Development of bioassay approaches to evaluate the impacts of pollution on New Zealand estuaries using the marine copepod *Quinquelaophonte sp.*

A thesis submitted in partial fulfilment of the requirements for the Degree of

PhD in Zoology

at the University of Canterbury

by

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2012

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Acknowledgments

First and foremost I would like to thank my three advisors; Dr. Louis Tremblay, Dr. Chris Glover, and Dr. Vaughan Keesing, for all of their expertise, support, and encouragement throughout the whole project. Without them this thesis would have not been possible.

I would also like to extend my gratitude to Dr. Grant Northcott and Dr. Sally Gaw for all of their help with the chemical analysis of sediment and pore-water contaminants; to Professor John Wells for always providing me assistance with copepod identification, taxonomic questions, and even going as far as naming a species after me (*Quinquelaophonte stringeri* in progress); to Professor G. Tom Chandler and Kate Washburn for hosting me at University of South Carolina and for their generous hospitality during my stay. I would also like to thank Ted Pickney for his assistance in building my culture system and for making it earthquake proof, to Kat Trought for all of her expertise and help in the laboratory over the last four years and Dr. Jamie Ataria for his help with the field sampling in Napier and Invercargill. I would also like to thank Assoc Professor Islay Marsden, Gavin Robinson, Rob Stainthorpe, Jane Arrow, Graeme Rodgers, Guy Forrester, Guadalupe Peralta, John Korsman and special thanks to the University of Canterbury for the Canterbury Doctoral Scholarship and the New Zealand Ministry for Science and Innovation supporting this research.

Finally, I would like to thank my family and friends for their support and relief during this PhD, especially to my wife Tatiana, whose support kept me motivated through the long days and nights working on my studies.

Abstract

Worldwide, estuaries are under increasing pressure from numerous contaminants. There is a need to develop reliable bioassay methodologies to assess the effects of these stressors on estuary health. This thesis aimed to develop and validate toxicity tests in a New Zealand marine harpacticoid copepod species for use in monitoring and evaluating the effects of estuarine pollution. A survey and toxicological assessment of a range of native copepod species resulted in the selection of *Quinquelaophonte* sp. as the ideal bioassay species. This selection was based on a broad regional distribution, ease of culture and high reproductive rate in the laboratory, sexual dimorphism, and sensitivity to contaminants. To validate the bioassay, spiked sediments were used to expose *Quinquelaophonte* sp. to three reference compounds representing important categories of estuarine chemical stressors: zinc (a metal), atrazine (a pesticide), and phenanthrene (a polyaromatic hydrocarbon). A method for spiking sediments that *Quinquelaophonte* sp. inhabit was developed to ensure even contaminant distribution in sediments. Two sediment bioassays using lethal and sublethal endpoints were validated, one acute (96 h) and one chronic (14 d). These assays incorporated both lethal and sublethal endpoints, which included reproductive output and mobility. Acute-to-chronic ratios were calculated for use in environmental risk assessment and to provide insight into the mode of action of the reference contaminants. The chronic sediment bioassay was used to assess sediment quality in three estuaries across New Zealand: Napier, Christchurch and Invercargill. This validated the bioassay for use with naturally-contaminated field sediments with varying mixtures of pollutants and sediment types (coarse sandy to fine silty organic rich sediments). *Quinquelaophonte* sp. was also tested to assess whether it can be used to characterise multi–generation impacts. After four generations of exposure to zinc, there were changes in acute sensitivity, indicating this species possesses mechanisms for acclimating or adapting to toxic stressors. Sediment bioassays in *Quinquelaophonte* sp. were successfully developed and validated, offering significant promise as a tool for monitoring effects of pollution in New Zealand estuaries.

List of Abbreviations

ACR – Acute-to-chronic ratio

ASW – Artificial seawater

d – Day

DNA – Deoxyribonucleic acid

DDT – Dichlorodiphenyltrichloroethane

EC_{50} – Median effective concentration

EC_x – Concentration that effects x% of the exposed individuals

EDC – Endocrine disrupting compound

ERM – Effects range median

GC – Gas chromatography

GCMS – Gas chromatography mass spectrometry

GLMM – Generalised linear mixed models

GPC – Gel permeation chromatography

h – Hour

ICP-MS - Inductively coupled plasma mass spectrometry

ISQG –Interim sediment quality guideline

LC_{50} – Medial lethal concentration

LC_x – Lethal concentration required to cause mortality in x% of the exposed individuals

LOEC – Lowest observed effect concentration

MATC - Maximum acceptable toxicant concentration

NOEC- No observed effect concentration

PAH – Polycyclic aromatic hydrocarbon

PEL – Probable effects level

PCB – Polychlorinated biphenyls

RCF – Relative centrifugal force

RSD – Relative standard deviation

SEM – Standard error of the mean

SQG – Sediment quality guideline

SQV – Sediment quality value

TIE – Toxicity identification evaluation

WQG – Water quality guideline

‰ – Parts per thousand

1.

Introduction

1.1 Introduction

Every ecosystem on our planet has been impacted in some way by anthropogenic processes such as global warming, chemical pollution, deforestation, and urbanisation. There will be an increasing number and magnitude of threats to the environment as the world's population grows, and with it, increased urbanisation and industrialisation occurs. However, accompanying this growth in potential impact is an enhanced awareness of our effects on the environment. This is reflected by the actions of governments all over the world imposing regulations to help protect the environment. This includes the creation of national parks, the protection of endangered species, emissions restrictions, and prohibition of harmful chemicals. It is becoming increasingly important to understand the impacts that humans have on the environment and to develop tools that enable us to monitor these impacts in ecological risk management strategies.

The marine environment is a valuable resource for humans, emphasised by the fact that over half of human settlements are located near coastal regions and estuaries (Kennish 1992, Kennish 2002). Historically, coastal regions have provided important food resources and a means of transportation, as well as convenient waste disposal sites (Kennish 2002). These provisions continue to the present day. Because of this, coastal regions and particularly estuaries have seen many centuries of human exploitation, habitat modification and pollution. This has left many of these regions degraded to an extreme level (Lotze et al. 2006). Human impacts have caused significant loss of wetlands, salt marshes, sea grasses and submerged aquatic vegetation. In fact, over 90% of recorded estuary species were depleted by the end of the 20th century (Lotze et al. 2006). Human-induced changes in the coastal environment have already led to fishery collapses in tropical and sub-tropical areas of the world and there is potential for further ecosystem collapses (Jackson et al. 2001). The degradation of estuarine

environments has significant impacts on biodiversity, the economies that depend on marine species as food sources and as a basis of income, as well as the ecosystem services that estuaries provide (Costanza et al. 1997). The increasing development and urbanisation of coastal areas will result in more land conversion, roadways, and other impermeable surfaces. This will increase non-point source pollution and runoff as well as reducing the natural filtering capacity of the land, thus increasing pollution in estuaries (Fulton et al. 1993, Kennish 2002). Consequently, while monitoring anthropogenic impacts on our environment is of critical importance in the present day, it will become more crucial in the near future. If policies and actions are established early enough, when contamination is at levels that have not severely impacted populations, it may be possible to salvage or avoid significant adverse ecological effects. Preventing significant ecological impacts is vital to preserving these ecosystems, and will mitigate further economic losses through collapsing fisheries as well as the large costs associated with clean up and rehabilitation of ecosystems after they have already degraded.

1.2 Estuaries

An estuary is a semi-enclosed body of water, where freshwater from rivers and streams meets the saltwater of oceans and seas. These regions are predominantly characterised by the mixing of salt and freshwater, and are subjected to the force of the tides (Prichard 1967, Fairbridge 1980). Estuaries are also regions where sediment and nutrients washed from the land are deposited in aquatic sediments on the ocean floor resulting in rich, productive ecosystems. In addition, estuaries are highly dynamic, with seasonal variation, tides, as well as fluctuating freshwater input. The severities of these dynamic forces within the estuary are affected by the type and shape of the estuary (Chapman and Wang 2001).

There are three general types of estuaries. The first is a salt wedge estuary, in which the river flow is dominant and the saltwater moves up the estuary in a wedge shape. The second general estuary type includes shallow, partly-mixed estuaries and fjords. These are similar to wedge estuaries with distinct layers of saltwater at depth and freshwater on the surface. However in these estuaries river flow is modified by tidal currents and there can be some vertical mixing of saltwater and freshwater. The third estuary is a vertically homogeneous estuary, which is characterised by low variation in vertical salinity (less than 1g/L), and is predominated by tidal currents, which cause mixing. In these estuaries there is still a horizontal salinity gradient (Chapman and Wang 2001). In general estuaries are influenced by seasonal variation such as river flow (e.g. snow melt periods), which can change the mixing of saltwater and freshwater leading to either increases or decreases in sedimentation.

Sediment in estuaries originates from the rivers that feed into the estuary as well as from the ocean. The relative contribution of these sources depends on the water circulation patterns in the estuary and the particulate load of the river. Estuaries with rivers that carry high particulate loads are often characterised by high fluvial silt content and significant sediment deposition. Sediments in estuaries fed by rivers with low particulate loads generally exhibit properties more similar to marine sediments (Guilcher 1967, Chapman and Wang 2001). The sedimentation characteristics of the estuary can change seasonally. The most common type of estuary sediment is fine grained, silty or muddy, especially in estuaries with little wave action. However, sediments can also be sandy and are often stratified throughout the estuary. Estuarine sediments are subject to complex and dynamic forces, which cause deposition and erosion (McManus 1998, Douglas et al. 2009). The transition from freshwater to saltwater and the rise and fall of water levels with the tides can increase particle settling in the estuary (Chapman and Wang 2001).

Estuaries have salinity variations ranging from 1 to 30‰ (where full strength SW is ~35 ‰). Within estuaries, regional differences in salinity can be quite extreme, depending on the type of estuary and the extent of mixing. This variation in salinity can make life extremely challenging for estuarine species due to pressure on osmoregulation from rapid changes in ambient osmotic pressure. It has been documented that distribution of benthic fauna changes seasonally due to variation in salinity in some estuaries (Chapman and Brinkhurst 1981, Chatzigeorgiou et al. 2011). There are also other gradients found in estuaries including temperature, pH, dissolved oxygen, redox potential, nutrients and composition of particulates (Chapman and Wang 2001), which can be influenced by regular fluctuations in salinity, tides, and wave action. All of these gradients can occur both vertically and horizontally and are controlled by the many complex physical characteristics of estuaries, such as shape, depth, river input, and currents. However, salinity is the most important factor in estuaries and is largely responsible for faunal distributions as well as contaminant bioavailability and partitioning (Chapman and Wang 2001).

The input of terrestrial nutrients from rivers into the estuary provides a high productivity habitat for many different species. Nutrients such as nitrogen and phosphorus are the limiting factors for primary production of algae (Howarth 1988, Rudek et al. 1991), and their introduction into estuaries permits high primary production that subsequently supports large and diverse food webs. Some of the many organisms that inhabit estuaries include species of shellfish, which live in sediments and on rocky substrates. A large variety of fish species are found in estuaries at some point during their life history, using it as spawning and nursery grounds, for migration, and feeding (Gillanders et al. 2003). Estuaries are also important habitat and feeding grounds for many species of birds, as well as being habitat for many species of mammals, insects, reptiles, and crustaceans (Chapman and Wang 2001, Lotze et al. 2006). This results in a

unique and diverse mix of marine, freshwater, and terrestrial species, and highlights the value of estuaries from a biodiversity perspective.

1.3 Pollution and Estuaries

Contaminants are substances that contaminate air, water, or soil. Contaminants can be artificial substances, such as pesticides and polychlorinated biphenyls (PCBs), or naturally occurring substances such as metals and oil. Contaminants have a variety of chemical properties, structures, and toxicities, which can impact estuarine and costal environments. Pollutants are mixtures of contaminants that can originate from one or from multiple sources and may include both chemical and physical stressors.

Influent rivers are major sources of contaminants that impact estuaries. Contaminants are washed off land from point and non-point sources or dumped into rivers and streams, which then transport the contaminants downstream, eventually depositing them in estuaries and estuarine sediments. Point sources, such as effluent discharges, have a discrete origin easily identified. Non-point source pollutants have no single point of origin and are deposited on the land from a wide range of anthropogenic activities. Agricultural runoff, storm water, industry waste, forestry, mining, land conversion, road runoff, sewage, and harbour activities all contribute to pollution in estuaries and coastal areas (Carpenter et al. 1998). Contaminants are often washed into rivers and streams during rain events. Depending on the amount of rain and the time between rain events, high concentrations of contaminants may wash into rivers during a single downpour (Carpenter et al. 1998, Mallin 2000).

1.3.1 Fate and distribution of contaminants in estuaries

Contaminants, once washed into rivers, bind to floating particulate and dissolved organic matter, which are then deposited in estuarine sediments (Droppo et al. 2001). These accumulate in the sediment matrix where they can remain for long periods. Concentrations of contaminants, especially metals, in sediments are usually three to five orders of magnitude greater than in the overlying water (Bryan and Langston 1992). These contaminants can potentially lead to significant adverse effects on biota that live within, or in contact with, the sediments.

Contaminants in the sediment matrix can additionally become re-suspended in the water column due to erosion, wave action, the tides and dredging (Droppo et al. 2001, Clement et al. 2004). Suspended metal concentrations have been found to be highest during the flood tide rather than the ebb tide in Manukau Harbour, New Zealand. This suggests that contaminants are likely to remain in some estuaries and not washed out to sea with the tides (Williamson et al. 1996). The importance of re-suspended contaminants and the deposition of new contaminants depend on the physical characteristics of the estuary and the complex forces that govern fate, distribution and the eventual bioavailability of these chemicals.

The fate and distribution of contaminants in estuaries is predominantly controlled by the salinity, be it via direct or indirect effects. The high ionic strengths of very saline waters can “salt out” organic matter and hydrophobic organic chemicals from the water column into the sediment. This would result in the removal of hydrophobic chemicals that sorb to organic particulates (Brunk et al. 1997, Chapman and Wang 2001). Water oxygen levels can also have an influence on toxicant distribution. Anoxic conditions, for example, can stimulate the mobilisation of some metals (e.g. iron and manganese) from sediments into

the water column (Kristiansen et al. 2002). Other metals (e.g. cadmium, copper, chromium and zinc) are removed from the water column by reduction or sulphate precipitation (Brugmann et al. 1992, Chapman and Wang 2001).

There are a number of other factors related to sediment type and composition that can also have important impacts on pollutant distribution, accumulation and eventually bioavailability within sediments. Grain size, for example, is particularly important, with smaller grain size sediments having higher concentrations of metals (Strom et al. 2011). For hydrophobic organic chemicals, the proportion of organic carbon within sediments seems to be a critical controlling factor (Di Toro et al. 1991, Chapman and Wang 2001). Organic carbon has also been shown to play a role in metal and polycyclic aromatic hydrocarbon (PAH) sequestration (Rockne et al. 2002). These contaminants bind to the organic carbon fraction within the sediment, which has implications for both their partitioning and bioavailability (see 1.3.2) (Luoma and Davis 1983, Rockne et al. 2002). Another factor influencing chemical fate and thus bioavailability is bioturbation, which is caused by meiobenthic fauna as they burrow, build tubes, irrigate their dwellings or scavenge for nutrients. Bioturbation affects the solute concentrations of contaminants and organic carbon in sediment pore-water (the water between sediment particles). Bioturbation is less prevalent in highly toxic sediments, potentially reducing contaminant concentrations in pore-waters (Aller and Aller 1992, Green and Chandler 1994, Hagopian-Schlekat et al. 2001). However, it is the bioavailable concentrations of contaminants, rather than their total concentrations, which are most relevant to sediment-dwelling organisms.

1.3.2 Bioavailability of contaminants

The forces that govern the fate and distribution of contaminants from the overlying water column into estuary sediments also modulate the bioavailability

of those contaminants (Chapman and Wang 2001). The bioavailable pollutant fraction is that which is available for absorption by the organism, and as such is a better representation of the potential toxic impact of a given contaminant compared to measures of total concentration (Di Toro et al. 2005). The amount of the contaminant that is bioavailable depends on a variety of factors that affect distinct contaminants differently, but is a measure that is almost always significantly less than the total sediment concentration. As a result different sediments potentially have markedly different bioavailable contaminant fractions even though they may have similar contaminant loads (Rockne et al. 2002, Strom et al. 2011).

Metal bioavailability is predominantly controlled by five factors: 1) speciation (e.g. Zn^{2+} is usually the most bioavailable form of zinc) and mobilisation into the interstitial water; 2) transformation into organo-species (e.g. methylmercury, alkyllead, and tributyltin); 3) control from major sediment metal-binding constituents (e.g. iron and manganese oxides and organic matter); 4) competition between sediment metals (e.g. copper and silver; zinc and cadmium) for uptake sites into organisms as well as binding to sediment components; and 5) environmental factors such as pH, redox potential, bioturbation and salinity (Bryan and Langston 1992, Simpson and Batley 2003, Di Toro et al. 2005, Atkinson et al. 2007, Hutchins et al. 2009, Simpson et al. 2011, Strom et al. 2011). The bioavailability of each metal is affected differently by these factors. For example, arsenic, mercury, lead, and tin have organo-species which are the most toxic and bioavailable form, while organic content and iron oxides have strong effects reducing lead, arsenic, copper, and zinc bioavailability (Bryan and Langston 1992). In pore-water concentrations of metals have been found to be affected by the presence of other metals, due to the different binding strengths of the metals to the sediment matrix, and competition for binding sites leading to displacement (Aller and Aller 1992, Hagopian-Schlekat et al. 2001).

PAH bioavailability is predominantly controlled by organic carbon content and solid organic matter. PAHs, particularly the higher molecular weight forms, sorb to the low-density components of the sediment more than they do to the high-density components (Rockne et al. 2002). Once PAHs are sorbed to the sediment, desorption and thus bioavailability is significantly affected by the molecular structure of the PAH. The preferential partitioning of PAH in the different sediment fractions is the main factor controlling bioavailability and exposure of organisms to PAHs (Rockne et al. 2002, Haftka et al. 2010). Bioavailability of other types of contaminants, such as pesticides, varies greatly depending on their chemical properties. However, organic carbon seems to be an important controlling factor in many contaminants, both inorganic and organic (Chapman and Wang 2001, Simpson et al. 2005).

Bioavailability of contaminants to sediment-dwelling organisms greatly depends on their life history and the predominant way that they come into contact with these chemicals. The routes of exposure and uptake are primarily sediment contact, sediment ingestion, or contact with interstitial pore-waters (Green et al. 1993, Simpson and King 2005). Uptake of the contaminant can also vary depending on the nature and concentration of the contaminant, the presence of other contaminants, as well as the potential mechanisms an organism may have to limit bioavailability. For example, the process of digestion can often enhance bioavailability by desorbing contaminants from ingested food/sediment, while body mucus secretion can prevent contaminants coming into contact with permeable body surfaces (Cajaraville et al. 1990, Triebskorn et al. 1998, Khan et al. 2008).

Even though bioavailability of contaminants is a critical aspect of toxicity, it is difficult to measure in sediments largely due to their heterogeneity (Chapman and Wang 2001, Simpson and Batley 2003). Luoma et al. (1995)

suggested that the use of a bioassay along with sediment chemistry could be an easy way to incorporate the bioavailability of contaminants into environmental risk assessments of toxicants to estuarine organisms.

1.3.3 Effects of pollution on estuarine fauna

Contaminants found in estuaries are of greatest concern to benthic species that live in, or are associated with, estuary sediments. These species are those more likely to be exposed to contaminants chronically, through contact and/or ingestion of the sediments. Another concern is with species that forage on sediment-associated organisms. These species may be subjected to elevated contaminant levels owing to biomagnification through the food chain, i.e. the increase of contaminant concentration in tissues with trophic level (Lester and McIntosh 1994, DiPinto and Coull 1997). Waterborne contaminants are often not as great of a concern as sediment-bound contaminants primarily due to the high fluctuations in concentration and distribution due to tidal mixing. In addition, the mixing of freshwater and saltwater can enhance the partitioning of contaminants into estuary sediments (see 1.3.2) where the contaminants can remain for extended periods.

Polluted sediments can have a variety of effects on estuarine fauna, including serious impairment and death. At low contamination levels sublethal effects are more prevalent over long exposure periods. These may include reproductive impairment, decreased growth rate, inhibited development, alteration of sex ratios, and intersex (Coull and Chandler 1992, LeBlanc 2007). These effects can be subtle and difficult to detect but have serious implications for the affected species and the ecosystem integrity.

The effects of contaminants on organisms can be characterised by laboratory tests, over short (acute) and long (chronic) time frames. The

sensitivity of the organism to a specific contaminant is measured by calculating a median lethal concentration (LC_{50}; concentration required to cause mortality in 50% of the exposed individuals) and/or a median effective concentration (EC_{50}; concentration required to effect a 50% change in a non-lethal endpoint). Another common toxicological measure is LOEC (lowest observed effect concentration) and NOEC (no observed effect concentration). However, the LOEC and NOEC have come under scrutiny owing to statistical concerns (Kooijman 1996) and their use is limited. These measures do, however, provide baseline data allowing for toxicity comparisons between species and contaminants. They also enable researchers to relate laboratory responses to effects found in the field.

The effects of contaminants such as metals, PAHs, and pesticides on estuarine systems are not well understood. This has obvious negative implications for effectively monitoring their impacts. Often contaminants can have greater toxicity when mixed, with additive or even synergistic (where toxicity is greater than that expected by adding the effects of toxicants) effects possible (Forget et al. 1999). This poor knowledge of the basic sensitivity of estuarine organisms to contaminants, coupled with a lack of general understanding of how chemical mixtures may affect toxicity, makes risk assessment of estuarine sediment toxicity difficult.

1.4 Estuarine Monitoring

Traditionally, estuarine monitoring has been performed by testing water and sediment for key contaminants. This involves chemical analysis of overlying water and/or sediments, using solid-phase or solvent extractions and then comparing those values to water or sediment quality values (SQVs) (Chapman and Wang 2001). SQVs are derived by integrating sediment chemistry, toxicology and biology to develop a threshold value for a particular contaminant. Concentrations that are above the SQV can potentially have negative biological

impacts (Abrahim et al. 2007). However, SQVs are only a numerical value for the level of contamination. It is difficult to translate those values into a meaningful evaluation of toxicity and environmental risk, especially considering the complex mechanisms controlling bioavailability of contamination, and the complicating effects of contaminant mixtures. SQVs are further compromised in that they are often based on limited toxicity data obtained from a small number of organisms (which may exclude highly sensitive species) and developed from international data (ANZECC 2000). This is particularly problematic in a setting such as New Zealand that has a unique indigenous estuarine fauna with poorly described sensitivity to environmental contaminants. These factors can reduce the efficacy of SQVs as tools for estuarine monitoring, and actual toxicity of sediments can potentially be greater than that predicted by the SQVs. In addition, it can be difficult to understand effects on the component species, communities and populations when simply monitoring for the presence and quantity of the pollutant itself. Better understanding of the responses of key species greatly heightens the value of monitoring and can allow a more timely and targeted response to issues otherwise missed by conventional monitoring systems.

There are increasing research efforts focussed on using estuarine species as indicators of estuarine pollution, and incorporating such species into bioassays. Bioindicator species are those that, due to their specific life histories, are advantageous for assessing toxicity. Ideally, they respond to contaminants in a predictable, observable, and quantifiable manner (Adams and Greeley 2000, Carignan and Villard 2002). The development of bioindicator species has allowed for improved methods of assessing sediment toxicity (Chandler and Green 2001). Species inhabiting the meiobenthos are particularly good candidates for assessing the health of estuarine sediments. These are generally small invertebrates (less than 1mm in length and therefore include the majority of estuarine copepods), and spend the majority of their life-cycle in sediments (Hicks and Coull 1983). Aside from meiobenthic copepods (see section 1.4.1),

amphipods, polychaetes, clams, oysters, and mussels are all being used as bioindicator species in a variety of bioassays to assess sediment toxicity (Greenstein et al. 2008).

Most bioindicator species are used in bioassays, which are controlled laboratory exposures where the bioindicator species are exposed to water or sediments to assess toxicity. Bioassays using whole sediment exposures allow for environmentally-realistic exposure conditions, similar to those that the organisms would be exposed to in an estuary, to be simulated in the lab (Chandler and Green 1996, Bejarano et al. 2004). In addition, these bioassays assess the toxicity of the complex assortment of contaminants in the sediment. SQVs can only assess the individual contaminant concentrations and do not account for the toxicity of pollutant mixtures (Chapman and Wang 2001).

1.4.1 Copepods and their use as bioindicators of pollution

Copepods (Subclass: Copepoda) are an important group of species that are increasingly being used to monitor estuarine sediment toxicity. They are a very diverse group of crustaceans, and can be found in both fresh and marine waters. They are the major component of zooplankton and are a vital link in the transfer of nutrients to higher trophic levels. Copepods are small (usually less than 10 mm length), dioecious, and reproduce sexually. Most are filter feeders and feed on phytoplankton and detritus (Hicks and Coull 1983). Copepods have a life cycle consisting of six naupliar stages, five copepodite stages, and an adult stage, which is sexually dimorphic. Females usually produce five to eight clutches in their lifetime, extruding egg sacs with between five and nine embryos per clutch (Hicks and Coull 1983, Huys et al. 1996). Of the three main orders of copepods, the Harpacticoida are predominantly benthic dwelling unlike the two other orders, Calanoida and Cyclopoida, which are largely pelagic. Harpacticoid copepods are important components of meiobenthic communities, being one the

most abundant taxa in sandy and muddy marine sediments (Hicks and Coull 1983).

Harpacticoid copepods are being increasingly used in bioassays to assess sediment toxicity. They are advantageous for this purpose in that they have short life spans (~28 days), high reproductive rates, and are easy to culture in the lab. These properties are ideal for assessing sub-lethal effects such as impaired reproduction, growth, and development (Kovatch et al. 1999, Chandler and Green 2001, Bejarano et al. 2004, Greenstein et al. 2008). They have close association with contaminated sediments, which makes them very susceptible to chronic exposure to contaminants (Coull and Chandler 1992, DiPinto and Coull 1997). Harpacticoid copepods and other meiobenthic species can be exposed to contaminants through pore-water or sediments. Pore-water is often characterised as having higher levels of contaminants than the overlying water column. Therefore pore-water exposure combined with exposure via the sediment means that harpacticoid copepod species are likely to be subjected to the full toxic impact of a contaminated sediment.

There are several species of harpacticoid copepods that are currently being used in sediment bioassays, including: *Amphiascus tenuiremis, Microarthridon littorale, Tigriopus brevicornis, Tisbe battagliai* (Forget et al. 1998, Kovatch et al. 1999, Hagopian-Schlekat et al. 2001, Bejarano and Chandler 2003). However, a bioassay using a New Zealand species of marine copepod has not been fully developed. Having a bioassay that uses a native species of copepod for monitoring estuarine contamination is important as this will give a better understanding of how pollution is affecting fauna in New Zealand's estuaries. This will complement the existing methods for estuarine monitoring currently used in New Zealand: SQVs and shellfish (e.g. cockles and mussels) monitoring. Having several different bioassay methodologies with

different species allows for a more accurate, sensitive, and robust assessment of estuarine contamination (Greenstein et al. 2008).

1.4.2 Bioindicators of pollution in New Zealand

There have been only limited efforts in establishing bioassays for aquatic sediments in New Zealand. There have been two studies that have used native amphipod species for monitoring estuary contamination. King et al. (2006) looked at the sensitivities of eight species of estuarine amphipods from Australia (6 species) and New Zealand (2 species), to zinc- and copper-spiked sediments. The authors suggested that the New Zealand amphipod *Melita awa* be used as a test species and that a whole sediment bioassay with this species should be developed. Another study using a native New Zealand species of amphipod, *Paracorophium excavatum*, as a bioassay species was that of Marsden and Wong (2001), who used copper-spiked sediments to develop the bioassay. Again, however, this study was only preliminary in nature and further testing is required to better evaluate the suitability of this species as a bioindicator. Dupree and Ahrens (2007) successfully used the amphipod *Melita* sp. to test PAH toxicity from contaminated Auckland estuary sediments. The results of this study indicate *Melita* is a potentially useful bioassay organism for New Zealand sediments. While these studies offer some promise as to the use of amphipods as bioassay organisms, there is still a lack of bioassays being conducted on field sediments in monitoring schemes. Additionally, in studies where responses of amphipods and harpacticoid copepod species have been tested under identical conditions, the copepods have been shown to be more sensitive than amphipods (Greenstein et al. 2008). Consequently copepods provided a more sensitive assessment of contamination (Greenstein et al. 2008).

Currently there is not a validated sediment bioassay using harpacticoid copepods to assess contamination of New Zealand estuaries. There has been

some preliminary work done by Hack et al. (2008a, 2008b) that identified a suitable New Zealand species of harpacticoid copepod, *Robertsonia propinqua*, and performed some initial experiments towards development of a bioassay for assessing toxicity of estuarine sediments. However, there has not been any research investigating the suitability of other New Zealand native harpacticoid species for estuarine settings, or the relative sensitivity of the array of native species to sediment contamination (an important consideration for bioassays). Willis (1999) examined the acute and chronic toxicity of pentachlorophenol with three native freshwater New Zealand copepod species, and later supplemented this study with a freshwater plankton mesocosm study (including copepods, rotifers, and phytoplankton) on the effects of pentachlorophenol (Willis et al. 2004). These studies provided very important information for the possible development of assessment tools for freshwater environments. However, the species used in this study are not found in estuaries and live in the water column, so they are not suitable test species for estuarine sediment contamination. Despite important advances in recent years, more work needs to be undertaken to provide substantial knowledge of the effects of pollution on native fauna, instead of relying on toxicity data from species not relevant to local settings to make management decisions (ANZECC 2000, King et al. 2006).

1.5 Pollution in New Zealand estuaries

New Zealand estuaries have been under increasing pressure from anthropogenic forces, especially from rapid urban growth in the major centres and primary industry processes in rural areas. In urban areas, the major sources of contamination are storm water and sewage effluent. Increased urban growth has, and will continue to, put more pressure on infrastructure (e.g. sewage treatment works and storm water drainage) which will result in increased contamination in estuaries and surrounding ecosystems (MFE 1997, Hack et al. 2008a). Rural areas are also of concern, due to runoff from agriculture,

commercial animal production, and forestry (ARC 2003, ECAN 2007, Hack et al. 2008a, 2008b). The rapid recent nationwide conversion of large areas of dry land farming to irrigated dairy production is a prime example of a future run-off contaminant increase. The contaminants that cause the greatest concern are metals and PAHs from storm water and industrial runoff, pesticides from agriculture and forestry, and endocrine disrupting compounds from sewage effluent (ARC 2003, ECAN 2007).

Although estuaries are established as at-risk ecosystems, there is relatively little information on the specific levels of contaminants and the degree of risk these pose in a New Zealand setting. However, New Zealand has adopted water quality guidelines (WQG) and sediment quality guidelines (SQG) for estuaries, developed by the Australia and New Zealand Environmental and Conservation Council (ANZECC 2000). Two interim sediment quality guideline (ISQG) values for each contaminant are linked to threshold concentrations for high (ISQG-H) and low (ISQG-L) biological impacts. Concentrations of contaminants below the ISQG-L value are considered acceptable (Abrahim et al. 2007). Table 1.1 lists reported concentrations of contaminants in estuaries around New Zealand. Most studies have focused on metal contaminants and have concentrated on the Auckland and Wellington regions. Even though these two regions have not been used as study sites for research in this thesis, a brief description of the extent of contamination in both of these regions follows as it provides information on the likely extent of contamination in other field locations as well as helping to identify priority pollutants.

Table 1.1 Concentrations of contaminants found in New Zealand estuaries.

Location	Contaminant	Max. Sediment Conc. µg/g	Source
Manukau Harbour	Cu	70	Aggett & Simpson 1986
	Cr	107	
	Pb	247	
	Cd	0.19	Williamson et al. 1996
	Cu	35	
	Mn	1220	
	Pb	57	
	Zn	166	
	Total PAH	1.52	Wilcock & Northcott 1995
Tamaki Estuary	Cd	1.49	Abrahim & Parker 2002
	Cu	60	
	Pb	200	
	Zn	366	
	Cd	0.24	Abrahim et al. 2007
	Cu	45	
	Pb	77	
	Zn	252	
Porirua Harbour	Cu	93	Glasby et al. 1990
	Co	20	
	Cr	101	
	Ni	27	
	Pb	170	
	Zn	435	
*Wellington Harbour**	As	14	Pilotto et al. 1998
	Cr	65	
	Cu	30	
	Ni	26	
	Pb	79	
	Zn	178	
Avon-Heathcote Estuary	Cu	23	ECAN 2007
	Cr	39	
	Pb	35	
	Ni	17	
	Zn	157	

* Values obtained from sediment traps sampling floating sediment particles in the water column, not benthic sediments.

In Auckland, the largest urban area in New Zealand, the development and expansion of the city has placed increased pressure on the three main waterways/estuaries: Manukau and Waitemata Harbours and the Tamaki Estuary (Williamson et al. 1996, Abrahim and Parker 2002). The two main metals of concern in the Auckland region are lead and zinc, with concentrations of lead as high as 247 µg/g being reported in Manukau Harbour sediment, which is above ISQG-H value of 220 µg/g (Aggett and Simpson 1986, ANZECC 2000). Zinc

and lead have both been found in concentrations above the ISQG-L values in the Tamaki Estuary (Wilcock and Northcott 1995).

In Wellington, contamination of copper, zinc, and lead has been found at high levels around storm water inputs (Pilotto et al. 1998). The lower reaches of Waiwhetu Stream, which flows into Wellington Harbour, have been shown to be highly contaminated with zinc and lead, metals enriched in storm water run-off (Deely et al. 1992), while Porirua Harbour, 20km north of Wellington, has been found to be moderately to strongly contaminated with lead and zinc (Glasby et al. 1990).While these two estuaries have not been assessed in this thesis the levels of contamination provide some background to the major contaminants of concern in New Zealand.

1.5.1 Christchurch estuary contamination

Since September 4th 2010 Christchurch has experienced an earthquake sequence unlike any other in recorded human history, with violent ground acceleration, vertical and horizontal displacements and widespread liquefaction. The earthquakes have considerably altered the city, with widespread destruction, abandonment of land, and closure of the central city. The city's sewage treatment works were shut down, and along with leaks in the sewer network, this resulted in raw sewage being be pumped directly into both the Avon and Heathcote Rivers and the Avon-Heathcote estuary. The rate of sewage input into these waters was determined to be as high as 50,000 m^3 per day (Zeldis et al. 2011).

The estuary itself saw major changes in topography and was largely covered with liquefaction, which added large amounts of archaic sediment to existing surface sediments. The earthquakes have impacted invertebrate community structure in the estuary, with reduced diversity of crabs and snails (Zeldis et al. 2011). In the Avon and Heathcote Rivers there have been

alterations noted in crustacean and caddis fly populations (James and McMurtrie 2011). The lasting effects of the earthquake on the estuary are unknown.

1.6 Types of Estuary Contaminants

Estuaries contain a complex mixture of contaminants, many of which can pose significant threats. The major types of contaminants can be classified into several categories: metals, pesticides, PAHs, polychlorinated biphenyls (PCBs), and endocrine disrupting compounds (EDCs). Each of these contaminant types can have significant and diverse adverse effects on exposed biota. However, as there are so many different contaminants that affect estuaries, reference contaminants are often used to assess the suitability of a bioassay species. Reference chemicals from a subset of these major groups of contaminants have been chosen for this thesis, each with a distinct mode of action and chemical structure. Furthermore, each of the reference contaminants chosen is a chemical of concern for estuaries worldwide (Bryan and Langston 1992, Kennish 1992, Ackerman 2007). The three reference contaminants are: zinc (a metal), atrazine (a pesticide) and phenanthrene (a PAH). Toxicological profiles of the contaminant categories, and of the selected reference contaminants, are detailed below.

1.6.1 Metal contamination

It is becoming increasingly common for estuaries to have high concentrations of metals, generally resulting from human activities. These high metal concentrations are a cause for concern. The most common anthropogenically-introduced metals in estuarine sediments are copper, cadmium, nickel, lead, and zinc (De Groot 1995). In addition, metals such as arsenic, chromium and mercury can also be of great concern to estuaries. Metals

eventually find their way into estuaries from processes associated with urbanisation, weathering, road and storm water runoff, anti-fouling paints and industry.

The toxicity of individual metals can vary significantly. Some metals are required by all organisms to survive. Known as micro-nutrients, metals such as iron, zinc, and copper, are necessary at low levels; however, when they are present in high concentrations they can have negative effects. There are also metals that have no positive actions at any concentration, these are sometimes termed the 'heavy metals' and include cadmium and mercury. The toxicity of different metals has been suggested to correspond to sulphur affinity, with metals that have a higher affinity for sulphides having a higher toxicity (Hook and Fisher 2002). These inherent differences in toxicity complicate monitoring approaches where the main information collected is regarding environmental metal burdens.

The bioavailability of metals is complex but plays an important part in metal toxicity, as discussed previously (see 1.3.2). In general, bioavailability dictates bioaccumulation, which, for most metals, is directly related to toxic impact (Hare 1992, Simpson 2005, Strom et al. 2011). The exposure of copepods to metals can occur via direct contact with sediments and water and also through food intake. Each of these exposure pathways will impact metal bioavailability and toxicity in a distinct manner. Studies in calanoid copepods have shown that assimilation of metals is greatest when ingested in algal food (Hook and Fisher 2001b, 2002), indicating a higher bioavailability of metals through the dietary pathway. The influence of direct contact and ingestion of contaminated sediment particles on metal bioavailability and toxicity in meiobenthic organisms, such as harpacticoid copepods, is complex (Hare 1992, Chandler et al. 1994, Di Toro et

al. 2005, Simpson 2005). The role and importance of sediment metal in terms of toxicity is likely to vary between species due to differences in lifestyles within sediments and the different composition of the sediments that they inhabit (Simpson 2005, Simpson and King 2005).

1.6.1.1 Zinc toxicity

Zinc is one of the major problematic metals in New Zealand's estuaries and estuaries around the world (MFE 1997, Chapman and Wang 2001, Lotze et al. 2006, MFE 2007). Zinc enters the environment through storm water, urban runoff and wastewater, and has been found in concentrations above the ISQG-L values in the Tamaki Estuary in Auckland (Abrahim and Parker 2002). Other areas that have been found to be severely contaminated include Waiwhetu Stream Estuary in Wellington Harbour (Deely et al. 1992) and Porirua Harbour (Glasby et al. 1990).

Zinc (as Zn^{2+}) can be very toxic to plants, fish and invertebrates. Zinc is thought to share absorptive pathways, and thus compete for absorption, with other nutrient ions, such as calcium, copper, and iron, potentially causing deficiency and imbalance of these elements (Santore et al. 2002). In crustaceans zinc can effect exoskeleton integrity (Ahearn et al. 1994, Poynton et al. 2007) and disrupt vitellogenesis (Hook and Fisher 2002). Zinc is problematic as it is used in many applications. In urban settings the most common source of environmental zinc is galvanised steel, which leaches zinc into rain runoff as it weathers.

1.6.2 Pesticide contamination

Pesticides pose significant risks to estuaries around the world, owing to important impacts on non-target organisms, including aquatic organisms exposed via agricultural runoff. In fact pesticides are often more toxic to aquatic organisms than to their target species (Naqvi and Vaishnavi 1993). Impacts in aquatic systems can extend to disruption of the food chain, leading to an altered food web and in serious cases can cause imbalance in entire ecosystems (Kennish 2002). In estuaries, micro-crustaceans and plankton can be the most affected organisms (Forget et al. 1998).

Pesticide contamination is often difficult to control due to the wide application and use of these chemicals in commercial agriculture and on private residential lands. Millions of kilograms of active pesticides, including multiple forms of insecticides, herbicides, and fungicides, are applied in coastal watersheds worldwide (Donaldson et al. 1999). Pesticides often do not remain where applied and instead run off with rain and irrigation into waterways, before being eventually transported into estuaries (DeLorenzo et al. 1999). Even though pesticides pose significant environmental risk, their use is unlikely to decrease. Pest-induced economic losses are in the billions of dollars worldwide and pesticides also perform roles in human health, eliminating vectors responsible for diseases such as malaria and West Nile virus (Karpati et al. 2004, Aggarwal et al. 2006, Ackerman 2007). This reliance on pesticides and the continuing development of new pesticides poses a ‘catch-22’ for environmental protection.

New Zealand, as an agricultural nation, uses pesticides extensively on pastoral land. However pesticides are also used in non-commercial environments for the control of pest species such as the Australian brush-tail possum, wasps, and the invasive weeds old man’s beard and gorse (Henderson et al. 1999, MFE 2007). The majority of pesticides used in New Zealand are herbicides (68%)

followed by fungicides (24%) and insecticides (8%) (MFE 2007). These pesticides may be leached into surface water and ground waters, posing risks to both the environment and human health (Sarmah et al. 2004).

Pesticides can vary in their mode of action. For example DDT (dichlorodiphenyltrichloroethane) and synthetic pyrethroids are neurotoxins (Costa et al. 2008). Organophosphorus and carbamate pesticides also impact the nervous system through a specific inhibitory effect on acetylcholinesterase (Fukuto 1990). Many rodenticides (such as warfarin) are anti-coagulants (Thijssen 1995), while herbicides often inhibit photosynthesis (Ensminger and Hess 1985). Mode of action can also vary between the target and non-target organisms. For example, DDT, while primarily a neurotoxin, can also be mutagenic and cause feminisation in birds (Fry and Toone 1981). Consequently pesticides may induce a wide variety of effects in exposed organisms.

Other factors will also impact toxicity of pesticides, and are of special relevance to the exposure of organisms in non-target environments such as estuaries. For example, different pesticides have distinct environmental half-lifes, different solubilities in water, and variable leaching potential to groundwater and rivers (Sarmah et al. 2004). Each of these factors will influence their bioavailability and toxicity to estuarine species. Modern pesticides tend to have shorter half-lives and are not as persistent as long-established pesticides such as DDT, but if used intensively can still pose a threat to estuarine organisms (Ongley 1996, Plimmer 2001). In addition, even though many pesticides are no longer in extensive use (often owing to environmental concerns), they can still leave a legacy. These pesticides, and particularly their metabolites and breakdown products, can remain in sediments long after they have been replaced by modern pest-control chemicals, and can therefore continue to have negative impacts on estuarine fauna (Plimmer 2001).

1.6.2.1 Atrazine

Atrazine (2-chloro-4-ethylamino-6-isopropyl-amino-s-triazine) is one of the most widely and heavily used herbicides in the world. It is applied in over 80 countries worldwide, and in the US alone, over 35 million kg is applied to agricultural land each year (Bejarano and Chandler 2003). In New Zealand, over 245,000 kg of triazine herbicides are used each year and this level of use has caused concerns regarding groundwater contamination (Close and Rosen 2001). For example, 76% of pesticides found in groundwater are triazine herbicides, and levels of atrazine up to 37 µg/l have been measured (Close 1993, Close and Rosen 2001). Atrazine is most commonly used for broad leaf and grassy weed control and is heavily used in agriculture, and turf and lawn care. It has been a cause for concern as it has high solubility in water (up to 28 mg/l), a half-life of 36-37 days in water, and has been found at concentrations up to 62.5 µg/l in estuaries in the US (Bejarano and Chandler 2003, Bejarano et al. 2005). Atrazine has been found to disrupt steroidogenesis in the frog *Xenopus laevis* causing demasculinisation at concentrations as low as 1 µg/l (Hayes et al. 2002). In rainbow trout (*Oncorhynchus mykiss*) concentrations of atrazine as low as 5 µg/l have been shown to cause inflammatory damage to gills and kidney (Davies et al. 1994).

As atrazine is a herbicide it is not unexpected that it has significant effects on estuarine plants, especially algal phytoplankton. A study by DeLorenzo et al. (1999) found that atrazine, and its metabolite deethylatrazine, reduced chlorophyll *a* and phototropic carbon assimilation in estuarine phytoplankton after 72 hours at concentrations as low as 50 µg/l. This subsequently impacted phototropic biovolume, and dissolved oxygen. In addition, six genera of algal phytoplankton were lost from the atrazine treatments. This reduction in primary production in estuarine food webs has implications for food and nutrient availability for higher trophic levels.

