Characterisation, potential toxicity and fate of storm water run-off from log storage areas of the Port of Tauranga.

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Abstract

Stormwater run-off from industrial sources can impact the receiving environment by the discharge of toxic substances, nutrients, sediments or fresh water (in marine environments). The Port of Tauranga is New Zealand's largest by cargo volume with untreated logs being one of the major exports. The port stores logs totalling up to 300,000 m^3 with an average residency time of 18 days. Runoff from log storage areas can cause toxicity to aquatic life due to low pH, high organic solids content and associated BOD, and chemicals leached from timber such as resin acids. Metals and PAH's from heavy vehicles and other machinery can also be present.

At the port, stormwater collects from the storage areas into slot drains and is screened for larger particulates in screening chambers before discharging into Tauranga Harbour. Large rainfall events produce a visible, highly coloured plume extending across the main harbour channel.

This study looks at compounds within the stormwater runoff and associated marine water samples and the toxicology of the effluent. It then focusses on the gradients of compounds found in sediments and biota in relation to the main discharge sources. This is followed with a more specific investigation of the bioaccumulation of resin acids in resident and transplanted mussels. It concludes with a comparison of low intertidal species assemblages within and outside the influence of the stormwater plume.

Findings indicate that there are high levels of wood derived chemicals in the stormwater runoff and a gradient of quantities of these can be detected in nearby sediments, decreasing with distance from the discharge point of the effluent. Those compounds, such as metals, able to be quantified against national and international guidelines were well within acceptable levels. Others, such as resin acids, were found in lower quantities than in a previous study. A correlation between organics related to leachate from the logs, inorganic compounds found in the effluent and sediment grain

size, indicates that some elements of the runoff may reach further into the harbour. The influence of dredging and disturbance of the seabed by shipping movements is considered in relation to this.

Levels of organic compounds, related to the log storage in transplanted mussel populations, were not detected spatially or temporally and no evidence of bio-accumulation of resin acids was found.

Based on the combined findings there are very low detectable effects on the marine environment from the runoff of the Port of Tauranga log storage areas. These come in the form of a gradient in chemical compounds related to the runoff and are well within the ANZECC (2000) Interim Guideline Levels.

This study adds to the limited knowledge on log storage runoff into the marine environment and incorporates elements which can be applied to many areas of research related to stormwater discharge. It uses the Port of Tauranga runoff as a relevant example of issues and environmental responses related to urban and industrial stormwater runoff.

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Chapter 1

Introduction

1.1 Introduction

Harbours and estuaries have historically been places of human development due to easy access to food resources, the sheltered aspect from exposed coasts, and connectivity between the land and ocean. As they have become more industrialised, ports and associated industries have evolved around the margins. Such developments have brought changes and impacts not only to the spaces they occupy but to the surrounding environments.

Stormwater runoff from urban and industrial sources into the marine environment are known to be a source of pollutants including heavy metals and polycyclic aromatic hydrocarbons (PAHs) (Brown *et al.*, 1985; Brown & Peake, 2006). It can also have negative ecological effects through changes in water quality such as reduced salinity and raised turbidity (Grant *et al*., 2003). Some effects are short term or episodic (Roberts *et al.,* 2007), others can accumulate over time changing species composition and preventing effective recovery (Williamson *et al.*, 1996).

Discharges related to log storage and wood treatment have been scrutinised where industrial activities such as logging and milling have impacted bodies of water (Pease *et al.*, 1974; Waldichuk, 1979; Jackson, 1986; Bailey *et al*., 1999; Taylor & Carmichael, 2003a). Contaminants found included metals, tannins and lignins (Bailey *et al*., 1999), suspended solids (Doig, *et al.*, 2006), pH (Taylor & Carmichael, 2003b) and high chemical oxygen demand (Hedmark & Scholz, 2008).

Resin acids are naturally occurring as a chemical defence against herbivore and pathogen attack in many conifer species (Keeling & Bohlmann, 2006). When leachate from felled trees enters aquatic systems the resin acid component is known to cause toxicity to aquatic life (Oikari

et al., 1982; Sierra-Alvarez & Lettinga, 1990; Peng & Roberts, 2000; Taylor & Carmichael, 2003b; Hernandez *et al*., 2008).

This chapter looks at the stormwater effluent in its raw form and identifies its key contaminants and potential for toxicity. It sets the scene for the possible routes by which these contaminants may enter and affect the Harbour ecosystem.

1.1.1 Tauranga Harbour

Tauranga Harbour, Te Awanui, is located on the east coast of the North Island of New Zealand in the Bay of Plenty region (Figure 1). It is large, approximately 200 km^2, and mainly shallow with over 60 percent exposed at low tide. Maximum tidal range is 1.98 metres with approximately 290, 000,000 tonnes of water exchange per tide creating a maximum flow of up to 7 knots at the southern entrance (Ellis *et al*., 2013).

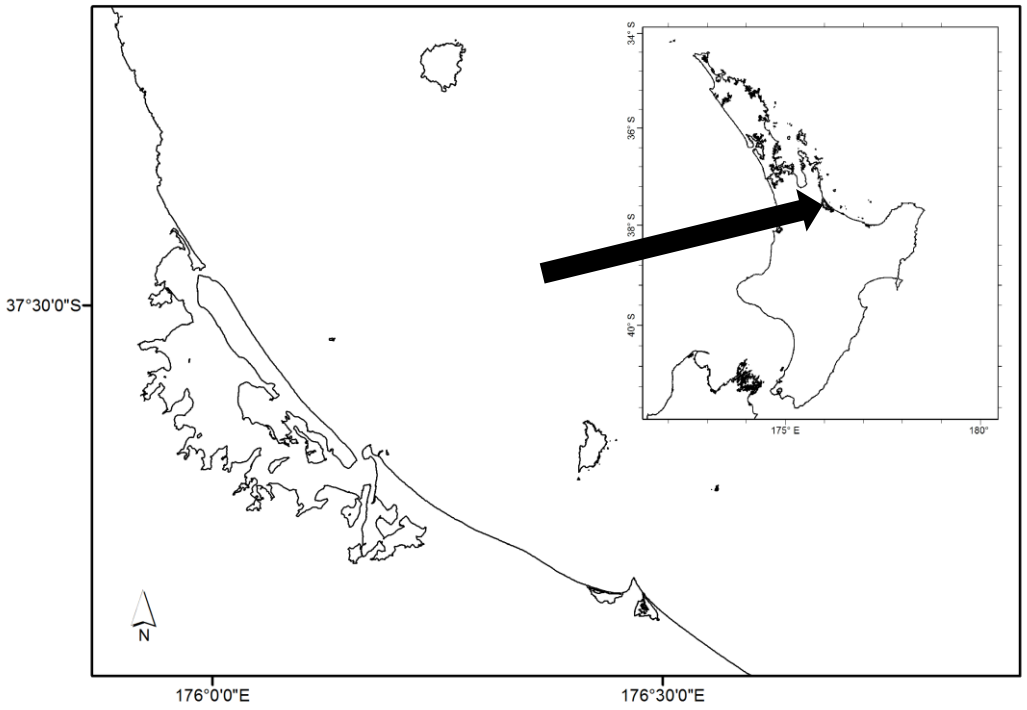


Figure 1. Tauranga Harbour overview and location map.

1.1.2 Study site

The study site encompassed the area from the harbour entrance (between Matakana Island and Pilot Bay) to part way along Town Reach in a north/south direction, and the width of the Stella Passage rounding Centre Bank to the edge of Matakana Island where some control sites were located (Figure 2).

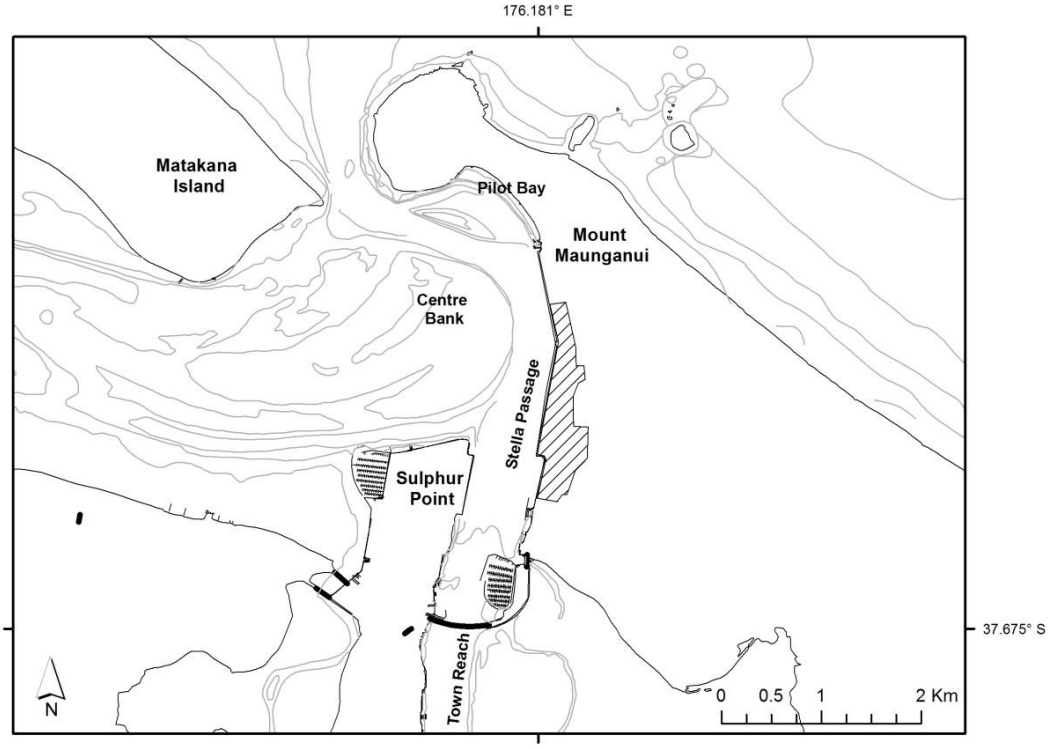


Figure 2. Overview of study site showing main features and approximate area of log storage (cross hatched).

1.1.2.1 Tidal Currents

Water movement in the harbour is dominated by strong currents. Tay (2011) found salinity and nutrient dynamics were influenced by tidal patterns. Residence time around the harbour entrance and well exposed regions were found to be low (Tay, 2011) indicating good flushing of the study site and therefore storm water inputs in that area. Sediment transport modelling shows sediment moving toward the harbour entrance via the upper harbour, over centre bank and through the Stella Passage at a rate of 10^2 to $>10^4$ grams per metre per tidal cycle (Davies-Colley & Healy, 1978b)

1.1.3 The Port of Tauranga

The Port of Tauranga was formed in 1857 and has expanded by reclaiming land along the edge of Mount Maunganui and Sulphur Point.

There is a long history of log exports from the Port. In 1957 the first log shipment of 158 tonnes went to Japan. Log exports totalled 6,296,000 tonnes for the year ending 2014 (Port of Tauranga, 2015). At any one time, up to 300,000 m^3 of logs, principally *Pinus radiata,* occupy the log storage areas. These logs have an average residence time of 18 days from delivery to loading aboard ships for export.

1.1.4 Resident Species Overview

Species within the study area have been recorded in detail subtidally as a result of baseline biodiversity surveys undertaken for Biosecurity New Zealand in 2002 and 2008 (Inglis *et al*., 2006, 2008). Intertidal studies of the area have been in a broader sense or outside of the study area of this work. A broad scale survey of Tauranga Harbour was undertaken by Manaaki Taha Moana in 2011/12 which identified many species on the intertidal sand flats (Ellis *et al*., 2013). The Bay of Plenty Regional Council have catalogued some harbour and coastal species, these were confined to the intertidal soft shores (Park, 2000, 2009). Species on rocky shores have also been investigated on a broad scale (Schiel, *et al.*, 2014).

A subtidal survey conducted in 1992 investigated species assemblages under the port wharves (Grace, 1993) and recently particular taxa such as sponges (McCormack, 2015) and larval fish (Brooke, 2015) have begun to be catalogued finding a number of unexpected and new species records.

There is very little literature related to species found in the harbour which have also been the subject of studies on effects of runoff from log storage or wood treatment. The Cawthron Institute in Nelson failed to find any adverse effects from log and wood chip storage at Port Nelson (Forrest & Roberts, 1995) and Grace (1993), mentioned above, failed to find differences in subtidal species assemblages related to runoff from the log storage areas, under the Port of Tauranga wharves.

One species which has been included in biodiversity surveys and impact studies in the region is the green shell mussel *Perna canaliculus* which was added to a wider study using freshwater mussels to evaluate accumulation of wood treatment derived contaminants (Hickey & Roper, 1992). Other mussels of the family Mytilidae (Levings, 1980; Fahræus-Van Ree & Payne, 1999) have been associated with these types of studies making mussels a good candidate for field based studies.

1.1.5 Sedimentary Regimes

Some studies were conducted on runoff from the log storage areas of the Port throughout the 1990's (Tian, *et al.*, 1994, 1995, 1998) which focussed mainly on suspended solids, light absorption and resin acids content of storm water runoff and sediments.

In the past large piles of bark and other debris were collected on the wharf and placed at the edge of the estuary at two sites in Waipu Bay (Healy, *et al.*, 1997). Debarking is now undertaken at a controlled area at the southern end of the log storage area in a shed to stop dust nuisance and material washing into the harbour. The bark is taken to a gardening supply company (Daltons in Matamata) and turned into landscaping products. This is likely to have reduced some effects from runoff, however, visible effects are still apparent during heavy rainfall in the form of a brown plume such as can be seen in Figure 3.

Figure 3. A highly coloured plume of runoff from the log storage area can be seen clearly as it enters the harbour around a berthed vessel being loaded with logs for export. Photo credit Nadine R. Brunschwiler.

1.1.6 Plume Characteristics

Stormwater related to this study enters Tauranga Harbour via a large log storage area. The runoff is mostly collected into slot drains that feed into 300, 600 and 900mm diameter pipes and into a screen chamber of 10mm mesh size. The slot drains are an alternative to the typical cess pit grates and are designed so that water can enter over a long strip rather than through one sink hole, effectively reducing blockages and lessening overland flow that can mobilise more material. The screen chamber can be likened to a large sieve which prevents coarser material from passing. After the screen chamber the collected stormwater enters the harbour through a 1200mm diameter pipe. At the point of discharge and some distance along the wharves the plume can be seen particularly in times of heavy rainfall.

A thesis investigating the log storage discharge plume characteristics was completed concurrently by Nadine Brunschwiler (Brunschwiler, 2015). She

states that the plume is thin and disperses quickly due to the harbour's strong tidal currents estimating that the plume could reach at least halfway across the Stella Passage. Beyond that point it was difficult to distinguish from other influences such as surface rainwater (Brunschwiler, 2015).

1.1.7 Aims and Objectives

The overall question that this work sets out to address is '*are there effects on the marine ecosystem of Tauranga Harbour from the runoff of the log storage area at the Port of Tauranga'*. The investigation was carried out to provide deeper insight into these highly relevant issues generally. To help answer this, the null hypothesis, *'there is no impact on the marine environment from the runoff from the log storage area at the Port of Tauranga'*, is tested through the work described in the following three chapters.

Chapter 2 investigates the chemical characteristics of the runoff, the potential for toxicity and attempts to define its presence once it has discharged into the harbour.

Chapter 3 assesses the potential for contaminants found in the runoff to bioaccumulate in marine biota and sediments.

Chapter 4 compares biological communities close to the area of discharge with those in a similar habitat, outside it.

A large number of compounds were either not included due to relevance to the study (e.g. Sodium) or, in the case of some organic compounds, were impossible to identify and quantify in the limited time available.

Chapter 2

Characterisation and Toxicity of the Port of Tauranga Log Storage Derived Stormwater

2.1 Introduction

The high concentration of urban and industrial activities around rivers, estuaries and harbours puts pressure on the surrounding environment. Stormwater from urban and industrial sources has long been known to have effects on aquatic ecosystems as pollutants wash into drainage systems along with runoff (Grant *et al*., 2003; Brown & Peake, 2006; Göbel, *et al.,* 2007). Studies on the marine environment are less numerous but effects can still be significant (Ahn *et al*., 2005).

Attempts to mitigate stormwater impacts are often made by legislation which attempts to place limits and controls on the levels of discharge. In New Zealand, consents to discharge stormwater are issued under the Resource Management Act 1991 (RMA) and administered by the Regional Councils. In these circumstances, the appointee of a consented discharge is often required to collect water, sediment or biota samples for independent chemical analysis. The results can help demonstrate whether consent conditions are being met.

This approach is sometimes criticised for taking a snapshot approach to environmental effects of discharges, rather than evaluating potential cumulative effects. Runoff influenced by storm events in particular, is highly variable making any assumptions about its toxicity or chemical composition difficult. Rainfall intensity and duration combined with wind, temperature, pH and many other co-discharged contaminants, both from the source in question and elsewhere, all influence any likely resultant toxicity of stormwater. Flushed contaminants in the ecosystem and the

potential at which these may also be accumulated either within organisms or habitats, increasing the overall potential to have a negative effect. The drainage system, often designed to carry rainfall from large impervious areas into concentrated drainage channels along with any treatment to remove contaminants, further affect this dynamic (Göbel *et al*., 2007).

Additionally, the chemistry that occurs once the stormwater enters the marine environment can be very complex. Dissolved organic and inorganic matter can flocculate increasingly with salinity resulting in dissolved pollutants precipitating into sediments (Sholkovitz, 1976; Webster, 1995). The presence of suspended particulates can also influence distribution and bioavailability of metals and other contaminants (Ackroyd *et al*., 1986).

As mentioned in the general introduction, the stormwater that discharges at the southern end of the Mount Maunganui wharves accumulates from a large log marshalling area. This discharge and its potential effects on marine life in the harbour are the main focus of this thesis. This chapter looks more closely at the properties of runoff in its undiluted form, its potential for toxicity and its immediate dilution upon entering the harbour waters.

Runoff from log storage and processing areas has been recognised as a possible source of contaminants with bark leaching responsible for some of the highest sources of environmental contamination (Jonsson, 2012).

Hedmark & Scholz (2008) reviewed literature on environmental effects of runoff from wood storage and include a study (Orban,*et al,.* 2002) which found volume of wood stored, frequency of runoff events and colour intensity were the top three factors influencing risk to the environment. Toxic discharges have been reported from log storage and wood processing areas, including those of the Port of Tauranga (Tian et al., 1995, 1998). Findings have pointed to metals, tannins and lignins (Bailey et al., 1999), suspended solids (Doig et al., 2006), pH (Taylor & Carmichael, 2003b) and high chemical oxygen demand (Hedmark &

Scholz, 2008) as sources of toxicity or environmental stress to marine species.

Resin acids have also been a contaminant of concern. Toxicity testing has found 96 hour LC50 to be as low as 20 µg/l on freshwater Daphnia magna and 400 µg/l for rainbow trout (Salmo gairdneri) for the most toxic individual resin acids (Peng & Roberts, 2000). Resin acids recorded in runoff from the Port of Tauranga by Tian et al. (1997) found total resin acids of 16 untreated runoff samples were between 72 µg/l and 2264 µg/l, averaging 1035 µg/l.

The specific aims of this component of the present study were to quantify the compounds present in the raw effluent and investigate its potential toxicity. Then, using this data, attempt to comment on the potential toxicity in the 'real world' by trying to detect effects from the discharge in the harbour waters.

The null hypotheses tested are:

The raw effluent is not toxic to a test marine species.

No presence will be detectable in the harbour upon dilution.

To assess the potential environmental toxicity of stormwater runoff, samples were collected of both raw effluent and from nearby harbour waters. Samples were then characterised and compared to relevant literature, national and international environmental standards.

Two of the samples of raw effluent were set aside and used for a series of simple toxicity bioassays using brine shrimp, *Artemia* sp. and locally sourced Mysid shrimp, *Tenagomysis* sp. to allow comparison with published results while additionally examining effects across an extended species range.

A series of transects were measured using a probe to examine water quality parameters around the discharge area, with reference to depth and distance from source. This was intended to investigate any water quality

gradients around the discharge points and infer at what distance from the outfall changes in water quality could be detected.

2.2 Methods

2.2.1 Water Chemistry

The water chemistry includes water sampling from a stormwater drain inside the log marshalling area (see 2.2.1.1, 2.4.1.1) and sampling of the harbour water adjacent to the discharge points of the same stormwater drain (see 2.2.1.1, 2.4.1.2). Water samples from the locations in the harbour, adjacent to the discharge point of the log marshalling area stormwater drain, were collected from the wharf at three sites on the 12-09-13. Sampling is summarised in Table 1.

On two of the sampling occasions from the stormwater drain inside the log marshalling area, additional water was collected for the toxicity experiments (see 2.2.2., 2.4.2). This was analysed via gas chromatography /mass spectrometry (GC/MS) and screened for 68 organic compounds (Appendix A). This was complimented using an Aquaread AP-2000-D water meter to read parameters during experiments.

Table 1. Summary of water sampling in the context of rainfall and other conditions.

Type	Location	Date	Time	Tide		Hourly rainfall (mm)	Rainfall 24h (mm)	Rainfall 7 day (mm)	Rain intensity (mm/h)	Wind Speed (Knots)	Wind Dir	Wind Dir
Marine	Outlet PC22	12/09/2013	11:31am	1.68	Fld	4.7	12.7	13.6	2.5	15	N	351
Marine	Outlet P16	12/09/2013	11:42am	1.7	Fld	4.4	13.2	14.1	2.5	12	N	341
Marine	Outlet P9	12/09/2013	12:01pm	1.76	Fld	5.7	15.3	16.3	2.5	13	N	354
Post rainfall	Log storage area	6/11/2013	08:50am	1.9	Fld	3.9	39	41.7	~~~	16	NE	52
First Flush Sample	Log Storage area	4/12/2013	03:47pm	0.3	Fld	1.2	2.9	28.6	0.7	17	NE	51
First Flush Sample	Log Storage area	10/06/2014	08:25am	0.76	Ebb	0.9	40.3	41.4	0.6	14	ENE	70
First Flush Sample	Log Storage area	28/11/2014	08:30am	1.27	Fld	0	0.4	0.5	0	4	W	279

Note: Locations mentioned above are as located on map in Figure 4.

2.2.1.1 Water Sampling of the Stormwater Drain and Harbour

Water samples were collected from the Port's log terminus drain by Port of Tauranga employees and the author between December 2013 and November 2014.

These were collected in bottles supplied by Hill Laboratories and pre-prepared appropriately for the type of chemistry intended. Samples for the toxicity experiments and further analyses were collected and stored in clean plastic water containers. There are access manholes for the main drains where runoff collects into a large chamber before being fed by a larger diameter pipe to the final discharge point into the harbour. The manhole used for the water sample collection is located at latitude S37.65312, longitude E176.18309 (WGS84, DD) and as described in Figure 4.

After lifting the manholes, samples were either collected by a pole with a bottle holder or by pumping using a manual bilge pump. Samples along the wharf were collected using a pole and bottle at locations P9, P16 and PC22 (Figure 4), as named on the Port of Tauranga's plans. Collected samples were kept chilled in polystyrene coolers. These were sent by courier to Hill Laboratories within 12 h of collection or, in the case of samples for the toxicity experiments, placed in a -20ºC freezer within one hour of collection. Hill Laboratories measured compounds as described in the analyses results example in Appendix B and results section (2.4.1).

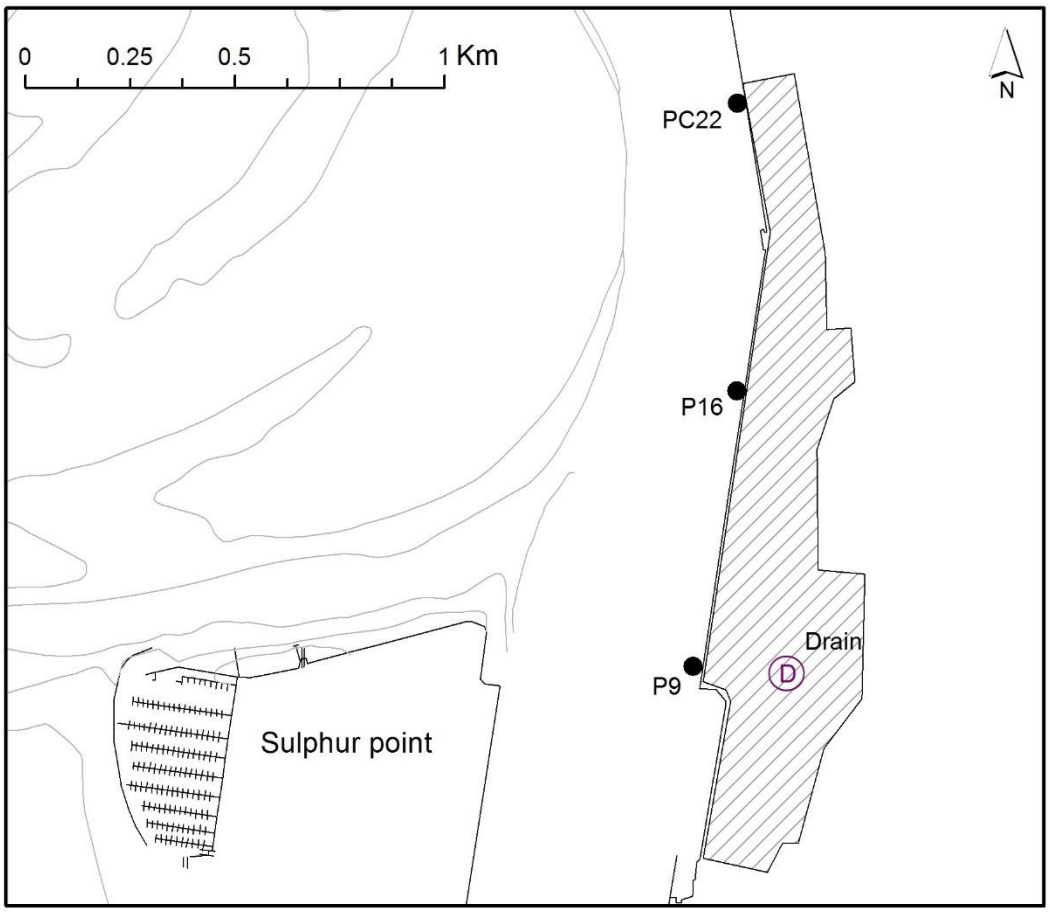


Figure 4. Drain (D) where raw effluent samples were collected and harbour sampling sites P9, P16 and PC22. Hatching indicates approximate area of log storage.

2.2.1.2 Water Samples Organic Assays

To evaluate levels of some of the common contaminants from wood storage (Waldichuk, 1979; Bailey *et al*., 1999), water collected on the 10 June 2014 and 27 November 2014 was also tested for monoterpenes, phenolics, fatty acids, resin acid neutrals, resin acids and phytosterols (Appendix A). These two samples were also used in the toxicity experiments (see 2.2.2, 2.4.2). This was undertaken at Scion using an in house method. Samples were extracted with dichloromethane, by continuous liquid/liquid extraction at pH 9 and silylated for analysis. Analysis was performed by GC/MS using scan acquisition (Robinson & Anderson, 2001).

2.2.2 Toxicity Experiments

Artemia are widely used as a subject for toxicity testing due to their resilience in a wide range of temperature, oxygen and salinity conditions. Its reliability, together with being a low cost alternative, in that they are easily stored and reared and are non-selective in their feeding methods, make them an excellent choice for baseline bioassays in ecotoxicology. The biggest criticisms against their use are the lack of presence in actual marine ecosystems and their resilience to extreme conditions. Its usual habitats are coastal lagoons and salt lakes (Nunes *et al*., 2006).

To put the results from the *Artemia* assays in context, a locally collected Mysid shrimp of the species *Tenagomysis* were used in a similar test to provide some comparative toxicity data.

2.2.2.1 Preliminary methods

Artemia spp., sourced from Brine Shrimp Direct, (2015) were hatched at the lab at 18°C, in saltwater (32ppt) and left undisturbed for 24 hours after hatching. Sensitivity for toxicology tests has been found to increase after this period (Nunes *et al*., 2006). To assess the suitability of *Artemia*, between 15 and 20 were put in 40 ml of filtered natural autoclaved seawater in a 60ml beaker in five replicates for five days. 98% surviving *Artemia* were counted at the end of this period.

For all experiments, a Fibox 3 AOT, fibre optic oxygen and temperature meter was used to record percentage dissolved oxygen (%DO) and temperature. An Aquaread AP2000D multi-parameter meter was used to record other parameters as detailed above.

The raw effluent used in the experiments was the same collected on 10 June 2014 (first set of tests, July 2014) and 27 November 2014 (second set of tests, January 2015) and chemically characterised as such by Hill laboratories and Scion. This adds useful detail about chemical composition for each experiment. Before use it was defrosted thoroughly, shaken and allowed to settle for one hour. As the effluent was of low salinity, low pH and high oxygen demand it was adjusted to match the

pH, %DO and salinity of the autoclaved seawater. This was to ensure that the percent concentration of the effluent was the main factor affecting survival in each treatment. Sodium bicarbonate was used to raise pH, an air stone and aquarium bubbler were used to raise the oxygen level, and red sea salt, purchased from a local aquarium supplier, was used to raise salinity.

The concentrations of the effluent for each treatment were selected using a geometric scale as shown in Table 2. Five replicates of each concentration and a control set of 100% autoclaved, filtered seawater were used.

Table 2. Log scale (log base 10), millilitres of effluent (ml) and percent concentration for each treatment.

Sample	Pot no.	Log	Effluent (ml)	% Concentration
A control	1TO 5	0	0	0
B	1TO 5	0.4	1	3
C	1TO 5	0.8	3	6
D	1TO 5	1.2	6	16
E	1TO 5	1.6	16	40
F	1TO 5	2	40	100

Treatments were labelled with the letters A to F. The five replicates of each treatment were labelled numerically. Each individual pot was assigned a number from one to 30 and positioned within the experiment according to a random matrix created using the following R code (R Core Team, 2013).

```
#Create vector A numbers 1 to 30#
A <- (1:30)

#Randomise order of A and create new Vector B#
set seed(1) B <-sample(A)

#Create matrix C#
C <- matrix(B,ncol=6) print(c)
```

2.2.2.2 Pilot *Artemia* Ecotoxicity Experiments July 2014

Pilot experiments were conducted in July 2014 in order to test the experimental design. Experiments were carried out in a temperature controlled room kept at 16°C. Artificial fluorescent light was constant throughout. To help buffer any changes in temperature from the thermostat cycling of room temperature control, a water bath was used in which the test containers floated in a polystyrene sheet with holes made for them. The containers were clear plastic 60 ml sampling pots obtained from Galantai Group Ltd. in Auckland. Pilot experiments were run for 12 and 24 hours.

Artemia were transferred to the treatments in a minimal volume of water by pipette. Rather than count individuals, a group was picked up and transferred, then counted. After the first pilot experiment it was decided to aim for lower numbers of *Artemia* per treatment which made counting faster upon completion. Jewellers' style magnifying glasses were used to help with counting.

2.2.2.3 Oxygen Depletion Experiment January 2015

The effluent was expected to have a high chemical and biological oxygen demand due to the widely recorded high solids content of runoff from log storage areas (Waldichuk, 1979; Bailey *et al*., 1999). For this reason oxygen depletion tests were conducted to understand any influence this may have on the experiments. These were carried out in 50 ml falcon tubes suspended in the temperature controlled bath. Using the FIBOX oxygen probe, the dissolved oxygen concentration was raised to 100% using an aquarium bubbler. The bubbler was then removed and oxygen was measured for at least one hour at one second intervals. Temperature was maintained at 18°C +/- 0.1°C throughout.

2.2.2.4 Further *Artemia* Experiments January 2015

A second set of experiments were run during January 2015. These experiments were run slightly differently to the pilot experiments in that temperature was controlled by floating the containers on a sheet of

polystyrene with holes cut through in a bath of water chilled to 18°C using a Hailea 220A aquarium chiller rather than using a temperature controlled room.

All treatments were also aerated for these experiments. A first experiment was run for 12 hours: because of the high mortality in the mid-range concentrations a second, identical experiment was run for quality control and continued for 24 hours, results were recorded at 12 hours to match the first experiment and at 24 hours.

2.2.2.5 Mysid Shrimp Experiments January 2015

To complement the *Artemia* experiments, a final experiment was carried out using *Tenagomysis* spp*.* These are a locally found species of Mysid shrimp and can be collected readily, using scoop nets, at the nearby Tauranga Bridge Marina. These were collected less than 24 hours before running the experiment and stored in water collected at the same time, with aeration. The experiment was run in the same manner as the preceding test and with the same replicate concentrations as the *Artemia* experiments described above, using the same equipment and conditions. As they are a larger size, only two mysid shrimps were placed in each pot. Counts and oxygen/temperature measurements were completed at 3, 6, 12 and 24 hours from the start of the experiment.

2.2.3 Water Quality Transects

A series of water meter readings were taken in the harbour, adjacent to the log storage discharge point (P9), along transects running from the wharf, across and along the channel and by depths of 02.m, 1m, 2m, 4m 6m and 10m or bottom. These were selected in order to examine water quality and how this may be influenced by stormwater runoff around this discharge point.

