Development and validation of the marine benthic copepod *Robertsonia propinqua* as a bioindicator to monitor estuarine environmental health

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A thesis submitted in partial
fulfilment of the requirements for the
Degree of Doctor of Philosophy
at
Lincoln University
New Zealand

By
L. A. Hack

Lincoln University
2008

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Abstract

Studies in the USA have reported that species of meiobenthic copepods can be used as bioindicators of sediment-associated contaminants. The main objective of this research project was to develop and validate methods to assess the effects of estuarine pollution, using the marine benthic copepod *Robertsonia propinqua* as a bioindicator of environmental health in New Zealand intertidal/estuarine areas. Cultures of *R. propinqua* were set up and maintained in the laboratory and individuals used in 96h acute and full life-cycle chronic bioassays using the pre-selected contaminants atrazine and zinc sulphate. From the 96h acute experiments it was found that the lethal doses at which 50% mortality occurred (LC_{50}) for exposed nauplii and adult individuals were 7.5 mg/L and 31.8 mg/L, respectively for atrazine and 1.7 mg/L and 2.7 mg/L, respectively for zinc sulphate. This indicated that the nauplii life stage was more sensitive than were the adult life stages for exposure to both contaminants. Based on the 'trigger' values reported (atrazine = 0.013 mg/L, zinc = 0.015 mg/L) in the Australian and New Zealand guidelines for fresh and marine water quality, which provide values at which concentrations of contaminants can occur in the environment before they begin causing effects on aquatic fauna, it is unlikely that the calculated LC_{50}s in the current research will induce biological effects in exposed copepods in the short-term. The calculated LC_{50} results were then used to further investigate the effects of chronic exposure of sediment-associated contaminants on the complete life-cycle (egg-reproductive adult) of *R. propinqua*. In a laboratory-based full life-cycle toxicity test, field-collected sediments from polluted sites in the Auckland and Bay of Plenty regions reduced reproductive output (nauplii and copepodite production) of *R. propinqua* individuals, but the number of males and females, gravid females, clutch size per female and the number of

eggs produced were not affected by either the polluted or non-polluted (reference) sediment samples from both field regions. Field investigations of meiofauna community composition in polluted and non-polluted field sites were carried out in 2004 in the Auckland and Bay of Plenty field regions in New Zealand. Greater sediment organic content and a correspondingly deeper redox potential discontinuity layer occurred in all polluted field sites compared with the non-polluted sites. However, species composition could not be used to characterise polluted and non-polluted sites, as there were no dominant taxa which were representative of these sites. The results presented in this thesis indicate that *R. propinqua* has strong potential to be a good candidate species as a bioindicator of environmental contamination. Furthermore, the full life-cycle toxicity test could be used as a rapid test to detect immediate changes in individual reproduction and development as well as long-term population effects. The technologies developed as part of this research may eventually provide additional tools for commercial environmental consultancies and may compliment existing standard operating procedures for environmental assessments involving pollution of estuarine ecosystems.

Keywords

Robertsonia propinqua, bioindicator, environmental health assessment, ecological risk assessment, atrazine, zinc sulphate, 96h acute and full life-cycle chronic toxicity bioassays, water quality guideline values, copepod life-cycle, copepod culture, standard operating procedures.

Acknowledgements

There are a great many people who deserve my most sincere appreciation and deepest gratitude for helping me progress through the past three years and assisting me with the completion of this thesis. Firstly, thank you to my supervisor Professor Steve Wratten (Lincoln University, Christchurch) without whom I would still be floundering in the quagmire of data processing and thesis drafts. I greatly appreciate his faith in me and providing me with so much guidance, support and encouragement when I was losing sight of the 'bigger picture'. Thank you to Dr. Louis Tremblay (Landcare Research Ltd) for initiating me into the field of Environmental Toxicology, for constantly challenging me to strive for higher goals. Thank you also to Dr. Vaughan Keesing (Boffa Miskell Ltd) for providing me with guidance and the opportunity to become involved in the application of science in a commercial context.

Secondly, special thanks to my colleagues at Lincoln University for their support and assistance, especially Alison Lister for statistical support. Boffa Miskell Ltd for providing logistical and financial support, encouragement and guiding me into the commercial world and Landcare Research Ltd for proving me with the facilities to undertake this project. I would also like to thank all the staff from these locations for the many light-hearted conversations and many jovial times.

Thirdly, thank you to Professor John Wells (Victoria University, Wellington) for identifying *Robertsonia propinqua*, Professor Tom Chandler (University of South Carolina, USA) for the many conversations which have led to my passion for meiofauna. Last but by no means least, Professor Duncan McLennan for his perseverance and kindness.

I would also like to acknowledge with gratitude the financial support given by the New Zealand Foundation for Research, science and Technology, AgResearch (Dr. Nigel Bell) and Environment Bay of Plenty (Dr. Stephen Park), Lincoln University, Boffa Miskell Ltd and Landcare Research Ltd, without which this project would never have succeeded. Thank you all for allowing me to contribute to the field of Environmental Toxicology.

I would like to thank my family and friends for providing me with strength and endless encouragement when I began to falter. Thank you for the many nuggets of happiness along the way which kept me sane and mindful that there is light at the end of the tunnel.

Finally, thank you to life, for giving one of the hardest roads to travel and for showing me that when the road becomes rough, to call on your strength and never give up.

"Hardship builds character, and a strong but humble character will guide you to fulfil your aspirations."

Table of Contents

List of Figures

List of Tables

CHAPTER 1. General introduction

1.1. Introduction

Throughout New Zealand, chemical pollution from urban and rural land uses is impacting on human and wildlife populations, with widespread effects such as soil contamination, aquatic algal blooms and shellfish poisoning. Estuarine areas are among the most productive ecosystems (Costanza et al. 1997), and are under increasing pressure from a variety of stressors including urbanisation and municipal and industrial waste effluents. The aquatic and, ultimately, the estuarine environments act as sinks receiving natural and anthropogenic contaminants, the potential impacts of which on exposed organisms are not well characterised.

To date, many environmental organisations in New Zealand, predominantly consultancies and unitary authorities use assessment of environmental effects (AEEs) as a framework to evaluate the risks of various activities (e.g., urban coastal development and road maintenance/development) to ecological resources. Accurate assessment of the biological and community level effects depends on the integration of laboratory based studies such as assessment of water quality using acute toxicity tests and chronic contaminated sediment studies using bioindicator species (Chapman & Hollert 2006). The overall assessment of the health of an ecosystem then depends on the holistic integration of components such as laboratory studies with field ecological assessments and the need to measure the state of the environment at any given time. Without these integral components, evaluation of the risks to populations and communities may be limited.

To determine the relationship between effects on benthic communities and contaminant concentrations, the Sediment Quality Triad (SQT) was developed 20 years ago (Long & Chapman 1985, Chapman 1990) and consisted of three components: sediment chemistry to determine chemical contamination, sediment bioassays to determine toxicity and benthic community structure to determine the status of resident fauna most exposed to sediment contaminants (Chapman & Hollert 2006). Since then, biomagnification (increasing concentrations of a few organic contaminants through food webs) has been added as a fourth

component resulting in a Sediment Quality Tetrad (Chapman & Hollert 2006). The use of the SQT can provide conclusive results regarding the pollution status of contaminated sediments. However, as causation is not determined using SQT and only correlations determined between parameters such as contaminated sediments and sediment organic composition, further studies are needed to ascertain why one parameter causes changes to the other parameter (Chapman & Hollert 2006).

