 30 secs, 72 °C 60 secs, and a final extension of 72 °C 7 mins. For DNA template samples which failed to amplify, the PCR reaction was again performed with addition of 4 μL BSA to the 50 μL mix and reduction of water to 12 μL. The PCR cycling conditions were changed to: 95 °C 10 mins, followed by 50 cycles of 95 °C 30 secs, 48 °C 30 secs, 72 °C 60 secs, and a final extension of 72 °C 7 mins. PCR products were visualised under UV illumination after 1% agarose gel electrophoresis with Red Safe DNA Loading Dye. PCR products were purified (Agencourt® AMPure® XP Kit; Beckman Coulter, USA), quantified (Qubit® 20 Fluorometer, Invitrogen), diluted to 10 ng/L and submitted to New Zealand Genomics Limited (Auckland, New Zealand) for library preparation. Sequencing adapters and sample-specific indices were added to each amplicon via a second round of PCR using the Nextera™ Index kit (Illumina™). Amplicons were pooled into a single library and paired-end sequences (2 × 300) generated on a MiSeq instrument using the TruSeq™ SBS kit (Illumina™). Sequence data were automatically demultiplexed using MiSeq Reporter (v2), and forward and reverse reads assigned to samples.

The MOTHUR work-flow was used to perform bioinformatics analysis of HTS data.[71] Contigs were assembled from paired-end reads. Duplicate sequences were removed and contigs length filtered. Chimeras were removed using the UCHIME algorithm[72] followed by sequence alignment to the SILVA bacteria reference[73]. Sequences were grouped in operational taxonomic units (OTUs) using 0.02 pairwise sequence distance cut off values. The number of reads in each sample was rarefied to account

for differential sequencing depth among samples. OTUs were classified to identify taxonomic annotation using the Ribosomal Database Project (RDP) taxonomic database Version 9.[74] Unknown chloroplast sequences were removed.

2.2.10 Statistical analysis

Differences between concentrations for detected EOCs, trace elements, nutrients and water quality parameters at upstream and downstream sites and between streams were tested using paired t-tests and t-tests respectively, conducted in R Studio.[75]

High throughput sequencing OTUs were 4th root data transformed. Differences in bacterial community structure between; sediment and biofilms, sites (sediment data only) and upstream and downstream were visualised using non-metric multidimensional scaling (nMDS) based on Bray-Curtis similarities with 100 random restarts and were plotted in two dimensions. Differences among the above groups and between sampling months were tested using permutational multi-variate analysis of variance (PERMANOVA; Anderson, 2005)[76] using PERMANOVA+ (Anderson et al., 2008)[77] and PRIMER v7 (Clarke and Gorley, 2015)[78].

Before testing correlations between bacterial community structure and environmental variables, a pollution index (P.I) was calculated based on the concentrations of elements detected in sediments using the following equation based on the method of Kalender et al, 2013:[79]

$$P.I = (CF1 \times CF2 \times CF3 \times \ldots\ldots CFn)^{1/n}$$

Where:

CF = Contaminant factor (the ratio obtained by dividing the concentration of each element in the sediment by the background value)[80].
N = The number of elements used in the equation.

The sums of total emerging contaminants (Total ECs) in water and sediment samples were used for correlations with biofilm and sediment bacterial communities, respectively. Total antimicrobial compounds in sediment samples (sum of mParaben and TCS) was also used for correlations with sediment bacterial communities.

The relationship between bacterial community structure and environmental variables was analysed using multivariate multiple regression using distance-based linear modelling (DistLM) in Primer 7. A marginal test was used where individual variables were fitted separately to test their relationship with bacterial OTU data (ignoring other variables), followed by a stepwise selection procedure, conditional on variables already included in the model and using the AICc selection criteria. The conditional test

identifies the subset of variables that best predicts the observed pattern in bacterial community structure. Both the conditional and marginal tests were undertaken with 9999 permutations using Bray-Curtis similarities. Draftsman plots were used to check collinearity and skewness among predictor variables. Most of the environmental variables used in the analyses were transformed to meet the assumption of homogeneity of dispersion. Where zero values were present, a pre-transformation factor of 0.1 was added. The variables used for correlations with sediment bacterial community structure were log (P.I), temperature, log10 (DO), log (conductivity), pH, log (NH_3 + 0.1), log (NO_3^- + 0.1), DRP, log (mParaben + 0.1), log10 (OPP + 0.1), log10 (OP + 0.1), log (BP3 + 0.1), log (BPA), log (OMC +0.1) log (total ECs), and log (total antimicrobials). All preceding PCP variables used sediment concentrations, the following PCP variables used stream water concentrations. The variables used for correlations with biofilm bacterial community structure were temperature, log10 (DO), log (conductivity), log 10 (pH), log (TOC), log (NH_3 + 0.1), log (NO_3^- + 0.1), DRP, log (Al), Zn, log10 (Cu), Ni, log10 (OP +0.1), log (BP3 +0.1), log (BP1 + 0.1), BPA, log (OMC + 0.1) and log (Total ECs).

The resulting model was visualised using DistLM, where the ordination axes are linear combinations of the environmental variables that maximally explain biotic variation.

A hierarchically-clustered shade plot (Primer7) using averages across sample replicates was used to visualise dominate bacterial class among samples. Species richness (R), Shannon-Wiener diversity index (H') and Pielou evenness index (J) were calculated in PRIMER 7 using the DIVERSE function and t-tests were used to determine differences between indices for Cross Stream and Dudley Creek Diversion.

2.3 Results

2.3.1 Personal care products and steroid hormones

The EOCs which were detected in stream water were BP1, BP3, BPA, mParaben, OMC and OP. Concentrations for detected analytes in stream water are presented in Table 2.3.1a. The UV-filter BP3 was detected in 18 out of 22 samples and BPA was detected in 20 out 22 samples. Concentrations were generally in the low ng/L range. The highest concentration detected was for OMC (17.9 ng/L). No significant differences were observed between upstream and downstream sites for the detected compounds. Higher concentrations of BPA stream water concentration were measured at Dudley Creek Diversion than Cross Stream ($p = \leq 0.05$). At both streams the UV-filter BP3 appeared to be present at lower concentrations in the winter months (June to August) than in March to April, while the UV-filter OMC was not detected in any samples from June to August.

Table 2.3.1a. Upstream and downstream stream water concentrations (ng/L) of emerging organic contaminants at Dudley Creek Diversion (DC) and Cross Stream (CS) from March – August 2015.

Compound	Site	March	April	May	June	July	August
BP1	DC upstream	ND	ND	1.6	<DL	ND	ND
	DC downstream	ND	ND	1	0.7	ND	ND
	CS upstream	NA	ND	ND	6	ND	ND
	CS downstream	NA	ND	ND	<DL	ND	ND
BP3	DC upstream	4.1	ND	2.9	1.7	<DL	<DL
	DC downstream	3.7	3.6	1.6	1.9	<DL	<DL
	CS upstream	NA	3.8	4.7	ND	ND	<DL
	CS downstream	NA	3.1	0.6	1.2	ND	<DL
BPA	DC upstream	7.6	8.1	5.8	6.5	4.8	3.2
	DC downstream	6.2	8.1	3.6	6.8	5	4.5
	CS upstream	NA	1.3	4	0.4	0.8	ND
	CS downstream	NA	<DL	0.5	ND	0.9	1.4
mParaben	DC upstream	1.9	ND	12.9	ND	ND	ND
	DC downstream	<DL	<DL	2.4	ND	ND	ND
	CS upstream	NA	ND	ND	ND	ND	ND
	CS downstream	NA	ND	ND	ND	ND	ND
OMC	DC upstream	9.5	9.8	9.6	ND	ND	ND
	DC downstream	17.9	6.4	ND	ND	ND	ND
	CS upstream	NA	4.9	9.6	ND	ND	ND
	CS downstream	NA	4.4	6	ND	ND	ND
OP	DC upstream	0.5	2.1	0.2	ND	ND	ND
	DC downstream	0.5	0.7	ND	ND	ND	0.3
	CS upstream	NA	ND	ND	ND	ND	ND
	CS downstream	NA	ND	ND	ND	ND	0.1

NA = Stream not sampled that month; ND = Not Detected; <DL = Signal observed but less than detection limit.

Compounds detected in the particulate phase samples (Table 2.3.1b) were similar to those in water samples except for the absence of BP1 and addition of OPP. Methyl paraben was detected more frequently in the particulate phase than in the stream water samples (detected in 50% of particulate phase samples) however most concentrations were below the limit of detection. Bisphenol A was detected at a similar frequency as in the water samples. Benzophenone-3 generally had higher concentrations in the dissolved phase (Figure 2.3.1a – b). Concentrations of BPA tended to be higher in the dissolved phase at Dudley Creek Diversion (Figure 2.3.1c) while the particulate phase was dominant at Cross Stream (Figure 2.3.1d). OMC was detected less frequently in the particulate phase samples than in stream water samples (14% and 41% respectively) but concentrations were similar. Concentrations of OP were also similar between the dissolved and particulate phases. In the same sample a particular compound was not always detected in both the water and particulate phase.

Table 2.3.1b. Upstream and downstream particulate concentrations (ng/L) of detected EOCs at Dudley Creek Diversion (DC) and Cross Stream (CS) from March – August 2015.

Compound	Site	March	April	May	June	July	August
BP3	DC upstream	1.1	1.2	< DL	0.5	0.8	0.3
	DC downstream	ND	1.4	< DL	0.9	0.5	0.4
	CS upstream	NA	1.0	1.2	0.4	0.8	ND
	CS downstream	NA	1.0	1.1	0.6	0.3	0.8
BPA	DC upstream	2.1	0.4	2.3	2.2	3.2	1.9
	DC downstream	ND	ND	3.8	2.9	2.4	2.2
	CS upstream	NA	ND	11.6	2.2	1.6	2.7
	CS downstream	NA	ND	1.3	4.1	2.0	1.5
mParaben	DC upstream	1.4	< DL	ND	ND	ND	ND
	DC downstream	< DL	< DL	ND	< DL	< DL	ND
	CS upstream	NA	< DL	ND	< DL	ND	ND
	CS downstream	NA	< DL	ND	< DL	ND	< DL
OMC	DC upstream	ND	ND	ND	ND	9.9	ND
	DC downstream	5.2	ND	ND	ND	ND	ND
	CS upstream	NA	ND	ND	ND	ND	2.1
	CS downstream	NA	ND	ND	ND	ND	ND
OP	DC upstream	ND	ND	ND	0.2	0.5	0.1
	DC downstream	0.2	ND	0.1	0.4	0.1	0.4
	CS upstream	NA	ND	1.0	ND	0.1	0.6
	CS downstream	NA	ND	0.3	0.5	0.1	0.6
OPP	DC upstream	ND	ND	2.3	1.2	1.5	1.3
	DC downstream	ND	ND	ND	1.4	1.1	1.4
	CS upstream	NA	ND	5.6	1.3	1.6	ND
	CS downstream	NA	ND	1.8	ND	1.2	0.6

ND = Not Detected; <DL = Signal observed but less than detection limit.

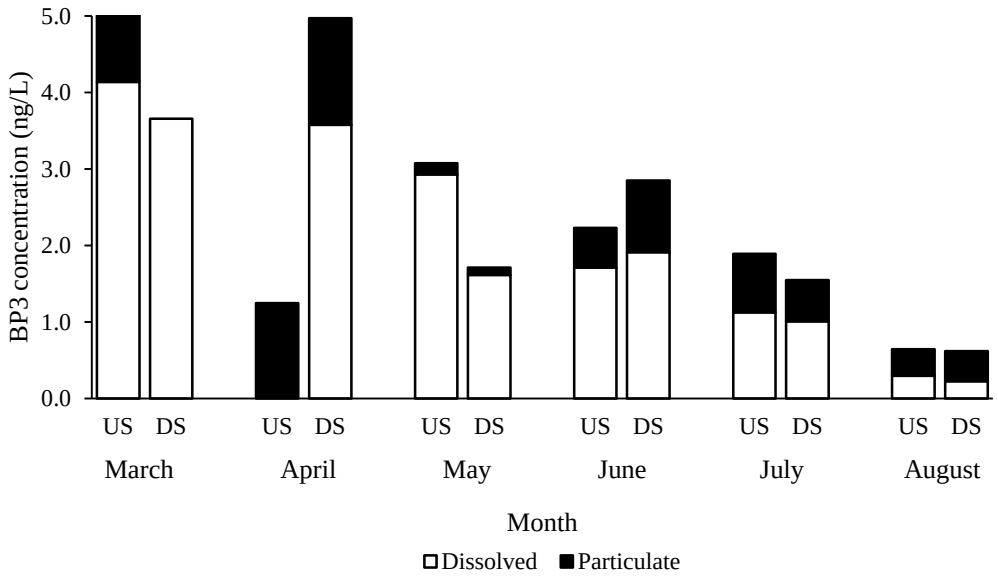


Figure 2.3.1a. Dissolved and particulate phase concentrations of BP3 at Dudley Creek Diversion (US = Upstream, DS = Downstream).

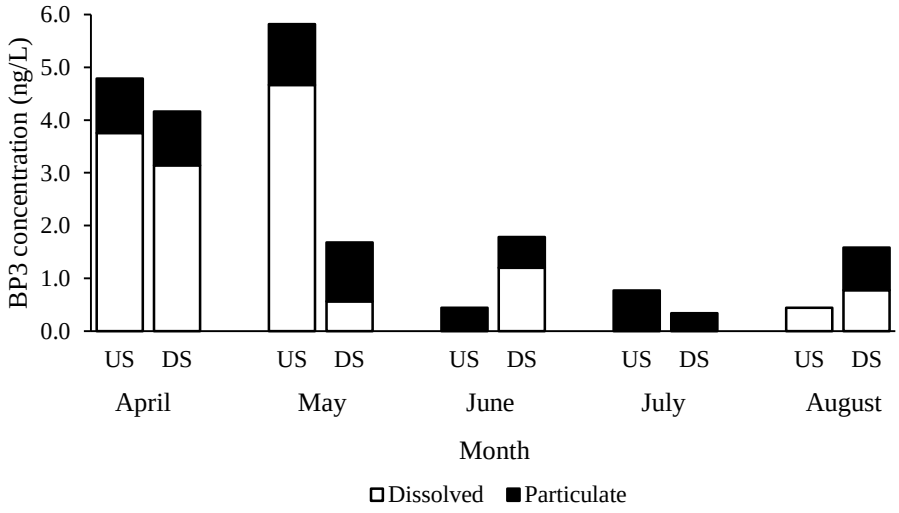


Figure 2.3.1b. Dissolved and particulate phase concentrations of BP3 at Cross Stream (US = Upstream, DS = Downstream).

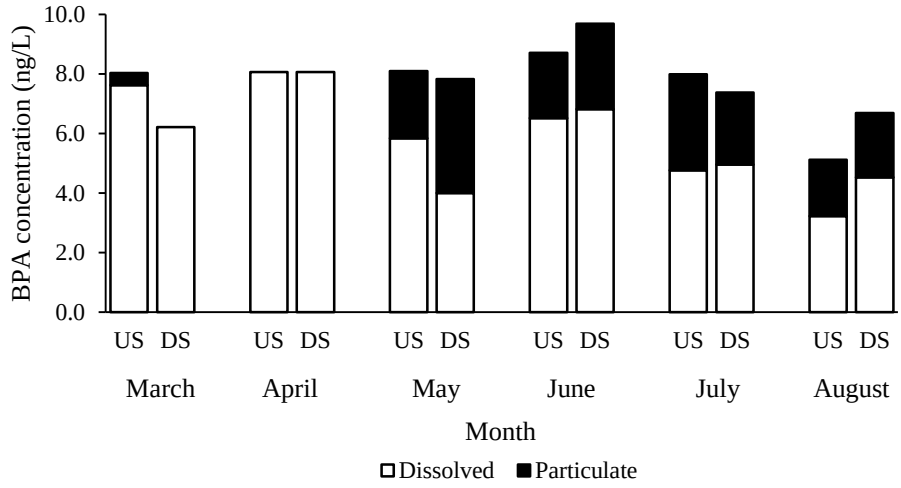


Figure 2.3.1c. Dissolved and particulate phase concentrations of BPA at Dudley Creek Diversion (US = Upstream, DS = Downstream).