A typical storm event on 12 March 2015, (Table 3) provided an opportunity to carry out a series of measurements which were taken from a small inflatable boat using an Aquaread 2000AP multi-parameter meter. This meter is capable of measuring date, time, temperature, barometric

pressure, depth, pH, oxidation-reduction potential (ORP, REDOX), dissolved oxygen (DO), electrical conductivity (EC in uS/cm), resistivity (RES in Ohms.cm), total dissolved solids (TDS in mg/L), salinity in parts per thousand (SAL in ppt), seawater specific gravity (SSG in st), turbidity (NTU) and recording a GPS position in WGS 84 decimal degrees (DD) (Aquaread Ltd, 2015).

Table 3. Rainfall at 08:30am on 12 March 2015.

Date	12 March2015
Time	08:30am
Tide	1.26 flood
Hourly rainfall (mm)	0.0
Rainfall 24hr (mm)	13.2
Rainfall 7 day (mm)	14.5
Rain intensity (mm/h)	0.0
Wind Speed (Knots)	16.0
Wind Dir	138
Wind Dir	SE

Based on rainfall data from Brunschwiler (2015) this would be a typical low intensity event with much greater flows and intensities possible.

Rainfall on the 12 March had started six hours prior to monitoring. Two sets of five readings were taken two hours before high tide. Three sets of four readings were taken two hours after high tide. Readings were taken in the direction of tidal flow in or out of the harbour and a set across the harbour from the northern outfall (Figure 5). In the afternoon a third opportunistic set of readings were taken from an outfall to the south in a westerly direction as a distinct brown plume was noticed as the team passed over it. This is the most southern transect in Figure 5. Position of the sampling points out from the northern outfall had to be adapted to shipping movements hence the slightly staggered nature.

For descriptive purposes each distance point is referred to in the results section by letters A to E, A being close to the wharf and E being furthest away. The approximate distance between each point is 30 m.

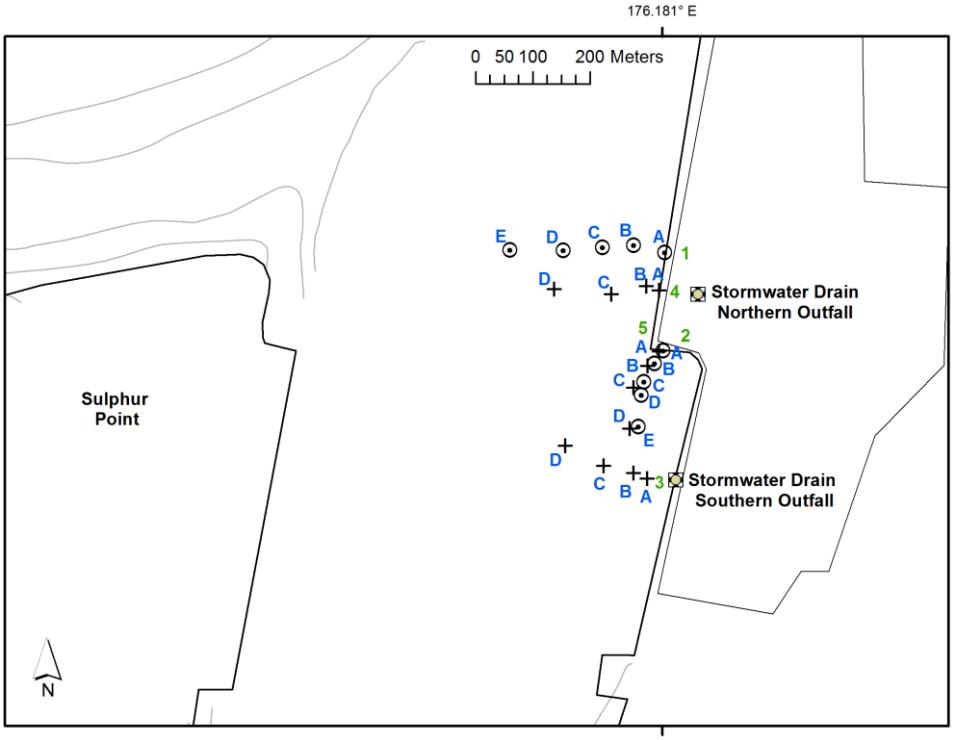


Figure 5. Locations of morning (circle) and afternoon (cross) sampling sites 12 March 2015 alongside locations of Northern and Southern Outfalls. 1-5 = transect numbers, A to D/E = locations at which readings were taken.

2.3 Statistical Analysis

Results were recorded into Excel spreadsheets and imported to R statistics (R Core Team, 2013) to return descriptive statistics and basic graphs. Water meter results were imported into Primer (Gorley & Clarke, 2015) and normalised. Resemblance analysis using Euclidean distance and principle components analysis (PCA) were performed on the data before permutational analysis of variance (PERMANOVA) (Anderson, Gorley, & Clarke, 2008) to test for significance and canonical analysis of principal coordinates (CAP) were completed. Analysis of variance and associated post-hoc tests on the toxicity experiments were also completed in R (R Core Team, 2013). Due to the variable numbers placed in each treatment and for ease of comparison, *Artemia* results were converted into percentage survival and mortality. Plots were completed in Excel and R using the graphics package ggplot2 (Wickham, 2009, p. 2).

Where used, example R code for many of the statistics methods is available via the links in APPENDIX C.

2.4 Results

2.4.1 Water Chemistry Results

2.4.1.1 Stormwater Drain Samples

Results from the stormwater drain samples are summarised below in Table 4. All compounds were assessed against the trigger values for species protection from the Australian and New Zealand Environment and Conservation Council guidelines for fresh and marine water quality (ANZECC, 2000). These set out guideline values, in µg/l, for percentage (99%, 95%, 90% and 80%) of species protection.

Those which exceed the 99% guideline are highlighted with blue. Those marked with a less than symbol (<) means that the chemical compound was found at levels less than indicated. Suspended solids in bold are those over the storm water consented limit (150 g/m^3).

Table 4. Summary of results of Hill Laboratories analyses of the stormwater samples taken from Drain (D) in Figure 4. Blue highlights indicate where value exceeds ANZECC guideline.

Individual Tests	Units	Drain 4/12/13	Drain 6/11/13	Drain 10/06/14	Drain 28/11/14	ANZECC (99%) FW
pH	pH	6.6	4.4	5.2	6.3	7.3
SS	g/m3	**440**	117	**340**	**310**	
Al	µg/l	8300	3600	8500	6000	27
B	µg/l	1380	35	58	1480	90
Cr(VI)	µg/l	<1.00	<1.00	<1.00	<1.00	10.00
Co	µg/l	6.50	1.93	3.20	10.80	
Mn	µg/l	1290.00	290.00	390.00	2500.00	1200.00
Hg	µg/l	<21.00	<0.08	<0.08	<0.08	0.06
Nitrite N	µg/l	5.00	3.00	6.00	5.00	
Nitrate N	µg/l	18.00	<2	16.00	24.00	17.00
cBOD5	g O2/m3	112.00		181.00	182.00	
COD	g O2/m3	350.00	840.00	850.00	690.00	
As	µg/l	21.00	1.10	2.70	<1.10	1.00
Cd	µg/l	1.10	0.06	0.10	<0.530	0.06
Cr	µg/l	14.00	2.50	5.40	7.00	0.01
Cu	µg/l	16.00	6.70	104.00	7.40	1.00
Pb	µg/l	20.00	1.85	4.50	3.10	1.00
Ni	µg/l	11.00	2.10	3.70	7.50	8.00
Zn	µg/l	540.00	104.00	116.00	350.00	2.40

2.4.1.2 Harbour Water Results

Harbour water results are summarised in Table 5. Levels of parameters measured in the samples taken at sites P9, P16 and PC22 were all lower than the same parameters recorded in samples taken from the stormwater drain. One compound was found to be significantly higher, which was boron. This was recorded as much as four times the levels as in the stormwater drain samples. In this table greyed out numbers with a less than symbol (<) indicates that the chemical compound was found at levels less than indicated. These should be read carefully as the minimum detection limits are high due to the need to dilute the sample. For example, the ANZECC (2000) 99% trigger value for copper, for marine water is 0.3 µg/l. Copper was not able to be detected below 53 µg/l due to dilution of the sample.

Table 5. Summary of results of Hill Laboratories analyses of the marine samples taken from P9, P16 and PC22 in Figure 4. Grey indicates chemical compound was found at levels less than indicated.

Individual Tests	Units	P9 12/09/13	P16 12/09/13	PC22 12/09/13
pH	pH	8.1	8.1	7.8
SS	g/m3	31	11	48
Al	µg/l	400	<320	440
B	µg/l	3800	4500	4300
Cr(VI)	µg/l	2.2	<1	1
Co	µg/l	<21	<21	<21
Mn	µg/l	<53	<53	<53
Hg	µg/l	<0.08	<0.08	<0.08
Nitrite N	µg/l	<10	<20	<10
Nitrate N	µg/l	<10	<20	11
COD	g O2/m3	240	240	280
As	µg/l	<110	<110	<110
Cd	µg/l	<5.3	<5.3	<5.3
Cr	µg/l	<53	<53	<53
Cu	µg/l	<53	<53	<53
Pb	µg/l	<11	<11	<11
Ni	µg/l	<53	<53	<53
Zn	µg/l	<110	<110	<110

2.4.1.3 Water Samples Organic Assays

The levels of organic compounds in those screened by GC/MS were quite different in the two collected samples. Total monoterpenes (Table 6) in the 10 June 2014 sample, used for the pilot toxicity experiments was much higher (293.9 µg/l) compared with the 27 November 2014 sample used for the *Artemia* and Mysid experiments in January 2015 (40.1 µg/l).

Table 6. Monoterpenes (µg/l) in 10 June 2014 and 27 November 2014 effluent samples taken from the stormwater drain.

Sample name	10/06/2014 Drain sample used in pilot tox. experiments	27/11/2014 Drain sample used in main *Artemia* experiments
Alpha-pinene	n.d.	n.d.
Beta-pinene	2.1	n.d.
Fenchone	n.d.	n.d.
Camphor	5.5	n.d.
Fenchol	n.d.	n.d.
Borneol	69.8	10.7
Terpinen-4-ol	40	n.d.
Alpha-terpineol	176.6	29.4
Total Monoterpenes	293.9	40.1

Results for phenolics are summarised in Table 7. Totals again were much higher in the 10 June 2015 sample (429 µg/l) then the 27 November 2014 sample (94.2 µg/l). In this case homovanillic acid made up for over half of that total.

Table 7. Phenolics (µg/l) in 10 June 2014 and 27 November 2014 effluent samples taken from the stormwater drain

Sample name	10 Jun 14 Drain sample used in pilot tox. experiments	27 Nov 14 Drain sample used in main *Artemia* experiments
Guaiacol	8.7	1.1
Eugenol	1.6	n.d.
Vanillin	10.9	4.3
Acetovanillone	7.4	5.1
Vanillic acid	8.8	2.1
Homovanillic acid	217.8	29.8
Ferulic acid	39.3	0.5
Gallic acid	n.d.	n.d.
Syringol	n.d.	n.d.
Acetosyringone	n.d.	n.d.
Syringylaldehyde	n.d.	n.d.
Syringic acid	n.d.	n.d.
Coniferyl alcohol	130.9	51.4
Coniferyl aldehyde	n.d.	n.d.
Pinosylvin, mono methyl ether	3.9	n.d.
Total Phenolics	429.2	94.2

Fatty acids totals (Table 8) were closer, 337.5 µg/l in the 10 June 2014 sample and 216.5 µg/l in the 27 November 2014 sample. Oleic, then palmitic acids were the dominant fatty acids in the 10 June 2015 sample and palmitoleic acid in the 27 November 2014 sample.

Table 8. Fatty acids (µg/l) in 10 June 2014 and 27 November 2014 effluent samples taken from the stormwater drain.

Sample name	10 Jun 2014 Drain sample used in pilot tox. experiments	27 Nov 2014 Drain sample used in main *Artemia* experiments
Decanoic acid (F10:0)	n.d.	n.d.
Dodecanoic acid (F12:0)	n.d.	n.d.
Tetradecanoic acid (F14:0)	n.d.	14.2
Palmitoleic acid (F16:1)	29.9	109
Palmitic acid (F16:0)	86.3	35.2
Margaric acid (F17:0)	n.d.	n.d.
Linoleic acid (F18:2)	15.8	n.d.
Oleic acid (F18:1)	102.9	12.1
Linolenic acid (F18:3)	1.8	n.d.
Elaidic acid (F18:1)	19.8	16.1
Stearic acid (F18:0)	23.9	14.8
Eicosanoic acid (F20:0)	9.2	2.7
Docosanoic acid (F22:0)	31.8	9
Tetracosanoic acid (F24:0)	16.2	3.5
Total Fatty Acids	337.5	216.5

Resin acid neutrals (Table 9) were not detected in the 10 June 2015 sample and small quantities of dehydroabietin (4.4 µg/l) and tetrahydroretene (4.5 µg/l) detected in the 27 November 2014 sample.

Table 9. Resin acid neutrals (µg/l) in 10 June 2014 and 27 November 2014 effluent samples taken from the stormwater drain

Sample name	10 Jun 2014 Drain sample used in pilot tox. experiments	27 Nov 2014 Drain sample used in main *Artemia* experiments
Fichtelite	n.d.	n.d.
Dehydroabietin	n.d.	4.4
Tetrahydroretene	n.d.	4.5
Retene	n.d.	n.d.
Methyldehydroabietin	n.d.	n.d.
Total Resin Acid Neutrals	n.d.	8.9

Many of the resin acids were found in significant quantities. Table 10 compares results from the two samples, 10 June 2014 and 27 November 2014, with samples taken from the same drain on the 5 December 2013

and analysed by Hill laboratories. The average of individual resin acids taken from Tian *et al*. (1997) is also added for comparison: those results were taken from runoff in the more southern log storage area in July 1995.

Table 10. Resin acids (µg/l) in 10 June 2014 and 27 November 2014 effluent samples taken from the stormwater drain, compared with results from sampling the same drain 5 December 2013 and averages from 16 samples (Tian *et al*., 1997). Grey background = highest figure for compound.

Project	This thesis	This Thesis	Port Tauranga	Tian 1997 Ave.
Sample name	Drain 10-Jun-14	Drain 27-Nov-14	Drain 5-Dec-13	from 16 samples
Pimaric acid	161.2	40.8	34	120.763
Sandaracopimaric acid	29.8	5.7	5	n.samp
Isopimaric acid	132.3	24.1	15	84.838
Palustric acid	250.9	17.9	6	n.samp
Levopimaric Acid	227.5	n.d.	n.samp	n.samp
Dehydroabietic acid	620.9	309.4	200	478.875
Abietic acid	300.3	36.9	22	72.527
Neoabietic acid	120.2	7.9	5	n.samp
Pimarenic acid	n.d.	n.d.	n.samp	n.samp
Sandaracopimarenic acid	n.d.	n.d.	n.samp	25.413
Isopimarenic acid	n.d.	n.d.	n.samp	n.samp
13-Abietenic acid	n.d.	13.1	n.samp	n.samp
Pimaranic acid	n.d.	n.d.	n.samp	n.samp
Isopimaranic acid	n.d.	n.d.	n.samp	n.samp
Abietanic acid	n.d.	1.6	n.samp	n.samp
Seco-1-dehydroabietic acid	11.9	6.3	n.samp	22.813
Seco-2-dehydroabietic acid	11	6.1	n.samp	10.700
12-Chlorodehydroabietic	n.d.	n.d.	2	n.samp
14-Chlorodehydroabietic	n.d.	n.d.	2	n.samp
12,14Dichlorodehydroabietic	n.d.	n.d.	2	n.samp
7-Oxodehydroabietic acid	25.8	11.6	33	157.667
de-DHAA	n.samp	n.samp	n.samp	76.019

2.4.1.3.1 In summary

The two sets of results are quite different in all organic compound groups, highlighting high temporal variability in composition of the storm water effluent. While not directly comparable, it may be of note the samples which contained the largest amounts of resin acids the Drain sample (10 June 2014) and Tian et al (1997) were taken in the winter (June 2014 and May to July 1995). Those two containing the lower amounts were taken November 2014 and December 2013.

Many more compounds were visible in the samples which as yet have not been identified and quantified (Appendix D).

2.4.2 Toxicity Experiments

The toxicity experiments results are treated as separate by the effluent used and date carried out as both were quite different. The pilot experiments undertaken in July 2014 (2.4.2) used the stormwater sample collected on 10 June 2014. The later toxicity experiments in January 2015 (2.4.3) are used the sample collected on 28 November 2014. These sample effluents are characterised in detail in sections 2.4.1.1 ‘stormwater drain samples’ and 2.4.1.3 ‘water samples organic assays’.

2.4.2.1 Pilot *Artemia* Ecotoxicity Experiments July 2014

Before the experiment was started, water quality parameters of the homogenised autoclaved seawater (control), effluent and adjusted effluent were recorded. These adjustments were to ensure all treatments had the same basic water quality parameters of oxygen, salinity, temperature and pH and were recorded as being within +/- 5% of the control solution and are displayed in Table 11.

Table 11. Water quality parameters of autoclaved seawater, effluent and adjusted effluent with percentage of increase or decrease after adjustment. O2 (%DO), Sal (ppt), Temp (ºC) and pH. Autoclaved seawater = control, Effluent Pre. = effluent before adjusting.

	Autoclaved SW	Effluent Pre.	Adjusted effluent	% Increase/Decrease
O2%	102.5	105.5	105.3	inc. 2.73
Sal	32.5	0.1	32.7	inc. 0.62
Temp	19.2	18.2	20	inc. 4.17
pH	8	5.2	7.9	dec. 1.25

After 12 hours, only one replicate (in the 40% treatment) had fallen below 50% mortality (Figure 6).

This result was considered a test run and, using the same autoclaved sea water and adjusted effluent, the experiment was run again for 24 hours.

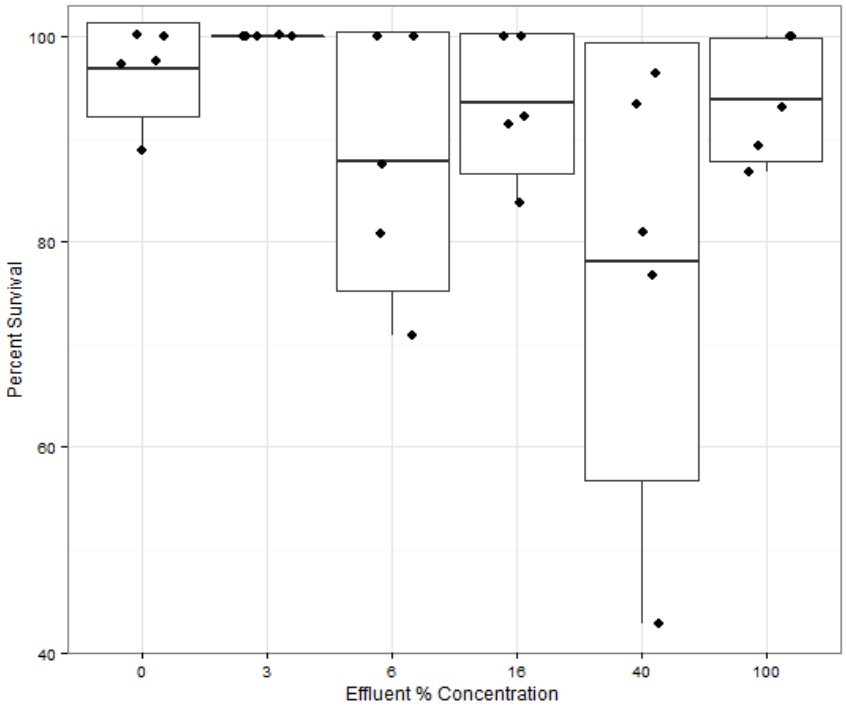


Figure 6. Boxplot *Artemia* % survival after 12 hrs. Horizontal line = mean % survival, Box= +/- S.D., vertical line = min/max, diamonds = data points

Results from the second pilot experiment, run for 24 hours (Figure 7) recorded greater effects on *Artemia*. Four of the five replicates exceeded

50% mortality in the 100% treatment, two in the 40% treatment, three in the 16% treatment and one in the 6% treatment. The 3% treatment had survival over 90% and the control had no mortalities.

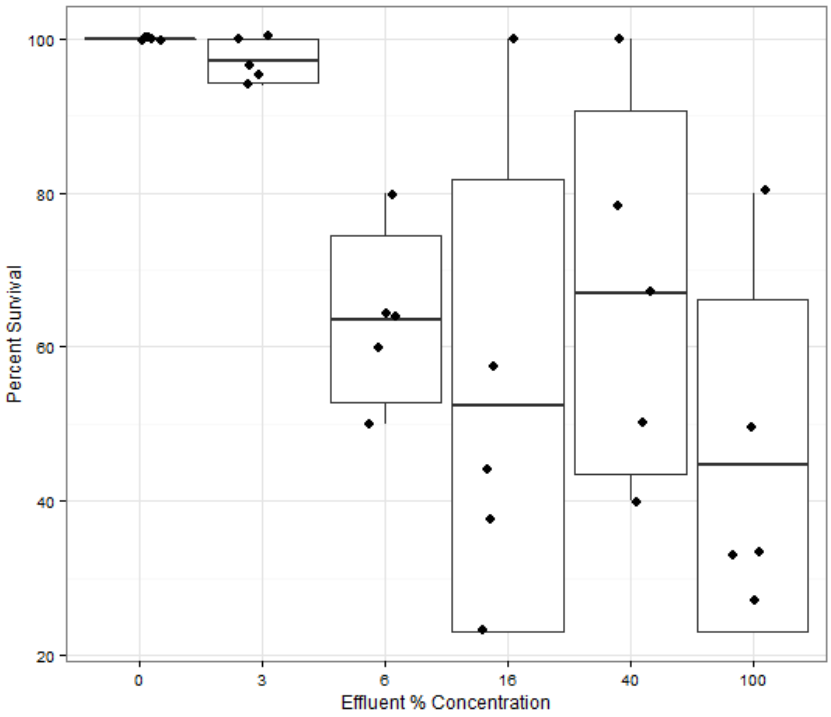


Figure 7. Boxplot *Artemia* % survival after 24 hrs. Horizontal line = mean % survival, Box= +/- S.D., vertical line = min/max, diamonds = data points.

Table 12 gives results of ANOVA for the 2 experiments. No significance was found in the first, 12h experiment (P<0.05). Significance was found between treatments in the second experiment, P = 0.000161.

Table 12. Table of ANOVAS for Experiment 1 (top) and 2 (bottom)

	Df S	um Sq	Mean Sq	F value	Pr(>F)	
Treatment	5	1522	304.4	2.549	0.055	
Residuals	24	2866	119.4			
Treatment	5	13204	2641	7.906	0.000161	* * *
Residuals	24	8016	334			

Oxygen and temperature were measured at 12 and 24 hours during the experiment. Temperature remained within +/- 0.5 °C throughout both

experiments with very little differences between treatments. Oxygen levels were noticeably lower in the higher (40 and 100%) concentrations (Figure 8). This raised questions on whether low oxygen was the reason for the higher mortalities in the 24 hour experiment. It was decided to run a more specific oxygen depletion experiment before conducting more of the toxicology work.

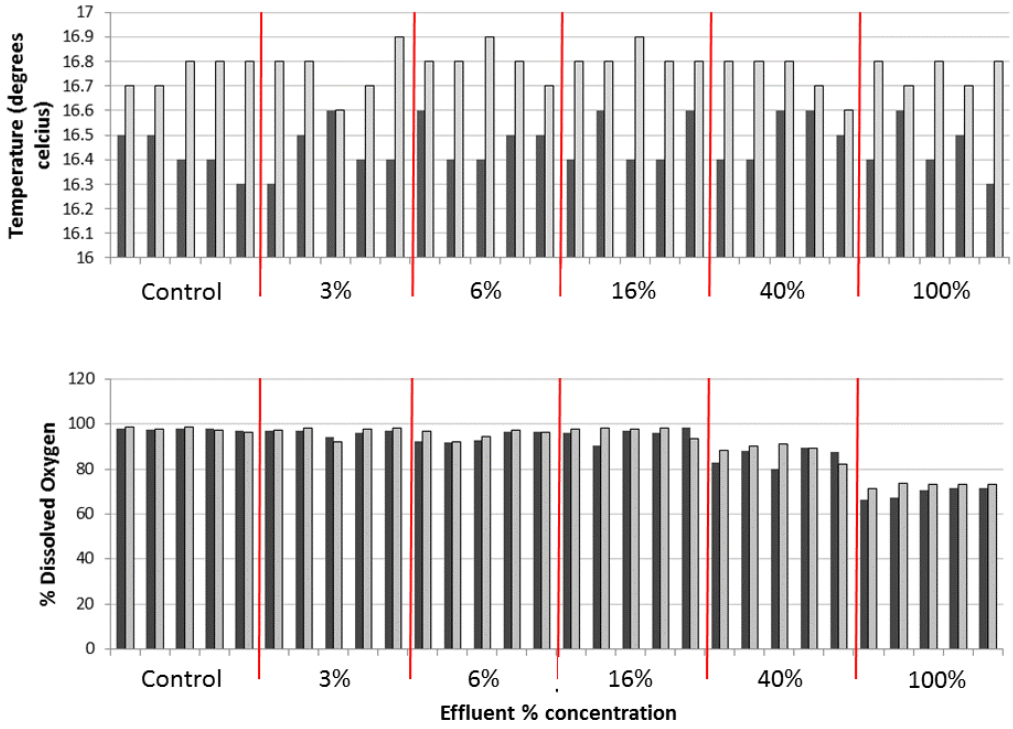


Figure 8. Temperature, °C (top), and oxygen concentration, %DO (bottom), measured at 12hrs (dark) and 24 hrs (light) in the second Artemia experiment for all replicates.

2.4.2.1.1 Summary of findings

At 12 hours *Artemia* showed little sign of acute toxicity even at 100% concentration. At 24 hours toxicity appears to increase with concentration of stormwater. Percent DO appears to decrease with increasing effluent concentration.

2.4.2.1.2 Oxygen depletion January 2015

In the specific dissolved oxygen experiment for each concentration, %DO decreased over one hour more rapidly with increased effluent concentration. The highest decrease was mainly recorded in the first 10 minutes in the higher concentration treatments E and F (Figure 9) before appearing to decrease at the same rate across all concentrations.

This result indicated that %DO may have influenced survival in the experiments and forced the decision to include a continuous oxygen supply for the final toxicity experiments.

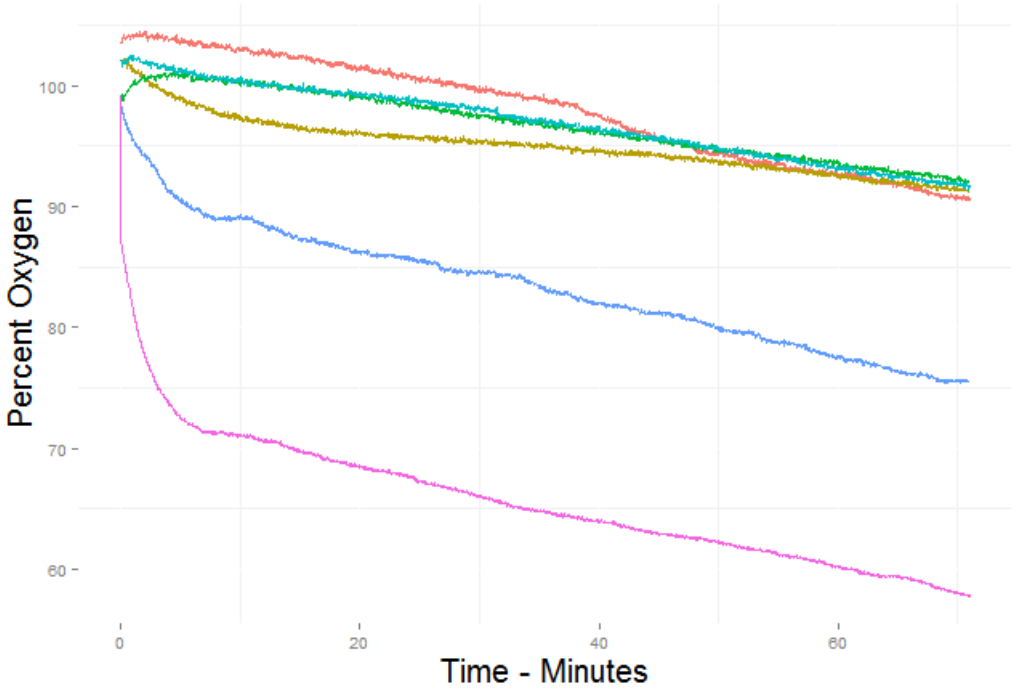


Figure 9. Oxygen depletion over time for each treatment. Orange = Control or 0%, Yellow = 3%, Green = 6%, Turquoise = 16%, Blue = 40%, Pink = 100%.

2.4.3 Further Toxicity Experiments January 2015

2.4.3.1 Characterisation and Preparation of Test Effluent

Test effluent is characterised in sections 2.4.1, organics parameters were substantially different from the first set of experiments in July 2014.

Effluent was added to autoclaved, filtered seawater in the same concentrations as for the pilot experiments in July 2014.

2.4.3.2 Artemia Experiments January 2015

Given results described above (2.4.2.1.2) indicating that the effluent will strip oxygen at a rate correlated to effluent concentration a second set of experiments were run with full oxygenation. Not surprisingly, considering this and the differences in characteristics of the effluents already described, the second batch of experiments using *Artemia* yielded different results than those conducted without oxygenation. The first 12 hour experiment (Figure 10) in the second batch of experiments suffered high mortality (>75%) in the 3, 6 and 16% concentrations. The 40 and 100% concentrations had some survival above 50%: in 4 of 5 treatments of the 40% concentration and 1 of the 5 treatments of 100% and less overall mortality than the 3, 6 and 16% concentrations. Control treatments were 100% survival in 4 replicates and 80% survival in the 5th replicate.

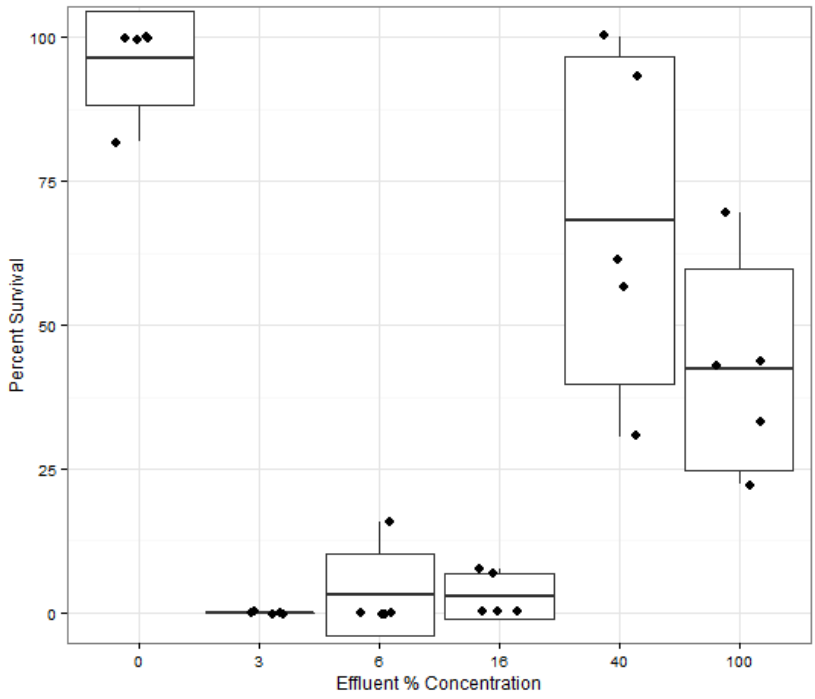


Figure 10. Boxplot *Artemia* % survival after 12 hrs. Horizontal line = mean % survival, Box= +/- S.D., vertical line = min/max, diamonds = data points.

Results were unexpected in that the mid-range concentrations had higher mortality than the higher concentrations of storm water. Due to the unexpected results the experiment was repeated. Very similar results occurred in the 3, 6 and 16% concentrations. The 40 concentrations suffered >50% mortality, the 100% suffered <50% mortality. The control treatment again had high survival (Figure 11).

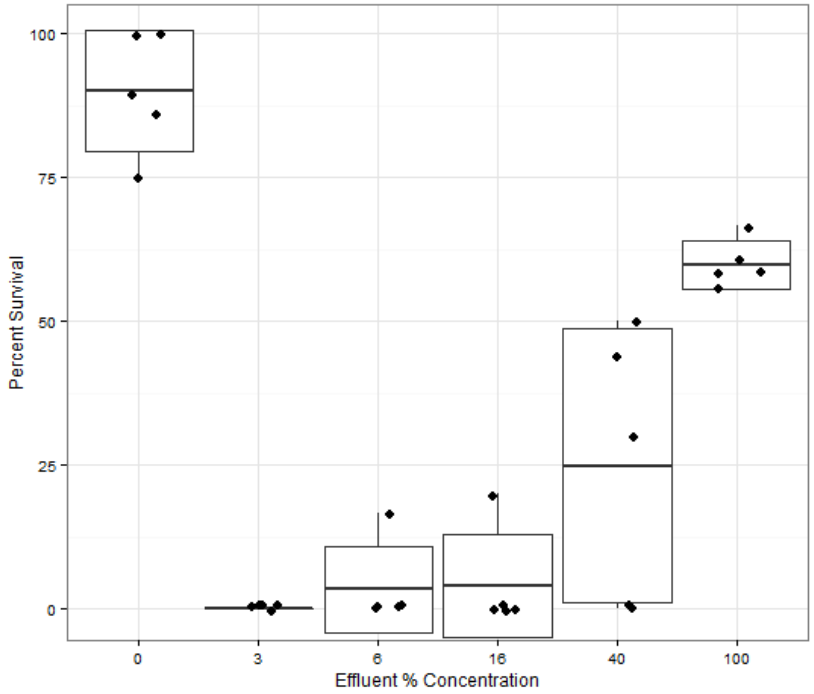


Figure 11. Boxplot *Artemia* % survival after 12 hrs. Horizontal line = mean % survival, Box= +/- S.D., vertical line = min/max, diamonds = data points

This experiment was left to run for a further 12 hrs. Mortality was 100% in all treatments of 3, 6, 16 and 40% concentrations and in all but one of the 100% treatments. Survival remained high for the control treatments (Figure 12).