1.2. The importance of estuaries

Estuaries are areas where fresh and marine waters meet. They are zones of transition for factors such as salinity and suspended organic matter and for the distribution of nutrients and oxygen. Fairbridge (1980) defined an estuary as:

> ….an inlet of the sea reaching into a river valley as far as the upper limit of the tidal rise, normally being divisible into three sectors: (a) a marine or lower estuary, in free connection with the open sea; (b) a middle estuary, subject to strong salt and freshwater mixing; (c) an upper or fluvial estuary, characterised by freshwater but subject to daily tidal action.

The boundaries between these sectors are not fixed as they are subject to tidal movements and seasonal variability.

Shallow inshore waters or estuaries, such as those of the Manukau and Tauranga Harbours in the North Island of New Zealand, are recognised worldwide for their high productivity. Such ecosystems are important for the maintenance of diversity and for the economies of countries with these resources, because of their ecosystem-service provision (Costanza et al. 1997). Human life is supported by natural ecosystems and the species that constitute them through conditions and processes known as ecosystem services or nature's services (Daily et al. 1997). Ecosystem services (ES) are the life-support systems of the planet (Myers 1996, Daily et al. 1997), and human life cannot exist without these services and functions. Māori, the indigenous people of New Zealand, value natural areas because these people have a strong spiritual connection with the land and sea, and view the environment as an interdependent entity. Some

Māori use estuaries and coastal areas as a source of income through harvesting shellfish and participating in commercial fisheries. New Zealand estuaries are important nursery grounds for bottom-feeding fish such as flounder (*Rhombosolea plebeian* Richardson) and mullet (*Aldrichetta forsteri* Valenciennes), and also provide resting areas for pelagic species such as kahawai (*Arripis trutta* Forster) and kingfish (*Seriola grandis* Castelnau). Apart from providing these important aquatic habitats, estuaries also support a large number of birds that use the intertidal areas as feeding grounds. Estuaries are, however, highly susceptible to a range of water-quality issues as they receive contaminants from many sources (e.g., Holland & Rahman 1999, Manktelow et al. 2005), and there is growing concern about the ways in which pollutants enter and are transported into estuaries where they may possibly accumulate in sediments (Kramer et al. 1994).

The majority of contaminants become biologically available through association with sediment and biological substrates. For example, many contaminants entering a river or estuarine system do so in conjunction with sediments. The latter can be derived from soil erosion, end of pipe inputs and stormwater and urban runoff. The contaminants are gradually bound to the suspended and benthic sediments within each system through flocculation (Chapman et al. 1982, Packman & Jerolmack 2004). The accumulation of contaminants in benthic sediments increases as salinity increases, and the eventual deposition of much of the suspended sediments and associated contaminants occurs in low depositional areas (Droppo et al. 2001). Therefore, the accumulation of contaminants into the sediment matrix of estuarine and intertidal environments has consequences for sediment-dwelling faunal communities and the overall health of the aquatic environment.

Estuarine intertidal areas are one of the most productive natural ecosystems on earth, with a gross productivity of up to 10 kcal/m^2 per year (Kennish 1995). Estuarine areas are under increasing pressure from a variety of sources, the most important being the effects of urbanisation, given that approximately 50% of the world's population now live close to the coast (GESAMP 1990). As a result of increasing soil erosion and runoff from land clearance, dredging and land reclamation, levels of sediment deposition and water turbidity have increased in coastal areas throughout the world (Ellis 1988, Vogt & Schramm 1991, Chou 1996). Sediment

accumulation and contamination tend to be most problematic in estuaries near human settlement and where there is poor tidal flushing.

It is a challenging task to monitor all the contaminants present in sediment and the marine environment and determine what possible impacts these may have, particularly as information on the persistence, bioaccumulation and toxicity of many contaminants is poorly known. The breakdown products of many contaminants also add to the effects of the bioaccumulated chemicals already present in the sediment (Coull & Chandler 1982, Kramer et al. 1994), which makes the identification of which chemicals have a disruptive effect on marine organisms and communities even more difficult (Coull & Chandler 1982, Kramer et al. 1994). These disruptive effects have a variety of consequences for organisms and the ecosystems of which they are a part and, ultimately, for human health.

There are a variety of sources of contamination such as stormwater, industrial sites, land clearance and agricultural and harbour activities. As these contaminants accumulate they can have a range of effects on organisms, such as small crustaceans living in the sediment, and their long-term effects on the ecosystem may be amplified by bioaccumulation in higher trophic levels, starting with those animals feeding on sediment-dwelling organisms (Kramer et al. 1994). Prolonged exposure of organisms to contaminants may cause sub-lethal effects, including decreased reproductive and growth rates and changes in sex ratio (Coull & Chandler 1982, Cary et al. 2004, Chandler et al. 2004). These sub-lethal effects have many consequences for the viability of a species and may provide useful indicators of environmental health. Throughout this introduction and the remaining thesis, the term ‘pollution’ and ‘contamination’ will be used to define any harmful or undesirable change in the physical, chemical or biological quality of the environment as a result of the release of e.g., chemicals, organic matter (e.g., sewage), particularly from human activity.

1.3. The state of estuaries in New Zealand

Rapid urban growth has put pressure on the capacities of natural resources and physical infrastructure, particularly around estuaries (Ministry for the Environment 1997). Urban retrofitting, infilling for agriculture and commercial land development has lead to increased demands on conventional infrastructure (e.g., stormwater piping) and has contributed to the decline of mangrove ecosystems this century (Ministry for the Environment 1997, 2002). The costs of maintaining existing and new stormwater and sewage systems using conventional design and engineering approaches are escalating. While extensive piped systems remove discharges from a site, urban stormwater discharges (flow peaks and contamination) are unpleasant and are degrading coastal and inland waterways (Curry 1981, Williamson 1991, Wilcock 1994, Snelder & Trueman 1995). After decades of debate over 'cause and effect' relationships, stormwater impacts remain an unresolved priority concern (Parliamentary Commissioner for the Environment 1998, Ministry for the Environment 2002). Rural areas are also of concern as runoff from these areas includes diffuse sources of pollutants from pastures, horticulture, agriculture and forestry and a number of treated effluents from piggeries and cowsheds (ARC 1994, Statistics New Zealand 2006).

Information on the state of contamination in New Zealand water environments is widespread (Hicks 1993, 1994, Boxall & Maltby 1995, 1997, Huser & Wilson 1997, Kingett Mitchell & Associates 1992, ARC 1994, 2002a, b, Macaskill et al. 1996, Mikkelson et al. 1996, NIWA 2001, Ministry for the Environment 1997, 2002, Parliamentary Commissioner for the Environment 1998, Robien et al. 1997, Snelder & Trueman 1995, Wilcock 1994, Williamson 1991), but there are limited publications providing specific information on the levels of sediment contamination in estuarine environments (Aggett & Simpson 1986, Fox et al. 1988, Hume et al. 1989, De Mora & Demeke 1990, Holland et al. 1993, Williamson et al. 1994, 1995, 1996, Wilcock & Northcott 1995, Bull & Williamson 2001, ARC 2003, and references therein). These monitoring reports have traditionally focused on community composition and the distribution and abundance of macrofauna species (e.g., the common oyster (*Saxostrea glomerata* Gould), estuarine mud crab (*Helice crassa* Dana), estuarine mud snail (*Amphibola crenata* Farnie) and some species of fish) (ARC 2003, and references therein).