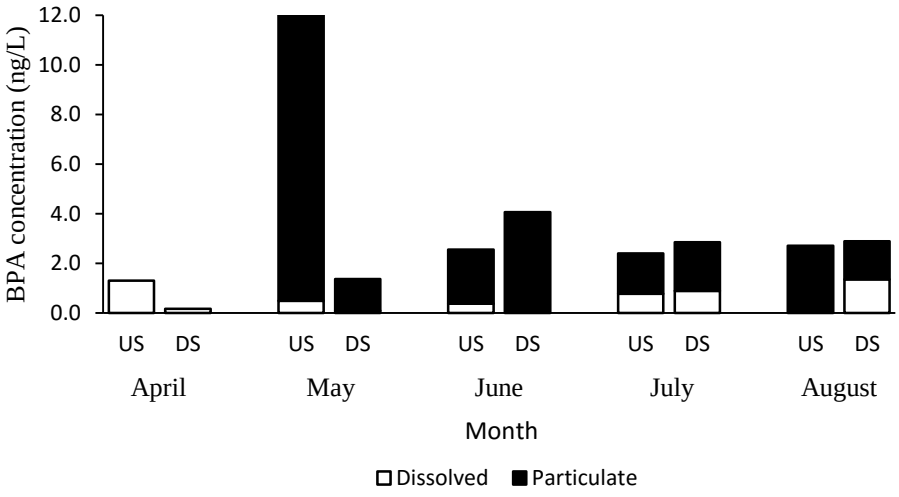


Figure 2.3.1d. Dissolved and particulate phase concentrations of BPA at Cross Stream (US = Upstream, DS = Downstream).

Reported concentrations for sediments are provisional due to the low recoveries of the ^{13}C surrogate compounds in sediment samples. The EOCs which were detected in sediment samples were BP3, BPA, mParaben, OMC, OPP, OP and TCS (Table 2.3.1c). Bisphenol A was detected in all sediment samples at concentrations ranging from 0.7 - 10.2 ng/g. The highest concentration detected was for mParaben (61.8 ng/g). The UV-filter BP3 was also frequently detected (13 out of 17 samples). Triclosan was detected at the downstream Dudley Creek Diversion site consecutively in March and April at 10.5 and 5.3 ng/g, respectively. Concentrations of BP3 were significantly higher at the downstream Dudley Creek Diversion site than upstream ($p < 0.05$). No significant differences were observed for the other

detected compounds between upstream and downstream sites for sediment samples. The sediment concentrations of mParaben at Dudley Creek Diversion were significantly higher than at Cross Stream, while the opposite trend was observed for OPP sediment concentrations ($p < 0.05$).

Table 2.3.1c. Upstream and downstream sediment concentrations (ng/g) of EOCs at Dudley Creek Diversion (DC) and Cross Stream (CS) from March – August 2015.

Compound	Site	March	April	May	June	July	August
BP3	DC upstream	NA	NA	NA	ND	NA	ND
	DC downstream	0.3	0.6	NA	1.9	1.3	0.7
	CS upstream	NS	0.3	0.7	ND	1.5	0.7
	CS downstream	NS	0.6	ND	0.9	0.8	1.6
BPA	DC upstream	NA	NA	NA	8	NA	7.1
	DC downstream	3.8	10.2	NA	4.1	8.2	6.2
	CS upstream	NS	0.8	1.7	6.4	8.1	1.8
	CS downstream	NS	0.9	2.6	0.7	2	6.2
mParaben	DC upstream	NA	NA	NA	61.8[a]	NA	ND
	DC downstream	5.1	20.7	NA	5.1	ND	7.4
	CS upstream	NS	2.6	ND	ND	7.9	ND
	CS downstream	NS	ND	ND	ND	ND	ND
OMC	DC upstream	NA	NA	NA	ND	NA	8.9
	DC downstream	ND	ND	NA	5.5	ND	ND
	CS upstream	NS	ND	ND	ND	ND	ND
	CS downstream	NS	ND	ND	<DL	<DL	ND
OP	DC upstream	NA	NA	NA	ND	NA	3.6
	DC downstream	ND	ND	NA	1.6	ND	2.4
	CS upstream	NS	ND	0.5	1.4	ND	0.5
	CS downstream	NS	0.3	ND	ND	ND	1
OPP	DC upstream	NA	NA	NA	ND	NA	0.7
	DC downstream	ND	ND	NA	ND	1.1	0.3
	CS upstream	NS	0.6	0.4	0.6	ND	1.6
	CS downstream	NS	0.9	1.1	0.9	0.8	0.7
TCS	DC upstream	NA	NA	NA	ND	NA	ND
	DC downstream	10.5	5.3	NA	ND	ND	ND
	CS upstream	NS	ND	ND	ND	ND	ND
	CS downstream	NS	ND	ND	ND	ND	ND

* NA = Insufficient material to analyse; NS = Stream not sampled that month; ND = Not Detected; <DL = Signal observed but less than detection limit.

[a]The value for mParaben of 61.8 ng/g was above the calibration curve. The sample was unable to be reanalysed due to instrumental issues and time constraints.

2.3.2 Trace elements

Measured concentrations for total trace elements and dissolved trace elements are presented in Table 2.3.2a and 2.3.2b. Aluminium, Fe, Mn and Zn were detected in all samples. While lead and copper were also consistently detected, about a third to a half of the measured concentrations were below detection limits. All cadmium and chromium concentrations were below the detection limit. Arsenic and nickel were detected in over half of the samples. For all elements there were no significant differences between upstream and downstream sites for both Dudley Creek Diversion and Cross Stream.

The Australian and New Zealand Conservation Council (ANZECC) provides guidelines for 95% freshwater species protection (Table 2.3.2) were used for comparison.[81] This level of protection was used to interpret stream water results for dissolved metals. In August, dissolved concentrations of aluminium and zinc at Dudley Creek Diversion exceeded their respective ANZECC guidelines (Figure 2.3.2a and 2.3.2b). All other metal concentrations were below their respective guidelines.

All elements were mainly associated with the dissolved phase. The average percentage of total concentration in the dissolved phase for Al, As, Cu, Fe, Mn, Ni, Pb, and Zn were 54.3%, 91.1%, 90.1%, 72.5%, 79.9%, 85.1%, 90 % and 78.7%, respectively.

Table 2.3.2. Australian and New Zealand Environment and Conservation Council guidelines for 95% freshwater species protection.

Element	Conc. (µg/L)
Al	55
Cr	1
Mn	1900
Ni	11
Cu	1.4
Zn	8
As (III)	24
Ag	0.05
Cd	0.2
Pb	3.4

Table 2.3.2a. Upstream and downstream total element concentrations for Dudley Creek Diversion (DC) and Cross Stream (CS) from March – August 2015 (µg/L). Values exceeding the ANZECC value are highlighted in bold.

Element	Site	March	April	May	June	July	August
Al	DC upstream	8.8	8.9	4.9	7.2	8.5	**64.3**
	DC downstream	5.8	8.7	1.8	7.1	7.9	**68.6**
	CS upstream	NA	3.6	1.0	2.3	2.1	6.8
	CS downstream	NA	3.2	1.3	2.7	1.8	8.6
As	DC upstream	< DL	0.2	< DL	0.3	0.3	0.2
	DC downstream	0.1	0.2	< DL	0.3	0.3	0.2
	CS upstream	NA	< DL	< DL	0.2	0.2	< DL
	CS downstream	NA	< DL	< DL	0.2	0.2	< DL
Cu	DC upstream	< DL	0.1	< DL	0.1	< DL	0.5
	DC downstream	< DL	< DL	0.1	0.1	0.1	0.5
	CS upstream	NA	0.2	< DL	0.3	0.3	0.3
	CS downstream	NA	0.3	0.2	0.3	0.3	0.3
Fe	DC upstream	86.4	110.3	59.6	99.8	110.8	334.2
	DC downstream	108.8	114.3	20.9	99.5	116.5	342.9
	CS upstream	NA	21.4	4.6	22.7	18.3	37.7
	CS downstream	NA	21.2	14.5	23.2	17.1	44.6
Mn	DC upstream	8.7	11.9	6.8	9.6	12.1	25.4
	DC downstream	11.8	12.1	4.2	11.1	14.5	25.8
	CS upstream	NA	1.0	0.2	0.8	0.7	0.9
	CS downstream	NA	1.0	0.8	1.0	0.7	1.1
Ni	DC upstream	ND	ND	< DL	0.2	0.2	< DL
	DC downstream	ND	ND	< DL	0.2	0.2	< DL
	CS upstream	NA	< DL	< DL	0.3	0.3	< DL
	CS downstream	NA	< DL	< DL	0.3	0.3	< DL
Pb	DC upstream	< DL	< DL	< DL	0.1	0.1	1.0
	DC downstream	0.1	< DL	< DL	0.1	0.1	1.1
	CS upstream	NA	0.2	< DL	0.1	0.1	0.3
	CS downstream	NA	0.2	< DL	0.2	0.1	0.3
Sb	DC upstream	< DL	< DL	< DL	< DL	< DL	< DL
	DC downstream	0.1	< DL	< DL	< DL	< DL	< DL
	CS upstream	NA	< DL	< DL	< DL	< DL	< DL
	CS downstream	NA	ND	< DL	< DL	< DL	< DL
Zn	DC upstream	5.4	6.9	2.2	7.6	2.6	7.8
	DC downstream	2.7	9.0	1.5	5.8	2.7	**8.1**
	CS upstream	NA	4.1	3.3	3.0	2.5	1.9
	CS downstream	NA	5.0	4.5	10.5	2.2	2.8

† NA: Not applicable as that site could not be sampled that month; <DL: Below the limit of detection for analysis.

Table 2.3.2b. Upstream and downstream dissolved element concentrations for Dudley Creek Diversion (DC) and Cross Stream (CS) from March – August 2015 (µg/L). Values exceeding the ANZECC guideline are highlighted in bold.

Element	Site	March	April	May	June	July	August
Al	DC upstream	0.8	3.5	2.6	2.7	3.5	**59.4**
	DC downstream	3.1	3.9	1.4	3.2	3.3	**61.8**
	CS upstream	NA	1.4	0.6	0.7	0.8	5.8
	CS downstream	NA	1.8	1.0	1.1	0.9	8.0
As	DC upstream	ND	0.1	< DL	0.2	0.3	0.2
	DC downstream	0.1	0.2	< DL	0.3	0.3	0.2
	CS upstream	NA	< DL	< DL	0.2	0.2	0.0
	CS downstream	NA	< DL	< DL	0.2	0.2	0.0
Cu	DC upstream	< DL	< DL	< DL	< DL	< DL	0.5
	DC downstream	0.0	< DL	< DL	< DL	< DL	0.5
	CS upstream	NA	0.2	< DL	0.2	0.2	0.3
	CS downstream	NA	0.2	0.2	0.3	0.2	0.3
Fe	DC upstream	21.3	65.0	34.9	52.1	68.5	324.1
	DC downstream	64.2	67.2	8.8	59.0	67.4	335.0
	CS upstream	NA	18.0	4.5	19.2	15.2	36.2
	CS downstream	NA	15.9	13.0	17.7	15.4	40.6
Mn	DC upstream	4.0	11.2	6.8	9.6	11.9	24.8
	DC downstream	11.4	11.7	4.4	10.6	13.7	25.7
	CS upstream	NA	1.0	0.2	0.8	0.6	0.8
	CS downstream	NA	1.0	0.8	0.9	0.7	1.0
Ni	DC upstream	< DL	< DL	< DL	0.1	0.1	<DL
	DC downstream	< DL	< DL	< DL	0.1	0.1	<DL
	CS upstream	NA	< DL	< DL	0.3	0.3	<DL
	CS downstream	NA	< DL	< DL	0.3	0.3	<DL
Pb	DC upstream	< DL	< DL	< DL	< DL	< DL	0.9
	DC downstream	< DL	< DL	< DL	< DL	< DL	1.0
	CS upstream	NA	< DL	< DL	< DL	< DL	0.2
	CS downstream	NA	< DL	< DL	< DL	< DL	0.2
Sb	DC upstream	< DL	< DL	< DL	< DL	< DL	< DL
	DC downstream	< DL	< DL	< DL	< DL	< DL	< DL
	CS upstream	NA	< DL	< DL	< DL	< DL	< DL
	CS downstream	NA	< DL	< DL	< DL	< DL	< DL
Zn	DC upstream	0.6	6.0	1.6	3.7	2.5	7.6
	DC downstream	2.6	7.2	1.0	5.5	2.6	**8.1**
	CS upstream	NA	3.5	1.3	2.7	2.1	1.9
	CS downstream	NA	4.5	3.1	4.2	2.1	2.6

† NA: Not applicable as that site could not be sampled that month; <DL: Below the limit of detection for analysis.

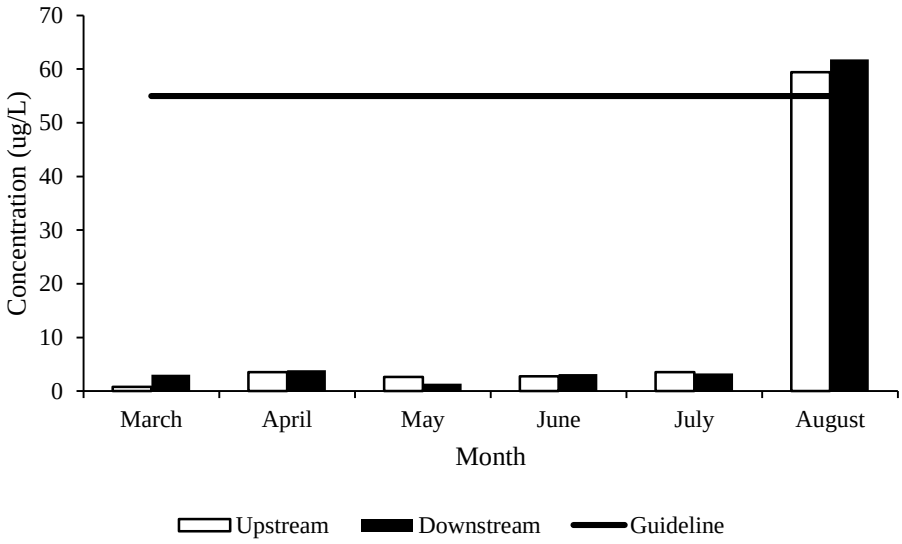


Figure 2.3.2a. Dissolved aluminium concentrations at Dudley Creek Diversion compared to the ANZECC guideline for 95% freshwater species protection.

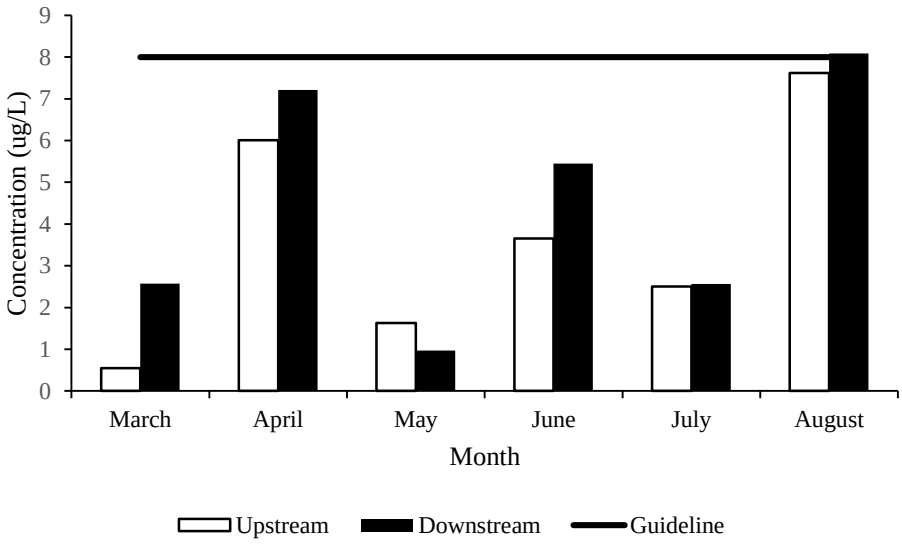


Figure 2.3.2b. Dissolved zinc concentrations at Dudley Creek Diversion compared to the ANZECC guideline for 95% freshwater species protection.