1.7 Polycyclic aromatic hydrocarbon (PAH) contamination

PAHs are some of the most common contaminants in aquatic systems. They are also among the most toxic, displaying potent mutagenic and carcinogenic effects (Kennish 1992). PAHs are a very diverse group of compounds structurally characterised by several joined aromatic rings, although the majority of PAHs that are found in sediments contain 2-5 aromatic rings. Of all contaminants that pose a threat to aquatic ecosystems it is often PAHs that are the most toxic (Kennish 1992, Pane et al. 2005). The three most common PAHs that are responsible for this toxicity are pyrene followed by flouranthene, and phenanthrene (Bellas and Thor 2007).

PAHs occur naturally from inefficient burning as well as being found in coal, tar, and crude oil deposits. However, PAHs pose a threat to the environment predominantly from human activities, especially those associated with crude oil, refined petroleum and diesel fuels (Latimer and Zheng 2003, Geffard et al. 2005), which reach waterways largely through road runoff and atmospheric deposition of incomplete combustion products (Kennish 1992, Carman and Todaro 1996, Lotufo 1998a, Lima et al. 2005). The hydrophobicity of PAHs determine their ultimate fate in the aquatic environment. As hydrophobic chemicals PAHs are sequestered by organic particles and eventually accumulate in sediments, and because they are fat soluble they also have the potential to bioaccumulate in exposed organisms (Lotufo 1998a). In industrial and urban areas PAH concentrations of 2 mg/kg have been found in estuarine sediments (Budzinski et al. 1997). PAH contamination can be lethal to benthic invertebrates, while sublethal effects include decreased growth, slowed development time, reduced reproduction, and decreased feeding rate (Lima et al. 2005, Bellas and Thor 2007, Fleeger et al. 2007, Silva et al. 2009).

PAH contamination of estuaries can have implications for the estuarine community structure. Studies using sublethal concentrations of PAH in a meiobenthic mesocosm have shown that PAHs affect meiobenthic communities by altering sex ratios in copepod species (Carman and Todaro 1996). Increased proportions of females, due to increased male mortality, have been noted. Stunted development of the nauplii has also been attributed to PAH exposure. PAH exposure also caused increased abundance of nematodes and reduced copepod abundance (Carman et al. 1995, Carman and Todaro 1996). Even these relatively subtle alterations in community structure can have important implications for estuarine benthic food webs. For example, reduced abundance of copepods could affect the juvenile fish that feed largely on these animals (DiPinto and Coull 1997).

In New Zealand PAH contamination has been noted adjacent to major urban areas. For example, Auckland's Waitemata and Manukau Harbours and Tamaki Estuary (Dupree and Ahrens 2007), and estuaries associated with Christchurch (Ermens 2007), Napier, and Invercargill (Dr. James Ataria, personal communication) have all reported elevated PAH levels in sediments. Concentrations as high as 3.7 µg/g total PAH have been measured (Holland et al. 1993).

1.8 Thesis Objectives

The overall goal of the thesis is to develop and validate toxicity testing methodologies to assess the impacts of pollution in estuarine sediments using a native New Zealand harpacticoid copepod species as a bioindicator. This copepod bioassay will help to assess estuarine and marine health in New Zealand and identify areas that are toxic and having adverse impacts on local fauna.

The central objectives and key research questions are:

1. Selection of a suitable native New Zealand harpacticoid copepod bioassay species:

   Which species of native harpacticoid copepod are the most sensitive to toxicants?

   Are these species able to be cultured in the laboratory?

   Do they have a life history that allows them to be a good bioassay species?

2. Validation of sediment bioassays:

   Does the bioassay species respond to exposed reference toxicants in a dose-dependent manner?

   How does the sensitivity compare to other bioassay species?

   Which endpoints are the most sensitive and how do they relate to each other (i.e. lethal v. sublethal endpoints)?

   How do the toxic responses vary between reference contaminants?

3. Laboratory testing of field-collected sediments:

   Is the bioassay able to be used on a variety of sediments?

   Is the bioassay able to identify toxic sediments?

4. Population level responses to low level contamination habitats:

   How do low levels of contamination affect population size and structure?

   Does the sensitivity to the exposed pollutant change?

   Does the sensitivity to an unexposed pollutant change?

1.9 Thesis Structure

This thesis is presented as six separate research chapters with a general discussion following in the 8th chapter. Because of the nature of each chapter there is some repetition in the methodologies.

Chapter 2 investigates the ecology of New Zealand harpacticoid copepod species and defines ranges of physical sediment matrix characteristics inhabitable by candidate bioassay species. Physiochemical parameters and species abundance were sampled along three transects in Portobello Bay, Otago Harbour. Generalised linear mixed models were used to correlate abundance to physiochemical parameters. This chapter has been accepted for publication to the *New Zealand Journal of Marine and Freshwater Research*.

Chapter 3 details the selection of a native harpacticoid bioassay species by a detailed comparison of life history and sensitivities to contaminants. This chapter also describes the methods for collection and culturing of harpacticoid copepods in the laboratory. Based on the multiple characteristics examined *Quinquelaophonte* sp. was selected as the best bioassay species. This chapter has been accepted for publication to *Ecotoxicology and Environmental Safety*

Chapter 4 describes the methods for spiking estuarine sediments for use in bioassays. The methods used to measure sediment and pore-water concentrations are also investigated. Zinc and phenanthrene are used as model spiking compounds as they required two different methods for spiking.

Chapter 5 details the methods and validation of the acute and chronic bioassays using three reference contaminants. Each bioassay uses two endpoints, mortality and a sublethal measurement of impact. Acute/chronic ratios are also examined. *Quinquelaophonte* sp. proved to be a very sensitive bioassay species, with chronic inhibition of reproduction being the most sensitive endpoint.

Chapter 6 shows the implementation of the bioassays to a variety of different estuaries around New Zealand. These estuaries consist of a range of sediment types from very fine silty sediments to low silt medium sand (300 µm) sediments. Whole sediment toxicity testing was successful in identifying toxic sediments, as well as proving the versatility of the bioassay species to different sediment types.

Chapter 7 explores the impacts of multiple generation exposures to zinc. Changes in population abundance and acute sensitivity to zinc and phenanthrene are examined. It was found that population numbers were significantly decreased at 134 µg/g zinc and changes in sensitivity were observed at concentrations of 82 µg/g zinc and above.

Chapter 8 is a general discussion of the findings of this thesis. It discusses how the bioassays described in this thesis can be incorporated into regulatory frameworks and environmental risk assessment. Suggestions for further research are also provided.

The Appendix includes an example of the application of the *Quinquelaophonte* sp. aquatic bioassay for the environmental risk assessment of oseltamivir (Tamiflu®) and its photodegradation products. The assessment of the ecotoxicity was conducted in conjunction with Alfred Tong of the University of Otago who examined the ultraviolet degradation of oseltamivir. This work has been published in *Environmental Chemistry* (2011, 8: 182-189).

2.

The effects of environmental gradients on the distribution of harpacticoid copepods in an intertidal flat, Portobello Bay, Otago Harbour, New Zealand

Adapted from:

Stringer TJ, Korsman JC, Peralta G, Keesing V, Tremblay LA, Glover CN. Effects of environmental gradients on the distribution of harpacticoid copepods in an intertidal flat, Portobello Bay, Otago Harbour, New Zealand. New Zealand Journal of Marine and Freshwater Research, in press.

Co-author contributions:

- J. Korsman played a significant role in the sampling and enumeration of the copepods as well as with sediment characterisation.
- G. Peralta assisted with the multivariate data analysis and interpretation.
- C. Glover, V. Keesing and L. Tremblay supervised the research and provided feedback on the manuscript

2.1 Introduction

Harpacticoid copepods are small (<1 mm in length), predominantly benthic-dwelling invertebrates and are among the most abundant of all taxa within estuarine and marine sediments (Hicks and Coull 1983, Coull 1999, Rombouts et al. 2009). They are globally distributed and ecologically important as a food source for fish (Coull 1990, Sarvala 1998, Rombouts et al. 2009). They exhibit a short life cycle (c. 21 - 28 days), show rapid reproductive rates and a high sensitivity to environmental contaminants (Huys et al. 1996, LeBlanc 2007). By virtue of these characteristics, harpacticoid copepods are being increasingly used as indicator species to monitor estuarine health (Chandler and Green 2001, Greenstein et al. 2008). This provides an impetus for understanding more about their general biology and, in particular, their ecology. A first step in this process is assessing the physical factors responsible for harpacticoid copepod distribution. Such knowledge will assist bioassay development and application by identifying optimum physicochemical sediment properties for cultured animals.

Factors responsible for harpacticoid distribution have been well documented (for a review see Hicks and Coull 1983). On a global scale, temperature is the key parameter that affects copepod diversity (Rombouts et al. 2009). However, at an estuary scale (m^2–ha), copepod distribution primarily depends on physical factors such as salinity, tidal exposure, sediment size and oxygen concentration (Findlay 1981, Brito et al. 2009). On a habitat scale (cm^2–m^2) biological variables increase in importance (Findlay 1981, Sandulli and Pinckney 1999, Azovsky et al. 2004), although these biological factors are often themselves shaped by physical factors (Hogue and Miller 1981, Hicks and Coull 1983, De Troch et al. 2006, Cacabelos et al. 2009). The factor which has been suggested to have the most influence on species distribution is sediment size (Findlay 1981, Hicks and Coull 1983, Azovsky et al. 2004); in particular the

proportion of silty, <63 µm, sediment fractions (Hicks and Coull 1983, Udalov et al. 2005, Cacabelos et al. 2009).

Harpacticoid copepods have affinities to certain substrate types due to life history traits (Chertoprud et al. 2007). There are species of copepods that are epibenthic and live on the surface of sediments, species that live in the interstitial space between sediment particles, and those that burrow through sediments (Hicks and Coull 1983, Walker–Smith 2004). Different sediment types will be preferred by some species over others and this is often reflected in their morphology and biotype (Chertoprud et al. 2007). For example epibenthic species are often larger with well–defined limbs and are usually bristly, whereas burrowing species are often smaller with more streamlined bodies, shorter limbs, and bristles (Chertoprud et al. 2007). There are also generalist intertidal species that are a mix of the burrowing, interstitial and epibenthic forms (Hicks and Coull 1983, Chertoprud et al. 2007). Most copepods are found in the first centimetre of surface sediment and this is especially true for finer sediments, where oxygen levels may limit copepod abundance and depth (Veiga et al. 2010).

Copepods are known to have a patchy distribution especially in muddy sediments where patch size can be smaller than in more sandy sediments (Sandulli and Pinckney 1999). Several studies have shown this patchy distribution to be related to benthic microalgae assemblages (Blanchard 1990, Santos et al. 1995, Sandulli and Pinckney 1999). However, this is only true for some copepods, due to variation in the ability of species to move between patches (Decho and Fleeger 1988, Sandulli and Pinckney 1999).

There is currently little known about most of the species of harpacticoid copepods in New Zealand. If the New Zealand species follow the trends exhibited by other species worldwide, then sediment size will be a dominant

factor affecting harpacticoid distribution. However, other factors, such as salinity, organic content, location in tidal reach, and pH also affect copepod distribution (Hicks and Coull 1983).

Gaining knowledge regarding the physical factors responsible for harpacticoid copepod distribution will assist bioassay development and application. This allows the optimum physicochemical sediment properties which support high densities of the copepod species of interest to be replicated in the laboratory for the culturing of copepods. This will also provide physical limits for bioassay application and allow targeting and sampling of field populations on the basis of sediment properties. This is a critical requirement for the development of a harpacticoid copepod bioassay as a sediment toxicity methodology. In addition, when utilising field contaminated sediments in laboratory bioassays, the test species is often not found at the location. This could be due to the fact that the sediment is contaminated or that the physical properties of the sediment are not favourable for the bioassay species. Understanding physical limits of bioassay species is vital to the outcome of the bioassay. If the sediment properties are not suitable for a species, and these limits are not understood, it could create false positives whereby toxicity is attributed to patterns shaped by simple physical sediment characteristics.

The main objectives of this chapter were to (1) describe the environmental gradients and the spatial distribution of harpacticoid copepods in a non–discharging, low energy New Zealand intertidal zone consisting of a relatively uncontaminated sand flat, and (2) determine factors potentially responsible for the copepod distribution. In addition the results from this study will help to develop sediment guidelines for copepod sediment bioassays, as two of the species found at the study location are candidate bioassay species.

2.2 Methods

2.2.1 Site description and sampling programme

The study area was in Portobello Bay, a gently sloping, non–discharging, low energy intertidal sand flat in Otago Harbour, New Zealand (45°50'S, 170°39'E; Figure 2.1), free from any known point sources of pollutants. The samples were collected at low tide in early spring 2009. The sampling strategy was to collect samples across the small bay to try to maximise the gradients present. A total of 23 samples were taken in three transects (eight samples per transect except in transect 3 where only seven were taken) with 100 m between each transect. Copepod and sediment samples were taken every 10 m along each transect from just below the high tide mark along the tidal reach to the low tide mark (70 m). Triplicate copepod samples were collected, with a single sediment sample taken for determining sediment characteristics (Li et al. 1997). A single pH and salinity measurement was recorded for each sample point.

2.2.2 Sampling procedures

The top 2 cm of the sediment layer was sampled for use in sediment analysis and for assessment of copepod densities. For the copepod densities the

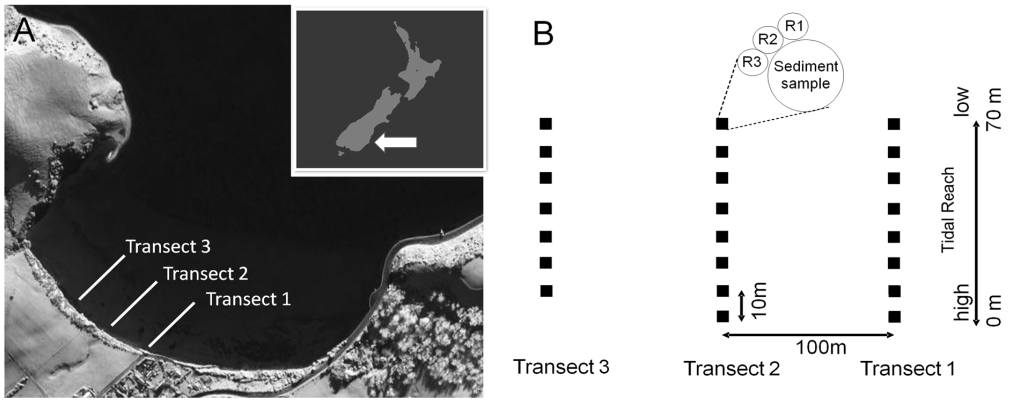

Figure 2.1 Portobello Bay. **A.** Position of transects. **B.** Sampling strategy of the 3 transects in the study including the distances between samples and how sediment samples were taken for the copepod abundance (R1-3) and sediment properties.

sediment was sampled using a small–bore plastic tube (a modified syringe; inner diameter 2 cm). A PVC sampling tube (inner diameter 8.7 cm) was used for collecting sediment for determination of sediment properties. Samples were immediately placed on ice, and then stored at 4°C until they were processed (within 3 weeks of collection). Copepods were extracted from the sediment by diluting the samples with tap water and subsequently pouring off excess water through a 55 µm mesh screen to trap copepods. This process was repeated 4 times to ensure that all copepods were collected. This method has been found to remove all copepods from sediments (Joint et al. 1982). Adult harpacticoid copepods were counted by hand at a magnification of 15x under a dissecting microscope (Leica MZ 12.5). Identification was based on species descriptions reported by Wells et al. (1982) and Hammond (1973). Copepods were identified to species level where possible, otherwise to genus. Two species of *Quinquelaophonte* genus were present. However, as one of the species has not been fully described and the two species are morphologically similar, these were pooled at the genus level. The identification of copepods to the genus (as opposed to the species level) is common practice, due to the majority of species within a genus being similar in terms of body form, life history, and response to environmental variables (Chertoprud et al. 2007). Copepod densities were standardised from individuals per sample to individuals per 10 cm^2 to enable comparison with other studies. Copepod abundance was averaged at each sample location for linear regression and summed for use in the models (see below).

2.2.3 Sediment analysis

Pore–water content in sediments was calculated by weighing sediment subsamples before and after drying for 72 h at 60°C (Ford and Honeywill 2002). Subsequently, the organic matter was measured as ash–free dry weight after 4.5 h at 450°C (Parker 1983). The rest of the sediment sample was used for determining grain size by sediment–sieving, using analytical sieves to evaluate

the proportion (% dry weight) of >1000, 1000–500, 500–250, 250–125, 125–63 and <63 µm size fractions. Sediments were classified into sediment type according to the Wentworth scale (Buchanan 1984). Salinity and pH of pore–water was directly measured during collection with a multi meter (WTW Multi 350i).

2.2.4 Statistical analysis

Linear regression models were used to determine distribution patterns of the environmental gradients along the tidal reach. The regression results were expressed in trend lines with their corresponding slope and intercept values. Additionally, the correlation coefficient (r^2) and standard deviation were derived. A 95% confidence limit (a *P*–value less than 0.05) was adopted for the results.

Generalised linear mixed models (GLMMs) with a Poisson error were used to estimate the effects of fine, medium and coarse sand, location in tidal reach, pH, salinity, mud, pore–water and abundance of other copepod species on the abundance of *Amphiascoides* sp., *Quinquelaophonte* and *Parastenhelia megarostrum* copepod species. For *Enhydrosoma* sp. and *Robertsonia propinqua*, the same variables were tested but using a negative binomial distribution, as the equidispersion assumption of the Poisson model was not achieved (Zuur et al. 2009). All the variables were incorporated into the models as fixed effects, whereas transect was incorporated as a random effect. The models were checked for overdispersion using the dispersion scale factor.

A maximal model containing all the variables was initiated, and this was then simplified by removing non–significant terms until no further reduction in residual deviance (measured using the Akaike Information Criterion, AIC) was observed (Bolker et al. 2009). Parameter estimates for fixed effects were tested for significance using a *Z* or *t* test (depending on the underlying error

distribution), as these provide a more robust test than the alternative likelihood ratio test when sample sizes are small (Bolker et al. 2009). Mixed models were conducted using the lme4 (Bates et al. 2011) and glmmADMB (Skaug et al. 2011) packages in the R 2.12.0 environment (R Development Core Team 2010), with glmmADMB being used in instances where a negative binomial distribution was needed.

2.3 Results

A total of five species groups were found in the study area. These were identified as: *Amphiascoides* sp., *Parastenhelia megarostrum*, *Enhydrosoma* sp., *Robertsonia propinqua* and *Quinquelaophonte.*

2.3.1 Description of sediment characteristics

Very fine sand (63–125 µm) was the dominant sediment size in the study area, followed by fine sand (125–250 µm) and muddy sediment (<63 µm) (Table 2.1). Linear regression models showed significant trends in environmental gradients for pH and several sediment size gradients. The pH slowly increased along the tidal reach (i.e. increased from high tidal zone to low tidal zone) in all transects (Figure 2.2A, Table 2.1). Significant gradients in mud proportion were found in transects 1 and 2 (Figure 2.2B, Table 2.1). Very fine sand proportions decreased along the tidal reach from 52% to 19% in transect 2 (Figure 2.2C; Table 2.1), whereas it increased in transects 1 and 3, albeit not significantly. Large gradients were found in percent of fine sand in all the transects, with the largest gradient found in transect 2 where this parameter increased along the tidal reach from 8% to 50% (Figure 2.2D, Table 2.1). A small gradient in medium sand was found in transect 2; this parameter decreased slightly along the tidal reach from 10% to 6% (Figure 2.2E, Table 2.1). No significant results were

Table 2.1 Physical sediment characteristics at habitat- and estuary-scale including r^2 values and *P* values of significant environmental gradients along the tidal reach.

	Transect 1				Transect 2				Transect 3			
Parameter	*Mean ±SD*	r^2	*Slope*	*Level of sig.*	*Mean ±SD*	r^2	*Slope*	*Level of sig.*	*Mean ±SD*	r^2	*Slope*	*Level of sig.*
pH	8.58 ± 0.4	0.68	0.015	*P* = 0.011*	8.57 ± 0.2	0.55	0.006	*P* = 0.035*	8.40 ± 0.3	0.74	0.01	*P* = 0.014*
mud	18.0 ± 6.6	0.59	-0.21	*P* = 0.026*	18.3 ± 7.8	0.94	-0.31	*P* < 0.001***	26.5± 8.3	0.31	0.02	*P* = 0.197
VFS	38.2 ± 10.3	0.09	0.12	*P* = 0.475	35.3 ± 12.6	0.86	-0.48	*P* = 0.001**	29.9± 5.7	0.54	0.19	*P* = 0.061
FS	17.8 ± 8.1	0.63	0.26	*P* = 0.019*	27.9 ± 16.3	0.82	0.60	*P* = 0.002**	25.7± 5.7	0.96	0.26	*P* < 0.001***
MS	11.1 ± 4.7	0.00	0.008	*P* = 0.919	9.31 ± 3.5	0.94	0.14	*P* < 0.001***	7.90± 2.9	0.25	-0.06	*P* = 0.258
CS	8.48 ± 7.7	0.25	-0.16	*P* = 0.200	4.58 ± 1.0	0.06	0.01	*P* = 0.565	4.62 ± 2.3	0.25	-0.05	*P* = 0.249
OC	1.56 ± 0.3	0.18	-0.005	*P* = 0.299	1.53 ± 0.3	0.16	-0.005	*P* = 0.314	1.49 ± 0.4	0.55	-0.013	*P* = 0.056
Cond.	54.3 ± 0.6	0.41	-0.06	*P* = 0.083	54.4 ± 0.6	0.30	-0.01	*P* = 0.159	49.0 ± 7.0	0.05	0.07	*P* = 0.639

Sediment parameters of mud, very fine sand (VFS), fine sand (FS), medium sand (MS), coarse sand (CS) and organic content (OC) expressed in percentage (dry weight) and conductivity (Cond.) in dS/m. * $P < 0.05$** $P < 0.01$, *** $P < 0.001$,

found for salinity, pore–water content, organic matter content, coarse sand (500–1000 µm) and very coarse sand (>1000 µm).

2.3.2 Copepod distribution

A number of copepod species had significant positive or negative abundance effects on other species (Table 2.2). *Amphiascoides* sp. abundances were positively affected by *Quinquelaophonte*, *P. megarostrum* and *Enhydrosoma* sp. abundances, whereas *Enhydrosoma* sp. abundance had a significant adverse effect on *Quinquelaophonte* abundance. *Enhydrosoma* sp. abundance was unaffected by the presence of other copepods. *R. propinqua* abundance was significantly negatively affected by *Enhydrosoma* sp. and *Quinquelaophonte* abundances.

Salinity significantly affected *Amphiascoides* sp. abundances in a positive way (i.e. an increase in salinity led to an increase in *Amphiascoides*) and this was the only copepod significantly affected by salinity (Figure 2.3A, Table 2.2). The proportion of mud significantly affected abundances of three copepod species (*P. megarostrum* negatively, and *Amphiascoides* sp. and *Quinquelaophonte* positively) (Figure 2.3B, Table 2.2). Fine sand was negatively related with *P. megarostrum* abundance (Figure 2.3C, Table 2.2); while medium sands were positively related with *Quinquelaophonte* and *R. propinqua* abundance (Figure 2.3D, Table 2.2). As the proportion of coarse sand increased, *Amphiascoides* sp. and *R. propinqua* abundances significantly decreased (Figure 2.3–E, Table 2). As location in tidal reach increased, i.e. towards the low tide mark, *Amphiascoides* sp. and *Quinquelaophonte* abundances significantly decreased (Figure 2.3F, Table 2.2). Habitat pH positively affected *Quinquelaophonte* abundance (Figure 2.3G, Table 2.2). Lastly, as organic

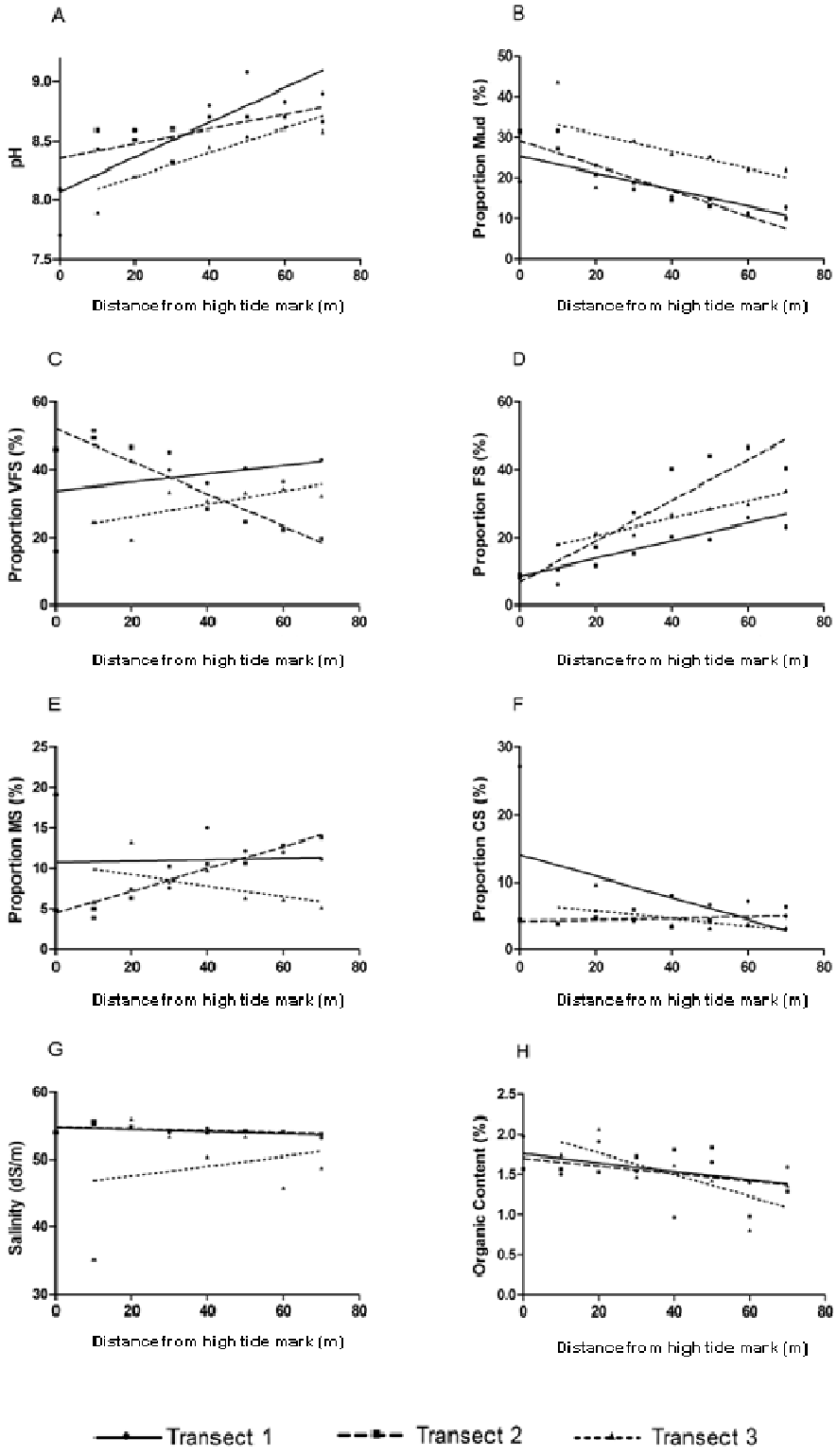

Figure 2.2 Changes in sediment properties along the three transects for **A.** pH. **B.** Proportion mud. **C.** Proportion very fine sediment (VFS). **D.** Proportion fine sediment (FS). **E.** Proportion medium sediment (MS). **F.** Proportion coarse sediment (CS). **G.** Salinity (dS/m). **H.** Organic content (%)

content increased, *R. propinqua* abundance increased (Figure 2.3H, Table 2.2). These results show that various sediment variables affect the abundance of different species differently, but that sediment grain size was a common factor affecting all species.

Table 2.2 Results of generalised linear mixed effects model for copepod species abundances: *Amphiascoides* sp., *P. megarostrum* and *Quinquelaophonte* with Poisson error (*Z* test), *R. propinqua* and *Enhydrosoma* sp. with negative binomial error (*t* test).

Response variable	Fixed effects	Estimate ± SEM	Z/t-value	*P*	Random effects (variance)
A *Amphiascoides* sp. abundance	Intercept	-7.58 ± 2.80	-2.71	0.007 **	0.275
	Quinquelaophonte abundance	0.053 ± 0.011	4.73	<0.0001***	
	P. megarostrum abundance	0.152 ± 0.068	2.22	0.026*	
	Enhydrosoma abundance	0.386 ± 0.062	6.22	<0.0001***	
	Salinity	0.157 ± 0.027	5.72	<0.0001***	
	Mud	0.059 ± 0.030	1.97	0.049*	
	Fine sand	0.037 ± 0.021	1.80	0.073	
	Coarse sand	-0.074 ± 0.032	-2.31	0.021*	
	Tidal reach	-0.033 ± 0.010	-3.33	0.001**	
B *Parastenhelia megarostrum* abundance	Intercept	12.69 ± 6.31	2.01	0.044*	1.316
	Amphiascoides abundance	0.055 ± 0.025	2.19	0.029*	
	Mud	-0.432 ± 0.201	-2.15	0.032*	
	Fine sand	-0.181 ± 0.074	-2.45	0.014*	
	Coarse sand	-0.408 ± 0.313	-1.30	0.193	
C *Quinquelaophonte* abundance	Intercept	-21.09 ± 6.10	-3.46	0.0005**	0.466
	Robertsonia abundance	-0.006 ± 0.003	-1.88	0.060	
	Enhydrosoma abundance	-0.226 ± 0.088	-2.57	0.010*	
	Organic content	0.679 ± 0.417	1.63	0.104	
	pH	2.500 ± 0.611	4.09	<0.0001***	
	Mud	0.085 ± 0.029	2.98	0.003**	
	Medium sand	0.122 ± 0.046	2.65	0.008**	
	Tidal reach	-0.050 ± 0.009	-5.78	<0.0001***	
D *Enhydrosoma* sp. abundance	Intercept	43.83 ± 32.82	1.34	0.207	0.003
	Amphiascoides abundance	0.283 ± 0.184	1.54	0.149	
	Quinquelaophonte abundance	-1.081 ± 0.741	-1.46	0.171	
	Parastenhelia abundance	-3.325 ± 2.104	-1.58	0.140	
	Robertsonia abundance	-0.082 ± 0.052	-1.58	0.141	
	Salinity	-1.339 ± 0.952	-1.41	0.185	
	Organic content	12.54 ± 8.05	1.56	0.145	
	Medium sand	1.72 ± 1.104	1.56	0.144	
	Coarse sand	-0.613 ± 0.323	-1.90	0.082	
E *Robertsonia propinqua* abundance	Intercept	1.398 ± 0.764	1.83	0.089	0.001
	Amphiascoides abundance	0.014 ± 0.007	2.02	0.063	
	Quinquelaophonte abundance	-0.032 ± 0.015	-2.12	0.052	
	Enhydrosoma abundance	-0.374 ± 0.140	-2.67	0.018*	
	Organic content	1.514 ± 0.441	3.43	0.004**	
	Medium sand	0.131 ± 0.055	2.39	0.031*	
	Coarse sand	-0.121 ± 0.039	-3.07	0.008**	

***$P < 0.001$, **$P < 0.01$, *$P < 0.05$

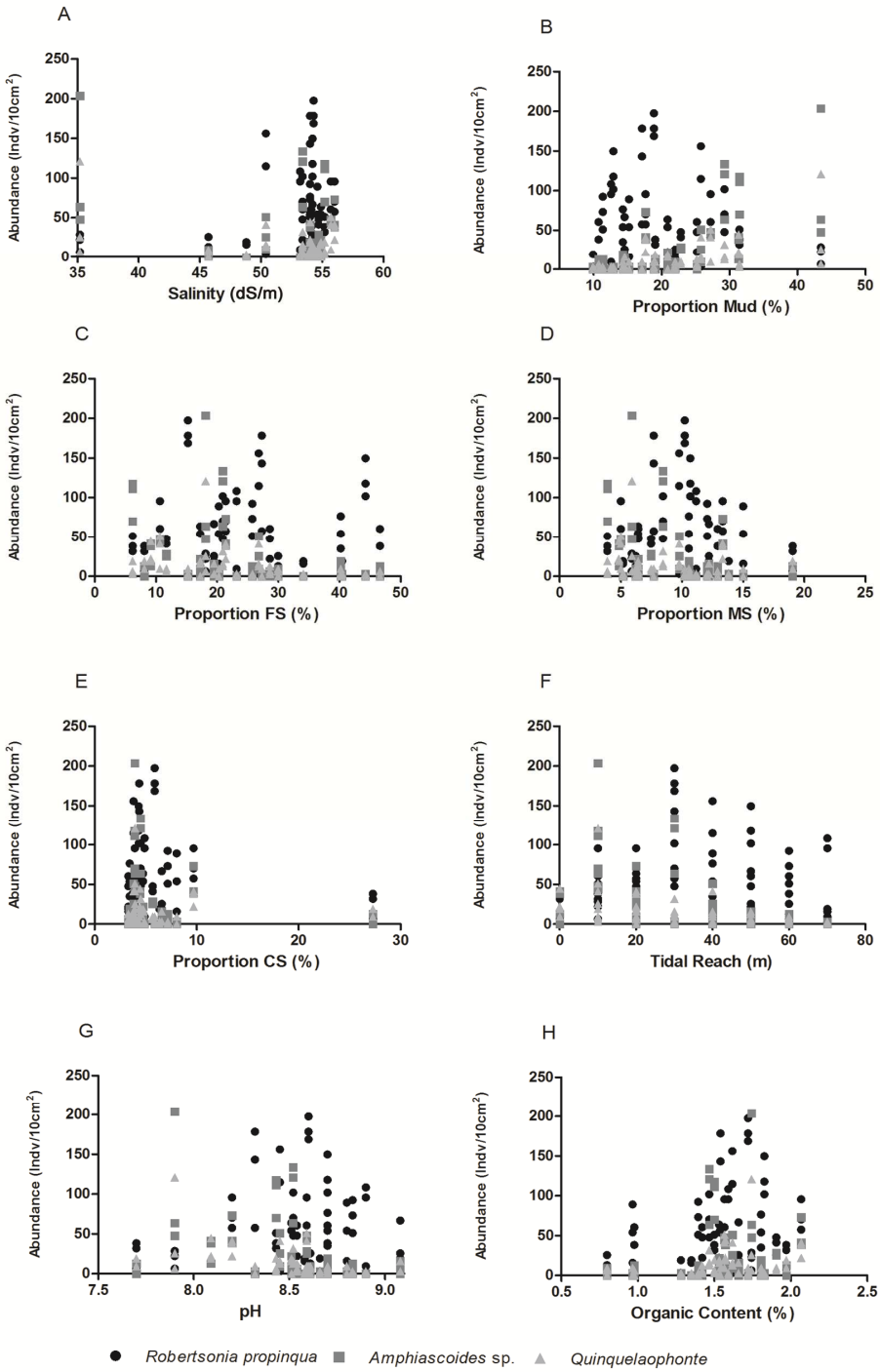

Figure 2.3 Scatter plots of densities of the three most abundant copepod species in relation to sediment properties. **A.** Salinity. **B.** Mud. **C.** Fine sediment (FS). **D.** Medium sand (MS) **E.** Coarse sand (CS). **F.** Tidal reach (location). **G.** pH. **H.** Organic content.

2.4 Discussion

The primary focus of this study was to describe the factors that govern copepod species distribution in a low energy tidal mud–sand flat. Data on how these physical factors affect copepod abundance and distribution can be used to inform sediment guidelines for a New Zealand native copepod bioassay.

Copepod densities up to 353 individuals per 10 cm^2 (total copepods) were recorded in Portobello Bay. Similar densities for harpacticoid copepods in undisturbed intertidal habitats in New Zealand were previously reported by Wells et al. (1982). In contrast, harpacticoid copepod densities of up to 3,387 individuals per 10 cm^2 were found in medium fine sand in South Africa (McLachlan 1977). These differences in copepod densities are most likely explained by global differences in temperatures (Palmer and Brandt 1981, Rombouts et al. 2009).

Sediment size was the predominant physical factor affecting the distribution of the five species groups found in Portobello Bay. The abundance of most species was positively affected by (i.e. correlated with) finer sediment fractions (mud, fine sand, and medium sand) and negatively affected by coarse sand, except for *P. megarostrum* where coarse sand had the opposite effect. Another factor that affected copepod distribution was location in tidal reach with *Quinquelaophonte* and *Amphiascoides* sp. abundance decreasing with increasing distance out to sea. Tidal reach has been shown to affect some species and not others and this can vary greatly between sites (Hicks and Coull 1983). This suggests that other factors (e.g. grain size, vegetation, salinity) are actually promoting the effect and that tidal reach is a covariate. Interestingly organic content, which has often been considered a driving force in copepod distribution (Hicks and Coull 1983), was found to only affect one species abundance in this study (that of *R. propinqua*). The study location had relatively low organic

content, which is common for sandy sediment types. However it also suggests that there may have been insufficient variation in organic content to affect distribution. The positive effect that pH had exclusively on *Quinquelaophonte* abundance suggests an avoidance of lower pH sediments by this species. This may be an aversion to anoxic sediments, which due to anaerobic sulphate–reducing bacteria, have lower pH (Ben-Yaakov 1973).

These results are similar to those of previous research, and indicate that of all the factors sediment size is the one that plays the most significant role in copepod abundance (Chertoprud et al. 2007). This is likely a consequence of the biotype of each species (Hicks and Coull 1983, Chertoprud et al. 2007). For example, *Robertsonia propinqua* has been found in coastal marshes (Coull et al. 1979), coastal muddy sediments (Soyer 1970), and salt water lakes in south east Australia (Hammond 1973), and is often found associated with sea grasses (Hicks 1977). In addition, *R. propinqua* has been seen to have a wide range of salinity tolerances (24–62‰ in some populations; Bayly 1970). These observations, combined with the findings of the current study, suggest that *R. propinqua* prefers muddy to sandy detritic sediments and has a tolerance to a range of tidal exposures and salinities. *Quinquelaophonte* have often been found in sandy sediments and in detritus-rich habitats, with some species associated with sea grass. The majority are within–sediment and sediment-surface-dwelling organisms (Hicks and Coull 1983, Walker–Smith 2004), which is consistent with the findings in this study. *Enhydrosoma* species have been observed to dominate in detritic muddy sediments (Soyer 1970). The absence of such sediments in this study likely explains the low abundance of *Enhydrosoma*. *Parastenhelia megarostrum* is an epibenthic copepod often dominating troughs in sediment ripples in sandy sediments around New Zealand (Hicks 1992). Though the sediments in this study are sandy, *P. megarostrum* was not found in high abundances in this study. *Amphiascoides* is an interstitial species showing a wide salinity tolerance of 11-45‰ (Ingole 1994), and is frequently found in medium

to coarse silty sands (Chertoprud et al. 2006, Chertoprud et al. 2009). In this study, salinity had a significant positive effect on *Amphiascoides* sp. abundance, with this species showing preference for the higher salinities. In addition, the current study also showed that when the amount of mud increased, the abundance of *Amphiascoides* sp. also increased. This suggests that there may be some variation in salinity and sediment preferences in this genus and further research investigating these differences could illuminate that preference.

Biotic factors can also influence copepod distribution. Aggregation of copepods is known to be caused by small–scale changes in topography (i.e. sediment ripple crests, grass shoots, feeding pit), food localisation, mating behaviour and interspecific competition (Findlay 1981). Species interactions were present in this study, especially in regards to *Amphiascoides* sp. whose abundance was positively affected by most of the other species. *Parastenhelia megarostrum*, *Enhydrosoma* sp. and *Quinquelaophonte* abundances predicted *Amphiascoides* sp. abundance and vice versa. This suggests that *Amphiascoides* sp. either has a preference to be around other species or, most likely, that there is a preference to a physical factor, which is shared by other species, and which was not elucidated by the model. The other main species–species interaction was a negative relationship between *R. propinqua* and *Quinquelaophonte* to *Enhydrosoma* sp., indicating there is sufficient interaction (possibly competition) to cause abundance differences. Further research investigating species–species interactions is needed to characterise the mechanisms affecting distribution.

When the biotic factors are combined with the abiotic information it seems that the three most abundant species follow a general pattern in this tidal flat. *Quinquelaophonte* dominate the upper tidal reaches with *Amphiascoides* sp. and *R. propinqua* being dispersed throughout the tidal reach. All three species are associated with finer sediment fractions (mud to medium sands, <500 µm).

Of the copepods present in Portobello Bay, two are of particular interest from a bioassay perspective. *Robertsonia propinqua* and *Quinquelaophonte* sp. have been identified as candidate species for estuarine sediment toxicity testing due their ability to be easily cultured in the laboratory and their sensitivity to contaminants (see Chapter 3). These two species have measurable correlations between abundance and the physical factors within estuaries. This allows sediment guidelines to be developed to determine if the sediment at a sample location is appropriate for use with these bioassay species. Both of these species were found to be positively affected by finer sediments that were smaller than 500 µm. *Robertsonia propinqua* was also impacted by organic content. *Quinquelaophonte* showed strong trends for tidal location preferring to be higher in the estuary and avoiding lower pHs. When all factors are considered for these two species the best–suited sediments are fine sandy to muddy sediments high in the tidal reach, which are generally of high salinity. Sediments that should be avoided are coarse sands >500 µm with low pH and those that are low in the tidal reach. However, the presence of both species throughout the tidal reach suggests that this is marginal factor.

2.5 Conclusions

Every tidal flat, estuary, or lagoon has a diverse range of habitat characteristics at a variety of scales. Except for sediment size there is no evidence of factors that entirely predict copepod distribution and abundance (Coull & Wells 1981). This study set out to examine physical sediment factors that may affect copepod distribution, in order to better understand the physical habitat requirements of copepods proposed for bioassays, and which will thus inform bioassay protocol requirements and limitations. The results of this study can be used to guide sediment sampling for assessment of sediment contamination with copepod bioassay test species. Further research is required to determine if New Zealand copepods are typically found in low densities (5–10%

of other reported regions), and to ascertain their exact role in the estuarine food chain (i.e. what species feed on them and at what rate or biomass). Such information will enable the results of laboratory sediment toxicity bioassays with harpacticoid copepods to predict contamination effects on the estuarine ecosystem as a whole.

3.

Development of an endemic harpacticoid copepod bioassay: selection of species and relative sensitivity to zinc, atrazine and phenanthrene

Adapted from:

Stringer TJ, Glover CN, Keesing V, Northcott GL, Tremblay LA. Development of an endemic harpacticoid copepod bioassay: selection of species and relative sensitivity to zinc, atrazine and phenanthrene. Accepted for publication in Ecotoxicology and Environmental Safety

Co-author contributions:

- C. Glover, V. Keesing and L. Tremblay supervised the research and provided feedback on the manuscript.
- G. Northcott assisted with the chemical analysis of atrazine and phenanthrene.

3.1 Introduction

Estuaries are productive ecosystems often impacted by human activity (Lotze et al. 2006), and the resulting degradation of these environments has significant impacts on biodiversity and the ecosystem services they provide (Costanza et al. 1997). Estuaries are unique in the fact that they are affected by marine, freshwater, and terrestrial pollution sources including agricultural runoff, storm water, forestry, mining, land conversion, road runoff, sewage, and harbour activities (Carpenter et al. 1998). Consequently, estuaries are a major sink where toxicants may accumulate. Pollutants are often trapped within estuaries due to the settling of particulates, partitioning from the water column into sediments, and binding to floating particulate and organic matter. The high ionic strengths of saline estuarine waters can promote the "salting out" of hydrophobic organic chemicals from the water into sediment (Brunk et al. 1997, Chapman and Wang 2001, Droppo et al. 2001).

Monitoring the impacts of anthropogenic activities on estuaries is crucial to prevent environmental and economic losses resulting from the collapse or contamination of the ecosystem (Chapman and Wang 2001, Lotze et al. 2006). Laboratory-based bioassays are powerful tools for assessing and monitoring ecosystem health, especially those that use key species naturally inhabiting estuarine sediments and which expose these animals to environmentally-realistic conditions (Chapman and Wang 2001). In general, marine and estuarine harpacticoid copepods are advantageous for this purpose owing to their short life cycles (~28 days), high reproductive rates, and their ease of culture in the lab. These properties are ideal for assessing sub-lethal effects such as impaired reproduction, growth, and development (Kovatch et al. 1999, Chandler and Green 2001, Bejarano et al. 2004, Greenstein et al. 2008, Perez-Landa and Simpson 2011). Harpacticoids have close association with contaminated sediments, which makes them very susceptible to chronic exposure to pollutants

(Coull and Chandler 1992, DiPinto and Coull 1997). Several species of harpacticoid copepods are currently being used in sediment bioassays around the world, including *Amphiascus tenuiremis, Microarthridon littorale, Nitocra spinipes, Tigriopus brevicornis, Tisbe battagliai* (Forget et al. 1998, Kovatch et al. 1999, Hagopian-Schlekat et al. 2001, Bejarano and Chandler 2003, Perez-Landa and Simpson 2011). While previous work identified *Robertsonia propinqua* as a suitable bioassay species (Hack et al. 2008a, b) there is no information on the sensitivity or utility of other native New Zealand harpacticoids as bioassay organisms, and a bioassay using a New Zealand species of marine copepod has not been developed and fully validated.

