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**Toitu Te Moananui a Toi –
The Effects of the MV *Rena* on the Water
Quality, Chemistry and Zooplankton of Otaiti
(Astrolabe Reef)**

by

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Nāu te rourou, nāku te rourou

Ka ora ai ngā tangata

With you contribution, and my contribution

the people will flourish

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Abstract

Humans have had a profound effect on the world's oceans, particularly through pollution. Marine pollution incidents are particularly important and increasingly involve both petrochemical and metal contamination. The effects of complex high level, point source pollution events such as ship wrecks on larval recruitment to reef environments is not well understood. Larval settlement is heavily influenced by chemical cues, hence pollution events could have a long term influence on reef ecology, and the likelihood that planktonic zooplankton can entrain contaminants into the reef food web is also of concern.

This thesis aims to address concerns from local Tangata Whenua, government, researchers, stakeholders and the public about the long term recovery of Otaiti following the MV *Rena* shipwreck and subsequent reef contamination. The incident is a complex one involving metallic container debris contamination along with oil. Research focussed on assessing the effects of water borne contamination surrounding the wreck site in order to examine acute and chronic responses of planktonic invertebrates: (1) the influence of *Rena* and associated debris to the chemistry and quality of water in the benthic zone around the wreck, (2) the toxicity of contaminants to survivorship of zooplankton, and (3) the influence on contaminant plumes to zooplankton recruitment behaviour of Otaiti.

There is a clear effect from the *Rena* and its associated debris field on the water quality and chemistry of Otaiti. Aluminium and copper for example, were consistently elevated in dissolved and total metal concentrations around the debris field. Laboratory based exposure of zooplankton to realistic concentration gradients of *Rena* contaminated sediments resulted in increased mortality with increased contaminant concentrations. Behavioural responses of pelagic and settling invertebrates to *Rena* pollution influenced sediment highlighted sensitivity to associated contaminant plumes. This could have significant ecological implications to the recruitment behaviour of other planktonic organisms that rely on reef conspecifics and chemical cues to initiate settlement.

Ecological concepts such as ecosystem function and biodiversity dynamics (including recruitment) relate the life force and longevity of a system. Key concerns within the scope of this research were highlighted that may have implications to Tangata Whenua decision making in assessing the Mauri of Otaiti. It can be implied that the *Rena* continues to impact the Mauri of Otaiti due to the presence of contaminants within the debris field.

Table of Contents

Acknowledgements.....	i
Abstract.....	iii
Table of Contents.....	v
List of Figures	viii
List of Tables.....	x
Glossary of Terms.....	xii
Chapter 1	1
General Introduction	
1.1 Introduction	2
1.2 The MV <i>Rena</i> Grounding.....	3
1.3 Otaiti (Astrolabe Reef)	3
1.4 In Response to the MV <i>Rena</i>	4
1.5 Restoration of Mauri.....	6
1.6 Review of Current <i>Rena</i> Research	7
1.7 Thesis Outline.....	9
1.7.1 <i>Research Limitations</i>	11
Chapter 2	12
Effects of the MV <i>Rena</i> debris field to dissolved metal content of reef boundary layer water of Otaiti (Astrolabe Reef)	
2.1 Introduction	13
2.2 Methodology	15
2.2.1 <i>Study site and field procedures</i>	15
2.2.2 <i>Sample Preparation</i>	18
2.2.3 <i>Calculations</i>	19

2.2.4	<i>Statistical methods</i>	19
2.3	Results.....	20
2.3.1	<i>DGT - dissolved metal concentrations</i>	20
2.3.2	<i>Water samples – total metal concentration</i>	24
2.4	Discussion	26
 Chapter 3		 30
Acute toxicity effects of copper and contaminated reef sediment on the early life stages of <i>Tenagomysis</i> sp. and <i>Ovalipes catharus</i>		
3.1	Introduction	31
3.2	Materials and methods.....	34
3.2.1	<i>Specimen collection</i>	34
3.2.2	<i>Experimental design</i>	35
3.2.3	<i>Statistical analysis</i>	36
3.3	Results.....	37
3.3.1	<i>Physico-chemical influences on mortality</i>	37
3.3.2	<i>The LC₅₀ experiment</i>	38
3.4	Discussion	41
3.4.1	<i>Goals and hypothesis testing</i>	41
3.4.2	<i>Physico-chemical factors</i>	42
3.4.3	<i>Biological impacts of metals</i>	43
 Chapter 4		 44
Behavioural responses of a planktonic mysid to waterborne contaminants		
4.1	Introduction	45
4.2	Methodology	46
4.2.1	<i>Collection of sample water and animals</i>	46

4.2.2	<i>Choice chamber</i>	47
4.2.3	<i>Statistical analysis</i>	49
4.3	Results	50
4.3.1	<i>Water chemistry</i>	50
4.3.2	<i>Choice experiment</i>	51
4.4	Discussion	53
 Chapter 5		55
General Discussion		
5.1	Thesis Design	56
5.2	Environmental contamination and reef ecology	56
5.3	Mauri of Otaiti post- <i>Rena</i>	57
5.4	Concluding Statement	59
 References		60

List of Figures

- Figure 1-1. Location of Otaiti (Astrolabe Reef). Source: Ministry for the Environment (2011) 4
- Figure 1-2. Chart of the Bay of Plenty showing the position of Otaiti (Astrolabe Reef), the exclusion zone, Mōtītī Island, and the mainland. Samples were taken from the within exclusion zone, and the northwest side of Mōtītī Island was the control location. Source: www.renarecovery.org.nz 6
- Figure 2-1. DGT and bulk water sampling sites on the outer reef (top right); and debris field (bottom right) of Otaiti (Astrolabe Reef) and the control site (Mōtītī Island). Red circles indicate sites sampled based on *Rena* Long Term Environmental Monitoring Programme 16
- Figure 2-2. A DGT-holding unit with three DGT samplers installed and attached to a 10kg vinyl coated plate on the seabed at a sample site. 17
- Figure 2-3. Overview of the water sampling regime over a 7 day period. DGT holding units were anchored to 10kg weights on the seafloor. Total water samples were taken on deployment and recovery of DGT holding units at each site. 17
- Figure 2-4a. Metal concentrations as measured by DGT for the 3 sampling areas; debris field, outer reef and Mōtītī Island (control site). Circles show mean concentration ($\mu\text{g/l}$), boxes represent standard (SE) and capped lines show confidence intervals (CI) of the DGT devices deployed together..... 21
- Figure 2-5. PCA ordination of water chemistry from sampling sites in the Otaiti debris field (=△), Otaiti outer reef (=■) and Mōtītī Island (control site) (=○) A total variance of the data of 63.5% was explained by PC1 and PC2. 23

Figure 2-6. Metal concentration as measured from bulk water samples for the 3 sampling areas on deployment and recovery. Circles show mean concentration (μg), boxes represent standard (SE) and capped lines show confidence intervals (CI)..... 25

Figure 3-1. *Rena* polluted reef sediment sourced from high impact area on Otaiti. Copper filings can clearly be seen. 35

Figure 3-2. Oxygen consumption for the *Rena*, copper and control treatments over 1 hour. There was a clear indication of stability in oxygen levels so depletion in oxygen was not a factor influencing mortality of test subjects 37

Figure 3-3. Comparison of a A) live paddle crab larva and B) dead paddle crab larva used in acute toxicity assay and infested with parasites. Arrow indicates a particularly large, unidentified parasitic organism. 38

Figure 3-4. Mean survival of mysid shrimp (A,B) and paddle crab larvae (C, D) when exposed to copper contaminant in solution (A, C) and on reef *Rena* contaminants in solution (B, D) over the experimental duration..... 39

Figure 4-1. Schematic of choice apparatus..... 47

Figure 4-2. Mean percentage ($\pm\text{SE}$) preference of mysid shrimp during the a) static and b) flow through treatment experiments 52

List of Tables

Table 2-1. One way analysis of variance results. Manganese was the only metal which indicated significance ($p < 0.05$).....	23
Table 2-2. Eigenvalues, percentage variation and cumulative percentage variation of normalised dissolved metal data explained by PC1 and PC2.....	23
Table 2-3. Schematic representing the concentration of measured total water to ANZECC marine water trigger values using the statistical distribution methods with 95% protection. Green = an order of magnitude below trigger value; blue= below trigger value; orange = above trigger value; red = exceedance of trigger value by an order of magnitude or more.	28
Table 3-1. Indicative LC ₅₀ percentage values derived from Figure 3-2 for mysid and paddle crab test organism at 4 key time frames	40
Table 3-2. Indicative LC ₅₀ values ($\mu\text{g/L}$) of copper derived from Figure 3-2 and calibrated with water sample analysis by McSweeney (2015, <i>in prep</i>) for mysid and paddle crab test organism at 4 key time frames.....	40
Table 4-1. Mean ($\pm\text{SE}$) values for 5 metals based on analysis undertaken by McSweeney (2015, <i>in prep</i>) comparing copper to <i>Rena</i> polluted sediments.	48
Table 4-2 . Comparison of mean ($\pm\text{SD}$) physico-chemical characteristics from the control vs. static and flow treatment choice trials.....	50
Table 4-3 . Mean percentage of time spent in the preferred test area in a static and flow through test. Mx= mix zone (unobservable), Ch=choice channel (treatment or control). Values in red show significance	51
Table 4-4 . Mean percentage of time spent in the preferred test water type in a static and flow through test. T= treatment , C=control, *=no significant preference.....	52

Glossary of Terms

Māori	English
Ahi kā	Local people who actively keeps the home fires burning
Aotearoa	"The land of the long white cloud", New Zealand
Hapū	Subtribe
Hawaiki	Ancient homeland
Hui	Gathering
Ika	Fish
Iwi	Tribe
Kaimoana	Seafood; shellfish
Kaitiaki	Custodian; guardian; caretaker
Kaitiakitanga	Active protection of Mauri
Karakia	Prayer or incantation
Kaumātua	Elder
Kawa	Customary protocol; ceremony
Mahinga kai	Food gathering place
Mamae	Hurt
Mana	Prestige
Mana Motuhake	Self determination
Manaakitanga	To show hospitality
Māori	Indigenous people of New Zealand
Mātauranga Māori	Māori knowledge and intergenerational way of life
Mauri	<i>refer page 6</i>
Moana	Ocean or sea
Mōtītī	An island within the Bay of Plenty region
Ngā Atua Māori	Gods of the natural environment
Ngāi Te Hapū/Patuwai	The subtribe of Mōtītī

Ngātoroirangi	Distinguished Te Arawa priest
Otaiti	Astrolabe Reef
Papatuanuku	Earth Mother
Rangatiratanga	Authority
Ranginui	Sky Father
Rawaho	Outsider; someone from another place
Tangata Whenua	Local people
Tapu	Sacred; restriction
Te Ao Mārama	The World of Light
Te Ao Māori	Māori worldview
Te Kore	Complete nothingness (stage of creation)
Te Moana-A-Toi	The Bay of Plenty region
Te Pō	Utter darkness (stage of creation)
Tikanga	Custom
Tino Rangatiratanga	Absolute authority
Tohunga	Expert; agent of the spiritual realm
Uri	Offspring
Waharoa	Gateway
Wāhi tapu	Sacred site; site of cultural significance
Whanaungatanga	kinship
Whenua	Land

Chapter 1

General Introduction

1.1 Introduction

Indigenous peoples worldwide share an intrinsic and spiritual connection with the natural environment. For coastal communities, the ocean is a source of spirituality, sustenance, and survival (ANZECC & ARMCANZ, 2000; Hamann, *et al.*, 2006; NOS, *et al.*, 2015). Māori have been concerned for some time about the degradation of coastal resources, the loss of *kaimoana* (seafood, and the increasing toxicity of waterways, sediments and marine fauna which have associated negative impacts on cultural identity (Hardy, *et al.*, 2011). An ongoing relationship with the natural environment and its resources is key to the cultural endurance of Māori and Māori values (Dick, *et al.*, 2012; Ellis, 2012). Basic exploration into the fundamental values, principles and concepts of the indigenous worldview stems from a shared creation story. Māori cosmogony originates in *Te Kore* (complete nothingness) and *Te Pō* (the utter darkness). From these devoid states of being, *ngā Atua Māori* (the gods) strategized on how to increase light and life in the world. The separation of the parents; *Ranginui* (Sky Father) and *Papatūānuku* (Earth Mother) leads to the emergence of *Te Ao Mārama* (The World of Light) (Marsden & Henare, 1992; Roberts, *et al.*, 1995; Himona, 2001; Ellis, 2012). *Ngā Atua* are the supernatural beings responsible for every aspect of our natural world and are acknowledged through *karakia* (prayer), *tikanga* (custom) and *kawa* (ceremony) (Himona, 2001; Ellis, 2012).

Te Ao Māori is a holistic world view that recognises the interrelatedness and interdependence of people (alive and deceased), resources, elements, landforms, flora and fauna, metaphysical beings and bodies of knowledge (Waitangi Tribunal, 2011; Ellis, 2012; Robb, 2014;). *Whanaungatanga* (kinship) is a key concept that was maintained throughout Māori evolution; from the period of creation to the oceanic migration from *Hawaiki* (the ancient homeland) to Aotearoa (Waitangi Tribunal, 2011). Principles such as *kaitiakitanga*, *mauri*, *tapu*, *tikanga*, *kawa*, *rangatiratanga*, *mana*, *mātauranga*, *whakapapa* and *manaakitanga* [refer Waitangi Tribunal (2011) for descriptions] are all vital within Te Ao Māori and are bound together in

mutual responsibility. Cultural consequences from the disassociation of the natural environment can include the deterioration of relationships between people and food species; severed transmission of traditional cultural knowledge; reduced connection between people and community; erosion of kinship; impaired health and impaired tribal development (Dick *et al.*, 2012).

1.2 The MV *Rena* Grounding

In the early hours of the October 5th, 2011, the 37,209t Liberian flagged container ship MV *Rena* ran aground on Otaiti (Astrolabe Reef) en route to Tauranga from Napier. The MV *Rena* was travelling at a maximum speed of 17 knots when it struck the reef following an alleged alteration to the ships course to meet the tidal window of opportunity to enter the Port of Tauranga at 3am. The vessel carried a crew of 25, with 1733 tonnes of heavy fuel oil (HFO) and 1368 cargo containers, including 11 containers of declared dangerous goods on board (Transport Accident Investigation Commission, 2012; Murdoch, 2013; Maritime New Zealand, 2014). The grounding was declared a tier three emergency by Maritime New Zealand (MNZ) and salvage operations commenced to remove oil form the stricken ship on the 9th of October 2011 (Maritime New Zealand, 2014).

1.3 Otaiti (Astrolabe Reef)

Otaiti (Astrolabe Reef) is located in the Bay of Plenty, approximately 7 km north of Mōtītī Island and 25km northeast of Tauranga Harbour (Figure 1-1, 37°32.4'S, 176°25.7'E). The reef is comprised of Miocene volcanic rocks located in a high energy environment where waves break the surface at low tide (Ngai Te Hapu Incorporated, 2013; Beca, 2014). Otaiti provides for approximately 49ha of valuable marine habitat to a depth of 60m; with the reef structure extending further to the sedimentary seabed at 75m. It is recognised as an “Area of Significant Conservation Value” as a haul out site for the New Zealand fur seal, *Arctocephalus forsteri*. The reef ecology is localised but famous for a diverse variety of biota including benthic and pelagic fish, sponges, molluscs, urchin, and algae (Robertson, 2014).