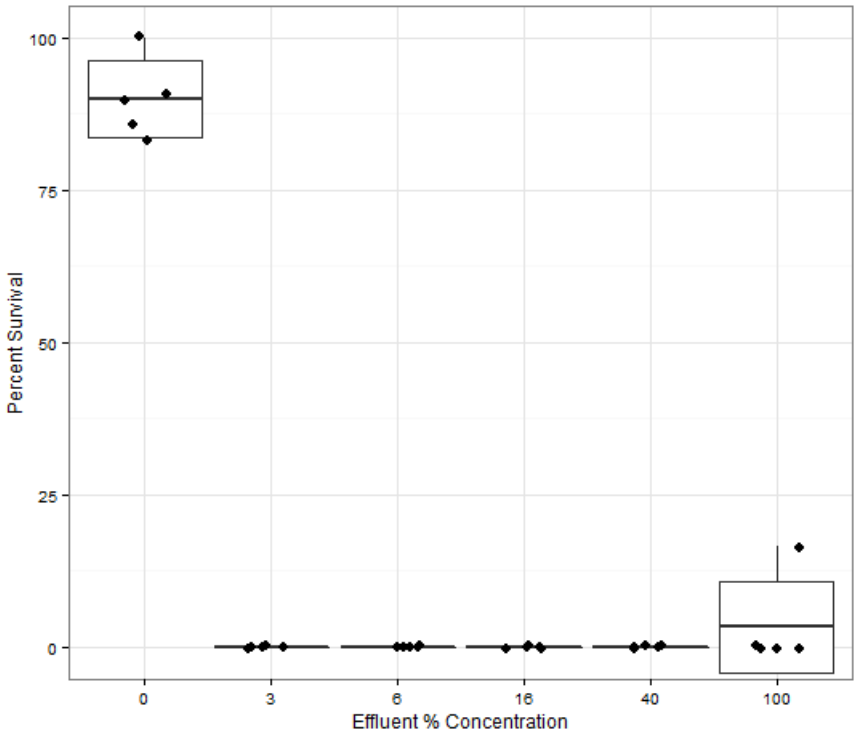


Figure 12. Boxplot *Artemia* % survival after 24 hrs. Horizontal line = mean % survival, Box= +/- S.D., vertical line = min/max, diamonds = data points.

ANOVA was run for the 3 experiments above and all were found to have very high statistical significant differences between treatments (Table 13).

Table 13. ANOVA results for the three *Artemia* experiments conducted in January 2015.

	Df S	um Sq	Mean Sq	F value	Pr(>F)
Trtmt	5	40980	8196	39.48	0.0000000000814
Residuals	24	4982	208		
Trtmt	5	34082	6816	49.08	0.00000000000796
Residuals	24	3333	139		
Trtmt	5	33290	6658	414.1	0.0000000000000002
Residuals	24	386	16		

2.4.3.2.1 Summary of Findings

In the second set of experiments, *Artemia* respond negatively to all concentrations of effluent, with acute toxicity apparent for all concentrations over 12 hours. Mortality was almost total, with only one

replicate having live animals after 24 hours. As described in section 2.4.1.4, the characteristics of the stormwater used in this second batch of experiments were quite different to those in the pilot. Survival was high in all control treatments.

2.4.3.3 Mysid Shrimp Experiments January 2015

The Mysid shrimp treatments were faster to count due to lower numbers of animals in each treatment and the larger size of the species. Numbers in the control treatment appeared to suffer mortality at similar rates to the other Mysid other treatments. In the 100% concentration mortality appeared to occur faster than all other concentrations having the highest percentage mortality each time counting took place (Figure 13).

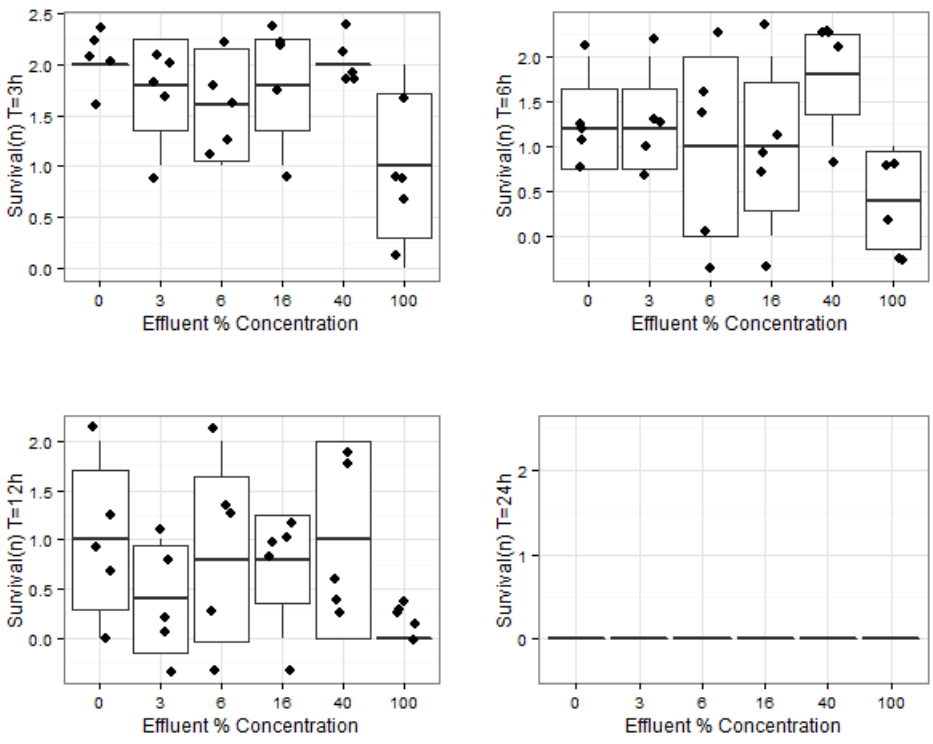


Figure 13. Boxplot of Mysid survival. Clockwise from top left after 3 =hrs, 6hrs, 12hrs, 24hrs. Horizontal line = mean % survival, Box= +/- S.D., vertical line = min/max, diamonds = data points.

Analysis of Variance (ANOVA) resulted in significant difference between treatments (P = 0.0162) after 3 hrs and no significant difference after 6 (P

= 0.0592) and 12 (P = 0.109) hrs. Post-hoc analysis (Tukeys HSD) of results after 3 hrs revealed the significant difference was between the 100% concentration and all other treatments.

Comparison of mortality over time for each treatment (Figure 14) does show an increasing trend and highlights the increased mortality of the higher concentration.

Most significant is the high mortality of the control rendering the test invalid. Some other factor of the water quality has clearly influenced survival of Mysids in these conditions.

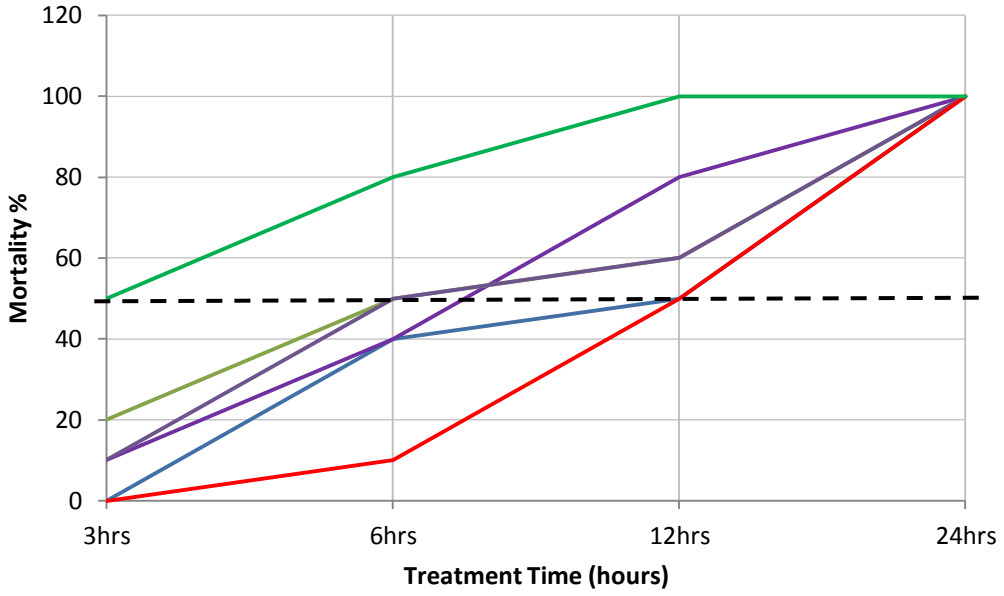


Figure 14. Mortality of Mysids for each treatment over time. Green = 100%, red = 40%, yellow = 16%, grey = 6%, purple = 3% and light blue = 0% concentrations. Dotted line = 50% mortality.

2.4.3.3.1 Summary of Findings

The second set of *Artemia* experiments yielded quite different results to the first. There is clearly an effect to them from both tests. Differences in stormwater effluent chemical characteristics are evident from the earlier analyses and other factors such as water quality used for the control may be a factor. This is demonstrated by the results of the Mysid experiment which was rendered invalid by high mortality in the control.

It does show a difference in sensitivities between the two species due to the faster mortality in all effluent concentrations and subsequent total mortality in the control treatment.

2.4.4 Water Quality Transects

As discussed a series of meter readings were carried out to investigate differences in water quality parameters with distance from the wharf and depth. These results were interpolated graphically and the more demonstrative results are included in the following Figures (15 to 19).

The following figures consist of four graphs: the first two are readings along the two transects taken in the morning the two below were taken in the afternoon. North to south and morning and afternoon are left top and bottom. East to west morning and afternoon are right top and bottom. The readings along the single transect taken from the southern outfall (as described earlier in Figure 5) had similar results and was left out for brevity. Each individual graph reads from close to wharf (distance A) to furthest from the wharf (distance E). The bars at each distance represent depth dark being the surface to light the maximum depth.

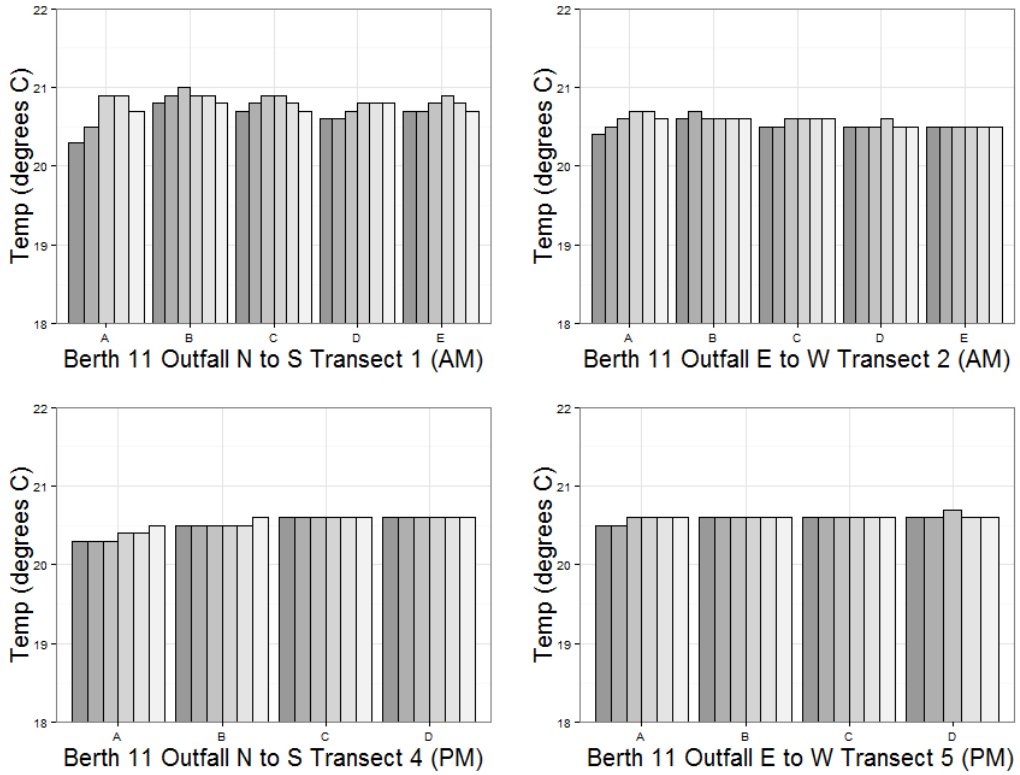


Figure 15. Temperature readings taken along transects from the wharf to central channel (A to E). Colour gradient represents depth, dark = 0.2m, 1m, 2m, 4m, 6m to light =bottom or 10m.

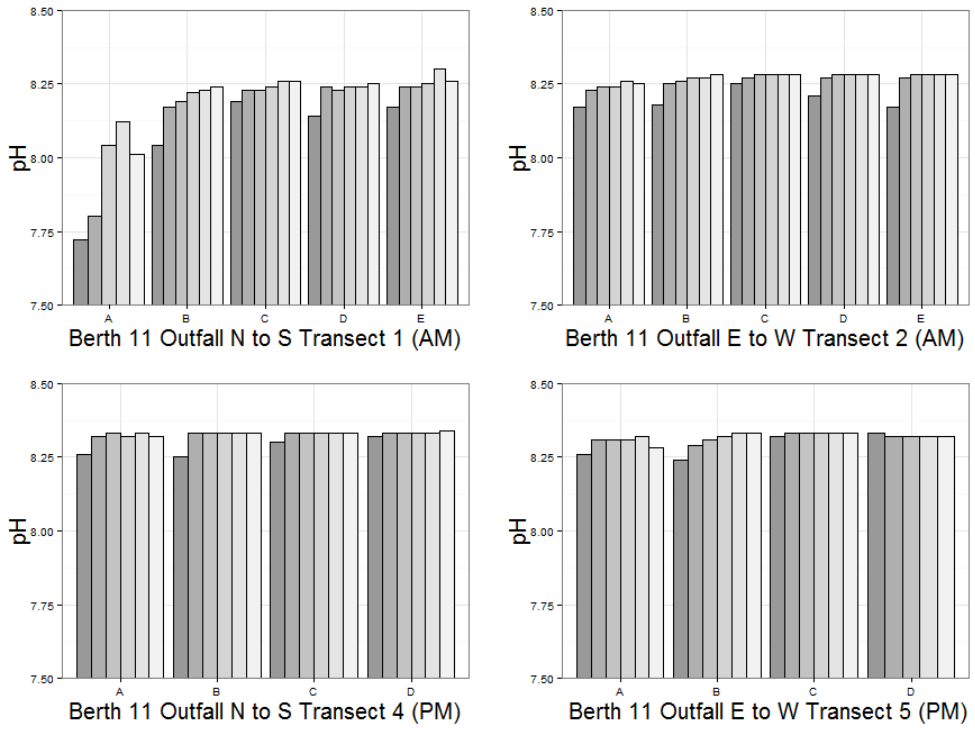


Figure 16. pH along transects from the wharf to central channel (A to E).

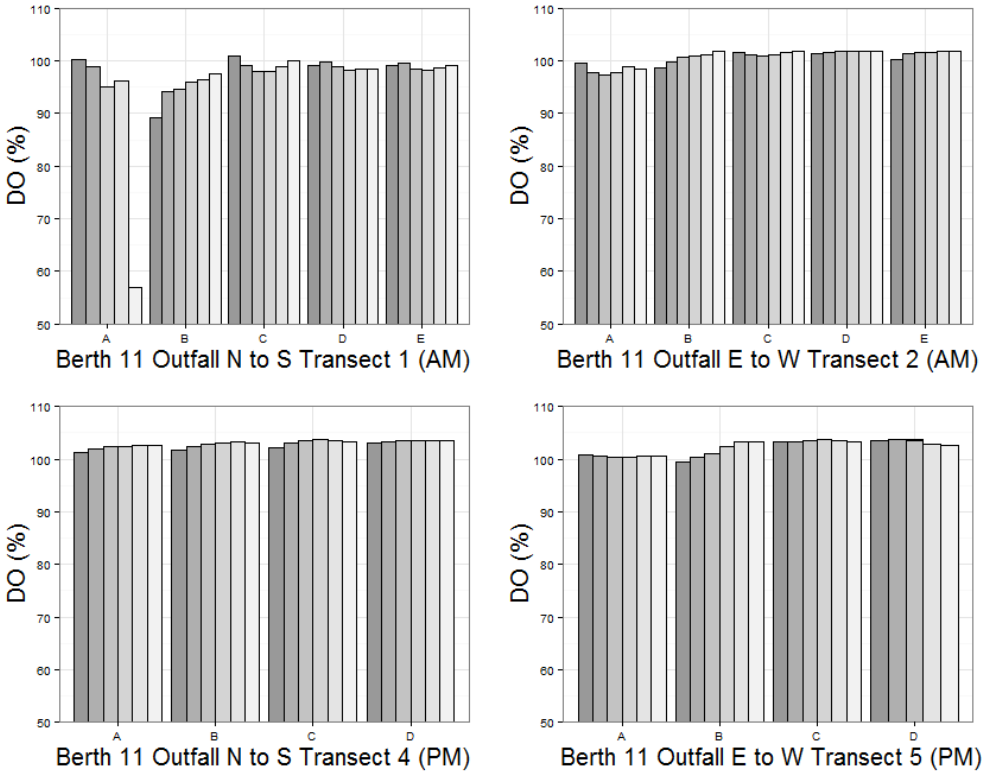


Figure 17. DO (%) taken along transects from the wharf to central channel (A to E). Colour gradient represents depth, dark = 0.2m, 1m, 2m, 4m, 6m to light =bottom or 10m.

Figure 18. ORP (REDOX) taken along transects from the wharf to central channel (A to E).

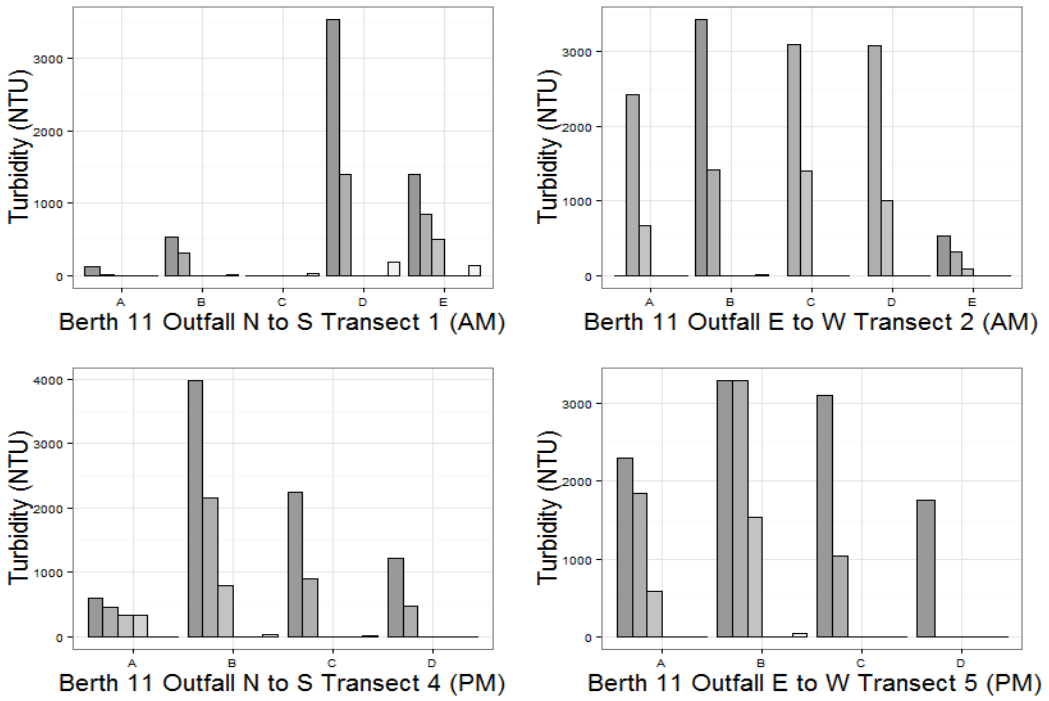


Figure 19. Turbidity (NTU) taken along transects from the wharf to central channel (A to E). Colour gradient represents depth, dark = 0.2m, 1m, 2m, 4m, 6m to light =bottom or 10m.

In summary the graphs in figures 15, 16 and 17 demonstrate subtle changes in temperature, pH and DO% both with change in depth and distance from the wharf. ORP, which measures oxidation-reduction reactions, has the greatest variation close to the wharf and on the incoming tide (Figure 18). This may indicate the edge of the mixing zone at this point in the tidal cycle. Interestingly, turbidity is not greatest at the closest point to the wharf but a little further out, then decreases with distance away from the wharf (Figure 20).

Principal components analysis (PCA) was run on normalised data (Figure 24): PC1 accounted for 48.5% of the variation and PC2 for 21%. Cumulatively 69.5% of the variation was explained by the ordination which is acceptable for this type of ordination but not outstanding. The different colours/shapes of the plot represent transects one to five. No definite clusters were immediately apparent, however readings ending with an 'S' indicating the shallow readings appear to the right of the ordination and that depth may have some influence on the distribution of the plot. The

overlay of base variables (water quality criteria) indicate Depth, TDS and EC positively influence variation across the ordination and vertically a positive influence of pH and DO.

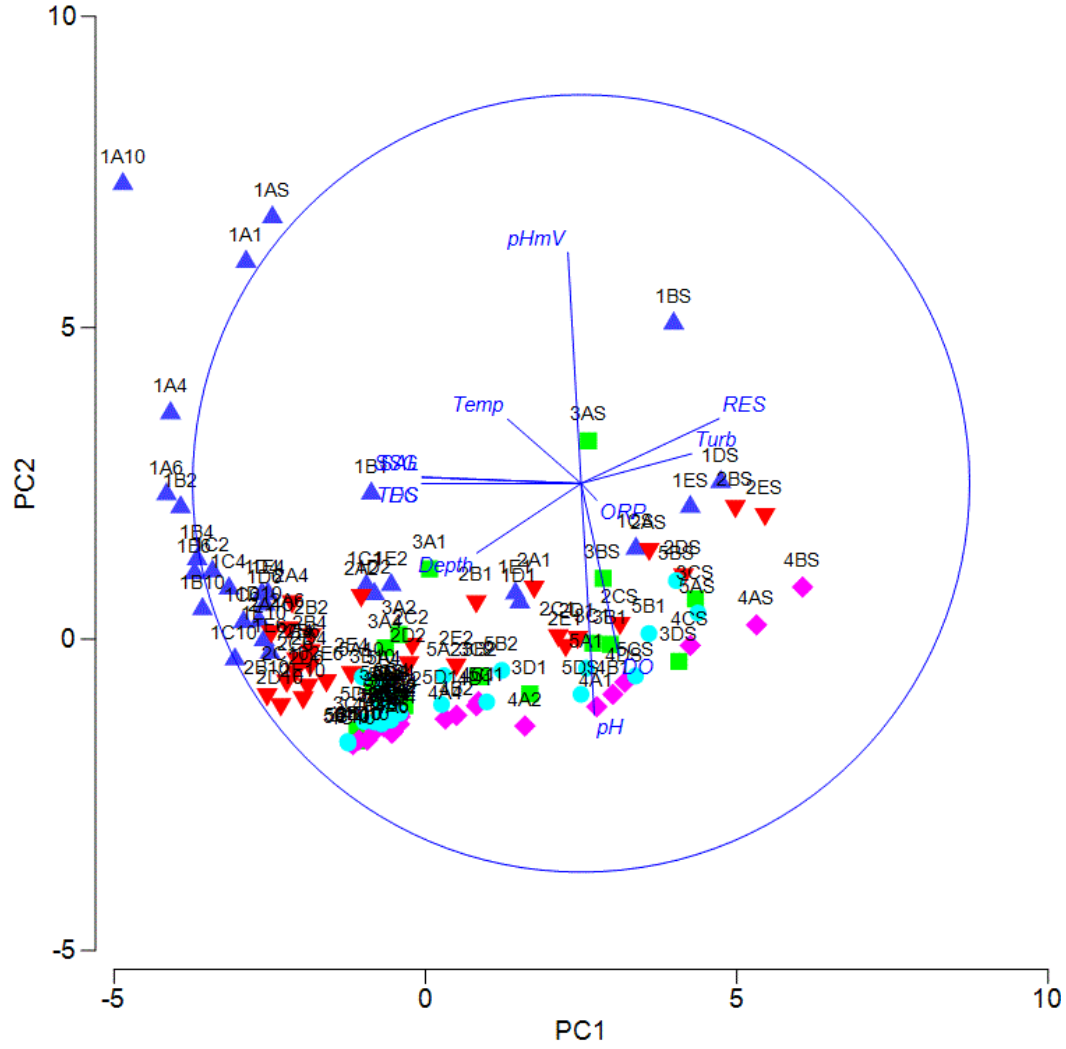


Figure 20. PCA of normalised meter readings data from 12 March 2015. Blue = Transect 1, red = 2, green = 3, pink = 4 and turquoise = 5. Labels (black text) = individual readings. Labels (blue text) = water quality criteria.

The data were analysed for resemblance by Euclidean distance and the resulting matrix was used for PERMANOVA to test for significance. Significance was found between Transects (P(perm) 0.001). Pair wise tests found significant differences between all transects excluding 3&4, 3&5 and 4&5 (P(perm) < 0.007). This did not help explain the spread in the PCA ordination so further PERMANOVA tests were run on depth and distance for each transect. Depth being depth of reading taken and

distance being distance along the transect from the outfall A (close) to E (distant).

Depth and distance were found to be significant (P(perm) 0.001 and P(perm) 0.002). Unsurprisingly, no significance was found from an interaction with depth and transect or distance and transect as each transect had multiple depths and drops. Pairwise tests of depth found all depths significantly different excluding 4&6m (P(perm <0.014). Pairwise tests for distance found significance between nearby readings (A) only (P(perm) <0.008).

To help visualise this, CAP analysis was performed on the resemblance matrix for Depth and Drop. The ordination for Depth (Figure 21) clearly separates the different depths and suggests an influence of resistance, turbidity, TDS, conductivity and, to a lesser degree, temperature and pH.

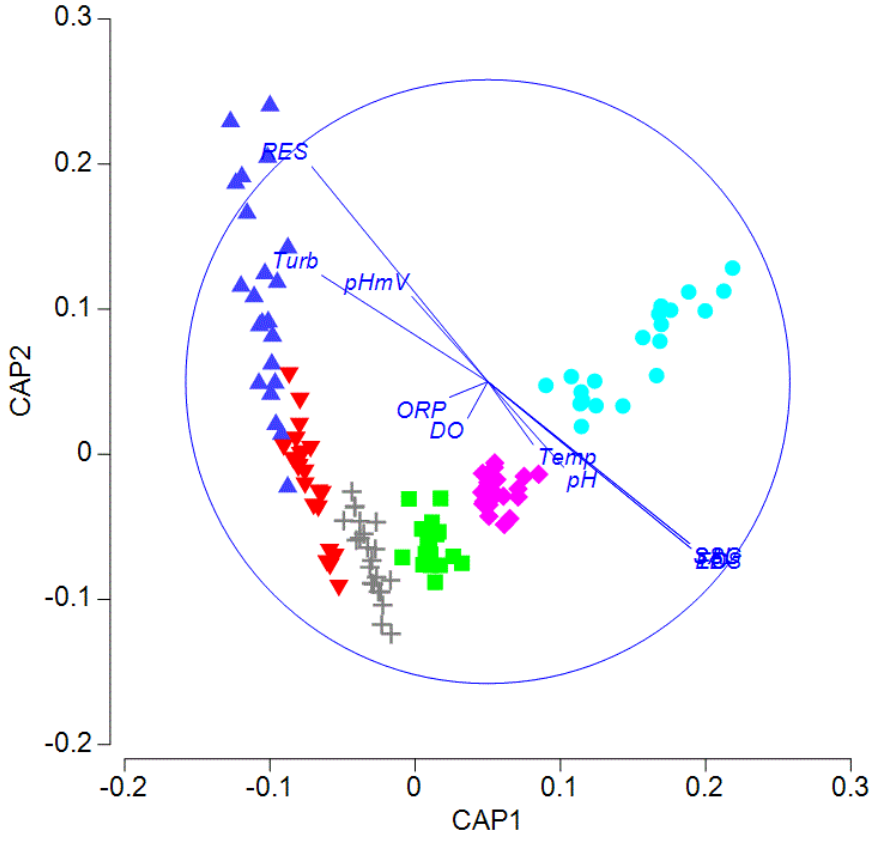


Figure 21. CAP analysis of resemblance matrix of normalised water quality data using Euclidean distance. Plots of Depth; Blue = Surface, red = 1m depth, grey cross = 2m depth, green = 4m depth, pink = 6m depth, turquoise = 10m depth.

The ordination for distance (Figure 22) appears to indicate that the 'A' (close) readings, influenced positively by ORP and conductivity, cluster separately from other distances influenced by pH, DO, and to a lesser degree temp.

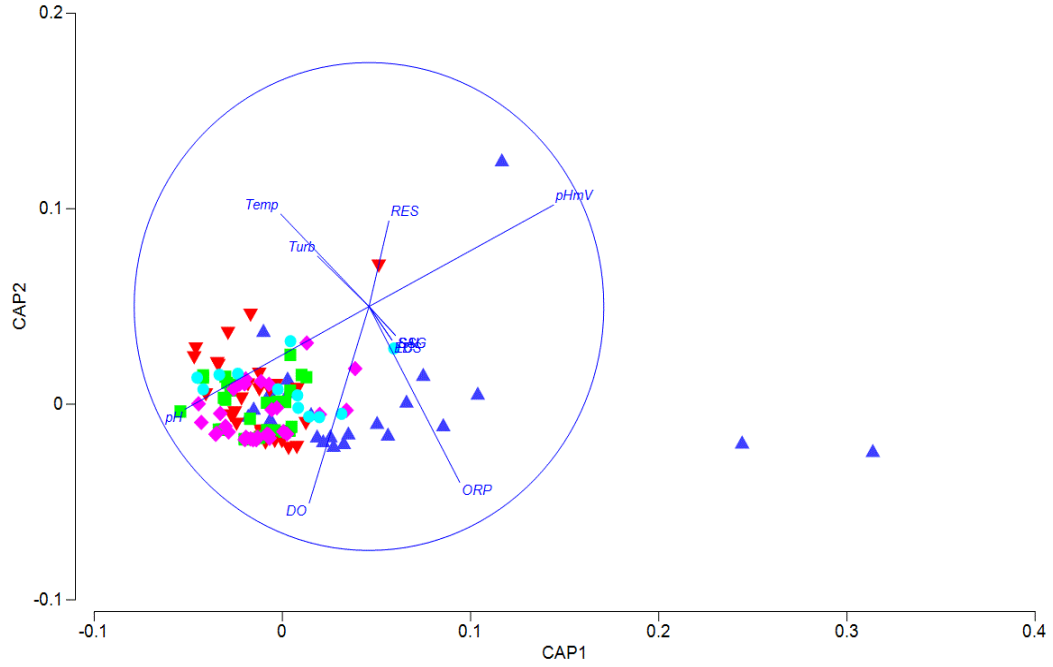


Figure 22. CAP analysis of resemblance matrix of normalised water quality data using Euclidean distance. Plots of distance from wharf; Blue = A (wharf), red = B (≈40m), green = C (≈80m), pink = D (≈120m), turquoise = E (≈160m or more).

2.4.4.1 In summary

Based on the meter readings taken during this one storm event, there appear to be definite detectable parameters related to the discharge from the main log storage area and also the more southern outfall. ORP and Turbidity seem most detectable but small changes in pH, DO and temperature with distance may also help to define the plume. This is not surprising as Brunschwiler (2015) found detectable changes in salinity and temperature also with distance from discharge points. The two theses also concur that mixing appears to take place fairly close to the point of discharge. In this case up to approximately 60 m influence of the plume

can be detected at different depths, beyond that variation can be detected in the surface layer only. This of course can also be influenced by rainfall and other freshwater discharges to the harbour.

2.5 Discussion

As set out in the introduction, the principle aims of this chapter were to characterise the stormwater runoff, undertake a preliminary investigation of its toxicity and describe the extent to which it may be detectable upon discharging into the harbour. There appear to be very few studies which specifically look at the impacts of stormwater on the marine environment.

On a large scale, stormwater has been found to impact coastal waters negatively both inshore and offshore (Ahn *et al*., 2005). Using bacteria and viruses linked to terrestrial stormwater plumes, evidence of stormwater was detected, not just in the visibly discoloured immediate vicinity as expected, but also offshore. Again level of detection varied with storm intensities and timing. The quantities detected were also associated with small particles in the stormwater.