The concentrations of trace elements measured in sediment samples are presented in Table 2.3.2d. Concentrations of Cd, Pb and Sb were significantly higher in downstream sediment samples at Dudley Creek Diversion ($p \leq 0.05$). The concentration for Cu exceeded the interim sediment quality guideline (ISQG; Table 2.3.2c) once at Dudley Creek Diversion and once at Cross Stream. The guideline for Pb was exceeded in every sample from Cross Stream, however there were no exceedances at Dudley Creek Diversion. Zinc concentrations exceeded the ISQG in three out five samples taken from the downstream site at Dudley Creek Diversion. All other values were below the ANZECC guidelines. Pollution indices were all below 1 except for the sample taken in March from the downstream Dudley Creek Diversion

site, indicating that the two streams are mostly unpolluted by metals (Table 2.3.2e). There were no significant differences between upstream and downstream values for either stream, or between streams ($p > 0.05$).

Table 2.3.2c. Australian and New Zealand Environment and Conservation Council interim sediment quality guideline (ISQG) trigger values for trace elements (µg/g).

Element	ISQG Trigger Value
As	20
Cd	1.5
Cr	80
Cu	65
Ni	21
Pb	50
Sb	2
Zn	200

Table 2.3.2d. Upstream and downstream sediment concentrations for Dudley Creek Diversion (DC) and Cross Stream (CS) from March – August 2015 (µg/g). Values exceeding the ANZECC interim sediment quality guideline Trigger Value are in bold. NA = Insufficient material to analyse; NS = Stream not sampled that month

Element	Site	March	April	May	June	July	August
Al	DC upstream	NA	NA	NA	4687	NA	6572
	DC downstream	5542	5240	NA	5724	5567	7701
	CS upstream	NS	4671	4721	4711	4663	5249
	CS downstream	NS	4723	4089	4642	5223	5121
As	DC upstream	NA	NA	NA	4.4	NA	6.7
	DC downstream	14.0	8.1	NA	3.6	11.8	14.8
	CS upstream	NS	2.2	1.8	1.9	1.9	2.2
	CS downstream	NS	1.7	1.6	1.6	2.1	2.0
Cd	DC upstream	NA	NA	NA	0.1	NA	0.0
	DC downstream	0.3	0.1	NA	0.1	0.2	0.2
	CS upstream	NS	0.1	0.1	0.1	0.1	0.1
	CS downstream	NS	0.1	0.1	0.1	0.1	0.1
Cr	DC upstream	NA	NA	NA	8.5	NA	15.0
	DC downstream	21.4	11.3	NA	10.6	22.3	29.0
	CS upstream	NS	9.0	8.3	8.4	8.6	9.9
	CS downstream	NS	8.4	8.0	8.0	9.5	9.7
Cu	DC upstream	NA	NA	NA	7.6	NA	13.7
	DC downstream	**207.5**	15.1	NA	18.2	17.2	29.9
	CS upstream	NS	31.1	21.3	**90.0**	20.4	16.9
	CS downstream	NS	16.5	19.9	19.6	24.5	19.2
Fe	DC upstream	NA	NA	NA	11206	NA	16953
	DC downstream	11981	11784	NA	12315	12363	14646
	CS upstream	NS	10562	9528	9470	8984	10675
	CS downstream	NS	9652	8742	9515	9325	9493
Mn	DC upstream	NA	NA	NA	183.0	NA	514.2
	DC downstream	191.0	245.1	NA	179.3	217.4	236.2
	CS upstream	NS	127.1	125.6	121.5	122.0	139.5
	CS downstream	NS	127.5	114.0	122.1	132.0	129.6
Ni	DC upstream	NA	NA	NA	6.3	NA	10.8
	DC downstream	7.9	7.3	NA	7.9	8.2	9.6
	CS upstream	NS	7.5	7.1	6.9	7.0	7.7
	CS downstream	NS	6.9	6.1	6.6	7.5	7.2
Pb	DC upstream	NA	NA	NA	19.4	NA	18.5
	DC downstream	47.8	26.7	NA	30.6	28.4	36.6
	CS upstream	NS	**199.2**	**115.0**	**156.9**	**68.6**	**51.5**
	CS downstream	NS	**70.6**	**73.7**	**69.6**	**60.8**	**82.5**
Sb	DC upstream	NA	NA	NA	0.1	NA	0.1
	DC downstream	0.5	0.3	NA	0.1	0.2	0.5
	CS upstream	NS	0.3	1.3	0.4	0.3	0.2
	CS downstream	NS	0.1	9.0	0.1	0.1	0.2
Zn	DC upstream	NA	NA	NA	93.4	NA	112.5
	DC downstream	**244.2**	119.1	NA	134.3	**213.2**	**205.7**
	CS upstream	NS	95.6	89.6	84.8	105.0	92.3
	CS downstream	NS	67.1	70.0	78.2	99.4	90.6

Table 2.3.2e. Pollution indices for sediment samples (Value >1 indicate metal pollution exists and are highlighted in bold).

Site	March	April	May	June	July	August
DC upstream	NA	NA	NA	0.37	NA	0.52
DC downstream	**1.09**	0.53	NA	0.48	0.70	0.85
CS upstream	NS	0.56	0.47	0.56	0.44	0.42
CS downstream	NS	0.39	0.38	0.40	0.45	0.43

* NA = Insufficient material to analyse; NS = Stream not sampled that month.

2.3.3 Nutrients

Concentrations for NH_4-N, NO_2-N and DRP were either below the detection limit or close to the detection limit (Table 2.3.3a). Nitrate concentrations were 1 – 2 orders of magnitude higher than the other nutrients. Average NO3-N concentrations ranged from 0.53 – 0.92 mg/L across the sites. There were no significant differences between upstream and downstream sites at both streams ($p < 0.05$).

Table 2.3.3a. Summary statistics for nutrients at upstream and downstream Dudley Creek Diversion and Cross Stream sites (mg/L).

	Ammonia		Nitrite		Nitrate		DRP	
	Range	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**
DC Upstream	0.030 - 0.049	0.039	<DL - 0.003	0.001	0.38 - 0.58	0.53	0.005 - 0.009	0.007
DC Downstream	0.028 - 0.052	0.042	<DL - 0.003	0.002	0.52 - 0.57	0.54	0.005 - 0.012	0.002
CS Upstream	<DL	<DL	<DL	<DL	0.87 -0.94	0.92	0.005 - 0.006	0.005
CS Downstream	<DL - 0.046	0.009	<DL - 0.003	0.001	0.57 - 0.94	0.84	0.004 - 0.006	0.005

2.3.4 Water quality parameters

Water quality parameters are summarised in Table 2.3.4. Ranges and averages for measured values for upstream and downstream were generally similar across both sites and no significant differences were observed. Dissolved oxygen concentrations were significantly lower at Cross Stream than Dudley Creek Diversion (p <0.05).

Table 2.3.4. Summary statistics for water quality parameters at upstream and downstream Dudley Creek Diversion and Cross Stream sites.

	DO (mg/L)		**Temperature (˚C)**		**Conductivity (mS/m)**	
	Range	**Mean**	**Range**	**Mean**	**Range**	**Mean**
DC Upstream	7.9 - 8.4	8.1	12.2 - 14.1	13.0	13.8 - 15.7	14.8
DC Downstream	8.0 - 8.6	8.3	12.1 - 14.0	12.9	12.7 - 15.8	14.7
CS Upstream	4.5 - 6.2	5.5	13.1 - 14.1	13.5	14.6 - 18.4	17.3
CS Downstream	4.8 - 6.2	5.6	13.1 - 14.1	13.5	14.5 - 18.4	17.2
	pH		**TOC (mg/L)**		**TSS (mg/L)**	
	Range	**Mean**	**Range**	**Mean**	**Range**	**Mean**
DC Upstream	6.8 - 7.4	7.2	9.4 – 15.0	12.5	0.6 - 1.3	1.0
DC Downstream	6.9 - 7.4	7.2	7.8 - 14.1	11.9	0.4 - 1.0	0.8
CS Upstream	6.2 – 7.0	6.7	15.1 - 18.4	17.0	0.8 - 1.3	1.1
CS Downstream	6.3 – 7.0	6.7	11.4 - 17.9	14.9	0.5 - 1.1	0.8

2.3.5 Benthic bacterial community composition

Classes of bacteria present in the biofilm and sediment were Acidobacteria, Actinobacteria, Alphaproteobacteria, Betaproteobacteria, Cyanobacteria, Gammaproteobacteria and Nitrospira. At Cross Stream the dominant Class of bacteria in biofilm were Alphaproteobacteria while Betaproteobacteria were most abundant in the sediment communities. Gammaproteobacteria were the dominant Class in sediment communities at Dudley Creek Diversion. Cyanobacteria were the least dominant class in sediment samples while Acidobacteria and Nitrospira were less dominant than Cyanobacteria in biofilm samples (Figure 2.3.5a).

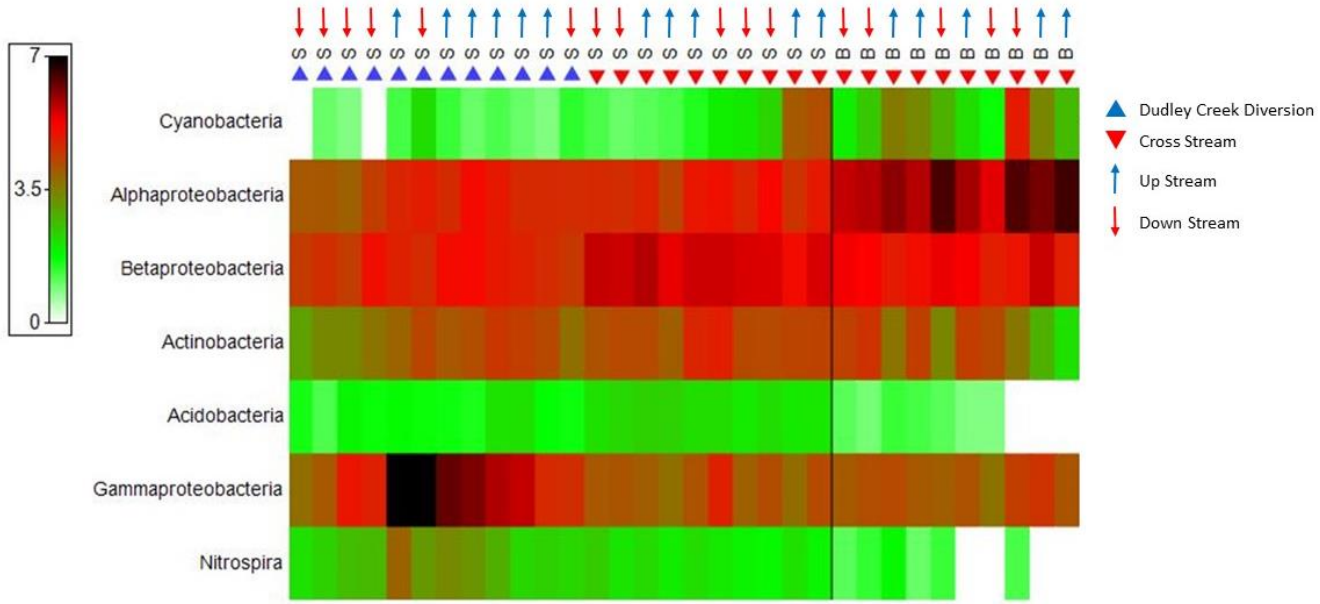


Figure 2.3.5a. Hierarchically-clustered shade plot illustrating the relative dominance of the seven identified Classes of bacteria among samples. The number of reads per samples were fourth root transformed.

Dudley Creek Diversion and Cross stream had very similar mean values and there were no significant differences for number of OTUs, species richness, Shannon-Weiner Index and Pielou Index however these values were more spread for Dudley Creek Diversion (Table 2.3.5a).

Table 2.3.5a. Summary of mean biological parameters for bacterial OTUs from sediment samples at Cross Stream and Dudley Creek Diversion.

Location		No. of reads	No. of OTUs	Shannon diversity (H')	Richness (S)	Evenness (J)
Dudley Creek Diversion	Mean	1686	79	3.76	10.63	0.86
	Std. Dev.	710	4	0.26	0.58	0.06
Cross Stream	Mean	1763	79	3.84	10.49	0.88
	Std. Dev.	300	3	0.09	0.40	0.02

Bacterial assemblages clustered according to their sample type (sediment verses biofilm; Figure 2.3.5b). PERMANOVA confirmed there was a significant difference between the two sample types (F = 45.463, $p \leq 0.001$). Bacterial community structure in sediment was also significantly different between the two sampling sites (PERMANOVA, F = 32.771, $p \leq 0.001$; Figure 2.3.5b). Samples taken from Cross Stream formed a tighter cluster separate from those from Dudley Creek Diversion. There was no significant difference between upstream and downstream sites at Cross Stream for either sediment or biofilm samples ($p > 0.001$). At Dudley Creek Diversion there was a significant difference observed for sediment samples between upstream and downstream sites (PERMANOVA, F = 5.2121, $p \leq 0.001$; Figure 1.2.5b).

A significant difference was identified among sampling months for sediment samples at Dudley Creek Diversion (PERMANOVA, F = 2.4484, $p \leq 0.001$) and Cross Stream (PERMANOVA, F = 1.869, $p \leq 0.001$). Pairwise tests identified among which months these differences occurred (Table 2.3.5b). In particular March was significantly different to all other months at Dudley Creek Diversion.

Table 2.3.2b Pairwise PERMANOVA P-values for bacterial sediment communities by month at Dudley Creek Diversion (DC) and Cross Stream (CS). Values in bold are significant (p<0.05).

Groups	P-Value (DC)	P-Value (CS)
March, April	**0.0083**	NA*
March, May	**0.0367**	NA
March, June	**0.0025**	NA
March, July	**0.0312**	NA
March, August	**0.0026**	NA
April, May	0.2297	0.6584
April, June	0.342	0.1348
April, July	0.2106	**0.0382**
April, August	0.3137	**0.0208**
May, June	0.0114	0.127
May, July	0.2526	0.4687
May, August	**0.0137**	**0.0018**
June, July	0.0513	0.1142
June, August	**0.0498**	**0.0145**
July, August	0.0825	**0.0032**

*NA = CS not sampled in March.