Multiple factors need to be considered in choosing a bioassay species. These factors include ease of culture, reproductive rate in laboratory culture, ease of distinguishing males and females (for assessment of gender-based toxic impacts), and sensitivity to contaminants. In most estuaries multiple sediment-dwelling copepod species will exist; however, there is no guarantee that any will meet all the essential criteria for a bioassay organism.

The aim of this study was to initially characterise and identify a suitable species of endemic harpacticoid copepod for use in estuarine bioassays. This was accomplished by: (1) characterising the suitability of five commonly occurring New Zealand harpacticoid copepods on the basis of factors such as ease of culture and reproduction rate, (2) using acute aquatic 96 h LC_{50}s to determine the relative sensitivity of the best-qualified species to key reference contaminants, and (3) use this information to identify the species with the greatest utility as a bioassay organism for assessing estuarine sediment health in New Zealand.

3.2 Materials and methods

3.2.1 Test species

Six commonly-occurring native New Zealand harpacticoid copepods were identified as potential bioassay species: *Amphiascoides* sp., *Stenhelia* sp., *Parastenhelia megarostrum*, *Quinquelaophonte candelabrum*, *Quinquelaophonte* sp. and *Robertsonia propinqua* (Figure 3.1). These species were selected as they have wide geographic distributions across New Zealand and are easily distinguishable from each other based on differences in overall body shape and defining characteristics of the species based on species descriptions in Wells et al. (1982) and Hammond (1973). The *Quinquelaophonte*

Figure 3.1 Copepods identified as potential bioassay spcies (A) *Amphiascoides* sp. (B) *Stenhelia* (C) *Robertsonia propinqua* (D) *Parastenhelia megarostrum* (E) *Quinquelaophonte candelabrum* (F) *Quinquelaophonte* sp. (Leica M125 microscope).

genus is characterised by serrated body segments and *Q. candelabrum* is distinguishable from the as yet unnamed *Quinquelaophonte* sp. by caudal rami morphology (pipette-shaped in *Q. candelabrum*) (John Wells, personal communication, *Quinquelaophonte* sp. currently being described). *P. megarostrum* has the defining characteristic of a large protruding rostrum (Wells et al. 1982), *R. propinqua* has a stout body shape with the prosome being the widest point, and abundant spinules on the abdomen (Hammond 1973) and finally *Amphiascoides* sp. has similar body shape to *R. propinqua* but is smaller, thinner, and does not display ornamentation on the abdomen. All species were identified by Prof. John Wells at the Victoria University Wellington.

Amphiascoides sp., *Quinquelaophonte* sp. and *Robertsonia propinqua* were sourced from Portobello Bay, Otago, New Zealand (45°50'S 170°39'E). *Parastenhelia megarostrum* and *Quinquelaophonte candelabrum* were sourced from Okains Bay, Canterbury, New Zealand (45°43'S 173°03'E). Copepods were isolated from collected surface sediments by gentle sieving and retaining copepods on a 125 µm sieve before being drawn out of detritus and large sediments by attraction to a light source. Copepods were then collected by means of a glass Pasteur pipette and transferred to Petri dishes where they were identified to species level, when sufficient taxonomic information was available, under a dissecting microscope (Leica M125). Sandy, silty sediments were also collected at both locations for use as the culture substrates. Sediments were cleaned and sterilised based on methods of Chandler and Green (1996). The sediments were washed with fresh water, passed through a 125 µm sieve, and autoclaved at 121°C for 15 min. Each copepod species was monocultured in a laboratory culture system modified from the recirculating seawater system designed by Chandler (1986). The system included a flow-through plastic aquarium (12 cm × 18 cm) containing approximately 1 cm of the <125 µm sieved and sterilised sediment overlaid with 5 cm of artificial seawater (ASW; Red Sea® sea salts, 30‰) under a dripping flow of 5 ml/min. Culture

inoculation consisted of >100 adult individuals. Cultures were monitored every 3 days for survival and reproduction (presence of females bearing egg sacs, hereafter referred to as “gravid” females) for approximately 4 weeks until it was determined that the species was taking to culture or not.

The culture system was maintained at 20°C and at 30‰ ASW, with a photoperiod of 12 h light: 12 h dark. Ammonia, nitrite, and nitrate were always below detection limit (determined by commercial aquarium test kits) and pH was maintained at 8.2. Water quality was monitored twice a week. The copepods were fed 40 ml of a concentrated mixed suspended algal diet of *Chaetoceros muelleri*, *Dunaliella tertiolecta* and *Isochrysis galbana* (1:1:1) twice a week (Chandler and Green 1996). Algal stocks were cultured in F_2 medium, which was sub-cultured every 7–10 days (Jeffrey and LeRoi 1997).

For the species that were able to be cultured, the culture density was estimated by counting adults in each of four replicate core samples (0.78 cm^2) taken from random locations within the individual culture aquaria. Only adults were counted, as this life stage is used in toxicity tests. Total adults, the proportion of gravid females, and reproductive rate (number of eggs per female × proportion of gravid females) were then calculated.

3.2.2 96 h acute toxicity tests

Three reference contaminants possessing a diverse range of structures and modes of action were chosen and included a heavy metal (zinc), a polyaromatic hydrocarbon (PAH; phenanthrene) and a pesticide (atrazine). These contaminants also provide the advantage of being commonly used as reference chemicals in a range of ecotoxicity test procedures, thereby providing opportunity to compare the performance of the copepod assay against other

organisms/species used in standardised toxicity assays. Aquatic 96 h LC_{50} tests (median lethal concentration after 96 h exposure to waterborne toxicant) was used as a rapid and technically more simplistic proxy to sediment toxicity testing. In addition, sediment toxicity can be heavily influenced by pore-water exposure, meaning that aquatic toxicity tests have some relevance to sediment-dwelling organisms (Simpson 2005, Simpson and King 2005, Hutchins et al. 2009).

Acute toxicity tests were performed with two candidate species, *R. propinqua* and *Quinquelaophonte* sp., as these two species performed the best in laboratory cultures. All of the LC_{50} tests used laboratory-cultured copepods, described above, and followed the methods outlined by Green et al. (1993) with modifications as noted below.

For each test, there were five geometrically-increasing concentrations (1.5 or 2 x concentration increases) of the chemical with four replicates for each treatment and control. Three of the replicates were used for biological enumeration at the termination of the test; the fourth was used for water quality and chemical analysis. Carrier controls were performed when a carrier was necessary for spiking atrazine and phenanthrene in the treatment groups (see below). Each LC_{50} test was repeated 3 times. Test vessels were acid-washed (min 24 h 10% nitric acid soak) and triple acetone-rinsed 50 ml borosilicate glass Erlenmeyer flasks (LabServ), containing 30 ml of aerated 30‰ ASW. Thirty adult copepods (15 males, 15 non-gravid females) were loaded at test initiation via pipette. The test vessels were loosely covered to prevent evaporation. The tests were static (no water exchange) and run for a period of 96 h. The test vessels were incubated in an environmental chamber at 20 ± 1°C, and under a 12 h light: 12 h dark cycle. Dissolved oxygen (DO), salinity, and pH were measured with a multi meter (WTW Multi 350i) at initiation and termination of the test.

3.2.3 Toxicant concentrations

Target toxicant concentrations were achieved by spiking ASW with aliquots of stock solutions. For zinc, a stock solution of $ZnSO_4 \cdot 7H_2O$ (BDH cat # 10299 AnalaR) was prepared in deionised water (Millipore, 0.22 µm filtered), while dimethyl sulfoxide (DMSO, BDH cat # 103234L AnalaR) and acetone (Mallinkrodt Nanograde) were used as organic solvent carriers for technical grade atrazine (A certified standard of atrazine (>98% purity) obtained from Dr. Ehrenstorfer GmbH) and phenanthrene (Sigma cat # 77470, 97%), respectively. The proportion of carrier solution in each test vessel was kept as low as possible to minimise the introduction of artefacts into the experiments. The final proportions of carrier solvent in the phenanthrene and atrazine test vessels were 0.1/1000 (v/v) and 0.5/1000 (v/v) respectively (ISO 14669:1999). Carrier-solvent-only controls were included in each schedule of testing to assess any toxic effect arising from exposure to the carrier solvents. There were solubility limits for the phenanthrene and atrazine of 1.1 mg/l and 33 mg/l, respectively, and the upper concentrations were deliberately kept below solubility limits in these tests to prevent precipitation.

Water samples (10 ml) were collected at the initiation and termination of each test to confirm the concentration of reference compound. The concentration of zinc in water test samples was determined by diluting samples 20 times with 10% nitric acid and analysing the prepared solutions by Inductively Coupled Plasma Mass Spectrometry (ICP-MS Agilent 7500 cx). QA/QC procedures incorporated into the schedule of analysis included the analysis of replicate samples and one spiked sample within each set of 20 samples. Recovery of the zinc spikes were 108 ± 12.1% (n=4) and the relative standard deviations (RSDs) for the mean concentration of duplicate treatment samples ranged from 0.95 to 1.03%.

Each atrazine and phenanthrene test solution was spiked with simazine and anthracene-d10 (CDN Isotopes) as surrogate spike compounds before extraction. The concentration of simazine added to the aqueous atrazine solutions varied for the different treatment concentrations: 0.05 mg/l for control treatments, 1 mg/l for 2 mg/l treatments, 2 mg/l for 4 mg/l treatments, 4 mg/l for 8 mg/l treatments, 8 mg/l for 16 mg/l treatments, and 16 mg/l for 33 mg/l treatments. Anthracene-d10, prepared as an acetone solution, was added to the aqueous phenanthrene solutions to provide a final concentration of 50 µg/l. Aqueous water samples of phenanthrene and atrazine were stabilised by the addition of 2 ml and 5 ml of dichloromethane, respectively, and stored under refrigeration prior to extraction and analysis. Phenanthrene and atrazine and their respective surrogates were extracted from the aqueous test solutions by liquid-liquid extraction using dichloromethane, and analysed by high resolution gas chromatography with mass spectrometric detection for phenanthrene and electron capture (ECD) and nitrogen-phosphorous detection (NPD) for atrazine. The 95% confidence intervals for the recovery of anthracene-d10 and simazine were 101 ± 3 (n=82) and 109 ± 4 (n=84) respectively. The reproducibility of analyses was excellent with RSDs for the mean concentration of triplicate treatment solutions ranging from 0.7 to 5.1% for phenanthrene and from 4 to 22% for atrazine, across the range of treatment concentrations. The excellent surrogate spike recovery and reproducibility data demonstrate the robustness of the sample analysis methods and the concentrations of atrazine and phenanthrene determined in the aqueous test solutions.

3.2.4 Toxicological and statistical analysis

At test completion the numbers of surviving individuals were enumerated (survival was assessed as any movement of the copepods in the 5 s following a

gentle prod with a needle) and the actual concentrations of the contaminants were used to generate dose response curves and estimate LC_{10}, LC_{20}, and LC_{50} values. The proportion of surviving individuals was regressed against log contaminant concentration using binomial regression. LC_{10}, LC_{20}, and LC_{50} values along with 95% confidence intervals were calculated using the FIELLER procedure (a generalised linear model with a binomial distribution and a logit link function) in the GenStat Statistical Package (12th edition) for each species (males and females combined as well as gender-specific) (GenStat Committee 2009). Differences in species responses to contaminants were tested using a parallel slope model of the log proportion dead v. log concentration and examining the intercepts and the slopes. Slope and intercept estimates were divided by the standard error of the differences and interrogated by *t*-test. Significant difference was established at the value of $P < 0.05$ (GenStat, 12th edition). Significant ($P < 0.05$) differences in culture density, egg number, and reproductive rate were determined using two-tailed *t*-tests (GraphPad Prism 5).

3.3 Results

3.3.1 Performance of all species in culture

Of the six species identified as potential bioassay species only *R. propinqua* and *Quinquelaophonte* sp. were able to reach densities in the lab cultures sufficient to support toxicity testing. The other four species, *Amphiascoides* sp., *Parastenhelia megarostrum, Stenhelia* sp., and *Quinquelaophonte candelabrum,* did not survive well in culture and populations died out after 3–4 weeks. *Quinquelaophonte* sp. produced the highest average density of 88.5 ± 43.1 (standard deviation) adults/cm^2, and *R. propinqua* had an average density of 17.2 ± 7.6 adults/cm^2. The average egg number for each gravid female was similar at 15.6 ± 2.5 eggs/female for *R. propinqua* and 16.6 ±

2.4 eggs/female for *Quinquelaophonte* sp. The percentage of gravid females in cultures of *R. propinqua* was 11.6% and for *Quinquelaophonte* sp. was 26.9%. Based on egg number per female and the percentage of gravid females in the population the calculated reproduction rates were 1.81 and 4.47 eggs/adult for *R. propinqua* and *Quinquelaophonte* sp., respectively. As with all members of the order Harpacticoida, both *R. propinqua* and *Quinquelaophonte* sp. are sexually dimorphic, and males are easily distinguished from females by the enlargement of the fourth segment of the antennules. Males are also distinguishable as being smaller in size, especially for *R. propinqua* (see Figure 3.2). These morphological differences between sexes is crucial for ease in distinguishing males and females, and is important for ensuring specific sex ratios in bioassays and for calculating sex-specific survival.

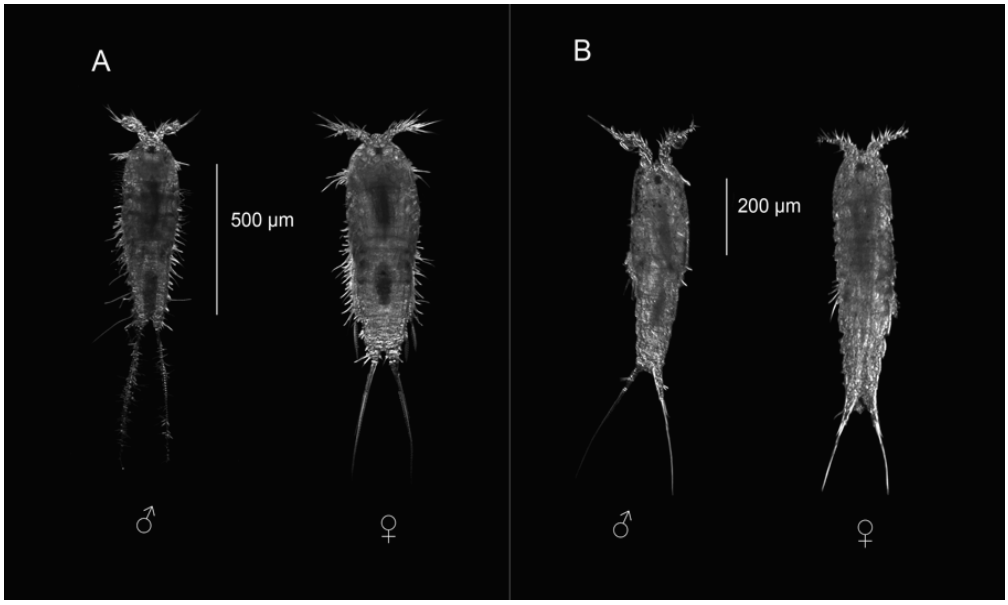

Figure 3.2 Dimorphism observed in male and female (A) *Robertsonia propinqua* and (B) *Quinquelaophonte* sp. harpacticoid copepods (Leica M125 microscope).

Table 3.1 Acute lethal concentrations (LC_x) values (mg/l) for the two harpacticoid copepods *Robertsonia propinqua* and *Quinquelaophonte* sp.

	LC_{10} (95% CI)	LC_{20} (95% CI)	LC_{50} (95% CI)
R. propinqua			
Zinc	0.86 (0.37 – 1.27)	1.16 (0.62 – 1.62)	2.0 (1.56 – 2.25)
Phenanthrene	0.43 (0.38 – 0.47)	0.53 (0.49 – 0.57)	0.89 (0.85 – 0.95)
Atrazine	1.4 (1.18 – 1.68)	2.65 (2.30 – 2.98)	7.58 (6.21- 9.39)
Quinquelaophonte sp.			
Zinc	0.24 (0.15 – 0.32)	0.34 (0.24 – 0.44)	0.64 (0.51-0.82)
Phenanthrene	0.28 (0.17 – 0.36)	0.41 (0.31 – 0.49)	0.75 (0.59-1.37)
Atrazine	7.11 (6.29 – 7.88)	10.6 (9.70 – 11.4)	20.8 (17.9-25.2)

3.3.2 Toxicity results

In all tests water quality parameters were maintained within optimal ranges (salinity 30‰, >7 mg/l DO, pH 8.0-8.5), and nitrate, nitrite, and ammonia concentrations were always below the method detection limits of 2.5, 0.05, and 0.25 mg/l respectively (RedSea® Marine Lab). Dissolved contaminant concentrations decreased during the tests, presumably due to combined processes of degradation, volatilisation (organic reference compounds), uptake by exposed copepods, and absorption to the surface of the glass test vessels. The latter is particularly important in the case of zinc (Batley and Gardner 1977). To account for the decrease in concentrations, the averages of the initial and final concentrations were used as the exposure concentrations. Reference toxicant concentrations were reduced by an average of 13.1, 11.9 and 9.2% for zinc, phenanthrene and atrazine, respectively. In all tests the mortality of test animals in control treatments was less than 10%, in accordance with ASTM (2008) recommendations.

Toxicity data (pooled data for both sexes) for both species are shown in Table 3.1 and dose-response curves are shown in Figure 3.3. LC_{50} values for *R. propinqua* were 2.0 (1.56-2.25) mg/l for zinc, 0.89 (0.85-0.95) mg/l for phenanthrene, and 7.58 (6.12-9.39) mg/l for atrazine. The corresponding LC_{50} values for *Quinquelaophonte* sp. were 0.64 (0.51–0.82), 0.75 (0.59–1.37), and

20.8 (17.9–25.2) mg/l for zinc, phenanthrene, and atrazine, respectively. Despite the fact that 100% mortality was not achieved in all tests, sufficient data were available for the calculation of LC_{50} values.

3.3.3 Comparative toxicity

Some significant differences in sensitivities to the reference compounds were exhibited by *R. propinqua* and *Quinquelaophonte* sp. While both copepods exhibited no statistically significant difference in sensitivity to the PAH phenanthrene (Figure 3.3A) this was not the case for the heavy metal zinc and triazine herbicide atrazine. *R. propinqua* and *Quinquelaophonte* sp. exhibited distinct mortality responses to zinc with significant differences in intercept recorded (diff intercept = 3.865, SE_{diff} = 0.468, $z = 8.26$, $P < 0.001$). This was reflected in toxicity values, with *R. propinqua* having an LC_{50} 3.1 times higher than that of *Quinquelaophonte* sp. (Figure 3.3B). *R. propinqua* and *Quinquelaophonte* sp. also exhibited distinct mortality responses following atrazine exposure as demonstrated by values for both intercept (diff intercept = 3.513, SE_{diff} = 0.718, $z = 4.50$, $P < 0.001$) and slope (diff slope = 4.94, SE_{diff} = 0.199, $z = 2.48$, $P = 0.02$). *Quinquelaophonte* sp. had an LC_{50} 2.7 times higher than that of *R. propinqua* for atrazine (Figure 3.3C).

3.3.4 Gender-specific toxicity

Robertsonia propinqua exhibited no gender-specific differences in sensitivity to any of the reference compounds. However, there were significant differences between male and female responses of *Quinquelaophonte* sp. to zinc and atrazine. For zinc, males were significantly more sensitive than females as

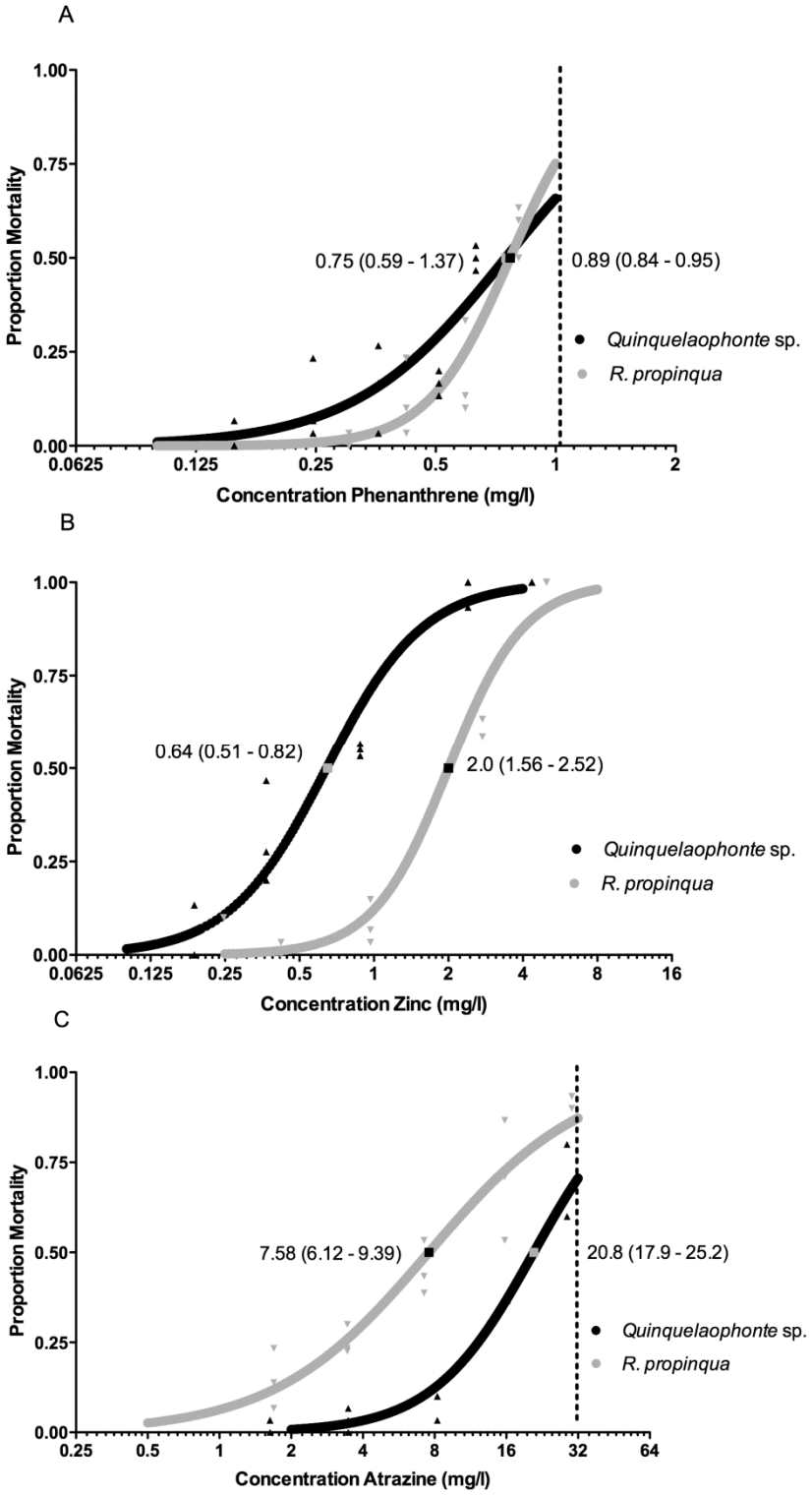

Figure 3.3 Dose-response curves for *Quinquelaophonte* sp. and *R. propinqua* to the three reference contaminants **A**. Phenanthrene, **B.** Zinc, and **C.** Atrazine. LC_{50} values and 95% confidence intervals are also given. Phenanthrene and atrazine both have solubility limits and are denoted by a dashed line.

demonstrated by differences in slope (diff. intercept = 3.126, SE_{diff} = 0.570, z = 5.49, $P < 0.001$) and with resulting LC_{50} values of 0.38 (0.32–0.46) mg/l for males and 1.1 (0.81–1.47) mg/l for females. Males of *Quinquelaophonte* sp. also exhibited increased sensitivity to atrazine compared to females as demonstrated by differences in slope (diff slope = 1.126, SE_{diff} = 0.441, z = 0.255, P = 0.02) and calculated LC_{50} values of 13.8 (10.8–16.2) mg/l for males and 44.0 (29.2–126) mg/l for females (Figure 3.4B). The sensitivity of *Quinquelaophonte* sp. to phenanthrene was independent of sex (males: 0.64 (0.51–1.21) mg/l, females 0.79 (0.63–1.65) mg/l).

3.4 Discussion

There is currently no copepod bioassay validated for the assessment of marine sediment toxicity in New Zealand. Preliminary studies have previously identified *R. propinqua* as a potentially useful bioassay species (Hack et al. 2008a, b). However, the suitability of other harpacticoid copepods has not been assessed. The ideal bioassay species is one that is easy to culture, has a high reproductive rate in laboratory culture, has easily distinguishable sexes, is sensitive to contaminants and is representative of species found in the wild.

3.4.1 Survivorship in laboratory cultures

Six species were assessed as potential bioassay organisms: *Parastenhelia megarostrum*, *Stenhelia* sp., *Amphiascoides* sp., *Robertsonia propinqua*, *Quinquelaophonte candelabrum* and *Quinquelaophonte* sp. Of these only *R. propinqua* and *Quinquelaophonte* sp. could successfully be cultured in the laboratory. In Chapter 2 it was shown that these two species have a preference for fine sandy sediments. The *Quinquelaophonte* genus is also known to often be found in detritus-rich sediments (Hicks and Coull 1983, Walker–Smith 2004),

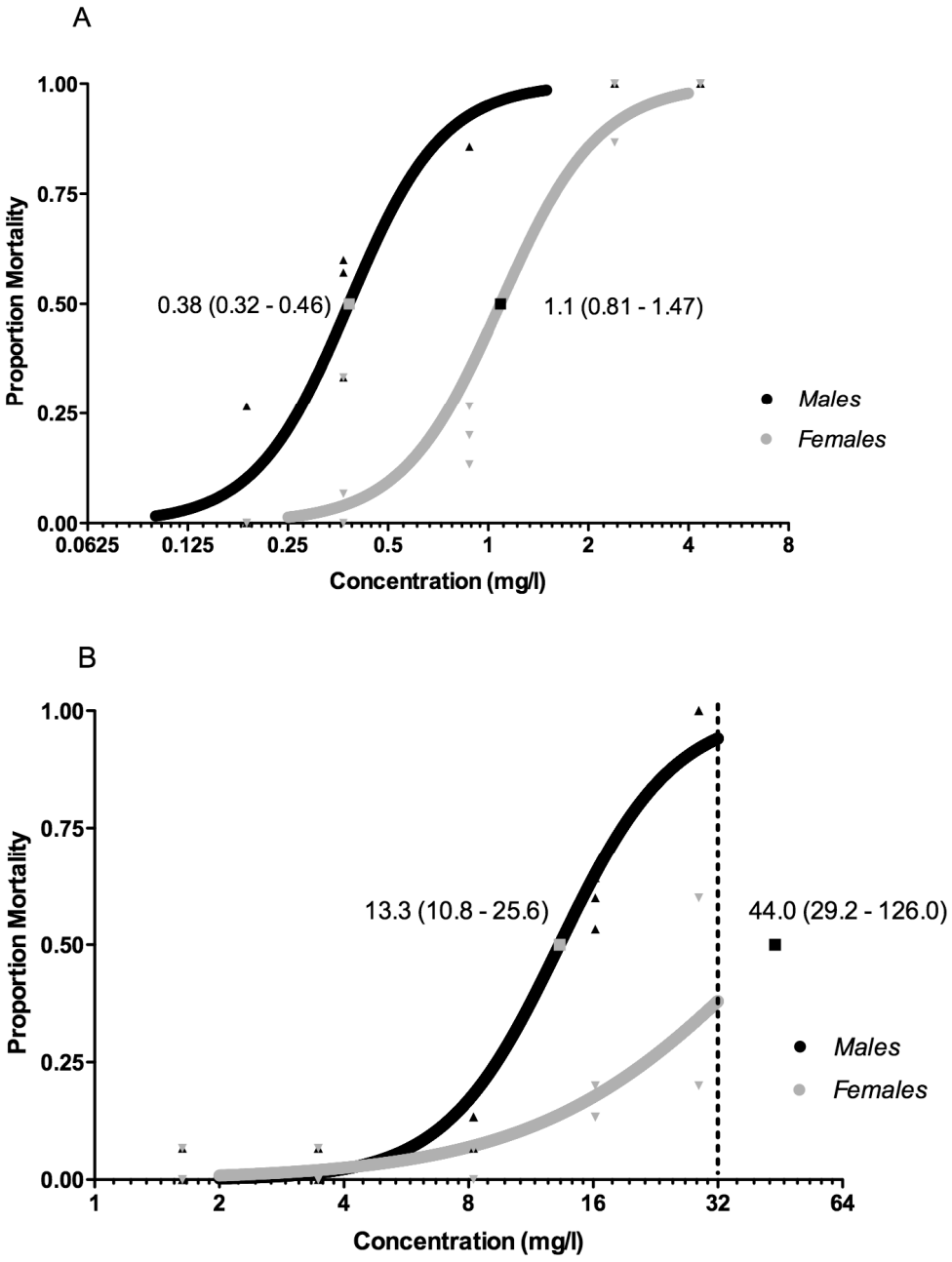

Figure 3.4 Sex-specific dose-response curves for *Quinquelaophonte* sp. to **A.** Zinc and **B.** Atrazine. Dashed line denotes solubility limit for atrazine.

and *R. propinqua* is also found in muddy coastal marsh sediments (Soyer 1970, Coull et al. 1979), sugesting that these species have a wide tolerance for sediment types. Failure of a given species to establish itself in culture may be related to changes in sediment characteristics owing to their processing and sterilisation. Alternatively, copepods may have been unable to withstand the stress of collection and transportation. Laboratory culture, rather than reliance on field-collected organisms, is essential for toxicity testing as it allows for assessment on naive individuals and ensures a more homogeneous genetic composition, which minimises variability and provides consistency within and between tests. The differences in sensitivity of field-collected and laboratory-reared animals have not been well studied. Some research shows that seasonal and dietary influences can cause variability in sensitivity (Sosnowski et al. 1979, Hedtke et al. 1986), promoting the concept that controlled laboratory conditions are required for consistency in toxicity assessments. Based on the findings of culture survivorship, *R. propinqua* and *Quinquelaophonte* sp. were assessed for their suitability as sediment toxicity organisms based on the remaining criteria: sensitivity to contaminants, reproduction rate, culture density, distribution and ecology.

3.4.2 Zinc toxicity

Zinc proved to be the most toxic of the contaminants to *Quinquelaophonte* sp. and the second most toxic to *R. propinqua*, with LC_{50} values of 0.64 and 2.00 mg/l respectively. These values are well above the current marine water quality guideline for Australia and New Zealand of 0.015 mg/l (95% species protection, ANZECC 2000). A comparison of the sensitivities of the two tested copepods to other estuarine and marine species is given in Table 3.2. *Quinquelaophonte* sp. is more sensitive to zinc relative to other evaluated copepod species. Other estuarine species have 96 h LC_{50} values

ranging from 0.032 mg/l for the estuarine crab *Scylla serrata* (Narayanan et al. 1997) to as high as 12.3 mg/l for shrimp *Palaemon elegans* (Bat et al. 1999) and 14.0 for the crab *Paragrapsus quadridentatus* (Taylor 1981). Tolerance of *R. propinqua* to zinc was significantly higher than that of *Quinquelaophonte* sp., and is similar to the estimated 96 h LC_{50} of 2.7 mg/l for adults and 1.7 mg/l for nauplii previously reported for this species (Hack et al. 2008b).

Zinc is known to have a range of impacts that contribute to acute toxicity. It has been shown to inhibit digestion and feeding in *Daphnia magna* and to interfere with exoskeleton maintenance and molting in crustaceans (Poynton et al. 2007). This latter effect is likely due to the potential for zinc to share and inhibit calcium uptake pathways in crustacean epithelia (Ahearn et al. 1994). Copepods have defence mechanisms that help them regulate internal metal concentrations and bioreactivity. One of these is metallothionein, a protein that binds and detoxifies metals. In copepods, as in other animals, the production of metallothioneins is induced in response to metal exposure (Barka et al. 2001, Poynton et al. 2007). The efficacy of metallothionein to detoxify toxic metals is hypothesised to vary between species (Hook and Fisher 2002). Differential binding capacity of metallothioneins may be an explanation for the large variation in sensitivities of estuarine species. This cellular mechanism may also explain the literature data showing that copepods can have increased tolerance to metals when exposed over long time-frames (Moraitou-Apostolopoulou 1978, Gardestrom et al. 2008), as metallothionein induction is likely to promote survivorship. Critically, the impacts of cellular defence mechanisms such as metallothionein show how life history affects sensitivity, and supports the use of lab-reared rather than field-collected individuals.

Table 3.2 Acute toxicity (96 h LC_{50}) data for estuarine species to zinc.

Group	Species	Life stage	96 h LC_{50} mg/l	Reference
Copepod (Calanoid)	*Acartia clause*	Adult	0.96	Taylor 1981
	Acartia tonsa	Adult	0.4	Taylor 1981
	Eurytemora affinis	Adult	6.0	Taylor 1981
Copepod (Harpacticoid)	*Amphiascus tenuiremis*	Adult	0.37 (pore-water)	Hagopian-Schlekat et al. 2001
	Robertsonia propinqua	**Adult**	**2.0 (1.56-2.25)**	**Present Study**
		Adult	2.7*	Hack et al. 2008a
		Nauplii	1.7*	Hack et al. 2008a
	Tigriopus brevicornis	Adult	0.715	Barka et al. 2001
	Tigriopus japonicus	Adult	3.2	Taylor 1981
	Quinquelaophonte sp.	**Adult**	**0.64 (0.51-0.82)**	**Present Study**
Amphipod	*Chaetocorophium* cf.*lucasi*	Juvenile	1.13	King et al. 2006
	Echinogammarus olivii	Adult	1.30	Bat et al. 1999
	Melita awa	Juvenile	0.71	King et al. 2006
	Melita matilda	Juvenile	0.65	King et al. 2006
	Melita plumulosa	Juvenile	0.64	King et al. 2006
Shrimp	*Palaemon elegans*	Adult	12.3	Bat et al. 1999
Crab	*Pagurus longicarpus*	Larvae	0.6	Taylor 1981
	Paragrapsus quadridentatus	Adult	14.0	Taylor 1981
		Larvae	1.3	
	Scylla serrata	Adult	0.032	Narayanan et al.1997
Bivalve	*Dreissena polymorpha*	Adult	0.054	ANZECC 2000
	Mysella anomala	Adult	4.40	King et al. 2004
	Soletellina alba	Adult	2.90	King et al. 2004
	Velesunio ambigua	Adult	11.2	ANZECC 2000

* Range-finder data, not definitive LC_{50}

Another factor affecting sensitivity of copepods to zinc is the thickness of the exoskeleton. Juvenile copepods are more sensitive to metals than adults as a result of having a thinner body casing, which facilitates metal absorption (Hagopian-Schlekat et al. 2001). Although only adults were used in this study, thickness of exoskeleton may vary between species, potentially affecting sensitivity. Zinc is also known to reduce chitinase activity and thus influence exoskeleton structural integrity (Poynton et al. 2007). These two mechanisms suggest there may be a strong link between zinc toxicity and exoskeleton integrity. Distinct physiological mechanisms associated with exoskeleton maintenance and development could be a contributing factor explaining the differences in sensitivity to zinc between *R. propinqua* and *Quinquelaophonte* sp.

3.4.3 Phenanthrene toxicity

On exposure to waterborne phenanthrene, *R. propinqua* and *Quinquelaophonte* sp. exhibited 96 h LC_{50} values of 0.89 and 0.75 mg/l, respectively. These values were not statistically significantly different. These two species of harpacticoid copepods are slightly less sensitive to phenanthrene than other copepod and estuarine species previously studied, with sensitivities varying from 0.360 mg/l for the shrimp *Palaemonetes pugio* (60 h exposure, Unger et al. 2007) to 0.522 mg/l for the cyclopoid copepod *Oithona davisae* (48 h exposure, Barata et al. 2005) (Table 3.3).

Non-polar narcosis is often considered the major overt toxic effect of PAH compounds (Ren 2002). Non-polar narcosis can be defined as non-specific disruption of the proper functioning of the cell membrane, which leads to behaviours such as inhibited locomotor activity and severely or totally inhibited

capacity to respond to external stimuli (Ren 2002). Bioaccumulation of PAHs can be very rapid, reaching steady state in body tissues in less than 12 h in harpacticoid copepods (Lotufo 1998a). Body lipid content is the best predictor of PAH accumulation, which in turn is the best indicator of toxicity (Lotufo 1998b). The lipid contents of *R. propinqua* and *Quinquelaophonte* sp. are unknown, although the similarity of the species' LC_{50} values suggests they may be similar, or that they have similar uptake rates.

3.4.4 Atrazine toxicity

Atrazine (2-chloro-4-ethylamino-6-isopropyl-amino-s-triazine) is one of the most widely and intensively used herbicides in the world. It is applied in over 80 countries worldwide, and in the US alone, over 35 million kilograms is added to agricultural land each year (Bejarano and Chandler 2003).

Both *R. propinqua* and *Quinquelaophonte* sp. were significantly more tolerant to atrazine than other previously tested copepods and estuarine species (Table 3.4), providing 96 h LC_{50} of 7.58 mg/l and 20.8 mg/l respectively. The sensitivity of other estuarine invertebrate species to atrazine range from as little

Table 3.3 Acute toxicity data for estuarine species to phenanthrene

Genus	Species	Life stage	LC_{50} mg/l	Reference
Copepod (Calanoid)	*Acartia tonsa*	Adult	0.422[a]	Bellas and Thor 2007
Copepod (Cyclopoid)	*Oithona davisae*	Adult	0.522[a]	Barata et al. 2005
Copepod (Harpacticoid)	***Robertsonia propinqua***	**Adult**	**0.89 (0.85-0.95)[b]**	**Present Study**
	Quinquelaophonte sp.	**Adult**	**0.75 (0.59-1.37)[b]**	**Present Study**
Shrimp	*Palaemonetes pugio*	Adult	0.360[c]	Unger et al. 2007

[a] 48h LC_{50}, [b] 96 h LC_{50}, [c] 60h LC_{50},

as 0.094 mg/l for the calanoid copepod *Acartia tonsa* (Ward and Ballantine 1985), to 9.0 mg/l for the shrimp *Palaemonetes pugio* (Ward and Ballantine 1985). In estuaries and coastal areas in the south-eastern US, environmental water concentrations of atrazine have been found to range from 90 ng/l to 62.5 µg/l (Bejarano and Chandler 2003). Atrazine concentrations in New Zealand estuaries are not well characterised, but they are unlikely to exceed the concentrations measured in south-eastern US waters. Consequently, acute effects of atrazine are unlikely to be observed for these two species in the New Zealand environment.

In vertebrates, atrazine is an endocrine disrupting compound, with several modes of action suggested. It has been shown to increase aromatase activity in humans (Sanderson et al. 2002); to disrupt the neuroendocrine control of ovarian function in female rats (Cooper et al. 2007); to increase plasma testosterone in male Atlantic salmon (Moore and Waring 1998); and to induce hermaphroditism in male American leopard frogs at concentrations as low as 0.1 µg/l (Hayes et al. 2003). As there is little known about invertebrate endocrine

Table 3.4 Acute toxicity (96 h LC_{50}) data for estuarine species to atrazine.

Group	**Species**	**Life stage**	**96 h LC_{50} mg/l**	**Reference**
Copepod (Calanoid)	*Acartia tonsa*	Adult	0.094	Ward and Ballantine 1985
Copepod (Harpacticoid)	***Robertsonia propinqua***	**Adult**	**7.58 (6.12-9.39)**	**Present Study**
		Adult	31.8*	Hack et al. 2008a
		Nauplius	7.5*	
	Tigriopus brevicornis	Adult	0.124	Forget et al. 1998
	Quinquelaophonte sp.	**Adult**	**20.8 (17.9-25.2)**	**Present Study**
Shrimp	*Palaemonetes pugio*	Adult	9.0	Ward and Ballantine 1985
	Penaeus duorarum	Adult	6.9	Ward and Ballantine 1985
Crab	*Uca pugilator*	Adult	>28	Eisler 1989
Fish	*Leiostomus xanthurus*	Adult	8.5	Ward and Ballantine 1985

* Range finder data, not definitive LC_{50}

systems it is unknown if atrazine affects similar pathways in copepods (Bejarano and Chandler 2003). Further research to elucidate atrazines mode of action and how it affects acute and chronic toxicity is required. Such knowledge would allow interpretation of the differential sensitivity to the two copepod species evaluated in the present investigation.

3.4.5 Differences in species sensitivity

There were significant differences in the sensitivity of response to the reference toxicants between the copepod species tested in this study, and further differences in the sensitivity of response reported for these copepods versus those observed in other studies. Some of these differences in sensitivity may be attributed to the physical size of the tested species. Smaller species such as *Amphiascus tenuiremis* (0.25–0.44 mm) (Chandler and Green 1996), generally exhibit higher sensitivity to toxicants than larger copepods such as *R. propinqua* (0.7–0.9 mm) and *Quinquelaophonte* sp. (0.6–0.8 mm). The larger surface area to volume ratio of smaller animals means they are more susceptible to exchanges with the environment, including the uptake of toxicants. Furthermore, smaller animals have a reduced "buffering capacity" to offset the impacts of bioaccumulated chemicals. It is noteworthy that the sizes of the two New Zealand species tested in the present study were very similar, and is thus unlikely to explain the differences between these two copepods in sensitivity to the reference toxicants.

In addition to size, there are other factors that can account for differences in the sensitivity of test species to toxicants. The physiology of different species may allow for one to be more tolerant to toxicants than the other. This may occur via processes causing an organism to metabolise a contaminant more efficiently or influence uptake and elimination of the contaminant, affecting

bioaccumulation, and thus toxic effect. These differences will be contaminant-specific and will depend on the specific mode of action of a contaminant (Escher and Hermens 2002, McClellan-Green et al. 2007). In this study *Quinquelaophonte* sp. was more sensitive to zinc than *R. propinqua*; however, the opposite was observed for atrazine. This exemplifies how dissimilar contaminants can affect species in different ways. In addition, there was a significant difference in the gender-associated toxicity of *Quinquelaophonte* sp. to zinc and atrazine, which may be due to body size, uptake and elimination, or physiological differences between males and females (McClellan-Green et al. 2007). Interestingly, the same pattern of sex-specific sensitivity was not seen in *R. propinqua* despite the size difference between genders in this species, suggesting that factors other than size difference are affecting gender-associated sensitivity. There is little information on the mechanisms that may be producing these marked differences in sensitivity. Consequently, caution should be exhibited when choosing a bioassay species based solely on its sensitivity to a single toxicant.

3.4.6 Bioassay species selection

Based on the results of acute waterborne toxicity tests there was no strong toxicological argument favouring the selection of either *R. propinqua* or *Quinquelaophonte* sp. as the bioassay organism-of-choice. Consequently, other considerations regarding life history and ecology were considered to select the species best suited for use in a bioassay for New Zealand.

Both species reproduced well in culture, with *Quinquelaophonte* sp. having a 2.5 times greater reproductive rate than *R. propinqua* (4.47 eggs/adult for *Quinquelaophonte* sp. and 1.81 eggs/adult for *R. propinqua*). In addition, *Quinquelaophonte* sp. reached higher densities during laboratory culture,

producing an average density of 88.5 adults/cm^2 compared with 17.2 adults/cm^2 for *R. propinqua*, providing an increased number of individuals for use in bioassays. Both species are sexually dimorphic, making it easy to distinguish males and females. Unfortunately, there is limited ecological and distribution information about these species, particularly for *Quinquelaophonte* sp. as it has only been recently discovered and is currently being described (John Wells, personal communication). *R. propinqua* is usually found in tropical to temperate silty sands. Both of these species were found to be of high abundance in Chapter 2 and associated with fine low organic content sands. However, for *R. propinqua*, there is evidence of an association with algae in shallow lagoons and it has often been found in plankton tows in the water column (Wells et al. 1982). The presence of *R. propinqua* in the water column is a potential issue when considering the use of this species for a sediment bioassay. It suggests this species could avoid contaminated sediments and produce misleading information regarding the toxicity of sediment-borne toxicants. In contrast, observations of *Quinquelaophonte* sp. suggest it is rarely seen swimming in the water column and is predominantly a benthic species (Stringer, personal observations). This suggests *Quinquelaophonte* sp. could be better suited for use in sediment bioassays.