Otaiti is the *waharoa* (gateway) to Mōtītī Island and a significant *wāhi tapu* (sacred site). Ngātoroirangi, a great Te Arawa *tohunga* (priest) performed a *karakia* (incantation) to render the reef *tapu* (sacred) as he made way for land on voyage to *Aotearoa* (New Zealand)(Ministry for the Environment, 2011; Ngai Te Hapu Incorporated, 2013). The reef has is a *mahinga kai* (food-gathering place) for all types of *kaimoana* (seafood) and *ika* (fish)for the descendants of Te Patuwai *hapū* (subtribe) of Mōtītī Island, and is a favoured recreational dive site (Ngai Te Hapu Incorporated, 2013; Beca, 2014). Otaiti is a significant area of cultural, environmental and recreational value.

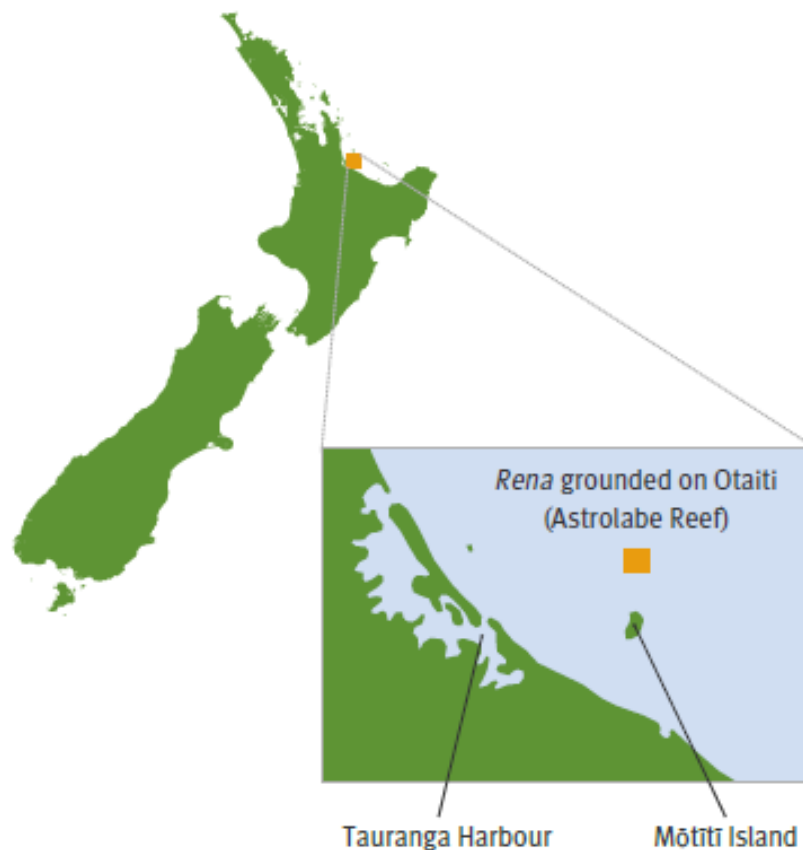


Figure 1-1. Location of Otaiti (Astrolabe Reef). Source: Ministry for the Environment (2011)

1.4 In Response to the MV *Rena*

In the days and months that followed the *Rena* grounding, container debris and 350-400 tonnes of oil spilt into the ocean. Due to the dynamic nature of

Otahi, tonnes of debris continued to fall from the steadily disintegrating ship. In January 2012 the ship split in half spilling a further 200-300 containers into the ocean. The force of the ocean and weather pushed the stern section off the reef in April 2012, sinking it to 23-65m deep (Maritime New Zealand, 2014).

The isolated predominantly Māori communities of Mōtītī, Matakana, and Maketu were hit hardest by the oil and debris. Immediately these communities felt the *Rena* had been to the detriment of significant cultural values which adversely impacted their social, cultural, environmental and economic well-beings. In response to the *mamae* (hurt), these communities acted in their role as *Kaitiaki* (custodians) and co-ordinated community driven *Rena* response units until official resourcing and assistance was available (Broughton, *et al.*, 2013; Ngai Te Hapu Incorporated, 2013; Hinemoana Associates, 2014). At the height of the response, around 9000 people were involved in elements of beach clean-up, wildlife, logistics, management, and salvage. Around 8000 of these workers were volunteers cleaning the beaches of oil and oiled debris (*Rena* Recovery Project, 2012).

Salvage operations continued as the bow section was cut down to one metre below low tide and a total of 1039 containers were eventually recovered. A remaining 329 containers are either trapped in the ship remains or contribute to the approximately 10,000m² debris field area that remains on the reef. This debris field is made up of scrap metals from the ship's structure, containers and cargo; together with non-recyclable materials like packaging (Maritime New Zealand, 2014). There are serious concerns from the Iwi, Hapū and wider community throughout the Bay of Plenty region around potential long-term effects and consequences of the oil, dispersant, debris and contaminant mixtures to the environment, ecology, kaimoana, wildlife and Mauri of the moana (Dickson *et al.*, 2012; Broughton *et al.*, 2013; Ngai Te Hapu Incorporated, 2013; Hinemoana Associates, 2014).

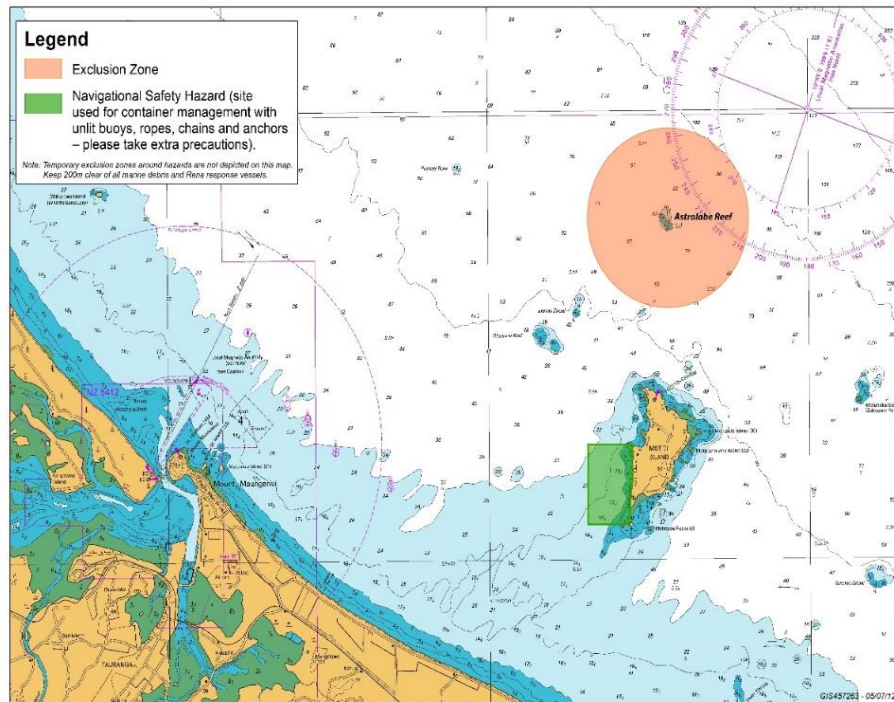


Figure 1-2. Chart of the Bay of Plenty showing the position of Otaiti (Astrolabe Reef), the exclusion zone, Mōtītī Island, and the mainland. Samples were taken from the within exclusion zone, and the northwest side of Mōtītī Island was the control location. Source: www.renarecovery.org.nz

1.5 Restoration of Mauri

The release of the *Rena* Long-Term Environmental Recovery Plan (RLTERP, 2011) had a key goal to restore the Mauri of the affected environment to its pre-*Rena* state. This directive (inspired by local Iwi) created a precedent in New Zealand environmental response action as directed by Government, and is one of the drivers for the work embodied here. It recognises the important meta-physical considerations which would not otherwise be included in a conventional assessment (Morgan, *et al.*, 2013).

Mauri is difficult to translate in wholeness as it is meta-physical, intrinsic and the core to many of the aforementioned Māori values. It can be described as the essential life force, the life supporting capacity of all things, and the fusion that holds the physical and meta-physical elements of being together to make existence possible (Durie, 1998; Hikuroa, *et al.*, 2011; Fa'au'i &

Morgan, 2014). Mauri is the central link that brings Māori values together. Kaitiakitanga is the active protection of Mauri (Fa'au'i & Morgan, 2014; Robb, 2014) to ensure longevity for future generations (Marsden & Henare, 1992; Hardy *et al.*, 2011; Robb, 2014). It can be specifically defined as:

“... the obligation, arising from the kin relationship, to nurture or care for a person or thing. It has a spiritual aspect, encompassing not only an obligation to care for and nurture not only physical well-being but also mauri” (Waitangi Tribunal, 2011, p. 24).

Mātauranga Māori is loosely defined as ‘Māori knowledge’ but is a broader concept that encompasses the Māori way of knowing, relating and interacting with the world (Mead, 2012; Robb, 2014) from inter-generational observations and experiences (Hardy *et al.*, 2011). As mentioned previously; Te Ao Māori principles are interrelated and holistic so cannot be isolated from one another. That is where the uniqueness and complexity of *Mātauranga Māori*, and Mauri, is derived.

The development of assessment tools such as the Cultural Health Index (Tipa & Teirney, 2006), the Mauri Model (Morgan, 2006) and others provides a means for evaluating the state of an environment utilising Kaitiaki perspectives and values. The concern for *Tangata Whenua* (local indigenous people) of Te Moananui a Toi is that the *Rena* grounding will have detrimental impact to the ongoing relationship and well-being of the *moana* (ocean).

1.6 Review of Current *Rena* Research

Pre-*Rena* environmental literature and data pertaining to Otaiti was sparse to non-existent. The *Rena* Recovery Programme set out to advance the understanding of the effects of the *Rena* contaminant mixtures and address the lack of information on the possible environmental effects of this event to the local environment. Research around the MV *Rena* was initially orientated towards environmental concerns associated with heavy fuel oils (HFOs) around the coast; and only in recent times has a focus on Otaiti eventuated.

There was very little documented baseline data for Otaiti and the surrounding marine-scape so monitoring programmes were implemented for the mainland soft shore, rocky shore, estuarine and subtidal reef areas around Tauranga and Otaiti. As access to Otaiti in the early stages of the *Rena* event was not possible (due to salvage operations), proxy reef systems were surveyed. These included Mōtītī, Tūhua, Kāwera, Okarapu *Motunau* (Plate) and *Motuhaku* (Schooner) reefs. Chemical analyses looked into the chemical fingerprint of *Rena* oil, the characterization of polyaromatic hydrocarbons (PAHs) in sediments and macro-fauna, and an interim analysis of metals in on-reef sediments. This included the assessment of natural sediment/rock metals content given the area is known to be volcanic (Battershill *et al.*, 2013).

Secondary research looked into the effects of heavy fuel oils (HFO), Corexit 9500 or a mixture, in ecotoxicology (Ling, 2013; Muncaster, 2013), the efficiency of cleaning techniques (Gaborit-Haverkort, 2013), initial mauri assessments (Stieger), and the mixing and modelling of oil and debris (de Lange, 2013)(Battershill *et al.*, 2013). Research continues with active projects looking at volunteer efforts and the effect of other *Rena*-related contaminants to bacteria and juvenile paua stocks.

It was clear, however, that there was an urgent need to refocus research and monitoring on Otaiti itself especially in the areas influenced by the now sunk and collapsed vessel. A new program of monitoring commissioned by the *Rena* owners and administered by BECA is current (Ross, Battershill, & Loombe, *in press*). Despite this comprehensive program, water quality associated with the debris field was not examined. This was seen as an important omission, given the directive from the Minister for the Environment concerning Mauri, and the possible longer term effects of the reef relevant to zooplankton as sources of food and recruitment to the reef. Zooplankton species are fundamental building blocks to the reef ecosystem, constituting the resource of important reef species in larval phases which are sensitive to the physicochemical characteristics of settling environments (Roberts, *et al.*, 2010). Ecological concepts such as ecosystem function and biodiversity

dynamics (including recruitment) can relate the life force and longevity of the reef system.

1.7 Thesis Outline

This thesis is multi-analytical, combining ecological, biological, toxicological, and chemical concepts to address the impact of the MV *Rena* grounding to the water quality, chemistry and zooplankton settlement on Otaiti (Astrolabe Reef). It employs three research chapters and a synthesis chapter;

Chapter 2 is field based and explores the chemistry of Otaiti and the *Rena* debris field (as it may affect surrounding water quality), focusing on metals. Metals are directly relevant as a major contaminant component, but also because little research has been undertaken to look at the chemical pollution of metals (apart from TBT) following a ship grounding. This chapter provides a platform for the following two chapters which are laboratory based assays on zooplankton species.

Chapter 3 reports at the acute toxicity of contaminants in solution to selected zooplankton species that have been chosen to represent both food items for reef associated fishes and invertebrates, as well as acting as proxies for settling phases (out of the plankton) of reef associated invertebrates such as important kaimoana species: kina (*Evechinus chloroticus*), paua (*Haliotis iris*) and crayfish (*Jasus edwardsii*). Though the contaminant mixtures locked in on-reef sediment are the focus, copper is used as a specific treatment control as it is known to be a prevalent metal in *Rena* polluted reef sediments. It provides a standard by which the reef collected contaminated sediments can be compared.

Chapter 4 focusses on the on-reef contaminated sediments which are a complex mix of metals and natural sediments, to examine how water-borne plumes emanating from the sediments may influence the behaviour of zooplankton species presumably through olfactory responses.

Chapter 5 is a synthesis of all work, drawing on the key points from the research chapters and perspectives from this chapter. Collectively the

components of research seek to address the key concerns from local Tangata Whenua and academics by providing a detailed biophysical picture of some aspects of coastal water quality and health of Otaiti.

All chapters, apart from introduction and synthesis components, are written as stand-alone papers for submission to peer-reviewed journals. For ease of reading they are written in the standard scientific paper format (abstract, introduction, methods, results, discussion and conclusion), with reference to scientific journals, official *Rena* web pages and associated reports.

Important note: Mauri, Mātauranga Māori and Western Science

This research is designed to straddle two cultural approaches to reviewing an important environmental issue, but for the purposes of the MSc Thesis, it is written in a traditional ‘western style’. Although the work acknowledges and incorporates cultural perspective, it is not written to incorporate a specific cultural framework (with which this author is familiar). Though there are many documented Māori frameworks available, the methodology and scope of this project isn’t focussed on Māori development or revitalisation, nor is it participatory in nature. A hui was held in 2012 at Tamatea Ki Te Huatahi Marae on Mōtītī Island to discuss the potential inclusion of Mōtītī whanau and Mōtītī Mātauranga as part of the research methodology. As kaitiaki of the whenua, moana and traditional knowledge of the area, the whanau of Mōtītī decided that in order to maintain, preserve and uphold their *Tino Rangatiratanga* (absolute authority), *mana motuhake* (self-determination) and *kaitiakitanga* (guardianship), their Mātauranga was to stay on the Island, for the *uri* (off-spring) of the Island to learn by experience and oral tradition. The pathway for this research was re-directed to embrace classic sciences to inform communities (Māori and non-Māori). The MV *Rena* event has set a precedence in acknowledging and incorporating Māori values in Euro-centric management process with a key goal of the *Rena* Long Term Environmental Recovery Plan (2011) being to “restore the Mauri of the affected environment to its pre-*Rena* state”. By no means is this research a comprehensive assessment of values such as Mauri. As a *rawaho* (outsider/someone from elsewhere), the author takes great care in

respecting the *rangatiranga* (authority) of *tangata whenua* in determining Mauri and other values. This work is designed to utilise science as a tool, and to provide a spring board for more comprehensive cultural analysis in another format.