Studies on the effects of runoff from log storage on the marine environment are less prevalent. A small number of studies indicate environmental impacts from pulp and paper mill effluent which contain some similar compounds (Ali & Sreekrishnan, 2001; McLeay, 1987; Milestone *et al*., 2012), these are made less relevant to this study by additional chemicals used in pulp and paper production such as bleaches, not found in wood leachate.

Resin acids have been associated with acute toxicity to aquatic species (Peng & Roberts, 2000) but not linked specifically to marine animals. Work has been done on bioaccumulation and sublethal effects and this will be dealt with in more detail in Chapter 3.

The Cawthron Institute has conducted a similar study investigating stormwater quality in the runoff from log and woodchip storage at the Port of Nelson in New Zealand (Forrest & Roberts, 1995). As in this thesis, that study found water quality to be highly variable with storm events. The

effluent was not characterised in that case, but some contaminants such as DO, conductivity, salinity and temperature were measured in situ; and pH, nutrients, SS, COD and colour were evaluated at the lab. Where these matched the parameters in this study, averages were similar.

As with this study, toxicity bioassays were conducted and ecological assemblages defined. Toxicity tests were carried out for algae, the marine diatom *Minutocellus ploymorphus*, bacteria (*Photobacterium phosphorus)* and amphipods *(Chaetocorophium lucasi)*. Of these the algae had an LC_{50} at a concentration of 6.3 and 24.5%, bacteria 23.2 and 26% and the amphipod 23.2 and 27%, to run off directly from log storage (Forrest & Roberts, 1995). The results for the ecological assemblages will be discussed in Chapter 4. One area not addressed in the Port Nelson study is potential for bioaccumulation and sublethal effects and this thesis investigates these impacts in the following chapter.

2.5.1 Log Storage Stormwater Water Quality

There are numerous methods of classifying water quality based on a range of water parameters and many variations on these (Bordalo *et al.* Chalermwat, 2001). For the purposes of this study, where possible, standards for individual parameters have been taken from the Australian and New Zealand Guidelines for fresh and marine water quality (ANZECC, 2000). In a few stated cases, others have been used where no suitable standards were found in ANZECC.

As stated in the results it is of importance to note that many of the minimum detection limits used were higher than the standard ANZECC protection levels for aquatic species (ANZECC, 2000). This highlights the need for accuracy when collecting and submitting samples to ensure correct analyses are used.

2.5.1.1 pH

An average pH of 5.6 is below the ANZECC (2000) lower guideline for freshwater systems but not extraordinary for a stormwater drain, natural rainwater has a pH of 5.5-6.0 and acidic rain can be lower (US EPA, 2015).

At the harbour sampling points along the wharf, pH averaged 8.0 which is lower than the mean pH of 8.3, recorded by University of Waikato staff at two other Tauranga Harbour sites (Waikareao Estuary entrance and Harbour entrance) bi-weekly over a twelve month period (Port, unpub. Thesis). There is no specific ANZECC (2000) recommendation for pH in the marine environment (indeed in an estuarine environment this will vary anyway). Normal variation of pH in sea water of 35 % salinity has been stated as 7.8-8.2 (Knutzen, 1981) which is in accordance with levels recorded along the wharf.

Low pH in the aquatic environment has been known to produce both lethal and sub-lethal effects in fish such as avoidance, disturbed social interactions, increased susceptibility to predation, reduced feeding and migration (Moiseenko & Sharova, 2006). Low pH is also an influencing factor in the toxicity of other contaminants such as aluminium, which can cause severe cardio vascular effects (Moiseenko & Sharova, 2006) and other metals which can bio-accumulate in aquatic organisms (Rand & Petrocelli, 1985a).

In the aquatic environment the toxicity of trace metals is directly linked to water quality criteria such as pH and water hardness (Rand & Petrocelli, 1985b). Low pH and calcium content can cause fish to accumulate Hg, Pb and Cd and other compounds from water even where concentrations are below detection limits for water analysis (Moiseenko & Sharova, 2006). The permeability of cell membranes increases with lower pH and this increases the accumulation of metals (Spry & Wiener, 1991).

The hydrophobicity of trace metals is also influenced by increasing acidity. Hydrophobic or lipophilic compounds are likely to cross membranes directly. It has been shown in octanol-water partitioning of trace metals that most metals partition into octanol with increasing acidity or reached a maximum under conditions close to neutral pH (Turner & Mawji, 2004).

This would be pertinent where low pH water containing a high ratio of dissolved metals was entering a higher or neutral pH environment (Davies *et al.*, 2011) and raises the question whether contaminants are bio-

accumulating in biota near the outfall or flocculating out of the low pH stormwater and precipitating onto sediments.

2.5.1.2 Salinity

Salinity has a significant effect on the toxicity of many metals: cadmium, chromium, copper, mercury, nickel, and zinc were all reported to increase with decreasing salinity. For most organics toxicity was not able to be linked to salinity, however, some insecticides have been found to become increasingly toxic as salinity increased (Hall & Anderson, 1995).

2.5.1.3 Suspended Solids

Suspended solids can affect available light and increase oxygen demand in aquatic environments. A figure used for suspended solids in stormwater discharge consents in the Bay of Plenty region is 150 g/m^3. Suspended solids in the drain samples averaged over 300 g/m^3 and 30 g/m^3 along the wharf again raising the question are the solids simply diluting or dropping out of suspension.

2.5.1.4 COD and $cBOD_5$

COD and $cBOD_5$ help indicate the amount of organic matter present in the sample and the depletion of DO related to these. COD uses a chemical oxidant to quantify the oxygen equivalent of organic matter susceptible to oxidation. Measurement of $cBOD_5$ measures molecular O_2 used in a time period for biochemical degradation of organic material and the O_2 used to oxidise inorganic material in a 5 day period ('Water Quality', 2001). ANZECC (2000) references COD only as a guideline for freshwater aquaculture production, at <40 mg/L. The $cBOD_5$ of 253.33 O_2/m3 probably reflects the high dissolved solids content of the effluent.

2.5.1.5 Organic Compound Content

The results from the organics assays indicate significant amounts of phenolics, fatty acids, and resin acids. Resin acids in the 10 June 2014 sample appear in similar quantities to previous sampling (Tian *et al*., 1997). Due to some of the compounds found, the sample taken on 27 November

2014 appears to represent a more degraded sample in accordance with the findings of Liss, *et al.*, (1997) linking degradation to compounds. This may be due to the sample being taken very early in the flushing cycle of the rain event and after a long dry period or, more unlikely, a possible degradation of the water sample after storage. The difference in the samples highlights the need for characterising test effluents and contextualising them to a wide range of conditions.

2.5.2 Toxicity tests

The results from the two sets of bioassays were highly variable and while it was initially suspected that DO concentrations may have had a strong influence, looking at the results in the context of the effluent characteristics shows a wide difference in them (chemistry results were not available until after the experiments were completed). The effluent from the first set of experiments was much higher in resin acids, phenolics and monoterpenes and, as such, higher toxicity would have been expected as indicated by research from McLeay (1987) and Ali & Sreekrishnan (2001).

The high variation between the two tests and the non-validity of the Mysid test make it difficult to draw any conclusions other than the fact that much more work is required to discern the potential acute toxicity of the effluent. The duration of exposure for organisms in the harbour is likely to be more variable, at lower concentrations and in quickly changing conditions.

While the reasons behind results are complicated and variable, some form of acute toxicity to the *Artemia* in all of the experiments is apparent, more so in the second batch of tests. In the case of the mysid shrimp a higher degree of sensitivity is apparent. This could tie in with questions raised around the use of *Artemia* as a test organism when looking at specific localised effects (Nunes *et al*., 2006). Mysid as a test organism may also not be ideal but demonstrates the need to use more than one test subject for this type of work and where possible a locally relevant species.

It would be highly recommended to do work with other relevant species as the harbour acts as a nursery for many stages of marine life, some of which have previously not been linked to the Tauranga Harbour such as

oceanic bluenose (Brooke, 2015). The experiments here only covered acute toxicity and further behavioural studies would be particularly relevant with migratory species such as whitebait which use the harbour and the Stella Passage as a transitory habitat (Brooke, 2015).

2.5.3 Water quality transects

Results from the water quality transects back up findings from Brunschwiler, (2015) which put the influence of the plume very close to the discharge points and greatest at the surface (0.2 m or above). The plume can be detected out into the middle of the channel by parameters such as temperature and salinity.

Some differences in the readings measured for water quality were picked up through all depths at the closest points but influence below the surface waters is difficult to detect 60m away from the wharf, again matching the more detailed findings of Brunschwiler, (2015). Concentration of the effluent at different distances is difficult to quantify as each storm event has different intensity and duration affecting the amount of runoff and speed of flow (Brunschwiler, 2015). Numbers of logs stored vary and the effects of wind and tide increase or decrease mixing of Harbour water.

2.6 In summary

The raw effluent has characteristics which would be considered toxic by freshwater standards. While it is specifically stormwater and not a freshwater body sustaining aquatic life, it is discharging into a marine environment and complicated chemistry is evident. There are also large amounts of compounds apparent from the GC/MS chromatograms many of which are as yet unidentified or quantified which may have additive, synergistic or antagonistic effects.

Water quality of the effluent samples tested was highly variable, dependent on factors such as the severity of the rainfall event and in which part of the cycle sampling occurred. Two of these factors are likely to increase. The number of log exports has continued to rise (Port of Tauranga, 2015) and a predicted likelihood in the escalation of frequency

and severity of rainfall events due to climate change. This underlines the need for continued temporal monitoring to better understand the intensities of discharges and related effects.

Toxicity testing indicates that there are potential harmful effects from the runoff once combined with salt water at the tested concentrations. These results should be considered preliminary and more lethal and sub-lethal studies are recommended. Based on the water testing, however, concentrations likely in the 'real world' would probably be much lower than those used in these experiments. Mobile animals would likely move away into less affected water if necessary and sessile ones such as mussels are able to close, minimising any negative effects during episodic events (Kramer, *et al*, 1989).

Due to the chemical processes at the interface between the stormwater and Harbour water, there is likelihood that some of the compounds are flocculating or attaching to organic particles and being deposited in the sediments. Those that remain in the water column could be ingested by filter feeding organisms. These are both possible routes for bio-accumulation of some of the contaminants found in the effluent.

Returning to the null hypotheses and aims of this chapter:

> *The raw effluent is not toxic to a test marine species*.
>
> This is rejected as some toxicity is measurable with both samples of the effluent tested.
>
> *No presence will be detectable in the harbour upon dilution.*

This is rejected as changes in water parameters are detectable 60m distance away from the discharge point at various depths and beyond that at the surface.

A variation was detected on a number of parameters including pH, DO, and temperature (within 100 meters) of the discharge point. This resulted in a gradient of increasing pH, DO and temperature with distance from the discharge point. The identified and quantified compounds in the raw

effluent are only detectable in small amounts or non-detectable in the harbour waters.

Appropriate detection limits on chemical analysis of water quality parameters and investigation of environmental fate will help to understand this better.

Chapter 3

Potential Bioaccumulation of Stormwater Derived Contaminants from Port of Tauranga Log Storage

3.1 Introduction

The preceding chapter addressed the chemical characteristics, possible interactions and toxicity of the effluent runoff from the log storage areas at the Port of Tauranga. Secondary to this, but by no means less important, is the environmental fate of such contaminants. Bioaccumulation and environmental degradation of compounds can have significant impact on marine environments (Long, *et al.*, 1995).

Estuarine habitats are known to be potentially extreme ecosystems where natural environmental pressures such as tidal exposure and freshwater input can be exasperated by anthropogenic inputs (Elliott & Quintino, 2007).

The toxicity of these inputs, at varying concentrations, can be assessed by characterisation and whole effluent testing as demonstrated in the previous chapter. This does not address the fate of the chemicals over time as once the runoff enters the marine environment, rapid changes in variables such as salinity and pH can affect the solubility and speciation of contaminants. Changes in these variables can affect the potential for dissolved contaminants to cross membranes, increasing toxicity (Rand & Petrocelli, 1985a). Such variation can also produce conditions where contaminants may come out of solution, causing them to become ingested by filter feeding organisms, or to come out of suspension into sediments (Sholkovitz, 1976).

A further important consideration is the subsequent movement of these compounds once initial equilibrium has been reached. Disturbance of the

sea bed by natural or anthropogenic processes may resuspend sediments changing chemical equilibrium and increasing the bioavailability of contaminants (Atkinson *et al*., 2007). Ingested compounds may be released back into the environment by natural processes, or the organism that has ingested compounds may in turn be predated upon, causing chemicals to move up through trophic levels and processes such as bio-magnification to occur (Kelly *et al*., 2004).

One solution to tracking the fate of these contaminants is to look at levels of key compounds in sediments and biota which relate back to the undiluted stormwater. Aspects of the fate and accumulation of contaminants such as metals has been covered extensively in estuarine and harbour environments, both internationally (Boyle *et al*., 1974; Elderfield & Hepworth, 1975; Bryan & Langston, 1992; Benoit *et al*., 1994; Chapman & Wang, 2001; Richard A. Feely, 2010; McKinley, Miskiewicz *et al*., 2011) and within new Zealand (Park, 2003; Thrush *et al*., 2003; Anderson, 2006; McConway, 2008). Runoff from log storage areas has received little specific attention in the marine environment.

3.1.1 Contaminants from Log Storage Runoff

Little literature exists on the effects of runoff from log storage areas into the marine environment but much has been done around the impacts of pulp and paper mill effluents (Levings, 1980; Owens, 1991; Wilkins *et al.*, 1997; Ali & Sreekrishnan, 2001; Meriläinen, 2007). The compounds common to log storage and pulp and paper mill effluent include metals, tannins and lignins (Bailey *et al*., 1999), suspended solids (Doig *et al*., 2006), pH (Taylor & Carmichael, 2003b) and high chemical oxygen demand (Hedmark & Scholz, 2008). Resin acids have also been a contaminant of concern with toxicity demonstrated to a range of freshwater and marine aquatic species (Oikari *et al*., 1982; Hickey & Roper, 1992; Croce *et al*., 1995; Burggraaf *et al*., 1996; Pacheco & Santos, 1999; Gravato, 2002; Hernandez *et al*., 2008).

Many of these compounds such as metals and inorganic compounds can also be common in urban and industrial storm water (Grant *et al*., 2003).

Resin acids are more specific to effluents from wood storage and industrial wood processing mills (McLeay, 1987; Forrest & Roberts, 1995; Bailey *et al*., 1999; Ali & Sreekrishnan, 2001; Hedmark & Scholz, 2008;) and as such make useful compounds for studies specifically examining potential effects of runoff from log storage and processing.

3.1.2 Resin Acids in Sediments

Previously Tian *et al*. (1995, 1998) had characterised and quantified resin acids in sediments adjacent to log storage storm water discharge points in Tauranga Harbour: a negative gradient in resin acids content was evident with distance from source. Some of the chemicals present have been shown to readily adsorb to sediments in freshwater systems causing negative effects on benthic invertebrates and fish species (Meriläinen, 2007). Resin acids and other contaminants have also been shown to desorb from sediments in a relationship dependent on severity of the disturbance (Meriläinen, 2007).

3.1.3 Port Dredging Program and Bottom Sediment Disturbance

Tian (1993) considered the impact that the port's dredging program may have on resin acids accumulation. Recent dredging events in 2013 and 2014 were considered in relation to the sites sampled (Figure 7). Tian's sampling locations (indicated by the triangles in Figure 23) certainly overlap dredging areas H1, H2 and H3 and would likely be disturbed by dredging activities.

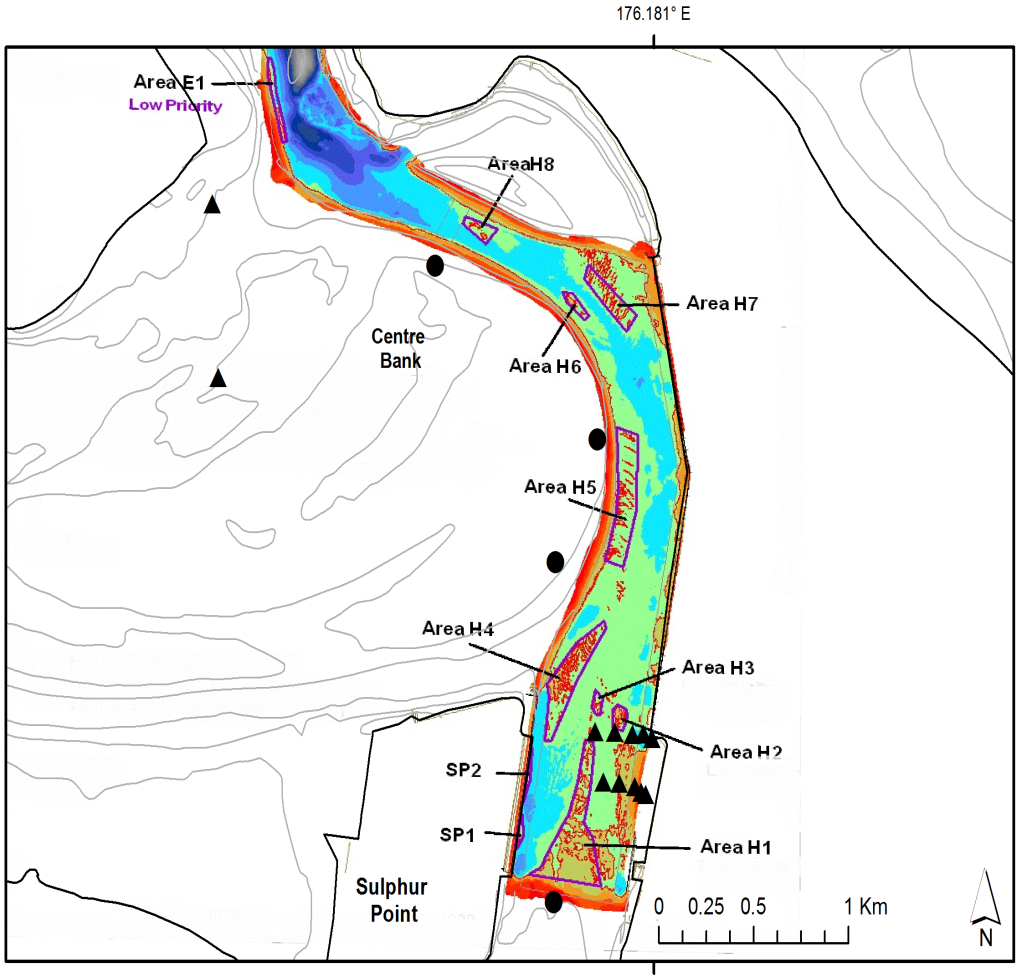


Figure 23. Sediment collection points (triangles and circles) overlaid on areas dredged in 2014, labelled and outlined in purple (layer supplied by Port of Tauranga).

Another cause of disturbance would likely be the activity of ships and tugs: bottom sediments can often be seen to be disturbed by the discolouration of the water during berthing activity (pers. obs.).

3.1.4 The Effects of Resin Acids on Marine Biota

Effects from resin acids have been recorded on a number of marine fish species including synergistic effects with other chemicals, causing disease in Atlantic salmon, *Salmo salar* (Croce *et al*., 1995) and liver biotransformation induction and erythrocytic genotoxic responses in juvenile sea bass, *Dicentrarchus labrax* (Gravato, 2002). The accumulation of resin acids has been recorded in two species, *Paralychtys*

adspersus and Paralychtys microps off the coast of southern Chile (Hernandez *et al*., 2008).

Some limited coverage of the accumulation of resin acids in mussels has been published, including a few studies conducted in New Zealand on the freshwater mussel, *Hydrella menziesi* (Hickey & Roper, 1992; Roper & Martin, 1993; Vascncelos, 1995; Burggraaf *et al*., 1996). In one of the studies, marine mussels, *P. canaliculus,* were deployed as a comparison to *H. menziesi* and concentrations of organic contaminants in both freshwater and marine mussels were found highly correlated (Hickey & Roper, 1992). Resin acids, along with chlorophenolics and mercury were found to accumulate, by analysis of homogenised tissues, in both freshwater and marine mussels over a six month period (Hickey & Roper, 1992).

Hydrella menziesi, were used to evaluate bioaccumulation and depuration rates of resin acids (Burggraaf *et al*., 1996). Bioaccumulation was found to reach equilibrium after approximately seven days at pH 7.3-7.6. Interestingly, depuration was fast (a biological half-life of three days).

Studies assessing the sub-lethal effects of resin acids on mussels detected oxidative damage to gills of Mediterranean mussels, *Mytilus galloprovincialis* (Gravato, Oliveira, & Santos, 2005) and blue mussels *Mytilus edulis* (Fahræus-Van Ree & Payne, 1999).

3.1.5 Use of Mussels for Biomonitoring

Mussels were considered an ideal species for this field study as existing literature on accumulation of anthropogenically produced runoff is widespread (Goldberg *et al*., 1978; Eisler, 2010; Chandurvelan *et al*., 2013). Mussels lend themselves well to studies of this kind and were particularly suited to this project due to the following characteristics.

- Sedentary, filter-feeding organisms that constantly sample the surrounding water.
- Available throughout the year, and are easy to collect and maintain under laboratory conditions.

- Tend to accumulate toxicants including trace metals and organic pollutants in their tissues.

(Adapted from Chandurvelan *et al*., 2013)

Although able to survive in a variety of environmental conditions, mussels are generally limited to an upper depth in the mid intertidal zone, limited by exposure above low tide of less than 40%, and perhaps salinity. A more flexible lower depth of up to 50 m is suggested, primarily limited by light and food availability (Morton, 1986; Jeffs *et al*., 1999). Salinity variation is also tolerable although varies with species. Decreasing salinity is least favourable especially for periods greater than a few days (de Bravo, Chung, & Pérez, 1998; de Bravo, 2003). *Perna canaliculus* has been found to survive at salinity 25 parts per thousand (ppt) and less for short periods (Jeffs *et al*., 1999). Temperature variation can be flexible with *P. canaliculus* tolerating temperatures from 5°C to over 27°C. Temperature was within these limits although lower salinity was recorded (Chapter 2, section 2.4.4 Water Quality Transects).

Mussels were also considered ecologically relevant to this project as they naturally occur in the Tauranga Harbour and are valued as a food source for humans and a variety of marine species. They were easily acquired, deployed and sub sampled.

Water temperature and salinity, total particulate matter, chlorophyll a and particulate carbon have been associated with condition index of *P. canaliculus.* Condition index (CI = (meat weight × 100)/(total weight − shell weight)) is widely used to measure the heath of mussel populations (Hickman, *et al.,* 1991). Length variation is considered a precise method of predicting total weight of *P. canaliculus* (Hickman, 1979) and was a fast, non-invasive method to use during deployment. It was therefore decided to use this method to compare mussel growth for this study.

3.1.6 Aims of this chapter

Resin acids are highly characteristic of the storm water effluent that emanates from log handling areas in Ports (Tian *et al*., 1998)and was backed up by the findings in Chapter 2. The fate of these compounds is related to complicated chemical processes. This chapter investigates the potential for key components of the runoff to bioaccumulate in sediments and biota in the Tauranga Harbour.

Experiments and sampling were conceived to detect resin acids accumulation near to and at increasing distances away from the main outfall from the log storage area. This was achieved by deploying caged mussels at the outfall and at increasing distances away and sampling sediments by similar design. The sediment sampling in this study incorporated a component which repeated aspects of a previous study carried out adjacent to the log storage area in Tauranga Harbour (Tian *et al*., 1998), allowing comparison over time as well as distance from source.

Mussels and sediments were screened for the same compounds as the water in Chapter 2. Sediments were additionally analysed for grain size and metals.

Considering these objectives, the following two null hypotheses are addressed:

1. *Contaminants from the log storage area storm water discharges are not accumulating in nearby sediments in Tauranga Harbour.*
2. *Contaminants from the log storage area storm water discharges are not accumulating in nearby filter feeding organisms*.

3.2 Methods

3.2.1 Sediment Sampling

The sediment sampling was designed to incorporate the sampling locations from a previous study (Tian *et al*., 1998), allowing a comparison over time, and to add some intermediate sites to deduce any wider influence from the log storage area discharge. The sampling locations used by Tian include the two main outfalls which discharge from the log storage areas. The northern one which discharges adjacent to Berth 11 and which was the focus of water sampling in Chapter 2; and the southern outfall which is located approximately 250 meters to the south (Figure 24). Tian also sampled two locations on the inside of Matakana Island as control samples.

The reason for choosing additional sites was to give more insight into potential gradients of contaminants with distance from the wharves. The criteria for choosing the additional sites were to provide the data at an increasing distance from the outfalls in the direction of incoming and outgoing tides and, where possible, out of the main dredged channel areas.

The samples were named N1 to N5 and S1 to S5 to define the northern and southern outfalls, N1 and S1 being close to the shore, numbers increasing in a westerly direction to N5 and S5. The control samples were named C1 and C2, and the additional sites X1 to X6. The N1 X2, X3 and X4 are also in approximately the same areas as the four closest mussel deployment sites described below.

On 25 March 2015, around the period of high tide, sediments were collected for the three analyses: organics, grain size and metals. Twelve sites, marked in Figure 24 by triangles, are those chosen to repeat and compare the results obtained by Tian *et al*., (1998). A further six locations, marked in Figure 24 by circles, are those selected to accompany these. Table 14 gives the coordinates for each sediment grab location.

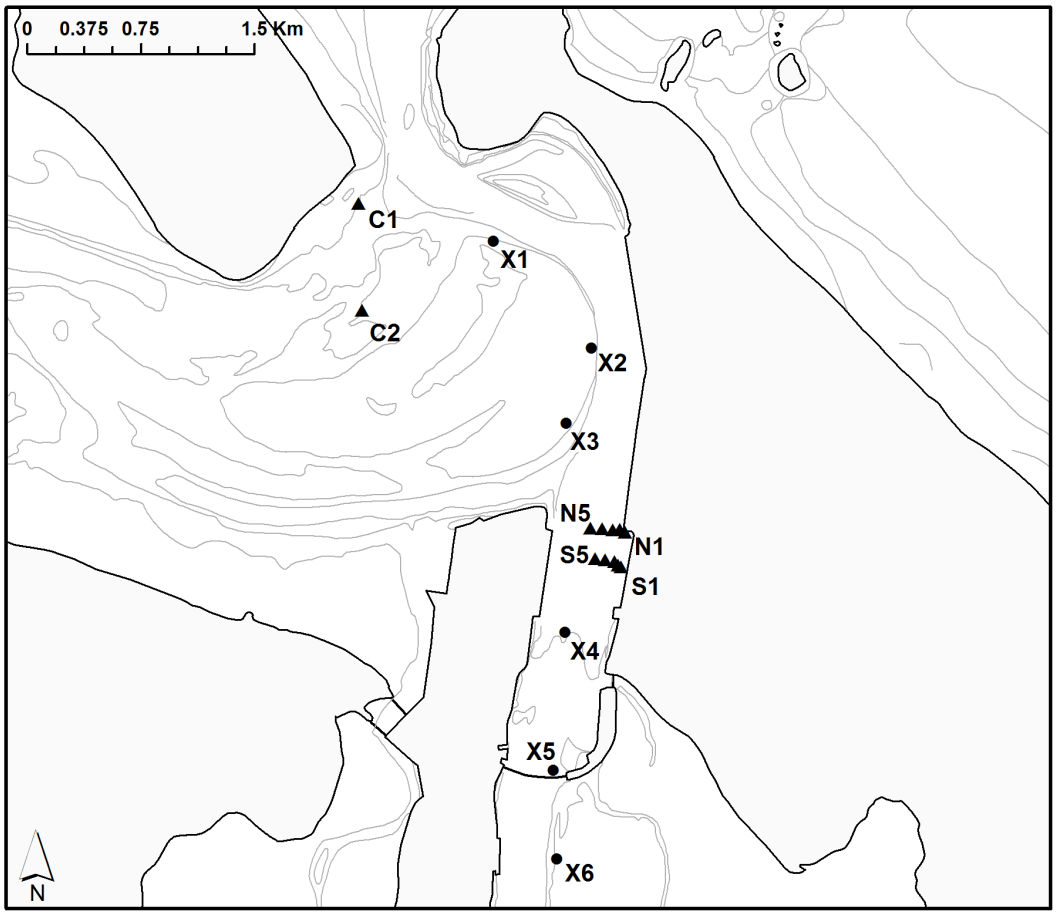


Figure 24. Sediment grab positions – approximate positions sampled by Tian et al 1998 indicated by triangles, additional grabs by circles. Note - N1 to 5 and S1 to 5 increase in a westerly direction from source to mid channel.

Table 14. Sediment collection positions (WGS84, DD) and times sampled.

Sample	Date	Time	Latitude	Longitude
C1	25/03/2015	10:56:49 a.m.	-37.63931	176.16031
C2	25/03/2015	10:56:49 a.m.	-37.64596	176.16060
X1	25/03/2015	11:13:15 a.m.	-37.64168	176.17091
X2	25/03/2015	11:18:44 a.m.	-37.64833	176.17862
X3	25/03/2015	11:23:27 a.m.	-37.65304	176.17662
X4	25/03/2015	11:28:17 a.m.	-37.66607	176.17653
X5	25/03/2015	11:38:21 a.m.	-37.67466	176.17563
X6	25/03/2015	11:42:58 a.m.	-37.68019	176.17588
N1	25/03/2015	12:18:19 p.m.	-37.65980	176.18121
N2	25/03/2015	12:20:19 p.m.	-37.65962	176.18081
N3	25/03/2015	12:25:38 p.m.	-37.65966	176.18028
N4	25/03/2015	12:29:12 p.m.	-37.65957	176.17944
N5	25/03/2015	12:34:53 p.m.	-37.65954	176.17851
S1	25/03/2015	12:38:43 p.m.	-37.66195	176.18090
S2	25/03/2015	12:42:21 p.m.	-37.66185	176.18069
S3	25/03/2015	12:43:36 p.m.	-37.66164	176.18038
S4	25/03/2015	12:47:11 p.m.	-37.66152	176.17965
S5	25/03/2015	12:49:57 p.m.	-37.66146	176.17890

A Ponar type grab (Figure 25) was used from a six meter aluminium pontoon boat to collect a minimum of 3 replicates from each sampling location. These were placed in pre-labelled Ziploc bags and stored in a large cooler on salt ice. These were then frozen to -20ºC within 2 hours of collection. At sites C1, C2, X1, X5 and X6 difficulty was experienced using the Ponar grab as shell debris caught in the jaws and allowed sediment to escape. At these sites a diver using SCUBA collected the sediments directly into 50 ml pots.

Figure 25. Ponar grab used for sediment sampling.

3.2.2 Sediments Grain size Analysis

Grain size analyses were undertaken as, although variation is likely to be related to environmental factors such as current and depth (Thrush *et al*., 2003a) and not distance from the discharge points of industrial runoff, relationships between grain size and chemical composition, especially contaminating compounds from discharges, can be useful to ecological effects investigations (Horowitz, 1985).

To analyse grain size the triplicate sediment samples were homogenised and oven dried at 80°C. Using an Endecott's EFL2000 sieve shaker (Figure 26) 100 g of each sample was separated into >2.0 mm, 1.999-1 mm, 0.9999 mm-500 µm, 499-250 µm, 249-125 µm, 124-63 µm and <63 µm.

After prioritising collected sediment for chemical analyses there was insufficient remaining to complete grain size analysis on the N2 sample.

Figure 26. Endecott's EFL2000 sieve shaker used for grain size analysis.

3.2.3 Mussel Deployment

The deployment of mussels was designed to allow spatial and temporal sampling and subsequent analysis. Locations were chosen close to and increasingly far from the northern discharge outfall of the log storage area which had been previously sampled for water. It was decided 200 mussels would be deployed at each of six locations, allowing a sub-sample of at least 20 to be collected on a number of occasions.

Two of the locations were chosen close to the outfall, one directly in front of the pipe itself and one slightly to the south. This was because the outfall is located under the wharf and receives little light, especially when shipping is berthed alongside and there was concern that this may affect the resilience of the mussels in some way. The second location was at the end of the log terminus and not obscured, but close enough to still receive significant effects from the discharge. Other locations were selected based on the availability of channel markers to attach the mussels.

Approximately 2000 Greenshell™ mussels were obtained from the Coromandel Peninsula (NIMPL personal Communication, May 14th 2014)) via North Island Mussel Processing Ltd. at Greerton, Tauranga (NIMPL) on 28 May 2014: these were sorted at the field station and broken or open mussels were removed. They were also sorted by eye into large and medium size groups to allow an even range of sizes to be deployed at each site.

Forty mussels were attached to a one meter long, 10 mm nylon rope in two bunches of 20 on top and 20 at the bottom (Figure 27, pic. 2). Approximately 10 large and 10 medium were included per group of 20. Mussel sock, supplied by Mussock International Limited, Christchurch, was used to ensure the mussels would remain in place while attaching to the rope (Figure 27). This method is commonly used to attach spat to lines in the commercial mussel industry. Five of these ropes were then attached to the inside of a folding crayfish pot for protection from predation (Figure 28). This enabled the total of 200 mussels to be deployed at each of the six locations.

Prior to collecting the mussels for the main experiment a pilot was conducted using locally sourced wild mussels in a marine aquarium. The biodegradable sock began to break down after seven days by which time the mussels had securely attached to the lines.