When considering the attributes required of an organism for use in sediment bioassays it is clear that *Quinquelaophonte* sp. is the superior harpacticoid copepod species of those assessed in this study (see Table 3.5). This is based on the observations that it is sensitive to contaminants; it reproduces well in culture and reaches high densities; has a short life cycle; and its toxic responses are likely to truly reflect sediment toxicity.

Table 3.5 Comparison of bioassay traits between *R. propinqua* and *Quinquelaophonte* sp. [(–) denotes no difference, (1) denotes the better suited species and (0) denotes the lesser suited species].

	Geo. Dist.	**Life History**	**Ease of culture**	**Life-cycle**	**Repro. Rate**	**Sexually dimorphic**	**Sensitivity** Zinc	Phenanthrene	Atrazine	Total
R. propinqua	-	0	-	0	0	-	0	-	**1**	1
***Quinquelaophonte* sp.**	-	**1**	-	**1**	**1**	-	**1**	-	0	**4**

Geo. Dist. – Geographical distribution

3.5 Conclusions

A bioassay species needs to be representative of the environment under investigation, it should be among the most sensitive of all organisms in that environment to concentrations of toxicants that cause biological harm, and it needs to be amenable to laboratory culture. This study showed that the harpacticoid copepod *Quinquelaophonte* sp. is the best suited copepod for use in a bioassay to study the effects of toxicants in New Zealand estuaries. *Quinquelaophonte* sp. was chosen based on a detailed investigation of critical bioassay characteristics, including the organisms sensitivity to three representative reference toxicants. This chapter identified a potential bioassay species and also showed that there can be significant differences in toxicological sensitivity between related animals, inhabiting similar habitats, and of similar size. This shows the importance of conducting robust assessments of potential bioassay organisms prior to selection. Critically, this chapter has provided toxicant sensitivity data for a New Zealand estuarine invertebrate species that has potential to be applied to the development of sediment and water quality guidelines.

4.

Development of a sediment spiking method for metal and organic contaminants in a harpacticoid copepod toxicity bioassay

4.1 Introduction

Harpacticoid copepod bioassays have been developed using a variety of different species and sediment types (Chandler and Green 1996, Simpson and Spadaro 2011). Copepods are a useful group for environmental risk assessment and monitoring because they reproduce and develop rapidly allowing for full life cycle testing of contaminated sediments. Furthermore, copepods are exposed to contaminants through pore-waters and via direct contact with, and/or ingestion of, sediment particles (Green et al. 1993, Simpson 2005). As such they are exposed to the full toxic extent of sediment-borne pollution, and therefore are subjected to a worst-case scenario of exposure.

The relevance of laboratory bioassays to natural environments relies on robust methods for spiking sediments, to provide homogeneous, reproducible, and accurate concentrations of tested contaminants. Methods for spiking sediments are varied, and include mixing sediments with a stir bar (Chandler and Green 1996, Hagopian-Schlekat et al. 2001), a rolling mill (Murdoch et al. 1997, Northcott and Jones 2000), hand mixing (US EPA 2001), and mechanical shaking (Stemmer et al. 1990). It is critical that reliable sediment spiking methods are used or the bioavailability of the contaminants may not reflect that of the environment and thus may impact the validity of the toxicity data (Simpson et al. 2004, Atkinson et al. 2007). The major consideration when spiking sediments is the composition of the sediment, particularly the factors that govern sequestration and equilibrium of spiked contaminants (Lee et al. 2004, Simpson et al. 2004). For example, physicochemical properties such as organic content, pH, and sediment size will all determine the success of the spiking method employed.

The type of contaminant to be spiked also influences the success of the spiking protocol. For example, metals in sediments are affected by particulate

organic content, acid volatile sulphides (AVS), and changes in pH and redox potential. The changes in pH and redox potential are due to the displacement of sediment-bound ferrous iron into the pore-water. When this is oxidised it catalyses hydrolysis reactions that result in a decreased pore-water pH, and increased redox potential (Simpson and Batley 2003, Simpson et al. 2005). Redox potential and pH govern metal-binding to sediments, and will therefore also influence the resulting pore-water concentrations of the spiked toxicant (Di Toro et al. 2005, Simpson and King 2005, Simpson et al. 2011, Strom et al. 2011). Chemicals that are hydrophobic pose greater challenges, particularly for aquatic sediments. As hydrophobic contaminants are not miscible in water, it is difficult to achieve an even distribution of the contaminant throughout the sediment without it precipitating out or binding in one localised area. This can lead to the uneven distribution of the contaminant in the sediment, resulting in variable toxicity. Similarly to metals, a key factor controlling binding of many hydrophobic organic chemicals is organic carbon (Di Toro et al. 1991). The extent that non-ionic organic compounds will bind to sediments is based on the partitioning coefficient of the compound to organic carbon (K_{oc}) and its water solubility (K_{ow}) (Di Toro et al. 1991, USEPA 1993b).

There are several methodologies used to overcome the challenge of spiking organic chemicals into aquatic sediments. The first approach is shell coating the contaminant on the inside of the spiking vessel prior to the addition of sediments, then allowing the contaminant to be distributed by the mixing of the sediment (USEPA 1993b, Murdoch et al. 1997, USEPA 2001). A second strategy involves spiking dry sediments using a solvent carrier, then allowing the solvent to evaporate leaving the contaminant behind (USEPA 2001). The sediments are then mixed and re-hydrated. A third technique entails spiking contaminants directly into sediments using a carrier solvent, and leaving traces of the solvent in the sediment (USEPA 2001). These methods all have different effects on contaminant dispersal through the sediment. Additionally, each of

these methods will have distinct effects on the properties of the sediments and could alter their “natural” chemistry (USEPA 2001, Simpson et al. 2005). It is important a sediment-spiking method is used that ensures a homogenous distribution of the contaminant in each sediment type.

The development of robust sediment-spiking techniques is an integral part of validating a copepod bioassay for marine sediments. The main objective of this chapter was to determine the best method for spiking fine silty-sandy sediments using two different contaminants: zinc, a metal highly soluble in water; and phenanthrene, a PAH, which is only minimally soluble in water. As these contaminants interact differently with sediment and water two methods have been developed to ensure homogeneous distribution. This was assessed by chemical analysis of the sediment- bound fraction of the pollutant and the aqueous fraction in the pore-water.

4.2 Materials and methods

4.2.1 Collection of sediments

Sediments were collected from Barry’s Bay in Akaroa Harbour (43°45'28.78"S, 172°55'3.17"E). About 15 kg of surface (0-2 cm) oxic sediments were collected by hand in a clean high-density polyethylene (HDPE) bucket. This location was chosen because of the presence of the harpacticoid copepod (*Quinquelaophonte* sp.) selected (Chapter 3) for subsequent bioassays (e.g. Chapter 5). Once collected, sediments were immediately transported back to the laboratory where they were processed.

4.2.2 Processing and preparation of sediments

Before the sediments were used for spiking and bioassays they were processed using a modification of the method by Chandler and colleagues (Chandler et al. 1986, Chandler and Green 1996). Sediments were homogenised with fresh water to remove any salts and therefore prevent abnormal salt concentrations after autoclaving (see below). The resulting slurry was wet filtered through a 125 µm stainless steel mesh sieve and collected in a HDPE bucket. The sediment and other materials larger than 125 µm retained by the sieve were discarded. Discarded material included any large indigenous fauna, pieces of shells, and large particulate organic matter.

The sieved sediment was re-suspended in freshwater and homogenised by hand mixing and left to settle overnight at 4°C in the dark. The following morning the overlaying water was decanted and the sediments were washed again re-suspending and re-homogenising with fresh water. The slurry was left to settle overnight and the supernatant decanted. This process was repeated 3 times in total. After the final wash the settled sediment slurry was homogenised by hand before being divided into clean acid-washed (10% nitric acid soak for a minimum of 12 h) and triple acetone-rinsed 1.5 l glass jars. Each jar containing approximately 1 kg of sediments was covered with aluminium foil and autoclaved for 15 min at 125°C and 130 psi. The glass jars of sterilised sediment were stored in the dark at 4°C until they were used in experiments (up to 6 months).

Before initiating an experiment the sediment stored in glass jars was reconstituted with artificial sea water (ASW; Red Sea® sea salts made up in deionised water). Approximately 400 ml of the “dry” sterilised sediments was added to an electric blender with 1 l of 0.22 µm filtered 30‰ ASW and homogenised for 30 s. The sediments were allowed to settle for an hour before

the supernatant was aspirated off. This process was repeated twice. The final reconstituted sediments maintained a constant 3:2 sediment:water ratio.

4.2.3 Determining sediment dry weights and volumes of spike

Two concentrations of each pollutant were chosen to assess the efficacy of the spiking methods: one very high concentration (1000 µg/g dry sed.) and one moderate concentration (100 µg/g dry sed.). The sediment spiking procedure was based on a dry sediment weight basis (Thomas Chandler, personal communication). The reconstituted sediment was poured into a 2 l beaker on an orbital shaker (Ratek orbital mixer incubator) at 200 rpm. Four 1 ml replicate sub-samples of the mixed reconstituted sediment were removed to determine the final density wet/dry weight ratio. The reconstituted sediment samples were weighed before being centrifuged (5,000 x *g* for 10 min). The resulting supernatant was then aspirated away and the sediment dried in an oven at 50°C for 24 h. The dry sediment samples were weighed. The following equations were then used to calculate the amount of the spike:

1. Density = average wet weight (g) of 1 ml sediment

2. Dry/wet ratio= average sediment dry weight (g) / average sediment wet weight (g)

$$\text{3. Vol. of spike } (\mu l) = \frac{(\text{conc. desired } (\mu g/g)) \times (\text{wt. sediment to be spiked } (g)/\text{density of sediment}) \times (\text{dry/wet ratio})}{(\text{conc. of stock solution of contaminant})}$$

4.2.4 Sediment spiking

Prior to spiking with the reference toxicants, approximately 100 g of the reconstituted sediment was transferred into a clean acid and acetone washed mixing jar (250 ml Schott Bottles, borosilicate glass). The volume of spike

solution to add to the sediment was determined using equation 3. A different spiking procedure was employed for the introduction of the two reference toxicants into sediment. To spike the sediment with zinc, a stock solution of $ZnSO_4 \cdot 7H_2O$ (100 mg zinc/ml; BDH cat # 10299 AnalaR) was prepared in deionized water (Millipore, 0.22 µm filtered), while two solutions of phenanthrene (100 mg/ml and 10 mg/ml; Sigma cat # 77470, 97%) were prepared in acetone (Mallinkrodt Nanograde) as an organic solvent carrier.

4.2.4.1 Zinc spiking

The glass jars of sediment were placed on an orbital mixer at a speed of 200 rpm to form a vortex in the centre of the mixing sediment. An aliquot (375 µl and 37 µl for the 1000 and 100 µg/g treatments respectively) of stock solution was added to the vortexing sediment slurry at a slow drip. A spiking control was prepared using the same methods with the addition of deionised water (375 µl) rather than zinc.

4.2.4.2 Phenanthrene spiking

Two methods were assessed for phenanthrene sediment spiking. In the first procedure the inside surface of the spiking jars was shell coated with the phenanthrene based on the methods of Northcott and Jones (2000). The glass jars were shell coated by adding the desired predetermined mass of phenanthrene in acetone solution to the mixing jar and evaporating the acetone while rolling the jars. This produced a thin coating of phenanthrene distributed on the inner walls of the glass jar. Next, sediment was added to the jars and mixed on an orbital shaker (Murdoch et al. 1997, Northcott and Jones 2000).

The second procedure, as with the zinc spiking protocol, involved adding an aliquot of the phenanthrene stock solution (in acetone) to the mixing sediment

(200 rpm) at slow dripping rate. This allowed the phenanthrene to be slowly mixed into the sediment, thus preventing phenanthrene from exceeding its solubility limit (1 mg/l) and precipitating out of solution. The volume of spike solution added to sediment was kept to a minimum to prevent acetone forming a co-solvent solution with sediment pore-water that could affect the partitioning and distribution of phenenthrene. In order to normalise any potential effects of acetone the same volume (3.7 µl/g sed.) was added to all phenanthrene treatments. A sediment acetone control (no added phenanthrene) was prepared by adding 370 µl of acetone to 100 g sediment.

4.2.5 Mixing and ageing

After spiking the sediments the head space in the jars was purged and filled with nitrogen gas to prevent oxidisation of the sediment and inhibit bacterial growth, which can alter sediment physicochemistry and the bioavailability of the contaminant. Each jar was vigorously shaken by hand for 30 s and mixed in the dark on the orbital shaker (Ratek orbital mixer incubator) at 20°C for 24 h at 200 rpm. After mixing for 24 h, the spiked sediments were rested for a further 24 h before being shaken by hand again for 30 seconds and mixed on the orbital shaker for a further 24 h. The sediment was aged in the dark at 20°C for 11 days, providing a total sediment ageing period of 14 days.

After the conclusion of the 14 d ageing period the spiked sediments were once again vigorously shaken by hand for 30 s before being split into 7 replicate 10 g sediment samples, which were frozen until chemical analysis. The remaining sediment was used for pH and redox measurements.

4.2.6 Physicochemical sediment properties

Sediments were analysed for grain size, total organic content (TOC), redox potential and pH. Sediment grain size was determined by separating sediments with analytical sieves to evaluate the proportion (% dry weight) of >125, 124–106, 105–90, 89–75, 74–63, 62 – 32 and <32 µm size fractions after being shaken for 15 min. TOC was measured as loss-on-ignition after 3 h at 500°C. Redox potential and pH were measured with a platinum tipped redox probe (Jenway 924 003), and gel-filled PEI pH combination electrode (Hanna Instruments HI-1230).

4.2.7 Analysis of sediment contaminant concentrations

4.2.7.1 Zinc analysis

The pore-water and sediment-bound zinc concentrations were analysed to provide complete information on the distribution of contaminants within each treatment and the potential effects they have on toxicity. The sediments were centrifuged at 4,500 x *g* for 15 min to separate pore-water and sediment particles. Pore-waters were decanted and filtered through a 0.22 µm filter to remove particulate and colloidal organic matter, acidified by the addition of 25 µl of concentrated nitric acid, and diluted with 2% nitric acid (20 – 205 x) to be within the optimal concentration range for analytical detection. The centrifuged sediments were dried at 50°C for 7 d to evaporate any remaining pore-water. After drying, the sediments were homogenised by hand mixing with an acid washed plastic spoon and a 1 g subsample was removed to determine the concentration of zinc. Residual zinc in the sediment samples was extracted using a modification of the USEPA 200.8 protocol. Four ml nitric acid (69% ultra pure) and 10 ml hydrochloric acid (10%) were added to 1 g sediment in an acid washed polycarbonate tube and digested by heating at 90°C for 20 min. The

acidic digest was diluted to a final volume of 20 ml with deionised water. In order to be within the optimal calibration concentration range the acid digests were further diluted with 2% nitric acid (10 – 50 x). The diluted acidic digest solutions were analysed for zinc using Inductively Coupled Plasma- Mass Spectrometry (ICP-MS Agilent 7500 cx). QA/QC procedures included the analysis of two duplicate samples and one spiked sample within each set of 20 samples, as well as the use of a certified reference material (National Institute of Standards and Technology #2702, inorganics in marine sediments). Final sediment concentration was calculated by subtracting the mass of zinc contributed from the pore-water during drying.

4.2.7.2 Extraction and analysis of phenanthrene residues

Similar to zinc the concentration of phenanthrene was determined separately in the sediment and pore-water phases of each treatment. The sediment in each treatment tube was separated from pore-water by centrifuging in borosilicate glass tubes (2,500 x *g* for 15 min; Hettich Rotanta 460R). After centrifugation the pore-waters were decanted off the sediment into separate borosilicate glass screw cap test tubes.

Quality assurance procedures for the phenanthrene analysis included the addition of a surrogate recovery compound, anthracene-d10, to pore-water and sediment samples prior to extraction. A separate assessment of the extraction efficiency of phenanthrene from control sediment treatments was also completed by spiking separately with phenanthrene. The mass of the recovery compound anthracene-d10 spiked into the pore-water and sediment samples varied with the concentration of phenanthrene within each treatment. The theoretical concentration of phenanthrene in sediment and pore-waters was calculated using the organic carbon normalised partition coefficient (K_{oc}) for phenanthrene, the average percent organic carbon for the test sediment, and the target concentration

of phenanthrene in each treatment. Anthracene-d10 was added at a corresponding rate equivalent to the theoretically calculated concentration of phenanthrene. This ensured the concentration of anatracene-d10 added as surrogate recovery compound was comparable to the concentration of phenanthrene in each sample treatment. Additionally, five of the control treatments were spiked with phenanthrene at a concentration of 0.009 µg/g sed. dry wt.

Following the addition of surrogate standard to each batch of pore-water samples 2 ml of pentane was added to the pore-water in the glass test tubes which were sealed with Teflon lined screw caps. The sealed test tubes were vigorously shaken for 2 min on a flat bed shaker (300 rpm; IKA KS50) to extract phenanthrene and anthracene-d10 into the pentane. The test tubes were left upright to assist the separation of the organic and aqueous phases. The upper layer of pentane was removed by glass pipette and transferred to 23 ml borosilicate glass vials. The residual aqueous solution was extracted a further two times with fresh 2 ml aliquots of pentane that were combined with the first solvent extract. The combined solvent extract was concentrated to dryness under a gentle stream of nitrogen gas. The dried sample extracts were reconstituted in a predetermined volume of toluene, the internal standard phenenthrene-d_{10} added, and the final extracts analysed by high resolution gas chromatography with mass spectrometric detection (GCMS).

Phenanthrene associated with the sediment phase was extracted using a modified method based on the US Environmental Protection Agency (USEPA) and the US National Oceanic and Atmospheric Administration (NOAA) protocols (Holland et al. 1993). Prior to extraction the centrifuged sediment solid samples were spiked with a predetermined mass of the surrogate spike recovery compound anthracene-d_{10}. A subset of the control treatments were also spiked with phenanthrene to provide an equivalent sediment concentration of 0.009 mg

phenanthrene/kg sediment (dry weight basis). Fifteen ml of 1:1 (v/v) pentane:acetone extraction solvent was added to sediment in each sample tube and the contents extracted in a sonication bath (Sonorex Digital 10P) for 10 min at 25^{o}C at 100% power. The tubes and contents were shaken for 10 min at 300 rpm on a flat bed shaker (IKA KS501). After shaking, the tubes were centrifuged at 2,500 RCF for 12 min at 15^{o}C (Hettich Rotanta 460R) to separate the sediment and solvent extract. The upper solvent extract was decanted into a 100 ml glass Schott bottle.

Another 15 ml aliquot of 1:1 (v/v) pentane:acetone extraction solvent was added to each sediment sample tube, the extraction repeated, and the solvent extract decanted and combined with the previous extract. Approximately 70 ml of Milli-Q water was added to the solvent extract to partition the PAHs into the pentane. An aliquot (5-10 ml) of the upper pentane layer was pipetted into a glass vial and concentrated to dryness under a gentle stream of nitrogen gas.

The sediment extracts required further purification before analysis by gel permeation chromatography (GPC). The dried sediment extracts were reconstituted in 0.5 ml of dichloromethane (DCM). GPC was carried out on a Shimadzu LC10AT liquid chromatography system fitted with a SIL 10AF autoinjector, SPD-10A UV-Vis detector and FRC-10A fraction collector. The DCM sample extract (0.5 ml) was injected onto two in-house prepared Biobead SX8 GPC columns (420 x 10 mm, glass column) connected in series and eluted with DCM at a flow rate of 1.5 ml/min. The fraction containing anthracene-d10 and phenanthrene was collected for analysis and concentrated to dryness under a gentle stream of nitrogen gas. The purified sediment extracts were reconstituted in a predetermined volume of toluene, the internal standard phenenthrene-d_{10} added, and the final extracts analysed by GCMS.

GCMS analysis of the extracts was carried out using an Agilent 6890 Network gas chromatograph coupled to an Agilent 5975 mass spectrometer detector (MSD) and ATAS GL Focus autosampler. Samples and calibration standards in toluene (1 µl) were injected into an Agilent split-splitless injector operated in purged splitless mode with a splitless time of 1.10 minutes, purge flow of 50 ml/min and temperature of 280^{o}C. The injected samples were chromatographed on an Agilent HP5MS glass capillary column (30 m x 0.25 mm ID x 0.25 µm). Helium was used as carrier gas and electronic pressure control used to deliver a pressure pulse of 30 psi (held for 1 min) followed by a constant flow of 1 ml/min. Separation of target analytes was achieved by implementing the following temperature programme. The temperature of the GC column was held constant at 90^{o}C for 1.5 min, increased to 130^{o}C (0 hold) at 20^{o}C/min, then to 280^{o}C at 6^{o}C/min (0 hold), followed by 40^{o}C/min to 320^{o}C where it was held for 5 minutes. The temperature of the MSD transfer line was held at 280^{o}C and the quadrupole and ion source temperatures were 150^{o}C and 230^{o}C, respectively. Compound-specific single ion monitoring (SIM) data was obtained for phenanthrene-d_{10}, phenanthrene and anthracene-d_{10}. Response factors for phenanthrene and anthracene-d_{10} were determined by running a range of calibration standards and the response compared against that of phenanthrene-d_{10} to construct calibration curves for internal standard quantitation.

Phenanthrene-d_{10}, phenanthrene and anthracene-d_{10} in the calibration standards and sample extracts were identified by a match of retention time and relative abundance of two compound specific mass ions. The identification of compounds was verified by comparison between the mass ion abundance ratios in the sample to that in the calibration solution using a quality acceptance criterion of 20%.

Table 4.1 Physicochemical sediment characteristics of the pre-spiked sediments. SEM: standard error of the mean.

Physicochemical property	**Average**	**SEM**
pH	7.32	± 0.11[a]
Redox potential	161 mv	± 3.68 mv[a]
TOC	1.03%	± 0.10%[b]
Sediment size	**Average (%)**	**SEM (%)[b]**
>125	0.6	± 0.05
124-90	4.5	± 0.15
89-75	22.8	± 0.72
74-63	43.8	± 0.60
62-32	25.1	± 1.04
<32	3.2	± 0.24

[a]n=4 [b]n=6

4.3 Results

4.3.1 Physicochemical sediment properties

A summary of the physicochemical characteristics of the sediment is presented in Table 4.1. Table 4.2 displays the background concentrations of selected metals in the sediment used in the spiking assessment. After spiking, the redox potential and pH remained stable in all spiked treatments except for the 1000 µg/g zinc treatment where the pH reduced by 1.24 units to 6.08 and redox potential rose by 50 mV to 210 mV (Table 4.3).

Table 4.2 Background metal concentrations for the sediments used in sediment spiking sourced from Akaroa Harbour. Averages and standard deviations (stdev) given in µg/g sed. dry wt. (n=25)

	Cd	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn***
Average	0.02	9.80	2.53	5920	103	2.46	3.87	18.5
± *stdev*	± 0.003	± 0.63	± 0.123	± 120	± 6.72	± 0.005	± 0.14	± 0.30

*n=10

Table 4.3 Redox potential and pH after sediment spiking and ageing (+ 14 days).

Pollutant	Redox (mv)	pH
Control	153	7.30
Acetone control	154	7.34
100 µg/g Zn	172	7.18
1000 µg/g Zn	210	6.08
100 µg/g phenanthrene	169	7.31
1000 µg/g phenanthrene	164	7.33

4.3.2 Zinc-spiked sediment

Sediments spiked with zinc demonstrated a homogeneous distribution of the metal through the sediments with little variation between samples as represented by the low relative standard deviation (RSD) (Table 4.4). The efficacy of the sample extraction was calculated based on percent recoveries of the certified reference material and spiked samples. The recovery of zinc from the certified reference material was an averaged 79.4% with an RSD of 3.2% (n=4) for zinc. Spiked recoveries in the QA/QC sample were an average 85% with an RSD of 3.8% (n=3), and the relative percent difference of the duplicate samples was an average of 1.5 ± 1.3% (n=6).

Table 4.4 Measured concentrations (average n=7) and relative standard deviation (RSD) of zinc and phenanthrene spiked sediments

Pollutant	Nominal	Average concentration (µg/g sed.)	RSD	Pore-water mg/l	RSD
Zinc	*Control (background)*	18.6	1.6%	0.005	11.1%
	100	71.0	2.8%	0.28	2.2%
	1000	490	2.2%	224	3.4%
Phenanthrene	*Acetone control*	<0.000	-	<0.000	-
	100	46.3	6.2%	0.80	53%
	1000	116.4	9.8%	9.58	71%

The processed sediments obtained from Akaroa Harbour had a background zinc concentration of 18.5 µg/g. The measured average concentration of zinc spiked into the sediments were 71.0 and 490 µg/g (n=7). The corresponding pore-water concentrations were 0.28 mg/l and 224.2 mg/l for the moderate and high zinc concentrations, respectively. The variation between the seven subsamples was low (an RSD of 2.8% and 2.2% for the sediment spikes) indicating good mixing and even zinc distribution throughout the sediments.

4.3.3 Phenanthrene spiked sediments

The shell coating spiking method proved to be inadequate for spiking as there were large flakes of phenanthrene left on the sides of the jar and in the sediment matrix. Due to the inadequate distribution of phenanthrene, the sediments were not further analysed.

For the direct spiked sediments, the 95% confidence interval for the mean recovery of the surrogate compound anthracene-d10 spiked into pore-water samples was 97.0 ± 0.05%. Similarly, the 95% confidence interval for the mean recovery of anthracene-d10 spiked into sediments was 89 ± 4%. The 95% confidence interval for the mean recovery of phenanthrene spiked onto the control treatments was 91 ± 6%.

The sediments spiked with phenanthrene provided an average sediment concentration of 46.3 and 116.4 µg/g sed. dry wt. (n=7). Variability between the samples showed an RSD of 6.2 and 9.8% for the 100 and the 1000 µg/g nominal treatments, respectively. The resulting sediment-bound concentrations of phenanthrene were significantly lower than target nominal concentrations. The

low rates of binding resulted in high corresponding pore-water concentrations of phenanthrene of 0.8 and 9.58 mg/l.

4.4 Discussion

4.4.1 Zinc

In the 100 µg/g zinc nominal concentration treatments the resulting sediment-bound concentration was calculated to be 71% of the nominal target concentration (71.0 µg/g zinc). This can in part be explained by partitioning of zinc into the pore-waters (average pore-water concentrations 0.28 mg/l) and loss by absorption to the borosilicate glass (Batley and Gardner 1977). The measured sediment concentration of zinc in the nominal 1000 µg/g zinc sediment was 490 µg/g zinc. This low binding can be explained by the saturation of the binding sites with zinc. This was confirmed from the extremely high pore-water concentrations of 224 mg/l.

There is a large variation (0.5-8%) in the organic content in spiked sediment studies and this can cause large variations in sediment toxicity (Strom et al. 2011). In the current study the organic content was low (1%) which is typical of sandy sediments. These types of sediment often have higher pore-water concentrations and higher toxicity, as demonstrated in this study, particularly in comparison to studies that have finer, more organic-rich, sediments (Chandler and Green 1996, Chandler and Green 2001). Organic carbon is the primary sediment constituent that binds contaminants (Chapman and Wang 2001, Rockne et al. 2002), and thus is an important factor in relating toxic effect levels across locations and sediment types.

Sediment size also plays a significant role in controlling bioavailability and toxicity. The majority of contaminants (especially metals) preferentially bind

to the smaller <63 µm sediment fraction over larger sediment particles, due to higher density and surface area of binding sites (Simpson et al. 2011, Strom et al. 2011). This leads to the potential for sandier sediments to sequester less contaminant than finer organic-rich sediments. This often can result in greater flux of contaminants into pore- and overlying waters from sediments (Simpson and King 2005, Strom et al. 2011). In this chapter, fine sandy sediments were used (44% of sediment in the 32-63 µm fraction), with a 1% organic composition. Although a large proportion of the sediments are <63 µm, the low organic content results in a low number of binding sites for metals, and because of this there was higher pore-water concentrations of contaminants relative to the sediment load. Prior to testing, the sediments were sieved through a 125 µm mesh to remove large debris that makes finding copepods in the sediment difficult. This should not affect the environmental relevance of the sediment type.

In sediments that have high pore-water concentrations, the main exposure route of the organism can be shifted from sediment contact or ingestion, to pore-water exposure (Simpson 2005, Simpson and King 2005). This can change the interpretation of toxic effect levels and highlights the importance of reporting not only the concentration of spiked metals in the sediment but also the pore-water concentrations of spiked metals (Simpson et al. 2005).

The changes in pH and redox potential observed in the high zinc spike concentration are most likely explained by the displacement of sediment-bound ferrous iron into the pore-water. When the ferrous iron is oxidised it catalyses hydrolysis reactions that result in the observed decreased pore-water pH, and increased redox potential (Simpson et al. 2005, Hutchins et al. 2007). The changes in pH and redox observed in the high zinc spike (1000 µg/g nominal) treatment were not abnormally high and remained within the ranges of acceptable pH (6-9) for toxicity testing. However, pH can be normalised by the

addition of NaOH (Hutchins et al. 2007). Normalisation of pH is recommended as changes in pH influence the partitioning of metals between sediments and pore-water, and hence the bioavailability of metals. Normalising pH in sediment toxicity assays to a specific value is desirable as it provides a better representation of the pH conditions experienced by organisms that are exposed *in situ* (Atkinson et al. 2007, Hutchins et al. 2007)

Overall the method for spiking sediments with zinc was successful. There was very low variability in the concentration of zinc between the seven replicate treatments, demonstrating that a homogeneous distribution of zinc was achieved in the spiked sediment. Changes in the measured physicochemical parameters were minimal in the high zinc spike treatment. However, the potential for high pore-water concentrations needs to be taken into account when using spiked sediment exposures and the potential effects that it can exert on the exposure pathways and resultant toxicity (Lee et al. 2004, Simpson and King 2005).

4.4.2 Phenanthrene spiked sediment

Two procedures were evaluated to spike sediment with phenanthrene. In the first procedure the inside surface of the spiking jars was shell coated with the phenanthrene. Shell coating is a common method used to spike sediments with organic chemicals as it eliminates the introduction of carrier solvent into spiked sediments (Northcott and Jones 2000). This method proved to be problematic as large flakes of residual crystallised phenanthrene were observed on the inside glass surfaces of the jar and in the sediment matrix. As a result this procedure was abandoned in preference to a direct spike method employing the use of a carrier solvent to deliver the phenanthrene. The carrier solution spike was accomplished by using a low volume of phenanthrene in acetone stock solution

added directly to mixing sediment. This method proved to be more successful with no obvious precipitation/crystallisation issues during spiking.

The concentration of phenanthrene measured in the spiked sediments was significantly lower than the target nominal concentrations. Analysis of the spiked sediments provided calculated recoveries of 46.3% from sediment spiked at a concentration of 100 µg/g and 11.6% from sediment spiked at 1000 µg/g sed. dry wt. The homogeneity of phenanthrene residues in the spiked sediments were acceptable as demonstrated by the relative standard deviation between replicates of 9.7% for the 1000 µg/g sed. dry wt. nominal concentration and 6.2% for the 100 µg/g sed. dry wt. nominal concentration. The higher variability between replicates obtained for phenanthrene spiked into the sediments compared to that obtained for zinc could result from the presence of localised crystalline residues of phenanthrene in the sediment. This has been reported in previous studies with hydrophobic organic compounds at high concentrations (Murdoch et al. 1997). The challenge of achieving a homogeneous distribution of hydrophobic organic chemicals spiked into soil and sediments is widely recognised (Northcott and Jones 2000). This is a result of the limited solubility of these chemicals in aqueous solution. In comparison the metal salts used to spike soil and sediment are readily soluble in water and are rapidly and evenly distributed throughout the solid phase. The results of this assessment suggest sediments should not be spiked with phenanthrene at concentrations exceeding 100 µg/g sed. dry wt. as this has potential to saturate binding sites and impact on equilibrium partitioning between the sediment and its pore-water in low organic content sandy sediments.

Phenanthrene sorption to sediments is controlled by organic carbon (Di Toro et al. 1991, Xia et al. 2011). The sediments used in this study had a relatively low organic carbon content (1%) which will have limited their sorptive capacity for phenanthrene. Phenanthrene is a highly hydrophobic chemical and may partially bind to glass and other substrates during sediment spiking.

Northcott and Jones (2000) reported that when phenanthrene was spiked into field wet soils it could crystallise onto glass surfaces, producing a less homogeneous distribution of spiked chemical and also a reduced concentration of toxicant in the spiked media. The sediments used in the current experiment had a high water content which will have promoted the crystallisation of phenanthrene in the sediment, and sorption to the walls of the glass spiking bottle. These combined processes could explain the low rate of phenanthrene recovery from the spiked sediments.

The concentrations of phenanthrene measured in the pore-water samples were higher than expected and exceeded the water solubility of phenanthrene in the 1000 µg/g sed. dry wt. nominal treatments. This solubility enhancement is most likely explained by the presence of dissolved organic matter and/or colloidal organic matter in the pore-water. The ability of these aqueous organic components to significantly increase dissolved phase concentrations of hydrophobic organic chemicals is widely acknowledged (Lassen and Carlsen 1997). In the 100 µg/g sed. dry wt. phenanthrene treatments the average pore-water concentration of phenanthrene was 0.8 mg/l which is just below the aqueous solubility limit of phenanthrene of 1 mg/l, suggesting the pore-water was close to saturation with phenanthrene. The ionic strength and temperature of aqueous solutions are other important factors affecting the solubility of phenanthrene in pore-water. Higher ionic strength solutions (e.g. seawater) act to decrease the aqueous solubility of phenanthrene (Lee et al. 2003). Temperature affects the sorption of organic chemicals to low organic carbon content sediments with increasing sorption as temperature decreases (Piatt et al. 1996). This is important when considering equilibrium conditions. It is common practise to equilibrate spiked sediments used in toxicity testing at 4°C prior to testing, which may result in increased partitioning to sediment during equilibration and a corresponding pulse or flush into pore-water when the temperature is increased to standard test conditions. These different factors

affecting sediment and pore-water concentrations of spiked contaminants need to be considered, and in some cases evaluated, prior to initiating bioassay testing. If artificially high pore-water concentrations are obtained in test procedures employing spiked sediments, the sediment pore-water mixtures can be centrifuged, the pore-water discarded, and the sediment and spiked contaminant re-equilibrated with fresh aqueous solutions of fresh or seawater (Simpson et al. 2005).

The results obtained from spiking phenanthrene highlighted the challenge of spiking hydrophobic organic compounds into marine sediments without introducing artefacts. The use of carrier solvents resulted in greater crystallisation/precipitation due to rapid dissipation of the water miscible solvent into the sediment and its pore-waters (personal observations). However, the concentrations used in this spiking assessment were one to two orders of magnitude greater (10-100 x) than those used in subsequent organism exposure experiments. The significantly reduced concentrations of phenanthrene used in subsequent organism exposure experiments minimised the potential of obtaining artificially high pore-water concentrations and low nominal recoveries of phenanthrene observed in these high spike concentration treatments.

4.5 Conclusions

The objectives of this study were to investigate and validate the spiking methods using zinc, a water soluble compound, and phenanthrene a hydrophobic compound. The spiking methods employed fine sandy sediments with low organic content and were found to be successful as demonstrated by the resultant homogeneous distribution of both contaminants. The limits of sediment saturation for zinc and phenanthrene were 490 and 116 µg/g, respectively. Pore-water concentrations were high, exceeding the aqueous solubility of

phenanthrene when phenanthrene saturated the sediment binding sites. The potential to produce this artefact needs to be considered in the design of toxicological assessments employing spiked test media, and in particular, low organic carbon content sediments with reduced sorption capacity for hydrophobic organic chemicals.

5.

Development of acute and chronic sediment bioassays with the New Zealand harpacticoid copepod *Quinquelaophonte* sp.

5.1 Introduction

Sediment bioassays are being increasingly used to monitor and test sediments to determine toxicity. These tests utilise a wide range of species from polychaete worms, amphipods, bivalves, chironomid larvae to harpacticoid copepods (Moore and Dillon 1993, Roper and Hickey 1994, Clement et al. 2004, King et al. 2006, Greenstein et al. 2008). The major strength of the bioassay is that it assesses the toxicity on the basis of biological effects, rather than by chemical characterisation and trigger values (as per ANZECC (2000) recommendations). Although trigger values are often based on testing in bioassays, the species used to establish the values are often not those of greatest relevance to the site of interest, and may therefore be inappropriate in predicting toxicity of a given ecosystem. Additionally, when sediment quality guidelines are applied, only abiotic parameters are considered and these may be poor predictors of toxicity. In part this is due to the inability of such approaches to account for any additive or synergistic interactions of contaminants, which can alter the toxicological impact of the sediment. A further weakness of these approaches is that they only test for the presence of a selected number of contaminants (Davoren et al. 2005). However sediments are very complex, with a large variation in chemical composition and substrate type, even within the same estuary. As these two factors contribute significantly to toxicity, this makes it even harder to predict potential adverse effects on the ecosystem (Simpson et al. 2011).

There has been a recent realisation that the use of acute lethal endpoints to assess toxicological risk is inadequate, and that sublethal endpoints and chronic tests allow for better assessments of toxicity (Brown et al. 2005, Greenstein et al. 2008, Kennedy et al. 2009, Simpson and Spadaro 2011). There have been a variety of sublethal endpoints used in sediment bioassays. These include developmental (Dahl et al. 2006), behavioural (Lotufo 1997, Silva et al. 2009), and reproductive (Ingersoll et al. 1998, Bejarano and Chandler 2003, Fleeger et al. 2007, Simpson and Spadaro 2011) endpoints. Of these, reproductive endpoints are most commonly employed in meiobenthic bioassays, primarily due to the difficulty in observing development and behaviour in a sediment-dwelling organism. Reproduction is an

important endpoint as it is sensitive and may have significant consequences at the population level. Inhibition of reproduction can be affected by toxicants via both direct and indirect effects. Direct effects are those where the toxicant acts on the reproductive system by either mimicking a hormone (endocrine disruption) or causing cellular damage to reproductive tissues (Hutchinson 2002). Indirect effects occur when the physiological balance of the organism is altered, or processes such as steroid and enzyme synthesis are disrupted (Mattison and Thomford 1989).

Although observations of behaviours *in situ* can be difficult for sediment-dwelling organisms, if behavioural assessments of sublethal toxicity can be tailored to the specific bioassay species, they can provide important insights into effects of contaminants. Examples of behavioural endpoints include respiration, locomotion, habitat selection, feeding, and predator avoidance. These parameters reflect a variety of genetic, neurobiological, physiological, and environmental effects (ASTM 2007). As behavioural effects are likely to reflect subtle changes in the biology of an organism, these endpoints provide more sensitive estimates of toxic effects that may occur in natural populations *in situ* (Ingersoll et al. 1998).

Before sediment bioassays are able to be used on field-contaminated sediments it is important to characterise the responses of the bioassay species to different toxicants. This provides insight into how the different bioassay endpoints vary with distinct modes of action and bioavailability within the sediments. This is accomplished by using single toxicant exposures. In addition to characterising bioassay species responses this information can be used in environmental risk management for the studied chemicals.

In environmental risk assessment there are two key factors involved in identifying risk. The first of these is the characterisation of the toxicant(s) and the nature of the exposure. This will include identifying the contaminant source, determining persistence in the environment, and the potential of the contaminant to bioaccumulate. The second factor involves identification of the contaminants potential ecological impacts. This involves assessment of level of toxicity, the mode of action, and any population effects. Ultimately, risk is determined by integrating these two elements (USEPA 1992). For example, if a pollutant is highly

toxic to an ecosystem or an organism, but degrades quickly, then the environmental risk may be relatively low. Conversely, a chemical which is less acutely toxic but persists in the environment for prolonged periods of time may be a greater environmental risk. In environmental risk assessment it is important to use local species wherever possible to best represent the ecosystems that are being evaluated. In Australia and New Zealand there is a lack of information using local species (ANZECC 2000). Increased use of native species will help to create better protection for the species that actually inhabit the areas of concern.

The previous chapters of this thesis have identified a harpacticoid copepod bioassay species, *Quinquelaophonte* sp. (Chapter 3), and have developed a method for spiking the sediments (Chapter 4) that were shown to be preferential for habitation by the *Quinquelaophonte* genus (Chapter 2). The objectives of this chapter are to create two sediment bioassay protocols, one acute and one chronic, using a New Zealand native harpacticoid species, *Quinquelaophonte* sp. The acute sediment assay examines a sublethal endpoint, which has been included to increase the sensitivity of the assay, and which may enhance the understanding of toxic effects aside from mortality. The chronic partial life cycle test has been developed to assess chronic effects of contaminants using mortality and reproductive responses (fecundity and total juvenile production). Consistent with the rest of the thesis, three reference contaminants (zinc, atrazine, and phenanthrene) will be examined. These contaminants are of environmental concern and have been used in other studies, allowing for the results attained in this chapter to be compared to literature findings.

5.2 Methods

5.2.1 Test species

Quinquelaophonte sp. were cultured in a laboratory culture system based on the recirculating seawater system designed by Chandler (1986) and modified for use with *Quinquelaophonte* sp. (Chapter 3). Copepods were mono-cultured in a flow-through plastic aquarium with approximately 2 cm of cleaned sterilised

sediments (size <125 µm), with 5 cm of overlying artificial seawater (ASW; RedSea® sea salts) under a dripping flow of 5 ml/min. The culture system was maintained at 20°C and at 30‰ ASW, with a 12 h:12 h light:dark photoperiod. The copepods were fed 40 ml of a concentrated mixed suspended algal diet of *Dunaliella tertiolecta, Isochrysis galbana,* and *Chaetoceros muelleri* (1:1:1) twice a week (Chandler and Green 1996).

5.2.2 Sediment preparation and spiking

Sediments were collected from Akaroa Harbour (43°45'29"S 172°55'5"E) at low tide by scraping the top 0-2 cm of sediments, and were processed according to the methods detailed in Chapter 4. Toxicant concentrations were achieved by spiking sediment with stock solutions of zinc, atrazine, and phenanthrene (see methods for zinc and phenanthrene in Chapter 4). Atrazine and phenanthrene were spiked using acetone as a carrier in the 14 d chronic bioassays, and in the 96 h acute test DMSO was used for atrazine. The solubility of atrazine in acetone was inadequate to generate the higher sediment concentrations required for the acute tests, hence the use of DMSO as a replacement carrier in these trials. Acetone is the preferable carrier as it is more likely to volatalise, thus reducing solvent concentrations in the sediment (Northcott and Jones 2000). Carrier controls were used in all tests where a carrier was used. The carrier/sediment concentration was consistent in all treatments and was below 3 µl/g sed.

5.2.3 Acute 96 h toxicity tests

Acute toxicity tests were performed with three reference contaminants (zinc, atrazine, and phenanthrene). All of the tests used laboratory-cultured copepods, described in Section 5.2.1 above, and followed the methods outlined by Green et al. (1993) with some modifications.

For each contaminant, there were five geometrically–increasing concentrations, with four replicates for each treatment and control. Three of the

replicates were used for biological enumeration at the termination of the test; the fourth was used for water quality and chemical analysis. Each test vessel consisted of a 50 ml Erlenmeyer flask with 10 ml clean or spiked control sediments, 20 ml of aerated 30‰ ASW, and 30 adult copepods (15 male, 15 non-gravid female) that were loaded at test initiation via Pasteur pipette. The test vessels were loosely covered with aluminium foil to prevent evaporation. The tests were static (no water exchange) and run over 96 h in an environmental chamber at 20 ± 1°C and a 12:12 light:dark cycle. Dissolved oxygen, salinity, and pH were measured at test initiation and at test termination (96 h).