1.7.1 Research Limitations

This study was limited in scope due to constraints on time, laboratory resources, and importantly due to the on-going MV *Rena* salvage operation which severely restricted time at sea on Otaiti. Compromises had to be made in the number of samples collected, the chemistry performed on them and other experimental work. As such, results are reviewed in a conservative fashion where limitations of methodological approach are acknowledged and the effects on conclusions stated.

Chapter 2

Effects of the MV *Rena* debris field to dissolved metal content of reef boundary layer water of Otaiti (Astrolabe Reef)*

*To be submitted for publication under the same title as: Dempsey, T.P.T., Hartland A., Ross P.M., Battershill C.N.

2.1 Introduction

Humans have had a profound effect on the world's oceans particularly through pollution. The development of an industrialised society has led to the significant increase in use and dependence of metal-based products (McLlorm, *et al.*, 2011; He, *et al.*, 2013). Metals exist naturally within the ocean environment, both in the water column and sediment (Dimitrakakis, *et al.*, 2014). Metals are typically sourced from mineralised rock of either volcanic origin, or from land based sources of metals (He *et al.*, 2013). The ambient levels of heavy metals within the marine environment are spatially variable due to the distribution of aforementioned sources, and differences in the magnitude of the various transport mechanisms. Many metals are biologically necessary in trace quantities (e.g. Cu, Ni, Fe). However, in excess, metals can cause lethal and sublethal effects in biota (Rainbow, 2002).

There is increasing concern about the consequences of anthropogenic heavy metal contamination on marine ecosystems. Anthropogenic metals can enter the marine environment from adjoining waterways such as rivers and canals through the water column or via sedimentation. This can occur from the intentional extraction and use through mining and manufacturing of metal-based products, the direct discharge of pollutants, wastewater and sewerage, or leaching from reclaimed coastal land (Birch & Taylor, 1999; Buzier, *et al.*, 2006; He *et al.*, 2013). These varied sources have the potential to combine to have major detrimental impacts on the health of aquatic environments (Birch & Taylor, 1999) and human health through consumption of contaminated seafood (Boyd, 2010; He *et al.*, 2013). Strategies to monitor bioavailable metal in aquatic environments surrounding sources of potential pollution are critical to evaluate and manage the impact to water quality, flora and fauna. Bivalve species such as mussels are widely used as a key monitoring species (Webb & Keough, 2002; Schintu *et al.*, 2008; Sondergaard, *et al.*, 2014) as they are an abundant sessile organism which is long-lived, robust and will bioaccumulate metals in their tissues to above ambient concentrations.

Passive samplers such as diffusive gradient in thin films (DGT) (Davison & Zhang, 1994) have been proposed as a chemical alternative to biomonitoring of trace metal pollution (Webb & Keough, 2002; Schintu *et al.*, 2008; Sondergaard *et al.*, 2014). DGT samplers have been used to quantitatively measure the labile, dissolved fraction of trace metals in various freshwater and marine environments (Zhang & Davison, 1995; Webb & Keough, 2002; Schintu *et al.*, 2008; Sherwood *et al.*, 2009; Hartland, *et al.*, 2011; Sondergaard *et al.*, 2014; Turner *et al.*, 2014). The DGT sampler is a simple device for in-situ passive sampling by controlling mass transport across a diffusion gel to enable the collection of quantitative data on concentration and speciation using simple, inexpensive and readily available equipment (Davison & Zhang, 1994). These properties are particularly useful when monitoring pollutants in environments where water chemistry is temporally variable, for example in estuaries (Turner *et al.*, 2014). DGT samplers work by permitting metal ions to diffuse freely through a polyacrylamide hydrogel layer and are captured in a cation-exchange resin selective for trace metals (Chelex 100) (Davison & Zhang, 1994; Schintu *et al.*, 2008; Hartland *et al.*, 2011; Sondergaard *et al.*, 2014). A time-integrated metal concentration from deployment can then be analysed from the pre-concentrate of dissolved trace metals (Schintu *et al.*, 2008; Sherwood *et al.*, 2009; Sondergaard *et al.*, 2014).

When the MV *Rena* ran aground [refer Chapter 1 for more details] it was carrying 1733 tonnes of heavy fuel oil (HFO) and 1368 cargo containers including dangerous goods such as cyrolite and chlorine on-board. Following the grounding, oil and debris spilled into the surrounding environment over several weeks, some washing ashore more than 7 km from Otaiti. In early 2012, the *Rena* broke in two. The aft section sank and remains on a 55-60 degree list in 23-65m of water (Beca, 2014; Maritime New Zealand, 2014). A halo of discharged debris up to 30m deep spanning 10,000m² remains on the seafloor. It is comprised of scrap metals from the ship's structure, containers and cargo; and non-recyclable materials such as timber and remnant packaging (Beca, 2014). The grounding of a ship in coastal environments can result in a severe localised physical and

ecological damage due to the impact of the grounding; the discharge of oil, fuel and debris; the abrading of antifouling (AF) paint; and the corrosion of ship structure (Jones, 2007). Metals are present in almost every element of a ship structure (hull and beams, oil, antifouling paints, electronic equipment and cargo) and can influence the physiochemical and biological parameters of the adjacent seawater (Dimitrakakis *et al.*, 2014). There have been many studies on the effects of oil, fuel and cargo released into the sea; but few have analysed the chemical pollution of metals following a ship grounding (Prego & Cobelo-Garcia, 2004; Jones, 2007; Dimitrakakis *et al.*, 2014). Ross and Battershill (2013) recorded elevated metal concentrations in on-reef and off-reef sediments, with on-reef sediments found to be an order of magnitude higher than off-reef samples for Mg, Se, Ba, Ni, Ti, Zn, Cu, Al and As. The effect of the MV *Rena* debris 'halo' to the water chemistry/quality of Otaiti is unknown. This chapter investigates the effects of the MV *Rena* shipwreck and its debris field on water chemistry of Otaiti, focussing on DGT-available metals, in comparison to the measured concentrations of total and dissolved metals in solution, both at impacted and control locations.

2.2 Methodology

2.2.1 Study site and field procedures

Surveys were carried out at three locations; the debris field, outer Otaiti (Astrolabe) reef; and Mōtītī Island (control site)(Figure 2-3). The Mōtītī Island site was chosen as a control site because it has a similar environment and is not in immediate proximity to the wreck. Within each location, three sample sites were selected based on the ongoing *Rena* Long Term Environmental Monitoring Programme (Figure 2-1). Sampling of dissolved metals using DGT samplers and total metals using spot samples of water occurred from 29th June to 6th July 2014 and the position of each site recorded using a Garmin Etrex 20 handheld GPS.

DGT devices purchased from DGT Research Ltd (Lancaster, UK) and impregnated with a Chelex-100 resin (0.40mm thick) were housed in piston

type Teflon holders and protected by a 0.45 μ m cellulose nitrate membrane filter as described by Zhang and Davison (1995). DGT samplers were installed in triplicate to a triangular polymethyl methacrylate holding units and weighted to 10kg vinyl plates (Figure 2-2). DGT housing units were deployed by divers within a depth range of 11.5 - 16.5 metres to the central-most sample sites in the debris field (E11, G13, H9), the north western sites on the outer reef (OTR1, OTR2, OTR3) and the north western side of Mōtītī Island (control site) (Figure 2-1). These sites were selected as they provided the least disruption to the ongoing salvage operation and were within a suitable depth band for divers.



Figure 2-1. DGT and bulk water sampling sites on the outer reef (top right); and debris field (bottom right) of Otaiti (Astrolabe Reef) and the control site (Mōtītī Island). Red circles indicate sites sampled based on *Rena* Long Term Environmental Monitoring Programme

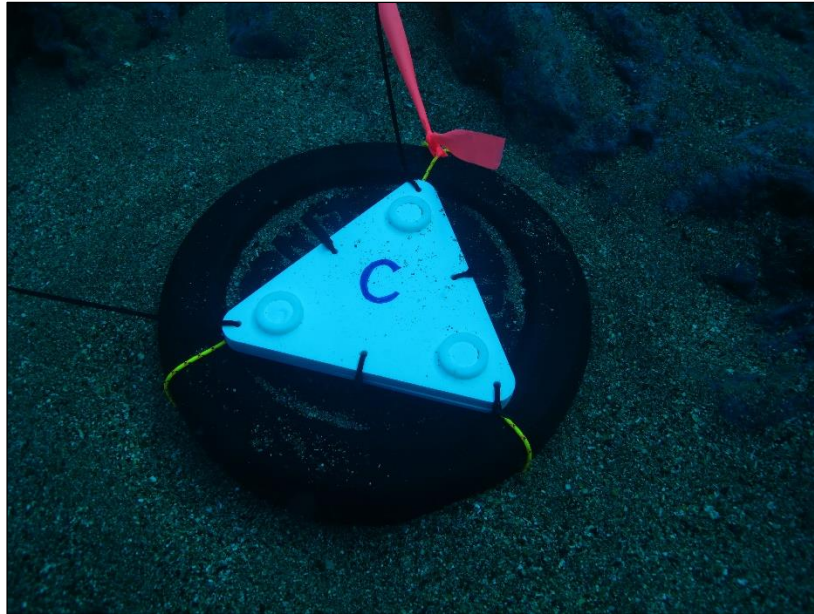


Figure 2-2. A DGT-holding unit with three DGT samplers installed and attached to a 10kg vinyl coated plate on the seabed at a sample site.

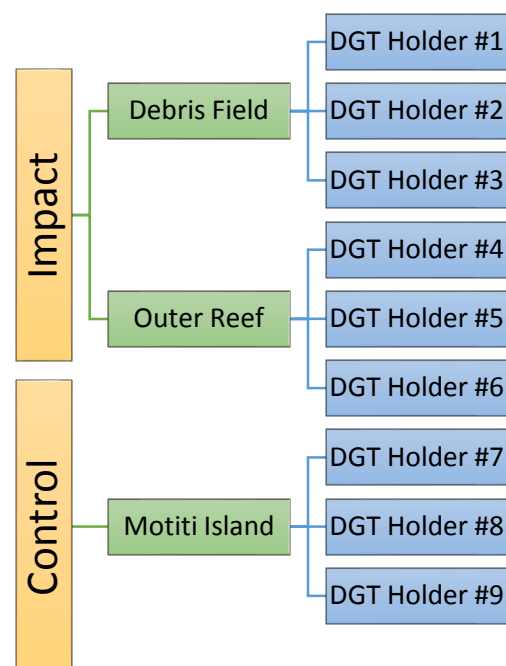


Figure 2-3. Overview of the water sampling regime over a 7 day period. DGT holding units were anchored to 10kg weights on the seafloor. Total water samples were taken on deployment and recovery of DGT holding units at each site.

After the 7 day sample period, DGT units were recovered, removed from the housing, rinsed with deionised water, placed in a clean polyethylene bag and stored in a cooler bin on salt ice until return to the mainland. Once on land, units were refrigerated at -4°C until laboratory processing was possible (within 48 hours).

Water samples were collected from the seafloor of each site by divers using a 1.2L Sistema container at the same time as deployment and recovery of DGT holding units. On return to the surface, a 10mL total water sample was filtered through a $0.45\mu\text{m}$ filter into a sterile 15ml falcon tube. The remaining sample water was transferred into 500mL sterilised Nalgene bottles. Equipment was rinsed with seawater thrice and then with deionised water before and after each sample was taken, and all equipment was handled wearing disposable latex gloves. As with the DGT units, water samples were stored in a cooler bin on salt ice until return to the mainland where samples were refrigerated at -4°C until laboratory processing was possible (all were processed within 8 days).

Water pH was measured using the 'DoubleTestr pH10', and water temperature, salinity, dissolved oxygen and conductivity were measured using the YSI Model 85 handheld multimeter on deployment and recovery of each DGT housing unit. Parameters ranged from 7.8–8.2 for pH; $15.1\text{--}16.4^{\circ}\text{C}$ for temperature; 31–32.4 ppt for salinity; 78.7–127.9% dissolved oxygen; and 38.5–47.6 ms for conductivity.

2.2.2 Sample Preparation

Within a laboratory environment, DGT caps were removed allowing the retrieval of the resin layer using instruments that had been soaked in diluted nitric acid (HNO_3). The resin gel was extracted with plastic tweezers and placed in a 15mL sterile falcon tube. Metals were eluted from the binding gel with 1mL of 1 M HNO_3 . This was left capped to elute metals for 48 hours at $<4^{\circ}\text{C}$. A further 4mL of deionised water was then added to dilute sample in preparation for ICP-MS analysis (Zhang & Davison, 1995).

A 0.4mL aliquot of each filtered water sample was diluted by 25% with deionised water (9.4mL) and left to acidify with 0.2mL HNO₃ for 24 hours. Samples were analysed using ICP-MS. Following ICP-MS analysis, water sample values were multiplied by 25 to account for the dilution factor to analyse the total metal concentration.

2.2.3 Calculations

The concentration of DGT-labile metals measured in the water column over the deployment period was calculated using the following equation (Zhang & Davison, 1995);

$$C_{DGT} = \frac{M\Delta g}{DtA} \quad (1)$$

where; M is the mass of metal absorbed on the resin (found by equating the analysis of an acid extract, and assuming the gel volume is 0.16mL with an elution factor of 0.8 for metals); Δg is the thickness of the diffusive gel (0.8mm) and the filter membrane (0.13mm); D is the diffusion coefficient of metal ions at mean water temperature during deployment; t is the deployment duration (in seconds) and A is the exposure area of the diffusive layer (3.14cm²). The diffusion coefficient was based on freshwater so it would be expected that seawater would have a lower value. A correction was not applied in this instance. Although it's not fully representative of marine environment, the estimate of concentration is conservative

2.2.4 Statistical methods

A principle component analysis (PCA) ordination was also used to test for dissimilarity in the DGT dissolved metal concentrations across sampling sites. The PCA technique for environmental data is correlation based, comparing the Euclidean distance between samples sites with the corresponding dissimilarity in variable structure (Clarke & Warwick, 2001).

Results for dissolved and total metal data were graphically presented as a mean \pm standard error (SE) of each metal for each sample location. One way ANOVA and Tukey's HSD tests were performed to gauge significance

from means from the various treatments. Due to atomical interferences, data for total iron and cadmium weren't presented. Significance levels were set $p < 0.05$ with all statistical analysis was performed using Microsoft Excel and Statistica (v12, Statsoft, USA).