The environmental industry is now moving towards integrated standard operating procedures (SOPs), but current SOPs have several significant technological gaps. A key one is their poorly developed ability to characterise effectively any adverse biological effects of exposure to chemicals in the environment. Although land-use practices involving the application of chemicals are heavily regulated, there are still problems associated with soil leaching and runoff from land. There is now a need to move towards a more detailed monitoring structure that will incorporate parameters such as levels of pollutants present at a particular site, measurements of both point source contaminant levels and the interaction of pollutants on estuarine ecology, effects on the biology of organisms, and implications for environmental health. Moreover, appropriate biological methods are needed to properly assess and provide 'early-warning' signals of potential biological effects resulting from chemical pollutants in intertidal/estuarine environments.

1.4. Ecological significance of chemical contamination

There is evidence that the effects of contaminants on marine invertebrates may have consequences on biodiversity and the structure and function of ecological communities. Exposure to low levels of toxicants can result in significant alterations to the function of the endocrine system (i.e., biochemical communication system that regulates body function and responses via chemical messengers, the hormones) in a wide range of species (Pesch et al. 1991, Dillon et al. 1993, Colburn et al. 1996, Depledge & Billinghurst 1999, Crane et al. 2000, 2002).

1.5. Chemical contaminants in sewage

Sewage is one of the most well-known examples of aquatic contamination of estuarine and coastal systems (Gross et al. 2001, Matthiessen & Law 2002, Zulkosky et al. 2002). Before the 1970s, poorly treated sewage water led to severe oxygen deficits, and asphyxiation of many estuarine organisms in the United Kingdom (Matthiessen & Law 2002). For example, contaminants, such as brominated diphenyl, from poorly treated sewage water were later discovered to originate from the Manchester Ship Canal and nearby textile industries (Matthiessen & Law 2002). Since the introduction of improved sewage treatment operations in the last 30 years, the true toxic effects of micro-contaminants have been recorded. Examples

include the poisoning of water through ammoniacal effluent from a nearby gas works, the reduction of the ability of migratory fish species to swim past affected areas, and the adverse effect of mercury inputs from chlor-alkali plants on the survival of organisms (Matthiessen & Law 2002). In a similar study, Kirby et al. (1998) investigated the water quality of estuaries and coastal waters in England and Wales. A simple bioassay using intermediate stages (naupliar and copepodite) of benthic copepod identified a toxicity trend: estuarine contamination is greater than near-shore contamination, which itself is greater than offshore contamination. The bioassays indicated a large difference in the toxic nature of samples taken over a tidal cycle. Toxicity was greatest at low tide, with samples collected at the high tide indicating a low level of toxicity. This cycle differed between estuaries. Bioassays are particularly valuable for detecting and quantifying toxicity where organisms are exposed to mixtures of compounds; although laboratory and semi-field biomarker assays can also establish physiological and biochemical evidence of contamination (McCarthy & Shugart 1990, Chandler & Scott 1991, Chandler & Green 1996, Anderson et al. 2001).

In Norway, male rainbow trout, affected by alkylphenols in discharges from wastewater treatment works, produced the female yolk protein (vitellogenin) and had inhibited testicular growth (Bechmann 1999). Furthermore, disruptions in the moulting process in invertebrates caused by interactions of toxicants with steroid hormone receptors have the potential to affect the survival of crustaceans (Bechmann 1999). Sex ratios of organisms within a community may be affected, resulting in changes to gender ratios. Insecticides produced to target specific endocrine functions, especially moulting and metamorphosis in arthropods, can disrupt the hormonal regulation of moulting in crustaceans, (e.g., harpacticoid copepods) (Fingerman et al. 1998, Oberdörster & Cheek 2000, Bejarano & Chandler 2003).

1.6. Metal contamination

Metal contaminants, whether at sublethal or lethal levels, reduce reproductive rates, increase development times and pre-spawning mortality, and reduce ovary growth and the net fecundity of marine harpacticoid copepod species and other marine species (Coull & Chandler 1982, Kluytmans et al. 1988, den Besten et al. 1991a, b, Lee & Noone 1995, DeWitt et al. 1996, Hutchinson et al. 1999a, b, Anderson et al. 2001, Callaghan et al. 2001, Curieux-Belfond et al.

2001, Hutchinson 2002). Furthermore, copepods are more affected by paired metal mixtures than by metals alone. For example, pollutant mixtures such as antifouling paints can inhibit the progression of life-history parameters *in vitro*, and in the field they can cause synergistic reductions in meiofaunal abundance and diversity (Coull & Chandler 1982, Luoma 1983, Alzieu 1991, 2000, Lee 1991, Chandler et al. 1997, Lotufo 1997, 1998, Horiguchi et al. 2002). The fact that contamination is often the result of exposure to multiple compounds exacerbates its effect on biodiversity.

Tributyltin (TBT) is a widespread contaminant of aquatic environments introduced through leaching from antifouling paints and via waste waters (Smith & McVeagh 1991, Ronis & Mason 1996). The compound is highly lipophilic, readily bioaccumulates, and is highly toxic to a wide range of marine and estuarine organisms. TBT was the causative factor in the decline of commercial oyster (*Crassostrea gigas*) populations in the Bay of Arcachon, France (Alzieu 1991). Abnormalities, consisting of wafer-like chambering of the shell with the formation of an interlamellar jelly, were first observed in Arcachon Bay in 1974 and were attributed to the influence of TBT on calcification. It was found that antifouling paints from recreational and commercial vessels were the primary contributing factors to TBT accumulation in the oysters, and regulations were therefore put in place for vessels under 25 m to restrict the use of antifouling paints on hulls.

Exposure of aquatic organisms to dissolved metals can result in metal deposition in the exoskeleton, although dietary exposure to metals can also result in metal accumulation in internal tissues (Schlekat et al. 1992, Hook & Fisher 2001). It is the concentration of metals in an organism's tissue that elicits a toxic effect, not the concentration in the surrounding water (Hook & Fisher 2002, Hutchinson 2002). Bioaccumulation of toxicants is governed by two factors: the concentration factors in the algal food; and the assimilation efficiency of metals in individuals (Hicks & Coull 1983, Chandler et al. 1997, Hook & Fisher 2001, 2002, Zulkosky et al. 2002). Some contaminants may be excluded from bioaccumulation because of molecule size, and some infaunal (those organisms living within the sediment matrix) organisms can construct burrows/barriers that limit contaminant exchange (Lake et al. 1990). Acclimation and/or adaptation of individuals to metal accumulation may occur as a result of long-term chronic exposures. The fact that sediment dwelling organisms can develop tolerance to toxicants is

important when assessing pollution effects such as altered reproductive output in natural sedimentary ecosystems (Matthiessen & Law 2002).