5.2.4 Chronic 14 d toxicity tests

The chronic partial life-cycle 14 d test was based on a method originally described by Chandler and Green (1996), but modified for *Quinquelaophonte* sp. In brief, each test included five geometrically-increasing concentrations of the three reference contaminants based on the results from the acute 96 h exposures described above. An overlap in concentrations was used to compare the differences in acute and chronic toxicity. Unspiked sediment was used as a control and carrier controls were implemented if a carrier was used to spike sediments. For each concentration there were four replicates. Test chambers consisted of a 50 ml Erlenmeyer flask with two opposing 1 cm diameter holes at the base of the neck of the flask which were covered with 55 μm nylon mesh. These apertures acted to maintain a constant level of overlying ASW (25 ml). Prior to water addition, 10 ml of sediment were added to each test chamber via syringe. After the sediments were added, the test chambers were inoculated with 30 copepods (15 male and 15 non-gravid females) via Pasteur pipette from the laboratory cultures. Test chambers were placed in a purpose built flow-through ASW system, under a dripping flow of approximately 1 ml/min. The tests were conducted for 14 d at 20 ± 2°C under a 12:12 dark:light photoperiod. Copepods were fed 1 ml (~$2 \cdot 10^6$ cells) of a concentrated 1:1:1 mixture of *Dunaliella tertiolecta*, *Isochrysis galbana*, and *Chaetoceros muelleri* algae twice a week. Water quality parameters such as salinity, dissolved oxygen, nitrate, nitrite, pH and ammonia, were monitored at the initiation of the test, after one week, and at test termination. Water quality was

maintained at salinity 30‰, pH 8.2, dissolved oxygen >6 mg/l, with nitrate, nitrite, and ammonia kept below detection limits (2.5, 0.05 0.25 ppm respectively, RedSea® Marine Lab).

5.2.5 Physicochemical sediment characteristics

The sediment was analysed for grain size, total organic content (TOC), redox potential, and pH using the same methods as described in Chapter 4. Redox potential of the sediment was determined by using a platinum-tipped redox probe (Jenway 924 003), and pH was measured using a gel-filled PEI pH combination electrode (Hanna Instruments HI-1230).

5.2.6 Toxicant concentrations

Sediment contamination concentrations were measured in pore-water and sediment-bound fractions at test initiation and termination. A subsample (c. 10 ml) of sediment taken at the beginning of the test, and sediment from one of the four replicates at test termination, was frozen at -20°C until analysis. The analytical methods for pore-water and sediment concentrations for zinc and phenanthrene are described in Chapter 4. Analyses were conducted concurrently with the samples in Chapter 4.

Similar to the phenanthrene analysis described in Chapter 4, atrazine concentrations in the sediment and pore-water were determined by first centrifuging sediments at 2,500 x *g* to separate pore-water and sediments. Pore-water was decanted into a borosilicate glass screw cap test tube, and both sediment and pore-water was spiked with the structurally-similar triazine herbicide simazine as a surrogate recovery compound. The mass of simazine spiked into the pore-water and sediment samples varied with the concentration of atrazine within each treatment. The theoretically expected concentration of atrazine in the sediment and pore-water of each treatment was calculated using the organic carbon-normalised partition coefficient (K_{oc}) for atrazine, the average percent organic carbon for the test

sediment, and the target concentration of atrazine in each treatment. Simazine was added at a corresponding rate equivalent to the theoretically calculated concentration of atrazine. This ensured the concentration of simazine added as a surrogate recovery compound was comparable to the concentration of atrazine in each sample treatment. The concentration of simazine used spanned 5 orders of magnitude in concentration. Five of the control treatments were spiked with desethyl-atrazine, desisopropyl-atrazine, simazine and atrazine at a concentration of 0.250 µg sed. dry wt. for spiked sediment (atrazine), surrogate recovery (simazine) and atrazine metabolite (desethyl-atrazine, desisopropyl-atrazine) analysis.

Following the addition of surrogate standard to each batch of pore-water samples, 2 ml of dichloromethane was added to each test tube. The test tubes were sealed with a teflon-lined screw caps and the contents shaken for 10 minutes at 300 rpm on a flat bed shaker (IKA KS501). After shaking, the tubes were left to stand upright to assist the separation of the organic and aqueous phases. The lower dichloromethane layer was removed by glass pipette and transferred into a glass vial. The residual aqueous solution was extracted a further two times with another 2 ml of dichloromethane. The combined solvent solutions were concentrated to dryness under a gentle stream of nitrogen gas. The dried sample extracts were then reconstituted in a predetermined volume of ethyl acetate and a predetermined quantity of the internal standard tetrachlorvinphos was added to the reconstituted atrazine sample extracts.

Sediment-bound atrazine was solvent-extracted after the addition of the simazine surrogate recovery spike using 15 ml of methanol. The sediment solvent mix was sonicated for 5 min at 25^{o}C at 100% power in a Sonorex Digital 10P ultrasonicating bath. The tubes and contents were shaken for 10 min at 300 rpm on a flat bed shaker (IKA KS501). After shaking, the tubes were centrifuged at 2,500 x *g* for 12 min at 15^{o}C (Hettich Rotanta 460R) to separate the sediment and solvent extract. The upper solvent extract was decanted into a 60 ml glass vial. Each sediment sample was extracted two more times using 10 ml of methanol and the three extracts combined. The methanol sample extracts were concentrated to dryness under a gentle stream of nitrogen gas using a Turbo Vap LP Concentration Work Station (Caliper Life Sciences). The dried extracts were reconstituted into a

predetermined volume of ethyl acetate. Extracts of the higher concentration atrazine treatments were diluted prior to analysis, and by doing so the effects of co-extracted matrix components were negated. Extracts obtained from the lower concentration treatments that could not be diluted for analysis required additional purification by GPC. The concentrated sample extract were reconstituted in 1 ml of ethyl acetate/cyclohexane (1:1, v/v), filtered through a 0.45 µm micro-filter, and transferred to an auto-sampler vial. GPC cleanup was achieved using a Shimadzu liquid chromatograph (LC-10) with SPD-10A UV detector and FRC-10A fraction collector, coupled to a Biobeads SX-3 column (45 cm x 1 cm diameter) using ethylacetate/cyclohexane (1:1 v/v) as mobile phase. One ml of the concentrated sample extract was injected onto the GPC column and the fraction containing the pesticide analytes collected in a glass vial. The cleaned-up sample extracts were concentrated, the internal standard tetrachlorvinphos added, and this extract transferred to an auto sample vial for analysis.

Residues of atrazine, its primary metabolites desethyl-atrazine and desisopropyl-atrazine, the surrogate recovery chemical simazine and internal standard tetrachlorvinphos were analysed by high resolution gas chromatography with electron capture (µ-ECD) and nitrogen-phosphorus detection (NPD) using an Agilent 6890 N gas chromatograph, 7683B series autoinjector and Programmable Temperature Vaporisation (PTV) injection. Five µl of sample extracts and calibration standards were injected by pressure-pulsed splitless mode onto a Hewlett Packard Ultra-2 capillary GC column (30 m x 0.25 mm i.d. x 0.33 µm) connected to a column effluent splitter (SGE VSOS) to split and transfer the column effluent to the two detectors. The PTV injector temperature was held at 70^{o}C for 0.1 min then increased at 200^{o}C/min to a final temperature of 250^{o}C for the duration of the run. The injected samples were chromatographed using an Agilent HP5 glass capillary column (25 m x 0.20 mm ID x 0.33 µm). Helium was used as carrier gas and electronic pressure control delivered a pressure pulse of 35 psi (held for 1 min) followed by a purge flow of 80 ml/min and constant column flow rate of 37 cm/s. After 2 min the injector purge flow was reduced to 20 ml/min. Separation of target analytes was achieved by implementing the following temperature programme. The temperature of the GC column was held constant at 70^{o}C for 2 min, increased to 130^{o}C (1 min hold) at 25^{o}C/min, then to 160^{o}C at

5°C/min (0 hold), followed by 30°C/min to 300°C (5 min hold). The temperature of the ECD and NPD was 300°C and 325°C respectively and nitrogen was used as the detector make-up gas.

Analyte peaks were identified and quantified by relative retention time against the internal standard and absolute responses to external calibration standards for both detectors. Calibration curves for the analysed compounds were prepared by injecting six calibration standards within the range of 0.02 to 2.0 µg/ml. Data analysis was carried out using the Agilent Environmental Chemstation chromatography package.

Test concentrations of contaminants in sediment and pore-water were calculated by averaging the initial and final concentrations to give an average exposed concentration over the test period. Percent losses were also calculated by dividing the difference of the initial and final concentrations by the initial concentration, to examine amount of contaminant losses to pore- and overlaying-water.

5.2.7 Toxicological analysis

5.2.7.1 Acute 96 h tests

At test termination, survival was determined by sieving replicates on a 55 µm mesh sieve. All individuals (alive or dead) were then gently washed into a Petri dish where survival was assessed and enumerated, and individuals were sexed. Copepods were considered dead if there was no response or movement in a 10 s period following being gently prodded with a needle. A sublethal endpoint, lethargy or lack of mobility (i.e. an inhibition of swimming) was also measured. Lethargy was defined as an inhibition of movement away from a stimulus (a gentle prod with a needle), after 10 s. Lethargic copepods were therefore distinct from dead copepods in that they did respond to the stimulus, however were unable to actively move from the stimulus. Copepods that moved away from stimulus were considered unimpacted by exposure.

5.2.7.2 Chronic 14 d tests

Copepods were enumerated, sexed, and a determination of mortality was conducted as described above for the acute test. Gravid females were isolated and preserved in 5% formalin for later fecundity assessment. After adult individuals were isolated the remaining sediment and juvenile copepods were preserved in 5% formalin and then stained with Rose Bengal to facilitate counting of nauplii and copepodites. Numbers of nauplii and eggs per female were then used to calculate the reproductive endpoints, defined below:

1. Total offspring = nauplii + copepodites

2. Potential offspring per female = (eggs + nauplii + copepodites) / surviving females

3. Realised offspring per female= (nauplii + copepodites) / surviving females

5.2.7.3 Statistical analysis

Measured concentrations of the contaminants in both sediment and pore-water were used to generate dose-response curves and estimate LC_x and EC_x values for acute and chronic endpoints. The proportion of individuals surviving ("unimpacted"; i.e. not lethargic), or total juveniles produced (as percent of control) were regressed against the log value of contaminant concentration using binomial regression. LC_x and EC_x (x = 50, 20, 10) values along with 95% confidence intervals were calculated using the FIELLER procedure in the GenStat Statistical Package (12^{th} edition). Means and standard deviations were calculated for the reproductive endpoints using GraphPad Prism (Version 5) and statistical significance was tested using one-way ANOVA with a Dunnett's post-hoc analysis to assess differences from controls. From the results of these analyses two values can be derived. The no observed effect concentration (NOEC) is the highest test concentration that was not statistically significantly different from the control value. The lowest observed effect concentration (LOEC) is the lowest concentration that showed a statistically significant difference to the controls. The maximum

acceptable toxicant concentration (MATC) was then calculated as the geometric mean of the LOEC and NOEC (Mebane et al. 2008). The LOEC, NOEC, and MATC were used in this study for calculation of acute to chronic ratios, and for comparison to previous research.

5.2.8 Acute to chronic ratios

Acute to chronic ratios (ACRs) were calculated using a variety of methods. In Europe, Australia and New Zealand it is common to calculate ACRs based on the acute LC_{50} divided by the chronic NOEC (Lange et al. 1998). In the United States ACRs are commonly calculated as the LC_{50}/MATC, however recently there has been a shift to acute LC_{50}/chronic EC_{20} (USEPA 2007, Mebane et al. 2008). In other parts of the world, acute LC_{50}/chronic EC_{10} is increasingly used as the NOEC and LOEC are becoming largely obsolete measures (Harrass 1996). All of these endpoints were calculated in the current study, along with the acute EC_{50}/chronic EC_{10} ratio, as a further ACR metric.

5.3 Results

5.3.1 Physicochemical sediment characteristics

Sediment characteristics are presented in Table 5.1, the sediments used in this chapter are identical to the sediments used in Chapter 4 (i.e. same collection and processing batch), and thus had the same organic content and particle size distribution. Sediments were found to have a total organic content of around 1%, consistent with fine sandy sediment types. The majority of the sediment was in the 32-62 µm sediment fraction (43.8%), with the 63-74 µm sediment fraction being the second largest. The pH and redox potential did not change after spiking and ranged from 7.7–8.3.

Table 5.1 Sediment pH, redox potential (mV), total organic carbon (TOC, %), and sediment particle distribution (%) in µm.

Physiochemical property	**Average**	**±SEM**
pH	8.07	± 0.24
Redox potential (mV)	140	± 5.3
TOC (%)	1.03	± 0.10
Sediment size (µm)	**Average (%)**	**SEM (%)**
>125	0.6	± 0.05
124-90	4.5	± 0.15
89-75	22.8	± 0.72
74-63	43.8	± 0.60
62-32	25.1	± 1.04
<32	3.2	± 0.24

5.3.2 Chemical analysis

Measured concentrations in sediment and pore-water for all three contaminants are listed in Table 5.2. The measured concentrations are an average of test initiation and termination concentrations, providing the average exposed concentration for each treatment (King et al. 2004). As the zinc and phenanthrene samples were analysed concurrently with those conducted in Chapter 4, they share the same QA/QC data. The recovery of zinc from the certified reference material was an average of 79.4 ± 3.2%, spiked sample recovery was an average of 85 ± 3.8%, and the relative percent difference of the duplicate samples was 1.5 ± 1.3%.

For phenanthrene the mean recovery of the surrogate anthracene-d10 spiked into pore-water samples was 97.0 ± 0.05%. The recovery of anthracene-d10 spiked into sediments was 89 ± 4%. The recovery of phenanthrene spiked into the control treatments was 91 ± 6%.

The mean recovery of simazine spiked into pore-water samples was 88 ± 6%. The recovery of simazine spiked into sediments was 88 ± 11%. The five control treatments that were spiked with desethyl-atrazine and desisopropyl-

Table 5.2 Measured sediment and pore-water concentrations for acute and chronic tests. Values are an average of the test initiation and termination concentrations. Sediment values are given in µg/g dry wt., and pore-water in mg/l. Percent losses over the test are also reported for the sediment concentrations of contaminants. Limits of detection were 0.01 mg/l for zinc and 0.001 for atrazine and phenanthrene

Pollutant	Test type	Average concentration µg/g sed.	Pore-water concentration mg/l	% lost
Zinc	*Acute*	89	0.30	6.7
		122	0.62	3.4
		172.	1.5	2.6
		255	4.9	3.3
		384	19	9.8
	Chronic	27	0.04	0.72
		37	0.05	1.9
		55	0.11	0.45
		95	0.27	0.92
		162	1.2	9.1
Atrazine	*Acute*	0.12	0.4	63
		0.35	1.3	60
		3.1	2.	69
		21	11	55
		176	18.	42
	Chronic	0.00005	0.002	>90
		0.0005	0.01	>90
		0.003	0.04	80
		0.05	0.11	85
		0.49	0.40	75
Phenanthrene	*Acute*	0.28	0.008	14
		2.1	0.04	11
		12	0.19	4.8
		25	0.3	8.0
		71	0.14	29
	Chronic	0.003	<LOD	33
		0.01	<LOD	29
		0.04	0.001	65
		0.3	0.009	57
		1.85	0.04	58

LOD – Limit of detection

atrazine, simazine and atrazine had a mean recovery of 85 ± 5%, 85 ± 6%, 91 ± 5%, and 100 ± 7%, respectively. The two primary metabolites of atrazine, desethyl-atrazine and desisopropyl-atrazine, were not detected (<0.001 mg/l) in the analysed pore-water or sediment samples.

Sediment concentrations were within expected ranges based on the spiking recovery results from Chapter 4. Pore-water concentrations of all three reference contaminants rose as spiked contaminant concentrations increased. Atrazine showed the highest pore-water concentrations relative to sediment burden, followed by zinc and phenanthrene (Table 5.2). The losses from the sediment over the test were low (less than 10%) in the zinc-spiked sediments and very high (up to 90% in chronic tests) in the atrazine-spiked sediments. Phenanthrene showed moderate losses over the test period of 4.8–65%.

5.3.3 Acute 96 h tests

Acute sediment toxicity of the three reference contaminants is shown in Figures 5.1 to 5.3 and the calculated LC_x and EC_x values, and corresponding 95% confidence limits, are given in Table 5.3. The average of the initial and final measured sediment concentration was used to generate the contamination concentrations incorporated into the calculation of the EC_x and LC_x values. Copepods in acute tests were assessed for inhibition of mobility (the sublethal endpoint, described by the EC_x data). Individuals displaying inhibited mobility were often incapacitated by the pollutant and would spasm and convulse after the stimulus. This effect was most prominent in the phenanthrene-exposed individuals. For zinc the sediment LC_{50} (95% confidence interval) value was 196 (184 – 209) µg/g sed. dry wt., with an EC_{50} for inhibited mobility of 137 (131 -142) µg/g sed. dry wt. (Figure 5.1, Table 5.3). Atrazine had an LC_{50} of 12.7 (15.1 – 47.0) µg/g sed. dry wt., and an EC_{50} for inhibited mobility of 5.35 (4.24 – 6.72) µg/g sed. dry wt. (Figure 5.2, Table 5.3). Sediment spiked with phenanthrene had a plateau in mortality at about 40% for concentrations greater than 12.3 µg/g sed. dry wt. (Figure 5.3, Table 5.3). This corresponded with a saturation in the pore-water concentration (see Table 5.2). This combination of mortality plateau and solubility

limit of phenanthrene (see Chapter 3) meant that even if higher concentrations of phenanthrene were used it is unlikely that 100% mortality would have been achieved. Consequently no phenanthrene treatment generated a sufficiently high enough mortality rate to facilitate the calculation of LC_x values (ASTM 2007). The EC_{50} for inhibited mobility was still able to be calculated, yielding a value of 2.56 (2.15 – 3.18) µg/g sed. dry wt. The inhibited mobility showed a typical dose response with effects seen at low concentrations (2.12 µg/g) and then complete population immobility at 12.3 µg/g and above.

Pore-water LC_{50} and EC_{50} values were calculated (Table 5.4) to provide insight into exposure pathways by comparison to the aquatic LC_x values calculated in Chapter 3. For zinc the pore-water LC_{50} was 2.05 (1.66 -2.47) mg/l, with an EC_{50} for lethargy of 0.85 (0.77-0.94) mg/l. For atrazine the calculated LC_{50} was 9.11 (8.44 – 9.84) mg/l and the EC_{50} was 4.10 (3.6 – 4.61) mg/l for pore-water exposure. Phenanthrene had a LC_{50} of 0.42 (0.29 – 0.81) mg/l and an EC_{50} of 0.046 (0.036 – 0.064) mg/l for pore-water exposure.

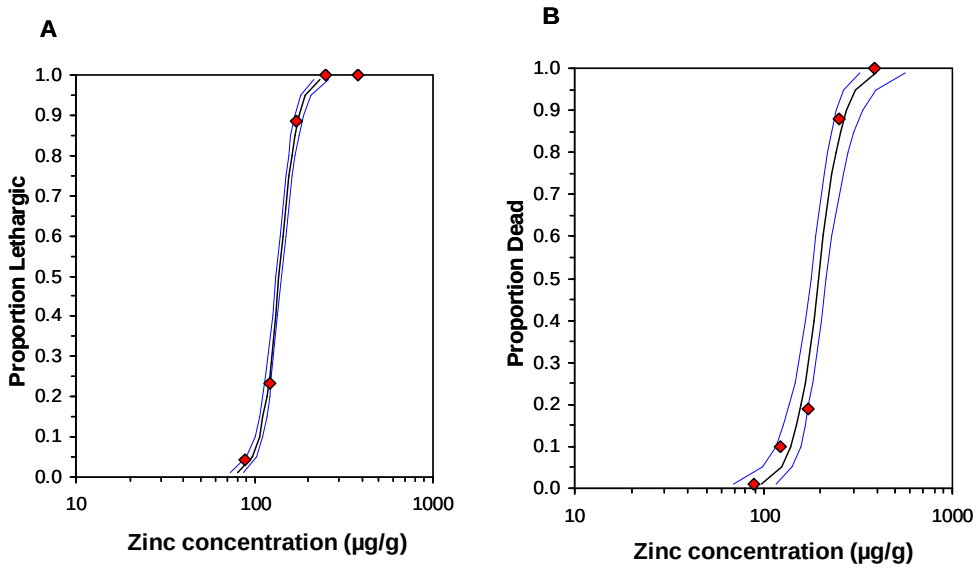

Figure 5.1 Dose-response curves (modelled response ± 95% confidence interval) for 96 h sediment exposures to zinc **A.** Lethargy EC_{50} **B.** LC_{50}. All values are given in µg/g dry wt. Average responses (n=3) for each concentration are also given.

Table 5.3 Comparison of test endpoints calculated for the acute and chronic sediment bioassays. Bracketed values represent 95% confidence intervals, or *P* values (for LOEC analyses). All values are given in µg/g dry wt. A (-) signifies an incalculable endpoint.

Test endpoint	Zinc µg/g	Atrazine µg/g	Phenanthrene µg/g
Acute bioassay			
LC_{10}	140 (116 – 157)	0.29 (0.057 – 0.54)	-
LC_{20}	159 (138 – 175)	1.27 (0.51 – 2.45)	-
LC_{50}	196 (184 – 209)	25.7 (15.1 – 47.0)	-
Inhibited mobility EC_{10}	106 (100 – 111)	0.0074 (0.0039 – 0.013)	1.20 (0.74 – 1.52)
Inhibited mobility EC_{20}	117 (112 – 121)	0.084 (0.054 – 0.13)	1.59 (1.15 – 1.91)
Inhibited mobility EC_{50}	137 (131 – 142)	5.35 (4.24 – 6.72)	2.56 (2.15 – 3.18)
Chronic bioassay			
Total offspring LOEC	36.8 (*P*<0.0001)	0.003 (*P*<0.0001)	0.037 (*P*<0.0001)
Total offspring EC_{10}	29.3 (21.9 – 35.2)	0.00022 (9.3E-5 – 0.00046)	0.0035 (0.0006 – 0.0088)
Total offspring EC_{20}	36.9 (29.6 – 42.7)	0.0020 (0.0011 – 0.0033)	0.010 (0.003 – 0.022)
Total offspring EC_{50}	54.5 (47.7 – 61.5)	0.083 (0.061 – 0.11)	0.067 (0.034 – 0.13)
Potential offspring LOEC*	55.2 (*P*=0.0033)	-	0.26 (*P*=0.0015)
*Potential offspring** EC_{10}	28.8 (15.1 – 39.6)	0.0049 (0.0021 – 0.009)	0.0048 (0.001 – 0.012)
*Potential offspring** EC_{20}	38.1 (23.3 – 46.4)	0.018 (0.010 – 0.029)	0.016 (0.005 – 0.033)
*Potential offspring** EC_{50}	61.7 (47.1 – 74.8)	0.17 (0.14 – 0.21)	0.13 (0.073 – 0.24)
*Realised offspring*LOEC*	55.2 (*P*<0.0001)	0.003 (*P*<0.0105)	0.037 (*P*<0.0001)
*Realised offspring** EC_{10}	22.4 (14.9 – 28.5)	0.00096 (0.00038 – 0.0020)	0.0056 (0.0012 – 0.013)
*Realised offspring** EC_{20}	30.8 (22.9 – 37.2)	0.0055 (0.0028 – 0.0092)	0.014 (0.0046 – 0.027)
*Realised offspring** EC_{50}	53.0 (45.1 – 62.1)	0.11 (0.080 – 0.14)	0.066 (0.035 – 0.12)
LC_{10}	97.7 (95.0 – 100)	-	-
LC_{20}	108 (105 – 110)	-	
LC_{50}	128 (126 – 130)	-	- -

*per female

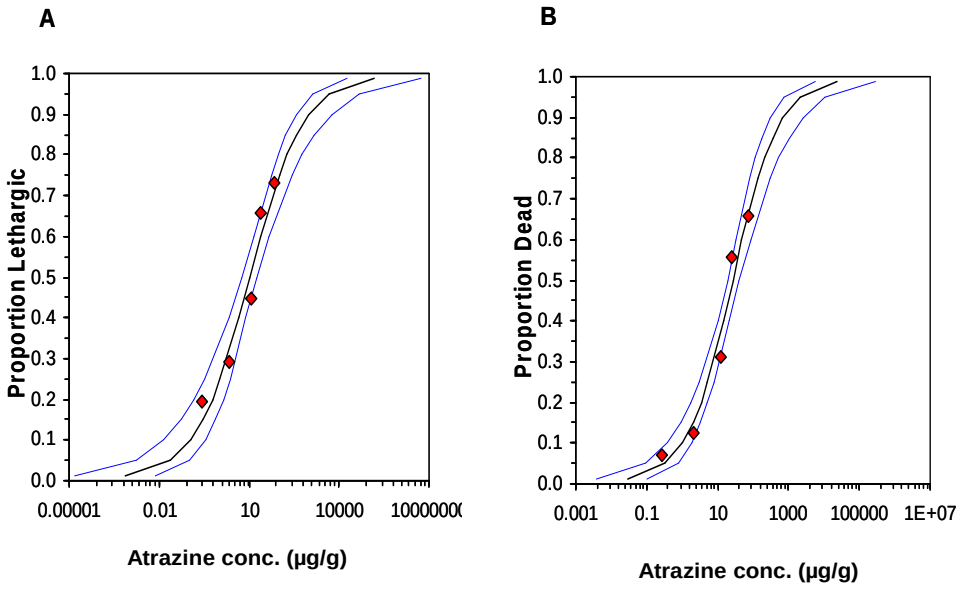

Figure 5.2 Dose-response curves (modelled response ± 95% confidence interval) for 96 h sediment exposures to atrazine **A.** Lethargy EC_{50} **B.** LC_{50}. All values are given in µg/g dry wt. Average responses (n=3) for each concentration are also given.

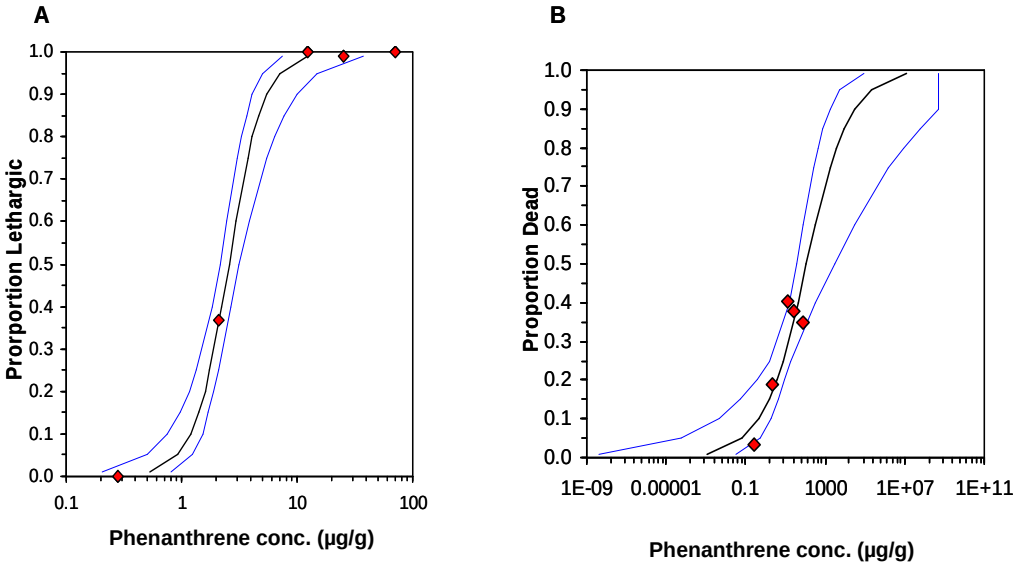

Figure 5.3 Dose-response curves (modelled response ± 95% confidence interval) for 96 h sediment exposures to phenanthrene **A.** Lethargy EC_{50} **B.** LC_{50} (data showed to illustrate plateau of mortality above 12.3 µg/g). All values are given in µg/g dry wt. Average responses (n=3) for each concentration are also given.

Table 5.4 Comparison of pore-water test endpoints calculated for the acute and chronic bioassays. Bracketed values represent 95% confidence intervals, or *P* values (for LOEC analyses). All values are given in mg/l. A (-) signifies an incalculable endpoint.

Test endpoint	Zinc mg/l	Atrazine mg/l	Phenanthrene mg/l
Acute bioassay			
LC_{10}	0.89 (0.59 – 1.17)	0.87 (0.73 – 1.02)	0.019 (0.007 – 0.032)
LC_{20}	1.26 (0.93 – 1.59)	2.08 (1.84 – 2.31)	0.059 (0.035 – 0.083)
LC_{50}	2.05 (1.66 – 2.74)	9.11 (8.44 – 9.84)	0.42 (0.29 – 0.81)
Inhibited mobility EC_{10}	0.45 (0.43 – 0.47)	0.14 (0.099 – 0.19)	0.02 (0.010 – 0.034)
Inhibited mobility EC_{20}	0.57 (0.55 – 0.59)	0.48 (0.38 – 0.60)	0.032 (0.018 – 0.040)
Inhibited mobility EC_{50}	0.85 (0.77 – 0.94)	4.10 (3.6 – 4.61)	0.046 (0.036 – 0.064)
Chronic bioassay			
Total offspring LOEC	0.05 (*P*<0.0001)	0.041 (*P*<0.0001)	-
Total offspring EC_{10}	0.037 (0.026 – 0.047)	0.0038 (0.0023 – 0.0056)	-
Total offspring EC_{20}	0.054 (0.042 – 0.066)	0.013 (0.0091 – 0.017)	-
Total offspring EC_{50}	0.11 (0.091 – 0.13)	0.10 (0.092 – 0.12)	-
LC_{10}	0.30 (0.24 – 0.36)	-	-
LC_{20}	0.39 (0.33 – 0.45)	-	-
LC_{50}	0.62 (0.54 – 0.69)	-	-

5.3.4 Chronic14 d tests

Survival of copepods exposed to zinc, atrazine and phenanthrene over the 14 d chronic test is illustrated in Figure 5.4. Chronic toxicity to the measured sublethal endpoints of total offspring, potential offspring per female and realised offspring per female for the three reference contaminants are shown in Figures 5.5-5.7). The calculated EC_x and LC_x values derived from the chronic assays are reported in Table 5.3. Mortality was minimal over the tests, only occurring at the highest tested concentrations of 162, 0.77, and 1.85 µg/g sed. dry wt. for zinc, atrazine, and phenanthrene respectively (Figure 5.4). Zinc significantly reduced the total number of offspring at concentrations of 36 µg/g sed. dry wt. and higher (*P*<0.0001) and reduced potential and realised offspring per female at concentrations over 55 µg/g sed. dry wt. (*P*=0.0033, *P*<0.0001 respectively; Figure 5.5). Atrazine caused a significant reduction in total offspring at sediment levels of 0.003 µg/g sed. dry wt. and above (*P*<0.0001, Figure 5.6). There was also a significant reduction in realised offspring per female at concentrations of 0.003

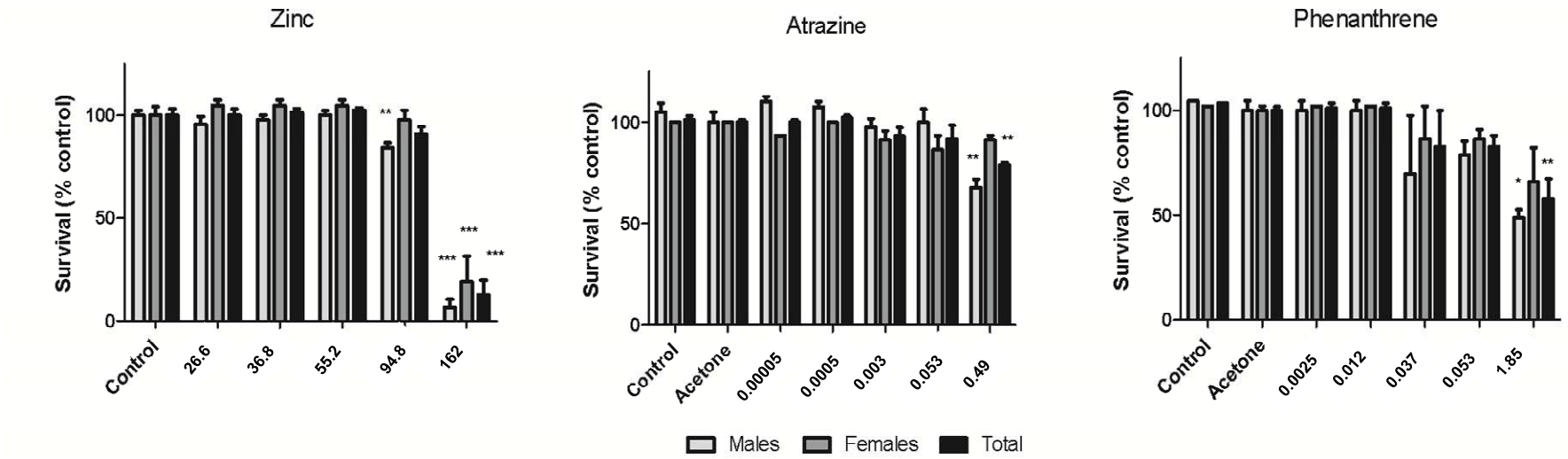

Figure 5.4 Survival of adult *Quinquelaophonte* sp. following 14 d chronic exposures to sediment spiked with **A.** zinc (µg/g sed.), **B.** atrazine (µg/g sed.) and **C.** phenanthrene (µg/g sed.). One-way ANOVA with a Dunnett's post hoc test was used to determine significant differences from controls (n=3). All toxicant values are given in µg/g dry wt.* $P>0.05$, ** $P>0.01$, *** $P>0.001$. 'Acetone' refers to the carrier controls.

µg/g sed. dry wt. and above (P=0.0150), and although there was a trend towards reduced potential offspring per female, this effect was not statistically significant due to high inter-replicate variability. Phenanthrene caused a significant reduction in total offspring at sediment levels of 0.037 µg/g sed. dry wt. and above (P<0.0001, Figure 5.7), with significant reduction in potential and realised offspring at 0.26 µg/g sed. dry wt. (P=0.0015, P<0.0001 respectively).

The calculated EC_{20} values for total offspring were 36.9 (29.6 – 42.7), 0.0020 (0.0011 – 0.0033), and 0.010 (0.003 – 0.022) µg/g sed. dry wt. for zinc, atrazine, and phenanthrene (Table 5.5, 5.6, 5.7). The LC_{50} values for the chronic sediment exposures were only able to be calculated in the zinc-spiked sediment, due to the low mortality in the atrazine and phenanthrene exposures. Zinc had a chronic LC_{50} of 128 (126 – 130) µg/g sed. dry wt.

Pore-water chronic endpoints for the three reference contaminants are given in Table 5.4. Zinc had an EC_{50} for total offspring of 0.11 mg/l and a LC_{50} of 0.62 mg/l. Atrazine had an EC_{50} for total offspring of 0.1 mg/l. LC_{50} values were unable to be calculated for atrazine due to insufficient mortality, and phenanthrene was not able have chronic pore-water endpoints analysed due to levels being below detection limits.

Of the five endpoints calculated for the three reference contaminants total offspring was the most sensitive, followed by realised offspring per female, potential offspring per female, EC_{50} of lethargy, and finally the LC_{50} value. Potential and realised offspring were the most variable endpoints.

5.3.5 Acute to chronic ratio

The different ACR metrics are listed in Table 5.5 and produced values that ranged from 1.53 to 7.37 for zinc and 1,660 to 117,000 for atrazine. Due to the inability to calculate an acute LC_{50} for phenanthrene, only the EC_{50}/EC_{10} ACR (731) was able to be determined for phenanthrene.

Zinc

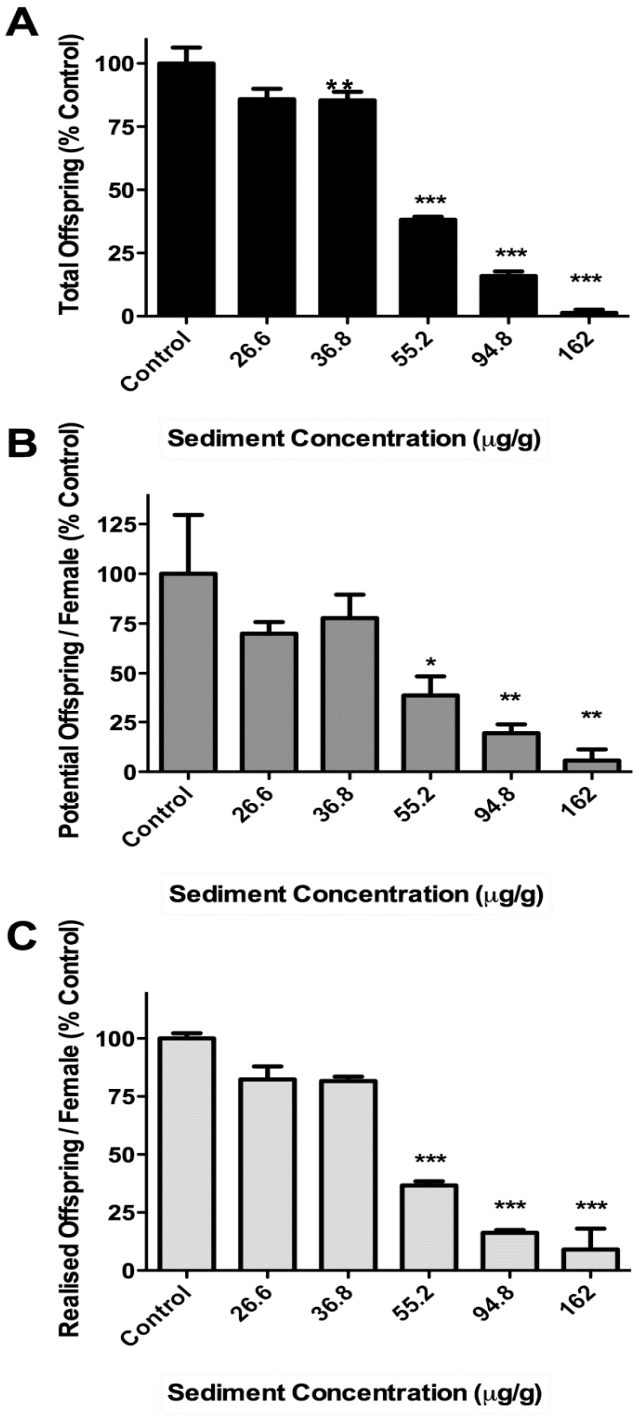

Figure 5.5 Reproductive endpoints of 14 d chronic exposures to zinc. **A.** Total offspring, **B.** Potential offspring per female, and **C.** Realised offspring are calculated as percent of control offspring. One-way ANOVA with a Dunnett's post hoc test was used to determine significant differences from controls (n=3). All values are given in µg/g dry wt. * $P>0.05$, ** $P>0.01$, *** $P>0.001$. 'Acetone' refers to the carrier controls.

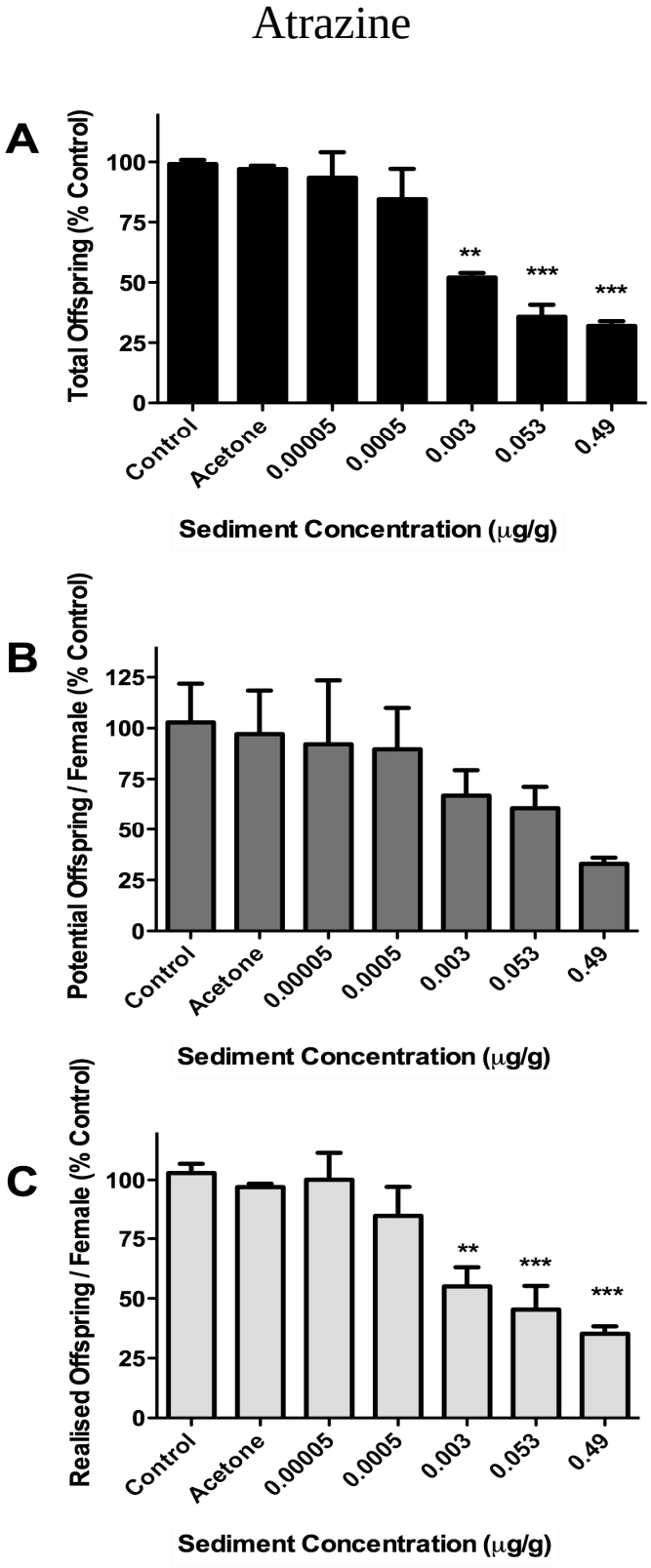

Figure 5.6 Reproductive endpoints of 14 d chronic exposures to atrazine. **A.** Total offspring, **B.** Potential offspring per female, and **C.** Realised offspring are calculated as percent of control offspring. One-way ANOVA with a Dunnett's post hoc test was used to determine significant differences from controls (n=3). All values are given in µg/g dry wt. * *P*>0.05, ** *P*>0.01, ****P*>0.001. 'Acetone' refers to the carrier controls.