2.3 Results

2.3.1 DGT - dissolved metal concentrations

The diffusion membranes showed light signs of biofouling by the end of the deployment period although water pH was within the range necessary for optimal performance of DGT using Chelax-100 binding resin (Zhang & Davison, 1995). Several DGT samplers were damaged, presumably due to the turbulent nature of the reef environment or grazing by invertebrates, and could not be analysed. On damaged samplers, diffusion membranes had been stripped from the housing unit. Two DGT samplers were damaged in the debris field (n=7); six in the outer reef (n=3) and five at control sites (n=4).

The overall range of concentrations of dissolved metals in seawater at all sites investigated in this study is 0.013-0.027 µg/L for Cu; 0.006-0.241 µg/L for Al; -0.022-0.352 µg/L for Fe; 0.016-0.044 µg/L for Mn; 0.036-0.186 µg/L for Zn; 0.0003-0.015 µg/L for Cd; 0.011-0.020 µg/L for Ni; 0.0006-0.0017 µg/L for Cr; 0.0003-0.0006 µg/L for Co; and 0.007-0.047 µg/L for Pb. The average concentration of metals is higher in the debris field compared to the outer reef or control site, with exception to Ni, Cd, Co, and Pb. It would be expected that the control site has the lowest mean concentration however Al, Mn, Zn, Cr, Co and Pb all recorded elevated concentrations at the control site, relative to the outer reef (Figure 2-4a & Figure 2-4b). Though trends were observed, only Mn showed a significant difference ($p < 0.05$) between the debris field and other sites (Table 2-1)

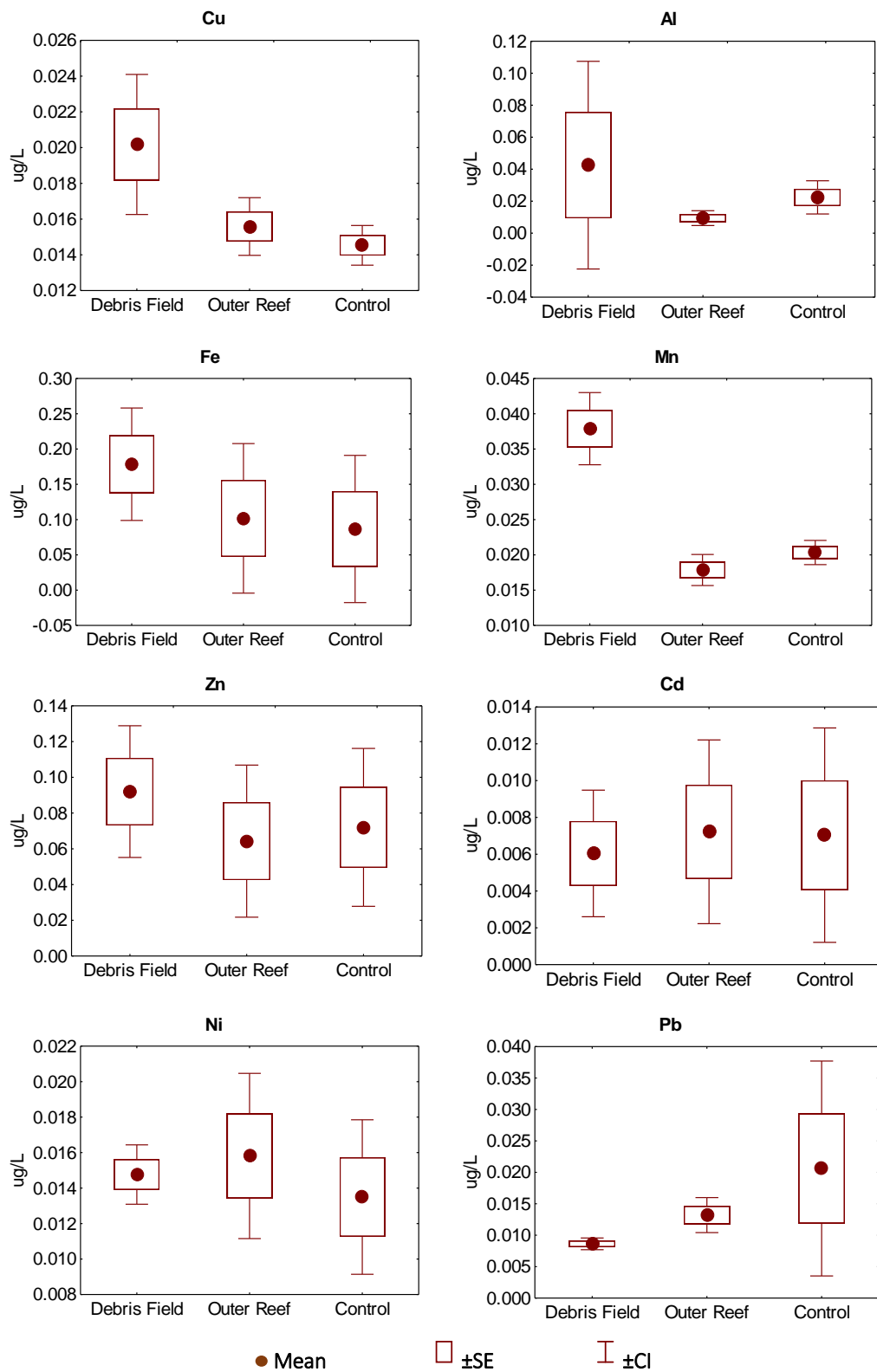


Figure 2-4a. Metal concentrations as measured by DGT for the 3 sampling areas; debris field, outer reef and Mōtītī Island (control site). Circles show mean concentration ($\mu\text{g/l}$), boxes represent standard (SE) and capped lines show confidence intervals (CI) of the DGT devices deployed together.

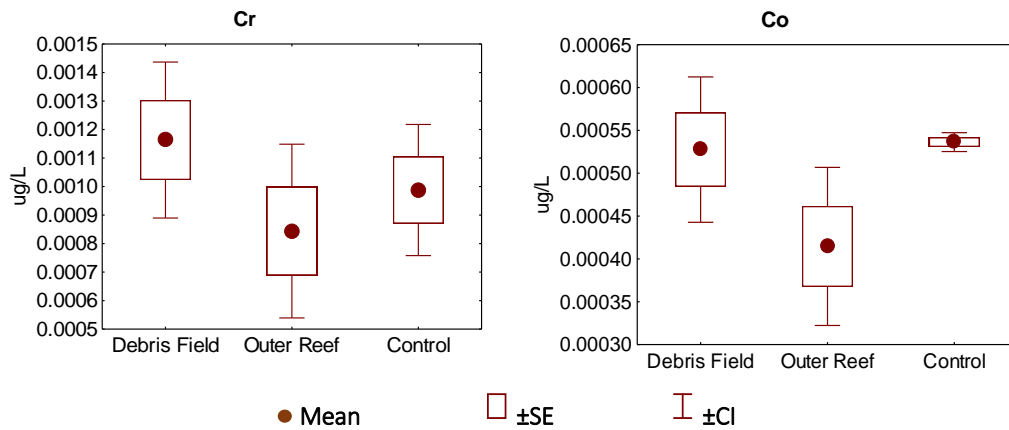


Figure 2-4b. Metal concentrations as measured by DGT for the 3 sampling areas; debris field, outer reef and Mōtītī Island (control site). Circles show mean concentration ($\mu\text{g/l}$), boxes represent standard (SE) and capped lines show confidence intervals (CI)

Principal component analysis (PCA) was performed on normalised data to explore the similarity of sites based on chemical properties and determine which metal variables are most influential (Figure 2-5). The PCA ordination of dissolved metals in solution accounted for 63.5% of the total variance in the data. The majority of variance (47.9%) was explained by the first principle component (PC1) and 15.6% explained by PC2 (Table 2-2). Cu was the variable of most importance ($r=0.91$); followed by $\text{Cd} > \text{Fe} > \text{Mn} > \text{Zn} > \text{Pb} > \text{Al} > \text{Co} > \text{Cr}$ and Ni ($r= 0.53 - 0.90$).

The majority of the metals cluster positively on the PC1 axis. This includes Cu, Cr, Fe, Mn, and Co, with Zn, Ni and Al influenced by the PC2 axis. PC2 is most correlated with Cd. An obvious clustering of four debris field samples separate to the remaining samples can be discerned. The major clusters contain some debris field, outer reef and control site samples. The isolated four samples relate to Otaiti sites G13 and E11 (Figure 2-1) which have a greater mean value than the average of all other sites in Co, Mn, Fe, Cu, Ni, and Al.

Table 2-1. One way analysis of variance results. Manganese was the only metal which indicated significance ($p < 0.05$)

<i>Cu</i>	<i>Al</i>	<i>Fe</i>	<i>Mn</i>	<i>Cd</i>	<i>Cr</i>	<i>Co</i>	<i>Ni</i>	<i>Zn</i>	<i>Pb</i>
0.09	0.74	0.34	0.000	0.92	0.36	0.19	0.67	0.64	0.16

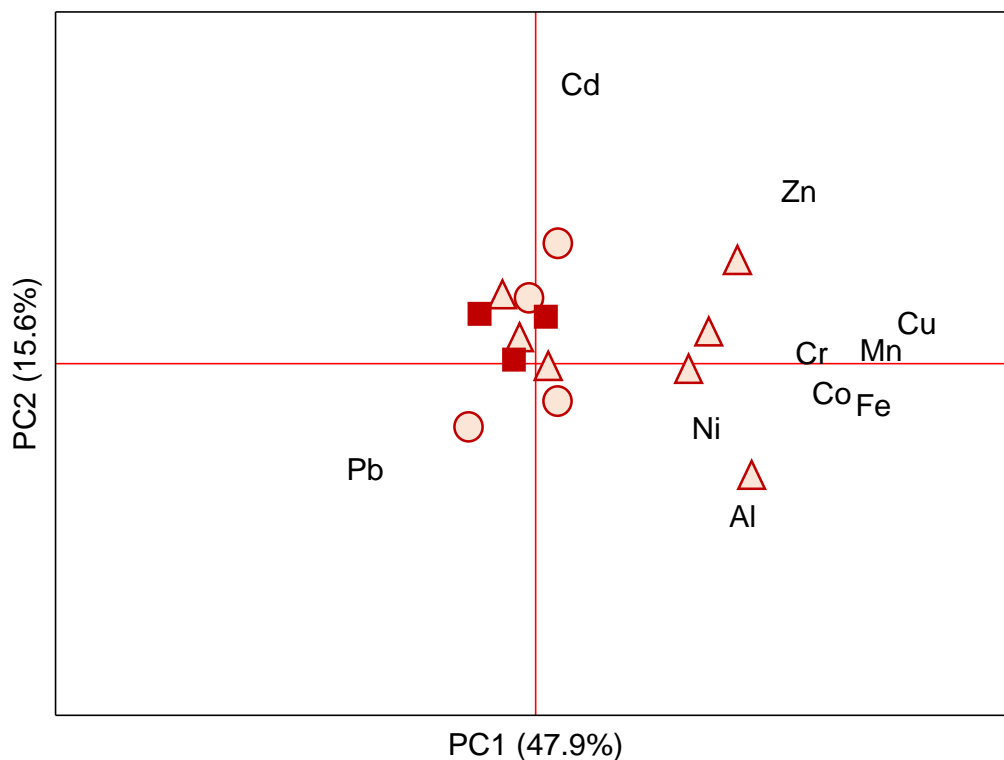


Figure 2-5. PCA ordination of water chemistry from sampling sites in the Otaiti debris field (=Δ), Otaiti outer reef (=■) and Mōtiti Island (control site) (=○). A total variance of the data of 63.5% was explained by PC1 and PC2.

Table 2-2. Eigenvalues, percentage variation and cumulative percentage variation of normalised dissolved metal data explained by PC1 and PC2

PC	Eigenvalue	% Variation	Cumulative % Variation
1	5.24	47.67	47.67
2	1.71	15.58	63.25

2.3.2 Water samples – total metal concentration

There was a negligible difference between the overall ranges of total metal concentrations measured during deployment vs recovery, so the mean values were used in this analysis. The total metal concentration range was 10.58 – 16.73 µg/L for Cu; 15.10 – 155.73 µg/L for Al; 0.63 – 1.53 µg/L for Mn; 11.06 – 28.45 µg/L for Zn; 12.97 – 15.49 µg/L for Ni; and 6.58 – 10.82 µg/L for Cr; 0.94 – 1.17 µg/L for Co; and 0.14 – 0.61 µg/L for Pb (Figure 2-6a & 3-6b). Although the data reported by ICP-MS facility at University of Waikato are discussed here, these values are not considered final since the ICMPS metal analysis in these samples was significantly influenced by interferences in the saline matrix (the samples are being re-analysed for publication write-up). Therefore, the relative differences between sites are considered here, rather than the absolute concentrations.

The mean concentration within the debris field was elevated for 5 of the 11 metals (Cu, Al, Mn, Co, Pb) when compared to the mean concentration for the outer reef and the control, with a further 3 metals indicating higher mean concentrations in the debris field versus the outer reef or control site only (Zn, Cd, Ag) (Figure 2-6). The control site indicated higher concentrations of Cu, Al, Fe, Zn, Ni, Co and Pb when compared to the outer reef of control site. The concentration of Fe in the debris field was significantly different ($p < 0.05$) to that of the outer reef and control site; as was Pb in the debris field versus the outer reef.