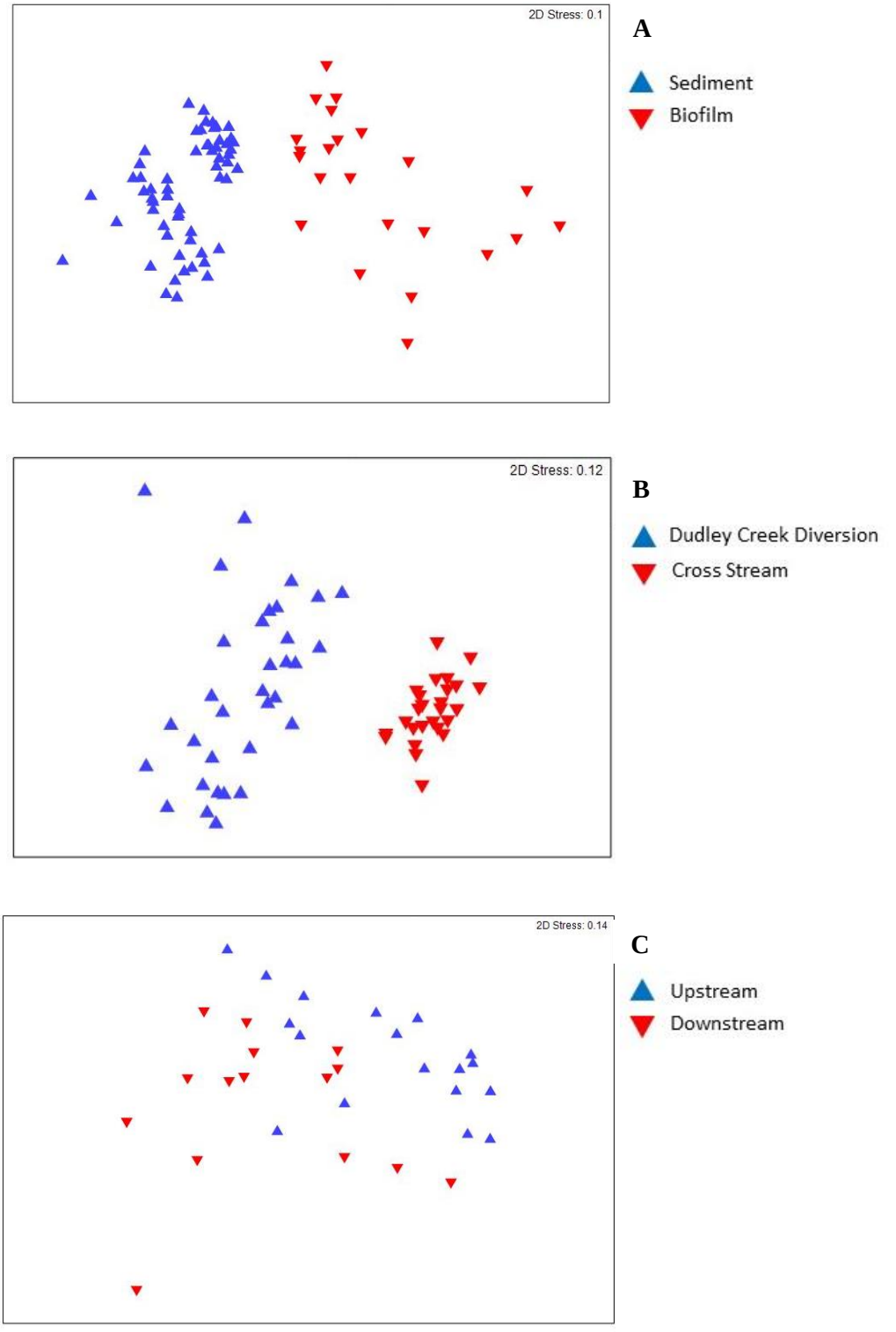


Figure 2.3.5b. Non-metric multidimensional scaling plots based on Bray-Curtis similarities of; **(A)** Bacterial communities in sediment and biofilm samples, **(B)** Bacterial sediment communities at Cross Stream and Dudley Creek Diversion, **(C)** Bacterial sediment communities upstream and downstream of the sewer overflow outfall at Dudley Creek Diversion.

2.3.6 Correlations between bacterial community composition and chemical parameters

The multivariate regression analysis showed that all environmental variables (water quality, nutrients, sediment P.I. and sediment PCP values) individually had a significant relationship with sediment bacterial community structure, except triclosan (Table 2.3.6a). The greatest amount of variation was explained by DO (28.7%) and NO_3-N (27.6%). The sequential model found that seven variables together explained 50.9% of bacterial community structure. Plots based on DistLM analysis indicate that OMC, triclosan and BPA were highly important in structuring the bacterial communities from Dudley Creek Diversion, and conductivity, BP3 and DRP were identified as strongly associated with samples from Cross Stream (Figure 2.3.6).

Table 2.3.6a. Results of multivariate multiple regression (DistLM) using bacterial 16S rRNA sediment data and environmental variables for; **(A)** each variable taken individually (ignoring other variables) and, **(B)** stepwise selection variables, where amount explained by each variable added to the model is conditional on variables already in the model (i.e. those variable listed above it). Prop: proportion of variance in species data explained by that variable; Cumul: cumulative proportion of variance explained.

(A) Marginal test	**F-Value**	**P-Value**	**Prop.**	
log(P.I)	3.27	0.0096	0.054	
Temperature	6.00	0.0005	0.095	
log10(DO)	22.94	0.0001	0.287	
log(Conductivity)	15.67	0.0001	0.216	
pH	12.75	0.0001	0.183	
log(TOC)	8.91	0.0001	0.135	
log(NH_3+0.1)	19.00	0.0001	0.250	
log(NO_3^-+0.1)	21.70	0.0001	0.276	
DRP	10.02	0.0001	0.150	
log(mParaben+0.1)	3.61	0.0068	0.060	
log10(OPP+0.1)	2.61	0.0288	0.044	
log10(OP+0.1)	6.71	0.0002	0.105	
log(BP3+0.1)	6.51	0.0003	0.102	
log(TCS+0.1)	2.00	0.07	0.034	
log(BPA)	12.31	0.0001	0.178	
log(OMC+0.1)	8.73	0.0001	0.133	
log(Total ECs)	18.78	0.0001	0.248	
log(Total Antimicrobials)	3.55	0.0056	0.059	
(B) Sequential tests	**F-Value**	**P-Value**	**Prop.**	**Cumul.**
log10(DO)	22.94	0.0001	0.287	0.287
log(BP3+0.1)	5.35	0.0001	0.062	0.349
log(Conductivity)	2.98	0.0053	0.033	0.383
DRP	3.01	0.0045	0.033	0.415
log(OMC+0.1)	3.09	0.0014	0.032	0.447
log(Tric+0.1)	3.78	0.0004	0.037	0.485
log(BPA)	2.47	0.0103	0.024	0.509

†P.I = Pollution index, DO = Dissolved oxygen, TOC = Total organic carbon, DRP = Dissolved reactive phosphorous, mParaben = Methyl paraben, OPP = O-phenylphenol, OP = Octylphenol, BP3 = Benzophenone-3, TCS = Triclosan, BPA = Bisphenol A, OMC = Octyl methoxycinnamate.

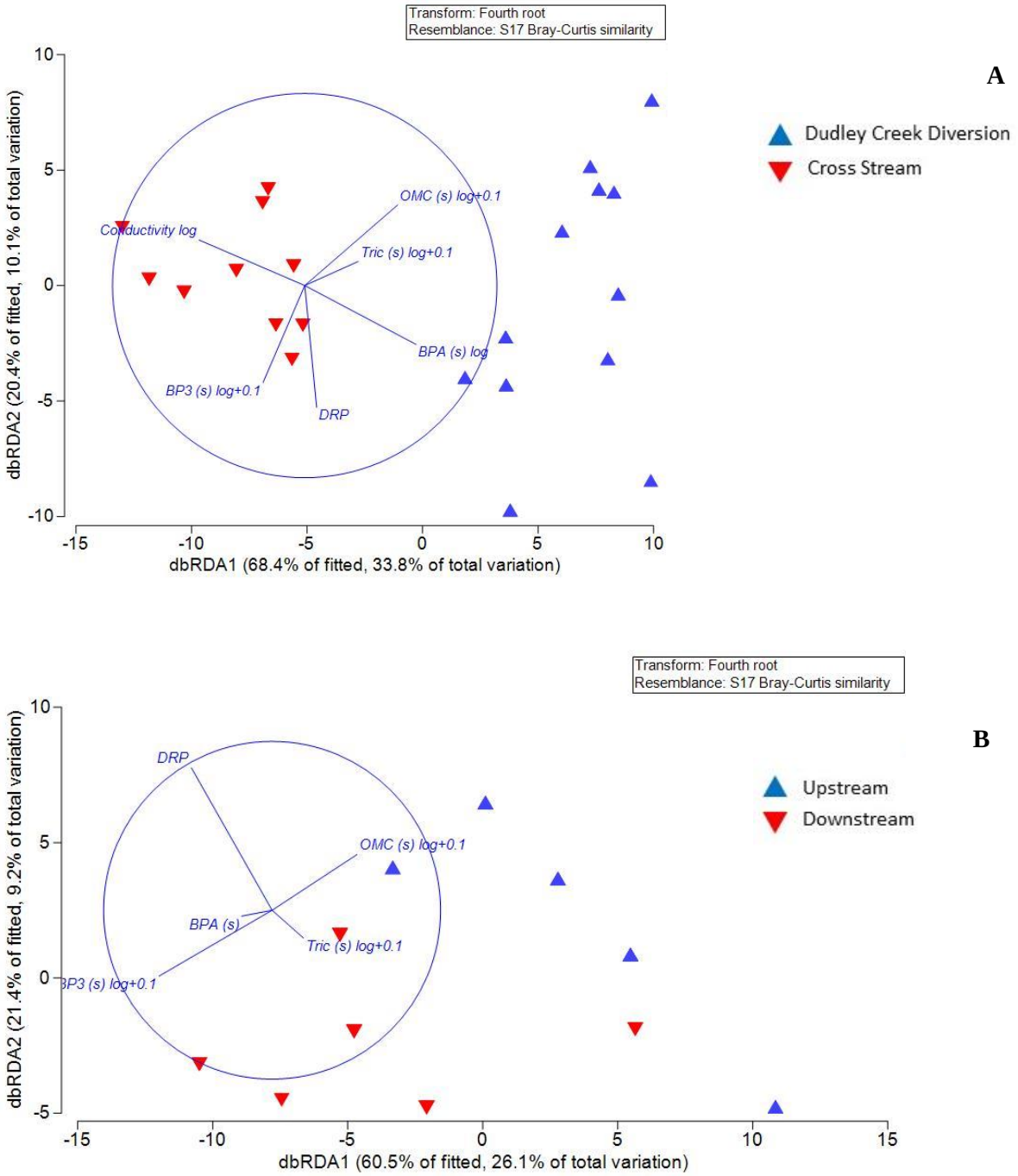


Figure 2.3.6. DistLM plots on the basis of Bray-Curtis similarities of bacterial OTUs DNA from; **(A)** sediment communities at Dudley Creek Diversion and Cross Stream, **(B)** sediment communities at upstream and downstream locations at Dudley Creek Diversion. Vectors showing the best environmental predictors are overlaid at a correlation level > 0.2 (Pearson correlation; BP3 = Benzophenone-3, BPA = Bisphenol A, DRP = Dissolved reactive phosphorous, OMC = Octyl methoxycinnamate).

For biofilm, seven environmental variables showed a significant relationship with bacterial community structure (temperature, DO, conductivity, TOC, Zn, OMC and total ECs). The sequential model found that two variables together explained 40.8% of bacterial community structure (Total ECs and DO, Table 2.3.6b).

Table 2.3.6b. Results of multivariate multiple regression (DistLM) using bacterial 16S biofilm data and environmental variables for; **(A)** each variable taken individually (ignoring other variables) and, **(B)** stepwise selection variables, where amount explained by each variable added to the model is conditional on variables already in the model (i.e. those variable listed above it). Prop: proportion of variance in species data explained by that variable; Cumul: cumulative proportion of variance explained. Significant values are bolded ($P \leq 0.05$).

(A) Marginal Tests	**F-Value**	**P-Value**	**Prop.**
Temperature	4.50	**0.0048**	0.177
log10 (DO)	3.09	**0.0255**	0.128
log (Conductivity)	3.24	**0.0192**	0.133
log (pH)	1.82	**0.1208**	0.080
log (TOC)	2.62	0.0448	0.111
log (NH_3 + 0.1)	1.34	0.2033	0.060
log (NO_3^- + 0.1)	2.17	0.0743	0.093
DRP	0.99	0.3704	0.045
log (Al)	2.37	0.0571	0.101
Zn	3.46	**0.0218**	0.141
log10 (Cu)	1.64	0.1447	0.072
Ni	1.83	0.1178	0.080
log10 (OP + 0.1)	1.04	0.354	0.047
log (BP3 + 0.1)	1.26	0.2515	0.057
log (BP1 + 0.1)	1.69	0.1409	0.075
BPA	1.20	0.2807	0.054
log (OMC + 0.1)	5.17	**0.0031**	0.197
log (Total ECs)	7.85	**0.0001**	0.272

(B) Sequential tests	**F-Value**	**P-Value**	**Prop.**	**Cumul.**
log (Total ECs)	7.85	0.0004	0.27207	0.27207
log10 (DO)	4.60	0.0008	0.13619	0.40826

[†]DO = Dissolved oxygen, TOC = Total organic carbon, DRP = Dissolved reactive phosphorous, OPP = O-phenylphenol, OP = Octylphenol, BP3 = Benzophenone-3, BP1 = Benozphenone-1, BPA = Bisphenol A, OMC = Octyl methoxycinnamate.

2.4 Discussion

2.4.1 Sewer overflow record

The last sewer overflows before the beginning of the field study occurred at Cross Stream in April 2014, and at Dudley Creek Diversion in June 2014. No sewer overflows occurred over the duration of the study. This is likely the reason that there were no significant differences in concentrations between upstream and downstream sites in stream water samples for all classes of contaminants analysed. It is therefore likely that the detected PCPs and metals in stream water were derived from stormwater inputs. Wastewater infrastructure was damaged in the Christchurch earthquakes and leaking pipes in the sewage network still occur.[82]

2.4.2 Commonly detected personal care products

As there is limited data available for urban waterways that do not receive WWTP discharges the results from the current study are useful to enable comparisons to concentrations of PCPs in water bodies of receiving a wide range of inputs.

2.4.2.1 Stream water and particulate phase samples

UV-filters were the most frequently detected compound class in stream water and particulate phase samples. Benzophenone-3, the most commonly detected UV-filter was measured in 55% of stream water samples (max. 4.1 ng/L) and in 82% of particulate phase samples (max. 1.4 ng/L). Concentrations of BP3 were generally higher in the dissolved phase than particulate phase. The log K_{OW} value of BP3 is relatively high (3.8),[83] hydrophobic organic contaminants with high log K_{OW} values are usually associated with the particulate phase of samples, especially particles high in organic carbon.[84] Though the organic carbon content of the particulate phase was not directly measured, total organic carbon (TOC) concentrations for stream water samples ranged from 7.8 – 18.4 mg/L in the present study. It is likely that a large fraction of the TOC content was associated with the dissolved phase in the samples as total suspended solid (TSS) concentrations were low (0.6 – 1.3 mg/L). This may have influenced the lesser partitioning to the particulate phase than expected, based on the log K_{OW} value of BP3. Stream water concentrations of BP3 (max. 4.7 ng/L) were in the lower range of those reported previously for freshwater including a Spanish River draining both agricultural and urban areas (max. 27 ng/L), in moderately polluted rivers in Japan (4 – 12 ng/L) and similar to the concentration detected in a moderately polluted river in Taiwan (3 ng/L) .[85] Higher concentrations have been detected in more heavily polluted rivers such as in Bangkok (116 ng/L).[86] In freshwater BP3 has been measured up to 125 ng/L.[87] Observationally, BP3 stream water concentrations appeared to be lower in the later winter months (all concentrations <DL for July and August). Higher concentrations of UV-filters in freshwater bodies have been reported for summer months compared with winter months in the literature, though

direct inputs due to recreational inputs can influence this trend. Overall the cause of higher concentrations in summer months is likely due to higher usage of products containing UV-filters.[27]

Eco-toxicological data for UV-filters is scarce,[29] however the levels of BP3 detected at Dudley Creek Diversion and Cross Stream are not likely to be toxic to algae (the EC_{50} for the microalge *Isochrisis galbana* was reported as 13.87 µg/L), or higher level organisms as microalgae have been shown to be the most sensitive class of organism to BP3.[26]

The UV-filter OMC was detected in 45% of stream water samples (4.4 – 17.9 ng/L) and in only 14% of particulate phase samples. The log K_{OW} value of OMC is 6.0 and thus it is expected that concentrations would be higher in the particulate phase than the dissolved phase.[27] This was the case at Dudley Creek Diversion in July and Cross Stream in August where OMC was detected in the particulate phase but not in the dissolved phase. However for the highest stream water concentration (17.9 ng/L), the corresponding particulate phase concentration was 5.2 ng/L. This result could also be influenced by the TOC content as described for BP3. Based on stream water concentrations, there also appears to be some temporal variation for OMC as it was not detected in the winter months (June to August). Frequently detected compounds in surface waters impacted by WWTP effluent may reflect the low removal rates of those compounds in WWTPs.[88] This is not applicable to the streams in this thesis which are impacted by stormwater and untreated sewage, thus the relative frequency of certain compounds detected may differ. In this study BP3 was detected more frequently than OMC. In contrast OMC was more frequently detected than BP3 in Korean Rivers,[88] as well as in heavily and moderately polluted rivers in Japan, possibly due to lower removal efficiencies of OMC in WWTPs.[85b] Stream water concentrations for OMC (4.4 – 17.9 ng/L) were comparable to measured concentrations in moderately polluted (12 – 91 ng/L), and background (0 – 18 ng/L) sites in Japan.[85b]

Bisphenol A was the most ubiquitous compound in stream water samples (91% detection frequency; max. 8.1 ng/L) and had the same detection frequency as BP3 in the particulate phase (82%; 0.4 – 11.6 ng/L). There is limited data on the distribution of BPA between the dissolved and particulate phase in urban stream water, however a study conducted in France found that across three catchments, the average amount of BPA in the particulate phase was 18%.[4] There was no significant difference between sites, indicating that the distribution was dependent on the physical and chemical properties of BPA such as its log K_{OW} value (3.4)[89] which indicates some partitioning to the particulate phase is likely.[4] This was not the case in the current study where BPA concentrations were generally higher in the particulate phase than the dissolved phase at Cross Stream, but lower at Dudley Creek Diversion. Total organic carbon was also generally higher at Cross Stream than Dudley Creek Diversion which could be a reason for the differences in distribution. Organic carbon has been identified as a possible key factor influencing the distribution between phases of EOCs, including BPA.[90] No temporal trends were observed in the present study which is not surprising since BPA is ubiquitous in the environment.[91]

Stream water concentrations of BPA in the current study (max. 8.1 ng/L) were similar to minimum detected concentrations in Japan (6.5 – 431 ng/L) and Korea (1.0 – 272 ng/L).[92] BPA has been detected in lakes along the Yangtze River in China up to 141 ng/L.[93] Concentrations in the Elbe River, Germany ranged from levels similar to those in the current study to much higher levels (9 – 776 ng/L).[94] A predicted no effect concentration (PNEC) of BPA for aquatic organisms, based on three trophic levels, has been estimated as 1 µg/L.[95] Therefore concentrations of BPA in Dudley Creek Diversion and Cross Stream are unlikely to result in negative effects on organisms.