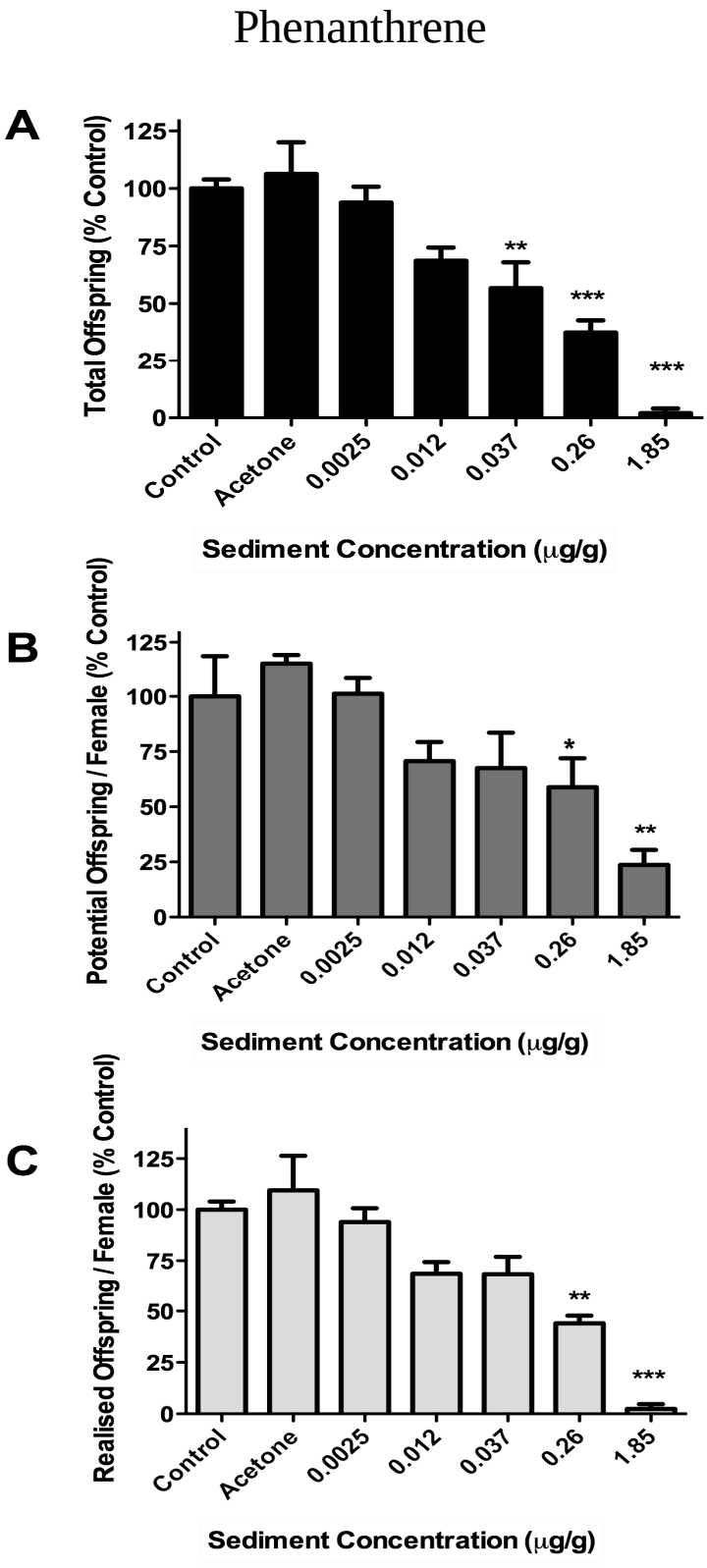

Figure 5.7 Reproductive endpoints of 14 d chronic exposures to phenanthrene **A.** Total offspring, **B.** Potential offspring per female, and **C.** Realised offspring are calculated as percent of control offspring. One-way ANOVA with a Dunnett's post hoc test was used to determine significant differences from controls (n=3). All values are given in µg/g dry wt. * $P>0.05$, ** $P>0.01$, *** $P>0.001$. 'Acetone' refers to the carrier controls.

Table 5.5 Acute to chronic ratios (ACRs) resulting from the most sensitive chronic endpoint, total juveniles produced. ACRs were calculated by dividing the 96 h LC_{50} or EC_{50} by a variety of calculated parameters from the chronic tests.

	LC_{50}/NOEC	LC_{50}/MATC	LC_{50}/EC_{10}	EC_{50}/EC_{10}	LC_{50}/EC_{20}	LC_{50}/LC_{50}
Zinc	7.37	6.20	6.69	4.68	5.31	1.53
Atrazine	25700	1660	117000	23400	12800	-
Phenanthrene*	-	-	-	731	-	-

* Due to the inability to calculate LC_{50} values for phenanthrene, only the EC_{50} was able to be used.

5.4 Discussion

The aim of this chapter was to validate bioassays for New Zealand's sandy estuarine sediments. These tests account for the bioavailability of environmental contaminants, and permit assessment of contaminant mixture toxicity. This provides more accurate information regarding environmental contamination pressures than traditional monitoring approaches using trigger values. Consequently, the ability to test the toxicity of field-collected sediments is vital to the protection of estuarine species. Furthermore, the combination of acute and chronic bioassays provides insight into how the different reference contaminants (and their varying modes of action) affect the toxic response thresholds of *Quinquelaophonte* sp. Information regarding how the mode of action interacts with bioassay response, in combination with chemical analysis through toxic identification evaluations (TIE), facilitates identification of the causes of toxicity.

5.4.1 Zinc toxicity

Zinc is a common toxicant in estuarine settings, owing largely to its high concentration in storm water runoff (Lotze et al. 2006). In this study zinc was found to have an acute LC_{50} of 196 µg/g and an EC_{50} for inhibited mobility of 137 µg/g. This characterises *Quinquelaophonte* sp. as more acutely sensitive to sediment Zn than several other common bioassay species. For example, the harpacticoid copepod *Amphiascus tenuiremis* exhibits an LC_{50} of 671 µg/g zinc (Hagopian-Schlekat et al. 2001), and the amphipods *Melita plumulosa* (King et al. 2006) and *Hyalella azteca* (Borgmann and Norwood 1997) display LC_{50} values of 1790 and

3530 µg/g zinc respectively (Table 5.6). Only one species has exhibited higher sensitivity to zinc than *Quinquelaophonte* and that was the amphipod *Corophium volutator* with an LC_{50} of 32 µg/g (Bat and Raffaelli 1998). Differences in metabolism, uptake, elimination (Lotufo et al. 2000, McClellan-Green et al. 2007), and exposure pathway (i.e. dietary, pore-water, or sediment contact; Simpson and King 2005) of the different species may impact sensitivity.

When the acute sediment pore-water and aquatic LC_{50} (from Chapter 3) values are compared for zinc, the pore-water LC_{50} is higher (2.1 mg/l) compared to the aquatic exposure value (0.9 mg/l). In sandy sediments it is believed that pore-water is the predominant exposure route for copepods (Strom et al. 2011), and therefore the sediment-exposure LC_{50} based on pore-water concentrations would be similar to that of the aquatic only exposures. One explanation for the difference in the toxicity is dissolved organic matter. Metals can bind to dissolved organic matter, which can reduce their bioavailability (Santore et al. 2002, Kramer et al. 2004, Di Toro et al. 2005). Sediment pore-waters have higher levels of dissolved organic matter compared to the overlying waters (Weston et al. 2006), thus making the bioavailable fraction of zinc much lower, raising the effect levels. In addition, as the aquatic tests were sediment-free and these copepods are sediment-dwelling organisms, the lack of sediment may have caused additional stress to the individuals, increasing sensitivity to exposed contaminants.

Another explanation for the differences in toxic effects level is the behaviour of the copepods. It is likely that copepods in sediments minimise their exposure to pore-water by staying near the sediment/water interface, where dilution of pore-water by the overlying water would occur, and/or by moving out of the sediment altogether (McMurtry 1984, Lotufo 1997).

The mode of action and acute toxicity for zinc has been discussed previously in Chapter 3. The main effects of zinc toxicity include ion imbalance (Santore et al. 2002), and interference with exoskeleton maintenance and moulting in crustaceans (Poynton et al. 2007). The inhibition of reproductive output in the chronic bioassays was found at very low concentrations, and may have resulted through several distinct mechanisms. There is some evidence that zinc can

Table 5.6 Comparison of acute and chronic bioassay responses of *Quinquelaophonte* sp. to zinc, phenanthrene, and atrazine with selected other estuarine species. LC_{50} and EC_{50} values presented from present chapter to allow for the easiest comparison between studies. All values are based on sediment contaminant concentrations given in µg/g dry wt.

Group	Species	Test type	Zinc µg/g	Atrazine µg/g	Phenanthrene µg/g	Reference
Copepod (Harpaticoid)	*Schizopera knabeni*	96 h LC_{50}			440	Fleeger 2007
		10 d Reduced reproduction			22	Lotofu and Fleeger 1997
		48 h decreased feeding rate			109	Silva et al. 2009
	Amphiascus tenuiremis	96 h LC_{50}	671			Hagopian-Schlekat et al. 2001
	***Quinquelaophonte* sp.**	**96 h LC_{50}**	**196**	**25.7**	**-**	**Present study**
		96 h EC_{50} – lethargy	**137**	**5.35**	**2.56**	**Present study**
		14 d EC_{50} - total offspring	**54.5**	**0.083**	**0.067**	**Present study**
		14 d LC_{50}	**128**	**-**	**-**	**Present study**
Amphipod	*Melita plumulosa*	10 d LC_{50}	1790			King et al. 2006
	Corophium volutator	48 h LC_{50}		>250		Hellou et al. 2008
		10 d LC_{50}	32			Bat and Raffaelli 1998
	Hyalella azteca	10 d LC_{25}	3530			Borgmann and Norwood 1997
Midge	*Chironomus tentans*	10 d LC_{50}		> 9		Douglas et al. 1993
Bivalve	*Mercenaria mercenaria*	10d LC_{50}		>20		Lawton et al. 2006

accumulate in reproductive tissues (De Schamphelaere et al. 2004), and disrupt vitellogenesis (Hook and Fisher 2002) which would result in direct effects on reproduction. Additionally, reproductive effects can be generated by more indirect causes including inhibition of feeding and digestion (De Coen and Janssen 1998), or interference with moulting through chitinase inhibition. Moult inhibition has been correlated with reduced reproduction in *Daphnia* (Poynton et al. 2007). A further possibility is that the effect on reproduction represents the high mortality of early life stages, as it is known that these are more sensitive than adult copepods (Brown et al. 2005).

In sediments the meiofauna can be exposed to metals through a number of different routes including diet, pore-water, or sediment (contact and/or ingestion)

(Green et al. 1993, Hook and Fisher 2001a, Wiklund and Sundelin 2002, Strom et al. 2011). In sediments it is most likely that there is a combination of all three exposure pathways for each organism (King et al. 2005). The relative importance of these pathways will vary with the different sediment partitioning coefficients (sediment properties) of metals between water and sediments, and the foraging behaviour of the exposed individuals (Simpson and King 2005). In this chapter it is hypothesised that in the chronic tests dietary exposure may play more of a role in toxicity as individuals are fed during the tests, whereas in the acute tests dietary exposure is not expected as individuals were not fed. Dietary exposure can occur through the biosorption of zinc by microalgae (Klimmek et al. 2001), which are then ingested by the copepods and cause a toxic effect (De Schamphelaere et al. 2008). Dietary exposure of metals causes reduced reproduction in copepods and in *Daphnia* (Hook and Fisher 2001a, De Schamphelaere et al. 2004, De Schamphelaere et al. 2008). When considering organisms *in situ* the heterogeneity of sediments will create variation between exposure pathways even over very small scales (Forbes 1999, Simpson and Batley 2003).

The concentrations at which toxic effects of zinc were noted are of concern as they are within the levels of contamination found in New Zealand estuaries. For example, zinc has been found at concentrations up to 435 µg/g in Porirua Harbour (Glasby et al. 1990), a level that is ten times higher than the EC_{10} for reduced offspring in the chronic bioassay and over twice that of the acute LC_{50} of 196 µg/g. Furthermore these toxic levels of zinc are below the ANZECC (2000) ISQV-Low of 210 µg/g. This is of concern as there is potential for many metropolitan areas in New Zealand to have adverse effects due to metal pollution (see zinc levels in Table 1.1). At contamination levels like these there is a potential for diminished copepod populations, which in turn could alter the structure of estuarine communities including effects on juvenile fish that rely on copepods as a food source (Hicks and Coull 1983, LeBlanc 2007).

It needs to be noted that sediment type determines pollutant bioavailability (Simpson et al. 2011, Strom et al. 2011). As the sediment organic carbon proportion increases, the sediment will bind higher levels of metals (Mahony et al. 1996,

Chapman et al. 1998, Machado et al. 2008). This means that the pore-water concentration of metals will be lower in sediments with higher organic content than those of a sediment with lower organic content (Simpson et al. 2004, Di Toro et al. 2005). This study used very fine sandy sediments with a low organic content of 1%, which can result in higher pore-water concentrations of zinc.

Hagopian-Schlekat et al. (2001) reported a zinc sediment 96 h LC_{50} of 671 µg/g with a corresponding pore-water LC_{50} of 0.37 mg/l in a bioassay with the copepod *A. tenuiremis* in sediments with 2.8% organic content. This compares to sediment and pore-water LC_{50} values of 196 µg/g and 2.0 mg/l, respectively, in this chapter for *Quinquelaophonte* sp. The higher sediment LC_{50} and low pore-water LC_{50} are good examples of the effect of organic carbon on toxicity results. If the LC_{50} value of 671 µg/g reported by Hagopian-Schlekat et al. (2001) is normalised to 1% organic carbon as suggested by Simpson and colleagues (2011), the resulting LC_{50} is 239 µg/g, which is similar to the 196 µg/g LC_{50} found in this study. This shows the necessity to account for organic carbon when interpreting these results, as the shift of metals from sediment to pore-water changes the predominant exposure route (Simpson 2005, Simpson and King 2005).

In this chapter the sediments were allowed to equilibrate for 14 d at 20°C to prevent abnormally high pore-water concentrations (Lee et al. 2004, Simpson et al. 2004, Simpson et al. 2005). However, due to the nature of the sediment, this did not preclude high pore-water concentrations of zinc. Thus pore-water may contribute more to toxic effects than sediment concentrations.

5.4.2 Atrazine toxicity

The toxic effects of atrazine have been controversial, mainly due to its connection to the global decline in frogs (Hayes et al. 2002, Hayes et al. 2003). Atrazine has been banned in the European Union due to its persistence in drinking water. It is, however, still heavily used in the US in corn and sugarcane agriculture and to a significant, but lesser, extent in Australia and New Zealand (Ackerman 2007). Atrazine is not very acutely toxic; however, it does have significant chronic

effects and is considered to be an endocrine disrupting compound in vertebrates. The mechanism of endocrine disruption is thought to occur via a mechanism that involves reduction in testosterone levels and an increase in oestrogen production, by promoting the conversion of testosterone into oestrogen (Hayes et al. 2002).

Quinquelaophonte sp. showed a large effect range in response to atrazine exposure, with an acute LC_{50} of 25.7 µg/g, inhibited mobility EC_{50} of 5.35 µg/g and an EC_{50} for total offspring of 0.083 µg/g. Similarly, in another copepod species, *Amphiascus tenuiremis*, inhibition of reproduction was observed at concentrations where there was no effect on development or survival (Bejarano and Chandler 2003). However, it is unknown through what mechanism atrazine is affecting reproductive function in invertebrates. It is possible that the effect is indirect, acting via impairment of behaviour and feeding. This has been seen in mussels, where atrazine was shown to cause starvation (Tuffnail et al. 2009). Effects similar to this could be causing the reduced reproduction in *Quinquelaophonte* sp. observed in this chapter. Further research into the effects of atrazine on invertebrate physiology is needed.

Atrazine showed high pore-water concentrations due to significant desorption from sediments. This strong dissociation from sediments into pore- and overlying water has been noted previously. Other studies have shown that up to 40% of sediment-bound atrazine can diffuse into the water column (Mersie et al. 1998, Mersie et al. 2000), resulting in high pore-water concentrations. In the chronic exposures between 75 and 90% of atrazine was displaced from sediments and resulted in pore-water concentrations as high as 0.4 mg/l. As with other organic contaminants, organic carbon also plays a major role in governing atrazine bioavailability and pore-water concentrations (Smalling and Aelion 2004, 2006). The high pore-water concentrations suggest that this is the main exposure route for atrazine in sediment exposures.

Inhibition of reproduction can have serious consequences for populations, especially at low, environmentally-realistic concentrations. A study by Bejarano et al. (2005) showed that at water concentrations at the USEPA seawater criterion for atrazine (26 µg/l), significant effects on mesocosm assemblages could be seen.

These could eventually translate to changes in overall community structure. Atrazine had effects at very low concentrations in the current chapter (0.003 µg/g sediment and 0.04 mg/l for pore-water concentrations), which creates concern that the adverse impacts of atrazine may be seen in wild populations. Atrazine has been found at concentrations as high as 49 µg/g in soils in the US (Douglas et al. 1993). In New Zealand there is little information on levels of atrazine in rivers or estuaries however it has been found in groundwater around the country (Close and Rosen 2001), with levels as high as 37 µg/l recorded (Close 1993). Adverse effects are unlikely to occur in New Zealand (owing to limited use of the pesticide in this country compared to other countries), or the EU (where atrazine is banned), but if similar sensitivities are exhibited by species where atrazine is extensively used (e.g. the US) then significant effects may be observed. Certainly this seems to be the case for some North American species (Bejarano et al. 2005).

There is limited data on sediment exposures of atrazine to marine invertebrates. This is a large knowledge gap, particularly in countries that still use atrazine extensively, and in nations where atrazine and its metabolites are persistent in the environment (Rice et al. 2004). There is also a lack of knowledge regarding the potential effects of sediment-bound atrazine (Douglas et al. 1993, Lawton et al. 2006). However, given the results of this study, sediment-bound atrazine desorbs very quickly, which means that levels of sediment-bound atrazine are likely to be low in estuaries. In contrast to the relatively low probability of toxic impact associated with sediment-bound atrazine, the reproductive effects seen at low pore-water concentrations (EC_{10} total offspring 0.0038 mg/l) are likely to be of greater concern.

5.4.3 Phenanthrene toxicity

Similar to the pattern observed for atrazine, phenanthrene showed a large difference between levels at which effects on reproduction and acute toxicity were noted. The predominant mode of action of phenanthrene is through non-polar narcosis (Ren 2002). This was evident in the present study by the inhibited mobility. Non-polar narcosis is a general mode of action shared by phenanthrene

and many other organic compounds including chlorobenzenes, alcohols, esters, ethers, and chlorinated alkanes (Roex et al. 2000). The lethargic effect was very pronounced in the acute test with an EC_{50} of 2.56 µg/g recorded. While these individuals are not dead, their incapacitation and failure to respond to stimuli results in them being 'environmentally dead'. In other words, due to their inability to move they are likely to be preyed upon, and if they avoid this fate, they are unlikely to recover (Brooks et al. 2009, Trekels et al. 2011). An acute LC_{50} was unable to be calculated due to the plateau in mortality at concentrations above 12.3 µg/g. This can be explained by saturation of the pore water at around 0.15 – 0.3 mg/l. Further increases in sediment phenanthrene led to no further increases in pore-water levels, corresponding with no increase in toxicity. This suggests that pore-water contaminants are the predominant exposure route for *Quinquelaophonte* sp., a finding that is consistent with literature on hydrophobic compounds (Di Toro et al. 1991, USEPA 1993b).

The EC_{10} of total offspring at 0.0035 µg/g is three orders of magnitude lower than the acute EC_{50}. This suggests that phenanthrene may have a second mode of action, other than non-polar narcosis, acting specifically on reproductive tissues. Non-polar narcotic compounds often have low ACRs (see section 5.4.4 for more on ACRs) due to their non-specific action, whereas chemicals with more defined modes of action (i.e. pesticides and metals) have higher ACRs (Roex et al. 2000, Paumen et al. 2008, Marinkovic et al. 2011). This suggests that phenanthrene may have a direct effect of reproductive function, i.e. a mode of action other than general narcosis inhibiting reproduction. Lotufo (1998a, b) suggested that sediment exposure to fluoranthene (a low molecular weight PAH, similar to phenanthrene) caused reduced reproduction in the copepods *Schizopera knabeni* and *Coullana* sp. by a mode of action other than general narcosis. Lotufo attributed this to the accumulation of the PAH in the lipid-rich tissues, especially eggs, which in turn inhibited hatching success and development of juveniles. Partitioning into the lipid-rich tissues occurs due to the hydrophobicity of the PAH (Van Wezel and Opperhuizen 1995). The passage of contaminant to the egg potentially assists with the detoxification of phenanthrene in females by reducing their body burdens (Lotufo 1998b). However, it could affect population growth as it results in reduced

offspring. Based on the large ACR, and the results from Lotufo (1998a, b), it is highly likely that phenanthrene inhibits reproduction through a direct effect.

The levels at which phenanthrene causes impairment of reproductive endpoints (EC_{50} of total offspring 0.067 µg/g) are lower than several SQVs, including the Effects Range-Median concentration (ERM) of 1.5 µg/g by Long et al. (1998), the Probable Effects Level (PEL) of 0.5 µg/g by MacDonald et al. (1996), and the ANZECC (2000) ISQG-low of 0.24 µg/g for sediment normalised to 1% organic carbon. This further emphasises that a trigger value is not a suitable replacement for a bioassay, as previously discussed (sections 1.4 and 5.1). This is especially relevant with PAHs as they are always found in mixtures (Latimer and Zheng 2003), and can have additive toxicity which is not predicted by trigger values (Landrum et al. 2003). Bioassays are able to assess the negative effects of the bioavailable fraction and the potential effects of mixtures.

Environmental concentrations of total PAH have been recorded over 10,000 µg/g (Huntley et al. 1995) and up to 3.7 µg/g in New Zealand (Holland et al. 1993), and are cause for concern worldwide (Kennish 1992). While this local level of phenanthrene is around that found to cause mortality (around 2 µg/g in the acute and chronic exposures); PAHs are most commonly found at levels where the potential for chronic reproductive effects is significantly more likely than acute toxicity (Maher and Aislabie 1992, Soclo et al. 2000, Mai et al. 2002, Readman et al. 2002). Potentially offsetting toxicological effects is the possibility of behavioural responses that might modify the exposure of organisms to contaminants. Studies have shown that copepods exhibit avoidance behaviour in response to PAH-contaminated sediments, and actively leave these sediments in search of less contaminated ones (Carman and Todaro 1996, Lotufo 1997). While this may be beneficial in terms of limiting the exposure to the toxicant, it can have additional negative implications for copepod populations. For example, copepods in the water column face the risk of increased predation by fish (Lotufo 1997, Brooks et al. 2009).

5.4.4 Acute to chronic ratios (ACRs)

Acute to chronic ratios (ACRs) were introduced in environmental risk assessment as a mechanism for predicting chronic toxicity from acute data. The impetus for this was due to the relatively large database of acute effects compared to the paucity of data on chronic exposures (Marinkovic et al. 2011). This is mainly owing to the difficulty in testing organisms over a chronic time-frame, especially in vertebrates that have long life cycles (Niederlehner et al. 1998, Raimondo et al. 2007). ACRs have been calculated for a multitude of species and toxicants, and have been used to develop the US ambient water-quality criteria and to predict NOEC's (Mount et al. 2003). The ACR can be calculated a number of different ways (Table 5.5), and this has resulted in an ACR that can vary significantly, even for the same toxicant. In the current study, for example, the ACR for zinc ranged from 1.53 to 7.37 depending on the metric used. Due to statistical issues with using LOEC/NOEC (and therefore MATC) it is strongly recommended that the LC_{50}/EC_{10} be used when calculating ACRs, primarily because the EC_{10} is a more accurate statistical method and is less likely to be affected by experimental design than LOEC/NOEC data (Harrass 1996). The LC_{50}/EC_{10} ACR is the metric from the current study used in the rest of this chapter.

There have been several studies that have linked ACRs to mode of action. Lange et al. (1998) showed that chemicals with specific modes of action have very large ACRs (Roex et al. 2000); whereas non-specific narcotic compounds have been shown to have low ACRs (Kenaga 1982). Non-specific narcotic compounds also show the lowest variation between species (Roex et al. 2000). Interestingly, phenanthrene, a non-specific narcotic compound which showed a prominent narcotic effect in the acute exposures, had a high ACR of 731. This ACR is in contrast to previous studies that examined phenanthrene and similar non-specific narcotic compounds. For example, Marinkovic et al. (2011) found an ACR of 1.5 for phenanthrene in the midge *Chironomus riparius*, while Roex et al. (2000) showed that ACRs in the non-polar narcotic class ranged from 1.2 – 6.7 in aquatic organisms. Other studies have looked at non-polar narcotic compounds as a group across different aquatic species and have found average ACRs of: 1.8 (Niederlehner

et al. 1998), 1.9 (Calamari et al. 1983), 2.6 (Roex et al. 2000), and 2.4 (Cowgill and Milazzo 1991). There have been a few studies that have measured higher ACRs with non-polar narcotic compounds, with Kuhn et al. (1989) reporting an average ACR of 41 in *D. magna*. The ACR found in this chapter is more similar to compounds that have a specific mode of action, suggest that phenanthrene may have a mode of action other than non-specific narcosis in copepods. As discussed previously (section 5.4.3), there is a precedence for this from previous studies (Lotufo 1997). Raimondo et al. (2007) showed a high variability in the ACR's of non-polar narcotic compounds, and suggested that the higher ACR are an indication of different modes of action in acute and chronic tests. Thus the findings of both these studies help to support the potential for two distinct modes of action for phenanthrene toxicity to *Quinquelaophonte* sp. in the acute and chronic tests.

Atrazine showed the highest ACR in this study of 117,000. Atrazine has been shown to be an endocrine disrupting compound (Hays 2002, 2003) with a specific mode of action. Studies have seen high ACRs in compounds with specific modes of action (Roex et al. 2000), and this is consistent with findings of this chapter. In studies examining acute and chronic toxicity of atrazine in amphipods, an ACR of 1.2 for *Hyalella azteca* and >12.5 for *Diporeia* sp., (96 h LC_{50} unable to be calculated) were discerned when comparing 96 h LC_{50} to 21 d LC_{50} (Ralston-Hooper et al. 2009). In the current study the acute LC_{50}/chronic LC_{50} was not able to be calculated due to insufficient mortality in the chronic tests; however, the other ACR metrics that were able to be calculated for atrazine are over 1,000 times greater than those for the amphipods *Hyalella azteca* and *Diporeia* sp. The reason for the large differences in ACR are unknown, however, body size and species-specific metabolism may be a factor as previously discussed (section 3.4.5).

In this study zinc had the lowest ACR relative to the other two reference contaminants. ACRs reported for zinc are quite variable, ranging from 1 for the trout, *Oncorhynchus mykiss* (Mebane et al. 2008), 2.9 for the cladoceran *Moina macrocopa* (Wong 1992, 1993) to 19.5 for a mysid shrimp (Gentile et al. 1982). ACRs for copper include 3.5 and 3.6 in midge *C. riparius* (Roman et al. 2007, Marinkovic et al. 2011), and 1.3 in the copepod *Eurytemora affinis* (Hall et al. 1997). In studies that have compared multiple metals and species, average ACRs of

15 (for aquatic invertebrates; Roex et al. 2000), and 9 (for a study with both aquatic invertebrates and vertebrates; Raimondo et al. 2007), have been reported. The results for *Quinquelaophonte* sp. are similar to these other studies with an ACR of 6.69.

In several studies examining a variety of different contaminants and species the average ACR appears to be around 5-10 (Roex et al. 2000, Ahlers et al. 2006, Raimondo et al. 2007). However, there is large variability in ACRs, with ACRs reported from 1 to 18,550 (Mebane et al. 2008; Raimondo et al. 2007). Differences in ACR between contaminants and tested animals have been attributed to variation in the toxicokinetics (the uptake and elimination of a contaminant of an organism) and the mode of action (Bonnomet et al. 2002, Duboudin et al. 2004). In addition, there are differences between studies in terms of the specific ACR metric used (e.g. NOEC, MATC, or EC_{10},) and the endpoints of chronic toxicity (reproduction, development, or emergence). This study only examined three reference contaminants, but also showed a wide variability of ACRs in *Quinquelaophonte* sp., with values ranging from 6.69 to 117,000.

ACRs provide useful information for environmental risk assessment, as well as characterising *Quinquelaophonte* sp. responses as a bioassay species, by helping to understand the relationships between acute and chronic toxicity. The relationships between contaminant mode of action and ACR will help to estimate chronic thresholds for SQG and in toxicity identification evaluation (TIE). However, as recommended by others, caution should be used when generalising ACRs and using them in environmental risk assessment as it is easy to under- or over-estimate potential chronic toxicity (Roex et al. 2000, Raimondo et al. 2007, Mebane et al. 2008).

5.4.5 Validation of bioassays

This study validated acute and chronic sediment bioassays by examining contaminant effects including mobility, reduced reproduction, and mortality. The acute 96 h sediment test was found to be more sensitive than many other bioassays

for the three reference contaminants, including 10 d amphipod survival tests (Table 5.6). The sublethal endpoint of inhibited mobility proved to be a very sensitive and effective means of measuring toxicity, and may have utility as an alternative to mortality. This endpoint is of ecological importance as individuals that are stunned or hindered by contaminants will be unable to escape predation and are essentially 'environmentally dead' (Brooks et al. 2009, Trekels et al. 2011). The inclusion of a sublethal EC_{50} in this acute bioassay not only increased the sensitivity of the test but also provided information on the mode of action and the physical effects of the contaminants. Phenanthrene acted through non-polar narcosis, and the lethargy endpoint was able to effectively elucidate this mode of action. Kenaga (1982) found that the majority of industrial organic compounds act through non-polar narcosis suggesting that lethargy can be used to detect a range of organic compounds. While this endpoint will not be able to provide definitive information on the contaminant mode of action it can help in TIE evaluations in field samples (Grote et al. 2005, Nendza and van Wezel 2006). Acute tests are not always suitable to characterise toxicity, however the more environmentally relevant endpoints that are used in acute tests the better they are at identifying toxic effects (Greenstein et al. 2008).

The chronic 14 d partial lifecycle test was a very sensitive test, especially with respect to the organic compounds tested. The total number of offspring produced was the most sensitive of the endpoints with the number of potential offspring per female the most variable. This could be due to variability in the production of a second clutch by females; however this is just from personal observations and has not been tested. However, in *Daphnia*, differences in time between extruding of clutches has been noted as an effect of exposure to toxicants (USEPA 2002). This can potentially cause the second clutch not to be produced in the test timeframe, introducing variability and a decrease in statistical sensitivity. The chronic test is a partial lifecycle assessment, only resulting in the production of naupliar stage juveniles and not copepodite life stages. This limitation has been shown not to affect the sensitivity of the bioassay in a study by Kovatch et al. (1999) which compared two harpacticoid bioassay species, *Amphiascus tenuiremis* and *Microarthridion littorale,* and two bioassay lengths, 14 and 21 days. Similar findings have been reported in other copepod species (Lotufo 1998a, b) supporting the utilisation of a partial life cycle test.

This chronic bioassay starts with adults not juveniles. This may reduce the mortality during the test, as juveniles are more sensitive than adult copepods (Brown et al. 2005). As the chronic assay requires individuals to mate during the test, the number of mating individuals will have an effect on the total juvenile production. Mating behaviour cannot be specifically measured in this bioassay, but it is possible, by analogy to other chronic bioassays systems (Blockwell et al. 1998), that toxicants could influence reproductive endpoints by an effect on this parameter. Consequently, this may enhance the sensitive of the assay by reflecting both behavioural and reproductive responses of toxicants on reproduction. The extent that any behavioural toxic effects will affect mating success will depend on the mode of action of the pollutant (Blockwell et al. 1998, Gray et al. 1999).

Another advantage of this chronic test is that it is a flow-through test. Such tests better mimic *in situ* exposures as overlying waters are continuously exchanged, preventing abnormally high pore-water concentrations in the sediment. However, this does result in decreasing sediment and pore-water concentrations through the test. In the current study up to 58% of phenanthrene was lost during the assay, although some of the loss may have been due to degradation.

Chemical analysis and trigger values can over- or under-predict toxic effects as they do not take into account bioavailability of contaminants in the sediments (Strom et al. 2011). Bioassays allow for biological effects to be assessed as a function of the total bioavailable contaminant fraction, which can vary greatly between sediment types (Simpson et al. 2011). The development of these two bioassays allows for flexibility in the application of a monitoring scheme. The acute test can be utilised as a rapid high-throughput assessment of potentially heavy levels of pollution. The chronic bioassay is much more sensitive and therefore would be better utilised in situations where the maximum environmental protection is required (i.e. near marine reserves or particularly sensitive environments). It requires a larger time and resource commitment, thus further suggesting that it has the greatest utility for particularly sensitive coastal regions. Simpson and Spadaro (2011) estimated that the costs of running their chronic tests with the copepod *N. spinipes* and the amphipod *M. plumulosa* were 1.5 times greater than an acute test.

For the current bioassays it is estimated that the cost was around two times greater for a chronic test with *Quinquelaophonte* sp. relative to the acute assay. This difference is due to the larger number of individuals and sediment volume used, adding additional time in enumerating juveniles.

5.5 Conclusions

This chapter has validated two sediment bioassays with multiple lethal and sublethal endpoints that can be applied in a wide range of sediment monitoring and risk assessment frameworks. Under the ANZECC (2000) guidelines local species are to be used wherever possible, and this is an element that has been lacking in New Zealand assessment frameworks. The harpacticoid copepod *Quinquelaophonte* sp. is a valuable local bioassay species and the incorporation of bioassays based on this species into environmental monitoring may provide the best local protection, and would subsequently reduce the reliance on trigger values derived from overseas species. This study showed that sublethal endpoints were valuable in detecting toxic effects at environmentally-relevant levels of contaminants. Analysis of acute and chronic toxicity helped elucidate the mode of action of phenanthrene through the use of ACRs, and the effects different modes of action had on the bioassay endpoints. Future research should focus on further developing complementary local bioassay species to be able to create a suite of bioassays to best assess contamination across taxa (Greenstein et al. 2008). This allows for multiple lines of evidence to assess pollution and permits causes of toxicity to be identified through patterns in the bioassay responses (USEPA 1993a, Burton et al. 2002a, Chapman and Anderson 2005).

6.

Toxicity assessment of sediment from Christchurch, Napier and Invercargill (New Zealand) estuaries using a harpacticoid copepod chronic bioassay

6.1 Introduction

Sediments vary in their capacity to bind and sequester contaminants (see Chapters 4 and 5). The binding properties of sediment control the bioavailability of contaminants, and consequently also mediate their toxicity. A whole sediment bioassay is advantageous over other mechanisms of toxicity assessment in that it provides an indication of the overall toxicity of a sediment, and can account for the presence of multiple stressors.

In the previous chapter the responses of single toxicant exposures were examined in the estuarine copepod bioassay. In the “real world" contaminants are rarely present as single toxicants, and instead occur in complex mixtures of multiple toxicants, which may vary in concentration and bioavailability. There can be large spatial variation in these mixtures depending on the surrounding land use. Sediments sourced from areas that are intensively used for industry, shipping, and urban land uses are likely to have different contaminant loads. In harbours typical contaminants include tributyltin (TBT), heavy metals, ammonia, phenols and oils (Young 1980), whereas typical farming (agricultural) contaminants include: fertiliser residues, insecticides, herbicides, animal manure, and farmyard wastes. Urban environments can have a large variety of contaminants that include polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), PAHs, and heavy metals (Kennish 2002).

Pollutant mixtures can have effects that are distinct from those of single contaminant exposures. One such effect of pollutant mixtures is additive toxicity. This is where the individual toxicities of specific contaminants add to each other generating greater toxicity than a single toxicant alone. This can result in mixtures of individual contaminants at concentrations that are each below their effect threshold (NOEC) causing toxic effects (Walter et al. 2002). This can occur through joint action (contaminants acting on the same pathway) or independent action (contaminants acting on different pathways) (SCHER 2011). Mixtures can also be more toxic than the predicted additive toxicity of the individual contaminants, a response known as synergistic toxicity (Forget et al. 1999).

Traditional monitoring using sediment quality guidelines and trigger values does not take mixtures of contaminants into account. Current regulations are based on single contaminant concentrations using threshold values created by mathematical models to predict effect levels of potential adverse contaminants on organisms. These values, such as the ISQG values in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC 2000), are limited in assessing potential risks to biota. These limitations are well recognised, even by the guidelines themselves (e.g. section 8.3.5.18 in ANZECC 2000). A key limitation is scenarios where additive or synergistic toxicity occurs, via contaminants that are present in the monitored environments below their trigger values (Kelly 2007). In addition to this problem in assessing additive and synergistic toxicity, SQVs do not take the bioavailability of contaminants into account. Bioavailability is a factor that can vary greatly between sediment types (Bryan and Langston 1992, Strom et al. 2011). For example, sediments with high organic matter bind significantly more metals than those with low organic contents, resulting in different bioavailability and thus different potentials for toxicity (Strom et al. 2011).

Trigger values and chemical monitoring do not account for the mode of organism interaction with a potential toxicant (and its surrounding medium). This is a factor that clearly affects its susceptibility to contaminants. Pathways of exposure such as ingestion, inhalation (gills, lungs) and surface absorption (through skin) regulate which toxicants the organism is exposed to, and the levels at which exposure occurs.

Relative to modelling approaches, bioassays are a more powerful tool for identifying and monitoring pollution effects (Davoren et al. 2005). Bioassays determine toxicity via direct experimentation, rather than by predicting an outcome, as for modelling approaches. Because sediment-bioassay species are in contact with the sediment and pore-water they are exposed to the bioavailable fraction of the contaminants in a situation not too unrealistic from a “natural” one (Chandler and Green 2001, Bejarano et al. 2004). The results from bioassays allow for sediments to be classified based on toxic effect, not contaminant load (Chapman et al. 1998,

Chandler and Green 2001, Davoren et al. 2005). A toxic result from a bioassay will highlight areas that require further investigation and potential regulatory action, while a non-toxic outcome suggests that no actions are likely to be required to identify a source of contamination or to remediate the sediment (Chapman et al. 1998). It is important to note that the bioassay being presented in this thesis is not a "one stop shop" for sediment quality assessment. For a more robust assessment, a suite of bioassay species should be used which have different life histories and exposure pathways (Greenstein et al. 2008, Simpson and Spadaro 2011). Once a potentially polluted area has been identified as a toxic risk then regulatory action can be taken to remediate the situation.

This chapter utilises the chronic 14 d reproductive bioassay developed with *Quinquelaophonte* sp. in the previous chapter (Chapter 5) on field-collected sediments. Sediments were sourced from three estuaries in different geographic regions in New Zealand: Napier on the east coast of the North Island, Christchurch on the east coast of the South Island, and finally Invercargill in the far south of the South Island. The focus of this chapter is to determine whether the bioassay can assess the toxicity of real sediment samples from a variety of sediment types. The levels of contamination were measured in sediments to be facilitate comparison to observed toxic effects. This is a mechanism of validating whether the bioassay provides an accurate portrayal of contamination.

6.2 Methods

6.2.1 Location of field sites

6.2.1.1 Avon-Heathcote (Christchurch)

Five sediment samples from three general areas were collected throughout the estuary on the 22nd October 2011 (Figure 6.1) (see 6.2.2 for method). Two samples were taken at Penguin Street, one at 1 m (PS 1m) from a waste water discharge point, and another sample taken 50 m (PS 50m) from this discharge. One sample was taken at Ferrymead (F) near the high tide mark. The final area was the causeway between Ferrymead and Redcliffs (near McCormack's Bay Reserve), and

consisted of two samples taken either side of the causeway (north (estuary) side (CW N/S) and south side (CW S/S)).

6.2.1.2 Invercargill

Sediment samples were collected from six different locations around the Invercargill Estuary in June 2011 (Figure 6.2) (see 6.2.2 for method). The sites included Omaui (OM), Oue (OU), Waipaka Stream (W), Kingswell (KW), Invercargill Dump (ID), and Steed Street Bridge (SB). Four sites (OM, OU, W, SB) had very sandy sediments with little silt. The remaining two sample locations (ID and KW) had very fine silty muds. As there were two very distinct sediment types,

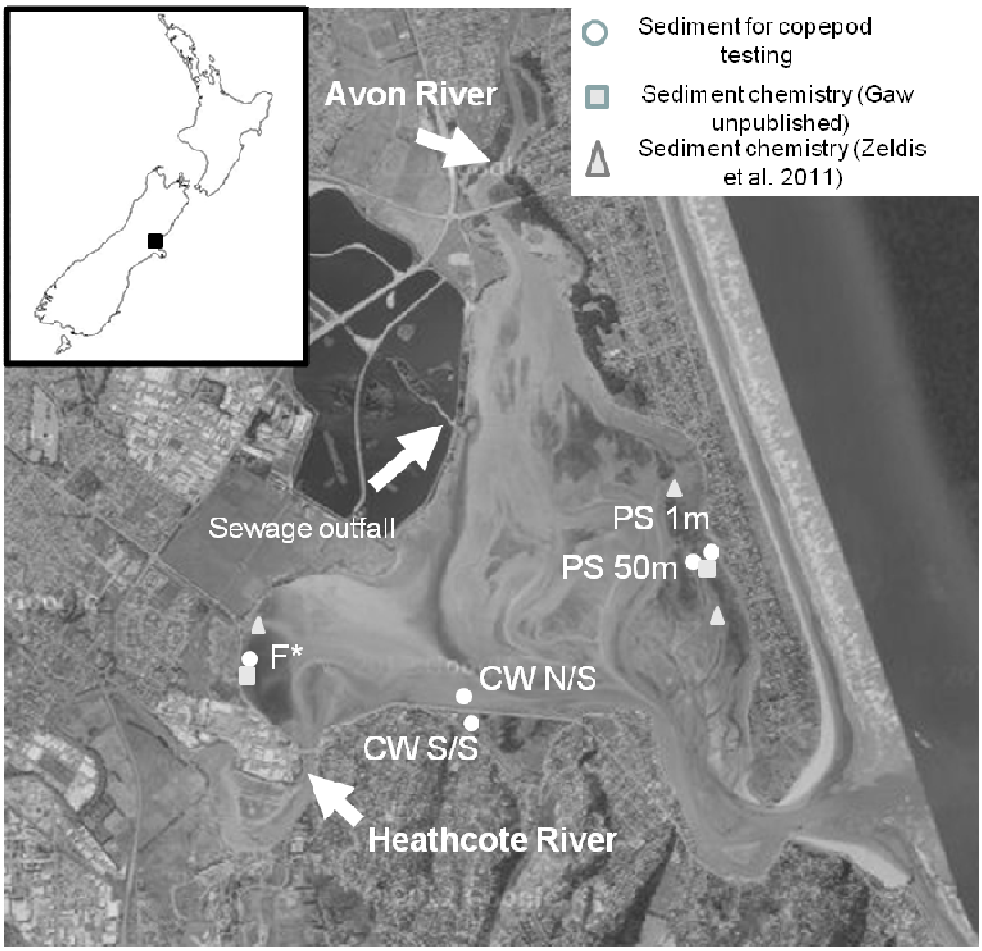

Figure 6.1 Sample locations for the 5 Avon/Heathcote Estuary sediment samples are given and include: Penguin Street 1 m (PS 1m) and 50 m (PS 50m), Causeway north side (CW N/S) and south side (CW S/S). Locations with corresponding sediment chemistry are also given.

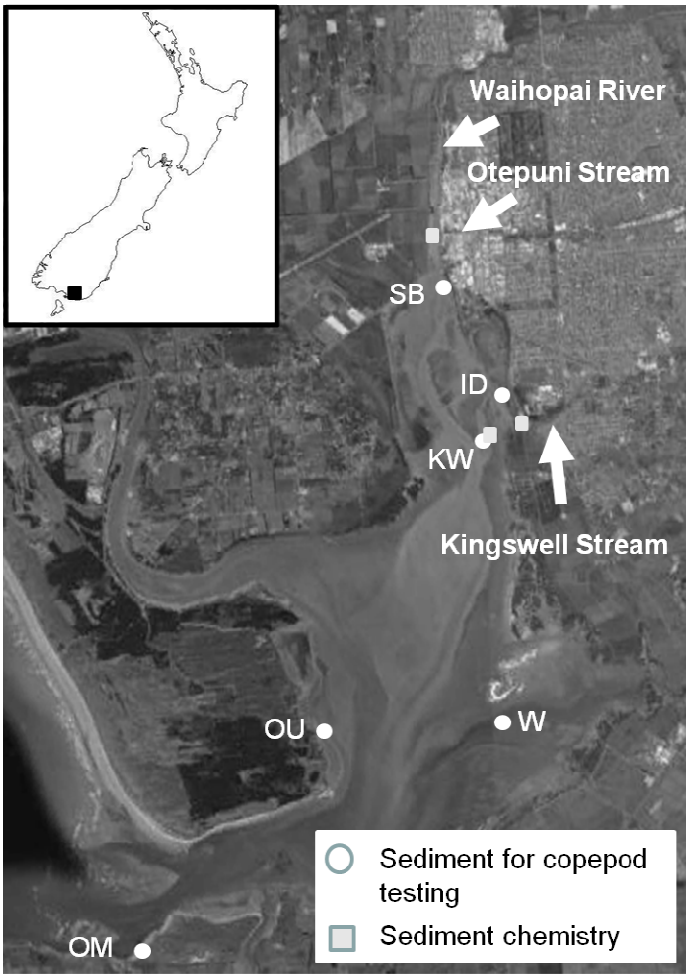

Figure 6.2 Sample locations for the 6 Invercargill Estuary sediment samples are given and include: Omaui (OM), Oue (OU), Waipaka Stream (W), Kingswell (KW), Invercargill Dump (ID), and Steed Street Bridge (SB). Locations with corresponding sediment chemistry are also given.

two control sediments were used: a <63 µm sediment high in silt and a <125 µm fine sand sediment (see 6.2.2 below).

6.2.1.3 Napier

The Napier samples were focussed on two main storm water inputs draining into the Napier Estuary (see 6.2.2 for method). Samples were collected on November 8th, 2011. Two samples were collected around the Plantation, County and George Drive Drain (GPC), one upstream (GPC U/S) and one downstream (GPC D/S). At the Tyne St Drain site samples were also taken upstream (TYN U/S) and downstream (TYN D/S). Another two samples (AHU and HUM) were taken

from the tidally-exposed sand bars between the two drains (Figure 6.3). Sediments were generally a fine sand with high organic component.