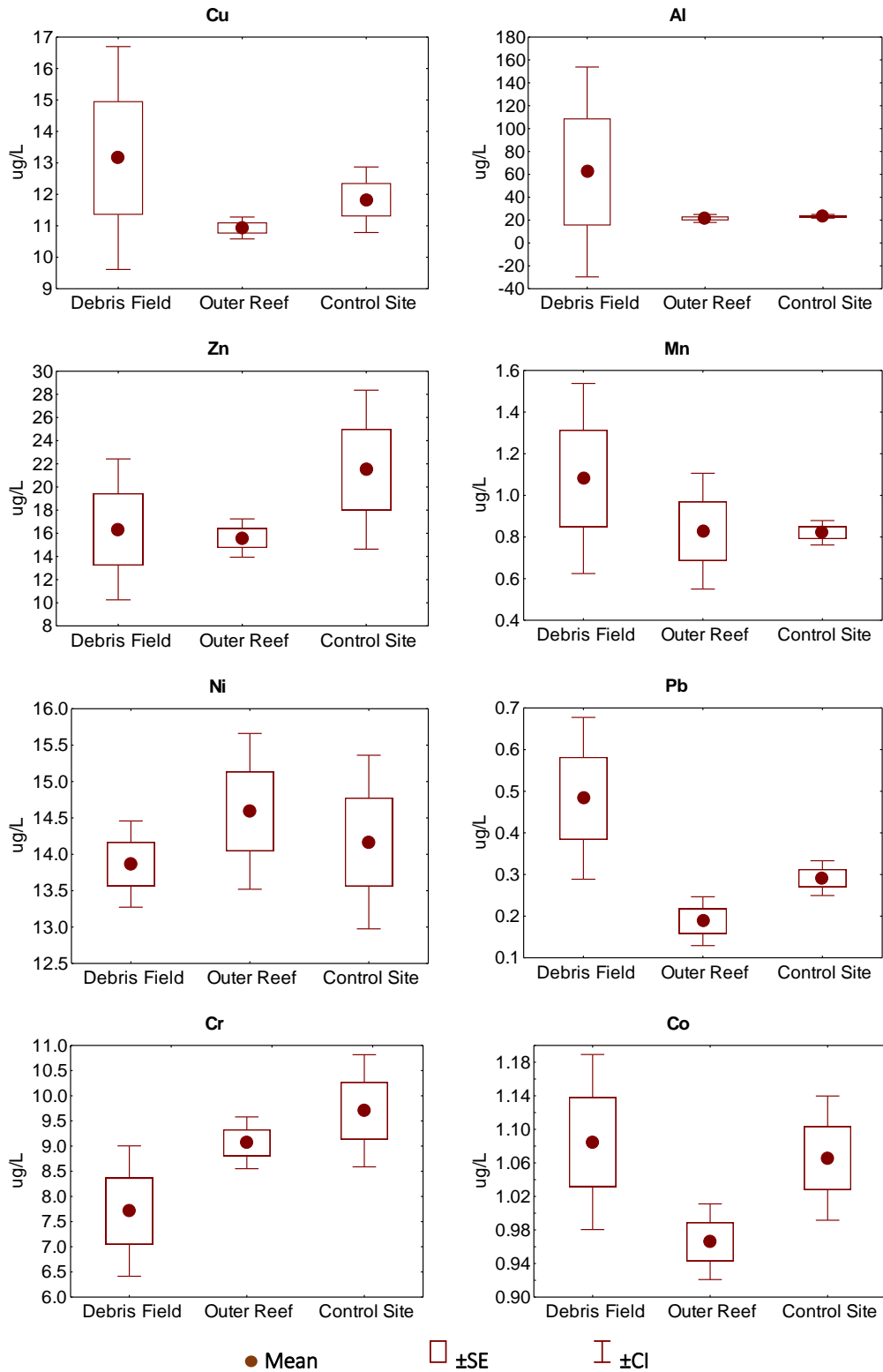


Figure 2-6. Metal concentration as measured from bulk water samples for the 3 sampling areas on deployment and recovery. Circles show mean concentration (μg), boxes represent standard (SE) and capped lines show confidence intervals (CI)

2.4 Discussion

Otaiti is a dynamic reef environment with currents up to 0.66m/s and significant wave action (Robertson, 2014). The probability of detecting significant contamination therefore seems unlikely, since metals released into the water column are rapidly mixed and diluted. Water sampling of dissolved and total metals using two methods, DGT and spot samples of water, within the debris field of Otaiti nevertheless demonstrated an effect of the *Rena* and its associated debris to water chemistry. Levels of copper, aluminium, manganese and zinc were elevated in the debris field relative to the control.

Sediment samples from the long term monitoring programme (Ross & Battershill, 2013) found enrichment of sediments with aluminium, zinc, copper, nickel and iron around Otaiti. Ross *et al.* (*in press*) found debris field sediments to still contain high levels of copper, nickel, zinc, lead, cadmium, chromium and tin which could be likely influencing higher concentrations in the adjacent water column. This could suggest a concentration gradient or plume of contaminated water is present around the debris field.

Debris field sites G13 and E11 had the greatest concentration in most of the metals, as indicated by the PCA ordination. However, the remaining debris field site (H9) was rather moderate in most metals. This suggests small scale spatial variability with localised effects relating to the types of debris at a given reef location.

All dissolved metals analysed were within the suggested background metal concentrations for New Zealand, Australian or world marine water using 'clean' techniques (ANZECC & ARMCANZ, 2000). The dissolved metal concentrations are ecologically relevant when looking at metal bioavailability and bioaccumulation as total concentration of metals is not a good predictor of effects such as acute toxicity (Meyer, 2002). Bioaccumulation refers to the uptake of chemicals from the surrounding environment in any way (e.g. ingestion, adsorption, etc.) and bioavailability refers to the portion of total metal in solution that is potentially available for uptake by biota, or any other biological action (Spacie, *et al.*, 1995; Meyer,

2002). This bioavailable fraction is potentially toxic (Rainbow, 1995) though metal uptake from the dissolved form is dependent on the physiology of the receiving organism (Rainbow, 2002) and the ambient geochemical conditions of a water body (Wang & Guo, 2000). For example, a study by Rainbow (2002) noted the concentration of zinc in an oyster that may be considered low, would be considered high in a mussel. Another study by Hutchins, *et al.*, (2008) highlighted the changes in copper bioavailability with small scale pH changes (pH 6.6, 7.2, and 7.6).

The Australia and New Zealand water quality guidelines suggested marine trigger values for of 1.3 µg/L for copper, 0.5 µg/L for aluminium, 80 µg/L for manganese, 5.5 µg/L for cadmium, 4.4 µg/L for chromium (VI), 1.0 µg/L for cobalt, 70 µg/L for nickel, 15 µg/L for zinc and 4.4 µg/L for lead. A deficiency in data meant no marine trigger value was developed for iron, but 300 µg/L was recommended as an indicative interim working level based on Canadian water standards. The trigger values for Al and Mn had low reliability as there was limited data and literature available to influence those guidelines. These are therefore used only as an indicative interim working level. The other metals had high reliability with 95% confidence (ANZECC & ARMCANZ, 2000). Total metals were elevated with reference to ANZECC guidelines, for some metals whereas others were low (Table 2-3). Mean total cadmium, manganese, and lead were all 1 order of magnitude below the guidelines. Mean total copper and aluminium were two orders of magnitude higher than the ANZECC marine trigger values at all sites (Table 2-3), however these values may be revised pending re-analysis. The increased level of copper could be due to an unrecovered container with 21 tonnes of scrap milled copper from within Hold 6 of the aft section (Faithful, 2014). Furthermore, it was estimated that 0.11 µg Cu/L would be the concentration in a plume resulting from the exposure of antifouling paint on the hull before dilution from mixing occurs (Elvines, *et al.*. Copper is an essential nutrient for aquatic biota (Levy, *et al.*, 2007) but is of key concern due to the known ecological effect to biota, especially for planktonic and larval life history stages (Prato, *et al.*, 2013).

Table 2-3. Schematic representing the concentration of measured total water to ANZECC marine water trigger values using the statistical distribution methods with 95% protection. Green = an order of magnitude below trigger value; blue= below trigger value; orange = above trigger value; red = exceedance of trigger value by an order of magnitude or more.

TOTAL WATER	Cu	Al	Fe	Cd	Mn	Cr	Co	Ni	Zn	Pb
Debris Field	Red	Red	Blue	Green	Green	Orange	Orange	Blue	Orange	Green
Outer Reef	Red	Red	Blue	Green	Green	Orange	Blue	Blue	Orange	Green
Control	Red	Red	Blue	Green	Green	Orange	Orange	Blue	Orange	Green

Given the highly volcanic nature of the region, it is important to review these levels in the context of the regions geology. Mōtītī Island for example is comprised of pliocene andersite (Henry, 1991). The heavy volcanic stimulus could influence ambient levels of metal and minerals in the area, as results from work on examining *Rena* influenced and control sediments from the wider Bay of Plenty would suggest (Battershill *et al.*, 2013). Results therefore suggest two patterns in water column metals chemistry; one as a response to the *Rena* debris field; and the second a background reflection of ambient metals regimes (Table 2-3). A broad scale regional assessment of coastal water quality and composition is needed to establish the baseline concentration for metals in Bay of Plenty waters.

The ongoing Otaiti Environmental Monitoring Programme will continue to investigate metals and other organic contaminants present in sediment and biota around Otaiti to determine what changes are occurring over time. The omission of water quality monitoring from this comprehensive programme is significant. This is the first research to conduct *in situ* water sampling around the wreck of the *Rena*.

There has been indication that the concentration of contaminants such as metals and organotins within sediment samples are not decreasing (Ross *et al.*, *in press*). The research in this thesis highlights the potential for

diffusion of metals contaminants from sediment sources on the reef into the adjacent water column. The following chapters explore the influence this chemistry could have to zooplankton (proxies for important food organisms and for recruiting larvae) in critical life history phases. It is recommended that water chemistry is further investigated to better understand the plume gradient above the seafloor, and away from the debris halo. If water column concentrations of bioavailable or interactive compounds are found to be significant; appropriate remedial action would be necessary to ensure longevity of the reef ecosystem

Chapter 3

Acute toxicity effects of copper and contaminated reef sediment on the early life stages of *Tenagomysis* sp. and *Ovalipes catharus**

*To be submitted for publication under the same title as: Dempsey, T.P.T, Ross P.M., Battershill C.N

3.1 Introduction

Planktonic organisms are a vital part of the marine ecosystem, making up more than 90% of the biological processes in the ocean (*et al.*, 2014). Zooplankton are pivotal to ecosystem functioning. They constitute the source of progeny for reef associated organisms as they settle and recruit from a pelagic phase and provide link between phytoplankton and primary productivity to higher trophic layers. They provide a rich source of organic carbon for detrital consumers and are the major consumers of marine phytoplankton. They are also a significant food source for larval fish and benthic invertebrates (Verslycke *et al.*, 2003; Zauke & Schmalenbach, 2006; Larkin, *et al.*, 2007; Lill, Lal, & Closs, 2010;). As such, zooplankton represent a key connectivity element for coastal and offshore island marine food webs. The sheer abundance and variety of zooplankton species provides a principal pathway for the transfer of energy to higher trophic levels (Hays, *et al.*, 2005; Almeda *et al.*, 2014). However, zooplankton can also contribute significantly to the transfer of trace metals and other accumulated contaminants to the higher trophic levels (Zauke & Schmalenbach, 2006; Rejomon *et al.*, 2008;).

Trace metals are metals that occur in trace amounts (typically <0.01% of an organism) within the environment or within biota (Marsden & Rainbow, 2004). Trace metal uptake can occur through ingestion and diffusion of dissolved sources, both of which can be affected by the physico-chemical properties of sediments for benthic invertebrates. The permeable surface such as that on the gills creates a major pathway for trace metal uptake. Many trace metals are necessary for biological function and all metals can be taken up by a marine organism. In moderation these metals can be regulated (to a degree) by the organism by excretion and/or detoxification. However, excess accumulation can be fatal (Rainbow, 2002; Marsden & Rainbow, 2004; Ferrer *et al.*, 2006).

Marine environments receive direct and indirect chronic pollution inputs, especially in industrialised coastal areas (Ferrer *et al.*, 2006) which can increase the bioavailable metal for marine invertebrates, promoting excess

metal uptake. Toxicity studies are used to evaluate the risk for an ecosystem exposed to an environmental stress such as contamination (Ferrer *et al.*, 2006) and are an integral element in pollution assessment and management. Bivalves are frequently used for coastal ecotoxicology and biomonitoring of water quality elements such as metal accumulation (Perez & Beiras, 2010), though there have been many studies on macro and meso-zooplankton organisms also (Zauke & Schmalenbach, 2006). Acute lethal toxicity bioassays are useful in providing a measurement of relative toxicity and assessing the sensitivity of an organism to a particular contaminant, or suite of contaminants (Ferrer *et al.*, 2006).

Information on the effect of heavy metals and contaminants to New Zealand marine zooplankton species is severely limited. Mysid species worldwide are used as a proxy species for acute and chronic toxicity testing (Nipper & Williams, 1997). *Americamysis* (= formerly *Mysidopsis*) *bahia* is a standard test organism in the US (Nipper & Williams, 1997; Perez & Beiras, 2010; Yan *et al.*, 2003), *Siriella armata* and *Neomysis integer* have been used in many European based studies (Perez & Beiras, 2010) and *Neomysis awatschensis* is a recommended standard test organism in China (Yan *et al.*, 2003). The advantage of mysid shrimps as toxicology test organism are the ease of handling and culturing, ecological relevance, relative sensitivity, short life cycle, size and direct relevance to larval development (Nipper & Williams, 1997; Yan *et al.*, 2003; Perez & Beiras, 2010). Specifically they are an important planktonic food source for reef associated predators and can be viewed as a surrogate for planktonic phase organisms that are about to recruit onto the reef system (e.g. rock lobster and crabs). Mysid shrimps (Family: Mysidae) are an abundant zooplankton invertebrate found in estuarine, lagoon and oceanic systems. New Zealand has many species of mysid shrimp with *Tenagomysis* being the dominant genus (Larkin *et al.*, 2007).

The New Zealand paddle crab *Ovalipes catharus* (White, 1843) is ecologically, commercially and culturally important. It is relatively well researched in aspects of life history and growth, reproduction, ecology and

behaviour (Armstrong, 1988; Osborne, 1987; Haddon, 1994), however, research on the ecotoxicological or larval development is missing from the published research. Paddle crabs inhabit sandy beaches, harbours and estuaries throughout New Zealand, the Chatham Islands and south-east Australia. They occur in the intertidal zone, but can also occupy deeper water. Paddle crabs are opportunistic predators that feed on a range of molluscs, other crustaceans, polychaetes, fish species and sometimes algae, *Paphies* species are a favoured diet (McLay & Osborne, 1985; Wear & Haddon, 1987).

The reef sediment of Otaiti was subjected to metal enrichment with levels measured above what would be considered natural for the area following the MV *Rena* grounding event in 2011 (Ross & Battershill, 2013). Contaminated sediments are recognised as a potential ecological hazard resulting in stress to benthic biota and the release of pollutants into interstitial waters and the water column that may potentially influence recruiting planktonic organisms or zooplankton in the boundary layers (Burgess *et al.*, 1993). The 10,000m² debris field that remains on the reef is comprised of a suite of scrap metals from the ships structure, containers and contents (Maritime New Zealand, 2014). Within Hold 6 of the aft section was a 20ft container with 21 tonnes of finely ground scrap milled copper filings that hadn't been recovered by November 2014 (Faithful, 2014). Copper is of key concern due to the known ecological effect of copper to biota, especially planktonic and larval species (Prato *et al.*, 2013).

This chapter aims to assess the acute toxicity of *Rena* contaminated on-reef sediment in solution and copper (as a positive control) in solution to paddle crab larvae and mysid shrimp. These species are being used as a proxy zooplankton to represent a range of invertebrate larvae that have relevance to coastal reef ecosystems either as food items or as recruiting organisms that will become the reefs next generation of crustacean residents. These species can be handled easily and arguably have attributes of other key reef species, and thus add to the New Zealand research portfolio of indicator species with respect to ecotoxicology. The null hypothesis for this research

is there is no significant difference in survival trends of test species when exposed to on-reef *Rena* contaminants, copper contaminants and a clean control treatment (no contaminant).

3.2 Materials and methods

3.2.1 Specimen collection

3.2.1.1 Mysid Shrimps

Mysid shrimp (*Tenagomysis* sp.) were collected using a fine-mesh dip net from the nearby Bridge Marina on an incoming tide. Collected organisms were placed in a clean 20L bucket containing seawater from the collection site. On return to the University of Waikato Coastal Marine Field Station, mysid shrimp were diluted with control test water and kept in a 20L aerated aquaria situated in a chill bath at $17^{\circ}\text{C} \pm 1^{\circ}$ with a photoperiod of 12 L:12 D.