Methyl paraben was less frequently detected in stream water (23% of samples; <DL – 12.9 ng/L) than the UV filters and BPA, and was detected in 50% of particulate phase samples, though only one particulate phase sample was above the detection limit and had a concentration of 1.4 ng/L. The higher concentrations measured in the stream water samples reflect the low log K_{OW} value of methyl paraben (1.66) which indicates that the compound is more likely found in the dissolved phase.[96] Concentrations were similar to surface water concentrations measured in Pittsburgh (2.2 – 17.3 ng/L),[97] Switzerland (3.1 – 17 ng/L)[98] and Portugal (3.3 – 16 ng/L).[99] Stream water concentrations have recently been measured an order of magnitude higher than those in the current study in a Brazilian river draining urban and rural areas which had an average concentration of 8.0 µg/L.[100] Methyl paraben was not detected in the winter months (June – August), a similar trend to the UV-filters in the current study. There is little comment in the literature on temporal variation of methyl paraben in stream water, however concentrations in sewage sludge have been reported to remain constant over a four year study period.[101]

Based on reported eco-toxicological data for methyl paraben (impacted reproduction of *Ceriodaphnia dubia* at 11 mg/L[102]; acute LC_{50} for *Daphnia magna* was 24.6 mg/L and >160 mg/L for *Pimephales promelas*[103]) , it is unlikely that aquatic organisms will be impacted by concentrations in Dudley Creek Diversion and Cross Stream. As no other parabens were detected in the current study, aquatic organisms at the study sites are not at risk of binary or synergistic effects of parabens, which is an area of growing concern.[104]

Octylphenol was also detected in both stream water (32% of samples; 0.1 – 2.1 ng/L) and particulate phase samples (68% of samples; 0.1 – 1.0 ng/L), with generally similar concentrations between phases. Octylphenol has been measured directly in stormwater at concentrations up to 72 ng/L in France.[4] The average percentage of total OP in the dissolved phase in the French study was 45%.[4] Reported concentrations elsewhere are orders of magnitude higher than in the current study, though the study sites tend to have more industrial land use in surrounding areas than in the present study. In Italy a river receiving textile factory waste had a measured OP concentration of 0.11 µg/L.[95] Average concentrations of OP in several Portuguese rivers impacted by agricultural and industrial runoff ranged from 0.04 – 2.13 µg/L.[105] Stream water concentrations were closer to those detected in the Yellow River in China

which is an important drinking water source (14.66 – 17.72 ng/L).[106] No seasonal trends were observed for OP concentrations, likely due to its wide spread use with sources including paint, concrete, building materials, asphalt and some vehicle parts.[4] The UK Environment Agency has issued a PNEC for OP of 0.12 µg/L for aquatic organisms, much higher than concentrations detected in the current study.[95]

O-phenylphenol was detected in particulate samples (0.6 – 5.6 ng/L) but not in stream water samples. Partitioning of OPP would be expected as it has a log K_{OW} value of 3.09.[107] It is somewhat surprising that the antimicrobial compound was not detected in stream water samples as other compounds in this study with higher K_{OW} values were. This could be due to the fast flowing nature of the streams and any pulses of OPP may have not been collected in stream water samples. There is limited data in the literature for comparison, however OPP had been detected in water samples from two Portuguese rivers with average concentrations of 6.6 and 32 ng/L.[99]

2.4.2.2 Sediment samples

Sediment concentrations are provisional to the low recoveries of the ^{13}C surrogate standards.

UV-filters were also the most frequently detected class of compound in sediment samples however there is limited data in the literature regarding the occurrence of UV-filters in sediment. Benzophenone-3 was the only PCP to have a significant difference in upstream and downstream sediment concentrations at Dudley Creek Diversion. As BP-3 was not detected in any of the upstream samples at Dudley Creek Diversion it is possible that sewage overflows in the previous year were the source of BP3. The detection frequency of BP3 (67%) was similar to that in Spanish river sediments (65%), however concentrations were generally lower in the present study compared with the Spanish concentrations (0.3 – 1.9 and <DL – 27 ng/g, respectively).[83] In Norway, BP3 was not detected in sediment samples from a lake and fjord receiving WWTP effluent.[26] Benzophenone 3 was also not detected in a recent study which measured sediment concentrations of UV-filters from polluted Iberian rivers.[108] Octyl methoxycinnamate was less frequently detected in sediment samples from the current study (24% of samples; <DL – 8.9 ng/g) than BP3, as was the case for stream water. Reports from the literature indicate that globally, OMC is more commonly found in freshwater sediment than BP3. In the Iberian study where BP3 was not detected, OMC was found in three sediment samples which were relatively similar to the concentrations measured in the current study (7.5, 22.9 and 18.9 ng/g).[108] Both the fjord and lake in the Norwegian study also contained OMC at concentrations ranging from 8.5 – 16.4 and 9.9 – 19.8 ng/g, respectively.[26] Further, OMC sediment concentrations in the current study were comparable to the concentrations reported for all classes of rivers in the Japanese study mentioned in section 2.4.2.1; heavily polluted (2.2 – 9.6 ng/g), moderately polluted (3.8 – 30 ng/g), and background (2.0 – 8.0 ng/g).[85b] The variation in water concentrations (section 2.4.2.1) but consistency in sediment concentrations between site class in the Japanese study may mean that OMC does not readily degrade

in sediment. The results from the current study reflect this as OMC was detected in sediment in the last three months of the study when it was not detected in surface water.

Bisphenol A was detected in every sediment sample (0.7 – 10.2 ng/g) in the current study, again this is likely due to the compounds ubiquitous nature. [91] Sediment concentrations in a German study ranged from 66 – 343 ng/g[94] while sediment concentrations from a large river in Italy were comparable to the current study ranging from 7 – 22 ng/g.[109] Concentrations of BPA can vary greatly depending on the site and its associated inputs. In Taiwan the concentration of BPA in sediments from 16 major rivers ranged from 0.37 – 491.54 ng/g.[110] Rivers with higher BPA concentrations in the study had received effluents from an industrial area as well as untreated municipal wastewater.[110]

Methyl paraben was more frequently detected in sediment samples (35% of samples; 2.6 – 61.8 ng/g) than stream water samples, though not as frequently as in other parts of the world. A study investigated sediment concentrations from several locations in the United States, Japan and Korea, and detected methyl paraben in 100% of the samples.[111] Concentration ranges were similar to the current study including 0.312 – 45.5 ng/g, 2.59 – 17.8 ng/g and 2.43 – 16.2 ng/g for the United States, Japan and Korea, respectively.[111] At Cross Stream, methyl paraben was only detected in upstream sediment samples indicating that sewer overflows are not the primary source of the compound at this site and stormwater may be more important in regards to methyl paraben inputs. In June at Dudley Creek Diversion, methyl paraben was detected at both upstream and downstream sites in sediment samples. The concentration measured in the upstream sample was much higher than in the downstream sample, indicating that non-point source stormwater may also be the primary source of methyl paraben at Dudley Creek Diversion. Potential sources of methyl paraben in stormwater include paint, varnishes, and pesticides.[112]

The concentrations of triclosan measured in the current study (10.5 and 5.3 ng/g) were in agreement with the concentration range in marine sediments in a South Australian Inlet impacted by a WWTP outfall (5 – 27 ng/g). [113] Triclosan has also been measured in marine sediment from Spain (0.27 – 130.7 ng/g)[114] and in estuarine sediments in the USA at concentrations up to 800 ng/g.[115] The last sewer over flow at Dudley Creek Diversion occurred 8 months prior to the detection of triclosan in sediments samples at the site in March and April 2015. The predicted half-life of triclosan in sediment is 240 days and a biodegradation study reported the half-life of triclosan in aerobic soil to be 18 days.[116] The initial sediment concentration of triclosan in the Dudley Creek sediment could have been approximately double the measured concentration in March 2015, had the last sewer overflow been the source of the compound, based on the half-life derived from computer modelling. Based on the sediment degradation half-life found in the biodegradation study for aerobic soil (18 days), the initial triclosan concentration could have been much more than double. This is possible as the measured concentration in the present study for April was approximately half of that measured in March.

Octylphenol was detected in 47% of sediment samples (0.3 – 3.6 ng/g). Studies reporting concentrations of OP in sediment are dominated by those originating from China. Comparable concentrations of OP have been reported in China at a riverine delta connecting an estuary to the South China Sea (0.4 – 3.0 ng/g).[117] Higher concentrations were measured in sediment from a river undergoing rapid urbanisation in China (15.9 – 49.6 ng/g).[118] The maximum OP concentration in the current study was similar to the minimum concentration detected in lake sediment from China where concentrations ranged from 2.69 – 166.87 ng/g.[93]

The sediment concentrations of OPP measured in the current study (0.3 – 1.6 ng/g) were lower than the mean concentration of OPP in river sediment from China (21.78 ng/g).[107] O-phenylphenol was detected at upstream and downstream sites at similar concentrations. Non-point source stormwater could therefore be a source of the contaminant which is used in a wide range of products including glues, concrete additives, leather, and as an active ingredient in disinfectants.[99]

2.4.3 Trace element concentrations compared to New Zealand and global trends

Maximum concentrations of Cu, Pb and Zn reported for other urban streams in the wider Avon Catchment (7, 3.7 and 190 µg/L, respectively) were higher than those in the current study (Table 2.4.3).[119] Dissolved element concentrations have been measured in rivers at their base flow in Wellington, New Zealand (Table 2.4.3). Zinc was measured up to 80 µg/L whereas the highest concentration in the current study was 8.1 µg/L. Copper concentrations in Wellington rivers were also higher than the two Christchurch streams (1.1 – 2.1 µg/L). Both Cr and Cd concentrations were also below the detection limits. The highest recorded Pb concentration in Wellington was 0.3 µg/L compared to 1.0 µg/L measured in Dudley Creek Diversion.[120] Trace element concentrations in the current study were generally below ANZECC guidelines for 95% species protection and are therefore not likely to impact organisms in Cross Stream and Dudley Creek Diversion.[81]

Globally, similar dissolved concentrations have been reported in England for Mn, Pb and Zn. Other elements tended to reach higher concentrations (Table 2.4.3).[121] However another river in the United Kingdom with urban inputs generally had higher mean concentrations for all trace elements.[122] Urban and Rural rivers in Ireland had an average Al concentration of 69 µg/L.[123]

Significantly higher concentrations have been reported internationally for polluted streams including in Turkey[124] and Houston where concentrations at four urban sites ranged from 0.29 – 0.98 mg/L for Fe and 0.03 – 0.22 mg/L for Zn (Table 2.4.3).[125]

Sediment concentrations for Cu, Pb and Zn (7.6 – 207.5, 18.5 – 199.2 and 67.1 – 244.2 µg/g, respectively) were generally higher than thos reported for another Christchurch urban stream (Cashmere Stream; 9.3, 16 and 98 µg/g, respectively).[126]

Table 2.4.3. Dissolved metal concentrations compared to the literature (concentrations are all µg/L except where denoted with *, the concentration is mg/L).

Element	Present Study Concentration Range (ug/L)	Literature Values (ug/L)	Location of Study
Al	0.8 - 61.8	3 - 831	England[121]
		55 (mean)	UK[122]
		69 (mean)	Ireland[123]
		0.17 - 133.5*	Turkey[124]
As	<DL - 0.3	0.68 (mean)	UK[122]
		10 - 290	Turkey
Cd	<DL	0.01 - 0.61	England[121]
		3 -15	Turkey[124]
Cr	<DL	0.34 (mean)	UK[122]
		16 - 110	Turkey[124]
Cu	<DL - 0.5	1.1 - 2.1	Wellington[120]
		0.1 - 3	England[121]
		2.43 (mean)	UK[122]
		11 - 94	Turkey[124]
		<DL – 7[a]	Christchurch[119]
Fe	4.5 - 335.0	142 (mean)	UK[122]
		0.25 - 48*	Turkey[124]
		0.29 – 0.98*	Houston[125]
Mn	0.2 - 25.7	0.2 - 73.3	England[121]
		86.7 (mean)	UK[122]
Ni	<DL - 0.3	0.1 - 1.2	England[121]
		4.0 (mean)	UK[122]
Pb	<DL - 1.0	0.3 (max.)	Wellington[120]
		0.05 - 0.63	England[121]
		2.7 (mean)	UK[122]
		11 - 370	Turkey[124]
		<DL – 3.7[a]	Christchurch[119]
Sb	<DL	0.50 (mean)	UK
Zn	0.6 - 8.1	80 (max.)	Wellington[120]
		0.2 - 11.8	England[121]
		21.60 (mean)	UK[122]
		150 - 4100	Turkey[124]
		0.03 – 0.22*	Houston[125]
		<DL – 190[a]	Christchurch[119]

[a] Concentrations are presented for urban streams in the Avon catchment only. Detection limits for Cu, Pb and Zn were 2, 1.5 and 1 µg/L, respectively, and therefore data from this report is difficult to compare to results from the current study.

2.4.4 Bacterial community composition and correlations with chemical data

Gammaproteobacteria were dominant in sediment at Dudley Creek Diversion and betaproteobacteria at Cross Stream. Alphaproteobacteria were dominant in biofilm. A study in South Australia has reported the presence of gammaproteobacteria in stream sediment at a site impacted by WWTP effluent while the same class of bacteria were not present at less impacted sites indicating that gammaproteobacteria are associated with streams polluted by wastewater.[127] Gammaproteobacteria have also been found to be dominant in river sediments of varying degrees of pollution.[127] Betaproteobacteria were also relatively abundant in the same sediments, while Cyanobacteria, Nitropirae, Actinobacteria and Acidobacteria were much less abundant, as was observed in the current study.[127] Alphaproteobacteria have also been reported as the dominant proteobacteria in Czech stream biofilms.[128]

Though DO, conductivity and DRP concentrations were not directly measured in the sediments, these water quality parameters could indirectly influence bacterial sediment community composition and thus were kept in the statistical analysis. In the stepwise DistLM analysis DO explained 28.7% of the sediment bacterial community structure. Dissolved oxygen concentrations were significantly lower at Cross Stream than Dudley Creek Diversion which may be why the water quality parameter explained a relatively large percentage of sediment community structure. It is also possible the lower DO at Cross Stream is one of the factors that influenced the significantly different sediment bacterial community structures between the two streams. Microbial community composition in Belgian river sediment was also positively correlated with DO after the introduction of a WWTP improving the water quality of the river which previously received untreated sewage.[129] Dissolved oxygen is also a known limiting factor for the growth of aerobic microorganisms.[130]

After DO, BP3 explained the second largest proportion (6.2%) of variation in sediment community composition. Data on the effects of BP3 and other UV filters on bacterial communities are very scarce, likely because UV filters are not designed to have antimicrobial properties. Other microorganisms such as microalgae have been shown to be more sensitive to BP3 than higher order species, though the EC_{50} value reported for the microalgae (13.87 µg/L) was much higher than concentrations detected in the current study.[26] There was also a significant difference between BP3 concentrations upstream and downstream of the sewer overflow outfall which may have influenced the bacterial community composition at the same site.