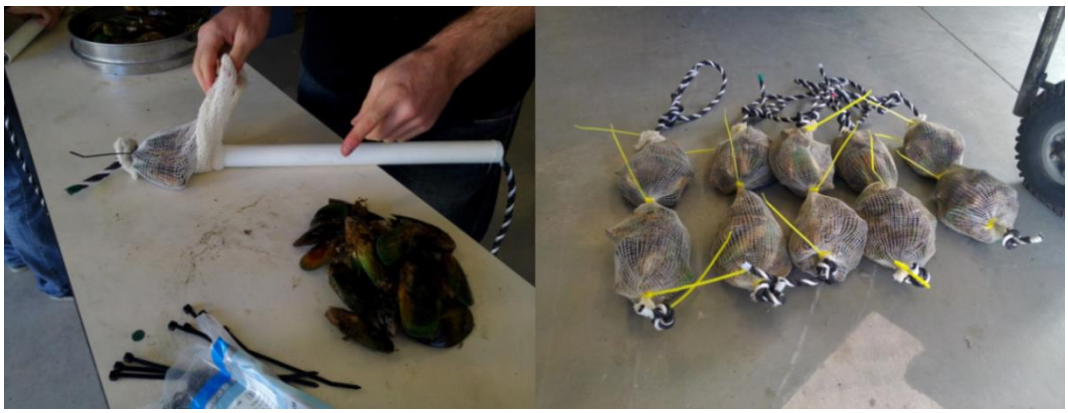


Figure 27. Mussels being attached to rope and example of completed site total

Figure 28. Folding crayfish pot for additional protection.

The bagged mussels were deployed on 15 May 2014 at the six locations shown below (Table 15, Figure 29). Number 21, Number 19 and Number 13 are channel markers along the shipping main channel, the cardinal marker near the harbour entrance was chosen as a control location. The two locations 'Under Wharf' and Outside Wharf' are located at Berth 11. The folding crayfish pot was attached to a rope between two log piles of the channel markers at a height just below the low tide mark and at the same height as wild mussels where present.

At the time of deployment 25 randomly selected mussels from each site were also measured length wise for later comparison.

Table 15. Deployment points (WGS84, DD) 15-05-14.

Location	Latitude	Longitude
Cardinal	-37.63779	176.16987
No. 19 (Leaner)	-37.65676	176.17637
No. 21 mark	-37.66605	176.17644
No. 13 mark	-37.64907	176.17931
Outside wharf	-37.65978	176.18137
Under wharf	-37.65923	176.18153

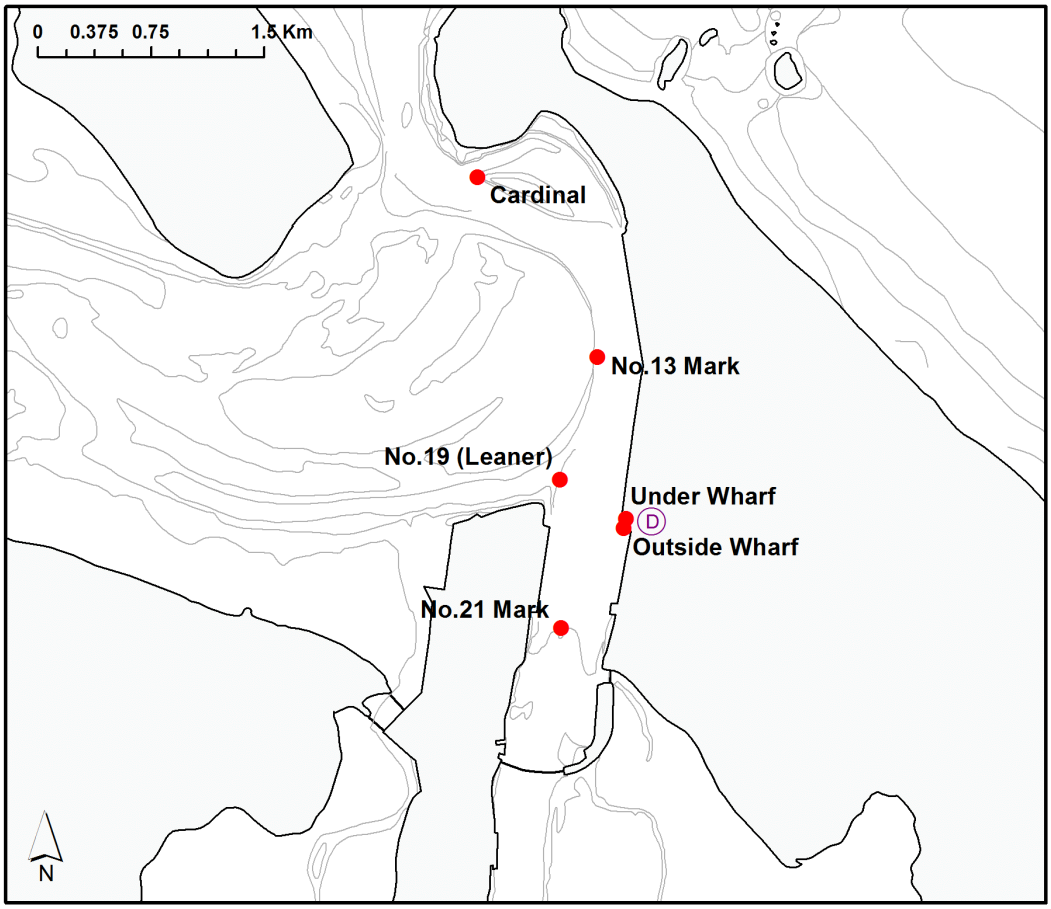


Figure 29. Deployment locations for mussel field deployment.

The morning after a rainfall event on 12 June 2014, 10 mussels were collected from each site and frozen to -20ºC to preserve the samples for later analysis. Note that no sample was collected from the Cardinal site as all gear (including mussels) was found to have been removed by persons unknown. Luckily a large sample of the original mussels had been frozen initially as a control sample. Further sampling was completed on the 18 December 2014 from each of the five remaining sites (No. 13, No. 19, No. 21, outside, and under the wharf) (Table 16).

A final sampling was conducted on 17 March 2015, at this time only mussels at the two wharf locations (‘under’ and ‘outside’) remained. To allow comparison with other sites, wild mussels were collected from channel marker Number 13, the wharf at the end of Sulphur Point near Number 19 and from channel marker Number 21

Table 16. Summary of sampling locations and dates sampled.

Sample	location	Date collected	Code	Event
1	Controls	28/05/2014	Cont	NA
2	Outside wharf	12/06/2014	OW	A
3	Under wharf	12/06/2014	UW	A
4	Leaner 19	12/06/2014	L19	A
5	No13	12/06/2014	N13	A
6	No21	12/06/2014	N21	A
7	Outside wharf	18/12/2014	OW	B
8	Under wharf	18/12/2014	UW	B
9	Leaner 19	18/12/2014	L19	B
10	No13	18/12/2014	N13	B
11	No21	18/12/2014	N21	B
12	Outside wharf	17/03/2015	OW	C
13	Under wharf	17/03/2015	UW	C
14	Leaner 19	17/03/2015	L19	C
15	No13	17/03/2015	N13	C
16	No21	17/03/2015	N21	C

3.2.4 Chemical Analyses of Sediments and Mussels

In the first instance, mussels were shucked while still frozen and five from each sampling location and event were transferred to 50 ml pots. Sub samples of sediments were also transferred to 50 mL pots. In the case of GC/MS analysis these were homogenised first.

All samples intended for organics analyses were again stored at -20ºC before and after they were freeze dried for five days in a Labconco Freezone6 freeze drier.

3.2.4.1 GC/MS Analyses for Resin and Fatty Acids

Mussels and sediments were taken to Scion in Rotorua, for organics extraction and analysis. Mussel extraction, subsequent analysis and identification and quantification of compounds was undertaken by Scion staff using an in house method:

‘Analysis of fish tissues for total resin acids, neutrals and sterols (including muscle extension’ (Robinson, 1997).

Each sample was first hydrolysed with methanolic potassium hydroxide. Neutrals/sterols were extracted from the hydrolysate with hexane/ether. The sample was then acidified and extracted with methyl tert-butyl ether. Fatty acids were removed from the resin acids by selective methylation and partitioning on a short florisil column. Resin acids and neutrals/sterols factions were then combined, derivatised and analysed using GC/MS.

The sediments chemistry was undertaken by the author under the guidance of Scion staff. The full method including lab notes is detailed in Appendix E.

3.2.4.2 ICPMS Analysis for Inorganics

Sub samples of sediments were prepared by transferring to 50 ml pots. These were transported to the University of Waikato laboratories in Hamilton. Samples were prepared by U.S. EPA method 3050b 'Acid Digestion Of Sediments, Sludges, And Soils 1.0 Scope And Application' (U.S. E.P.A, 1996) and analysed by ICPMS.

3.3 Statistics

Descriptive statistics were run and 'sparkline's' used in excel to identify any trends of the grain size. Compound or sieve size groups were then graphed. Chemical data was added to Primer e (Gorley & Clarke, 2015) where data were normalised, and a distance matrix created using Euclidian distances. For each analysis and collectively cluster analysis and PCO were used to explore any groupings and PERMANOVA (Anderson *et al*., 2008) to investigate statistical significance.

3.4 Results

3.4.1 Sediments Analysis Results Organics by GC/MS

The method used for analysis of the sediments considered the same groups and individual compounds as targeted for the water sampling by GC/MS and referred to in detail in Chapter 2 (2.1.4.3). These are all related to residue from wood and include monoterpenes, phenolics, fatty acids, resin acid neutrals, resin acids and phytosterols. As per the method

for organics in water, a total of 68 compounds were quantified as per the extraction methods (Appendix A). Many more were present but time needed to identify and quantify them did not allow their inclusion in this work.

3.4.1.1 Total Extractives by Sample

The total organic compounds (Figure 30) were greater in the northern (N1 to N5) and southern (S1 to S5) sampling locations adjacent to the outfalls. At the southern outfall site total organic compounds decline as sampling locations move away from the wharf (S1 to S5). This is not the case at the northern outfall as total organic compounds increase again at N4 and 5.

The next highest level of organic compounds were found as sampling location X2, then X3 and X6. X4, X5 and C2 had a similar quantity, X1 and C1 had the lowest.

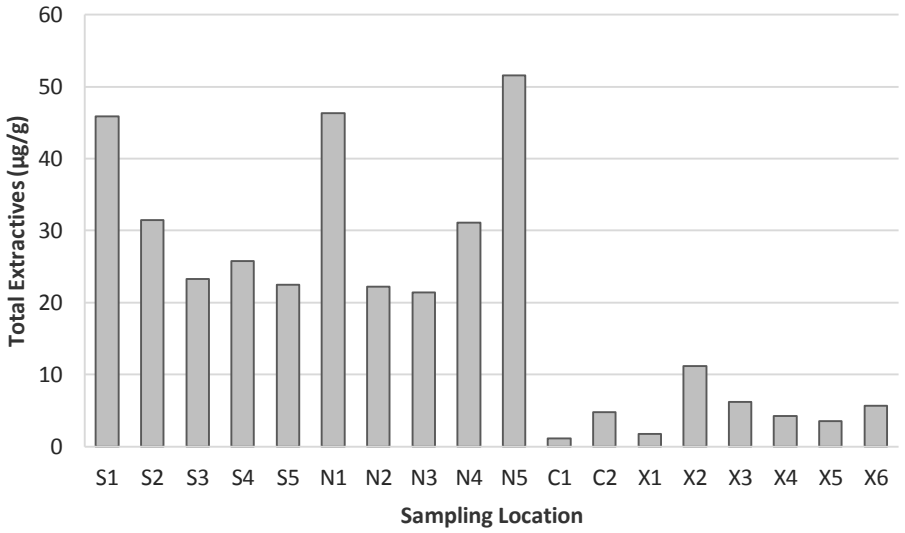


Figure 30. Total organic compounds for each sampling location (µg/g). S1-5 = southern transect, N1-5 = northern transect, C1&2 = control sites, X1-6 = additional individual sites.

No samples had any detectable measurements of monoterpenes. Phenolics were only detected in sites S1 and S2, N1 and N3. Fatty acids were present in all samples (Figure 31) and all of the fatty acids, apart

from linoleic acid, were detected at most sites. These were again found in larger quantities at the north and south transects with a similar gradient from the wharf and substantially more detected at N5. Fatty acids were the compound group found in the highest quantities overall.

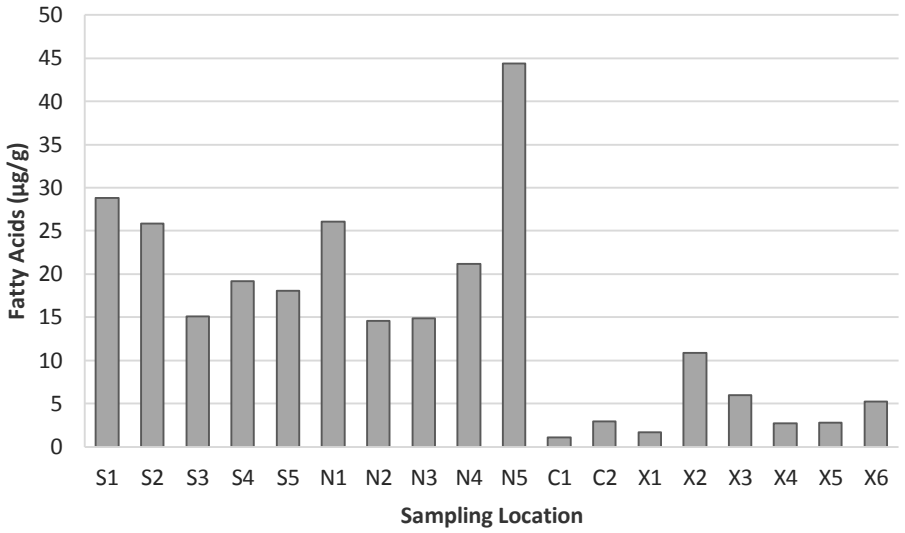


Figure 31. Fatty acids detected at each sampling location (µg/g). S1-5 = southern transect, N1-5 = northern transect, C1&2 = control sites, X1-6 = additional individual sites.

No samples had any detectable measurements of resin acid neutrals. Of the resin acids (Figure 32) only abietic acid was found at all sites. The greatest amounts of total resin acids were again found at site N5. The southern site had a noticeable gradient with distance from the wharves. Very low levels of resin acids were detected at the control and 'X' sites.

Not all resin acids screened for were detected (Table 17), indeed only abietic acid was detected at all sites, including the controls C1 and C2

Table 17. Detected resin acids (ave. per sample) µg/g. Pim = Pimaric acid, Sand = Sandaracopimaric acid, Iso = Isopimaric acid, DHAA = Dehydroabietic acid, Ab = Abietic acid. <0.01= below detection limit.

Resin Acids	Pim	Sand	Iso	DHAA	Ab
S1	0.018423	0.006889	0.092895	0.463799	0.227825
S2	<0.01.	<0.01.	<0.01.	0.275747	0.196558
S3	<0.01.	<0.01.	<0.01.	0.184026	0.202508
S4	<0.01.	<0.01.	<0.01.	0.098981	0.140187
S5	<0.01.	<0.01.	<0.01.	<0.01.	0.093468
N1	<0.01.	<0.01.	<0.01.	<0.01.	0.144069
N2	<0.01.	<0.01.	<0.01.	<0.01.	0.083492
N3	<0.01.	<0.01.	<0.01.	<0.01.	0.048331
N4	0.003839	0.018619	<0.01.	<0.01.	0.086446
N5	<0.01.	<0.01.	<0.01.	1.07612	0.186272
C1	<0.01.	<0.01.	<0.01.	<0.01.	0.004171
C2	<0.01.	<0.01.	<0.01.	<0.01.	0.003666
X1	<0.01.	<0.01.	<0.01.	<0.01.	0.004149
X2	<0.01.	<0.01.	<0.01.	<0.01.	0.005137
X3	<0.01.	<0.01.	<0.01.	<0.01.	0.00293
X4	<0.01.	<0.01.	<0.01.	0.014846	0.036533
X5	<0.01.	<0.01.	<0.01.	<0.01.	0.007403
X6	<0.01.	<0.01.	<0.01.	<0.01.	0.005825

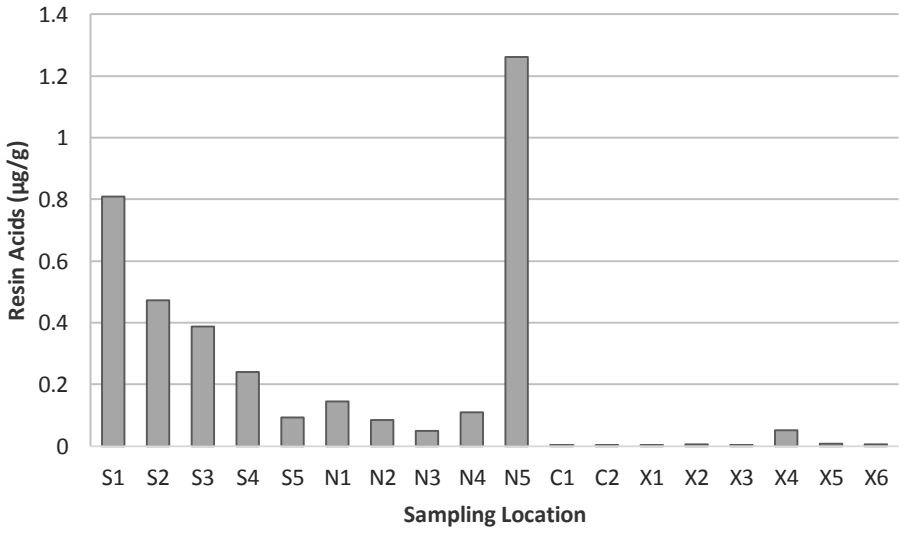


Figure 32. Total resin acids at each sampling location (µg/g). S1-5 = southern transect, N1-5 = northern transect, C1&2 = control sites, X1-6 = additional individual sites.

Phytosterols were found at all sites apart from C1 (Figure 33). The greatest amounts were again at the southern and northern sites with a

partial decreasing gradient as sampling moved away from the discharge points.

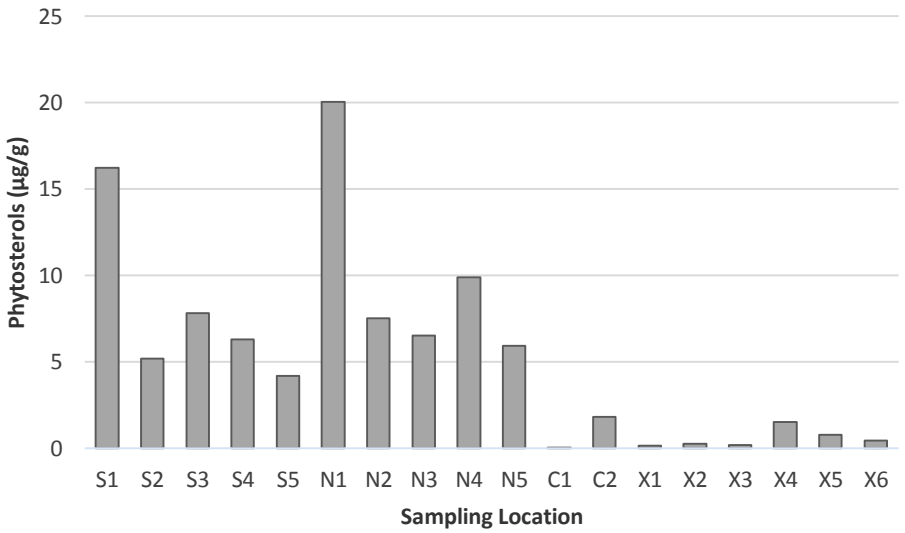


Figure 33. Phytosterols at each sampling location (µg/g). S1-5 = southern transect, N1-5 = northern transect, C1&2 = control sites, X1-6 = additional individual sites.

To investigate differences between the locations further, data were entered into Primer e (Gorley & Clarke, 2015). Here it was normalised and a resemblance matrix using Euclidean distances formed. Cluster analysis of these data (Figure 34) revealed samples N1 and S2 diverge at the first node forming their own cluster, distinct from other samples. Other N and S samples branch at the second, third and fourth nodes. The X and C samples, apart from X2 cluster together in the centre of the dendrogram.

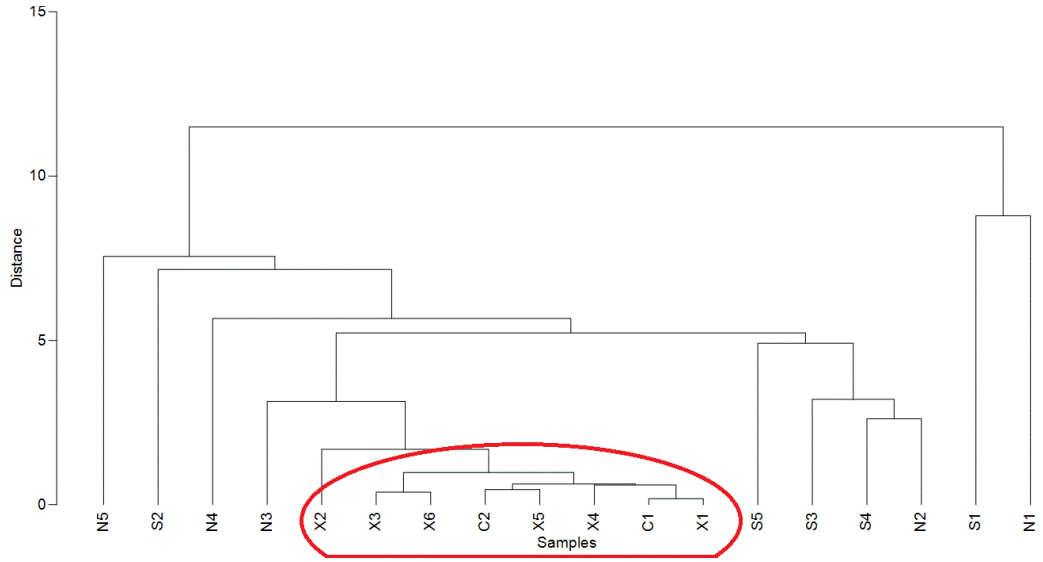


Figure 34. Cluster dendrogram of all samples. Normalised data, distance = resemblance: euclidian distance. S1-5 = southern transect, N1-5 = northern transect, C1&2 = control sites, X1-6 = additional individual sites.

PCO analysis of the same matrix (Figure 35) further explores the relationships between the sites graphically with PC1 and PC2 explaining 70.78% of the total variation. The overlay of chemicals is difficult to read on the graph but the main influences on the variation between sampling locations were the resin acids dehydroabietic acid and abietic acid and the fatty acids dodecanoic acid, tetradecanoic acid, palmitoleic acid, palmitic acid, margaric acid, linoleic acid, and oleic acid. The phytosterols had some influence across PCO1. Sample S1 was strongly influenced by the fatty acids - eicosanoic acid, docosanoic acid tetracosanoic acid - most likely related to larger amounts of these than in other samples.

PERMANOVA between sampling locations showed significant differences (P(perm) 0.031).

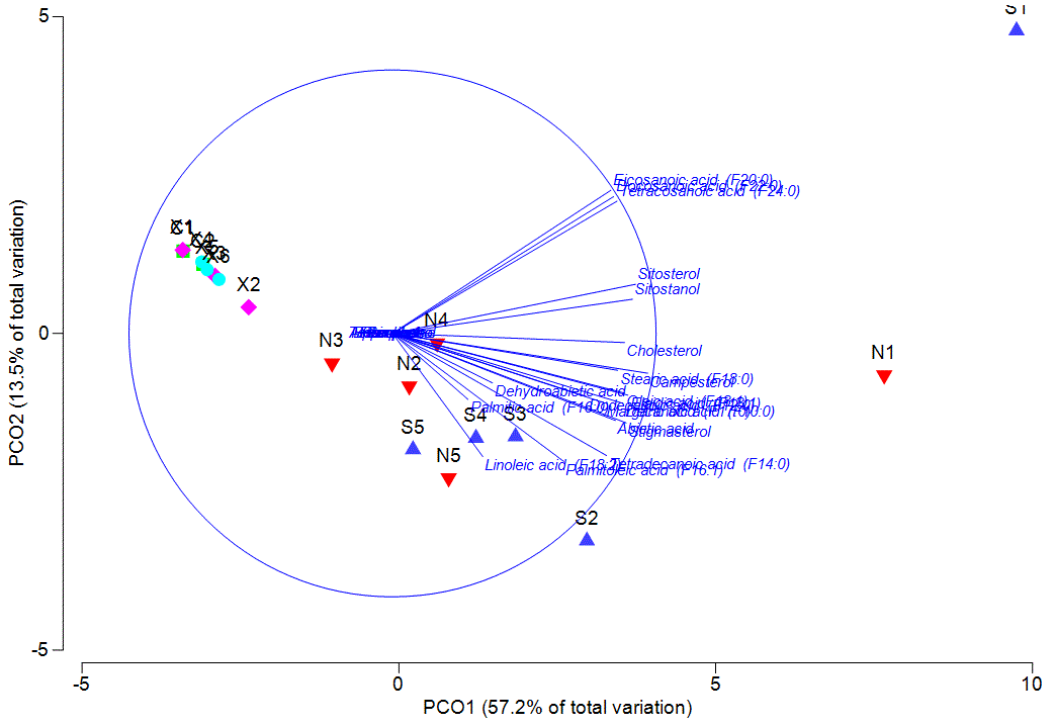


Figure 35. CO graph of sample locations coloured by location group. S = blue, N= red, XN = pink, XS = turquoise, green = control.

3.4.1.2 In summary

Gradients are evident for detected compounds. With a decrease in detectable amounts with increasing distance away from the wharf and further, less obvious gradients on a larger spatial scale. Some of the compounds, such as resin acids which were detected in previous studies, such as Tian (1995), were screened here and detected in lower quantities or not detected. This may be related to extraction method as well as environmental reasons such as differences in rainfall event intensity.

Multivariate analyses indicate that the sampling locations S1 and N1 are strongly influenced positively by the organics (Appendix A) screened.

3.4.2 Sediment Grain Size Analysis Results

A summary of sediment grain size for each sampling location is included in Table 18. The samples S1 to S5 show a similar make up with the most weight recorded in the 125-249.9 micron group. This was also true for the N1 to N5 samples, N2 was not analysed. The northern and southern sites have a higher proportion of the 125-63 micron grain size and are the only sites that contain over 2 g of the <63 micron size. The X1 to X3 sites indicate a gradient of larger grain size as samples become more distant from the point of discharge. X4 and X6 again had the most weight in the 125-249.9 micron group with X5 more in the 250-499.9 micron group. The C sites both had the most weight recorded in over 500 microns, C1 having much more of the larger grain sizes 2.0mm, 2-1mm, 0.5 - .99 mm.

Table 18. Sediments grain size results in grams with spread of size classes (mm = millimetres).

Dish ID	Total	>2mm	1.9-1.0	0.9 -0.5	0.49-0.25	0.249-.125	.1249-.063	<.063	Final	Spread
S1	100.02	2.53	3.82	10.04	23.41	31.90	18.83	8.96	99.48	
S2	100.96	0.15	0.29	1.28	9.08	38.16	31.08	20.03	100.07	
S3	100.01	0.27	0.63	3.36	13.04	45.63	24.90	12.16	99.99	
S4	87.30	0.41	1.04	3.34	11.08	39.21	21.35	10.69	87.12	
S5	100.38	1.88	0.57	3.40	17.85	49.59	20.68	6.02	99.98	
N1	100.12	1.16	1.72	6.59	17.13	42.13	20.40	10.38	99.51	
N2									0.00	
N3	100.11	0.80	0.64	3.32	14.05	43.11	25.23	12.72	99.86	
N4	100.09	0.85	1.53	7.81	24.25	41.21	17.38	7.04	100.08	
N5	100.70	6.79	1.24	7.76	26.62	41.71	12.55	3.30	99.97	
X1	100.28	20.55	30.66	44.70	3.21	0.62	0.14	0.04	99.90	
X2	100.18	0.68	1.08	9.33	51.19	35.82	1.59	0.13	99.83	
X3	100.02	0.80	0.41	1.24	12.25	67.23	17.28	0.80	100.00	
X4	100.07	1.16	1.74	2.82	17.56	62.71	11.80	1.76	99.54	
X5	100.07	12.96	7.29	14.67	44.27	19.90	0.07	0.31	99.48	
X6	100.16	10.32	1.56	2.03	13.83	67.67	3.38	0.14	98.92	
C1	100.06	13.79	11.20	24.08	35.28	15.06	0.59	0.03	100.04	
C2	100.02	2.86	0.92	8.11	73.28	14.69	0.18	0.01	100.05	

A stacked bar graph shows the proportions of each grain size by percentage at each sampling location (Figure 36). At the northern and southern outfalls sediments of the 499 to 250 microns bin increase steadily with distance from the wharf while the smallest < 63 bin decreases. The control and X sites have very little of the smallest < 63 bin.

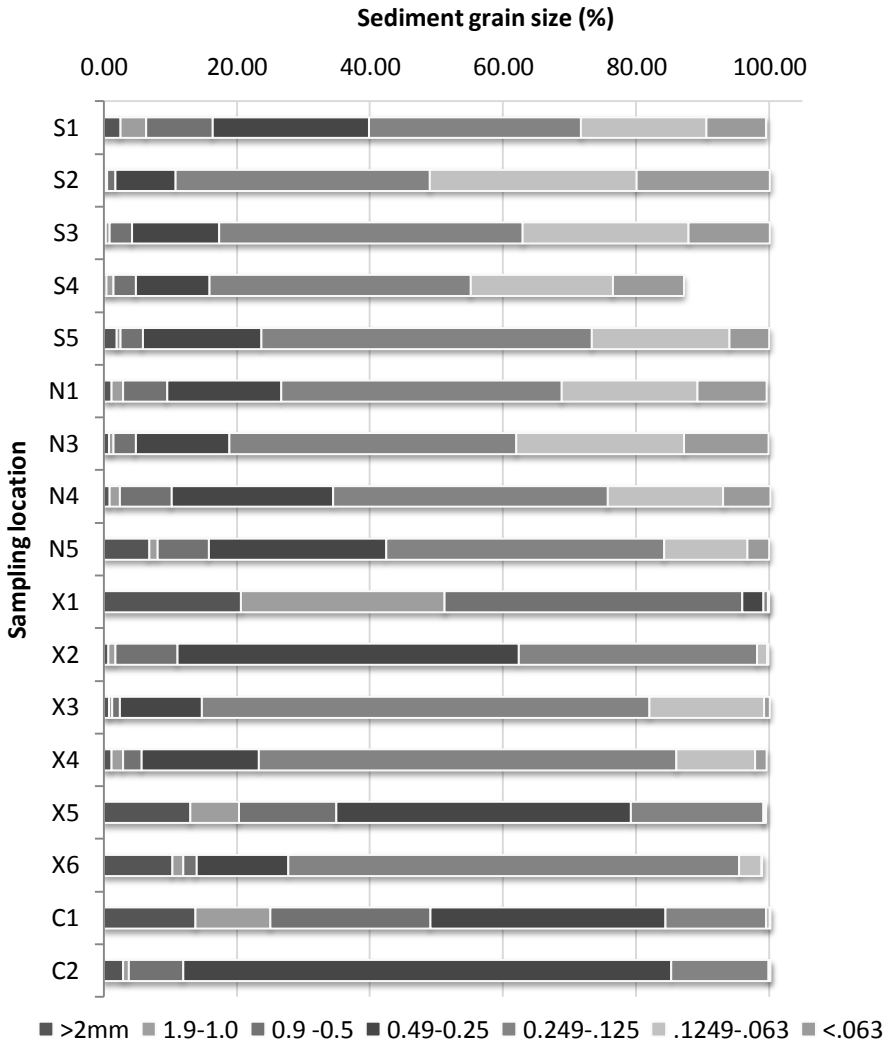


Figure 36. Stacked bar chart of sediment grain size x axis = % of each size faction, y axis = sampling location.

3.4.3 Metals Analysis Results

As a comparison with quantities of compounds sampled by Hill Laboratories in the water sampling results (Chapter 2) a number of compounds were quantified in sediments (Appendix F). Of these boron (B), aluminium (Al), chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) were evaluated (Table 19) to investigate any relationships with quantities in the water samples and sediments.

Table 19. Selected compounds in sediments for comparison with water sampling (µg/g dry weight). Each compound colour coded by quantity, red = highest to green = lowest. Final column = relative amounts of each compound by sampling location. Arrows = direction from wharf for s = southern transect, n = northern transect, x = wider more distant sites, c = control sites.