1.7. Environmental risk assessment techniques

Within the broad Assessment of Environmental Effects (AEEs) and Sediment Quality Tetrad (SQT) assessments, laboratory based studies such as water and sediment quality assessments, acute (partial life-cycle) and chronic (full life-cycle) toxicity tests, chemical and geochemical analysis, biodegradation and toxicity testing, ecological survey procedures for identifying changes in community structure and biomonitoring procedures using biomarkers, support the development of contaminated sediment management frameworks. However, many of these tests are expensive to perform and require advanced laboratory facilities and may not be applicable in countries with limited financial resources.

1.7.1. Methods for the assessment of contaminants in estuarine water

Water quality tests such as solid-phase or solvent extraction, in which contaminants are extracted from a water sample and the concentrated extract used for conducting toxicity tests, provide a 'snap-shot' of environmental contamination. However, methods for assessing contaminated water nevertheless provide important information such as the area of contaminant dispersal and potential toxic effects to aquatic species (Kirby et al. 1998). The acute effects of water borne contaminants to aquatic biota can then be measured by employing lethal concentration studies investigating the concentrations at which fifty percent of test organisms are killed (LC_{50}). These results provide the basis for undertaking full life-cycle tests using bioindicator organisms (e.g., copepods, oligochaetes) to determine reproductive and developmental effects arising from contaminant exposure. However, these studies are not able to identify the effects of sediment-associated contaminants on benthic dwelling communities or the individual organisms.

1.7.2. Methods for assessing contaminated sediments

Sediments are recognised as a sink for many contaminants discharged into surface water bodies. Contaminated sediments can result in adverse ecological effects to sediment-associated biota (e.g., macrophytes, benthos, and demersal fish) and to higher-level biota (e.g., pelagic fish and aquatic birds). Assessing potential impacts of contaminated sediments has traditionally relied on

comparison of sediment chemistry to sediment quality values (SQVs) (ANZECC 2000) and/or laboratory studies. Most of the current Australian and New Zealand Environment Conservation Council (2000) SQVs are conservative and therefore resulting management decisions using these values will also be conservative. However, in the absence of sufficient biological effects data (e.g., biomarker development) these SQVs provide baseline effects/no-effects threshold values for a range of contaminants and provide protection to aquatic organisms.

1.8. Meiofauna as bioindicators of contamination

To date, the species used most often for testing the effects of contaminated sediments on life history parameters have been nereid and capitellid polychaetes, sand-dwelling amphipods and chironomid larvae are often used, however these species may not be the most ideal to use as they may not be represented in the area of interest (Chandler & Green 1996, Chapman 2001).

Meiobenthic taxa (i.e., invertebrates passing through a 1 mm sieve but retained on a 0.063 mm sieve) have characteristics such as spending most of their life-cycle within the sediments, short generation time, high reproductive output and can be easily cultured, making these taxa ideal to develop as bioindicators (Chandler & Green 1996). Copepods as a member of the meiofauna occur throughout freshwater, brackish and marine ecosystems and are an essential component of the marine and freshwater benthos. This assemblage of meiofauna or microscopic invertebrates can be defined as organisms between 53 and 500 μm in length (Wells 1971, 1976, Wells et al. 1982, Higgins & Thiel 1988). Harpacticoid copepods are particularly susceptible to the bioaccumulation of toxicants due to the organisms close association with sediments, sessile life cycle, benthic offspring and high reproductive rates (Strawbridge et al. 1992). As a result, the sublethal effects of contaminants on reproduction, growth, development and behaviour can be rapidly assessed, typically within weeks, enabling the prediction of long-term population effects. Transfer of bioaccumulated toxicants to higher trophic levels can occur through the ingestion of contaminated particulate matter by meiofauna and the subsequent feeding of juvenile fish on these. Because bottom-feeding fish must graze the sediments and filter quantities of sediment, indirect ingestion of contaminated sediment occurs in addition to the consumption of contaminated prey (DiPinto & Coull 1997).

As meiofauna provide one of the most important food sources for juvenile fish, trophic toxicant transfer can occur rapidly and affect community structure and diversity (Higgins & Thiel 1988, DiPinto & Coull 1997). Apart from juvenile fish feeding on benthic copepods and nematodes, many larger crustaceans such as sandy shore crabs often feed on the copepods and nematodes (Higgins & Thiel 1988, Hicks & Coull 1983). Bioaccumulation of toxicants can lead to alterations in the internal regulation of physiological parameters during reproduction and development, and may affect the survival of the organism and its ability to contribute to subsequent generations.

To date, organisations such as the Organisation for Economic Co-operation and Development (OECD) has given high priority to the development of acute and chronic tests with species of marine Crustacea (OECD 1995). A new guideline for testing chemicals using life-cycle of the calanoid copepod *Arcartia tonsa* Dana has recently been developed and submitted to the OECD for review, however the guideline is still in draft format (OECD 2005a). Furthermore, three species of benthic harpacticoids have been suggested as test organisms (OECD 2005b) and include *Nitocra spinipes* Boeck, *Tisbe battagliai* Volkmann-Rocco and *Amphiascus tenuiremis* Sars (ASTM 2004, OECD 2005b). A guide for conducting renewal microplate-based life-cycle toxicity tests with the marine copepod *Amphiascus tenuiremis* has been developed and published as a standard by the American Society for Testing and Materials (ASTM E2317-E2324 2004). However, as previously mentioned the species may not be present in an area of interest and therefore not relevant to testing but the methods may be applicable to other species.

1.9. The usefulness of *Robertsonia propinqua* as a bioindicator of environmental contamination

The estuarine harpacticoid copepod *Robertsonia propinqua* (T. Scott 1894) occurs in both the Northern and Southern Hemisphere's and is abundant in sandy/mud sediments of intertidal habitats of Auckland, New Zealand (Wells et al. 1982, Wells pers. comm.). *R. propinqua* also has been described from Queensland, Argentina, the Andaman archipelago and Culcutta, as well as the original description from the Gulf of Guinea. The species has also been reported (but not described) from Bermuda, Ghana, Belgium, Mediterranean France, the Adriatic, Suez Canal, Aldabra, Mozambique, Maldive Islands and Puget Sound (Wells pers. comm.). *R. propinqua*

have a life cycle consisting of six naupliar and five copepodite stages (copepodite VI being the adult) with sexually dimorphic adults (Huys et al. 1996). Gravid females produce multiple clutches (five to eight) in 14 to 20 days postinsemination and clutches are extruded as dual egg sacs each with five to nine embryos in a planar layout (Huys et al. 1996). *R. propinqua* was polyxenically cultured in a recirculating sediment microcosm at Landcare Research Ltd (Auckland) and individuals used in biological and toxicological studies (Hack unpublished data). Furthermore, *R. propinqua* possesses a generation time of 25 days at 21^{o}C (Hack 2002), allowing for simple and accurate assessment of reproductive and developmental endpoints.