3.2.1.2 Paddle Crabs

Ovigerous female *Ovalipes catharus* were collected from Pilot Bay, Tauranga, in August-September 2014. Mating occurs throughout winter-spring with bulk of spawning occurring in early spring and extending to later summer in some instances (Haddon & Wear, 1993). Animals were transported back to the Bay of Plenty Polytechnic aquaculture laboratory and placed in a recirculation system under environmentally controlled conditions. Each specimen was individually housed in 10L plastic containers with a 250 μm mesh bottom within a 1500L circular tank. Air was readily pumped into the container to ensure water flow and aeration. Crabs were fed mussels *ad libitum* every other day and water changed on alternating days. Seawater was maintained at a constant temperature of $17.45 \pm 0.56^{\circ}\text{C}$; salinity of 35.69 ± 0.41 psu; pH of 7.28 ± 0.12 and oxygen saturation of $89.92 \pm 4.75\%$. Water was recirculated through a UV filter to mechanical and bio filtration membranes and animals were exposed to a 3L:21D (fluorescent light) photoperiod. The difference in photo period was due to the different ambient conditions of the two laboratories utilised in this study.

Ovigerous females were monitored daily to detect signs of upcoming egg release, hatching of eggs, signs of stress, or mortality in which females were removed from the experiment. Immediately after hatching, zoea were removed for acute toxicity testing.

3.2.2 Experimental design

Contaminant mixtures locked in on-reef sediment were the focus for this study and copper was used as a positive treatment control as it is known to be a prevalent metal in *Rena* polluted reef sediments (see Figure 3-1). It provides a standard by which the reef collected contaminated sediments (derived by diver collections from high impact areas within the debris field where copper and other metal contamination of sediments could be clearly seen) can be compared.



Figure 3-1. *Rena* polluted reef sediment sourced from high impact area on Otaiti. Copper filings can clearly be seen.

Acute toxicity tests were conducted on one group of wild caught mysid shrimp, and the larvae of three different paddle crabs. As ovigerous females

released their larvae at different times, the offspring of each female was considered its own test group. This automatically provided for an experimental 'control' for age, development and genetic history of test subjects. Each group was exposed to two treatment solutions; *Rena* derived reef contaminants and copper. Both treatments were delivered in 3 concentrations (100%, 50%, 5%) diluted with autoclave seawater, with a 0% control. Treatment solutions were created by weighing out 2.5 ± 0.1 mg of copper shavings or contaminated reef sediment into a 500mL Nalgene bottle filled with autoclaved seawater. This was shaken and left for 48 hours. The treatment concentrations were replicated four times, with 5 mysid shrimp (n=35) per replication in a semistatic system, and 10 paddle crab larvae (n=210) per replication in a static system. Only individuals that demonstrated active swimming movements were used in the test.

Physico-chemical parameters were measured for each water type prior to experimental commencement. A secondary control trial on oxygen consumption in each treatment concentration was extensively measured every 2 seconds for one hour using a Fibox 3 optical oxygen meter. Mortality is a valid endpoint for acute assays (Nipper & Williams, 1997). The criteria for mortality was the absence of movement observed under a 10x compound specific microscope, even when prodded with a plastic rod. Mortality was recorded every 12 hours until all experimental individuals were recorded as deceased (or for 1 week).

3.2.3 Statistical analysis

Trends are graphically presented for the percentage survival over the entire experimental duration. This experiment was formulated based on standard methodology for acute toxicity tests (Ferrer *et al.*, 2006; Nipper & Williams, 1997), however, specific elements of an LC₅₀ assay design were not achieved from this experiments design. This was largely due to infrastructure limitations in the initial period of commencement. Indicative LC₅₀ values were calculated, but need to be treated with some caution. In this regard, results are discussed in a conservative manner.

3.3 Results

3.3.1 Physico-chemical influences on mortality

The overall mean (\pm SD) water chemistry prior to experimentation was 8.19 ± 0.18 for pH; $20.34 \pm 0.84^\circ\text{C}$ for temperature and 29.76 ± 0.24 ppt for salinity. Little variance in characteristics was observed across each treatment. There was a clear indication of stability in oxygen levels across treatment concentrations so depletion in oxygen was not a factor influencing mortality of test subjects (Figure 3-2). It can be noted that copper maintained a lower oxygen percentage throughout compared to *Rena* treatments. This could be due to the fact that copper in the *Rena* treatment was bound in other materials including highly weathered oil (mousse) that may have influenced its reactivity with oxygen.

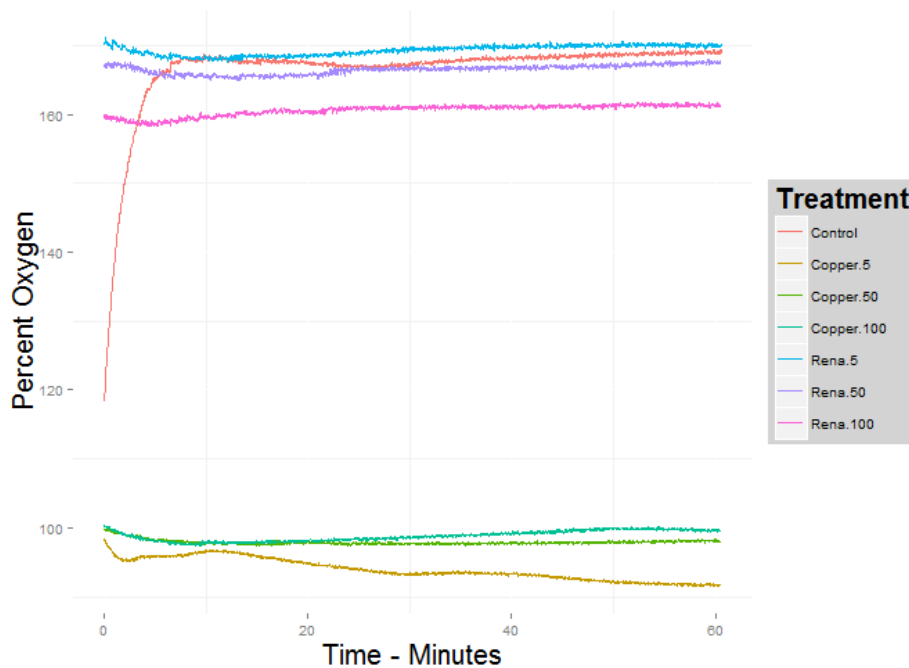


Figure 3-2. Oxygen consumption for the *Rena*, copper and control treatments over 1 hour. There was a clear indication of stability in oxygen levels so depletion in oxygen was not a factor influencing mortality of test subjects

3.3.2 The LC₅₀ Experiment

The exposure of paddle crab larvae and mysid shrimp (as a proxy for zooplankton) to the copper and reef mixture treatments indicate an increased mortality with decreased dilution of treatment solutions. At 12 hours, the 100% treatments for both assays showed strong signs of mortality. Incipient mortality was observed for the 50% treatment groups with signs of lethargy, erratic swimming movements, and wafting of the pereopods at 12 hours. The same behaviour wasn't observed in 5% treatment groups until 24-48 hours for mysid shrimp and 36-48 hours for paddle crab larvae. Some signs of obvious mortality included the disintegration of a deceased individual, a translucent colouration, and the consumption of the body by infection or parasitic beings (Figure 3-3).

Complete mysid group mortality was featured at only 96 hours (Figure 3-2 A, B). Survival for paddle crab larvae extended to over one week for the control treatment. Treatment survival was drastically reduced for the 100% and 50% treatment groups, with complete mortality featured at 24-48 hours (Figure 3-4 C, D). For this reason, the results from the first 24 hours of the experiment may be viewed as valid, given the control survivorship across all tests. As it was not possible to feed test animals in this work, the extended experimental mortality across all treatments is to be expected.

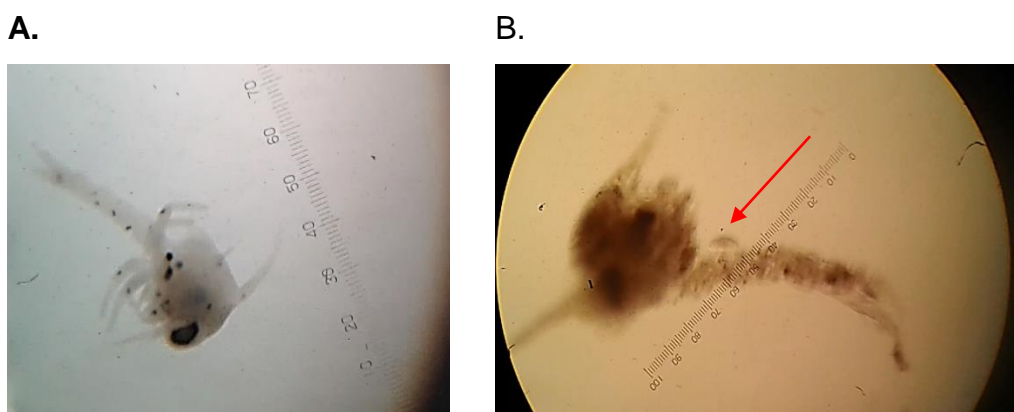


Figure 3-3. Comparison of a A) live paddle crab larva and B) dead paddle crab larva used in acute toxicity assay and infested with parasites. Arrow indicates a particularly large, unidentified parasitic organism.

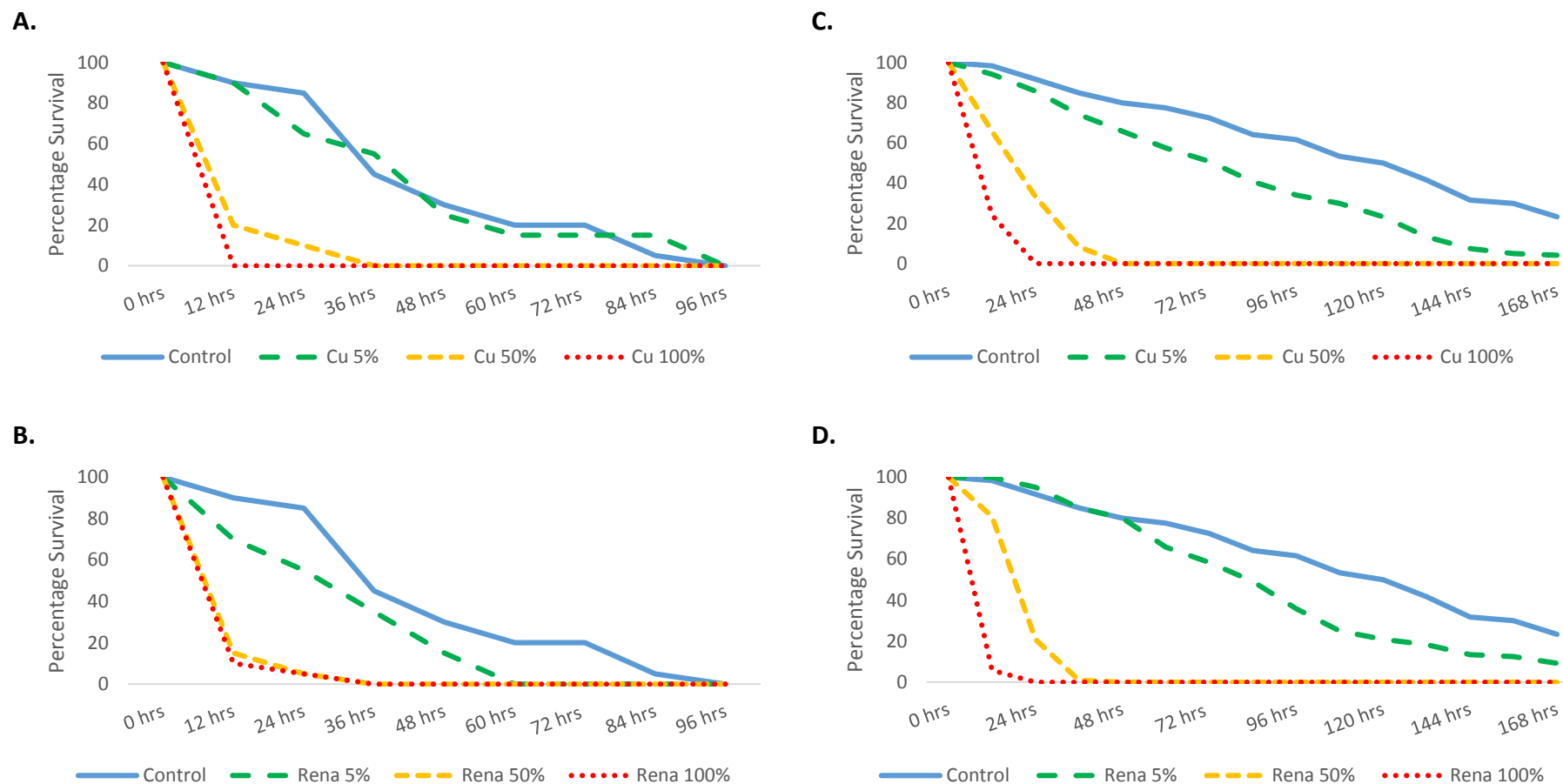


Figure 3-4. Mean survival of mysid shrimp (A,B) and paddle crab larvae (C, D) when exposed to copper contaminant in solution (A, C) and on reef *Rena* contaminants in solution (B, D) over the experimental duration.

3.3.2.1 Copper

Indicative LC₅₀ values were initially calculated as a percentage ratio (Table 3-1). An experiment by McSweeney (2015, *in prep*) found the mean concentration (\pm SE) of copper in solution is $1158 \pm 62.28 \mu\text{g/L}$ and $1240 \pm 47.61 \mu\text{g/L}$ for contaminated *Rena* sediment and copper treatment solutions respectively. Those concentrations were created using the same materials and ratios as used in this experiment. As water samples taken during the experiment haven't yet been analysed, the assumed maximum concentration for this acute study are based of the analysis undertaken by McSweeney (2015, *in prep*). The assumed concentrations for copper in this acute toxicity study would suggest an indicative 24 hr LC₅₀ within *Rena* treatments of $267.30 \mu\text{g/L}$ and $69.06 \mu\text{g/L}$ for first stage paddle crab larvae and mysid shrimp respectively (Table 3-2).

Table 3-1. Indicative LC₅₀ percentage values derived from Figure 3-2 for mysid and paddle crab test organism at 4 key time frames

Species	Mysid		Paddle Crabs	
Treatment	Copper %	Rena %	Copper %	Rena %
12 hrs	18.78	12.21	67.09	63.88
24 hrs	9.50	5.96	17.63	23.07
48 hrs	0.28	0.01	8.92	12.98
96 hrs	-	-	1.26	1.52

Table 3-2. Indicative LC₅₀ values ($\mu\text{g/L}$) of copper derived from Figure 3-2 and calibrated with water sample analysis by McSweeney (2015, *in prep*) for mysid and paddle crab test organism at 4 key time frames

Species	Mysid		Paddle Crabs	
Treatment	Copper ($\mu\text{g/L}$)	Rena ($\mu\text{g/L}$)	Copper ($\mu\text{g/L}$)	Rena ($\mu\text{g/L}$)
12 hrs	233.04	141.47	832.50	740.17
24 hrs	117.88	69.06	218.77	267.30
48 hrs	3.47	0.12	110.69	150.39
96 hrs	-	-	15.64	17.61

3.4 Discussion

3.4.1 Goals and Hypothesis Testing

This research assessed the acute toxicity of *Rena* contaminated on-reef sediment in solution and copper (as a positive control) in solution to paddle crab larvae and mysid shrimp. The null hypothesis for this research was that there would be no significant difference in survival trends in animals exposed to on-reef *Rena* contaminants, copper contaminants or a control treatment. The 24hr LC₅₀ experiment is viewed as valid with most control organisms surviving through this period. The survivorship curves for the dilution treatments for *Rena* sediments matched those for the copper (positive control) treatments indicating good alignment of copper concentrations in these two treatments.