Triclosan did not have a large impact on bacterial community composition as observed in other studies,[51, 59] only explaining 3.2% of sediment bacterial community structure in the stepwise analysis and was not significant individually. Triclosan was only detected in two sediment samples which is likely the reason for this, whereas BP3 was more frequently detected.

Trace elements did not contribute to the structure of bacterial communities in step-wise analyses for either sediment or biofilm. Another New Zealand study using stream water amended with Zn, Cu and Pb in an artificial flow chamber reported a significant change in biofilm bacterial community structure after just 3 days of exposure.[131] However concentrations in the flow chamber experiment (500 µg/L Zn, 50 µg/L Cu and 50 µg/L Pb) were much higher than concentrations measured at Cross Stream where biofilm samples were taken from in the current study.[131] Biofilms in the same study were shown to recover, becoming more similar to unexposed populations once transferred to uncontaminated water.[131] It is therefore possible that biofilms in the environment, such as in the current study may be able to recover from pulses of increased metal contaminants. Most trace element concentrations in the current study were also below ANZECC guidelines and therefore may not have been high enough to induce change in bacterial community structure.

Bacterial sediment communities at Dudley Creek Diversion in March were significantly different from those sampled in all other months. The only pollution index value greater than 1 (indicating metal pollution) occurred in March at Dudley Creek Diversion which could be a reason behind the bacterial community trend. However, as discussed above, metals were not found to be important in structuring community composition. The lowest BP3 and BPA sediment concentrations measured at Dudley Creek Diversion were both recorded in March. Both of these compounds were important in the sequential DistLM analysis and could be a factor in the temporal difference in community composition.

2.5 Conclusion

Compounds detected in stream water samples included the UV-filters BP1, BP3 and OMC along with the most ubiquitous compound BPA, and less frequently detected mParaben and OP. All compounds that were detected in stream water were also detected in the particulate phase except BP1. Concentrations of the detected PCPs were in the low ng/L range and were below concentrations reported to be toxic to aquatic organisms. The highest concentration measured in stream water was for OMC (17.9 ng/L) which was similar to concentrations measured in moderately polluted rivers in Japan. Observed temporal patterns included lower concentrations of UV-filters form June to August, possibly caused by lower usage of products containing these compounds during winter. There were no significant differences in upstream and downstream concentrations for any compounds likely because no sewer overflows occurred during the 6 month study.

In the sediment samples, BP3 was significantly higher at the upstream Dudley Creek Diversion site than downstream indicating sewer overflows as a potential source of the UV-filter. Bisphenol A was also the most frequently detected compound in sediment samples, representing its widespread inclusion in a range of products and industrial sources. Triclosan, the antimicrobial compound studied in Chapter Three, was detected in two downstream sediment samples in March and April at Dudley Creek

Diversion at concentrations of 10.5 and 5.3 ng/g. The results from this study contribute to the very limited data on concentrations of PCPs in New Zealand waterways.

Several PCPs were shown to influence bacterial community structure including BP3, OMC, TCS and BPA. Triclosan did not impact bacterial community structure to the same degree as reported in other studies, though this could be because the compound was only detected in two samples. Trace elements were not found to play a role in bacterial community structure, however concentrations detected in this study were generally below ANZECC guidelines and were therefore unlikely to impact organisms at Cross Stream and Dudley Creek Diversion.

3 Effects of triclosan on photosynthetic microorganisms

3.1 Introduction

Triclosan is frequently detected in the aquatic environment and further information is required to determine its potential impacts on aquatic life.[132] As triclosan is an antimicrobial compound there is particular concern over the effects on aquatic microorganisms such as algae.[51] Algae play an important role in aquatic ecosystems as primary producers and impacts on these organisms may effect higher trophic levels.[17] Triclosan was detected in stream sediments in this study (section 2.3.1) indicating that aquatic organisms in New Zealand waterways are exposed to this antimicrobial compound.

The toxicity of triclosan to aquatic organisms has been investigated in several studies. A 2002 study by Orvos et al examined toxicity to a range of organisms including fish, invertebrates, and higher order plants.[20] Reported EC_{50} values for triclosan included invertebrates (*Daphnia magna* mortality 390 µg/L, 48hrs and *Ceriodaphnia dubia* survival and reproduction 184.7 µg/L, 48hrs)[20], fish (*Pimephales promelas* and *Lepomis macrochirus* mortality 260 and 370 µg/L, respectively, 96 hrs)[20] and Macrophytes (*Lemna gibba* growth inhibition >62.5 µg/L, 7 d)[20]. Certain species of microalgae have been shown to be the most sensitive organisms to triclosan. The green algae *Scenedesmus subspicatus* had an EC_{50} for inhibition of biomass of 1.4 µg/L whereas a second green alga, *Selenstrum capricornutum* had an EC_{50} for growth inhibition of 4.46 µg/L.[20, 135] Both of their values were significantly lower than the EC_{50} values reported for other aquatic organisms.[20] The diatoms *Skeletonema* and *Navicula*, however, showed a greater resistance to triclosan compared with other microalgae with EC_{50} values of 66 and 19.1 µg/L, respectively.[20] In a mesocosm study filamentous green algae in a biofilm community decreased in abundance and cellular integrity was impacted after exposure to triclosan while diatoms in the community recovered after a decrease in abundance.[58] Other studies have documented impacts on river biofilms including inhibition of biofilm development and reduced species diversity,[59] and decreased photosynthetic activity.[136]

There is ongoing discussion as to the reason behind the significant differences in sensitivity of microalgae to triclosan.[135] The variation in sensitivity to triclosan of microalgae could have implications for aquatic ecosystems. Species that are more tolerant to triclosan could become dominant in environments where this compound is present.[51] Changes in community diversity may have implications on community function in higher food web levels, for example if less nutritious organisms increase in abundance.[51] Drury et al showed that after exposure to triclosan there was a 6-fold increase in the relative number of cyanobacterial sequences, increasing from <1% of the total community to just over 5% while there was a clear reduction in abundance of green algae.[51]

Contrary to Drury et al's findings, reductions in both algal and cyanobacterial biomass have been observed in other river biofilm communities, despite no significant impact on biomass of bacteria.[137] Other studies also suggest that algae are more sensitive to triclosan than bacteria despite this compound being used for its antibacterial properties. Triclosan was not found to be toxic to bacteria in marine periphyton communities where algae were impacted in a recent Swedish study.[138] The microalga *Selenastrum capricornutum* was also shown to be 30-fold more sensitive to triclosan that the bacterium *Vibrio fisheri.*[139]

In this study the effect of triclosan on the photosynthetic activity of a green alga *Stigeoclonium* sp. and the benthic cyanobacteria *Phormidium autumnale* was monitored over 96 hrs. *Stigeoclonium* is a common genus in New Zealand waterways and is known to dominate periphyton.[140] The effect of metals on algae has been well studied and metals are known to be toxic to algae. For example, Copper (Cu) is an essential nutrient for aquatic life, however at concentrations as low as 1 µg/L it can be toxic.[141] Copper was used in this experiment to establish its toxicity to *Stigeoclonium* sp. and *P. autumnale* to enable comparison to other species' sensitivity in the literature. As there is limited information on the toxicity of triclosan to New Zealand algae species, this study provides insight into the potential impacts of triclosan on primary producers in New Zealand waterways.

3.1.1 Pulse amplitude modulated chlorophyll fluorometry

In recent years toxicity tests using algae have progressed to include the use of Pulse Amplitude Modulated (PAM) flurometers.[142] The development and use of PAM technology is due to enhanced understanding of chlorophyll fluorescence.[143] The efficiency of photosynthetic activity at Photosystem II (PSII) reaction centres in algal cells is associated with chlorophyll fluorescence.[144] Reaction centres in dark adapted algae are open and available to receive and process a photon. A saturating pulse (>3000 µmol photon $m^{-2}s^{-1}$) closes all reaction centres so that additional photons absorbed will be reemitted as fluorescence or heat.[144] Heat generation is favoured when the reaction centre is under stress while fluorescence is favoured when the reaction centre is not stressed. The maximum quantum yield (F_v/F_m) is a measure of the potential of a cell to undergo photosynthesis if all reaction centres are open at PS II.[144] Thus PAM allows measurements of photosynthetic activity as an indicator of cell health.

3.1.2 Objectives

The objectives of this experiment were to:

- Determine the concentrations of triclosan that inhibit the photosynthetic activity of *Stigeoclonium* sp.and *P. autumnale.*
- Determine whether *Stigeoclonium sp*. was more sensitive to triclosan than *P. autumnale.*
- Determine the sensitivity of *Stigeoclonium* sp. and *P. autumnale* to copper to enable comparison to literature.

3.2 Methods

3.2.1 Culturing and bioassay design

Isolation and culturing

A biofilm sample was taken from a cobble in Cross Stream using a Whirl-pak™ Speci-sponge™ and placed in a clean container. Any long algae filaments were scraped directly into the container which was filled with stream water and capped. Filaments were isolated from samples by micro-pipetting and were transferred to 24-well plates (Becton Dickinson, USA) containing 500 µL of growth medium (MLA medium) per well.[145] Successfully isolated strains (*Stigeoclonium* sp.*)* were incubated under standard conditions (100 ± 20 µmol photons $m^{-2}.s^{-1}$; 12:12 hour light:dark; 18 ± 1°C) and maintained in 50 mL plastic bottles (ThermoFisher Scientific, New Zealand). The *P. autumnale* strain CYN50 from the Cawthron culture collection (http://cultures.cawthron.org) was also grown under the same conditions.[145] Both cultures were grown until sufficient biomass was obtained for the experiment.

Range finding experiment

The concentrations of triclosan and copper used in the current study were determined based on the results of two initial range finding experiments exposing *Stigeoclonium* sp. to triclosan and one experiment exposing *Stigeoclonium* sp. to copper. Nominal triclosan concentrations in the first range finding experiment were 0, 0.05, 0.5, 5 and 50 µg/L (no observed effects), and 0, 0.005, 0.5, 0.5 and 5 mg/L for the second experiment where no effect was observed at 0.5 mg/L and below but *Stigeoclonium* sp. were killed at 5 mg/L. Concentrations used in the Cu range finding experiment were 0.0001, 0.001, 0.01, 0.1 and 1 mg/L.

Media preparation

Stigeoclonium sp. and *P. autumnale* were exposed to triclosan and Cu separately in the Organisation for Economic Co-operation and Development (OECD) artificial freshwater medium.[146] In this experiment, $CuCl_2.2H_2O$ was emitted from the OECD medium as it could not be sourced in the

laboratory. Copper is an essential element for growth but at higher concentrations can impact cell function.[141] Therefore the absence of $CuCl_2.2H_2O$ may have affected the potential growth of triclosan exposed algae but for the Cu treatments any potential effect would have been insignificant compared to the levels of Cu added to the artificial freshwater (prescribed concentration of $CuCl_2.2H_2O$ in the OECD medium is 0.012 µg/L)[147]. This experiment did not test growth as an end point so results should not have been affected.

Five variations of triclosan spiked OECD medium were prepared (Table 3.2.1). Due to the low solubility of triclosan in water, five standards of triclosan were prepared in acetone at necessary concentrations so that when 25 µL of each standard was diluted to 250 mL with OECD medium the desired triclosan concentration was obtained. The standards in acetone were prepared in amber glass vials on ice to prevent photo-degradation of triclosan and evaporation of acetone. The final concentration of acetone in each of the OECD medium variations was 0.1% as recommended by the OECD to minimise solvent effects and a 0.1% acetone blank was included in the experiment to account for any effects.[147] For each triclosan spiked medium preparation, the volumetric flask was chilled on ice before dispensing 25 uL of the triclosan standard into the flask. The OECD medium was immediately added with constant shaking until a volume of 250 mL had been reached. All glassware and vials used in the triclosan exposure experiment were solvent rinsed with HPLC grade dichloromethane (DCM), methanol (MeOH) and acetone prior to use.

All glassware and vials used for Cu exposures were acid washed over night in 10% HNO_3 and rinsed thoroughly with Milli-Q water. Five Cu concentrations were prepared by diluting the 100 mg/L (Cu) stock standard of $CuSO_4.5H_2O$ with OECD medium in 250 mL volumetric flasks (Table 3.2.1).

Experimental setup

The *Stigeoclonium* sp. culture was transferred into four 50 mL Falcon tubes and centrifuged (20 min, 3, 200 × g). The MLA media was removed using a pipette and the algal suspensions were combined into a single Falcon tube. The process of spinning down and pipetting off MLA media was repeated until a concentrated suspension of algae was obtained in about 5 mL of residual MLA medium. A homogenous algae suspension was made up to 45 mL with OECD artificial freshwater medium.

The *P. autumnale* culture formed a mat within the flask. The mat was carefully teased apart into small pieces with sterilised tweezers. The size of the pieces was similar to the base of a well in a 96 well plate to enable sufficient coverage when carrying out PAM measurements. The *P. autumnale* culture was then centrifuged (10 min, 3, 200 × g) and the MLA medium removed and made up to 45 mL with OECD medium.

Aliquots (20 mL) of each OECD medium variation were pipetted in two sets of triplicates into pre-labelled, glass scintillation vials. An aliquot (1 mL) of *Stigeoclonium* sp. suspension (Figure 3.2.1) and of *P. autumnale* was added to the two separate sets of test vials in triplicate.

Table 3.2.1 Nominal triclosan and copper concentrations used for the exposure experiment.

TCS (mg/L)	Cu (mg/L)
0	0
0.70	0.05
1.12	0.09
1.79	0.16
2.87	0.29
4.59	0.52

Triplicate control samples (no algae) were set up for each concentration to monitor toxicant concentration over the duration of the experiment. Each triplicate was sampled for both triclosan and Cu analysis at each sampling time (10 mL aliquots each). On the final day, aliquots (10 mL) were taken from replicates containing *Stigeoclonium* sp. to determine whether there was any microbial degradation or sorption of copper. Samples for Cu analysis were stored in 10 mL centrifuge tubes and acidified (< pH 2) in the University of Canterbury clean room with quartz distilled ultra-pure HNO_3. Samples were then left to sit for one week before analysis. Samples for triclosan analysis were stored in solvent washed 15 mL glass vials. Dichloromethane (2 mL) was added to each water sample for triclosan analysis immediately before shaking the vial to extract the triclosan from the water into the dichloromethane fraction. All samples were stored refrigerated until analysis.

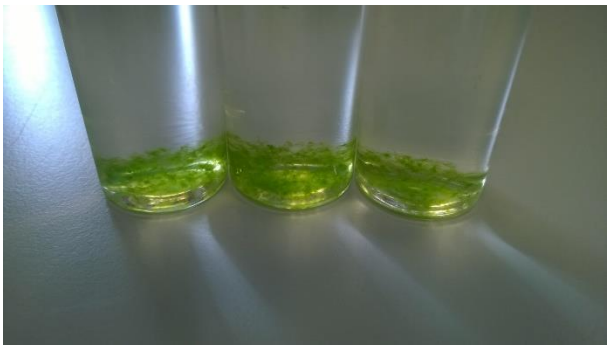


Figure 3.2.1. *Stigeoclonium* sp. suspension in glass scintillation vials.