	S1	S2	S3	S4	S5	N1	N2	N3	N4	N5	X1	X2	X3	X4	X5	X6	C1	C2	Trend by Sampling Location
B	25.25	20.67	24.72	16.96	11.90	16.62	8.70	18.32	13.77	11.06	4.51	6.82	7.51	7.87	8.59	6.89	4.16	4.41	
Al	8283.44	8785.70	8599.43	7473.46	5615.72	6457.07	3655.22	9308.69	5687.26	5047.31	1305.66	2295.38	2604.59	2718.85	3100.40	2532.43	1137.01	1332.16	
Cr	5.77	6.00	5.78	6.48	4.34	4.54	2.15	4.86	4.25	3.94	1.80	3.34	3.73	2.72	3.70	3.10	1.96	2.01	
Mn	54.77	60.87	51.32	49.92	46.61	38.93	277.17	38.89	49.69	50.97	66.00	20.09	17.74	111.85	46.17	21.98	33.27	38.74	
Co	1.14	1.21	1.08	1.02	0.88	0.96	2.75	0.95	0.92	0.90	0.59	0.39	0.47	1.07	0.76	0.46	0.33	0.41	
Ni	2.28	2.54	2.32	2.06	1.55	1.79	0.86	1.82	1.73	1.65	1.84	1.02	1.02	1.21	1.51	0.78	0.76	1.11	
Cu	5.06	4.60	5.06	3.65	2.25	3.73	2.42	4.06	2.90	2.26	0.31	0.45	0.63	0.70	2.18	0.72	0.23	0.32	
Zn	34.85	27.18	26.57	21.87	17.02	21.50	17.50	24.34	18.05	27.99	5.84	8.62	8.66	18.59	17.80	11.00	5.32	5.06	
As	5.98	6.03	5.21	5.69	4.95	4.40	3.07	3.89	4.99	4.93	10.47	7.42	4.44	7.14	5.81	5.06	5.26	5.73	
Cd	0.10	0.07	0.09	0.06	0.04	0.08	0.03	0.06	0.05	0.04	0.01	0.01	0.02	0.01	0.03	0.01	0.01	0.01	
Hg	0.04	0.06	0.05	0.04	0.03	0.03	0.03	0.04	0.02	0.02	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.01	
Pb	6.09	6.04	5.98	4.89	3.92	4.50	3.89	6.61	4.05	6.11	1.04	1.56	1.75	2.67	2.90	2.07	1.02	1.14	
Total	8424.76	8920.97	8727.62	7586.11	5709.21	6554.16	3973.78	9412.54	5787.68	5157.19	1398.06	2345.09	2650.56	2872.70	3189.86	2584.51	1189.34	1391.10	

A trend in decreasing amounts for most compounds, with distance from the wharf is observable at the southern transect (Figures 41, 42, 43). Amounts are variable with distance from the wharf at the northern transect. A trend is also apparent for the 'X' sampling locations where compound levels increase from X1 to X4 at the closest point to the outfalls, then decrease at X5 and X6 as distance increases again to the south of the log storage areas (Figures 37, 38, 39).

Copper (Figure 37), lead (Figure 38) and zinc (Figure 39) were found at 6.1 µg/g , 13 µg/g and 46 µg/g respectively, at the most impacted site (Te Puna) in the broad scale survey of Tauranga Harbour (Ellis *et al*., 2013). Maximum values in this study were 5.06 µg/g for copper at S1 and S3, 6.61 µg/g for lead at N2 and 34.85 µg/g for zinc at S1. These are below interim sediment quality guidelines, low trigger values of 65 µg/g, 50 µg/g and 200 µg/g respectively (ANZECC, 2000).

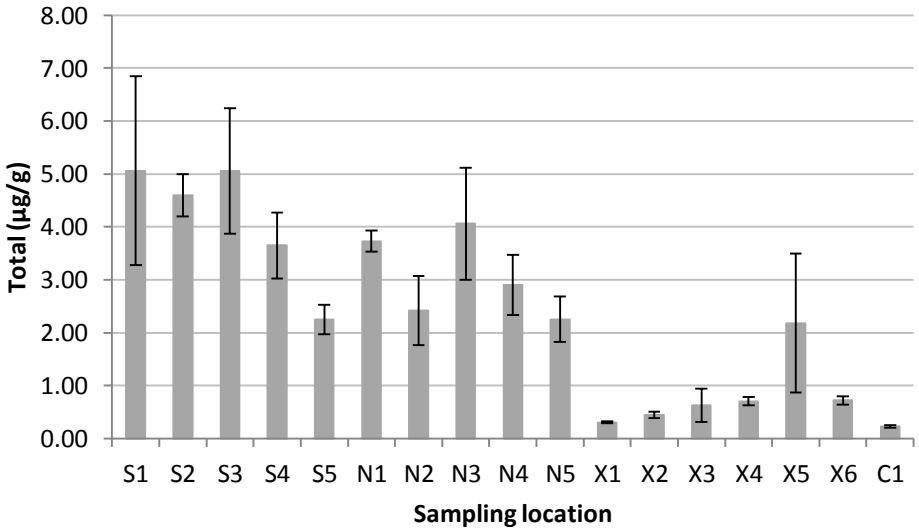


Figure 37. Total copper at each sampling location (µg/g). Error bars = standard deviation.

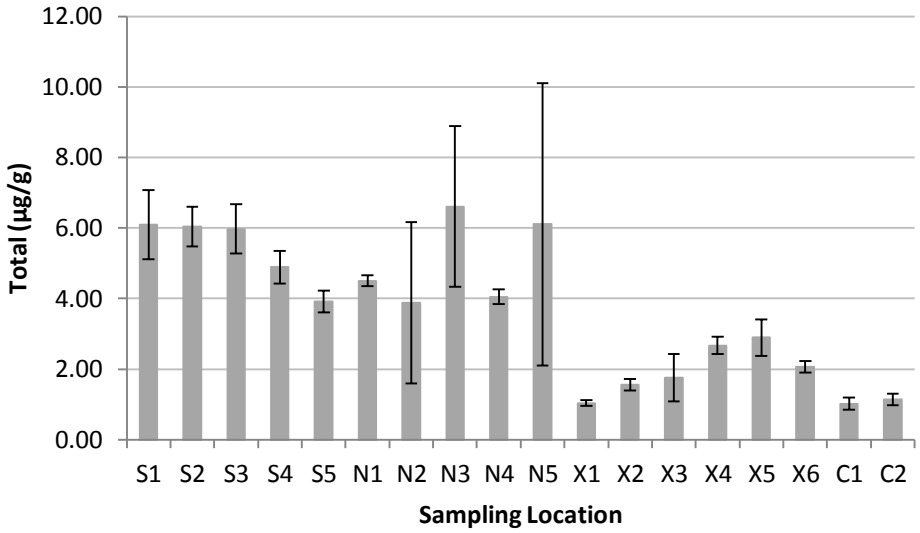


Figure 38. Total lead at each sampling location (µg/g). Error bars = standard deviation.

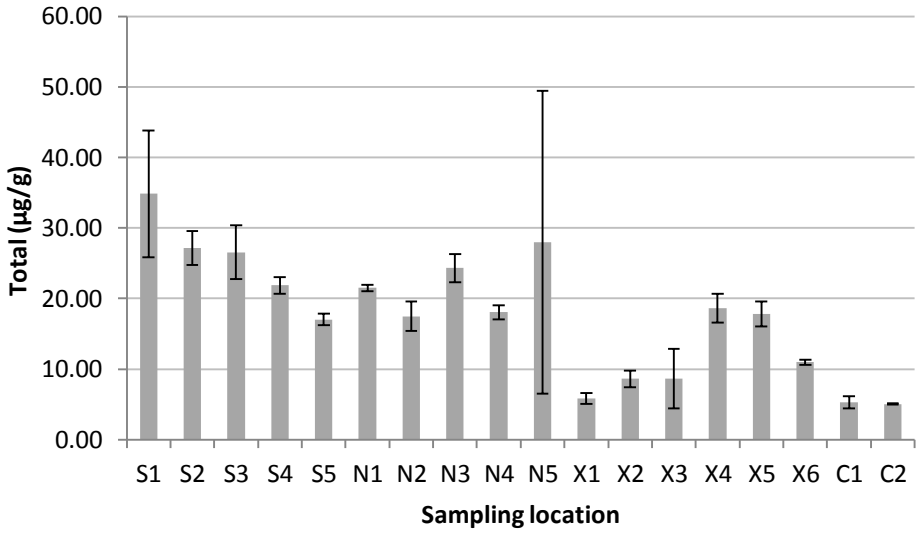


Figure 39. Total zinc at each sampling location (µg/g). Error bars = standard deviation.

3.4.4 Relationships between Grain Size, Organics and Metals

Relationships were explored between fatty acids, resin acids, phytosterols, the smallest grain size grouping and total metals. Data were log transformed, graphed (Figure 40) and the correlation function performed in excel (Table 20).

All variables were well correlated with lowest R_2 value .806 for grain size and phytosterols and highest value .964 for grain size and total metals. This is unsurprising as metal content and lower grain size are known to be well correlated (Krumgalz *et al*., 1992; Chapman & Wang, 2001; Thrush *et al*., 2003).

Figure 40. Graph of relationships between the variables fatty acids, resin acids, phytosterols, grain size <63µ and total metals by sampling location. Data log transformed.

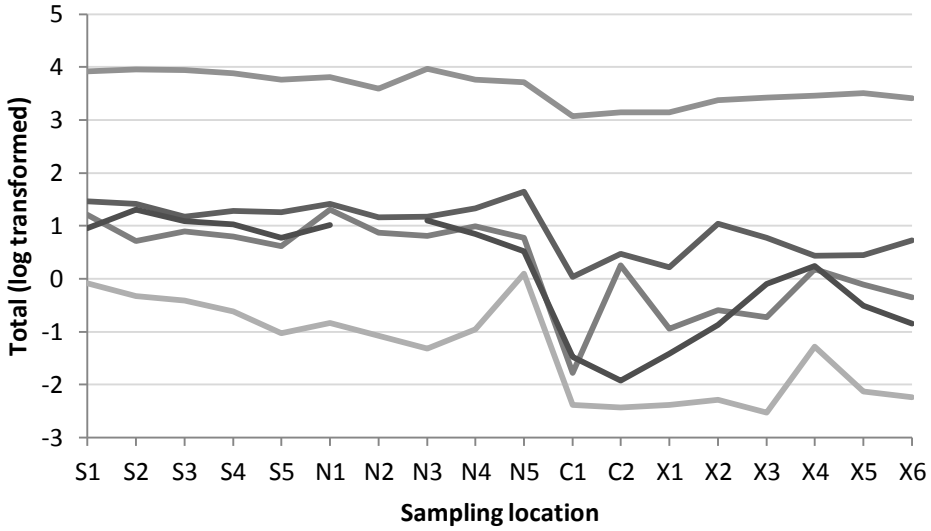


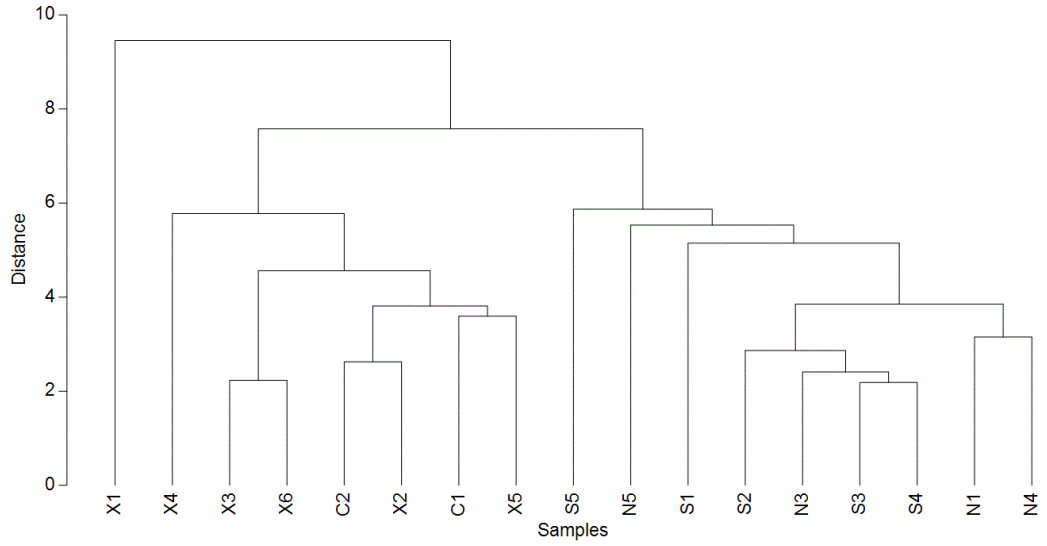
Table 20. Pairwise correlation coefficients for variables fatty acids, resin acids, phytosterols, grain size <63µ and total metals.

	F. Acids	*R. Acids*	*Phytos*	*Grn Sze <63*	*Total Metals*
Fatty Acids	1.000				
Resin Acids	0.827	1.000			
Phytosterols	0.826	0.819	1.000		
Grain Size <63µ	0.822	0.849	0.806	1.000	
Total Metals	0.857	0.846	0.846	0.964	1.000

These results supported further exploration of the data so combined variables were re-entered into Primer e (Gorley & Clarke, 2015) where data were normalised and a resemblance matrix was formed by Euclidian distance. The location N2 was removed as the effect of ‘no grain size’ could not be accounted for in the analysis.

The cluster dendrogram (Figure 41) shows clustering of the northern and southern transects from the X and control sampling location, locations X1 is distinct from these groupings.

Figure 41. Cluster dendrogram from normalised data, resemblance matrix using Euclidian distance, of sampling locations.



PCO analysis (Figure 42) demonstrated similar clustering but explained only 65.16% of the variation. The northern and southern transects appear to be influenced positively by most compound groups and grain size <63µ, the controls positively by grain size 500-250µ. X1 appears positively influenced by other grain sizes and As.

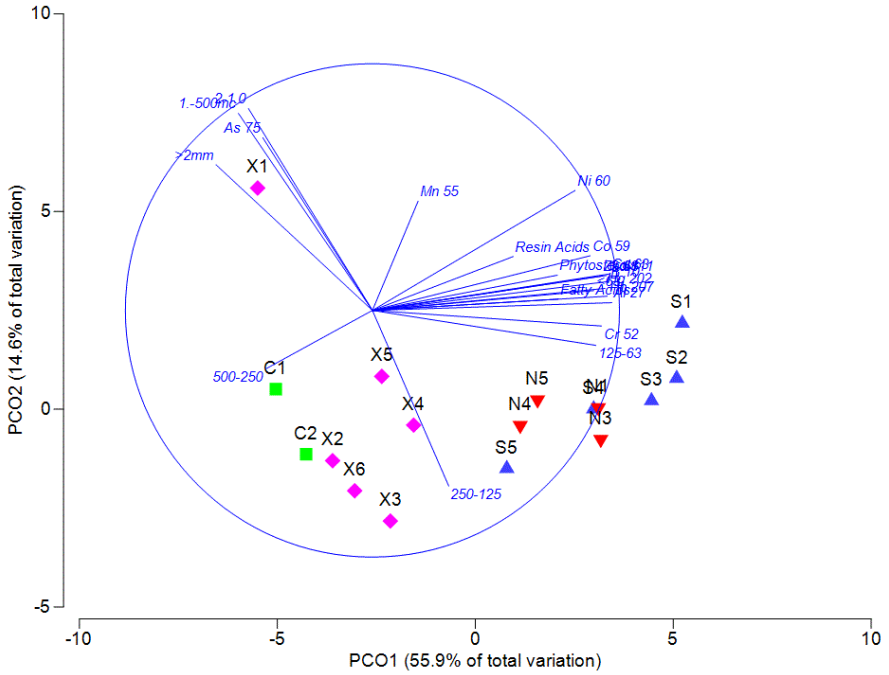


Figure 42. PCO of normalised data, resemblance matrix using Euclidean distance. Blue = southern transects, red = northern transects, pink = 'x' locations, green = control sites.

To further investigate significant differences between the sampling locations CAP analysis was performed on the same data (Figure 43). Clear separation of the sample groups are apparent with the southern transects positively influenced by the metals and smaller grain size groups <63µ, 63-124.9µ and 125-249.9µ. The northern transect locations, while similarly influenced, also appear to be negatively related to Arsenic. Control sites are positively influenced by the larger grain sizes.

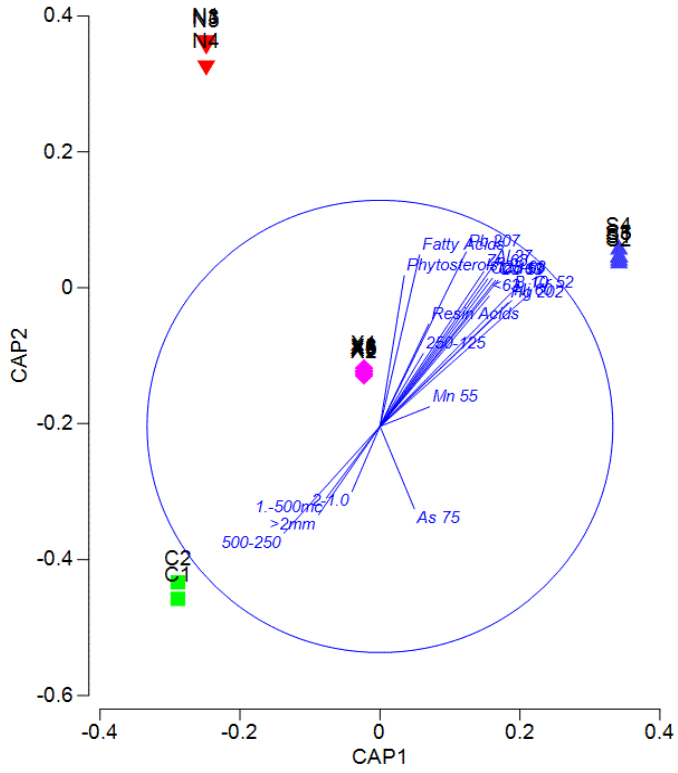


Figure 43. CAP analysis of sampling locations. Normalised data, with resemblance matrix using Euclidean distance. Blue = southern transects, red = northern transects, pink = 'x' locations, green = control sites.

PERMANOVA for groups found significant differences (P(perm) 0.001). Pairwise test between groups (Table 21) found significance (p>0.05) between southern and control groups, southern and 'X' groups and northern and 'X' groups.

Table 21. PERMANOVA pairwise analysis for groups of sampling locations.

Groups	t	P(perm)	Unique perms
S, N	1.0584	0.341	125
S, C	3.0376	0.047	21
S, X	2.8497	0.006	410
N, C	2.8131	0.069	15
N, X	2.2269	0.008	207
C, X	0.93502	0.493	28

3.4.5 In summary

Results from organics, grain size and metals all indicate a strong gradient of decreasing quantities of compounds along transects from the wharf with distance from the discharge points of storm water runoff from the log marshalling areas.

Some gradient is also apparent from the additional sites on a larger and less direct spatial scale. This may be influenced by other inputs but the strong correlation with organics may indicate at least some of this is directly related to the storm water runoff from the log marshalling areas.

Levels of compounds appear to be relatively low where comparable with guidelines and related literature, however this may be a positive influence from the dredging program. Dredging and disturbance may be removing the surface sediment layer in some cases but may also be responsible for resuspension and redistribution of some contaminants.

3.4.6 Mussel Analysis Organic Compounds and Length

Most of the screened compounds (Appendix A) were non-detectable in mussel samples (minimum detection level 0.01µg/g d.w.). Fatty acids and phytosterols were the only organic compounds found. Total extractives of fatty acids and phytosterols showed no decrease with distance from the discharge point. There was also no increase in these compounds found in the mussel samples over time (Figure 44). Highest total extractives were recorded at No.21 Mark from the 18 December 2014 sample. The No.19, No.21 and No.13 samples on 17 March 2015 were from wild populations as those deployed were no longer available for sampling. It was assumed that any long term build-up of contaminants would be similar by this time.

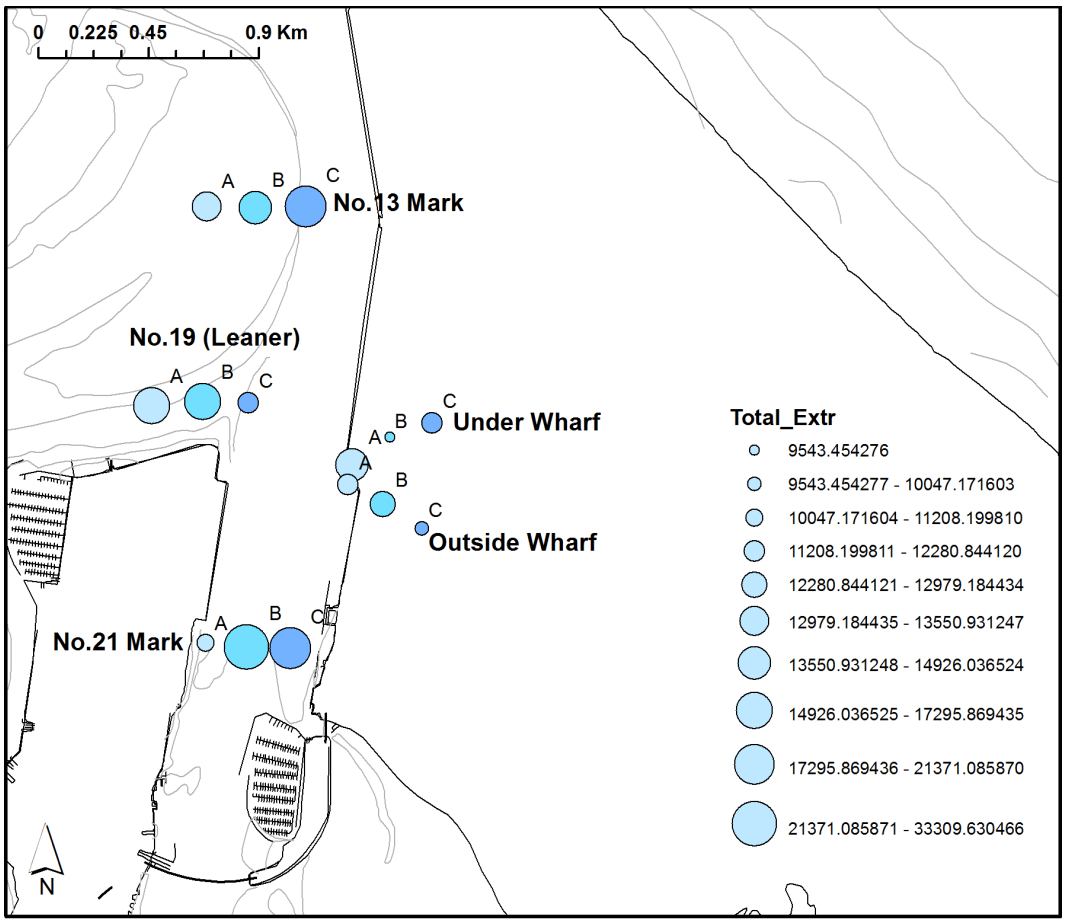


Figure 44. Total organic compounds extracted at each sampling point over time A = 12 June 2014, B = 18 December 2014, C = 17 March 2015. The No.19, No.21 and No.13 samples on 17 March 2015 were from wild populations.

Quantities for each individual organic compound over the three sampling events are summarised in Table 22 alongside the control sample.

Table 22. Fatty acids and phytosterols in mussels by location and sampling event (A, B or C). Colour map = highest (red) to lowest (green)

location	Controls	Outside	Under	No13	Leaner 19	No21	Outside	Under	No13	Leaner 19	No21	Outside	Under	No13	Leaner 19	No21
Date collected	28/05/14	12/06/14	12/06/14	12/06/14	12/06/14	12/06/14	18/12/14	18/12/14	18/12/14	18/12/14	18/12/14	17/03/15	17/03/15	17/03/15	17/03/15	17/03/15
Event	NA	A	A	A	A	A	B	B	B	B	B	C	C	C	C	C
Decanoic acid	2.59	2.30	3.25	2.27	4.68	2.98	1.76	0.99	3.62	1.92	2.87	2.32	1.17	2.83	2.33	2.19
Dodecanoic acid	12.45	8.99	9.82	6.77	20.63	8.72	10.15	4.26	24.37	18.99	11.16	11.91	8.41	9.47	10.88	13.44
Tetradecanoic acid	2219.57	715.51	846.37	871.58	1182.79	599.36	664.73	378.01	1174.15	682.44	1729.70	571.18	559.03	1204.39	1173.49	949.30
Palmitoleic acid	7088.09	1570.57	1986.52	1999.51	2964.87	1156.46	1853.73	747.08	2222.94	1507.15	10580.00	1309.50	1485.27	7153.24	3312.97	7358.88
Palmitic acid	6631.42	2744.77	3136.60	3524.96	4378.59	2674.09	2796.18	1765.58	3516.33	2569.10	6272.17	1914.03	2336.33	3659.30	3860.93	3286.47
Margaric acid	382.43	243.41	218.08	223.53	299.97	276.91	155.13	189.27	269.21	256.19	183.76	209.99	208.76	139.80	227.57	148.41
Linoleic acid	346.17	121.86	157.34	138.87	210.02	95.75	254.56	108.99	139.22	148.19	1001.33	108.31	175.95	551.40	215.12	597.50
Oleic acid	1101.07	362.39	591.34	461.55	832.09	291.52	712.70	262.70	445.06	386.69	3279.43	290.26	445.96	1902.67	705.85	1579.71
Linolenic acid	116.52	38.26	50.37	48.85	80.83	31.38	95.75	28.15	39.17	43.59	448.55	24.49	46.71	207.11	78.73	236.45
Elaidic acid	2151.41	632.45	880.92	724.66	1308.67	567.54	810.67	360.49	716.78	687.93	3915.09	506.45	564.69	2316.61	1119.77	1925.64
Stearic acid	1900.70	900.85	1145.87	1050.06	1414.57	1012.79	814.66	705.09	1097.00	974.45	1289.78	789.28	863.77	1021.18	1007.83	882.91
Eicosanoic acid	169.75	26.35	30.34	31.76	53.05	26.55	n.d.	7.54	23.30	20.43	n.d.	12.49	14.00	58.31	12.79	n.d.
Docosanoic acid	56.87	20.84	n.d.	19.28	26.73	23.77	n.d.	9.86	24.78	33.16	n.d.	7.43	6.35	n.d.	17.46	n.d.
Tetracosanoic acid	78.12	30.23	n.d.	18.69	27.86	39.90	n.d.	15.67	24.62	39.48	n.d.	10.39	8.89	n.d.	20.95	n.d.
Total Fatty Acids	22257.14	7418.78	9056.81	9122.33	12805.33	6807.72	8170.02	4583.69	9720.54	7369.72	28713.85	5768.03	6725.30	18226.32	11766.67	16980.90
Cholesterol	3529.22	4390.27	5468.27	4024.52	4103.62	3994.80	4456.15	4556.37	4236.42	4127.18	4255.94	3936.77	4689.80	4960.97	3714.84	4122.37
Campesterol	65.43	107.88	98.86	97.95	96.60	96.21	96.21	100.98	115.47	125.23	80.17	80.70	97.35	93.84	70.96	68.16
Stigmasterol	24.36	98.50	102.07	86.53	70.14	88.36	75.13	72.40	88.72	115.00	73.44	70.84	53.55	89.82	56.63	49.18
Sitosterol	139.28	265.41	200.02	219.60	220.17	221.12	181.67	230.02	276.69	316.72	186.23	187.21	159.84	247.97	210.68	150.48
Sitostanol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.63	1.89	n.d.	n.d.	n.d.
Total Phytosterols	3758.30	4862.06	5869.23	4428.60	4490.54	4400.48	4809.16	4959.76	4717.30	4684.14	4595.78	4279.15	5002.44	5392.59	4053.11	4390.19
Total Extractives	26015.44	12280.84	14926.04	13550.93	17295.87	11208.20	12979.18	9543.45	14437.84	12053.86	33309.63	10047.17	11727.74	23618.91	15819.77	21371.09

Highest levels for half of the fatty acids were found in the control sample. Most of the highest levels of the other fatty acids were found in the No21 sample from the 18th December 2014. Lowest levels of fatty acids were recorded in the 'Under Wharf' sample (closest to the stormwater discharge point) on the same sampling date.

Phytosterols were all lowest in the control sample. The highest total of phytosterol content was in the 'Under Wharf' sample from 12 June 2014. Highest individual phytosterols were cholesterol, found in the 'Under Wharf' sample from the same date. Other phytosterols were highest in the Leaner No.19 sample from the 18 December 2014, apart from sitostanol which was only detected in the 'Outside Wharf' and 'Under Wharf' samples from the 17 March 2015. Cholesterol was found in largest quantities in all samples.

Percentages of each organic compound were also investigated, there was very little variation in percentage of phytosterols (Figure 45), between sites or over time. This was found to be the same for fatty acids.

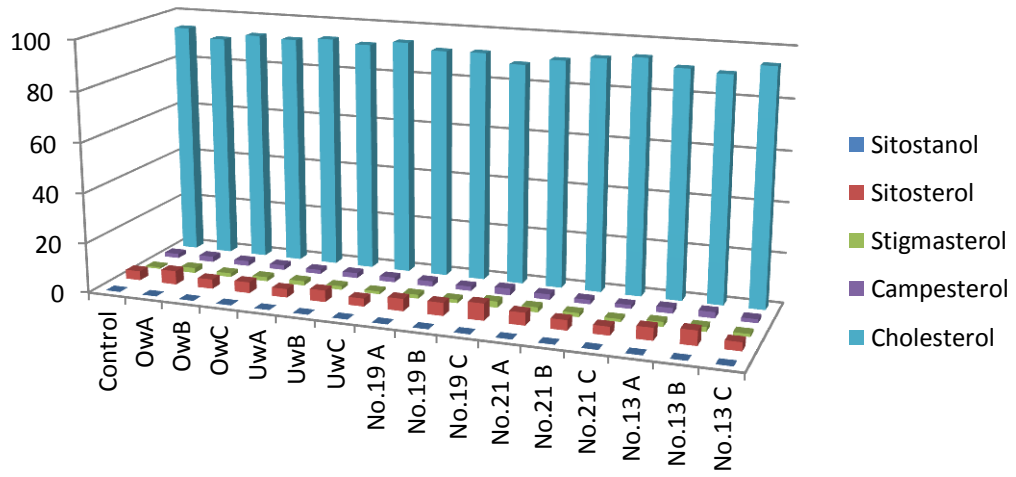


Figure 45. Percentage of individual phytosterols between sites (Ow, Uw, No.19, No.21 and No.13) and sampling event (A, B or C).

3.4.6.1 Rainfall Around Sampling Periods

Rainfall events are summarised for each sampling period in Table 23.

Table 23. Summary of rainfall during and before each mussel sampling period.

Date	12/06/2014	18/12/14	17/03/2015
Time	11:48am	8:00am	9:30am
Tide	0.5	0.81	0.76
Hourly Rainfall (mm)	0.2	0.0	0
24h Rainfall (mm)	50.7	105.7	2.3
7 Day Rainfall (mm)	261	188.9	53
Rain intensity (mm/h)	1	0	0
Wind Speed (Kts)	20	4	8
Wind Dir.	128 SE	320 NW	241 WSW

No statistical correlation was found between rainfall prior to sampling and quantities of organic chemicals in mussel samples.

3.4.6.2 Mussel Length

Mussels were measured at deployment, after the second sampling event on 18th December 2014 and after the final sampling on 25th March 2015. Only 'Outside' and 'Inside Wharf' samples were measured on 25th March as others were sampled from the wild populations.

From deployment on 29th May 2014 to 18th December 2014, a period of seven months and ten days, the average mussel length showed a small decrease at No.19, No.21 and No.13 and an increase at the 'Outside' and 'Under' wharf locations (Figure 46).

The 'Outside' and 'Under Wharf' sites also showed an overall increase in size over the entire period from deployment (29th May 2014) until retrieval (17th March 2014) (Figure 47). This was despite a decrease from the 18th December measurements.

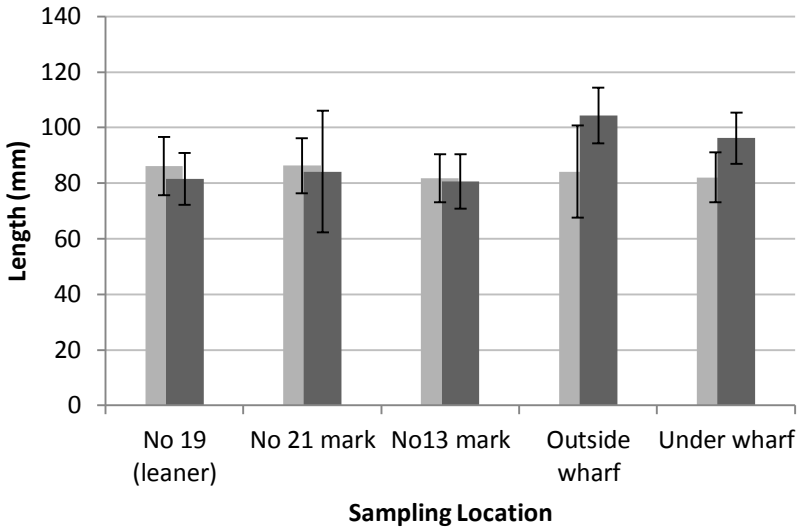


Figure 46. Average length (mm) of mussels between deployment (29th May 2014) and sampling on the 18th December 2014 (left to right).

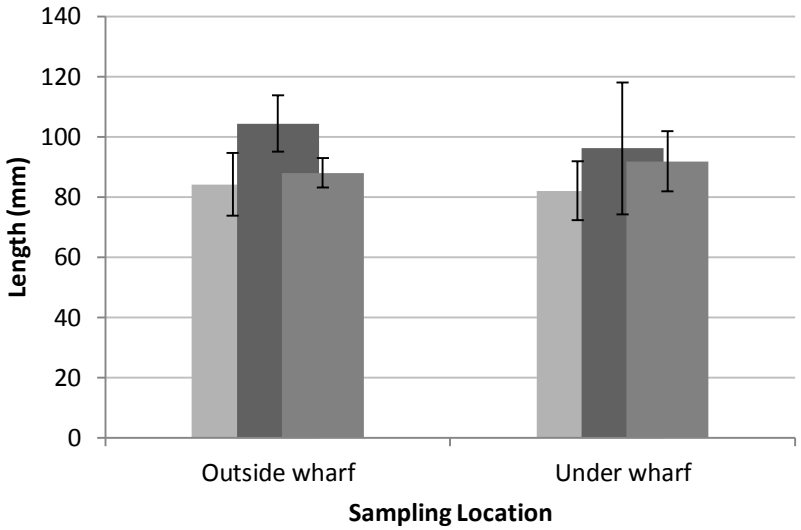


Figure 47. Average length (mm) of mussels between deployment, 29th May 2014, sampling on the 18th December 2014 and retrieval, 17th March 2015 (left to right).