A study investigating *R. propinqua*, a native marine benthic harpacticoid copepod as a bioindicator species for ecosystem health, has been undertaken and results are presented in this thesis. Harpacticoid copepods have not been used for this purpose in New Zealand to date and the development of biomonitoring methods using short-generation and culturable meiofauna may enable environmental consultancies and other authorities to offer new services on pollution characterisation at contaminated and estuarine sites. Furthermore, by using *R. propinqua*, the new methods will be designed for and relevant to, New Zealand but will also provide potential applications in Australia.

1.10. Aims and hypotheses

The aim of the present work was to develop and validate methods to assess the effects of pollution in intertidal areas using a marine benthic copepod as a bioindicator. This aim was addressed by setting the task of using whole organism life cycle effects approach, using acute bioassays (Chapter 4) and sediment toxicity tests (Chapter 5).

Key hypotheses to be tested in this work were:

- **Species composition of benthic meiofauna communities differs according to sediment contaminant levels**

Based on the levels of contaminants known to be present within field site sediments, it was hypothesized that there would be successive reductions in benthic meiofauna community composition proportional with increasing field site contaminant levels (Chapter 2).

● **A mixed microalgal diet reduces the developmental time of *R. propinqua* juveniles to reproductive adult compared with a single microalgal diet**

Because diet is an important component in the successful laboratory culture of copepods, it was expected that a mixed microalgal diet would promote the fastest developmental time of *R. propinqua* compared with a single species diet (Chapter 3).

● ***R. propinqua* exposed to aqueous contaminants leads to reduced survival and individual reproductive output and increased juvenile maturation time**

Based on studies with other copepod species, including different developmental stages (Kovatch et al. 1999, Hagopian-Schlekat et al. 2001, Bejarano & Chandler 2003), it was expected that adults and juveniles exposed to aqueous contaminants would exhibit signs of chemical induced stress (Chapter 4).

● **Copepods exposed to polluted sediments provides a predictive measure with which to assess potential long-term population changes**

Because *R. propinqua* is benthic dwelling (including all life stages) and is in direct association with the sediments, it was hypothesized that exposure of mature adults to sediment associated contaminants would reduce the number of offspring capable of maturing to the reproductive adult (Chapter 5).

The thesis is structured as follows:

Chapter 1. General introduction.

Chapter 2. Benthic meiofauna community composition at polluted and non-polluted sites in New Zealand intertidal environments.

This assesses the distribution and abundance of intertidal meiofauna communities at polluted and non-polluted sites within pre-selected field sites in the Auckland and Bay of Plenty regions.

Chapter 3. Evaluation of cultured microalgae for the rearing of the copepod *R. propinqua* and for use in bioassays.

This evaluates combinations of cultured microalgae that promoted the fastest growth rates for the laboratory rearing of *R. propinqua*.

Chapter 4. Range-finder tests of acute and chronic toxicity of zinc sulphate and atrazine to nauplii and adults of the marine harpacticoid copepod *R. propinqua*.

This evaluates the acute sensitivity of zinc sulphate and atrazine to nauplii and adults using 96h range-finder bioassays. It also examines the chronic full life-cycle reproductive and developmental effects from long-term exposure to the contaminants.

Chapter 5. Toxicity of estuarine sediments using a full life-cycle bioassay with the marine copepod *R. propinqua*.

This examines the chronic reproductive and developmental effects resulting from continued exposure to contaminated sediment using a full life-cycle bioassay while also providing information on an initial pilot study.

Chapter 6. General discussion.

Appendix 1. Assessment of the nematode-copepod ratio as an indicator of pollution in New Zealand intertidal environments.

This examines the applicability of the nematode-copepod ratio as an index of pollution in New Zealand intertidal environments.

CHAPTER 2. Benthic meiofauna community composition at polluted and non-polluted sites in New Zealand intertidal environments

In part adapted from:

Hack, L. A., Tremblay, L. A., Wratten, S. D., Lister, A., Keesing, V. 2007. Benthic meiofauna community composition at polluted and non-polluted sites in New Zealand intertidal environments. *Marine Pollution Bulletin* in press.

Co-author contribution

This chapter is in press as a paper in the *Marine Pollution Bulletin*. Anne Austin is thanked for editorial services.

2.1. Abstract

Meiofauna composition was investigated for six field sites, including polluted and non-polluted sites, within two regions (Auckland and Bay of Plenty) during winter (July–August 2004) in the North Island of New Zealand. Physico-chemical parameters were measured during the sampling period and meiofauna distribution and abundance were compared with these measured parameters. Analysis of meiofauna abundance indicated that foraminiferans, nematodes and ostracods were the taxa that contributed to the variability between field sites within the Auckland region. However, no clear taxa dominance was seen in the Bay of Plenty region. Comparison of meiofauna abundance and physico-chemical parameters was done using multivariate analysis (PRIMER). However, no clear relationships between the parameters were observed in any field site in either region. The Shannon-Weiner index of diversity did not show any clear differentiation between polluted and non-polluted field sites. Therefore, from the present study, the taxa or physico-chemical parameters used could not effectively characterise pollution at the investigated field sites.

2.2. Introduction

Estuarine areas are among the most productive ecosystems, and are under increasing pressure from a variety of stressors including urban and industrial waste effluents. Contaminants introduced into the marine environment by human activities, such as heavy metals, polycyclic aromatic hydrocarbons (PAHs) and organochlorines, tend to adsorb to the surface of fine particles and because contaminants typically bind to the finer silty material of estuarine sediments, this then creates a reservoir of contaminants becoming a source of pollution to the water column and associated organisms (Oberholster et al. 2005). The primary contaminants of concern in New Zealand estuaries are zinc (e.g., galvanised steel, tyres), copper (vehicle wiring), lead (vehicle emissions) and PAHs (also originating from vehicle emissions) and have accumulated in estuarine sediments (Williamson & Wilcock 1994, ANZECC 2000, ARC 2003). Environmental response criteria for these contaminants have been developed and are often used to categorise a site as having either low, elevated or high sediment contaminant concentrations (ARC 2003). The criterion is analogous to traffic lights with green representing low, amber: elevated, and red: high contaminant concentrations (Table 2.1). This response criteria was used in the present study to assist in selecting polluted and reference sites. Furthermore, studies are beginning to indicate that these contaminants are having an effect on the fauna, particularly sediment dwelling organisms by reducing species abundance, increasing contaminant accumulation in shellfish and crustaceans and causing changes in growth and reproductive rates (Ministry for the Environment 1997, and references therein).

Table 2.1. Contaminant concentrations and traffic light colours assigned to the environmental response criteria (source: ARC 2003). Concentrations for zinc, lead and copper are in mg/kg and PAHs in mg/kg.

Traffic Light Colour	Zinc	Lead	Copper	PAH
Green	<125	<30	<19	<0.66
Amber	125-150	30-50	19-34	0.66-1.7
Red	>150	>50	>34	1.7-3

Intertidal areas are also heavily influenced by environmental factors, including the erosion and deposition of sediments from seawater currents and the diffusion of nutrients and air into the sediment matrix, which contribute to the changes in distribution and abundance of benthic

organisms. Studies have investigated the effects of these parameters on benthic dwelling organisms (Dyer et al. 1983, Rees et al. 1999, Schratzberger et al. 2006) but have predominantly investigated the larger macrofauna as they are easily sampled and identified compared with the smaller meiofauna (crustacea, worms etc. between 63 and 500 μm in length).