3.2.2 Analysis of health and survival by use of a pulse-amplitude modulated chlorophyll fluorometer

A subsample (200 µL) of the homogenous *Stigeoclonium* sp. and a piece of *P. autumnale in* 200 µL of media were taken from each scintillation vial and dispensed into individual wells in a 96 well plate (OptiPlate-96, Black Opaque 96-well Microplate, Perkin Elmer) at 0, 24, 48, 72 and 92 hrs.[148] A black

polythene cover was attached to the bottom of the plate to prevent light leakage between wells. The black frames of the wells also prevented light leakage.[148]

A Walz Phyto-PAM instrument with an emitter detector fibre-optic (EDF) probe was used to carry out Pulse Amplitude Modulated (PAM) fluorometry on the samples at each sub-sampling period.[148] The plate was dark adapted for at least 20 minutes before beginning measurements. The EDF probe was secured in place on a stand to maintain the same distance from algae cells for each measurement. The maximum quantum efficiency of the PSII photochemistry (Fv/Fm) and a rapid light curve (RLC) were determined for each sample by saturating the algae with a pulse of light at 2600 µmol quanta $m^{-2}s^{-1}$, pulse length 200 ms.[148] The photon flux was then increased for 10 second pulses (PAR = 0, 1, 16, 32, 64, 90, 120, 180, 295, 405 µmol photons$^{-1}m^2s^{-1}$) respectively. The auto-gain function was used to optimise readings from samples with varying culture densities.[148]

3.2.3 Analysis of water from bioassays

Copper

Samples were analysed for Cu concentration by Agilent 7500 series ICP-MS as per section 2.2.5 of this thesis. A water CRM was included in the analysis (Synthetic 1643 CRM, Inorganic Ventures; CRM Cu recovery = 105%). A duplicate sub-sample was analysed after every 10 samples, followed by a duplicate and 3 mL triplicate sub-sample sample spiked with 30 µL of a 1000 µg/L standard after every 20th sample. Duplicate percentage difference and spike recovery results are presented in Table 3.2.3. Before each run, the instrument was calibrated with a blank and standards ranging from 0.1-1000 µg/L. The detection limit for Cu was 0.1 µg/L.

Table 3.2.3. Duplicate percentage difference (n = 9) and spike recovery (n = 5) for copper analysis (C.I = confidence interval).

QA/QC	Average	Std. Dev.	Lower C.I	Upper C.I
% Difference	3.3	3.3	1.5	5.1
% Spike Recovery	88.4	2.6	86.7	90.0

Triclosan

The artificial freshwater samples were extracted by liquid-liquid extraction. The DCM fraction for each sample was removed from the amber glass vial and transferred to another pre-weighed amber glass vial with a Pasteur pipette. An additional 2 mL of DCM was added to the water sample and vortexed before leaving the sample to sit for 5 minutes. The DCM was removed from the sample and combined with the original DCM fraction. This was repeated once more. The combined extracts were dried under nitrogen gas at 40 °C before re-weighing the vials to determine the amount of water transferred. The dried extracts were quantitatively transferred with MeOH (1×500 µL, 2×250 µL) to an amber glass High performance liquid chromatography (HPLC) vial to be analysed by HPLC.

The method of analysis was modified from that of Ricart et al 2010.[149]Samples were analysed by a Dionex HPLC fitted with an Ultimate® 3000 pump and autosampler coupled to an Ultimate® 3000 diode array detector. Samples were injected (20 µL) onto a 150 × 2 mm Phenomonex Gemini® column (5 µm particle size, C18 packing material, 110A particle pore size) with a Gemini® security guard column (C18 packing material, 4×2 mm internal diameter). The mobile phase was 90% MeOH, 10% Milli-Q water. Samples were run at a column temperature of 40 °C and an isocratic flow rate of 0.2 mL/min for a total run time of 10 minutes. The detection wavelength was 280 nm, the retention time for triclosan was 3.8 minutes and 4.7 minutes for methyl triclosan.

Seven calibration standards were prepared for triclosan (0, 3, 5, 10, 25, 50 and 75 µg/L) and methyl triclosan (0, 0.5, 1, 2, 5, 10 and 15 µg/L), and were run at the beginning and end of each run. Every ten samples 0, 3 and 50 and µg/L standards were run as a calibration check and to check for any carry over. Lower standards than those used for the calibration were used to calculate the LOD. The LOD for triclosan was 0.3 µg/L and 0.06 µg/L for methyl triclosan.

3.2.4 Statistical analysis

A three-parameter log-logistic model was fitted and EC_{50} and EC_{10} values for the *Stigeoclonium* sp. and *P. autumnale* were calculated using the drc package in R Studio.[75, 150] The ratio of the EC_{50} values were calculated also using the drc package to determine whether there was a significant difference between the values for the *Stigeoclonium* sp. and *P. autumnale*.[150]

3.3 Results

3.3.1 Concentrations of water samples from bioassays

Triclosan

Nominal and analysed triclosan concentrations are presented in Table 3.3.1a. Lower concentrations detected for the samples taken at 96 hrs from the vials containing algae compared with the samples containing no algae indicate that there was some microbial degradation. Samples from vials containing *Stigeoclonium* sp. were only taken at 96 hrs therefore the exact concentrations the algae were exposed to throughout the experiment cannot be known.

Table 3.3.1a Average media triclosan concentrations (mg/L).

Nominal	0 hrs	24 hrs	48 hrs	72 hrs	96 hrs	96 hrs*
0	0	0	0	0	0	0
0.7	0.62	0.66	0.68	0.67	0.65	0.53
1.12	0.82	0.92	0.88	0.91	0.89	0.9
1.79	1.95	1.98	2.09	2.01	1.99	0.98
2.87	2.59	2.78	2.83	2.85	2.78	1.3
4.59	3.79	4.36	4.35	4.23	4.02	1.82

*Concentrations of samples taken at 96 hrs from vials containing *Stigeoclonium* sp.

The measured 0 hr concentration were used for EC_{50} and EC_{10} calculations because there was an effect seen at 0 hrs and there was the largest decrease in photosynthetic activity between 0 and 24 hrs.

Methyl triclosan

Methyl triclosan was only detected in samples with the initial concentration of triclosan of 0.82 mg/L (the second lowest concentration). Measured concentrations were 0.03 mg/L at 72 hrs and 96 hrs and the compound was not detected at any other time period.

Copper

Nominal and analysed Cu concentrations are displayed in Table 3.3.1b. There was some loss of Cu in the vials with *Stigeoclonium* sp. as indicated by the concentrations of the media at 96 hrs. Analysed 0 hr concentrations were used for EC_{50} and EC_{10} calculations.

Table 3.3.1b Average media copper concentrations (mg/L).

Nominal	0 hrs	24 hrs	48 hrs	72 hrs	96 hrs	96 hrs*
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.050	0.043	0.042	0.043	0.075	0.041	0.031
0.090	0.080	0.057	0.060	0.083	0.059	0.045
0.16	0.16	0.16	0.15	0.16	0.14	0.097
0.29	0.28	0.28	0.27	0.26	0.22	0.15
0.52	0.49	0.46	0.41	0.36	0.30	0.26

*Concentrations of samples taken at 96 hrs from vials containing *Stigeoclonium* sp.

3.3.2 Triclosan Study

Stigeoclonium sp. was more sensitive to triclosan than *P. autumnale* (Figure 3.3.2a). Triclosan had an effect on the *Stigeoclonium sp.* from 0 hrs while *P. autumnale* was not affected until 48 hrs. By 48 hrs the *Stigeoclonium sp* treatments exposed to the highest two concentrations of triclosan (2.59 and 3.79 mg/L) had died with Fv/Fm values of 0 (Figure 3.3.2b).

The EC_{10} and EC_{50} values for the *Stigeoclonium* sp. and *P. autumnale* are presented in Tables 3.3.2a – 3.3.2b. The largest decrease in photosynthetic activity for the *Stigeoclonium* sp. was from 0 to 24 hrs where the EC_{50} value decreased from 3.71 mg/L to 1.93 mg/L. From 24 to 72 hrs the EC_{50} values remained relatively constant (1.90 – 1.97 mg/L). There was a further decrease at 96 hrs with a final EC_{50} value of 1.23 mg/L. The EC_{10} values for *Stigeoclonium* sp. were similar from 0 to 72 hrs ranging from 1.65 to 1.89 mg/L before decreasing to 0.69 mg/L at 96 hrs.

The EC_{50} values for *P. autumnale* were similar at 48 and 72 hrs with values of 3.73 and 3.86 mg/L, respectively. The final EC_{50} value at 96 hrs also decreased slightly to 3.17 mg/L. The EC_{10} values for *P. autumnale* gradually decreased from 48 hrs with values of 3.31, 3.68 and 2.21 mg/L for 48, 72 and 96 hrs, respectively.

The ratio of 96 hr EC_{50} values for the *Stigeoclonium* sp. and *P. autumnale* was calculated to be 0.39 (Lower limit = 0.31, Upper limit = 0.47), therefore the EC_{50} values were significantly different as neither the ratio nor the lower or upper limits of the ratio were equal to one.

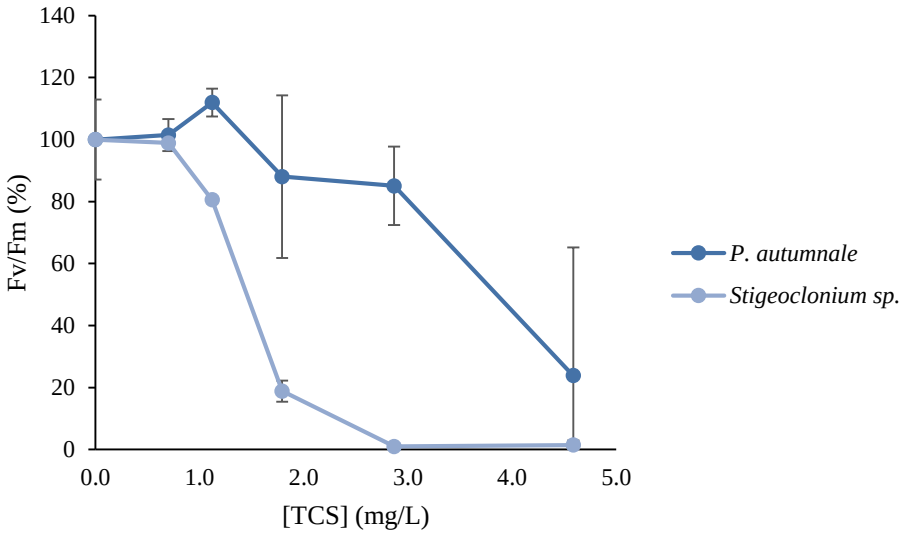


Figure 3.3.2a. Percentage yield (Fv/Fm; relative to the control) of *Stigeoclonium* sp. and *P. autumnale* after exposure to triclosan at 96 hrs (error bars are the standard error of the replicates).

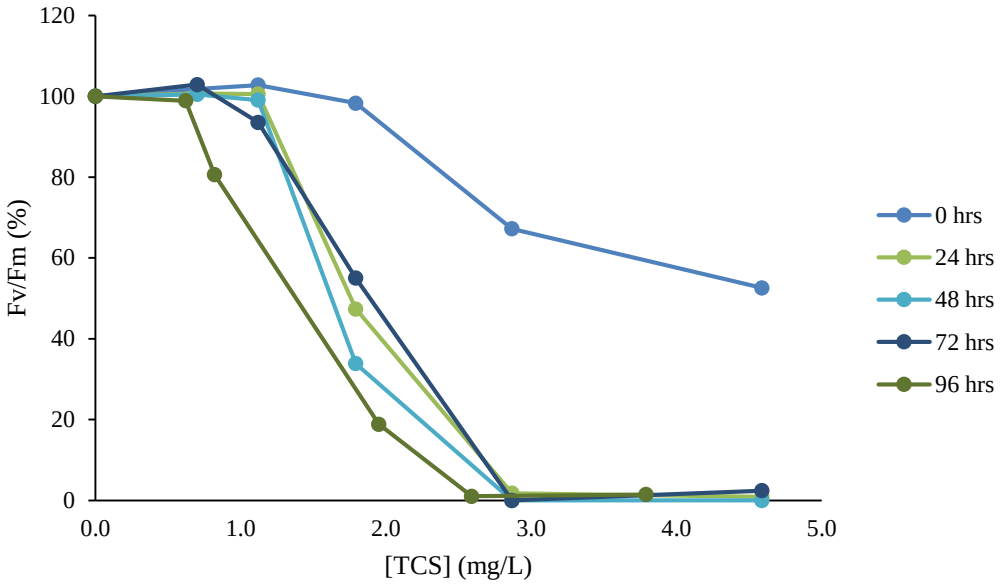


Figure 3.3.2b. Percentage photosynthetic yield (Fv/Fm; relative to the control each day) of *Stigeoclonium sp.* after exposure to triclosan over 96 hrs.

Table 3.3.2a EC_{50} values for *Stigeoclonium* sp. exposed to triclosan from 0 – 96 hrs.

Time (hrs)	EC_{50} (mg/L)	Std. Error	Lower C.I	Upper C.I
0	3.71	0.31	3.08	4.34
24	1.93	0.03	1.88	1.98
48	1.90	0.09	1.84	2.03
72	1.97	0.1	1.77	2.16
96	1.23	0.06	1.11	1.36

Table 3.3.2b EC_{10} values for *Stigeoclonium* sp. exposed to triclosan from 0 – 96 hrs.

Time (hrs)	EC_{10} (mg/L)	Std. Error	Lower C.I	Upper C.I
0	1.77	0.31	1.14	2.4
24	1.65	0.13	1.38	1.96
48	1.75	0.42	1.24	2.3
72	1.89	0.73	0.30	3.3
96	0.69	0.06	0.56	0.8

Table 3.3.2c EC_{50} values for *Phormidium autumnale* exposed to triclosan from 0 – 96 hrs.

Time (hrs)	EC_{50} (mg/L)	Std. Error	Lower C.I	Upper C.I
0	NE[a]	NA[b]	NA	NA
24	NE	NA	NA	NA
48	3.73	0.2	3.32	4.15
72	3.86	0.47	2.9	4.83
96	3.17	0.25	2.65	3.68

Table 3.3.2d EC_{10} values for *Phormidium autumnale* exposed to triclosan from 0 – 96 hrs.

Time (hrs)	EC_{10} (mg/L)	Std. Error	Lower C.I	Upper C.I
0	NE[a]	NA[b]	NA	NA
24	NE	NA	NA	NA
48	3.31	1.24	0.78	5.84
72	2.68	1.00	0.64	4.77
96	2.21	0.48	1.27	3.14

[a] No effect was observed for this associated time period.
[b] Not applicable as no EC_{50} value reported.

3.3.3 Copper Study

Stigeoclonium sp. was also more sensitive to copper than *P. autumnale.* The EC_{50} and EC_{10} values for *Stigeoclonium* sp. are presented in Tables 3.3.3a and 3.3.3b. There was no observed effect on *P. autumnale* for the tested concentration range over the 96 hrs. *Stigeoclonium* sp. was not affected until 48 hrs with an EC_{50} value of 1.20 mg/L. This decreased to 0.56 mg/L at 96 hrs. The EC_{10} values decreased from 48 hrs from 0.37 mg/L to 0.21 mg/L at 96 hrs. Photosynthetic yield (Fv/Fm) for the highest treatment of copper showed the largest decrease from 0 to 96 hours (0.77 to 0.42 mg/L; Figure 3.3.3).

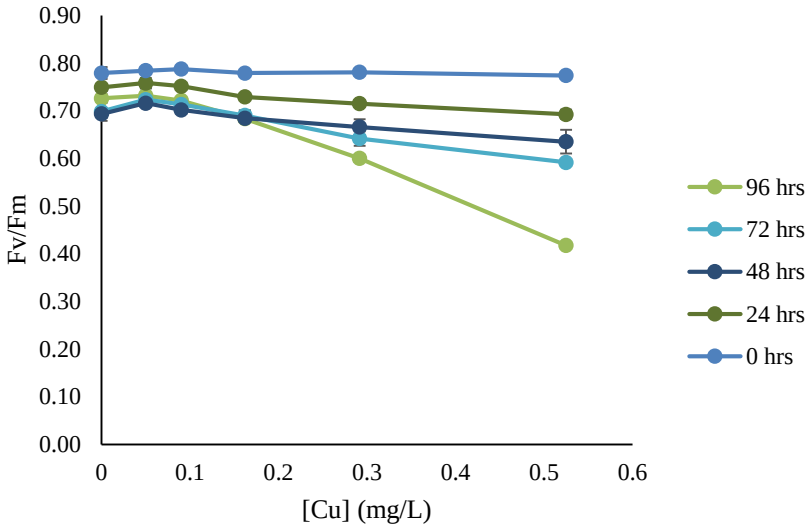


Figure 3.3.3. Photosynthetic yield (Fv/Fm) of *Stigeoclonium* sp. and after exposure to copper sulfate [Cu] over 96 hrs (error bars are the standard error of the triplicates).