6.2.2 Sediment sampling and processing

The methods for the bioassays have been already described in detail in Chapter 5. Briefly, samples were collected from the field by hand. This was done by scraping off the top 1-2 cm of oxidised sediments at each sample location. Approximately 500 g of sediment was collected at each sample site. Samples were then kept on ice and transported to the lab. Sediments were kept at 4°C for at least 24 h and up to two weeks prior to testing. Refrigerating sediments has been previously shown to kill all resident meiofauna (Kovatch et al. 1999). Sediments were homogenised and press-sieved through 125 µm or 300 µm sieves depending on sediment size, prior to being added to each test chamber. The coarser sediments:

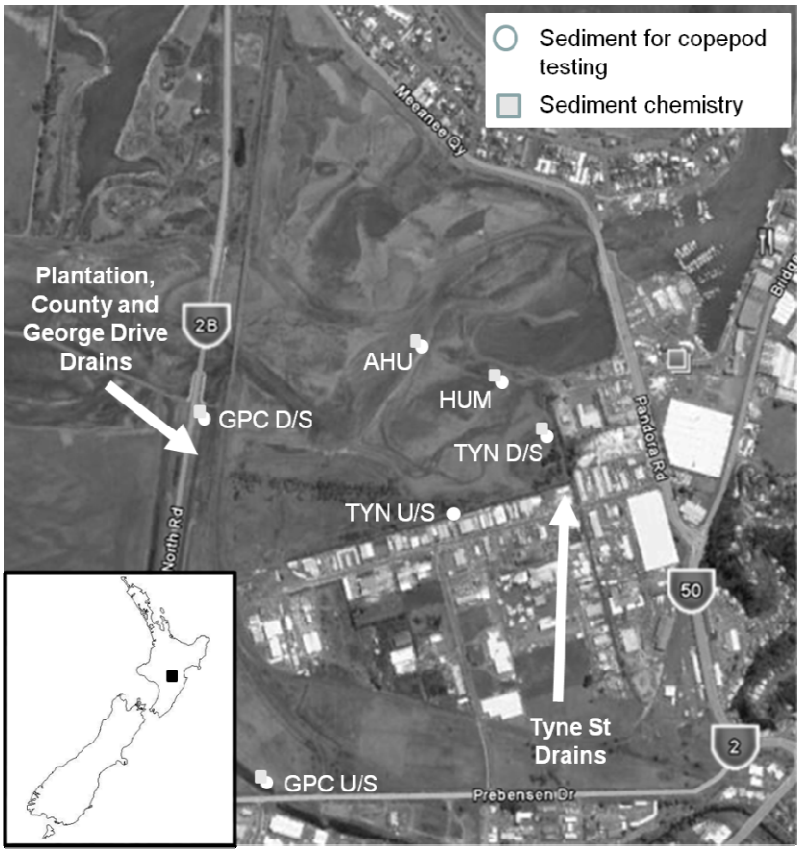

Figure 6.3 Sample locations for the 6 Napier Estuary sediment samples are given and include: County and George Drive Drain upstream (GPC U/S) and downstream (GPC D/S), Tyne Street drain upstream (TYN U/S) and downstream (TYN D/S) as well as the estuarine locations HUM and AHU. Locations with corresponding sediment chemistry are also given.

OM, W, SB, OU, CW N/S, PS 1m, and PS 50m were sieved through a 300 µm mesh, whereas KW, ID, CW S/S, GPC U/S, GPC D/S, TYN U/S, TYN D/S, AHU, HUM were sieved through a 125 µm mesh. For each sediment sample three replicates, sub-sampled from collected and sieved sediment, were included in each test. Control sediments were sourced from Akaroa Harbour (described in Chapter 4) and Blueskin Bay (45.74S, 170.58E, processed according to methods in Chapter 4). This latter sediment was a high silt (<63 µm) sediment and was used in the Invercargill sediment bioassay to matrix match two of the sample sites (KW and ID) that were very silty. A minimum of 80% survival in control sediments was set as the requirement for a valid test (ASTM 1992).

6.2.3 Chemical analysis of sediments

The trace metals for the Christchurch samples were analysed according to the modified USEPA 200.8 protocol described in Chapter 4. Samples were analysed for arsenic, cadmium, copper, lead and zinc via ICPMS (Agilent 7500 cx). QA/QC procedures included the analysis of a certified reference material (NIST #2702, inorganics in marine sediments), with recoveries ranging from 80.2 – 93.6%.

Invercargill and Napier sediments were analysed for total recoverable cadmium, copper, mercury, lead, and zinc at RJ Hill Laboratories (Hamilton), using the USEPA 200.8 protocol and ICP-MS (similar to the method used in Chapter 4). The limit of detection (µg/g) for trace levels of each metal were: cadmium, 0.02; copper, 0.2; mercury, 0.01; lead, 0.04; and zinc, 0.4. PAHs, nitro musks and polycyclic musks in sediments were analysed by first taking a subsample of field wet unsieved sediment (equivalent to 5 g dry weight) which was weighed, mixed with Celite, and packed into 22 ml stainless steel Accelerated Solvent Extraction (ASE) cells. The sediments were extracted twice for 5 min using acetone/hexane (1:1, v/v) at a temperature of 100^{o}C and pressure of 1500 psi. Acid-activated copper granules were added to the extract to remove coextracted residues of sulphur and the extract concentrated to approximately 1 ml under a stream of nitrogen gas (Turbo Vap LP Concentration Work Station Caliper life Sciences). The

concentrated solvent extracts were cleaned-up using florosil adsorption chromatography and gel permeation chromatography (GPC). A range of deuterated PAHs and musk compounds were added to the purified extracts for use as internal standards and the extracts transferred to vials for analysis. Purified extracts were analysed by GCMS using an Agilent 6890N gas chromatograph (GC) coupled to an Agilent 5975A inert XL mass spectrometer (MS) and CTC autosampler. PAHs and musk fragrances were detected using single ion monitoring of target compound specific mass ions and quantified by internal standardisation using Chemstation data analysis software.

6.2.4 Statistical analysis

Survival and total number of offspring were calculated using the methods described in Chapter 5 and normalised to percent of controls. Means and standard deviations were then calculated for each endpoint using GraphPad Prism (Version 5) and statistical significance ($P < 0.05$) was tested using one-way ANOVA with a Dunnett's post-hoc analysis to test for significant differences from controls. Control sediment was that which best matched the test sediment type. For example, the fine high silt sediments of ID and KW were normalised to the <63 µm sediment control.

6.3 Results

6.3.1 Avon-Heathcote

The Ferrymead sample location was unable to be tested as the sediment turned anoxic after 4 d at 4°C. Three of the remaining four sample locations were found to be toxic, reducing survival or reproduction (Figure 6.4). The south side of the causeway (CW S/S) was the only location that demonstrated no significant impacts on survival or reproduction. The north side (CW N/S) of the causeway showed an approximate 20% reduction in survival and total number of juveniles, however only the effect on survival was statistically significant. The Penguin Street sampling locations were significantly toxic (i.e. negatively affected survival and fecundity). The PS 1 m sample location reduced survivorship and reproduction by

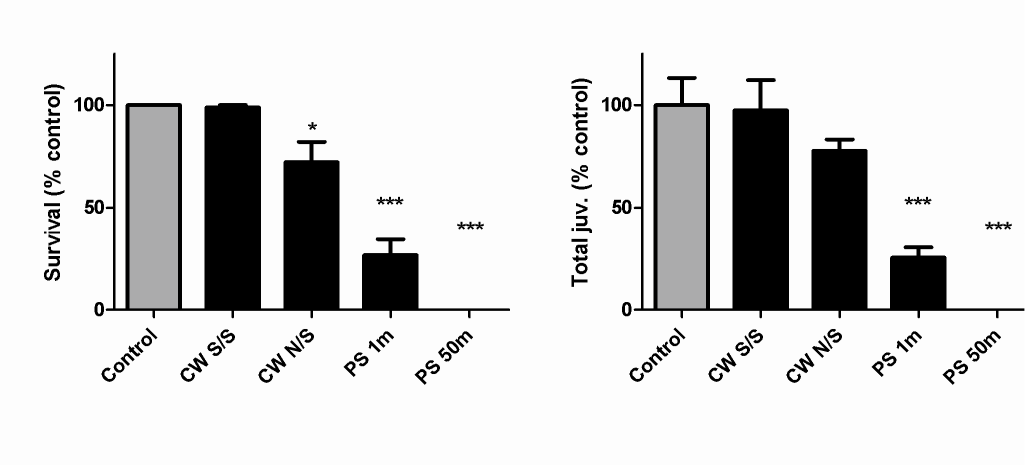

Figure 6.4 Results of 14 d chronic reproductive test with *Quinquelaophonte* sp. after exposure to Avon-Heathcote sediments **A.** survival and **B.** total juveniles produced during the test, given as percent of controls. Causeway South side (CW S/S), Causeway North side (CW N/S), Penguin Street 1m (PS 1m) and Penguin Street 50m (PS 50m). One-way ANOVA with a Dunnett's post-hoc analysis was used to test for significant differences from controls (n=3).
** P>0.05, ***P>0.001*

approximately 70% and the PS 50 m sample caused complete mortality. Both of these samples started to shows signs of anoxia by the end of the test even though the sediment was very sandy with little silt. Control survivorship was greater than 90%.

Metal sediment concentrations are given in Table 6.1 for the F and PS 1 m locations, as well as the results from the similar sample locations from Zeldis et al. (2011). All metal levels were below the ANZECC (2000) ISQV low trigger values. The two studies showed similar levels of metals in the overlapping sample areas. The F site and the Humphrey Drive location had the highest levels of metals while the sites on the East side of the estuary, Penguin Street, Herron Street and Plover Street, had relatively low values in both studies.

Table 6.1 Heavy metal levels in the Avon-Heathcote estuary for two of the sites sampled. Metal residues from similar sites from Zeldis et al. (2011) are also given as a reference. All concentrations are given in µg/g.

			Zeldis et al. (2011)		
	Ferrymead (F)	**Penguin St. (PS 1m)**	**Humphrey Dr.#**	**Heron St.***	**Plover St.^**
Arsenic	4.24	1.73	6.4	2.2	3.3
Cadmium	0.098	<0.04	0.14	0.024	0.044
Copper	11.8	3.46	13.0	3.0	4.4
Lead	17.6	7.62	20.5	6.5	9.3
Zinc	85.8	33.9	94.3	31.0	40.0

Similar location to Ferrymead (reference only)
* North of Penguin St. (reference only)
^South of Penguin St. (reference only)

6.3.2 Invercargill

The sediments in Invercargill were not found to be toxic except at the Invercargill dump (ID) site (Figure 6.5). This site had extreme growth of a red slime algae or bacteria which appeared to have suffocated the sediments causing complete mortality of the copepods. In both the <125 µm and <63 µm control sediments survival was greater than 80%. Sediment contaminant burdens are given for the KW location and Otepuni Stream, a location close to the SB sample site (Table 6.2). The KW location demonstrated low levels of contamination with total PAHs 84.3 µg/g, and all metals below ANZECC (2000) ISQV low values. The Otepuni Stream site which is upstream of the SB site showed zinc at the ANZECC (2000) trigger value of 200 µg/g.

6.3.3 Napier

The *Quinquelaphonte* sp. chronic bioassays with Napier sediments were run in an identical manner to those for the Invercargill and the Avon-Heathcote samples. Unfortunately, however, the reproductive endpoint was unable to be quantified. This was due to inadequate refrigeration during the storage of samples resulting in the resident copepod meiofauna persisting in the sample. As this was a reproductively viable population, and distinguishing resident nauplii from assay-species nauplii was not possible, total juvenile production was not able to be assessed.

There was no reduction in survival in any assays of Napier sediment samples relative to control sediments (Figure 6.6). All sediments tested and controls had greater than 90% survival. Sediment chemistry is shown in Table 6.2 for the GPC U/S, GPC D/S, TYN D/S, HUM, and AHU sample locations.

Table 6.2 Sediment chemical residues of PAHs, musk compounds and heavy metals from the Napier and Invercargill Estuary sites.

Sample location	NAPIER GPC U/S	GPC D/S	TYN U/S	TYN D/S	HUM	AHU	INVERCARGILL OM	W	SB*	OU	ID	KW
Chemistry data for tested sediments	*Yes*	*Yes*	*No*	*Yes*	*Yes*	*Yes*	*No*	*No*	*Otepuni data*	*No*	*No*	*Yes*
PAH concentration ug/kg (DW)												
Naphthalene	23.5	2.43		14.1	168	7.98			8.68			1.17
2-methylnaphthalene	42.0	1.93		2.94	19.0	2.87			4.86			1.09
1-methylnaphthalene	36.2	1.21		2.81	14.2	1.81			4.57			0.54
Biphenyl	17.9	1.91		3.04	8.39	2.13			3.14			2.22
2,6-dimethylnaphthalene	62.9	2.54		4.03	19.6	2.47			5.24			1.05
1,6-dimethylnaphthalene	36.4	1.51		3.39	16.4	2.26			5.68			0.82
2,3-dimethylnaphthalene	7.50	0.61		1.40	5.67	0.71			1.85			0.3
1,5-dimethylnaphthalene	5.85	N.D.		N.D.	6.67	N.D.			1.86			0.61
Acenaphthylene	19.3	1.59		3.28	20.3	N.D.			8.88			0.53
1,2-dimethylnaphthalene	4.99	0.73		1.44	5.76	N.D.			2.1			0.33
Acenaphthene	3.00	1.28		7.23	14.7	N.D.			4.14			0.48
4-methylbiphenyl	7.00	3.03		3.90	5.13	2.76			4.01			3.45
2,3,6-trimethylnaphthalene	6.27	0.96		2.24	8.73	1.15			3.16			N.D.
Fluorene	11.4	4.20		8.22	17.6	3.87			8.17			3.31
1-methylfluorene	9.97	2.10		3.67	9.97	1.83			6.76			N.D.
Dibenzothiophene	10.7	3.09		7.92	24.9	2.11			10.3			0.38
Phenanthrene	82.1	21.3		111	262	21.0			93.6			2.01
Anthracene	13.4	2.91		20.9	43.3	2.12			16.3			0.38
3-methylphenanthrene	18.9	4.78		18.8	52.1	4.16			15.2			0.41
2-methylphenanthrene	21.5	5.76		21.9	61.0	6.17			18.5			0.45
2-methylanthracene	6.92	1.29		11.3	24.5	1.15			7.45			N.D.
4H-cyclopenta[def]phenanthrene	19.7	3.19		23.8	57.6	3.59			18.20			0.81
9-methylphenanthrene	19.1	4.50		18.7	42.1	4.88			13.5			N.D.
1-methylphenanthrene	15.5	3.51		15.5	40.0	3.61			13.9			0.36
9-methylanthracene	N.D.	N.D.		0.27	N.D.	N.D.			N.D.			N.D.
2-phenylnaphthalene	14.9	3.35		14.1	41.1	3.52			12.50			0.42
1,7-dimethylphenanthrene	13.4	2.20		10.1	26.2	2.27			11.4			0.27

Table 6.2 Continued

Sample location	NAPIER GPC U/S	GPC D/S	TYN U/S	TYN D/S	HUM	AHU	INVERCARGILL OM	W	SB*	OU	ID	KW
PAH concentration ug/kg (DW)	**Continued...**											
Fluoranthene	200	39.4		277	623	44.3			244			5.89
Pyrene	244	40.8		246	704	49.0			242			6.3
1&3-methylfluoranthene	40.9	6.96		51.1	130	7.83			43.4			0.91
Retene	27.2	7.41		18.5	34.0	4.23			27.1			2.27
4-methylpyrene	37.4	4.89		21.0	82.4	5.17			19.7			0.71
1-methylpyrene	23.3	3.87		16.5	54.7	3.68			16.8			0.56
Benzo[ghi]fluoranthene	22.1	3.19		18.3	53.5	3.46			16.8			0.53
Benzo[c]phenanthrene	17.1	2.70		20.3	53.5	3.41			16.7			0.59
9-phenylanthracene	1.35	N.D.		0.28	2.6	N.D.			0.29			N.D.
Cyclopenta[c,d]pyrene	6.32	1.66		6.80	11.4	1.64			10.1			0.37
Benzo(a)anthracene	80.8	15.1		104	220	16.9			83.3			2.12
Chrysene & Triphenlyene	85.2	13.3		136	251	15.1			106			2.32
3-methylchrysene	47.7	8.16		37.8	95.2	5.89			32.2			0.62
6-methylchrysene	10.1	1.61		5.97	18.0	1.33			5.53			N.D.
Benzo(b)fluoranthene	116	18.3		120	254	22.1			88.1			3.06
Benzo(j&k)fluoranthene	102	17.3		111	257	22.1			102.00			3.02
Benzo(a)fluoranthene	35.1	4.88		31.2	74.0	5.30			28.4			0.78
Benzo(e)pyrene	117	15.6		86.5	252	18.8			74.5			2.62
Benzo(a)pyrene	133	17.3		125	366	21.4			118			2.79
Perylene	43.6	10.4		38.5	87.8	9.84			53			10.8
9,10-diphenylanthracene	N.D.	N.D.		N.D.	N.D.	N.D.			N.D.			N.D.
Dibenz(a,j)anthracene	41.5	6.28		26.7	83.7	6.51			26.5			1.1
Indeno(1,2,3-c,d)pyrene	201	25.0		141	328	27.3			134			4.78
Dibenz(a,h & a,c)anthracene	32.0	4.69		22.7	74.0	4.44			22			0.81
Benzo[b]chrysene	35.3	6.18		30.1	79.2	7.22			31			1.51
Picene	57.2	8.67		38.2	109	9.77			70.4			2.75

Table 6.2 Continued

Sample location	NAPIER GPC U/S	GPC D/S	TYN U/S	TYN D/S	HUM	AHU	INVERCARGILL OM	W	SB*	OU	ID	KW
Benzo(ghi)perylene	131	17.4		71.9	177	18.4			71.5			3.04
Anthanthrene	27.0	5.42		17.9	61.9	4.13			19.5			0.66
Dibenzo[b,k]fluoranthene	55.1	10.9		39.5	70.4	10.5			41.8			1.63
Coronene	24.5	5.3		14.1	41.9	4.96			15.8			0.88
Dibenzo[a,e]pyrene	15.8	3.19		11.8	31.7	3.30			11.4			0.53
Dibenzo[a,h]pyrene	12.7	N.D.		6.36	28.9	N.D.			6.68			N.D.
Total PAHs	**2554**	**408**		**2228**	**5725**	**442**			**2082**			**85.3**
Sum of 16 EPA PAHs	**1475**	**241**		**1512**	**3765**	**276**			**1347**			**41.5**
Benzo[a]pyrene Equivalents	**218**	**30.1**		**199**	**555**	**35.3**			**184**			**4.98**
Total recoverable metals µg/g (DW)												
Cadmium	0.35	0.127		0.6	0.53	0.161			0.23			0.041
Chromium	35	14.5		51	123	35			33			13.6
Copper	61	14.9		16.2	43	16.5			29			8.1
Lead	68	18.6		40	77	24			38			4.7
Mercury	0.158	0.053		0.063	0.13	0.092			0.082			0.023
Zinc	590	280		240	370	166			200			42

N.D. = not detected,

16 EPA PAHs – naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]flouranthene, benzo[*a*]pyrene, dibenz(ah)anthracene, benzo[*ghi*]perylene, and indeno(1,2,3-cd)pyrene

Highlighted cells represent values that exceed the ANZECC (2000) ISQG Low trigger values.

* Chemistry data from an upstream location has been shown to illustrate the contamination profile in the stream.

There were elevated PAH concentrations in the GPC U/S, TYN D/S, and HUM sample locations with the highest being 5725 µg/kg (HUM). Metal concentrations were also high, with sediment concentrations higher than the ANZECC (2000) ISQG low values in 4 of the locations. Sediment concentrations exceeding ISQG low values in several locations. At GPC U/S lead, mercury, and zinc (68, 0.158, and 590 µg/g respectively), at GPC D/S and TYN D/S zinc (280 and 240 µg/g respectively); and at HUM copper, lead, and zinc (43, 77, 370 µg/g respectively) were all measured at levels over trigger values.

6.4 Discussion

A total of 16 estuarine sediment samples from three distinct geographical locations were tested. The bioassay was successfully able to test a variety of

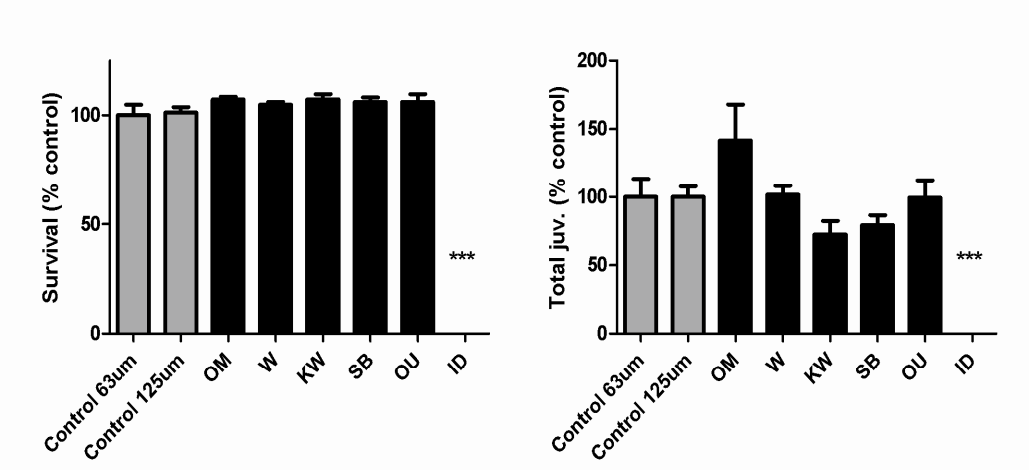

Figure 6.5 Results of 14 d chronic reproductive test with *Quinquelaophonte* sp. after exposure to Invercargill sediments **A.** survival and **B.** total reproduction (total juv.) given as percent of controls. Omaui (OM), Waipaka (W), Steed Street Bridge (SB), and Oue (OU) are percent of the <125 µm sediment control. Invercargill Dump (ID). Kings Well (KW) are percent of the <63 µm sediment control. One-way ANOVA with a Dunnett's post-hoc analysis used to test for significant differences from controls (n=3). ****P>0.001*

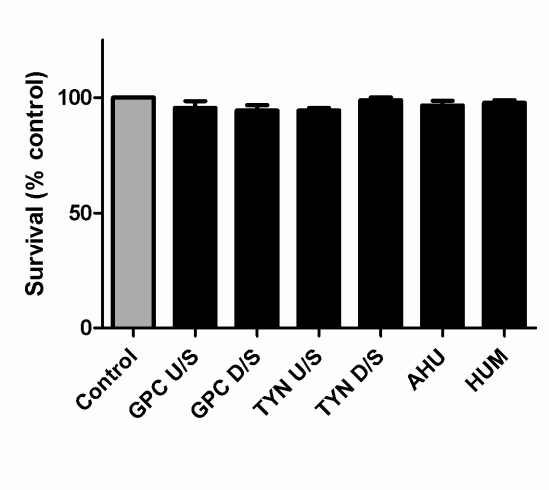

Figure 6.6 Survival of *Quinquelaophonte* sp. after 14 d chronic exposure to Napier sediments. One-way ANOVA with a Dunnett's post-hoc analysis used to test for significant differences from controls (n=3).

different sediment types and sizes, from medium sandy sediments low in organic content (particle size ~300 µm) to very silty, high organic content sediments (particle <63 µm). Of the 16 sediment samples analysed, four were found to be toxic. Three of these sediments were found in the Avon-Heathcote Estuary and one from Invercargill.

Some of the Napier locations displayed metal values above the ANZECC (2000) ISQG low values, although these values were insufficient to produce toxic effects in the bioassay. While the Napier sediments had high levels of contaminants, based on the bioassay these contaminants were not toxic. This may be related to the bioavailability of the metals in these sediments; for example, the binding of metals to organic carbon (Di Toro et al. 1991, Mahony et al. 1996, Simpson 2005, Strom et al. 2011). See sections 1.3.2 and 4.4 of this thesis for discussion of bioavailability of contaminants and organic carbon.

6.4.1 Avon – Heathcote Estuary

Historically the Avon-Heathcote Estuary, located adjacent to the city of Christchurch, has been associated with a variety of potentially toxic inputs including moderate to light industry effluents, sewage, and residential and urban runoff (ECAN 2007). This contamination profile was drastically changed by the 2011 earthquake events. The February 22nd earthquake caused major changes in the estuary structure with the north end of the estuary subsiding by 0.5m, while the south side rose by 0.5m. The earthquakes also caused a massive failure of sewage treatment throughout the city as well as broken pipes in the reticulated sewer network (Zeldis et al. 2011). This resulted in the release of raw sewage directly into the Avon-Heathcote Estuary, as well as its tributaries. In addition to sewage contamination, the liquefaction, which covered 20-40% of the estuary (Measures et al. 2011), caused buried contaminants to resurface, adding to the contaminant load in the estuary. This effect has been seen previously in Izmit Bay after the 1999 Turkish earthquake (Tolun et al. 2006, Tolun et al. 2008).

Two locations, Penguin Street and Ferrymead, were chosen as sampling sites as these locations are being used in another study on emerging contaminants and metal loads in sediments and waters (Dr. Sally Gaw, personal communication; See Fig 6.1). The other sampling site was the causeway between Ferrymead and Redcliffs and consisted of samples taken either side of the causeway. Owing to its close proximity to a main road, runoff is likely to have contributed to sediment contaminant profile with elevated metals and PAHs (Boxall and Maltby 1995). There is also the possibility of industrial waste inputs from the Heathcote Valley being an issue at this site (ECAN 2007). This location is of particular importance as there are concerns regarding contaminant transfer into the local biota (especially cockles) that are being collected and consumed by locals (ECAN 2007). On the south side of the causeway the estuary is bound by

two drainage pipes and retains water over the tidal cycle. This potentially prevents contaminants from being flushed out to sea.

Three of the four Avon-Heathcote sediments tested were found to be toxic to the bioassay species *Quinquelaophonte* sp. This may reflect the impacts of seismic activity on the estuary. For example, sewage pipes around the city were broken, and sewage treatment plants were shut down. This resulted in 50,000 m^3 of raw sewage to be dumped into the estuary per day (Zeldis et al. 2011).

Sewage contamination is probably the main contaminant source in the Avon-Heathcote and may be the cause of the anoxic conditions seen at the Penguin Street sample location. The combination of bacterial contamination and organic enrichment associated with the raw sewage input could have resulted in very high levels of eutrophication throughout the estuary, leading to the anoxic conditions. Zeldis et al. (2011) found that the organic content in the estuary had risen from 0.5 to 2.0% (depending on location) pre-earthquake to 1.7 to 3.0% following the February 22nd earthquake. They attributed this rise to new organic content from sewage, and sedimentation from slips on the river banks. They also found there was enrichment of pore-water nutrients, which adds to the eutrophication threat. This may be the cause of the anoxia and potentially the toxicity (reduced survival and reproduction) seen in the bioassays. The anoxia seen in the tests could result from the addition of sewage-derived organic matter to sediments which is then oxidised to CO_2 by microbes, resulting in a decreased dissolved oxygen and causing anoxia (Cloern and Oremland 1983). Additionally, the anoxia could be caused by rapid algal growth, due to the increased nutrients, which depletes dissolved oxygen resulting in anoxia (Valiela et al. 1997). A study by Tinson and Laybourn-Parry (1985) with cyclopoid copepods showed that exposure to anoxic conditions caused mortality in as little as 6 h. Behavioural changes and mortality were also seen in a variety of benthic

crustaceans exposed to anoxic sediments *in situ* (Haselmair et al. 2010). These effects are consistent with those observed in this bioassay.

In the Zeldis et al. (2011) report the eutrophication effects were highest in the Humphreys Drive location which is a site similar to Ferrymead in the present study. This sediment was unable to be tested due to it becoming anoxic during storage at 4°C prior to testing. This perhaps provides some anecdotal evidence, especially combined with the anoxic conditions seen in the bioassay in the PS 1 m and PS 50 m samples, that eutrophication is a serious threat to the estuary. Another study modelled the effects of the sewage contamination on the post-earthquake Avon and Heathcote rivers and estuary. The results of this study suggested that low dissolved oxygen and increased ammonia concentrations were expected throughout the rivers and estuary due to the bacterial breakdown of biodegradable organic material, and highlighted this as a potential cause of toxicity (Rutherford and Hudson 2011). This lends further support for anoxia as a major environmental stressor in the post-earthquake Avon-Heathcote Estuary.

Heavy metals were found to be at concentrations below the ANZECC (2000) ISQG low values at the Avon-Heathcote sites sampled (Dr. Sally Gaw, personal communication, Table 6.1). The toxicity at the PS 1 m and 50 m sites was unlikely to be caused by the metal levels as they are below the LOEC for zinc contamination in Chapter 5, while all of the other metals are well below the ANZECC (2000) ISQG low values. The levels of metals in these sites are similar to those that Zeldis et al. (2011) measured at similar locations in the estuary. Interestingly, they found that the metal concentrations in the liquefaction sediment mounds in the estuary had lower heavy metals than in the surrounding mud flats. They attributed this to the unearthing of archaic sediments with lower levels of metal contamination than more recent sediments. The sediments in this chapter were not collected from liquefaction mounds, however due to the large

impact of liquefaction it is highly likely that some of the sediments could have been derived from liquefaction.

Zeldis and colleagues (2011) found levels of 94.3 µg/g for zinc at the Humphreys Drive location, near the Ferrymead (F) location in this study, which had similar levels for zinc (85.8 µg/g). In Chapter 5 reproductive effects were seen at similar concentrations, in very similar sediment type to that of the Avon-Heathcote Estuary. However due to the anoxia in this sample prior to testing, it is unknown what effect the mixture of metals in this sample would have had on toxicity. Clearly, however, the levels of metals measured in the Avon-Heathcote Estuary have the potential to cause toxic effects.

Zeldis et al. (2011) did not assess organic contaminants such as PAHs or PCBs, which were found to be elevated in Izmit Bay after the Turkish earthquake of 1999 (Tolun et al. 2006, Tolun et al. 2008). Consequently it is, as yet, unknown if organic contaminants were elevated after the earthquake and whether the levels reached could cause toxicity.

It is difficult to quantify the effects of the sewage contamination seen in these samples because of the complication of many different types of contaminants and the additional pressures of anoxic conditions. Anoxia could have interacted with the contaminants present in a sediment to cause additive or synergistic toxicity. For example, copepods and other benthic meiofauna have been shown to alter their behaviour in anoxic conditions (Powers et al. 2001). This could cause physical exhaustion in the tests as they try to avoid anoxic sediments by swimming in the water column. If this effect is combined with stress from pollutant exposure it could lead to greater sensitivity and reduced survival or reproduction.

The results from the Avon-Heathcote suggest that the estuary currently suffers from very serious contamination, whether through sewage contamination and/or other unknown pollution sources. Further monitoring and recovery should be instigated, both from the perspective of estuary health, but also as a learning opportunity regarding the recovery of ecosystems impacted by earthquakes and their direct and indirect impacts. The estuary had been used extensively for recreational fishing and shell-fishing, sporting activities such as windsurfing, sailing, and kayaking, and in addition, two major swimming/recreational beaches of Sumner and New Brighton flank the estuary mouth (ECAN 2007). Monitoring of the estuary will allow for accurate assessment of potential adverse effects to estuarine ecology, will serve as a proxy for potential human health effects, and will allow for targeted rehabilitation and closures to preserve and/or promote ecological and human health.

6.4.2 Invercargill Estuary

The Invercargill and Napier locations were selected as a collaborative project with Dr. James Ataria (Lincoln University) examining the potential effects of contamination to areas of significance to Maori communities. The sites selected (Figure 6.2) have a wide range of potential contaminant issues from multiple point and non-point sources. In Invercargill, the Stead Street Bridge was selected as a sampling site owing to its location downstream of the Waihopai River and Otepuni Stream. The Waihopai River drains an area dominated by agricultural land use as well as having several point source discharges into the river from residential and light-medium industry. The Otepuni Stream receives storm water from residential, city and light-medium industry. The Invercargill City Dump site is about 800 m downstream of the decommissioned city refuse station that has been capped with soil. The Kingswell site is where the Kingswell Stream, which drains the south Invercargill residential area, discharges into the

estuary. The Waipaka Stream has a short catchment but runs past a meat processing plant (the site of a recent effluent spill) and a milk solids drying plant. The Waipaka sample site is near the input of the stream. The Oue site is on the western shores of the Oreti River discharge into the estuary and drains predominately agricultural land. The Omaui site is located at the opening of the Invercargill Estuary to Foveaux Strait and could potentially be subjected to cumulative inputs from activities upstream (Dr. James Ataria, personal communication).

The only Invercargill Estuary location that showed toxicity in the bioassay was the Invercargill dump (ID). This result is not surprising due to the potential runoff and leaching of waste from the Invercargill dump. Landfill leachate is known to contain potentially high levels of heavy metals, aromatic hydrocarbons, phenols, chlorinated aliphatics, pesticides, and plasticisers, in addition to organic matter and bacteria (Kjeldsen et al. 2002). Unfortunately there is no chemistry data for this site so conclusions are unable to be drawn regarding the causes for the toxicity. This sediment was, however, notable for the appearance of a red algal or bacterial slime. Further research will be needed to determine if this directly caused the toxicity or if other contaminants are responsible. This area should be reinvestigated and the source of toxicity identified to guide potential remediation of the site, and to mitigate any environmental harm.

Only one of the sediments tested, KW has available sediment chemistry data and the lack of toxicity in this sediment is consistent with chemistry results. This site had low PAH and metal concentrations (Table 6.2) which are all below ANZECC (2000) guidelines. This predicts that there is very low chance of adverse effects being seen in the exposed populations, a prediction confirmed by the results of the bioassay.

The SB sediment, which exhibited no toxicity, does not have chemistry data available. However, there is data for an upstream location, Otepuni (Table 6.2). Zinc at Otepuni was 200 µg/g, a value that is at the ANZECC (2000) ISQG low value, and which caused acute mortality in Chapter 5. It would appear that the contamination levels further downstream at the sample site (SB) are either at a lower level or that they are not sufficiently bioavailable to cause toxicity. Future monitoring should be used in this case to ensure that there are no toxic effects.

The OM site showed around 40% greater reproductive output than the control sediments. Even though there is no chemistry for this site, the location (at the mouth of the estuary) suggests that it would have the lowest contamination due to the most exposure to "clean" seawater (Figure 6.2). Standardised sediment was used for the control sediments and the OM sediment may be providing conditions that are more favourable to the growth of the copepod, potentially thorough the presence of micronutrients (Anderson and Pond 2000).

The sites, OU and W, had slightly greater reproduction than controls and are also located in the middle of the estuary and so are further away from the potential pollution sources. The results from the bioassay suggest that these areas of the estuary are unlikely to have pollutant levels that would be adverse to local biota.

6.4.3 Napier Estuary

In Napier two inputs that drain directly into the estuary were the focus of attempts to assess potential impacts of contaminants on the estuary fauna. The Plantation, County and George Drive Drain receives water runoff from the city of Napier and surrounding residential areas including some parts of the Hastings

district municipality. The Tyne St. Drain collects runoff from a light-medium industrial park (Pandora industrial area) that contains a galvanising plant, automobile wreckers, painter's yards, fertiliser storage facility, and a cement plant. Historically this area also housed a timber treatment yard and sheep pelt tanneries. This drain is also known to have unconsented storm water discharges (Dr. James Ataria, personal communication).

None of the Napier Estuary sites tested showed any toxic effect on mortality over 14 d. As mentioned above (section 6.3.3), and discussed below (section 6.4.4), these sediment samples were unable to be analysed for reproductive effects due to presence of resident harpacticoid copepods. This is an unfortunate outcome as these sites showed elevated contamination (although no mortality). The site with the highest contamination levels was the HUM site which showed a total PAH level of 5.7 µg/g (not nomalised to 1% sediment organic carbon), and exceeded the ANZECC (2000) ISQG low values for chromium, at 123 µg/g, and zinc, at 370 µg/g. PAH- and heavy metal-contaminated sediments have been shown to show synergistic toxicity in experiments with harpacticoid copepods (Fleeger et al. 2007). While there was no effect on survival in this chapter, there is concern for potential reproductive effects.

The lack of overt toxicity (i.e. mortality) at these concentrations can be potentially attributed to the high organic content of these samples. The three sites with the highest PAH contamination had high organic contents, the main controlling factor of PAH bioavailability (Di Toro et al. 1991). For example, the GPC U/S site had an organic carbon proportion of 4.6% (Dr. James Ataria personal communication). This highlights the importance of accounting for organic carbon when examining chemistry data. The ANZECC (2000) ISQG values for organic compounds are based on normalisation to 1% total organic carbon as this is the predominant factor governing their bioavailability

(especially for PAHs). If the levels of total PAH contamination for the GPC U/S site are normalised to 1% organic carbon the resulting value would be 555 µg/kg sed., which is below the ANZECC (2000) ISQG low value for total PAH.

The organic content of the sediments also explains the lack of toxicity from the level of metal contamination in the GPC U/S, GPC D/S, TYN D/S and HUM locations, all of which were over the ANZECC (2000) ISQG low (Simpson 2005, Strom et al. 2011). The high organic content of these sediments will bind metals much more strongly than the more sandy sediments found in some of the Invercargill and Christchurch sample locations, which is consistent with the lack of toxic effects seen. Retesting these sediments with the full bioassay (i.e. including reproductive endpoints) is required before these contamination levels, and the potential for synergistic effects between the metals and the PAHs, can be fully appreciated.

The presence of a resident harpacticoid population in the Napier sediments does not suggest that these sediments were non-toxic. The resident populations may be adapted or acclimated to the pollution levels, or may be a more tolerant species. The bioassay species *Quinquelaophonte* sp. is not acclimated to contaminant exposure as they have not been exposed to contaminants in culture. While the reproduction of native copepods provides some anecdotal evidence that there is little likelihood of adverse effects in the sediments, more investigation is needed to determine if this is the case.

6.4.4 Performance of bioassay

In the present chapter the copepod chronic sediment bioassay developed in Chapter 5, was successfully applied to a variety of real-world sediments. Sediments with low levels (i.e. below ANZECC (2000) trigger values) of

contamination had similar reproductive outputs to control sediments which were consistent across tests and sediment types. Two control sediments were used to matrix-match several sediment locations that were fine silty sediments. The 125 µm control (Akaroa) sediments were the same as that used in sediment spiking (Chapter 5), as well as in the maintenance cultures (Chapter 3). Copepods in the <63 µm sediment, sourced from Blueskin Bay, showed similar survivability and reproductive output as the 125 µm control sediments (Figure 6.5; $P>0.05$). In the field sediments, there was a wide range of particle sizes and organic contents (personal observations). For example, sediments such as OM was a very sandy sediment, low in organic carbon, whereas KW and GPC U/S sites were organic carbon-rich. Sediments were from very different geographic regions further showing the ability of the bioassay to test sediments New Zealand-wide. This indicates that the copepod assay has the ability and scope to address a variety of sediment types, increasing its utility as a monitoring tool.

With the sediments tested in the present chapter, the most prominent source of toxicity appeared to be anoxia. Whether anoxia interacted with chemical contaminants to alter toxic impact is unknown, but both of these stressors are environmental concerns (Desprez et al. 1992, Elliott and de Jonge 2002, Lotze et al. 2006), and may co-occur in many settings (e.g. Kristiansen et al. 2002). Alone, anoxia has been known to alter benthic community structures (Moodley et al. 1997), and to cause fish kills, which have been an increasing threat to ecosystems worldwide (Paerl et al. 1998, Camargo and Alonso 2006). However, anoxic conditions can "hide" more subtle chemical effects (i.e. reduced reproduction relative to overt mortality; (Gorokhova et al. 2010). This can make management and remediation decisions difficult. The results from this chapter, although limited in terms of sample size, suggest that eutrophication may be a more important environmental concern than metals or PAH contamination. This is especially relevant in Christchurch due to the sewage contamination of the estuary and other waterways. Additional field testing with

contaminated sediments will help to further elucidate the responses to anoxia/chemical combinations.

Interestingly, three of the four toxic sediments, were found to be toxic by the mortality endpoint, not reproductive endpoints. This is not an uncommon occurrence in sediment bioassays. For example, in the American amphipod *Leptocheirus plumulosus*, survival is a more sensitive endpoint than reproduction (McGee et al. 2004, Kennedy et al. 2009). In the present study, mortality was statistically different from controls, but reproduction, while reduced, was not statistically different from control reproduction. This may be an interesting anomaly. Alternatively there may be a methodological explanation; when testing field sediments there is potential for larger variation in reproductive endpoints between replicates, than in spiked sediment studies. In field collected sediments the number of replicates can be increased to four or five replicates per concentration. This has been shown to help in detection of toxicity in other studies as it gives more statistical power when testing for significant differences from control, therefore increasing the sensitivity of the bioassay (Simpson and Spadaro 2011).

In all of the sediment spiking studies in Chapter 5, reproduction was a more sensitive endpoint than mortality. Additionally, the majority of bioassays use reproductive endpoints as they are a more sensitive endpoint both logically, and as seen in practice (Kovatch et al. 1999, Chandler and Green 2001, Bejarano and Chandler 2003, Perez-Landa and Simpson 2011). This bioassay (in general) has shown that using the reproductive endpoints is more sensitive than using mortality alone.

The use of just the survival endpoint in the Napier sediments due to the contamination by resident copepods highlights several factors critical for sediment bioassays. These sediments were tested less than 48 h after field

collection, and despite transport on ice via air freight, refrigeration overnight once received at the lab, and sieving through a 125 µm mesh prior to testing, the resident copepods survived. Refrigeration and sieving of sediments has been shown to kill the majority of sediment meiobenthos in previous studies (Kovatch et al. 1999). This method was successful in testing of the Christchurch and Invercargill sediments; however, it is believed that the short time between collection and testing, while good for reducing the changes in sediment chemistry was inadequate to remove resident meiofauna. Based on these findings it is recommended that sediments are kept at 4°C for longer than 48 h prior to testing.

The modification of the bioassay to focus on survival as the sole endpoint brings significant reduction in sample analysis time, at the cost of a possible reduction in sensitivity. This is an important aspect to consider when implementing pollution monitoring schemes. The time invested in counting juvenile copepods incurs greater costs, and a balance needs to be struck between the number of sites that can be tested and the sensitivity of the endpoint used. In this chapter survival was the most sensitive endpoint but this is not expected to be a common occurrence, and the reproductive endpoint is generally likely to be the more sensitive parameter. However, the inability of the survival endpoint to classify the Napier sediments as toxic, despite contamination above ANZECC (2000) trigger values, suggests caution should be applied if using mortality alone.

6.5 Conclusion

This chapter has shown the versatility of the *Quinquelaophonte* sp. bioassay that has been validated in the previous chapters (see the Appendix for another example of the application of the bioassay). A variety of natural

sediments were tested with the 14 d chronic bioassay and it proved successful in its ability to test a range of sediment types. Several sediments were identified as toxic in Christchurch and in Invercargill. Interestingly, with regard to the Christchurch toxic samples, the bioassay appeared able to detect effects from sewage contamination that resulted in anoxic conditions. Anoxia is a stressor that is very different from the ones that are included in traditional monitoring of estuaries (PAH and metals predominantly). The bioassay was able to successfully test a range of sediment sizes and organic contents, showing versatility in testing different substrate types. This is important as a bioassay limited to a very specific sediment type lacks utility. The results from this chapter and that of the previous chapters solidify the use of *Quinquelaophonte* sp. bioassay as a successful monitoring tool for New Zealand estuaries.

7.

Multiple generation exposures of a harpacticoid copepod to zinc: Changes in sensitivity and population structure

7.1 Introduction

In earlier chapters of the thesis it has been established that many contaminants represent a serious concern for the health of estuarine sediment biota. In particular, a variety of contaminants have the potential to cause acute and chronic toxicity to copepods themselves, and ultimately may cause effects on the wider food chain. In Chapter 5 the three reference contaminants reduced the number of total offspring produced at concentrations much lower than those required for acute mortality. These levels were also lower that the ANZECC (2000) sediment quality guidelines (ISQG-Low). The observed mortality, reduced reproduction, and inhibited mobility could potentially have population-level effects. Relatively subtle population effects include altered sex ratios, reduced number of juveniles, and changes in the genetic makeup of the population. Multiplied over several generations these subtle changes can have severe consequences, and could lead to populations losing the ability to adapt to novel pressures such as diseases and habitat changes (Bickham et al. 2000, Medina et al. 2007).