Meiofauna are an important component of marine benthic communities providing ecosystem services including sediment bioturbation and recycling of organic matter (Higgins & Thiel 1988, Nozais et al. 2005). In intertidal systems meiofauna provide an important link between primary producers as they are consumers of microphytobenthos (Pace & Carman 1996, Pinckney et al. 2003) and provide an important food source to juvenile fish that use intertidal habitats as a nursery ground (Nakagami et al. 2000, Reichert 2003, Uye et al. 2004). Because of their small size and intimate association with the sediments, meiofauna are rapidly affected by changes in abiotic and biotic environmental parameters, therefore resulting changes in community structure can directly affect higher trophic organisms which depend on the meiofauna as a source of food.

The purpose of the present study was to, 1) examine the distribution and abundance of benthic meiofaunal communities at different field locations exposed to known levels of sediment associated contaminants, 2) to investigate relationships between physico-chemical sediment characteristics (redox potential, grain size composition and sediment organic content) and meiofauna distribution and abundance and 3) identify whether abundance of key taxa can be used to characterise polluted and non-polluted sites.

2.3. Materials and methods

2.3.1. Field site locations

2.3.1.1. Auckland region (Figure 2.1)

The Manukau harbour is the second largest coastal harbour on the west coast of the North island of New Zealand, adjacent to the city of Auckland and is the sixth largest harbour in the world. Tidal influences range from a mean high water spring (MHWS) value of 4.17 m to a mean low water spring (MLWS) value of 0.45 m (LINZ 2006). The harbour covers an area of approximately 350 km^2 and is surrounded with extensive urban, industrial and rural land uses. The area supports a population of around 1.3 million (Statistics New Zealand 2006). Previous studies have shown that parts of the harbour close to urban and industrial areas are moderately to

heavily polluted by metals (Williamson et al. 1992) and organic pollutants (Fox et al. 1988, Holland et al. 1993, Williamson & Wilcock 1994, Hickey et al. 1995). Okura estuary is located near the northern edge of the North Shore area of Auckland, New Zealand and is under increasing pressure from urbanisation (Ford et al. 2003). Tidal influences in this area range from MHWS 3.26 m to MLWS 0.45 m (LINZ 2006).

2.3.1.2. Bay of Plenty region (Figure 2.1)

The Tauranga harbour is located on New Zealand's northeast coast in the Bay of Plenty and experiences a MHWS level of 1.86 m and a MLWS level of 0.16 m (LINZ 2006). The harbour catchment covers an area of approximately 200 km^2 and is well developed with extensive horticultural, agricultural, urban and commercial uses. Tauranga city supports a population of around 110 000 (Statistics New Zealand 2006). Ohiwa harbour (2872800E, 6346725N, MHWS 1.9 m, MLWS 0.3 m) is located in the Whakatane region, south of Tauranga city with a population around 33 000 (LINZ 2006, Statistics New Zealand 2006). The harbour catchment covers an area of approximately 27 km^2 and is surrounded by rural pastures and low density housing, mainly in the Ohope Beach area.

2.3.2. Field site classification (Figure 2.1)

Contaminant information for each field site detailed in Table 2.6 was taken from available Regional Council publications and does not represent contaminant analysis undertaken as part of this study. However, Auckland Regional Council as part of a long-term monitoring programme for chemical contaminants in marine sediments of the Auckland urban area has collected data since 1998 which enabled changes in contaminant concentrations to be recorded (ARC 2002c, 2003). The present Auckland field sites were selected based on the published reports and ensured the multi-year contaminant data could be used to determine changes in community composition in the present study. Sites within the large metropolitan Auckland region included two polluted sites, Hastie Ave (2670560E, 6471585N, Manukau harbour) and the Railway Yards (2673345E, 6472140N, Manukau harbour) and one reference site within the Okura estuary (2663765E, 6501480N). Both sites within the Manukau harbour have been classified as having 'unhealthy' benthic communities and the area surrounding the Okura estuary as having 'healthy' benthic communities (ARC 2003). Okura estuary is surrounded by a small urban area and rural pastures and levels of contaminants have been recorded at or below the ANZECC (2000) water quality

guideline values. The primary contaminants of concern for the urbanised Auckland region are zinc, lead, copper and polyaromatic hydrocarbons (PAHs) (ARC 2003). The selected sites within the harbour have recorded contaminants above the ARC (2003) high contaminant guideline values for zinc, lead and copper (i.e., >150 mg/kg, >50 mg/kg and >34 mg/kg respectively), but below the PAH guideline value (i.e., <0.66 mg/kg) (Table 2.6). Lead concentrations in the Railway Yard field site have recorded levels below the guideline value. Conversely, contaminant levels recorded within the Okura estuary have been recorded at levels at or below the low contaminant guideline values; <125 mg/kg, <30 mg/kg, <19 mg/kg and <0.66 mg/kg, for zinc, lead, copper and PAHs, respectively (ARC 2003) (Table 2.6).

Sites within the smaller metropolitan Tauranga region included two polluted sites, Waikareao Foreshore Reserve (2788350E, 6384875N, Tauranga harbour) and Te Puna estuary (2779155E, 6388855N, Tauranga harbour) and one reference site within the Ohiwa harbour (2866425E, 6347630N, Whakatane). These sites were selected based on areas investigated as part of a three year marine sediment contaminant survey undertaken by Environment Bay of Plenty (2003). As for the Auckland field sites, the multi-year contaminant data could be used to determine changes in community composition in the present Bay of Plenty field sites. Both polluted sites have lower levels of sediment associated contaminants compared with those recorded in the Auckland field sites, namely, zinc, lead and copper (25-40 mg/kg, 4-6 mg/kg, 1-3 mg/kg, respectively), but these contaminants and their respective values are nevertheless a cause of concern for the region. Polycyclic aromatic hydrocarbons (PAHs) compounds exceeded the analytical detection limits (2 µg/kg) within the Waikareao Foreshore reserve field site (32 µg/kg, Environment Bay of Plenty 2003) indicating a low to moderate environmental impact, however this recorded value was lower than the ARC (2003) low contaminant guideline value (i.e., <0.66 mg/kg). No data for PAH levels was available for Te Puna. Contaminants in the Ohiwa harbour (Whakatane) have been recorded well below the low contaminant guideline values: <125 mg/kg, <30 mg/kg, <19 mg/kg and <0.66 mg/kg, for zinc, lead, copper and PAHs, respectively (ARC 2003) (Table 2.6).