The mysid shrimp within a 'non-contaminated' control treatment only survived for 96 hours (Figure 3-2 A, B). This would likely be due to the lack of food to maintain the test population and was not unexpected. Acute 96-hr mortality tests undertaken by Nipper and Williams (1997) and Verslycke *et al.* (2003) used wild caught mysid shrimp that were then cultured in a laboratory for an extended period prior to toxicity testing. These were feed *Artemia* nauplii at a rate of 100-150 nauplii/mysid/day. The presented study could not accommodate generation of food for experimental animals in these bioassays, therefore did not examine a pre-test culture regime.

Larval development for paddle crabs lasts approximately 8 weeks (Haddon, 1994) and progresses through 8 zoea stages and a megalopa stage. Larvae are thought to stay in deeper water (~200m) until the megalopa stage where they migrate back inshore (Haddon, 1994). This study was undertaken on first stage larvae, which underwent metamorphosis into second stage zoea based on unpublished observations by Sayers (2011). These can be described as the development of a mouth and light sensitive eye, movement by tail thrusts and a curved dorsal spin at 1mm length. Control larvae survivorship was prolonged when compared to *Rena* and copper treatment groups. Other larval crab species are known to experience high larvae

mortality between the first and third zoeal stages (Ramachandran, Patel, & Colbo, 1997). As there was no feed regime included in this experimental design, it would be assumed that larvae have remaining yolk reserve from the egg and pre zoea stage, though no yolk was observed in test organisms.

First stage zoea are known to be more sensitive than juvenile crabs when exposed to metals in acute toxicity studies (Ferrer *et al.*, 2006). A study looking at acute toxicity to first stage *Chasmagnathus granulata* larvae found Cu to have a 24hr LC₅₀ of 666.0 µg/L (Ferrer *et al.*, 2006) and 80 µg/L at 48hr for the third stage *Scylla seratta* larvae (Ramachandran *et al.*, 1997). The 24hr LC₅₀ of 267.30 µg/L observed in this experiment highlights relative sensitivity. This is the first ecotoxicological research on the New Zealand paddle crab. There is an indication for potential impact to larval communities; though further research is required.

3.4.2 Physico-chemical factors

There could be a number of interacting physico-chemical events that influence the resultant toxicity, especially with regard to the *Rena* sediment samples which is a mixture of metals (mainly copper) and hydrocarbons (mainly weathered heavy fuel oil HFO380) (Battershill *et al.*, 2013). Metal bioavailability is affected by abiotic factors such as the partial pressure of oxygen and salinity (Ferrer *et al.*, 2006; Marsden & Rainbow, 2004). A study by De Wolf *et al.* (2004) utilised a time to death ecotoxicological assay and found periwinkle mortality increased with lower salinity levels (De Wolf, *et al.*, 2004). This acute toxicity assay was performed with 29.76 ± 0.02 psu salinity which is higher for mysid shrimp and lower-within the range for crab larvae compared to other literature in the field.

Optimal salinity for the survival of a New Zealand variety of mysid; *Tenagomysis novae-zealandiae*, in a laboratory environment is 15 – 25 psu. This lower salinity is expected as many mysid species are estuarine based. Outside this salinity range, survival of 70% or less is expected (Nipper & Williams, 1997). The relationship between metal toxicity and salinity should be carefully considered, however salinity levels in this research were

deemed appropriate for the experiment as it was based on ambient levels from freshly collected seawater in the same environment that the test organisms were collected.

3.4.3 Biological impacts of metals

Excess metal uptake in biota can have high energy costs associated with the attempt to excrete and/or detoxify incoming metals. The result of such energy expenditure is a reduction in growth, reproduction and development (Marsden & Rainbow, 2004). Useful endpoints for mysid toxicity tests could therefore be reproductive success, growth over a given period and survival-mortality (Nipper & Williams, 1997).

The use of cultured mysid targeting specific age classes gives added control to sensitivity between different life stages and allows for standardisation within experimentation (Verslycke *et al.*, 2003). *Tenagomysis novae-zealandiae* has a 4 week lifecycle (Nipper & Williams, 1997) therefore chronic life cycle assays are viable and would derive far greater information to develop the New Zealand ecotoxicology literature. It would also further aid in the development of research to better understand the generational impacts of metal contamination to sensitive organisms in a critical life phase. The use of mysid shrimp as a standardised species is hugely beneficial, but more information is required for indigenous and endemic marine organisms in New Zealand.

It is important to understand the interaction of different pollutant mixtures under environmental relevant conditions to better understand the effect to marine environments. There is an obvious effect from sediment derived *Rena* contaminants and copper shavings to mysid shrimp and paddle crab larvae as proxy species for zooplankton in a laboratory based experiment. Further research into the composition of contaminant mixtures, the dilution rate, and dilution gradient from the sediment into the water column is needed to better understand the effect of the debris halo to important ecosystem functions such as larval recruitment.

Chapter 4

Behavioural responses of a planktonic mysid to waterborne contaminants*

*To be submitted for publication under the same title as: Dempsey, T.P.T; Ross, P.M.; Battershill, C.N.

4.1 Introduction

Larval recruitment plays a critical role in coastal ecosystems by contributing to community structure and regulating population dynamics (Caley *et al.*, 1996; Krug & Zimmer, 2000). Most invertebrates are sessile or have limited mobility as adults, so dispersal is primarily achieved during the larvae life phase (Atema, *et al.*, 2002; Roberts, *et al.*, 2008; Radford, *et al.*, 2012). Marine invertebrates disperse numerous swarms of offspring that eventually settle and metamorphose. Habitat selection and settlement of planktonic marine larvae was once considered to be a submissive process where larvae were at the mercy of the hydrodynamics of the natural environment. Research into larval recruitment and settlement has demonstrated that waterborne signals originating from other larvae, adult populations of the same or different species, and habitat conspecifics can induce active swimming and orientation behaviour in planktonic larvae towards said suitable habitats (Krug & Zimmer, 2000; Atema *et al.*, 2002; Gerlach *et al.*, 2007; Elbourne & Clare, 2010); or away from predators (Hamren & Hansson, 1999; Campbell *et al.*, 2001).

Degradation of marine water quality is a growing concern within communities and can have substantial impacts on marine organism behaviour. Contamination of established benthic communities and habitats is known to influence the settlement behaviour of recruiting larvae. It can lead to a reduction in the recruitment of some species or alter the community structure to promote recruitment for more tolerant species. The flow on effects of this to other fauna can be both detrimental and beneficial (Roberts *et al.*, 2008). A study by Banks and Brown (2002) found recruitment of a settling bryozoan larvae to be significantly reduced on clay tiles exposed to crude oil, however, there was enhanced response from barnacle and oyster larvae. It was suggested that hydrocarbons facilitated the occurrence of biofilms to the benefit of said larvae (Banks & Brown, 2002).

Choice chamber experiments are a way of investigating the active behavioural responses of recruiting biota. The focus of most documented choice experiments are towards the olfactory response in fish (Baker &

Montgomery, 2001; Atema *et al.*, 2002; Gerlach *et al.*, 2007; Radford *et al.*, 2012); or response to predatory conspecifics (Hamren & Hansson, 1999; Campbell *et al.*, 2001).

The grounding of the MV *Rena* container ship on Otaiti (Astrolabe Reef), Bay of Plenty, New Zealand, on 5th October 2011, led to the discharge of more than 350 tonnes of heavy fuel oil (HFO) and 10,000m² of debris (scrap metal, cargo and non-recyclable materials) into a pristine coastal marine environment (Beca, 2014; Maritime New Zealand, 2014). There has been no research published that looks into the potential effect this event could have to larval recruitment or population longevity of the reef community on Otaiti. This chapter aims to investigate the influence of contaminated on-reef sediment to zooplankton behaviour, using mysid shrimp as a proxy, in a Y-maze choice chamber.

4.2 Methodology

4.2.1 Collection of sample water and animals

Experiments were conducted at the University of Waikato Coastal Marine Field Station in Tauranga, New Zealand, in January 2015. The laboratory is situated approximately 100m from the Otumoetai channel of the Tauranga Harbour (S37° 39.36', E176° 9.82'). Experimental seawater was obtained from the Otumoetai Channel on an incoming tide and stored onsite in a 1000L storage tank.

Mysid shrimp (*Tenagomysis* sp.) were collected using a fine-mesh dip net from the nearby Bridge Marina on an incoming tide. Collected organisms were placed in a clean 20L bucket containing seawater from the collection site. In the laboratory, mysid shrimp were diluted with control test water and kept in a 20L aerated aquaria situated in a chill bath at 17°C ± 1° with a photoperiod of 12 L:12 D. All test animals were used within 72 hours of collection.

4.2.2 Choice Chamber

Pairwise, choice chamber experiments were conducted using a two-channel choice flume based on the design by Atema *et al.* (2002) which allows test subjects to freely choose between water from two sources. The rectangular flume (25cm x 122cm) contained two receiving inflow compartments. These compartments contained inverted funnels to discharge water into the choice channels with minimal disturbance to the distinct and parallel flow. The mix zone at the end of the choice channels was covered with black plastic to shut out light and induce a stimuli up the choice channels. Water was discharged via a bevelled outfall weir (Figure 4.1). Both static and flow through experiments were trialled using this system.

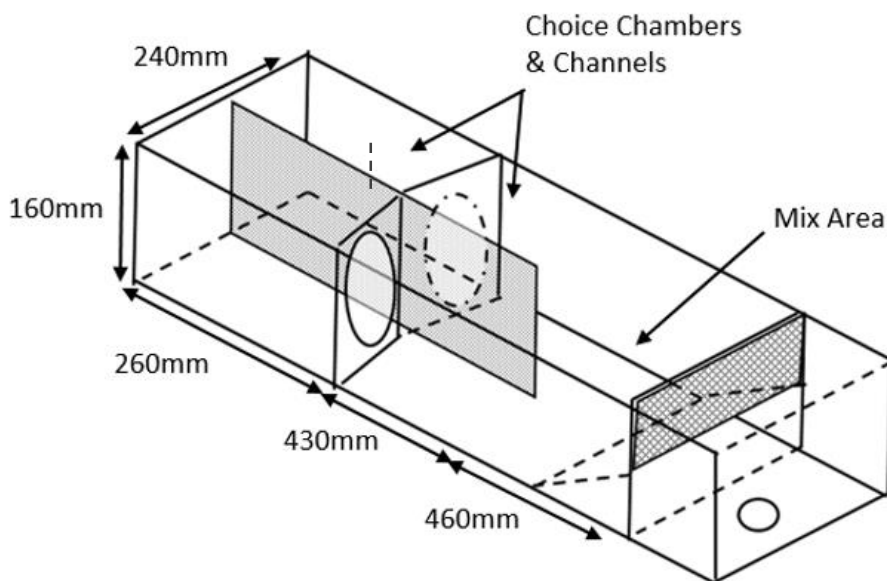


Figure 4-1. Schematic of choice apparatus

Water quality parameters were maintained at 8.12 ± 0.01 pH; 20.45 ± 0.25 °C temperature; 21.54 ± 1.12 ppt salinity; and $101.41 \pm 0.19\%$ dissolved oxygen. These parameters were tested for before, during and at the end of each trial with the monitoring areas being the control bucket, the treatment bucket and the mixing zone. Each trial ran for up to 60 minutes, with 15mL water samples also being obtained using a clean syringe in the aforementioned areas of the flume system for subsequent analysis

The static experiment was designed to examine the diffusion gradient of sediment bound contaminants as it may influence zooplankton behaviour. For each static experiment, the choice chamber was filled with filtered 1 μ seawater through the receiving compartments and given ten minutes to allow the water to settle. A 200gm mesh bag of contaminated Otaiti reef sediment was placed in one of the receiving compartments. The contaminant side was randomly selected for each trial with control (containing no contamination bag) also being undertaken.

The flow experiment provides two water sources to encourage test animals to choose a preferred water source. For flow through choice experiments, filtered 1 μ seawater was maintained in two 40L header tanks at 17°C \pm 1°C. Water was gravity fed through two isolated 6mm hoses into the flume. Flow rates were maintained at 300mL min⁻¹ per channel throughout all experiments. Flow rate and tracer dye tests were undertaken at each water change to ensure the flow rate and hydrology remained constant. Only three replicates of treatment and control flow trials were able to be undertaken as the channel divide developed a very slight bend, creating an eddy that disturbed the water flow distinction.

As water samples taken during the experiment haven't yet been analysed, the assumed maximum concentrations for this study are based on the analysis undertaken by McSweeney (2015, *in prep*)(Table 4.1). A 200gm bag of copper shavings or on-reef *Rena* sediment was placed in 40L aerated aquaria and left to circulate for 6 hours. This has real world relevance in that an area of seawater could hover over a contaminated seabed site for a tidal cycle. Figures need to be treated with some caution. In this regard, results are discussed in a conservative manner.

Table 4-1. Mean (\pm SE) values for 5 metals based on analysis undertaken by McSweeney (2015, *in prep*) comparing copper to *Rena* polluted sediments.

$\bar{x} \pm SE$	Cu (μ g/L)	Al (μ g/L)	Zn (μ g/L)	Mn (μ g/L)	Ni (μ g/L)
Rena	254.07 \pm 31.96	9.59 \pm 0.13	80.03 \pm 5.30	7.96 \pm 0.14	10.26 \pm 0.68

Mysid shrimp (*Tenagomysis* sp.) were placed centrally into the downstream end of the flume and given 10 minutes to acclimatise. Mysids were able to move about freely and allow for olfactory behaviour responses to initiate. The number of mysids which moved up each choice channel was then recorded at 10 minute intervals for 60 minutes, with sampling and parameter checks occurring at 0mins, 30mins, and 60mins. The number of mysids per group were counted at the beginning and end of each experiment to ensure all were accounted for. Each trial was run in triplicate and the flume was thoroughly rinsed with fresh seawater between each experimental procedure.

Control trials were run for both static and flow through experiments to test for side preference choice by mysids. Mysids were not reused for any other experiment. The proportion of non-observed mysids (i.e. mysids which stayed in the mix zone and were unseen for the experiment duration) remained included in the results of this study.

4.2.3 Statistical Analysis

Due to the variances in sample sizes across the different treatment replicates, observations were converted to percentage ratios and the mean overall observed percentage was used in analysis. The significance of the collected data was examined in two ways using chi-squared analysis alongside single factor ANOVA. The first analysis looked at the number of mysid that moved up the choice channels (control or treatment) compared to the number of mysid that remained in the mix zone. This was to show whether a significant number of mysid would move in response to the stimuli.

The second procedure examined sensitivity and avoidance of mysid shrimp to the contaminant stimulus. The percentage ratios for these analyses were created by removing the un-observed mysid from the previous data set to gain a new percentage relative to active participation of test animals. The null hypothesis predicted a percentage result of 50:50 or both analysis types. The critical level of statistical significance for all tests were $\alpha = 0.05$.