3.5 Discussion

3.5.1 Sediments Analyses

The results of analyses for resin acids and associated compounds do reflect the trends found in earlier studies regarding accumulation in sediments such as Tian (1998).

Overall there is evidence of a gradient of the screened compounds in the sediments related to the raw effluent. There are differences in the gradients between the compound groups and individual chemicals which could be related to characteristics of the chemicals, such as solubility (Peng & Roberts, 2000) and water chemistry as discussed in Chapter 2. Equilibrium partitioning between the sediment organic carbon, interstitial water and organisms (Hansen *et al*., 2003) is known to be a strong factor in the way chemicals interact with sediments, biota and surrounding water. Currents have also been shown to affect the distribution of compounds into sediments (Osborne, 1991).

Resin acid contamination is where most work has been done regarding the accumulation of wood derived and associated discharges in sediments. In the samples for this study abietic and dehydroabietic acid were found in the highest quantities. The high presence of abietic acid is at odds with data reported by Tian *et al*. (1998) who found it in only one sample. Dehydroabietic acid was the next highest in quantity, but not found in many of the samples from this study, interestingly this was found in all of Tian *et al's* samples and in the greatest amounts. It is often noted as the most prevalent in studies of resin acids accumulation in sediments. Tian *et al* (1998) also found pimaric acid, sandaracopimaric acid and 7-hydroxy-dehydroabietic acid in significant amounts in most of the samples.

While there seems to be no single clear reason for the differences there are many possibilities. Sampling techniques may have been different to Tian's, who note that shallow samples at the wharves were collected by hand, at low tide and from under the wharves. The samples collected here were all done by grab at the wharves. Tian *et al*., (1998) also started and ended further out. The chemistry methods used for this study also vary

from Tian's, from the extraction solvents used through to GC/MS method. Tian used a SIM method to target resin acids rather than the screen method used here.

The influence of the dredging program is another factor which needs to be taken into account. Tian *et al*. (1998) discussed the influence of dredging and hypothesised that dredged areas would effectively return to zero for the accumulation of resin acids after each dredging event. In the sampling for this thesis, the northern transects outer samples N4 and N5 are close to the dredged areas H2 and H3 in the dredging plan. The outer samples of the southern samples may be impacted by activity in the H1 area. For the samples which were in addition to Tian's, X1 to X4 may be impacted by dredging, X5 and X6 are out of the dredging zone. However it is also worth considering the association with an area which needs regular dredging makes it an area where sediments accrue more quickly than other areas.

While dredging is likely to remove contaminants with the surface sediments, disturbance may increase bioavailability to marine life. Dredging has occurred regularly and will influence surface sediments greatly, and together with the disturbance by repeated propeller wash, should probably be considered as an area for further study.

While some research points to rapid desorption of resin acids into sediments in freshwater (Osborne, 1991; Tavendale *et al.*, 1997), the higher pH and salinity of saltwater may complicate this. Resin acids have been shown to become more soluble in increasing pH, this was observed with decreasing lipophilicity. This could decrease association with sediments and biota. Salting out of chemicals, which is actually used to remove resin acids from solution in the lab, would likely be of influence in estuaries, the influence of this is not well understood but may increase association with sediments (Turner, 2003).

The high occurrence of fatty acids in marine sediments is not unusual and can be linked to both marine inputs such as plankton and terrestrial inputs such as plant matter. Due to the correlation with the resin acids of

decreasing amounts with increasing distance from the wharves it is likely to be related to terrestrial influence. The type of fatty acids found also occur widely in *Pinus radiata* (Hemingway *et al*., 1971).

Phytosterols in the sediments are not a surprising occurrence. Cholesterol is known to be found in marine water and along with sitosterol is important to phytoplankton and zooplankton. Campesterol and stigmasterol are found in marine algae but also associated with higher plants which can indicate a terrestrial influence (Saliot & Tusseau, 1984; Volkman, 1986).

The results from the inorganics analyses also reveal a gradient in concentrations in sediments with distance from the wharf. Compared to other analyses such as Ellis et al., (2013); Park, (2003), some of the metals appear elevated for the harbour in general but not beyond ANZECC standards (ANZECC, 2000).

3.5.2 Mussels Organics and Size Comparisons

The fatty acid and sterol composition of Perna canaliculus is well known due to its commercial popularity in New Zealand (Murphy, et al., 2003; Miller, et al., 2014). It has been sampled in a number of locations and fatty acid and sterol composition compared with other New Zealand Mollusca (K. J. Murphy, et al., 2002; K. Murphy et al., 2003).

Fatty acid and phytosterol composition in the experimental samples reflect percentages of those found in other studies such as those above by Murphy et al. (2002; 2003). Totals of these extractives decline at the ‘Under’ and ‘Outside Wharf’ locations and at ‘No.19’ and increase at the No.21 and No.13 locations. The three outer locations were sampled from wild populations in the final sampling event.

While specifics of dietary intake have not been investigated here the results infer that growth of mussels, deliberately placed in perceived low quality environment, was not affected negatively compared to mussels from the same original source placed further away from the stormwater discharge.

3.6 In summary

Rejecting the original null hypothesis '*Contaminants from the log storage area storm water discharges are not accumulating in nearby sediments in Tauranga Harbour*' is not straightforward. While contaminants are certainly detectable in the sediments, compounds are able to be cross-referenced temporally, such as resin acids, are clearly less numerous than in the study by Tian *et al.* in 1998.

It is probable that dredging and other activities such as prop wash from shipping movements are affecting the distribution of surface sediments. This raises the question of possible desorption during resuspension and increased bioavailability of some contaminants.

The transplanted mussels did not appear to be accumulating resin acids over time or related to specific storm events. The null hypothesis '*Contaminants from the log storage area storm water discharges are not accumulating in nearby filter feeding organisms'* can therefore be accepted as there is no evidence to suggest any spatial or temporal impacts.

Chapter 4

Comparison of Intertidal Assemblages Near and Distant to Port of Tauranga Log Storage Derived Stormwater Discharge

4.1 Introduction

Studies which attempt to define estuarine environments and related species composition, often look at large scale influences such as latitude, climate and tidal influence (Hume *et al*., 2007). Further classification relates community structure to environmental factors such as sediment characteristics, salinity and freshwater inputs (Ellis *et al*., 2006).

Assessment of estuarine habitats often reveals changes in epifaunal community composition, this is generally termed patchiness (Kotliar & Wiens, 1990). While changes in wind, wave and temperature climate can influence species composition profoundly (Schiel, 2011), the loss of epifaunal species has also been attributed to anthropogenic influences (Bennington, 1979; Thrush *et al*., 1994, 2003). This loss can influence food webs where species at higher trophic levels, such as putting pressure on feeding of fish which rely on epifaunal species as the main part of their diet, possibly causing the decline of other higher level species (Gaston *et al*., 1998).

Smaller scale studies of community composition have been conducted in New Zealand to quantify effects from anthropogenic sources such as pollution (Roper, 1990; Nipper *et al*., 1998; Hack *et al*., 2007;). Such studies are becoming more common as resource consent requirements dictate this type of monitoring work.

To probe the effects of the outfall plume on the marine life of Tauranga Harbour, a baseline survey was conducted to compare species composition within and outside of the plume's principal area of influence as defined from the earlier work in this thesis and Brunschwiler, (2015).

4.2 General Biodiversity

There is little literature available on the intertidal rocky shore sites within Tauranga Harbour due in part to the limited extent of such habitats. Some species lists were available on rocky shore sites in the wider Bay of Plenty (Schiel *et al*., 2014) and give an indication of main species assemblages, but these predominantly focus on open coastal habitats. Detailed monitoring of the Port of Tauranga has been conducted by Biosecurity New Zealand but was almost exclusively subtidal (Inglis *et al*., 2006, 2008).

The Port of Tauranga is described as having a larger than average diversity of native and cryptogenic species compared to 13 other ports in New Zealand (Inglis *et al*., 2006). Schiel *et al*. (2014) described Bay of Plenty rocky shore sites as falling into two categories: small rocky outcrops among sandy beaches, and larger rocky platforms. The sites used in this study did not fall into these naturally formed categories as they consist of small boulders placed as riprap to prevent shoreline erosion. Schiel *et al*. (2014) found less variation in species compositions at small rocky outcrops compared to larger rocky platforms. Numbers of individual taxa were also less at these sites, especially where rocky outcrops ended directly onto a sandy low tide zone, as is the case at the port sites in this study. While it would be a stretch to compare a modified harbour environment directly with one from the rocky shore, it may help in understanding some aspects of the species composition.

4.3 Methods

The two areas for this study were selected to be very similar in habitat (Figure 48) with one site inside, and the other outside, the main area of influence of the stormwater plume (Figures 48, 49 & 50). The Berth 11 (B11) site is located between the northern and southern outfalls which have been a focus of the wider study in Chapters 2 and 3. To the south of this area a riprap wall extends from a boat ramp close to Butters Wharf which services the Matakana Island ferry; this is the location of the Butters Wharf (Butt) site.

Figure 48. Overview of the B11 (top) and Butt (above) sites

A 50 m transect reel was laid out along the areas to be surveyed. One end was secured with an electric fence post. At 5 m intervals a 0.25 m^2 quadrat was placed on the substrate. This was repeated eight times at each site. The day of the survey was chosen as one coinciding with a 0.1 m low tide, fine weather and light winds to make visual identification as easy as possible. Areas were sampled either side of low tide (13:52 hrs.) on 19 May 2015; Butt approximately 30 minutes before, and the area adjacent to B11, 15 minutes after this low tide.

A pre-prepared sheet was used to record site, date, quadrat number, species, number of species and notes relevant to each quadrat. A GPS (Garmin 64s) position was recorded and a photograph was taken of each quadrat (Appendix G). Notes were also made of other species observed outside the quadrats. If identification was not 100% positive, samples were photographed and collected where possible for subsequent assignment to species or operational taxonomic units (OTU's).

Figure 49. Overview of the two sites.

Figure 50. Quadrat locations. Top = B11 site, bottom = Butt.

4.3.1 Statistics

Data were organised and boxplots graphed in R statistics (R Core Team, 2013). To further investigate species composition by site, data were imported to Primer 7 (Gorley & Clarke, 2015). The data were transformed by square root and a Bray–Curtis similarity resemblance matrix was applied. A dendrogram was produced from cluster analysis of the data. Non-metric multidimensional scaling (nMDS), Principle coordinates analysis (PCO), permutational multivariate analysis of variance (PERMANOVA) (Anderson *et al*., 2008) and canonical analysis of principal coordinates (CAP) was undertaken.

4.4 Results

The habitat at both sites was dominated by riprap boulders placed as protection from erosion. Between the boulders, at the very lowest part of the tide, course sand was present. In the splash zone, barnacles and occasional rock oysters were observed. The total height of the boulder protection at the Berth 11 site (B11) was greater (at ≈ 7 m) than at the Butters Wharf site (≈ 2 m).

A total of 16 species were recorded in the quadrats. Species richness at the B11 site was 12 and at Butt, 10. Of those 16 species, 8 were macrofauna and 8 were algae. Seven of the 8 macrofauna species were recorded at B11, and 3 at Butt.

Density of macrofauna was highest for *Lunella smaragda* at both sites: 18.5 individuals per m^2 (Butt) and 31.5 per m^2 (B11). The only remaining macrofauna species recorded at both sites was *Patiriella regularis* having a density of 0.5 per m^2 (Butt) and 0.5 per m^2 (B11) (Table 24).

Table 24. Macrofauna species list and density (per m2) at Butt and B11.

Species	Butt	B11
Lunella smaragda	18.5	31.5
Patiriella regularis	0.5	0.5
Anthothoe albocincta	0	13.0
Oulactis sp.	0	1.0
Sypharochiton pelliserpentis	0.5	0
Buccinulum linea	0	0.5
Evechinus chloroticus	0	0.5
Dicathais orbita	0	0.5

Of the 8 algae species, all were found at Butt and 3 at B11. Algae were recorded as percent cover. Larger green and brown algae were absent from within the quadrats and adjacent areas at B11, Table 25. Large brown and green algae were also absent from the species observed outside the quadrats at the time of the survey, a full species list is available in Appendix G.

Table 25. Algae species list and percent cover (per m2).

Species	Butt	B11
Carpophyllum maschalocarpum	10.00	0.00
Corallina officinalis	15.00	37.50
Dictyota ocellata	1.00	0.00
Red rugged algae UNID	50.00	72.50
non geniculate coralline algae	58.50	0.00
Zostera muelleri	0.25	3.50
Colpomenia claytoniae	5.50	0.50

The cluster dendrogram (Figure 51) using Bray Curtis similarity did not reveal any obvious groupings attributable to site location. BQ3 branches at the top of the dendrogram which indicates a high degree of dissimilarity from other quadrats from both sites. Some of the Butt (Bu1, 2, 3, and 7) quadrats also cluster independently from other quadrats at the top of the first node, again indicating dissimilarity from other quadrats across both site locations. Two groups form at the second node made up of a mix of quadrats from both site locations, (BQ1, 2 and 5; BuQ4 & 6) and (BQ4, 6, 7 and 8; BuQ 5 and 8)).

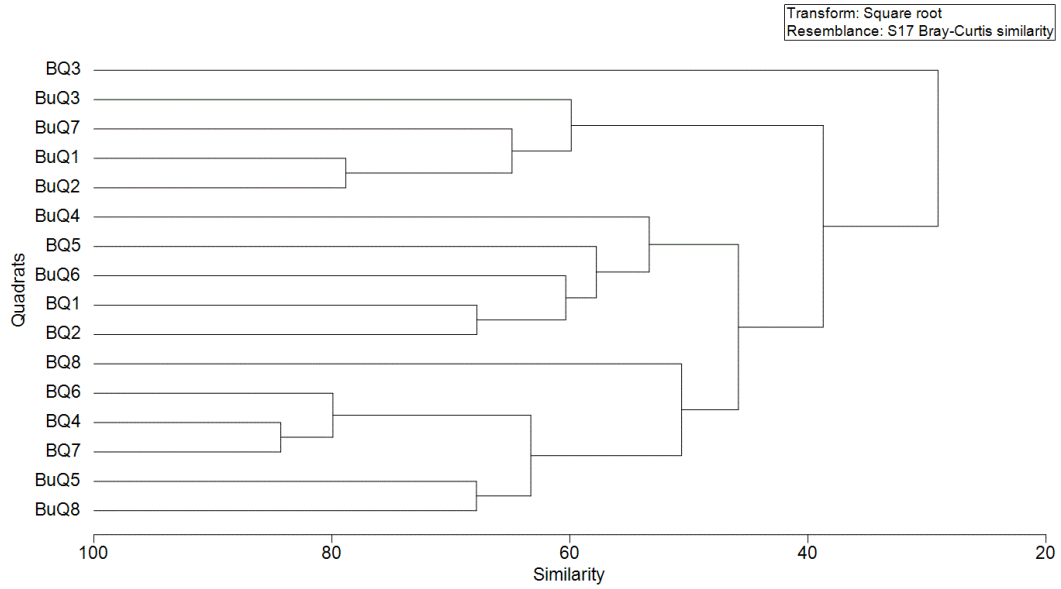


Figure 51. Cluster dendrogram of quadrats. Data square root transformed, resemblance = Bray Curtis similarity. B = B11 quadrats, Bu = Butt quadrats.

Box plots of average numbers of macrofauna species did not highlight any large differences in species numbers between the two sites (Figure 52). Larger numbers of each species were recorded at the B11 site with the exception of *Sypharochiton pelliserpentis.*

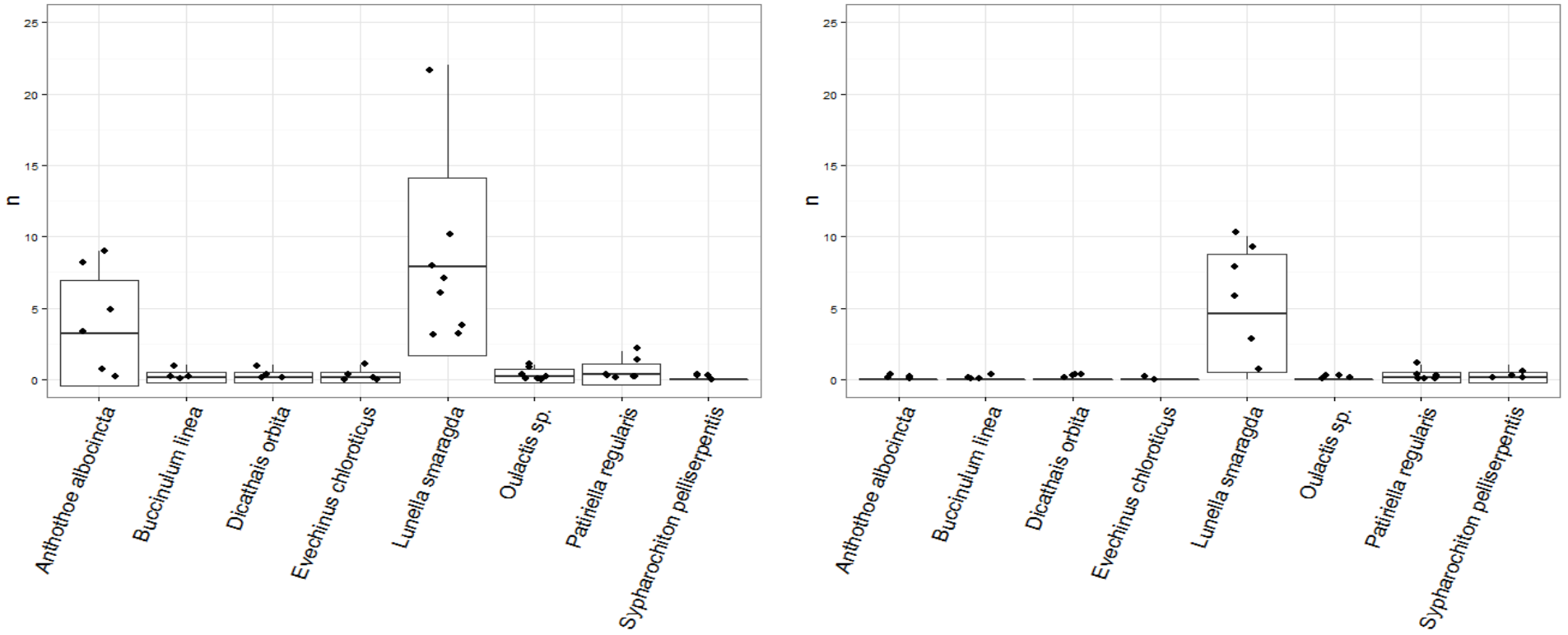


Figure 52. Macrofauna species counted at sites Berth 11 and Butters Wharf on 19 May 2015. Horizontal line = mean n, Box= +/- S.D., vertical line = min/max, diamonds = data points + jitter.

Graphed nMDS showed a fairly even division of individual quadrats (Figure 53), clustering was slightly tighter for the Butt sites. Sites were dispersed by Butt quadrats being in the upper and right side of the ordination and B11 quadrats to the lower and left side of the ordination. The stress level of 0.17 indicates a just adequate but not strong representation of the data.

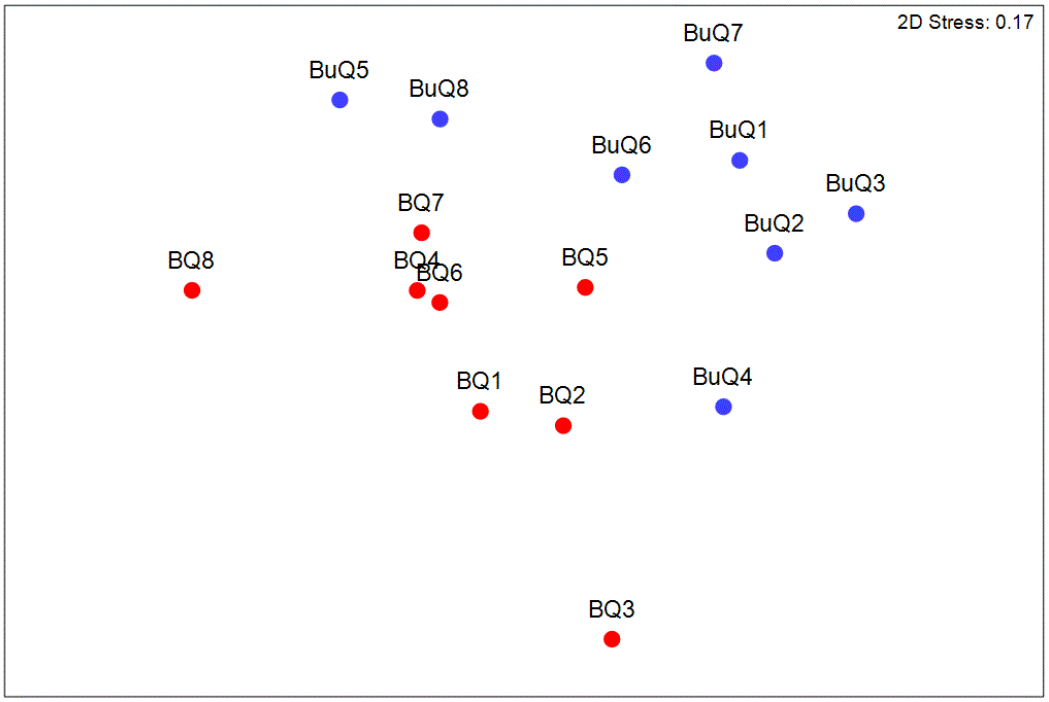


Figure 53. nMDS ordination of biological data by quadrat number (Q) and site. Data square root transformed, resemblance = Bray Curtis similarity. Bu (Blue) = Butters Wharf, B (red) = Berth 11. Stress = 0.17.

The PCO plot in Primer (Figure 54) indicated algae species *C. maschalocarpum, C. claytoniae*, non-geniculate coralline algae and the chiton *S. pelliserpentis* influenced variation in quadrats from the Butt site. The B11 site was associated with the species *D. orbita, C. officinalis, Z. muelleri* and the anemone *Oulactis* sp. The variation explained by the principle coordinates was not high: PCO1 was 37.4% and PCO2 27.8%. A cumulative total of 65.2% variation was explained

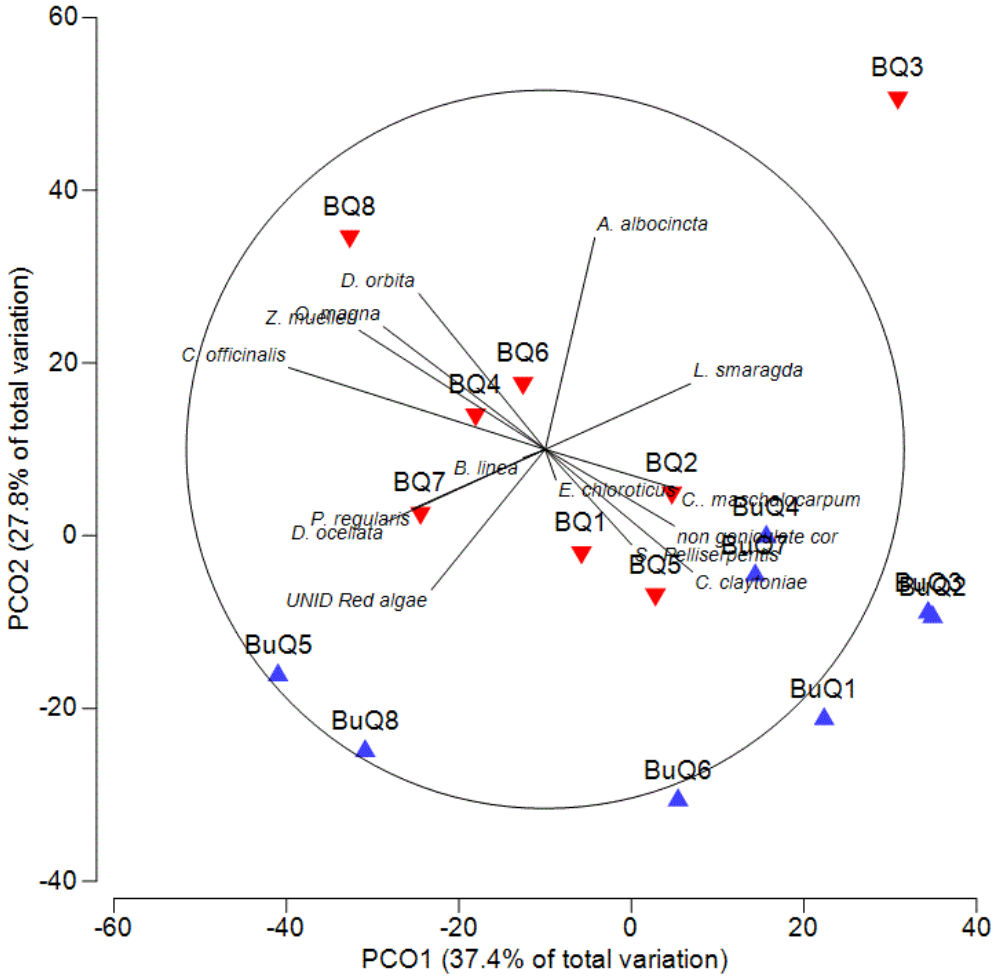


Figure 54. PCO plot of biological data by quadrat and site. Data square root transformed, resemblance = Bray Curtis similarity. Blue Triangle = Butters Wharf, red cross = Berth 11. Text in graph = species.

PERMANOVA found significance P(perm) 0.006 in the difference between the two sites (Table 26).

Table 26. Results table for PERMANOVA of sites.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Si	1	4977.6	4977.6	3.5608	0.006	926
Res	14	19571	1397.9			
Res	14	19571	1397.9			

4.5 Discussion

The general species composition appears to reflect other studies done on the Bay of Plenty region's low intertidal zones. *Lunella smaragda* and *S. pelliserpentis* were among the species recorded in the Mount Maunganui rocky intertidal zone by Schiel *et al*. (2014). Algae species *C. maschalocarpum, D. ocellata and C. officinalis* were also recorded as dominant species alongside non-geniculate coralline algae (Schiel *et al*., 2014).

Significant difference was found between the two study sites from the PERMANOVA. PCO results did not show strong explained variation or MDS enough stress value to understand what the significance might have been. However, from the raw data and graphical interpolation it appears that lower species numbers at the Butters Wharf site may be the greatest influence. This finding would indicate no discernible negative effects in the area of the plume's influence.

As this was a very small scale study, it would be difficult to draw any major conclusions from the lack of larger green and brown algae present at the B11 site. Only a little was found at the Butt site and a lack of algal matrices was representative of the sites with a sandy low shore in Schiel *et al*.'s (2014) study, this therefore may relate more to habitat type.

A study on the effects of log and wood chip storage at Port Nelson also surveyed intertidal assemblages at that port and found no discernible effect from log water discharge (Forrest & Roberts, 1995). Interestingly, they noted an increase in some species close to the point of discharge.

As in the case of this study, Forrest & Roberts (1995) argued that intertidal assemblages were the most relevant as likely to be most affected by the surface stormwater plume. In light of the preceding chapters it could also be argued that, due to salting out of chemicals and processes like flocculation, benthic organisms some distance away from the discharge could be affected by the runoff from the Port of Tauranga log storage areas.

In summary, differences were found in low intertidal species composition in relation to the stormwater plume adjacent to the Berth 11 and the Butters Wharf sites. These were principally lower numbers of macrofauna at the Butters Wharf Site and some macroalgae species absent from the Berth 11 site which may not be attributable to the plume.

A study of benthic organisms would be a recommended focus for a future investigation. Other recommendations would include intensification of this survey design by seasonal repetition and expansion of the survey area to include the shoreline immediately adjacent to the southern outfall.

Chapter 5

Final Discussion and Conclusions

This thesis has taken a journey from the Port of Tauranga log storage areas, through the ports storm water system, into the Tauranga Harbour and beyond.

The fact that contaminants found in the runoff exceed trigger values set for freshwater protection should not necessarily be of concern, however, the limited amount of water sampling in the marine environment and high minimum detection levels sometimes used in testing, make it difficult to discern any impact in the marine environment from water sampling alone.

The variation between sample results due to storm intensity and time of sampling in relation to the storm event underline the need for regular and varied temporal monitoring to allow a realistic modelling of contaminant levels. Future increase in log exports and the influence of climate change are factors which may increase this variability.

Toxicity testing indicates that some elements of the effluent are toxic to marine species. Again the variation between the two samples of the stormwater was of importance here. While the test on the Mysid was rendered invalid, a greater sensitivity of local species should still be considered possible. This emphasises the need for using multiple species to evaluate toxicity.

Water meter readings in the field indicate a high degree of chemical reactivity occurring. This appears to be taking place close to the wharves at the interface where discharged runoff meets the harbour waters. The differences in some of the master variables such as temperature, salinity and pH can affect solubility and other characteristics, causing changes in bio-availability of the compounds in the effluent.

Compounds detected in the raw effluent are found in sediments nearby. Quantities decrease with increasing distance from the point of discharge and levels are well within ANZECC (2000) Interim Sediment Guidelines. There is likely to be some effect on remobilisation and subsequent transport of some of these contaminants due to the regular dredging program and disturbance of the seabed by shipping movements. This may increase in the future with continued expansion of shipping movements.

Temporal and spatial accumulation of the screened organic compounds were not detected in transplanted mussels, it can therefore be concluded that there is little or no effect to these types of organisms from the log storage stormwater runoff.

No adverse effects were discernible on intertidal assemblages around the area influenced by the runoff from the log storage areas. However, based on the findings from this and Brunschwiler's (2015) work, it is more likely that any possible effects are occurring subtidally and close to the wharves.

While the influence of the plume is detectable and some impact on the harbour is evident in sediments, adverse effects on intertidal marine life were not apparent. It is suggested that any further study be conducted on direct toxicity to benthic organisms and avoidance by larval species which may use the harbour as a transitory habitat. Additionally more investigation at the interface between the stormwater and marine water will add to the understanding of the chemical processes at work.