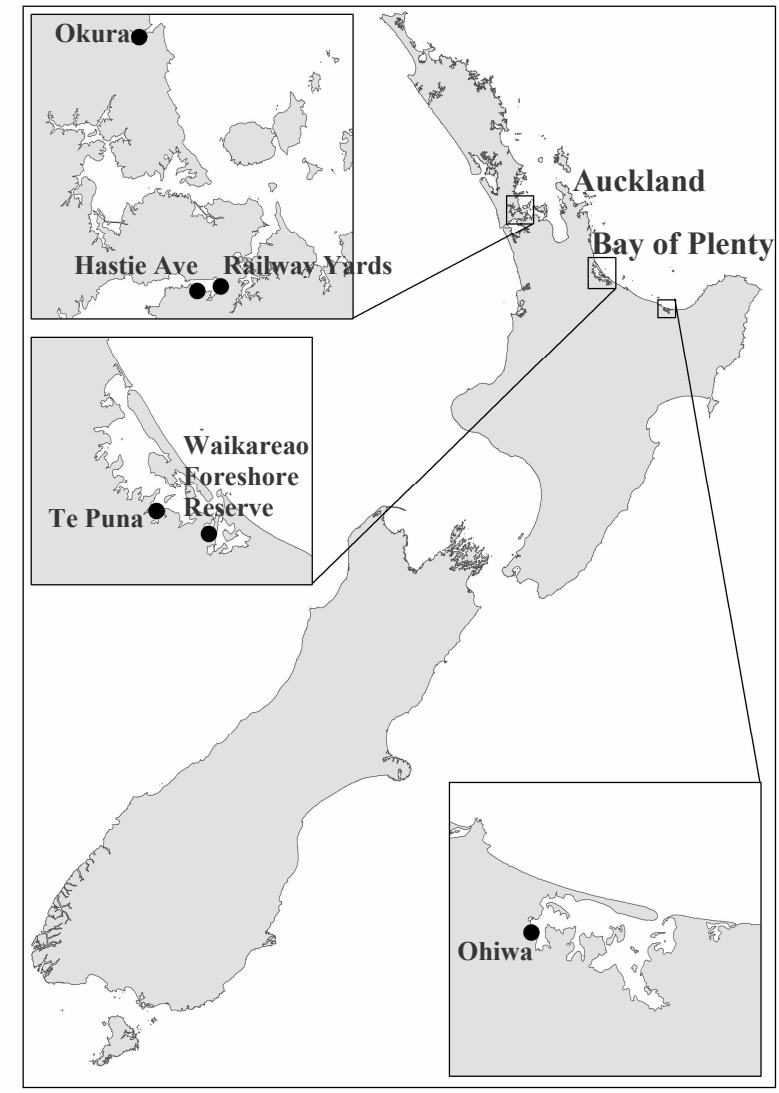

Figure 2.1. Map of New Zealand indicating location of field sites within the Auckland and Bay of Plenty field regions.

2.3.3. Experimental procedure

Sediment samples for physico-chemical and meiofauna community analyses were collected at each of the six field sites (Okura estuary (reference), Hastie Ave, Railway Yards, Ohiwa harbour (reference), Waikareao Foreshore Reserve and Te Puna) during winter (July-August) 2004. Three 30 m transects were laid perpendicular to the shoreline and located below mean high water spring level at all field sites. Samples for biological and physico-chemical analyses were collected at 0, 10, 20, 30 m intervals and sampled from within two 1 m^2 quadrats. The sampling procedure included the collection of three haphazard 30 ml sediment samples for meiofauna

composition analysis and two 30 ml sediment samples for organic analysis. Meiofauna and organic samples were collected by scraping the upper oxic sediment layer. The samples were analysed for total organic carbon and calcium carbonate composition by Landcare Research Ltd, New Zealand using the methods outlined by Leco (Laboratory Equipment Corporation) and Blakemore et al. (1987). Redox potential discontinuity (RPD) depth was recorded from within each replicate quadrat and consisted of three measurements of the organic (oxic) layer. Samples for mean sediment grain size analysis were collected at 15 m from each transect and analysed by Auckland University, New Zealand using a Malvern Mastersizer 2000 laser diffraction particle analyser (Allen 1992).

2.3.4. Statistical analysis

Meiofaunal total abundance data for each site and field region were analysed using multi-dimensional scaling (MDS) analyses using the PRIMER (Plymouth Routines in Multivariate Ecological Research) statistical package. The MDS plots were carried out on totals over the quadrats and cores, giving 12 samples (3 transects and 4 distances) for each of the six field sites. Similarity matrices for each region were calculated using the Bray Curtis similarity measure and to reduce the influence of taxa with very large abundances, data was square root transformed prior to calculating the similarities.

Principal component analysis (PCA) was also undertaken to explain the variation in the data and identify which taxon contribute to the variation. However, as results were consistent with the SIMPER and ANOSIM analyses, results have not been included. Analysis of similarity (ANOSIM) was used to test for differences between the sites (data were averaged over transects and distances) and also between each transect distance combination. Bray Curtis was used as a similarity (SIMPER) measure to identify those taxa primarily responsible for the differences between the sites (Clarke & Warwick 1994). The Shannon Weiner Index of Diversity was also used to investigate the biodiversity of the meiofauna community at each distance and site combination in addition to average field site calculations.

2.4. Results

2.4.1. Multivariate analysis of meiofaunal composition

The stress values (degree of correspondence between the distances among points reflects the similarity to other points/samples) of 0.05 and 0.09 for both the Auckland and Bay of Plenty regions respectively, corresponds to the data having "excellent" or "good" ordination in 2-dimensional space, respectively, with "no real prospect of a misleading interpretation" as stated in Clarke & Warwick (1994). Based on the rank similarities between the samples, the closeness of the points on the Auckland and Bay of Plenty MDS plots reflects how similar the field sites are. The current results indicate that predominantly all field sites both reference and polluted, within both regions are different because of the clustering of data points, however, there was some overlap with the two polluted Auckland region field sites, Hastie Ave and the Railway Yards (Figure 2.2A, B).

Analysis using ANOSIM indicated that there were differences between all Auckland field sites when averaged over transects and distances (Table 2.2A). Further investigation showed that in the Okura (reference) and Hastie Ave (polluted) field sites, samples from 0 and 10 m were more similar to each other in meiofaunal composition than to the 20 and 30 m samples. For the remaining Railway Yard (polluted) site, distances were indistinguishable from each other (Table 2.3A). The field site differences were investigated using similarity percentages (SIMPER) which showed that the taxa foraminifera, nematodes and ostracods accounted for between 33% and 85% of the dissimilarity between the three field sites (Table 2.4A). Furthermore, these taxa groups showed the greatest abundances in the Hastie Ave (polluted) field site in comparison with the Railway Yard (polluted) and Okura (reference) field sites (Figure 2.3A, B, C).

ANOSIM analysis indicated differences between all Bay of Plenty field sites (reference and polluted) when averaged over transects and distances (Table 2.2B). Furthermore, Ohiwa (reference) and Waikareao Foreshore Reserve (polluted) samples from 0 and 10 m were more similar to each other than to the 20 and 30 m samples (Table 2.3B). Within the Te Puna field site (polluted), 0 m was different to all other distances (Table 2.3B). Using SIMPER to analyse the differences between field sites it was found that no particular taxa groups consistently accounted

for the dissimilarity between the reference and polluted sites in the Bay of Plenty region (Table 2.4B).

Table 2.2. Analysis of similarity (ANOSIM) values for differences between each field site within the **A)** Auckland and **B)** Bay of Plenty field regions.