4.3 Results

4.3.1 Water Chemistry

The target water parameters for the choice chamber trial were based on the observed oceanic measurements of approximately 8.09 pH; 15.76°C temperature; 31.82 *psu* salinity; and 93.04% dissolved oxygen (refer Chapter 2). Operationally, it was found to be more realistic to maintain the animals and perform the trial with locally sourced water from the Otumoetai Channel. This therefore had slightly lower pH and salinity. The temperature was difficult to reduce below 20°C due to the high ambient temperature in the summer months.

Physico-chemical characteristics of the control and treatment trials are summarised (Table 4-2). Little variance of characteristics was observed within each trial treatment. There was no significant difference between the control and flow through treatment trial for the physico-chemical characteristic of pH; temperature; salinity and dissolved oxygen (Table 4-2). It should be noted that the 'Aquatech multimeter' was unavailable during the static trial which meant a YSI Model 85 handheld multimeter was employed to measure the water parameters of test solutions. Feasible salinity readings were unobtainable and slight variance between equipment readings appeared present. However the variance within just the static trial is insignificant.

Table 4-2 . Comparison of mean (\pm SD) physico-chemical characteristics from the control vs. static and flow treatment choice trials

Trial Batch	pH	Temperature °C	Salinity ppt	Dissolved Oxygen %
Control	8.14 \pm 0.02	18.43 \pm 0.14	28.08 \pm 0.63	101.14 \pm 0.31
Static*	7.93 \pm 0.03	22.62 \pm 0.51	-	102.35 \pm 2.23
Flow	8.17 \pm 0.04	18.56 \pm 0.32	30.15 \pm 0.65	101.47 \pm 1.04

* The Aquatech multimeter was unavailable during the static trial which required the use of a YRI probe in its place.

4.3.2 Choice Experiment

Mysid behaviour during the experiments varied from between trial replicates, with some groups showing active swimming up and down channels and between treatments; and others settling in an area of the flume and remaining there throughout the experiment.

In the first set of analyses, the Chi-squared test found that only the flow through experiment initiated a significant response with mysid participants utilising the choice channels. Over half of the test animals in the control and static trials preferred to remain in the mix zone and hence were unobservable throughout the experimental duration (Table 4-3).

Table 4-3 . Mean percentage of time spent in the preferred test area in a static and flow through test. Mx= mix zone (unobservable), Ch=choice channel (treatment or control). Values in red show significance

Test Type	Preference	\bar{x} (\pm SE) % time in preference	χ^2	<i>n</i>	<i>p</i>
Control	Mx	54.39 \pm 2.12	0.77	230	0.02
Static Ch v Mx	Mx	69.53 \pm 4.38	15.26	93	8.8x10 ⁻⁵
Flow Ch v Mx	Ch	69.42 \pm 0.90	15.09	57	3.2x10 ⁻¹¹

On removal of the unobserved counts, the second set of analyses displayed significant behavioural response away from the contaminant stimuli ($p < 0.05$), see Table 4-4, Figure 4-2. This highlights a level of sensitivity for mysid shrimp to water borne *Rena* contaminants. The control tests showed no preference for either side of the choice flume ($\chi^2 = 0.04$, $p > 0.05$). This indicates the mysid didn't have a bias towards either side of the flume channels.

Table 4-4 . Mean percentage of time spent in the preferred test water type in a static and flow through test. T= treatment , C=control, *=no significant preference

Test Type	Preference	\bar{x} (\pm SE) % time in preference	χ^2	p
Control	*	50.99 \pm 2.59	0.04	0.600
Static T v C	C	63.72 \pm 4.73	7.53	0.002
Flow T v C	C	52.33 \pm 0.93	0.22	0.005

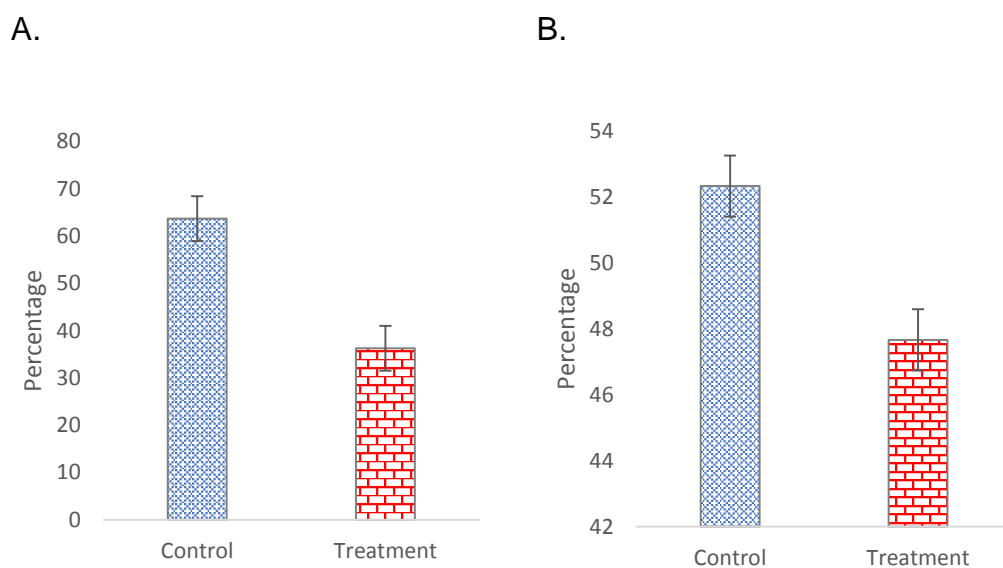


Figure 4-2. Mean percentage (\pm SE) preference of mysid shrimp during the a) static and b) flow through treatment experiments

4.4 Discussion

Larval recruitment to reef environments is heavily influenced by chemical cues which induce settlement (Roberts *et al.*, 2010). Mysids have been known to display positive rheotactic responses to varying current velocities (Roast *et al.*, 2000, 2001). Chemical interference could therefore adversely affect important ecological functions important to the longevity of a reef ecosystem. Results indicate that wild caught mysid shrimp (*Tenagomysis* sp.) show significant sensitivity to *Rena* contaminants derived from the on-reef sediment. This has implications for the settlement behaviour of other planktonic organism that rely on reef conspecifics and chemical cues to initiate settlement behaviour.

Classical survival-mortality acute and chronic toxicity assays are commonly used for experimentation on mysid shrimps (Nipper & Williams, 1997; Verslycke *et al.*, 2003; Yan *et al.*, 2003; Perez & Beiras, 2010). Sublethal endpoints, such as choice preference and behavioural disruption to swimming ability for example, are increasingly being studied to assess effects of contaminants to biota (Roast *et al.*, 2000). The measure of an organism's behaviour following contaminant exposure at environmentally-realistic concentrations provides a better understanding of environmental consequences.

The use of flume technology in ecotoxicological research is relevant as it examines chronic behavioural response at critical stages of an organism's life history. Swimming behaviour variations such as position in the water column, speed and orientation can be observed with different current velocities, substrate type and salinity levels. Roast *et al.* (2000) and Roast *et al.* (2001) investigated the swimming behaviour effects on *Neomysis interger* in an annular flume when exposed to cadmium and organophosphate pesticide respectively. Under control conditions, *N. interger* could sustain a swimming speed 6cm s^{-1} in a laboratory based annular flume and showed better positioning with a mud substrate rather than a sandy substrate (Roast *et al.*, 1998). Exposure to cadmium caused significant disruption to hyperbenthic behaviour, and exposure to

organophosphates lead to reduced ability to maintain position (Roast *et al.*, 2000, 2001).

A 'y' maze choice chamber used in this study is more commonly used for reef fish research (Atema *et al.*, 2002; James *et al.*, 2008; Radford *et al.*, 2012) as allows the test subject the chance to swim through zone of partial mixing to make a preference choice.

The range of behavioural traits displayed by the different mysid trials could be due to the contaminant stimuli; or the overall health of the wild caught test subjects. To replicate this experiment with laboratory raised animals where age and condition could be controlled for produce would a stronger result.

The statistical methodology used in this research was unique in that previous literature replicated trials with one test subject at a time (Campbell *et al.*, 2001; Atema *et al.*, 2002; James *et al.*, 2008; Radford *et al.*, 2012). That was not feasible with mysid shrimp, so percentage ratios were employed gauge the movement of test animals.

To the researcher's knowledge, this is the only assay that utilises a choice flume to observe mysid sensitivity to waterborne contaminant plumes. Further research into the levels of similarity between *N. interger* and *Tenagomysis* sp. behavioural response under environmentally relevant conditions would be of benefit for further understanding the role of sublethal contaminant concentrations to zooplankton communities, and recruitment behaviour in high impact areas. This technique is therefore a highly relevant test procedure for marine toxicology.

Chapter 5

General Discussion

5.1 Thesis Design

This thesis aimed to address the concerns from local Tangata Whenua, government, researchers, stakeholders and the public about the long term recovery of Otaiti following the MV *Rena* shipwreck and subsequent reef contamination. The three objectives for this research were to;

1. Examine the influence of *Rena* and its associated debris field to the chemistry and quality of the water column adjacent to Otaiti;
2. Assess the toxicity of *Rena* contaminants to survivorship of zooplankton
3. Assess how *Rena* contaminants might influence zooplankton behaviour and therefore recruitment to Astrolabe Reef.

By addressing uncertainties around the consequences of *Rena* contaminants for survivorship and behaviour of planktonic and early life phases, the research provides a clearer picture of the effects of *Rena* on coastal water quality and the environmental health of Otaiti. This research will inform communities about the potential impacts to the longevity of the Otaiti system and inform resource management decision making around the continuing salvage operation.

5.2 Environmental contamination and reef ecology

There is a clear effect from the *Rena* and its associated debris field on the water quality and chemistry of Otaiti (Chapter 2). The metals present in almost every component of a ship structure and can influence the physiochemical and biological parameters of the adjacent seawater (Dimitrakakis *et al.*, 2014). Aluminium and copper for example, were consistently elevated in dissolved and total metal concentrations around the debris field. Previous *Rena* research had highlighted the enrichment of these metals within Otaiti reef sediments in 2013 and 2014 (Ross & Battershill, 2013; Ross *et al.*, *in press*). Exposure of zooplankton to concentrations of these *Rena* contaminated sediments resulted in mortality with increased contaminants concentrations increasing rates of mortality

(Chapter 3). An indicative 24hr LC₅₀ percentage ratio of 5.96% and 23.07% dilution exposure to *Rena* derived contaminant solutions was expressed for mysid shrimp and first stage paddle crab larvae respectively. This translates to approximately 69.06 µg/L and 267.30 µg/L for mysid shrimp and first stage paddle crab larvae respectively, based on solution analysis by McSweeney (2015, *in prep*) (awaiting reanalysis of water samples).

Zooplankton, although small and less charismatic than some members of temperate reef communities, are ecologically important. The term zooplankton refers to both organisms that spend their entire life in the water column as well as the pelagic life history phases of important reef associated taxa. Many of these species are sensitive to the physicochemical characteristics of the environment. Alterations to water chemistry as a result of the *Rena* grounding could alter recruitment patterns to Otaiti (Roberts *et al.*, 2010). The mysid shrimp used here as a proxy for zooplankton demonstrated the potential sensitivity of pelagic and settling invertebrates to *Rena* contaminants derived from the reef sediment (Chapter 4). This could have significant ecological implications to the recruitment behaviour of other planktonic organism that rely on reef conspecifics and chemical cues to initiate settlement.

5.3 Mauri of Otaiti post-*Rena*

A key goal of the *Rena* Long-Term Environmental Recovery Plan (RLTERP) (2011) was to restore the Mauri of the affected environment to its pre-*Rena* state. The reference to Mauri recognises the important meta-physical considerations which would not otherwise be included in a conventional assessment (Morgan *et al.*, 2013). The measurement of Mauri is difficult to determine as it is so closely linked to a suite of other values holistically (refer chapter 1 for details).

A range of tools are being developed to support the integration of Mātauranga Māori and Western Science for environmental management. A Cultural Health Index (CHI) is one such tool, which uses key indicators to evaluate the health of a waterway and its surrounds from both worldviews

using a variety of tools specific to each case. The first CHI (Tipa & Teirney, 2006) compared indicator components such as the status of the site (i.e. traditional or contemporary), valued species and their uses (i.e. mahinga kai species and the accessibility to them), and the overall health (i.e. physical parameters and intuitive relationship with the site) for two significant streams in the Ngāi Tahu region. A Stream Health Monitoring Assessment Kit (SHMAK) and the Macro-Invertebrate Index (MCI) was also used to support the CHI components for the overall assessment (Tipa & Teirney, 2006). As a framework, Coastal CHI are being/have been developed for Ngāi Tahu, Tauranga and Hawkes Bay (Ngāti Kere) as an evaluation and implementation tool for cultural environmental management (Moller & Schweikert, 2010; Taiapa *et al.*, 2013; Wakefield & Walker, 2005).

The Mauri Model (Morgan, 2006) can be utilised as an assessment tool to aid in decision making from an Iwi and Hapū perspective. The simplicity and intuitive design of Mauri Model is its strength. It is participatory in nature and allows for the integration of Mātauranga Māori and Western Science in a simple framework to determine absolute sustainability for a proposed activity (Hikuroa *et al.*, 2011; Morgan *et al.*, 2013). A non-participatory Mauri Model assessment of Mōtītī Island undertaken on 2012 highlighted a 3.8 negative change (+1.8 to -1.8) in Mauri. This clearly highlighted the mauri had diminished as a result of the *Rena* incident. The loss of marine life was the greatest influencing indicator to this assessment, with recommendations made for further scientific analysis to further inform the Mauri Model (Steiger, 2012).

Otaiti is a dynamic reef environment (Robertson, 2014) and the probability of detecting significant contamination was therefore unlikely. This research was designed 'have a peep' at an important element of ecosystem function that otherwise would not have been investigated as part of the *Rena* Recovery Programme. The ocean is a source of spirituality and sustenance, providing a suite for valued resources. High quality health and well-being is therefore of upmost importance. The investigation into the foundations of the reef ecosystem as a part of coastal water quality and larval recruitment

highlighted an effect to water quality and a result for recruitment sensitivity suggests the potential impact health, well-being and longevity of the reef ecosystem. These are tangible elements that effect mauri. The extent of this effect is difficult to quantify from an ecological perspective within the methodology used.

Mauri is relative to other values such as kataitiakitanga and tino rangatiratanga, it cannot be assessed without the direct guidance and input of *tangata whenua ahi kā* (local people with active and continuous occupation). Ecological concepts such as ecosystem function and biodiversity dynamics (including recruitment) relate the life force and longevity of a system. Keys concerns within the scope of this research were highlighted as they may have implications to Tangata Whenua decision making in assessing the mauri of Otaiti.

5.4 Concluding Statement

The *Rena* debris field has an effect on the water quality of Otaiti. The contaminants contained within have an effect to zooplankton as an ecosystem foundation. Though this is not a Mauri assessment, it can be implied that the *Rena* continues to impact the Mauri of Otaiti due to the presence of contaminants within the debris field.

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