Table 3.3.3a EC_{50} values for *Stigeoclonium* sp. exposed to copper sulfate from 0 – 96 hrs.

Time (hrs)	EC_{50} (mg/L)	Std. Error	Lower C.I	Upper C.I
0	NE[a]	NA[b]	NA	NA
24	NE	NA	NA	NA
48	1.20	0.33	0.53	1.87
72	1.34	0.47	0.37	2.31
96	0.56	0.06	0.42	0.68

Table 3.3.3b EC_{10} values for *Stigeoclonium* sp. exposed to copper sulfate from 0 – 96 hrs.

Time (hrs)	EC_{10} (mg/L)	Std. Error	Lower C.I	Upper C.I
0	NE[a]	NA[b]	NA	NA
24	NE	NA	NA	NA
48	0.37	0.10	0.18	0.57
72	0.31	0.06	0.19	0.43
96	0.21	0.05	0.096	0.31

[a] No effect was observed for this associated time period.
[b] Not applicable due as no EC_{50} value reported.

3.4 Discussion

3.4.1 Tolerance of the species

In this study *Stigeoclonium* sp. was more sensitive to triclosan than *P. autumnale.* This finding corroborates the results of Drury et al who showed there was a 6-fold increase in the relative number of cyanobacterial sequences, increasing from <1% of the total community to ca. 5% and the abundance of green algae decreased after exposure to triclosan.[51] The 96 hr EC_{50} value for *Stigeoclonium* sp. (1.23 mg/L) was however much higher than values given for other species of green algae in the literature. Reported EC_{50} values for other green algae exposed to triclosan are one to three orders of magnitude lower, for example 1.4 µg/L (biomass inhibition, *Scenedesmus subspicatus*) and 4.46 µg/L (growth inhibition, *Selenstrum capricornutum*).[20, 135] Previous studies on the effects of triclosan on photosynthetic activity of algae are limited, however the EC_{50} values reported in a short term study by Franz et al, for photosynthetic inhibition ranged from 3.7 µg/L for the chlorophyte *Scenedesmus vacuolatus* to 900 µg/L for a periphyton community dominated by diatoms.[139a] This implies that even within an algae class, such as green algae, there can be different responses to triclosan depending on the species tested. Challenges arise from this finding in regards to predicting the effects of triclosan to a specific waterbody.

The periphyton community dominated by diatoms in Franz et al's study was grown from a sample taken from a river in Germany and had an EC_{50} value comparable to the EC_{50} value obtained for the green alga in this study.[139a] Triclosan has been detected in urban streams globally,[7, 51] and was detected in stream sediment in this study (though not in Cross Stream where the green alga was obtained). It is therefore possible that the green alga in this study and the periphyton community in the German study had prior exposure to triclosan and may have developed resistance. Resistance to triclosan has been reported for bacteria in clinical settings with mechanisms including non-susceptible enoyl reductase enzymes, and cellular impermeability alterations.[134] There is little known regarding the causes behind differences in species sensitivity to triclosan and the mechanism of action in algae is unclear.[51] In *Escherichia coli*, triclosan specifically inhibits the enzyme enoyl-acyl carrier protein reductase (ENR), leading to blockage of lipid synthesis. Here triclosan mimics the enzymes natural substrate and acts site-specifically.[139a] The existence of ENR in bacteria, fungi and higher plants has been observed. Therefore the sensitivity of a particular algae species could be due to the presence or absence of ENR.[139a]

The EC_{50} value for the *Stigeoclonium* sp. exposed to Cu after 96 hrs (0.56 mg/L) was an order of magnitude higher than reported (EC_{50} values) for other green algae in growth inhibition tests, 0.047 mg/L (*Tetraselmis* sp.),[151] 0.046 mg/L (*Chlorella* sp.),[152] and much higher than the EC_{50} value for *Pseudokirchneriella subcapitata* (0.8 µg/L).[152] Reasons for differences in metal sensitivity between species is also an ongoing area of research. It is proposed that the uptake of copper into algal cells is a

two-stage process.[151] The exterior of the cell membrane consists of metal binding sites which are metabolically active, where copper may enter the cells, and non-active sites where copper entry to the cell does not occur.[151] The first step is fast adsorption to these sites.[151] In marine microalgae it has been shown that inter-species differences in sensitivity to copper were not related to adsorption of copper to different algal cell walls.[151] Internalisation of the metal across the cell membrane is the second step in metal uptake. It is thought that the process in the algal cell membrane occurs through ion pore channels or transporters. This is generally considered rate-limiting in the uptake of charged metal ions and therefore a significant factor in metal uptake and toxicity.[151]

Levy et al reported that the sensitivity of algal species to copper was not related to external binding or only due to intracellular copper concentrations and uptake rates.[151] They suggested that information on the localisation and form of copper within the cell is more important than total intracellular copper concentrations for predicting toxicity.[151] For example, metals bound to heat sensitive proteins or to organelles may be more likely to be toxic. Species sensitivity may also depend heavily on cell detoxification mechanisms.[151] A similar study measuring the relationship between metal-algal cell binding and copper sensitivity on a range of species found there was no correlation between differences in sensitivity and cell size, cell wall type, taxonomic group or solution-cell partition coefficients.[141] This study also suggests that internal binding mechanisms or detoxification mechanisms may influence difference in sensitivity for different microalgal species.[141] Therefore *Stigeclonium* sp. may have detoxification mechanisms better equipped to minimise Cu toxicity than many other microalgae species.

Phormidium autumnale was not affected by any of the Cu concentrations (the highest concentration was 0.49 mg/L). A previous long term study also using *P. autumnale* reported that growth was significantly affected by Cu at 0.25 mg/L though not all cells were killed.[153] The concentration found to have a significant effect on growth for *P. autumnale* is about half the value of the top concentration used in the current study where no effect was observed. To gain a better understanding on the sensitivity of *P. autumnale* to Cu, the current study would need to be repeated at higher concentrations. For the cyanobacteria *Anabaena torulosa*, EC_{50} values for photosynthetic oxygen production were between 0.5 and 0.7 mg/L depending on cell age.[154]

A disadvantage of ecotoxicity assays such as this experiment is that experimental conditions are different to real ecosystems.[155] Natural algae assemblages usually contain many species competing for light and nutrients. This experiment used unialgal cultures. The presence of other species could be linked to an increased sensitivity to toxicants.[155] A study examining the impacts of algacides in unialgal and bialgal cultures found that the growth of the green alga *Pseudokirchneriella subcapitata* was impacted by the presence of the cyanobacteria *Aphanothece. clatharata* while the cyanbacterium had similar growth rates in both the presence and absence of *P. subcapitata.*[155] Further, the presence of the cyanobacterium was shown to be a more significant growth inhibiting factor for the green alga than the

algacide itself in treatments with a lower tconcentration.[155] It may be possible to test these findings in a study similar to the current experiment by growing *Stigeoclonium* sp. in the same vial as *P. autumnale.*

3.4.2 Microbial degradation of triclosan and loss of copper

Decreased concentrations of triclosan and copper were observed in samples taken from vials containing algae at 96 hrs compared with vials containing no algae. This indicates that triclosan underwent microbial degradation possibly by both algae and any bacteria present in the culture. As the concentrations of triclosan in vials without algae were relatively constant over time, photodegradation was likely not a major factor. Bacteria have been shown to degrade triclosan in wastewater,[156] pure cultures, soils and activated sludge.[157] It is possible that some triclosan accumulated in *Stigeoclonium* sp. as bioaccumulation of triclosan has been reported in other algal species.[17] Photodegradation was not likely to contribute to the degradation of triclosan as concentrations in the vials without algae were relatively constant over the 96 hours. The loss of Cu could have been caused by sorption of Cu to the cell wall, or uptake into the cell as described in section 3.4.1.[151]

Triclosan can undergo methylation by microorganisms, an important biotransformation process for the compound.[158] Methyl triclosan was only observed in samples taken from the second lowest triclosan concentration. Higher concentrations of triclosan may have killed any bacteria present in the media, preventing biotransformation.

3.4.3 Environmental implications

Due to its higher resistance to triclosan, *P. autumnale* may out compete green algae in water bodies receiving discharges containing this antimicrobial compound. As primary producers, algae play a crucial role in aquatic food webs,[17] disruption to the composition of algae in aquatic communities could impact community function in higher food web levels.[17]

It is plausible that an increased resistance to triclosan is one of the factors explaining the increase of *P. autumnale* proliferations in New Zealand. Although most blooms do not occur in urban streams, there have been reports of increased blooms downstream of wastewater treatment plants[159] and at these sites triclosan may contribute to the increased abundance of *Phormidium. Phormidium autumnale* produces a range of neurotoxins in New Zealand, and ingestion of mats has led to many canine deaths and health warnings been issued for numerous New Zealand rivers.[52] An increase in abundance of *P. autumnale* may increase the risk of exposure of humans and animals to cyanotoxins.

3.4.4 Future studies

Due to the high EC_{50} value for the green algae, further work could be undertaken using other species of green algae to determine how triclosan affects a wider range of species. The experiment could also be conducted again for *P. autumnale* at higher concentrations of triclosan as a smaller effect was observed for this species. The Cu study should also be repeated with higher concentrations to ascertain the toxicity of Cu towards *P. autumnale*. Diatoms were unable to be cultured in this experiment but should be included in future studies as they are common in New Zealand rivers.

3.5 Conclusion

The green alga *Stigeoclonium* sp. was more sensitive to both triclosan and copper than the cyanobacteria *P. autumnale.* This could have implications for the environment where *P. autumnale* could become more abundant than green algae in waterways impacted by triclosan. *Stigeoclonium* sp., which is commonly found in New Zealand waterways, was however more tolerant to triclosan than other species of green algae previously studied, with an EC_{50} value of 1.23 mg/L, three orders of magnitude higher than those reported in the literature. This result creates challenges in predicting the effects of triclosan in waterways as it appears there can be species specific responses to the antimicrobial compound, even within similar groups of organisms, such as green algae. The results of the study provide important information that may be required for any future risk assessment of triclosan in New Zealand.

4 Conclusions and recommendations

4.1 Key findings

4.1.1 Occurrence of personal care products in urban streams and impacts on bacterial community structure

UV-filters (BP1, BP3 and OMC) were the most frequently detected class of compounds in stream water, particulate phase, and sediment samples. Benzophenone-3 was the most commonly detected UV-filter in all types of samples with concentrations measured up to 4.1 ng/L, 1.4 ng/L, and 1.9 ng/g in stream water, particulates, and sediments, respectively. The UV-filters tended to be present in stream water at lower concentrations in the winter months. Similar trends have been observed in the literature where higher concentrations of UV-filters were detected in summer months compared with winter months due to higher use of the compounds and recreational inputs in summer.[27]

Bisphenol A was the most frequently detected compound in this study. Concentrations ranged from <DL to 8.1 ng/L in stream water which was similar to concentrations detected in Japan[92], Korea[92] and Germany[94]. No temporal patterns were observed for BPA likely due its ubiquitous nature in the environment.[91] Personal care products that were less frequently detected in stream water included mParaben and OP which were measured at concentrations up to 12.9 ng/L and 2.1 ng/L, respectively. For all detected compounds in stream water, there were no significant differences between upstream and downstream concentrations of the sewer overflow outfall at either Cross Stream or Dudley Creek Diversion. No sewer overflows occurred over the six month study which is likely the cause of the lack of significant differences between upstream and downstream sites.

It is possible however that sewer overflows were the source of BP3 in Dudley Creek Diversion sediments as concentrations downstream of the outfall were significantly higher than upstream ($p < 0.05$). Triclosan was also detected in two downstream samples in March and April at Dudley Creek Diversion at concentrations of 10.5 and 5.3 ng/g. The last sewer overflow at Dudley Creek Diversion occurred 8 month prior to the study. It is therefore possible that initial concentrations of the antimicrobial compound where much higher if the degradation rate was similar to that reported in the literature for triclosan in aerobic soil (18 days).[116] Other compounds detected in sediment (BPA, mParaben, OMC, OPP, and OP) did not show a significant difference in upstream and downstream sediment concentrations indicating that stormwater is the likely source of these of these compounds in the studied streams. These compounds are ingredients in a range of products, including mParaben in

paint, varnishes, and pesticides,[112] and OPP in glues, concrete additives, leather, and as an active ingredient in disinfectants.[99]

Though concentrations of PCPs were below reported PNECs and concentrations reported to be toxic to aquatic organisms, several compounds were identified as contributing to the structure of bacterial communities. The compounds BP3, OMC, TCS and BPA influenced sediment bacterial community structure while the sum of the detected PCPs in stream water were identified using DistLM modelling as playing an important role in structuring biofilm communities. Trace elements were not identified using the statistical analysis as influencing bacterial community structure in either sediment or biofilm despite reports in the literature of Zn, Cu and Pb impacting bacterial biofilm communities after just 3 days.[131] This finding could be because concentrations of trace elements were generally low. All concentrations were below ANZECC guidelines.

4.1.2 Effects of triclosan on benthic photosynthetic microorganisms

The green algae *Stigeoclonium* sp. was more sensitive to triclosan than the cyanobacteria *P. autumnale.* This finding is in agreement with that of Drury et al who showed that cyanobacteria increased from <1% of the total bacterial community in the study to ca. 5% while green algae decreased in abundance after exposure to triclosan.[51] Despite the relative sensitivity of *Stigeoclonium* sp. to triclosan compared to *P. autumnale,* the 96 hr EC_{50} value for *Stigeoclonium* sp. (1.23 mg/L) was three orders of magnitude higher than values reported in the literature including 1.4 µg/L (biomass inhibition, *Scenedesmus subspicatus*) and 4.46 µg/L (growth inhibition, *Selenstrum capricornutum*).[20, 135] *Stigeoclonium* sp. was cultured from a sample taken from Cross Stream. As triclosan was detected in stream sediment in this study and in urban streams globally, [7, 51] it possible that *Stigeoclonium* sp. had prior exposure to triclosan and may have developed resistance. It is also possible that even within an algae class, such as green algae, triclosan has species specific responses. This highlights the challenge in predicting the effects of triclosan on algae in waterways.

Stigeoclonium sp. was also found to be more sensitive to Cu (96h hr EC_{50} of 0.56 mg/L) than *P. autumnale* (no observed effect). *Stigeoclonium* sp. was more tolerant to Cu than other green algae studied in the literature. Reported growth inhibition test EC_{50} values include, 0.047 mg/L (*Tetraselmis* sp.),[151] 0.046 mg/L (*Chlorella* sp.),[152] and 0.8 µg/L *(Pseudokirchneriella subcapitata).*[152] As noted above, within an algae class it is evident that contaminants can exhibit different responses depending on the species.

4.2 Implications and recommendations

There is limited data on the presence and effects of PCPs in New Zealand waterways. The results from Chapter Two indicate that stormwater may be a source of PCPs for New Zealand waterways as compounds were detected in stream water despite there being no sewer overflows. The data obtained in this study will assist in risk assessments and the development of any future regulations regarding acceptable levels of PCPs. The lack of regulations in place for PCPs creates challenges for managing these compounds.[5] To increase the knowledge of the occurrence of PCPs in New Zealand, similar studies to the field study presented in Chapter Two should be undertaken throughout the country. The analysis of PCPs in sediment samples from the current study should also be revisited due to the difficulties encountered with low ^{13}C surrogate recoveries.

Personal care products were shown to influence benthic bacterial community structure. As bacteria can play important roles in aquatic ecosystems in the cycling of nutrients and carbon, changes to community structure could impact organisms of higher trophic levels.[160] Regional councils do not currently monitor for PCPs but do monitor trace elements and nutrients in urban streams.[126] As trace elements did not influence bacterial community structure, monitoring of PCPs is suggested to increase knowledge on response of microbial communities to these contaminants.

Stigeoclonium sp. and *P. autumnale* are both commonly found in New Zealand waterways. Both of these species were tolerant to high concentrations of triclosan and Cu. In order to gain a greater understanding on the potential effects of triclosan in New Zealand waterways, ecotoxicity experiments should be carried out on a wider range of organisms, including diatoms which were unable to be cultured in this experiment. The experiment presented in this thesis could also be conducted again for *P. autumnale* at higher concentrations of triclosan as there was only a small effect observed for this species. The Cu study should also be repeated with higher exposure concentrations to ascertain the toxicity of Cu to *P. autumnale.*

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