This work in total adds to the limited store of knowledge around the effects of log storage and resin acid contamination in the marine environment. It better helps to understand the impacts from stormwater discharge from the Port of Tauranga and the wider field of industrial and urban stormwater discharge

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Appendix A

Organic Compounds Screened by GC/MS

Monoterpenes
Alpha-pinene
Beta-pinene
Fenchone
Camphor
Fenchol
Borneol
Terpinen-4-ol
Alpha-terpineol

Phenolics
Guaiacol
Eugenol
Vanillin
Acetovanillone
Vanillic acid
Homovanillic acid
Ferulic acid
Gallic acid
Syringol
Acetosyringone
Syringylaldehyde
Syringic acid
Coniferyl alcohol
Coniferyl aldehyde
Pinosylvin, mono methyl ether

Fatty Acids
Decanoic acid (F10:0)
Dodecanoic acid (F12:0)
Tetradecanoic acid (F14:0)
Palmitoleic acid (F16:1)
Palmitic acid (F16:0)
Margaric acid (F17:0)
Linoleic acid (F18:2)
Oleic acid (F18:1)
Linolenic acid (F18:3)
Elaidic acid (F18:1)
Stearic acid (F18:0)
Eicosanoic acid (F20:0)
Docosanoic acid (F22:0)
Tetracosanoic acid (F24:0)

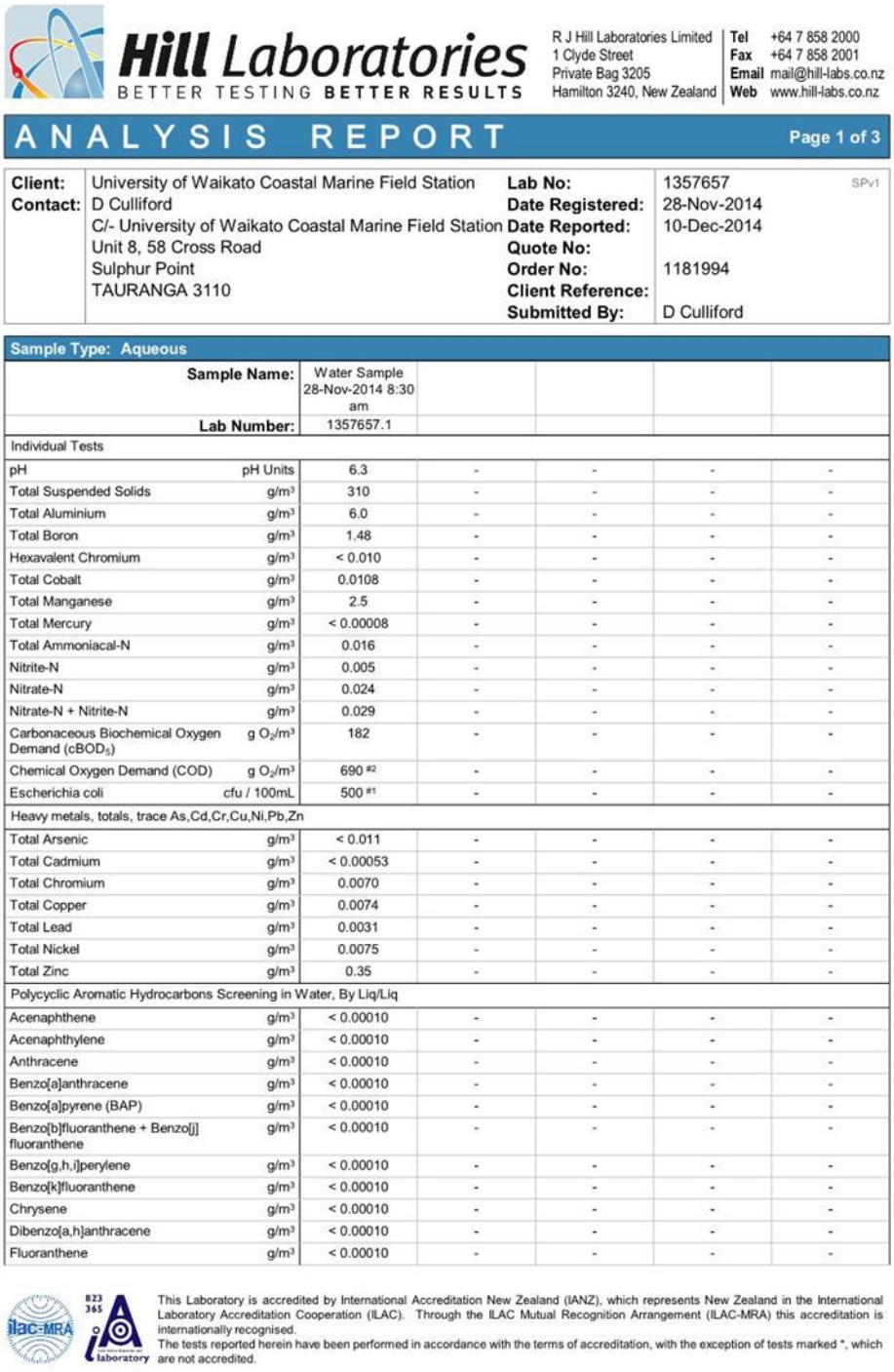
Resin Acid Neutrals
Fichtelite
Dehydroabietin
Tetrahydroretene
Retene
Methyldehydroabietin

Resin Acids
Pimaric acid
Sandaracopimaric acid
Isopimaric acid
Palustric acid
Levopimaric Acid
Dehydroabietic acid
Abietic acid
Neoabietic acid
Pimarenic acid
Sandaracopimarenic acid
Isopimarenic acid
13-Abietenic acid
Pimaranic acid
Isopimaranic acid
Abietanic acid
Seco-1-dehydroabietic acid
Seco-2-dehydroabietic acid
12-Chlorodehydroabietic acid
14-Chlorodehydroabietic acid
12,14-Dichlorodehydroabietic
7-Oxodehydroabietic acid

Phytosterols
Cholesterol
Campesterol
Stigmasterol
Sitosterol
Sitostanol

Appendix B

Example of Analysis Report from Hill Laboratories



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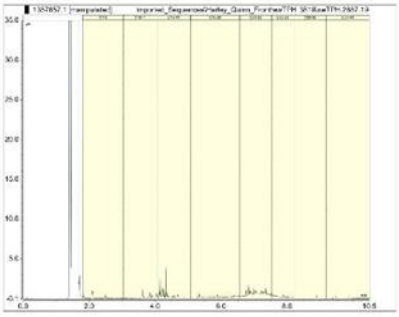
ANALYSIS REPORT Page 1 of 3

Client:	University of Waikato Coastal Marine Field Station	**Lab No:**	1357657	SPv1
Contact:	D Culliford	**Date Registered:**	28-Nov-2014	
	C/- University of Waikato Coastal Marine Field Station	**Date Reported:**	10-Dec-2014	
	Unit 8, 58 Cross Road	**Quote No:**		
	Sulphur Point	**Order No:**	1181994	
	TAURANGA 3110	**Client Reference:**		
		Submitted By:	D Culliford	

Sample Type: Aqueous						
Sample Name:		Water Sample 28-Nov-2014 8:30 am				
Lab Number:		1357657.1				
Individual Tests						
pH	pH Units	6.3	-	-	-	-
Total Suspended Solids	g/m³	310	-	-	-	-
Total Aluminium	g/m³	6.0	-	-	-	-
Total Boron	g/m³	1.48	-	-	-	-
Hexavalent Chromium	g/m³	< 0.010	-	-	-	-
Total Cobalt	g/m³	0.0108	-	-	-	-
Total Manganese	g/m³	2.5	-	-	-	-
Total Mercury	g/m³	< 0.00008	-	-	-	-
Total Ammoniacal-N	g/m³	0.016	-	-	-	-
Nitrite-N	g/m³	0.005	-	-	-	-
Nitrate-N	g/m³	0.024	-	-	-	-
Nitrate-N + Nitrite-N	g/m³	0.029	-	-	-	-
Carbonaceous Biochemical Oxygen Demand ($cBOD_5$)	g O_2/m³	182	-	-	-	-
Chemical Oxygen Demand (COD)	g O_2/m³	690 #2	-	-	-	-
Escherichia coli	cfu / 100mL	500 #1	-	-	-	-
Heavy metals, totals, trace As,Cd,Cr,Cu,Ni,Pb,Zn						
Total Arsenic	g/m³	< 0.011	-	-	-	-
Total Cadmium	g/m³	< 0.00053	-	-	-	-
Total Chromium	g/m³	0.0070	-	-	-	-
Total Copper	g/m³	0.0074	-	-	-	-
Total Lead	g/m³	0.0031	-	-	-	-
Total Nickel	g/m³	0.0075	-	-	-	-
Total Zinc	g/m³	0.35	-	-	-	-
Polycyclic Aromatic Hydrocarbons Screening in Water, By Liq/Liq						
Acenaphthene	g/m³	< 0.00010	-	-	-	-
Acenaphthylene	g/m³	< 0.00010	-	-	-	-
Anthracene	g/m³	< 0.00010	-	-	-	-
Benzo[a]anthracene	g/m³	< 0.00010	-	-	-	-
Benzo[a]pyrene (BAP)	g/m³	< 0.00010	-	-	-	-
Benzo[b]fluoranthene + Benzo[j] fluoranthene	g/m³	< 0.00010	-	-	-	-
Benzo[g,h,i]perylene	g/m³	< 0.00010	-	-	-	-
Benzo[k]fluoranthene	g/m³	< 0.00010	-	-	-	-
Chrysene	g/m³	< 0.00010	-	-	-	-
Dibenzo[a,h]anthracene	g/m³	< 0.00010	-	-	-	-
Fluoranthene	g/m³	< 0.00010	-	-	-	-

Sample Type: Aqueous						
Sample Name:		Water Sample 28-Nov-2014 8:30 am				
Lab Number:		1357657.1				
Polycyclic Aromatic Hydrocarbons Screening in Water, By Liq/Liq						
Fluorene	g/m³	< 0.0002	-	-	-	-
Indeno(1,2,3-c,d)pyrene	g/m³	< 0.00010	-	-	-	-
Naphthalene	g/m³	< 0.0005	-	-	-	-
Phenanthrene	g/m³	< 0.0004	-	-	-	-
Pyrene	g/m³	< 0.0002	-	-	-	-
Total Petroleum Hydrocarbons in Water						
C7 - C9	g/m³	< 0.10	-	-	-	-
C10 - C14	g/m³	< 0.2	-	-	-	-
C15 - C36	g/m³	< 0.4	-	-	-	-
Total hydrocarbons (C7 - C36)	g/m³	< 0.7	-	-	-	-

1357657.1
Water Sample 28-Nov-2014 8:30 am
Client Chromatogram for TPH by FID



Analyst's Comments

Please interpret these microbiological results with caution as the sample temperature was > 8 °C on receipt in the lab. Samples are required to be less than 8 °C (but not frozen).

#1 Statistically estimated count based on the theoretical countable range for the stated method.

#2 Severe matrix interferences on sample 1357657/1 for Total COD required that a dilution be performed prior to analysis, resulting in a detection limit higher than that normally achieved. Sample 1357657/1 was tested using a MERCK Merckoquant Chloride Test Strip which indicated that the sample contained greater than or equal to 3000 mg/L Chloride. The Total COD method is not suitable for samples containing greater than 2000 mg/L Chloride and hence the need to dilute the samples prior to analysis.

SUMMARY OF METHODS

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively clean matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis.

Sample Type: Aqueous			
Test	**Method Description**	**Default Detection Limit**	**Sample No**
Heavy metals, totals, trace As,Cd,Cr,Cu,Ni,Pb,Zn	Nitric acid digestion, ICP-MS, trace level	0.000053 - 0.0011 g/m³	1
Polycyclic Aromatic Hydrocarbons Screening in Water, By Liq/Liq	Liquid / liquid extraction, SPE (if required), GC-MS SIM analysis [KBIs:4736,2695]	0.00010 - 0.0005 g/m³	1
Total Petroleum Hydrocarbons in Water*	Hexane extraction, GC-FID analysis US EPA 8015B/MfE Petroleum Industry Guidelines [KBIs:2803,10734]	0.10 - 0.7 g/m³	1
Filtration, Unpreserved	Sample filtration through 0.45µm membrane filter.	-	1
Total Digestion	Boiling nitric acid digestion. APHA 3030 E 22nd ed. 2012 (modified).	-	1
pH	pH meter. APHA 4500-H+ B 22nd ed. 2012.	0.1 pH Units	1

Sample Type: Aqueous			
Test	**Method Description**	**Default Detection Limit**	**Sample No**
Total Suspended Solids	Filtration using Whatman 934 AH, Advantec GC-50 or equivalent filters (nominal pore size 1.2 - 1.5µm), gravimetric determination. APHA 2540 D 22nd ed. 2012.	3 g/m³	1
Total Aluminium	Nitric acid digestion, ICP-MS, trace level. APHA 3125 B 22nd ed. 2012 / US EPA 200.8.	0.0032 g/m³	1
Total Boron	Nitric acid digestion, ICP-MS, trace level. APHA 3125 B 22nd ed. 2012.	0.0053 g/m³	1
Hexavalent Chromium	Diphenylcarbazide colorimetry. Discrete Analyser. APHA 3500 Cr B (modified from manual analysis) 22nd ed. 2012.	0.010 g/m³	1
Total Cobalt	Nitric acid digestion, ICP-MS, trace level. APHA 3125 B 22nd ed. 2012 / US EPA 200.8.	0.00021 g/m³	1
Total Manganese	Nitric acid digestion, ICP-MS, trace level. APHA 3125 B 22nd ed. 2012 / US EPA 200.8.	0.00053 g/m³	1
Total Mercury	Bromine Oxidation followed by Atomic Fluorescence. US EPA Method 245.7, Feb 2005.	0.00008 g/m³	1
Total Ammoniacal-N	Filtered sample. Phenol/hypochlorite colorimetry. Discrete Analyser. (NH_4-N = NH_4+-N + NH_3-N). APHA 4500-NH_3 F (modified from manual analysis) 22nd ed. 2012.	0.010 g/m³	1
Nitrite-N	Automated Azo dye colorimetry, Flow injection analyser. APHA 4500-NO_3^- I 22nd ed. 2012.	0.002 g/m³	1
Nitrate-N	Calculation: (Nitrate-N + Nitrite-N) - NO2N. In-House.	0.0010 g/m³	1
Nitrate-N + Nitrite-N	Total oxidised nitrogen. Automated cadmium reduction, flow injection analyser. APHA 4500-NO_3^- I 22nd ed. 2012.	0.002 g/m³	1
Carbonaceous Biochemical Oxygen Demand ($cBOD_5$)	Incubation 5 days, DO meter, nitrification inhibitor added, dilutions, seeded. Analysed at Hill Laboratories - Microbiology; 1 Clow Place, Hamilton. APHA 5210 B (modified) 22nd ed. 2012.	2 g O_2/m³	1
Chemical Oxygen Demand (COD), trace level	Dichromate/sulphuric acid digestion in Hach tubes, colorimetry. Trace Level method. APHA 5220 D 22nd ed. 2012.	6 g O_2/m³	1
Escherichia coli	Membrane filtration, Count on mFC agar, Incubated at 44.5°C for 22 hours, MUG Confirmation. Analysed at Hill Laboratories - Microbiology; 1 Clow Place, Hamilton. APHA 9222 G, 22nd ed. 2012.	1 cfu / 100mL	1

These samples were collected by yourselves (or your agent) and analysed as received at the laboratory.

Samples are held at the laboratory after reporting for a length of time depending on the preservation used and the stability of the analytes being tested. Once the storage period is completed the samples are discarded unless otherwise advised by the client.

Ara Heron BSc (Tech)
Client Services Manager - Environmental Division

Appendix C

Links to Examples of R code used

General link to folder of all files:

https://drive.google.com/folderview?id=0B9SDhZA4tgN3RmRzR2RNeVRMWHc&usp=sharing

Chapter 2 – Bar and box plots water results:

Inorganics box plots
https://drive.google.com/open?id=0B9SDhZA4tgN3bFZYbFNteHQtQWs
Bar plots combined to grid
https://drive.google.com/open?id=0B9SDhZA4tgN3UExxRUNvT1dPNUU
Heavy metals box plots
https://drive.google.com/open?id=0B9SDhZA4tgN3NF9Ub3VNbWVrZ2M

Chapter 2 – Box plots, multi-line graphs, ANOVAS:

Multi line graph of O_2 concentration
https://drive.google.com/open?id=0B9SDhZA4tgN3N1czNXotNF9JY1E
Mysids box plots
https://drive.google.com/open?id=0B9SDhZA4tgN3SV9oT0c2ZGdFZkE
ANOVA and post hoc
https://drive.google.com/open?id=0B9SDhZA4tgN3c2E5WlVVTkZGdTg

Chapter 2 – Multi bar plots, distance and depth:

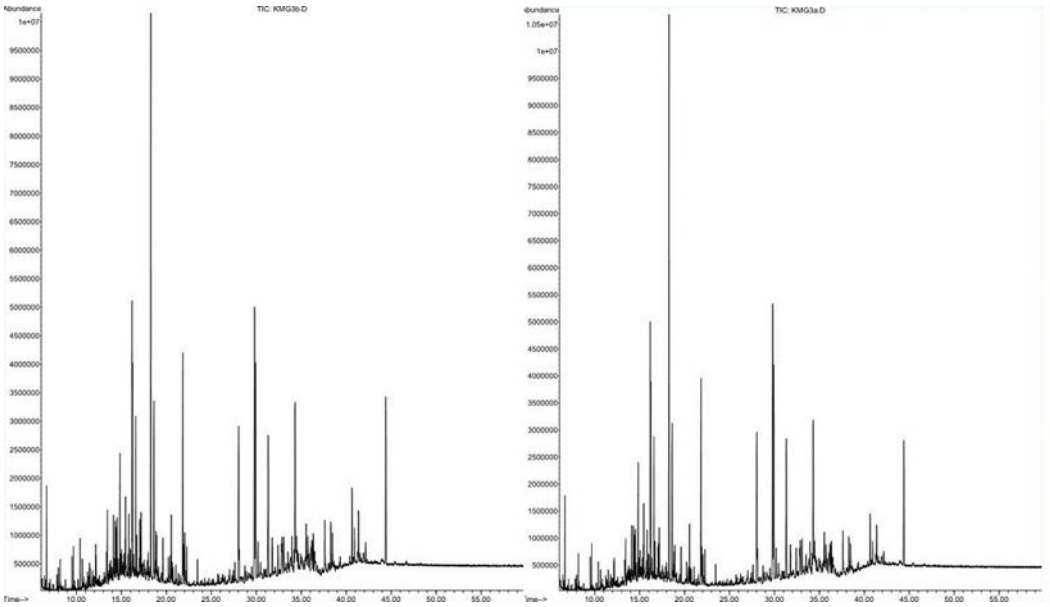
Bar plots of water meter readings
https://drive.google.com/open?id=0B9SDhZA4tgN3dmRiRlBVTUdSQnc

Chapter 4 – PCA:

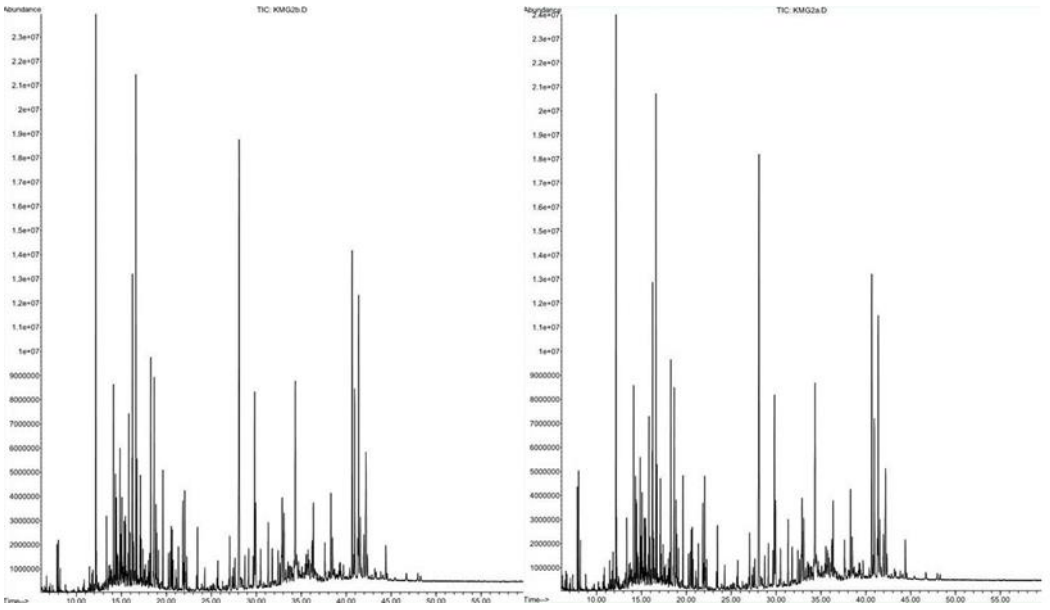
PCA of intertidal species survey
https://drive.google.com/open?id=0B9SDhZA4tgN3RXlPejRmQzBuUVU

Appendix D

Chromatograms of Water Samples Analysed by GC/MS for Organics



Chromatograms of water sample collected 10-06-14.



Chromatograms of water sample collected 28-11-14.

Appendix E

Lab Notes of Sediments Extraction for GC/MS

Sediments analysis Lab notes

Sediments methods were adapted from SCION's 'in house' method 'Analysis of Sediments and Soils for Resin Acids by Soxhlet Extraction'.

Glassware and Apparatus

All glassware and apparatus apart from measuring cylinders were muffled at 450°C. Condensers were rinsed with acetone. Measuring cylinders rinsed with acetone and isopropanol hexane (1:1). Once clean all glassware and accessories were covered with aluminium foil to minimise potential build-up of dust or other contaminants. Initially mortar and pestle were cleaned thoroughly with detergent, rinsed very well in hot water then acetone. Sodium sulphate was then added and ground to give a final clean and remove any traces of moisture. Between samples it was rinsed 3 times with acetone, sodium sulphate was ground inside it. It was then re-rinsed with acetone.

Apparatus list per extraction

- 250mL Round bottomed flask
- Soxhlet
- Funnel
- Small beaker
- Zymark tube (200mL)
- Drying tube
- 2 small beakers

Standards

A blank and blank plus standard was run using standards prepared at SCION. Syringes were rinsed three times in acetone and once in the standard before use.

Surrogate Standard Solution

2,4,6-Tribromoanisole

2,4,6-Tribromophenol

D10-Anthracene

D31-Palmitic acid

8(14)-Abietenic acid

Dihydrocholesterol

Internal Standard Solution

Primary stock solution: Accurately weigh (+/- 0.1 mg) approximately 25 mg Dibromoanthracene into 40 ml. reagent bottle and dissolve in 25 mL pyridine. Record date and concentrations in standards logbook.

Extractives Screen standard solution

Alpha-pinene

Beta-pinene

Fenchone

Camphor

Fenchol

Borneol

Terpinen-4-ol

Alpha-terpineol

Guaiacol

Eugenol

Vanillin

Acetovanillone

Vanillic acid

Homovanillic acid

Ferulic acid

Gallic acid

Syringol

Acetosyringone

Syringylaldehyde

Syringic acid

Coniferyl alcohol

Coniferyl aldehdye

Pinosylvin, mono methyl ether

F10:0

F12:0

F14:0

Palmitoleic acid (F16:1)

Palmitic acid (F16:0)

Margaric acid (F17:0)

Linoleic acid (F18:2)

Oleic acid (F18:1)

Linolenic acid (F18:3)

Elaidic acid (F18:1)

Stearic acid (F18:0)

F20:0

F22:0

F24:0

Fichtelite

Dehydroabietin

Tetrahydroretene

Retene

Methyldehydroabietin

Pimaric acid

Sandaracopimaric acid

Isopimaric acid

Palustric acid

Levopimaric acid

Dehydroabietic acid

Abietic acid

Neoabietic acid

Pimarenic acid

Sandaracopimarenic acid

Isopimarenic acid

13-Abietenic acid

Pimaranic acid

Isopimaranic acid

Abietanic acid

Seco-1-DHA acid

Seco-2-DHA acid

12-ChloroDHA acid

14-ChloroDHA acid

12,14-DichloroDHA

7-OxoDHA acid

Cholesterol

Campesterol

Stigmasterol

Sitosterol

Sitostanol

Sample Preparation

Standards and sediment sample were removed from the fridge or freezer and brought to room temperature (approximately 1 hour).

Sample Extraction Sediments

Each sample was transferred to mortar and pestle and homogenised. Sample was then split approximately into four. Pre-muffled (450°C) sodium sulphate was mixed with one sample of the sediment (1:1) in mortar and pestle. Small beakers were pre-weighed then reweighed with sediment sample (+/- 0.1mg).

Soxhlet extractor was prepared in the following order. A layer of glass wool was followed by a layer of sodium sulphate. The sample was then added followed by 50 µL of surrogate standard solution. This was followed by a final layer of sodium sulphate. A blank and blank plus extractive standard was also prepared

Approximately 150 mL of isopropanol hexane (1:1) and anti-bumping granules were added to the round bottomed flask and attached to the soxhlet. Condensers were connected and water turned on. Heating mantels were turned on and extraction run for 12-16hrs.

Once cooled soxhlets were dismantled and condensers rinsed with acetone. Extract was transferred from round bottom flask to a Zymark tube which was pre-prepared with glass wool to the first shoulder and sodium sulphate to the second shoulder. Extract was concentrated to 5 mL on the Zymark then rinsed with hexane and re concentrated.

Extract was finally transferred to GC Vials through a micro dying tube (glass dropper) again pre-prepared with glass wool to first shoulder and

sodium sulphate to approximately 20 mm from the top. Zymark tube was rinsed twice with hexane to ensure all extract was transferred. At this time a blank vial containing hexane and hexane plus extractive standard were also prepared. To all vials 50 µL of injection standard and 50 µL of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA + TMCS, 99:1) for derivatisation, was added. Tops were screwed tightly and placed in a drying oven at 70°C for one hour. Vials were analysed by GC/MS as soon as possible once taken out of the oven.

Once analysis was complete identification and quantification was carried out by the author, under the guidance of staff from Scion, using Agilent MSD Chemstation version G1701DA D.01.02.16 enviroquant software. The most conservative methods were applied and compounds only included where certainty was 100%.

Appendix F

Complete Results of ICPMS Analyses of Sediments

		S1	S2	S3	S4	S5	N1	N2	N3	N4	N5	X1	X2	X3	X4	X5	X6	C1	C2
Boron	B 10	25.25	20.67	24.72	16.96	11.90	16.62	8.70	18.32	13.77	11.06	4.51	6.82	7.51	7.87	8.59	6.89	4.16	4.41
Sodium	Na 23	10227.95	8651.01	9386.67	8372.25	7300.51	9343.71	6972.20	9711.90	7035.85	8205.68	2614.61	5006.40	5433.90	5378.49	5282.01	4468.22	4845.92	5131.04
Magnesium	Mg 24	2684.26	2590.04	2651.88	2325.06	1963.95	2237.39	1450.26	2216.84	1917.04	1922.97	1166.11	1269.92	1332.11	1607.30	1608.10	1152.83	739.56	804.20
Aluminium	Al 27	8283.44	8785.70	8599.43	7473.46	5615.72	6457.07	3655.22	9308.69	5687.26	5047.31	1305.66	2295.38	2604.59	2718.85	3100.40	2532.43	1137.01	1332.16
Phosphorous	P 31	294.53	261.84	252.97	204.91	169.95	238.94	124.84	168.30	193.97	157.87	140.72	119.00	121.30	90.15	114.02	67.97	83.66	90.56
Potassium	K 39	1171.82	1199.27	1254.67	1082.22	878.42	1059.97	752.64	1102.46	910.49	915.72	449.66	588.57	711.28	606.73	668.51	559.79	528.54	599.47
Calcium	Ca 43	7373.02	9949.36	8659.95	8660.94	8694.13	9951.75	3861.66	6821.71	7629.97	13563.03	81440.49	11987.35	5922.47	35423.59	36735.93	1910.90	23790.28	50994.54
Vanadium	V 51	13.23	13.47	12.88	10.60	8.28	9.69	5.88	9.35	8.99	7.88	5.50	6.42	5.58	5.06	6.64	5.36	3.94	4.52
Chromium	Cr 52	5.77	6.00	5.78	6.48	4.34	4.54	2.15	4.86	4.25	3.94	1.80	3.34	3.73	2.72	3.70	3.10	1.96	2.01
Iron	Fe 54	7232.91	7474.28	7170.64	6107.61	5142.89	5734.72	6816.20	5245.98	5209.00	4715.99	5226.00	3011.94	3244.61	5780.71	4630.32	3053.25	4522.87	4838.85
Manganese	Mn 55	54.77	60.87	51.32	49.92	46.61	38.93	277.17	38.89	49.69	50.97	66.00	20.09	17.74	111.85	46.17	21.98	33.27	38.74
Cobalt	Co 59	1.14	1.21	1.08	1.02	0.88	0.96	2.75	0.95	0.92	0.90	0.59	0.39	0.47	1.07	0.76	0.46	0.33	0.41
Nickle	Ni 60	2.28	2.54	2.32	2.06	1.55	1.79	0.86	1.82	1.73	1.65	1.84	1.02	1.02	1.21	1.51	0.78	0.76	1.11
Copper	Cu 63	5.06	4.60	5.06	3.65	2.25	3.73	2.42	4.06	2.90	2.26	0.31	0.45	0.63	0.70	2.18	0.72	0.23	0.32
Zinc	Zn 68	34.85	27.18	26.57	21.87	17.02	21.50	17.50	24.34	18.05	27.99	5.84	8.62	8.66	18.59	17.80	11.00	5.32	5.06
Arsenic	As 75	5.98	6.03	5.21	5.69	4.95	4.40	3.07	3.89	4.99	4.93	10.47	7.42	4.44	7.14	5.81	5.06	5.26	5.73
Selenium	Se 82	0.77	0.75	0.80	0.69	0.54	0.70	0.48	0.80	0.54	0.51	0.22	0.25	0.27	0.37	0.38	0.27	0.15	0.22
Strontium	Sr 88	61.87	81.76	72.09	72.82	72.76	79.08	38.35	62.30	60.86	112.62	612.31	76.98	43.02	197.39	215.12	14.62	111.37	227.96
Silver	Ag 109	0.03	0.03	0.03	0.02	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01
Cadmium	Cd 111	0.10	0.07	0.09	0.06	0.04	0.08	0.03	0.06	0.05	0.04	0.01	0.01	0.02	0.01	0.03	0.01	0.01	0.01
Barium	Ba 137	19.09	24.83	23.24	19.12	16.31	21.52	44.05	55.76	14.61	15.94	4.22	4.07	5.39	5.68	8.96	3.67	2.11	2.93
Hg	Hg 202	0.04	0.06	0.05	0.04	0.03	0.03	0.03	0.04	0.02	0.02	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.01
Thallium	Tl 205	0.04	0.07	0.05	0.04	0.03	0.04	0.03	0.03	0.08	0.07	0.03	0.03	0.05	0.08	0.10	0.08	0.02	0.03
lead	Pb 207	6.09	6.04	5.98	4.89	3.92	4.50	3.89	6.61	4.05	6.11	1.04	1.56	1.75	2.67	2.90	2.07	1.02	1.14
Uranuim	U 238	0.70	0.61	0.67	0.56	0.37	0.61	0.33	0.98	0.46	0.42	0.14	0.18	0.18	0.23	0.41	0.33	0.09	0.13

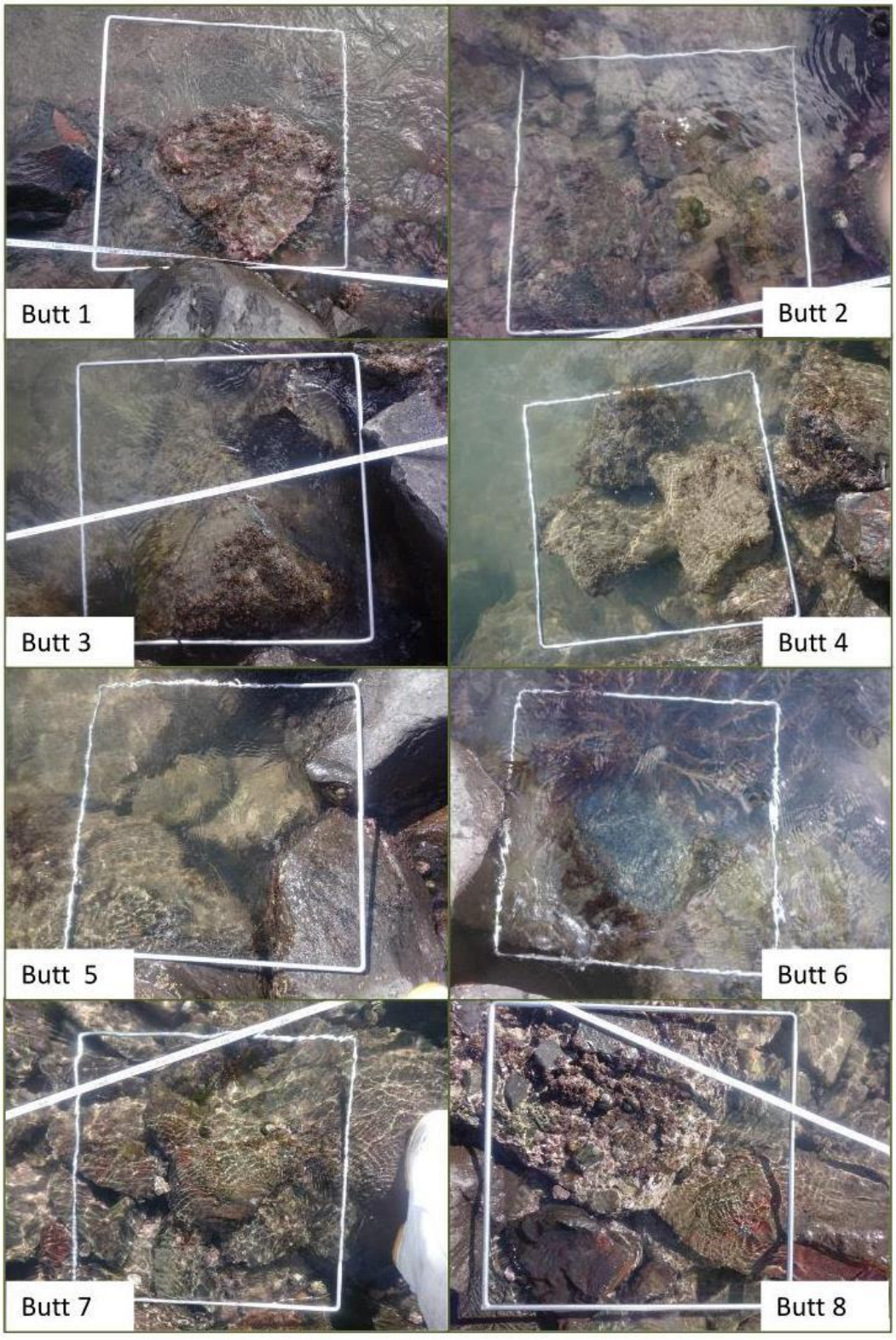
Appendix G

Low Tide Survey Results and Photos

All species observed during low tide surveys (Chapter 4) with common name and presence at each site.

Species observed	Common name	Butters	Berth 11
Lunella smaragda	Cats eye	Y	Y
Dicathais orbita	White rock whelk		Y
Cominella virgata	Red mouthed Whelk	Y	
Buccinulum linea	Lined whelk		Y
Saccostrea glomerata	Rock oyster	Y	Y
Sypharochiton pelliserpentis	Snakeskin Chiton	Y	Y
Patiriella regularis	Cushion star		Y
Evechinus chloroticus	Kina	Y	Y
Anthothoe albocincta	Anemone		Y
Actinia tenebrosa	Wharatah anemone	Y	Y
Oulactis sp.	Large shore anemone		Y
Elminius modestus	Barnacle	Y	Y
Carpophyllum maschalocarpum		Y	
Corallina officinalis		Y	Y
Dictyota ocellata		Y	
UNID red algae		Y	Y
Non geniculate coralline alge	pink paint	Y	Y
Zostera muelleri	Seagrass	Y	Y
Colpomenia claytoniae		Y	
Undaria pinnatifida		Y	
Codium fragile		Y	
Orange encrusting sponge			Y

Butters Wharf Site Quadrat Photos



Berth 11 Site Quadrat Photos