Recently, there has been increased research into sublethal effects and chronic exposures in order to better assess toxicity (see Chapter 5). These effects are more focused on individuals. However, the most important, profound and persistent effects of environmental contamination are at the population and community levels of biological organisation (Bickham and Smolen 1993, Belfiore and Anderson 1998, Bickham et al. 2000, Belfiore and Anderson 2001, Medina et al. 2007). Studying the transgenerational effects of contaminants at the population level are challenging and have not been well researched. It has been suggested that pollution and other anthropogenic stressors are the strongest of current evolutionary forces (Palumbi 2001, Salice et al. 2010), and understanding the effects that pollution can have on the long term viability of

populations is vital. These effects, while potentially more prevalent *in situ*, are harder to measure than acute effects, and thus to best understand how these effects can change a population, a model system has to be used.

Recent findings show that changes in populations can accrue in only several generations of exposure to contaminants (Gardestrom et al. 2006, Medina et al. 2007, Gardestrom et al. 2008). A laboratory study by Vogt et al. (2007) exposed midges (*Chironomus riparius*) to sublethal concentrations of tributyltin (TBT) over eleven generations. They found that development and reproduction were affected, as well as significant changes in the acute tolerance (24 h LC_{50}), with a four-fold increase after nine generations, and an eight-fold increase after ten generations. Another study with midges (Vedamanikam and Shazilli 2008) found that tolerance (96 h LC_{50}) to nine metals, including zinc, increased when populations were chronically exposed for six generations. They also found that the tolerance to zinc remained higher than initial sensitivity after three generations in clean conditions. These findings suggest that there can be lasting genetic effects in populations, and that there can be relatively rapid changes in populations, when exposed to pollution pressures.

These effects have caused changes to exposed populations and while initially this may seem beneficial, it is not always the case. For example, in a study by Gardestrom et al. (2008) the harpacticoid copepod *Attheyella crassa* was exposed to copper-spiked sediment for 60 and 120 days, after which the authors found a significant reduction in genetic diversity. They attributed this as a direct selection for copper-tolerant genotypes. This suggests that latent costs may be involved in adapting to a toxicant. This is when one adaptation results in reduced fitness for another trait (Salice et al. 2010). Such changes can potentially have serious consequences for the ability of a population to cope with the occurrence of new stressors, be they chemical, biological or physical. As an example of latent costs, snails exposed to cadmium for three generations increase

their cadmium tolerance, but developed a decreased tolerance to high temperatures (Salice et al. 2010). Another study on *Daphnia*, showed that increased tolerance to cadmium led to increased tolerance to lead, but a reduced tolerance to phenol (Ward and Robinson 2005). Other examples of latent costs of adaptation to metals include reduced temperature tolerance in killifish with increased cadmium tolerance (Xie and Klerks 2003) and an inhibited ability of metal-tolerant plants to compete with normal plants (Hickey and McNeilly 1975).

Changes to population size or genetics due to exposure to pollutants, can be subtle and are not detected by bioassays. Chapters 5 and 6 of this thesis have described in detail the 14 d chronic sediment bioassay which is based on mortality and reproductive performance. These endpoints are widely used in bioassays; however, they are limited in their ability to predict more subtle and long-term effects.

The major issue with studying transgenerational effects of populations is that there are limited methodologies available that can be used in a timely manner. This is especially true for those species that have long life spans (e.g. vertebrates). To characterise these effects a model organism must have the following characteristics: 1) a short life cycle, 2) high reproductive rate, and 3) be easily cultured in the laboratory. Harpacticoid copepods meet all those requirements and could provide effective for testing transgenerational effects of pollution.

The ability of *Quinquelaophonte* sp. to act as a model for determining multiple generation effects of pollutants was assessed by: (1) exposing the copepod to zinc-contaminated sediments for 90 days; (2) examining the structure of exposed populations (sex-ratio and abundance); and (3) determining the changes in sensitivity in the exposed populations to zinc and phenanthrene, a

non-exposed contaminant, using aquatic 96 h LC_{50} tests. Zinc was chosen as a model contaminant because, as a metal, it does not degrade over time like some organic contaminants. It is also a contaminant of concern worldwide, particularly in estuaries (Chapman and Wang 2001).

7.2 Methods

7.2.1 Test species

The harpacticoid copepod *Quinquelaophonte* sp. was cultured in a laboratory culture system as described previously in Chapter 3.

7.2.2 Sediment preparation and spiking

Sediments were collected from Portobello Bay (45°50'S, 170°39'E) at low tide by scraping the top 0-2 cm of sediments and were then processed according to Chapter 4. Sediments were then stored at 4°C until use, approximately 4 months prior to spiking.

Toxicant concentrations were achieved by spiking sediment with zinc stock solution ($ZnSO_4 \cdot 7H_2O$ in deionised water) following the methods outlined in Chapter 4. Sediments were then aged for 40 d at room temperature in the dark. Prior to testing, sediments were mixed for 1 h and then distributed into test vessels (see 7.2.3 for description). Sediment samples were taken at test initiation and termination for chemical analysis of zinc pore-water and sediment concentrations.

7.2.3 Experimental design

Spiked sediments with nominal concentrations of 0, 20, 60, and 120 µg Zn/g sed. dry wt. were distributed between four replicate test aquaria. Test concentrations were based on the results of Chapter 4 which showed considerable reductions in reproduction starting at concentrations as low as 36 µg/g, while at 120 µg/g there was an approximately 80% reduction in offspring.

Test aquaria consisted of a 9 cm diameter plastic container with two 2 cm opposing windows cut into the side covered with 55 µm mesh. A total of 60 ml of sediment, approximately 1 cm deep in the aquaria, was added to each replicate. At test initiation 100 copepods (50 males and 50 non-gravid females) were added to each replicate from laboratory cultures. The replicates were then placed into the flow-through ASW culture system under a dripping flow of ~1 ml/min. Each replicate was fed 10 ml of a concentrated mix algae diet ($\sim 1 \cdot 10^6$) of *Dunaliella tertiolecta, Isochrysis galbana,* and *Chaetoceros muelleri* (1:1:1) twice a week. Three of the replicates were used for biological endpoints and the fourth was used for water quality and sediment chemistry. Water quality was monitored twice a week in the overlying water and care was taken not to disturb sediments. Water quality parameters remained stable throughout the test with only salinity varying due to evaporation and small changes in drip rate due to salt build-up on drippers. The test was conducted in a temperature-controlled room at 20°C with a 12 h:12 h light:dark photoperiod.

After 90 days of exposure (approximately 3-4 generations) each replicate was carefully sieved through a 175 µm mesh to collect adults. The remaining sediments and juvenile copepods were collected on a 55 µm mesh sieve and preserved in a 5% formalin and 0.5% Rose Bengal solution. The adult copepods were then sorted into male and female for use in toxicity testing. Only adult copepods were used in population structure analysis as juveniles were too

numerous to count individually and sub-sampling proved to be inconsistent and unreliable for population estimates (personal observations).

7.2.4 Adaptive changes in sensitivity

Adaptive changes in sensitivity were tested by aquatic LC_{50} tests using the two reference contaminants, zinc and phenanthrene. Phenanthrene was chosen as a non-exposed stressor due to its different mode of action to zinc. As such the responses to phenanthrene will provide insight into the potential for latent costs of increased tolerance to zinc. Individuals from each treatment were pooled to achieve sufficient numbers (390 adult copepods per treatment) for toxicity testing. The aquatic LC_{50} tests followed the method in Chapter 3, with the addition of the behavioural sublethal endpoint described in Chapter 5. The only difference was that only 20 adult copepods (10 male, 10 female) were used per replicate.

7.2.5 Chemical analysis

Zinc concentrations in sediment, pore-water and in the aqueous phase from aquatic LC_{50} exposures were measured by ICP-MS. Methods describing the analytical procedure for the sediment chemistry are outlined in Chapter 4. Waterborne levels were analysed according to the methods in Chapter 3. Aqueous phenanthrene concentrations were analysed by high resolution gas chromatography with mass spectrometric detection (Chapter 3).

Table 7.1 Initial and final sediment bound and pore-water zinc concentrations. All values are given in µg/g dry wt.

Sediment			
Nominal (µg/g)	***Initial (µg/g)***	***Final (µg/g)***	***Average (µg/g)***
Control	39.0	33.3	36.2
20	48.5	46.1	47.3
60	85.5	79.1	82.3
120	133.8	134.2	134
Pore-water			
Nominal (µg/g)	***Initial (mg/l)***	***Final (mg/l)***	***Average (mg/l)***
Control	0.014	0.029	0.022
20	0.034	0.054	0.044
60	0.063	0.088	0.076
120	0.16	0.11	0.13

7.2.6 Toxicological analysis

Mean population abundance in the three zinc treatments was compared to the zinc-free control using a one-way ANOVA and significance analysed using a Dunnett's post-hoc test at $P < 0.05$ (GraphPad Prism, Version 5). LC_{50} and EC_{50} values were generated using the measured concentrations of zinc and phenanthrene and calculated following the methods described in Chapter 3 using the GenStat Statistical Package (12^{th} edition).

7.3 Results

7.3.1 Chemical analysis of sediments

Concentrations of sediment-bound zinc are given in Table 7.1. The average of the initial and final sediment concentrations were 47, 82, and 134 µg /g sed. dry wt. The background zinc concentration in control sediments was 36 µg/g sed. dry wt. zinc. The rate of loss of zinc over the 90 day test period was very low (<10% in treatment groups).

7.3.2 Effects on population structure

Optimal culture conditions (Chapter 3) were maintained during the tests. Salinity ranged between 30 – 33‰, and pH 8.1 ± 0.2. Nitrate, nitrite, and ammonia were below detection levels during the entire test (Red Sea® commercial aquarium test kits).

After 90 d of exposure to zinc-spiked sediments there were statistically significant changes in population size at the highest contaminant concentration, 134 µg/g sed. dry wt. zinc (Figure 7.1, Table 7.2). The total adult population size was reduced at the highest test concentration to an average of 57% of the control population (P=0.0138). This effect was not sex-specific as the numbers of males

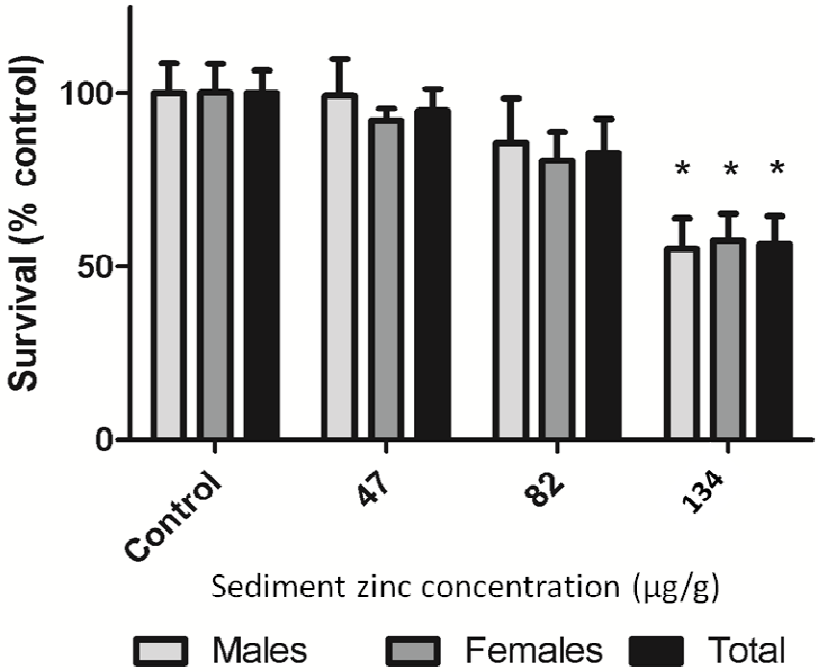

Figure 7.1 Survivorship (normalised to control population) after a 90 d exposure to sediment zinc. Averages and standard deviations are percent of controls. One-way ANOVA with a Dunnett's post-hoc analysis used to test for significant differences from controls (n=3). * *P>0.05*

Table 7.2 Average final population sizes of adult *Quinquelaophonte* sp. (n = 3) in the control and treatment groups following a 90- d exposure to zinc, One-way ANOVA with a Dunnett's post-hoc analysis used to test for significant differences from controls (n=3). * *P>0.05*

	Control	**47 µg/g**	**82 µg/g**	**134 µg/g**
Population size ± SD	549.0 ± 63.4	522.3 ± 58.1	454.3 ± 93.4	310.3 ± 75.7*

SD: Standard deviation of the mean,

and females were equally reduced by 55% and 57% respectively (P = 0.0221, P = 0.0203). There were no significant changes in population size or evidence of changes in sex ratio in any other treatment.

7.3.3 Adaptive changes in sensitivity

7.3.3.1 Zinc sensitivity

The sensitivity of copepods to zinc decreased in groups exposed to 82 and 134 µg/g zinc compared to controls for both the LC_{50} and EC_{50} values (Table 7.3). The change in sensitivity was also seen as significant differences in the intercepts and slopes of the modelled responses. In the 82 µg/g treatment group there was a significant change in the intercept of the modelled dose responses for mortality (Diff = 1.413, SE_{diff} = 0.347, Z = 4.07, P< 0.001) and lethargy (Diff = 3.02, SE_{diff} = 0.909, Z = 2.73, P<0.001). There was weak evidence for a change in slope for mortality (Diff = 0.898, SE_{diff} = 0.476, Z = 1.89, P= 0.06), and significant change in slope for lethargy (Diff = 3.64, SE_{diff} = 1.33, Z = 2.73, P= 0.006). For the 134 µg/g sediment treatment there was a significant change in modelled intercept for mortality (Diff =3.59, SE_{diff} =0.605, Z =5.94, P<0.001), and for lethargy (Diff =3.48, SE_{diff} = 0.907, Z = 3.84, P<0.001). There was no significant change in slope for mortality (Diff = 0.038, SE_{diff} = 0.589, Z = 0.06, P= 0.9), however, there was a significant difference in slope for the lethargic response (Diff = 4.04, SE_{diff} = 1.3, Z = 3.11, P= 0.002). There was not any significant differences in intercept or slope in the 47 µg/g zinc exposed population or between the control and the starting population.

Table 7.3 Toxicity of aqueous zinc and phenanthrene to *Quinquelaophonte* sp. populations after 90 d of exposure to sediment zinc. 96 h LC_{50} and EC_{50} for lethargy are given along with the 95% confidence values.

	Zinc mg/l		Phenanthrene mg/l	
Treatment group	***EC_{50} (95% CI)***	***LC_{50} (95% CI)***	***EC_{50} (95% CI)***	***LC_{50} (95% CI)***
Start population	0.76 (0.69 – 0.88)	0.97 (0.88 – 1.06)	0.38 (0.37 – 0.40)	0.96 (0.86 – 1.11)
Control	0.74 (0.69 – 0.80)	1.06 (0.96 – 1.17)	0.35 (0.32 – 0.38)	1.29 (1.21 -1.40)
47 µg/g	0.76 (0.71 – 0.81)	1.08 (0.96 –1.22)	0.36 (0.34 – 0.37)	0.92 (0.80 – 1.13)
82 µg/g	0.98 (0.90 – 1.04)	1.69 (1.47 – 1.95)	0.35 (0.33 – 0.37)	0.96 (0.93 – 1.0)
134 µg/g	1.07 (0.98 – 1.18)	2.34 (2.12 – 2.59)	0.37 (0.35 – 0.39)	1.04 (0.86 – 1.46)

7.3.3.2 Phenanthrene sensitivity

No changes in phenanthrene sensitivity of copepods were observed after exposure to sublethal levels of zinc after 90 days (no changes in intercept or slopes of modelled responses). EC_{50} and LC_{50} values ranged from 0.35 to 0.38 mg/l and from 0.92 to 1.29 mg/l, respectively (Table 7.3).

7.4. Discussion

This chapter utilises the chronic 14 d bioassay in order to better understand the effects that pollution has on *Quinquelaophonte* sp., by assessing whether this copepod is a suitable model species to test multigenerational effects of low levels of contaminants. It also further characterises the species response to sediment-borne contaminants. In addition, the results generated in this chapter further adds to data for the response of *Quinquelaophonte* to reference contaminants.

The decrease in population size at the highest concentration tested, 134 µg/g zinc, was of note as this concentration of zinc is below the ANZECC (2000) ISQV-low of 200 µg/g zinc. When the reduced population size in the 134 µg/g zinc treatment is compared to the results with zinc from the 14 d chronic bioassay in Chapter 5, an inconsistency becomes apparent. In the 14 d bioassay there was significant reduction in total offspring at concentrations as low as 55 µg/g zinc (>60%), with 85% reduction in the test population at 95 µg/g zinc, and almost complete shutdown of reproduction at 163 µg/g zinc. These data would have predicted that in 82 µg/g zinc treatment there would have been significant reductions in population size (even potential for population collapse), and in the 134 µg/g zinc the treatment group would have been expected to collapse and have no individuals remaining after 90 d. However, it was observed that after 90 d exposure to 82 µg/g zinc, there was no statistical change in the population size, and at 134 µg/g zinc there was a statistically significant reduction (44%) in population size (Figure 7.1, Table 7.2). This suggests that these populations were able to acclimate or adapt to the zinc exposure, to be able to reproduce, sustain and grow the population to an average size of 310 ± 76 adults (from the initial population of 100) for the 134 µg/g zinc exposed population. However, as this population size was reduced relative to the control population size (549 ± 63), it does suggest that this acclimation came at a cost.

In the exposed populations there were changes in the acute sensitivity to zinc. The populations exposed to 82 and 134 µg/g zinc saw an increase in the EC_{50} for lethargy to 1.0 and 1.1 mg/l zinc, respectively. The LC_{50} values also increased to 1.7 and 2.3 mg/l zinc for these two groups. This indicates that the zinc-exposed copepods were more tolerant to zinc compared to the control population (EC_{50}: 0.8 mg/l, LC_{50}: 1.0 mg/l). This change in sensitivity explains the ability of the populations at these concentrations to be at higher abundances than those predicted from the results of the 14 d chronic bioassay. These populations have therefore adapted/acclimated to zinc exposure.

Population changes can be enacted through several genetic and non-genetic mechanisms. Genetic mechanisms may include direct mutations to DNA, reduction in genetic diversity, and via the selection of specific "favourable" genes. The main genetic driver for adaption of the population to pollution pressures is through individuals with greater resistance having better survivorship and/or higher rates of reproduction (Bickham et al. 2000, Belfiore and Anderson 2001). Non-genetic mechanisms can also help individuals adapt to pollution. These non-genetic mechanism include altered behaviour, different metabolic rates, or increases in regulatory mechanisms (i.e. metallothionein for metal exposure) to alleviate toxic affect and/or increase tolerance to the exposed pollutant (Maltby and Crane 1994, Vedamanikam and Shazilli 2008). Obviously it is arguable that these "non-genetic" mechanisms still have a genetic root. That argument aside, it is unknown though which mechanism or mechanisms the apparent acclimation/adaptation to zinc observed in this chapter has occurred.

Increased tolerance through any mechanism can be beneficial to a population in that it allows that population to persist in polluted areas, or persevere long enough to move or for the pollution to be removed. However, the population may also become more sensitive to other factors, such as other pollutants or changes in environmental conditions (e.g. temperature), which may reduce overall fitness (Bickham et al. 2000, Medina et al. 2007). The ability of a population to handle different stressors ultimately affects the viability of the population in response to other pressures. In the current study the exposure of copepods to zinc was shown to result in population changes, presumably through enacting mechanisms that increased tolerance. It was of interest to determine whether that tolerance came at a cost in terms of sensitivity to another pollutant, phenanthrene. There was, however, no observed change in phenanthrene sensitivity. Zinc and phenanthrene have very different modes of action and so it is perhaps not surprising that there was not a change in phenanthrene tolerance.

Phenanthrene was chosen as it had been used in previous chapters of this thesis (Chapters 3 and 5) and the biological effects on *Quinquelaophonte* sp. are known. Testing of other contaminants, specifically those with similar modes of action, such as metals, may be more fruitful in future studies.

This aim of this chapter was to assess the suitability of *Quinquelaophonte* sp. as a model organism to study the effects of multigeneration contaminant exposure on populations. The results of the study showed that *Quinquelaophonte* sp. is a suitable model species to be used in these kinds of studies. As discussed, there was a change in the tolerance of the exposed copepods, shown both as an increase in acute LC_{50} values, and final population sizes that were greater than that which would have been predicted from the 14 d chronic bioassays (Chapter 5). However, there was no within-generation sensitivity control and this resulted in an inability to differentiate between within- and trans-generational changes of the populations. The addition of genetic and physiological measures of pollution exposure will increase the power of this method, and be able to detect within- versus trans-generational changes in populations. For example, measures of genetic diversity can provide information as to whether genes are being enriched in exposed population through the selection of specific traits or through genetic drift (Gardestrom et al. 2008). Gene expression (mRNA) can also be examined at different time-points of the exposure using microarrays or quantitative real-time PCR, to better understand the physiological responses at the genetic level (Ki et al. 2009). Also simple generational comparisons of sensitivity can also be used to examine physiological versus generic changes in tolerance (Vedamanikam and Shazilli 2008).

Future research using this test system to assess adaptive tolerance should focus on the mechanisms that allow for the adaptation, whether genetic or physiological. Further studies could also focus on the latent costs of the

adaptation and how persistent they are in the population. The following questions could be addressed: How will the population recover if the pollution pressure is removed? Will the population still be able to persist? Will populations be able to return to a pre-exposure state? Will they be able to compete with other species, or will they be outcompeted? The answers to these questions are critical to being able to protect populations that are threatened, and to provide the most effective protection for pristine environments.

It is important to acknowledge that this chapter, like many other studies, has used only single toxicant exposures. As discussed previously in this thesis (Chapter 6), mixtures of contaminants can be more toxic than single contaminant exposures. Pollutant mixtures are thought to be harder to adapt to due to multiple levels of stress acting on different biological pathways (Gardestrom et al. 2008). This is an important area where more research is needed, as exposure to pollutant mixtures is the most likely environmental exposure scenario.

7.5. Conclusions

This chapter has shown that *Quinquelaophonte* sp. is an ideal species to investigate multigenerational effects of pollution. It validated a method that provides a framework for future studies to quantify the effects that low levels of pollution (i.e. those that are not causing overt effects in short exposures) have on population parameters. For the protection and conservation of natural environments it is vital that population-level effects of pollutants are understood. There are potential "time bombs" of latent costs due to micro-evolution and adaption to pollution. The more that is understood regarding how low-level pollution pressures effect populations, and the timescales that they occur over, the better the prevention of negative environmental impacts. There are still many unanswered questions as to how changes in population abundance, structure, tolerance to pollutants, and genetic diversity effect populations in the long-term.

Future research should focus on answering these questions and *Quinquelaophonte* sp. is an excellent model species to be used to examine these effects.

8.

General Discussion

8.1 Overview of research

This thesis has detailed the development of three bioassays using a native New Zealand copepod. Two of these bioassays allow for the testing of marine and estuarine sediments on a local species that is likely to be found in sediments from around New Zealand, and the third is an aquatic test. These tests allow for the effects of pollution to be identified and quantified based on adverse biological effects. This is an advance on monitoring methods that rely solely on chemical measurements of contamination levels, and toxicity prediction based only on these measures. Chemical characterisation of estuarine sediments and the derivation of SQV values such as the ISQG from the ANZECC (2000) guidelines, the PEL, and ERM from Long et al. (1998) and MacDonald et al. (1996), have important roles in sediment quality assessment. However contaminant levels exceeding these trigger values do not necessarily mean that there will be biologically-adverse effects (Hubner et al. 2009). Conversely, adverse biological effects can occur at levels below the trigger values as seen in Chapter 5 and by Kelly (2007). In Auckland's Manukau and Waitemata Harbours Kelly (2007) found that benthic community structures were altered in similar ways to communities in contaminated areas, despite levels of pollution below trigger values. The use of the *Quinquelaophonte* sp. bioassay in these sediments may have been able to determine if these adverse effects were caused by pollution. Thus the use of the bioassay in conjunction with sediment chemistry/trigger values is required for a better assessment of environmental effects of toxicants.

Bioassays are also advantageous in that they can account for the complex assortment of chemical mixtures that may occur in natural sediments. The variable composition of such sediments can have complex and unpredictable impacts on pollutant bioavailability, and subsequently, toxicity. Bioassays, such as those developed here, and when used in conjunction with chemical analyses, are consequently the most effective way to assess pollution risk to the local biota. When used in a tiered approach, such as that outlined in the ANZECC (2000) guidelines,

which uses multiple lines of evidence, the bioassay/chemical characterisation employed in the current thesis provides the best approach to monitoring potentially contaminated environments and informing management decisions.

This thesis has carefully selected the most suitable New Zealand estuarine bioassay species for use in a wide range of applications. Amphipods have been used in several studies as a local bioassay species in New Zealand (Marsden and Wong 2001, King et al. 2006, Dupree and Ahrens 2007). Although combining both amphipods and copepod bioassays has been very successful (Simpson and Spadaro 2011), harpacticoid copepods exhibit greater sensitivity than amphipods (Greenstein et al. 2008). This provided a large part of the rationale for choosing a harpacticoid copepod to develop a new sediment bioassay for monitoring estuarine pollution. Harpacticoids are also advantageous in that they are present at the freshwater-marine interface, occupy a position at the base of many important marine and estuarine food webs, and have short life cycles (Ward et al. 2011). They are consequently found in high densities where terrestrial pollutants are often accumulated, and are an ecologically significant species. Their ecological importance makes the extrapolation of laboratory effects to native *in situ* species easier (Coull and Chandler 1992).

The rationale for choosing *Quinquelaophonte* sp. over other harpacticoids was based on a variety of different factors, including life-history traits, cultivability and sensitivity. The sediment factors governing its distribution in a sand-mud flat were examined to provide more information regarding the ecology and habitat preferences of this and other potential bioassay species (Chapter 2). This is an important aspect of a bioassay that is often overlooked. The more information that is known about the "test" species, and its environmental requirements, the more confidence there is in the results derived from the bioassay. An understanding of the ecology of a species identifies the factors other than pollution that can affect behaviour, reproduction, or development, and which could be mistaken as a toxic effect.

The main products of this thesis are the sediment bioassays. The two sediment bioassays (acute and chronic) were validated using spiked sediments. To this end a method was developed for spiking of fine sandy sediment (that which was found to be preferred by *Quinquelaophonte* sp.) to ensure that there was adequate distribution of contaminants through the sediment. The spiked sediment bioassays were used to classify the responses of *Quinquelaophonte* sp. to sediment-bound contaminants. Mortality (both acute and chronic) was measured as this is the classical endpoint used in toxicity testing. The sublethal endpoints of mobility inhibition (acute) and reduced reproduction (chronic) were also measured. These bioassays can be used in both monitoring and environmental risk assessment to assess both current impacts of pollution as well as mitigating the risk of new compounds (see the Appendix).

The three different reference contaminants (zinc, atrazine, and phenanthrene) showed markedly different effects on exposed individuals. Zinc showed a very narrow range of effects with an ACR of 6.7. This small ACR signals some concern for estuarine copepods, as it indicates that only relatively minor increases in zinc concentration will move toxicity from an individual-level acute effect to a more "serious" population-level chronic effect.

Of all the reference contaminants tested, atrazine showed the widest range in effect concentrations with an ACR of 117,000. This large difference in toxic effects is most likely due to the specific endocrine disrupting effect of atrazine (Hayes et al. 2002). The low levels at which atrazine showed reproductive effects (total offspring EC_{50} of 0.83 µg/g) are of concern as they are within the range of environmental concentrations (Rice et al. 2004).

Phenanthrene showed adverse reproductive effects at very low concentrations (EC_{50} 0.67 µg/g), with an ACR that was significantly higher than that predicted by its mode of action (Roex et al. 2000). This suggests that there may be another mode of action of phenanthrene, one that is acting directly on the reproductive tissues or affecting juvenile development (Lotufo 1997). This variety of effects at different

contaminant exposure levels shows that the bioassay is able to elucidate adverse effects of low level contamination, as well as effects from contaminants with different modes of action. This suggests that the bioassay will be useful in identifying levels of contamination that might lead to subtle food web and community perturbations.

The chronic bioassay was used to test field samples from a variety of estuaries that have different contaminant profiles. Napier and Invercargill estuarine sample sites are mainly affected by urban, industrial, and agricultural pollutants. During the testing of sediments the bioassay showed its versatility in being able to assess very different sediment types. This included very sandy sediments from the mouth of the Invercargill estuary (Omaui) to the fine organic-rich sediments near the river input (Invercargill dump) and in Napier. This is a vital characteristic of this bioassay as it allows for wide application in monitoring schemes throughout New Zealand.

An interesting outcome of the research is that the bioassay picked up some non-chemical causes of toxicity. In Christchurch sediments, anoxic conditions were observed during the bioassay, likely the result of sewage contamination resulting from earthquake-impacted infrastructure. This, while not a chemical effect, is important as adverse effects related to non-chemical stressors may occur frequently in wild populations, and the bioassay is able to detect this deterioration in habitat quality.

Native indigenous copepods also had effects on the bioassay. Due to inadequate refrigeration the native copepods survived when testing the Napier sediments. This prevented the ability to use the reproductive endpoints in the testing. Due to this adequate refrigeration and sieving is recommended to remove the majority of native fauna prior to testing.

Quinquelaophonte sp. was further characterised in terms of its response to pollution by looking at multigeneration exposures. The results of this study showed that in response to toxicant exposure the tolerance of the population, as measured by a 96 h LC_{50}, changed. The increase in tolerance illustrated that there is potential for populations to adapt or acclimate, through either genetic selection or physiological mechanisms, to pollution pressures. While adaptations to pollution can be beneficial to populations in exposed areas it can make them more vulnerable to reduced genetic diversity and latent costs. For example, while they may appear to adapt, the latent cost of adaptation/acclimation may reduce life span, fecundity, tolerance to other stressors, competitiveness, or some other aspect of the organisms biology. Consequently biological adjustment to pollutant exposure may negatively impact the individual itself, but also may eventually result in adverse changes at the population level.

8.1.1 Challenges and limitations

During this thesis there have been many challenges which are worth highlighting. The first was the culturing of harpacticoid copepods. Establishing a laboratory culture of a bioassay organism is an essential step in ensuring that the results of the bioassay are not impacted by prior exposure history. A total of six species were bought into the laboratory as potential bioassay organisms. Only two species were able to be cultured successfully. The factors that were found to be the most important in culture survival were sediment type and processing, confirming findings of previous studies (Chandler 1986, Nipper and Roper 1995). Laboratory populations of *R. propinqua* and *Quinquelaophonte* sp., were the healthiest (highest density, highest proportions of gravid females) when cultured in the sediments that they were initially sourced from. While they were able to sustain populations in other sediments, they were never as healthy as when cultured in native sediments. The sediment used in cultures is therefore vitally important for having large healthy populations of copepods in the lab and so provides easy and effective testing of contaminants (Chandler 1986). Processing sediments (i.e. washing, sieving and sterilising) removed the majority of

sulphides and detritus which could reduce water quality, and allowed for monocultures of each copepod species by removing any native fauna (Chandler 1986).

The other main challenge that was not able to be resolved was the extraction of DNA from individual copepods. This was planned as a major component of the multigeneration exposure in Chapter 7, and would have facilitated an analysis of genetic diversity during the exposures to zinc. It would have permitted an assessment as to whether observed changes in sensitivity were the result of genetic change. Multiple attempts were made to extract DNA from both individual and pooled individuals using a variety of different methods such as that of Schizas et al. (1997), as well as using commercial kits such as Qiagen® DNeasy and Sigma® RED extract N amp. There are several reasons that may explain why this was unsuccessful. Firstly these copepods are very small (<1 mm long), do not have a lot of DNA, and any DNA that is extractable may be contaminated with DNA from sediment bacteria and fungi. Furthermore they have a chitinous exoskeleton which is also thought to make DNA extraction difficult (Schizas et al. 1997). There are several methods for extracting DNA from eggs (Montero-Pau et al. 2008, Xu et al. 2011), and eggs may not have the same issues associated with adult individuals that interfere with DNA extraction. Future studies may have better success using mitochondrial DNA, and primer amplification as this has been successfully used in other studies (Durbin et al. 2008). This lack of genetic endpoint was a short-coming of the multigenerational study as the exposed populations could not be assessed for reduced genetic diversity due to zinc exposure. This is an effect that has been seen in other studies (Gardestrom et al. 2008). Future research is needed to better assess the effects of long term pollutant exposure on genetic diversity, as reduced diversity can impair population fitness and ability to adapt (Staton et al. 2001, Morgan et al. 2007).

The bioassays developed in this thesis are a very useful tool for environmental monitoring in New Zealand. While not the first harpacticoid bioassay (Chandler and Green 1996, Brown et al. 2005, Raisuddin et al. 2007, Perez-Landa and Simpson 2011), it is the first with applicability for New Zealand that has been fully evaluated

and validated. This allows for a native species to be used to detect adverse effects on the local biota. Despite the versatility of the bioassay it is not a “one stop shop” for sediment toxicity evaluation. Different bioassay species have different exposure routes including particle ingestion, contact with sediment, and pore-water exposure (Green et al. 1993, Chandler et al. 1994, Simpson and King 2005, King et al. 2006, Greenstein et al. 2008). Therefore single species testing to evaluate sediment toxicity cannot be recommended (Greenstein et al. 2008, Simpson and Spadaro 2011). It is suggested, however, that this bioassay can form a nucleus of tests that incorporate a suite of bioassay species to fully evaluate potential risks from pollution. Other test species that would work well in conjunction with this copepod bioassay include an amphipod bioassay, such as that used by Marsden and Wong (2001) or Dupree and Ahrens (2007), or a bivalve or polychaete worm bioassay (Moore and Dillon 1993). A suite of species such as these will give the most accurate risk assessment of contaminated sediments as it would evaluate pollution through different exposure mechanisms and types of organisms, which is a better representation of a benthic community than a single species. When toxicity is found it is recommended that chemical characterisation be undertaken for TIE analysis to identify the potential causes of the toxicity.

8.2 Objectives revisited

The main objective of this thesis was to validate a marine and estuarine sediment bioassay with a native New Zealand harpacticoid copepod, in order to develop a tool for monitoring environmental impacts and conducting environmental risk assessment. This overall objective was split into distinct sub-objectives to ensure that the bioassay was well validated, and was a tool with real-world utility. These objectives, and how they were accomplished, are discussed below.

Objective 1: Selection of a suitable native New Zealand harpacticoid copepod bioassay species.

An ecological assessment of several candidate copepod species was undertaken to understand as much as possible about the environment that they live in, and how environmental factors impact their distribution *in situ* (Chapter 2). Little is known about the ecology of many species of copepods. This chapter contributed novel understanding regarding aspects of native copepod ecology and assisted with the development of culture conditions by defining the most suitable sediment types.

Two species were able to be cultured: *Robertsonia propinqua* and *Quinquelaophonte* sp. These two species were then compared across multiple traits, including culture density, reproductive rate in laboratory culture, ease of distinguishing males and females (for assessment of gender-based toxic impacts), geographic distribution, and sensitivity to contaminants. This then permitted selection of the species that would be best-suited for testing in a range of estuarine sediments, and which would also be sensitive to pollution, and thus act as a suitable sentinel of environmental impact (Chapter 3).

Objective 2: Validation of sediment bioassays.

In total there were three bioassays used and validated. First the aquatic 96 h acute test was used to assess and compare the sensitivity of *Robertsonia propinqua* and *Quinquelaophonte* sp. (Chapter 3). This method is a standard test that is used with species worldwide (ISO 1999), and was used with the New Zealand harpacticoid species with only minor modifications. This technique allows for rapid assessment of a variety of different contaminants or pollutant mixtures and permits easy comparison between species. Two sediment copepod bioassays were also validated using sediments spiked with three reference contaminants: zinc, atrazine and phenanthrene (Chapter 5). This required the validation of a sediment spiking method to ensure that there was an even contaminant distribution though the sediments (Chapter 4).

The sediment bioassays included an acute 96 h test that, in addition to mortality, included a sublethal endpoint (inhibition of mobility). This is an important addition to an acute test as it allows for increased test sensitivity (Dhawan et al. 1999, Wallace and Estephan 2004). It is also an environmentally relevant endpoint as an inhibited individual is more likely to have reduced survivorship (Brooks et al. 2009, Trekels et al. 2011). This endpoint can also be used in the aquatic tests. The chronic bioassay, using reproduction as an indicator of toxic effect, was the most sensitive, capable of detecting atrazine toxicity at a levels two orders of magnitude lower than those of acute tests (Chapter 5).

Objective 3: Laboratory testing of field collected sediments.

Sediments from three different estuaries from around New Zealand were tested. These included a variety of sediment types with differing organic content and grain size. The bioassay proved to be very versatile and differences in reproduction or survivorship were not influenced by sediment type. Toxic effects were seen at several of the sampling sites. Sites within Christchurch's Avon-Heathcote Estuary were identified as being most toxic, likely owing to the effects of the Canterbury earthquakes and the dumping of sewage (Chapter 6).

Objective 4: Population-level responses to low level contamination habitats.

Population-level responses to contaminants were tested by exposing copepods to zinc-spiked sediment for several generations. Population size, sex ratios and aquatic LC_{50}s to zinc and phenanthrene were used to assess the effects on the population. Testing sensitivity to both the tested contaminant (zinc) and a novel contaminant after generational exposure to zinc allowed for insight into the tradeoffs that populations potentially make as a consequence of adjusting to one stressor. There was a change in zinc sensitivity showing a response to the pollution pressure, either through changes in population genetic structure or individual physiological plasticity.

8.3 Future research

Future research should include a continuing assessment of the predictive ability of the bioassay. This will add significant value to the utility of this tool. In particular, examining how the results of the bioassay translate to toxicity and changes in the ecosystem structure and functionality would be of interest. This could be accomplished by integrating the results of the bioassay with ecological assessments. This might include species diversity (Rodrigues and Quintino 2001), food web (Pojana et al. 2003), biomass (Joseph and Joseph 2001), and contaminant transfer (Lawrence and Mason 2001) studies. The increased knowledge gained by better understanding the changes in ecology at contaminated locations, and how that relates to the results of the bioassay, will allow for better management decisions, as well as a better estimation of environmental impacts (Solomon et al. 1996).

Additional methodologies to assess the long term effects of pollution will also strengthen the bioassay predictability. The multigenerational exposures showed changes in tolerance after four generations of exposure but there was no indication as to whether this was a genetic or a physiological effect. Understanding the nature of this effect will help to better integrate the ecological effects of wild populations exposed for generations and the results of the bioassay. Future research examining the transgenerational effects could include correlative approaches where sensitivities of the F_1 generation (first generation exposed throughout life-cycle) are compared to the F_3 generation potentially giving insight into physiological tolerance (no difference between F_1 and F_3) and potential genetic adaptation (F_3 more tolerant than F_1 generation) (Vogt et al. 2007). Alternatively, after multigenerational exposure, populations can be moved to clean sediments and after several generations tested again for sensitivity. If tolerance was still higher than the starting population then it might be concluded that genetic adaption had occurred through inherited tolerance (Vedamanikam and Shazilli 2008). It is also possible to examine the mechanistic

reasons for changes in sensitivity. This can include changes in expression of genes such as cytochrome P450, heat shock proteins, and vitellogenin, following contaminant exposure. This is just a small subset of genes that are known to change with toxicant exposure (Ki et al. 2009). Measures of genetic diversity with techniques such as amplified fragment length polymorphism (AFLP), microsatellites, or random amplification of polymorphic DNA (RAPD) allow for assessment of lost genetic diversity and population divergence, which can affect the long-term viability of a population (Medina et al. 2007, Gardestrom et al. 2008). Further investigation of the latent costs of adapting to a variety of different pollutant and environmental stressors will help to better understand the effects of adaptation to pollution and the potential reduced viability of the populations (Ward and Robinson 2005, Salice et al. 2010). Being able to adapt to, and survive in, polluted environments is not always beneficial in the long run (Medina et al. 2007). The loss of the ability to adapt to other changing forces in the environment, other pollution sources, climate change, and/or disease, can severely impact populations (Boulding 2008). This is an area of research that should increase in importance across all ecosystems, as the majority of contamination is at low levels and this is the level where potential subtle effects can be overlooked.

Creating additional bioassays with different estuarine species would complement the copepod bioassay and allow for optimal environmental protection in monitoring schemes. Having a suite of several bioassay species with different exposure mechanisms would provide a far more robust assessment of sediment toxicity (Davoren et al. 2005, Greenstein et al. 2008). This provides multiple lines of evidence to support management decisions and improves understanding of the causes of toxicity through the patterns in responses (Burton et al. 2002b, Chapman and Anderson 2005). This is a more thorough way to monitor environments, and to determine the best environmental safety procedures to minimise negative impacts that human activity has on New Zealand's unique estuarine environment. In addition to developing more bioassays with local species, bioassays need to be wisely used. Monitoring schemes should be implemented in areas of high risk or in areas where conservation is paramount (ANZECC 2000).

8.4 Conclusion

The marine environment is an important resource for humans, emphasised by the fact that over half of human settlements are located near coastal regions and estuaries. Estuaries are functionally special, biologically diverse ecosystems. That they are also one of the ecosystems most impacted by pollution, is a significant ecological issue. Pollution pressures are only going to become greater in the future and there needs to be monitoring schemes that help to prevent adverse effects. Such monitoring should be particularly adept at recognising early indicators of adverse effects.

In this thesis a robust set of sediment and aquatic bioassays has been developed with the harpacticioid copepod, *Quinquelaophonte* sp. These bioassays have defined endpoints that encompass a variety of toxic impacts from reproductive effects, to immobility and mortality. There are a vast number of biological monitoring tools for assessing environmental impacts, however these biological tools are underused in environmental management and regulatory contexts (Van der Oost et al 2003). This thesis strongly recommends that sediment bioassays play a more critical role in future assessments of environmental quality, both in New Zealand and worldwide.

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Appendix

A.Y. C. Tong et al., *Environ. Chem.* **2011**, *8*, 182–189. doi:10.1071/EN10095 www.publish.csiro.au/journals/env

UV-induced photodegradation of oseltamivir (Tamiflu) in water

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Environmental context. Oseltamivir (Tamiflu) is widely used to prevent and treat influenza but conventional wastewater processes involving sedimentation and biotic oxidation do not appear to significantly remove it from sewage, leading to its discharge into the environment. A range of advanced oxidation processes (AOPs) involving photolysis of aqueous solutions of oseltamivir with UV alone, UV/H_2O_2 and UV/H_2O_2/Fe^{II} is demonstrated to lead to photodegradation of oseltamivir to products with no ecotoxicity observed. These AOPs may therefore offer potentially environmentally friendly sewage water treatment options.

Abstract. Aqueous solutions of the antiviral drug oseltamivir phosphate (OSP, Tamiflu, (3*R*,4*R*,5*S*)-ethyl 4-acetamido-5-amino-3-(pentan-3-yloxy)cyclohex-1-enecarboxylate) were degraded using advanced oxidation processes (AOPs) involving photodegradation with UV alone, UV/H_2O_2 and UV/H_2O_2/Fe^{II} (photo-Fenton reaction). The photodecay of the parent OSP in all three cases followed first-order kinetics with respective rate constants of 0.21, 1.56 and 1.75 min^{-1} at 208C in pH 7 phosphate-buffered Milli-Q water. The rate of UV/H_2O_2 photolysis in the presence of 2-methylpropan-2-ol was significantly slower with an approximate first-order rate constant of 0.13 min^{-1} suggesting the involvement of $^{\bullet}OH$ in the degradation process. NMR spectroscopy, mass spectrometry and high-performance liquid chromatography (HPLC) with UV diode array detection were used to identify the crude photoproduct as the hydroxylated OSP derivative (3*S*,4*R*,5*S*)-ethyl 4-acetamido-5-amino-2-hydroxy-3-(pentan-3-yloxy)cyclohexanecarboxylate that occurs by an unknown mechanism. OSP and this crude photoproduct demonstrated no effect on the survival of *Quinquelaophonte* sp. over 96 h.

Additional keywords: advanced oxidation process, antivirals, ecotoxicity, pharmaceuticals.