A)

Global Test
Sample statistic (Global R): 0.507
Significance level of sample statistic: 0.1 %

Pairwise Tests

Groups	R Statistic	P-value
Okura, Hastie Ave	0.84	0.001
Okura, Railway Yards	0.29	0.001
Hastie Ave, Railway Yards	0.37	0.001

B)

Global Test
Sample statistic (Global R): 0.75
Significance level of sample statistic: 0.1 %

Pairwise Tests

Groups	R Statistic	P-value
Ohiwa, Waikareao Foreshore Reserve	0.85	0.001
Ohiwa, Te Puna	0.55	0.001
Waikareao Foreshore Reserve, Te Puna	0.84	0.001

Table 2.3. Analysis of similarity (ANOSIM) values for each field site within the **A)** Auckland and **B)** Bay of Plenty field regions. Note: 3-decimal places are given for the R-statistic where numerical information would otherwise have been lost.

A)

OKURA (reference)			**HASTIE AVE**			**RAILWAY YARDS**		
Global Test			Global Test			Global Test		
Sample statistic (Global R): 0.043			Sample statistic (Global R): 0.089			Sample statistic (Global R): -0.003		
Significance level of sample statistic: 0.1 %			Significance level of sample statistic: 4.7 %			Significance level of sample statistic: 45.0 %		
Pairwise Tests			**Pairwise Tests**			**Pairwise Tests**		
Groups	**R Statistic**	**P-value**	**Groups**	**R Statistic**	**P-value**	**Groups**	**R Statistic**	**P-value**
0, 10	-0.01	0.503	0, 10	0.01	0.363	0, 10	-0.02	0.568
0, 20	0.08	0.041	0, 20	0.06	0.069	0, 20	0.02	0.215
0, 30	0.08	0.042	0, 30	0.21	0.001	0, 30	0.05	0.102
10, 20	0.03	0.13	10, 20	0.05	0.123	10, 20	-0.04	0.934
10, 30	0.05	0.077	10, 30	0.14	0.003	10, 30	-0.003	0.396
20, 30	-0.002	0.385	20, 30	0.07	0.045	20, 30	-0.02	0.635

B)

OHIWA (reference)			**WAIKAREAO FORESHORE RESERVE**			**TE PUNA**		
Global Test			Global Test			Global Test		
Sample statistic (Global R): 0.09			Sample statistic (Global R): 0.193			Sample statistic (Global R): 0.051		
Significance level of sample statistic: 0.3 %			Significance level of sample statistic: 0.1 %			Significance level of sample statistic: 1.3 %		
Pairwise Tests			**Pairwise Tests**			**Pairwise Tests**		
Groups	**R Statistic**	**P-value**	**Groups**	**R Statistic**	**P-value**	**Groups**	**R Statistic**	**P-value**
0, 10	-0.02	0.682	0, 10	0.001	0.458	0, 10	0.07	0.046
0, 20	0.11	0.022	0, 20	0.16	0.004	0, 20	0.07	0.031
0, 30	0.23	0.001	0, 30	0.31	0.001	0, 30	0.15	0.001
10, 20	0.04	0.127	10, 20	0.29	0.001	10, 20	-0.03	0.746
10, 30	0.15	0.003	10, 30	0.38	0.001	10, 30	0.04	0.142
20, 30	0.03	0.192	20, 30	0.04	0.164	20, 30	0.01	0.326

Table 2.4. Similarity percentages (SIMPER) identifying those taxa that are primarily responsible for the differences between the sites from the ANOSIM analyses within the **A)** Auckland and **B)** Bay of Plenty field regions. Note: average dissimilarity is a measure of dissimilarity between the field sites, dissimilarity standard deviation is a measure of the variability, cummulative % is the number of observations that lie above a particular value in the data set.

A)

Groups Okura and Hastie Ave				Average dissimilarity = 36.09	
Taxa	**Okura average abundance**	**Hastie Ave average abundance**	**Average dissimilarity**	**Dissimilarity S.D.**	**Cummulative %**
Foraminifera	63.94	452.74	18.47	4.12	51.16
Ostracods	22.11	90.88	6.58	3.52	69.38
Nematodes	79.5	121.88	4.85	1.37	82.83
Gastropods	0.42	3.14	1.94	2.55	88.21
Polychaetes	2.92	0.36	1.47	1.46	92.28
Groups Okura and Railway Yards				Average dissimilarity = 30.03	
Taxa	**Okura average abundance**	**Railway Yards average abundance**	**Average dissimilarity**	**Dissimilarity S.D.**	**Cummulative %**
Foraminifera	63.94	181.1	10.04	1.26	33.43
Nematodes	79.5	65.94	6.55	1.37	55.24
Ostracods	22.11	49.75	3.45	1.26	66.75
Gastropods	0.42	4.07	3.05	1.74	76.91
Polychaetes	2.92	0.18	2.31	1.6	84.6
Copepods	5.44	7.01	2.2	1.72	91.93
Groups Hastie Ave and Railway Yards				Average dissimilarity = 30.50	
Taxa	**Hastie Ave average abundance**	**Railway Yards average abundance**	**Average dissimilarity**	**Dissimilarity S.D.**	**Cummulative %**
Foraminifera	452.74	181.1	15.77	1.61	51.7
Nematodes	121.88	65.94	6.09	1.7	71.66
Ostracods	90.88	49.75	4.32	1.8	85.83
Copepods	4.4	7.01	1.62	1.72	91.15

B)

Groups Ohiwa and Waikareao Foreshore Reserve				Average dissimilarity = 28.38	
Taxa	**Ohiwa average abundance**	**Waikareao average abundance**	**Average dissimilarity**	**Dissimilarity S.D.**	**Cummulative %**
Amphipods	0.24	14.97	7.09	5.3	24.96
Nematodes	14.83	44.21	5.25	1.95	43.47
Polychaetes	0.69	10.54	4.71	3.11	60.06
Foraminifera	31.54	57.07	4.17	1.45	74.75
Ostracods	18.13	37.33	3.54	1.98	87.22
Copepods	8.22	10.78	1.77	1.25	93.47
Groups Ohiwa and Te Puna				Average dissimilarity = 22.55	
Taxa	**Ohiwa average abundance**	**Te Puna average abundance**	**Average dissimilarity**	**Dissimilarity S.D.**	**Cummulative %**
Foraminifera	31.54	66.39	6.36	1.69	28.19
Copepods	8.22	0.29	5.87	2.54	54.22
Ostracods	18.13	36.67	4.18	1.36	72.74
Nematodes	14.83	23.03	2.48	1.33	83.75
Polychaetes	0.69	1.57	1.22	1.49	89.16
Bivalves	0.85		1.15	1.16	94.26
Groups Waikareao Foreshore Reserve and Te Puna				Average dissimilarity = 27.39	
Taxa	**Waikareao average abundance**	**Te Puna average abundance**	**Average dissimilarity**	**Dissimilarity S.D.**	**Cummulative %**
Amphipods	14.97	0.01	7.36	6.58	26.88
Copepods	10.78	0.29	5.48	4.17	46.88
Polychaetes	10.54	1.57	3.66	2.41	60.26
Formainifera	57.07	66.39	3.57	1.43	73.29
Nematodes	44.21	23.03	3.43	1.38	85.81
Ostracods	37.33	36.67	2.53	1.38	95.03

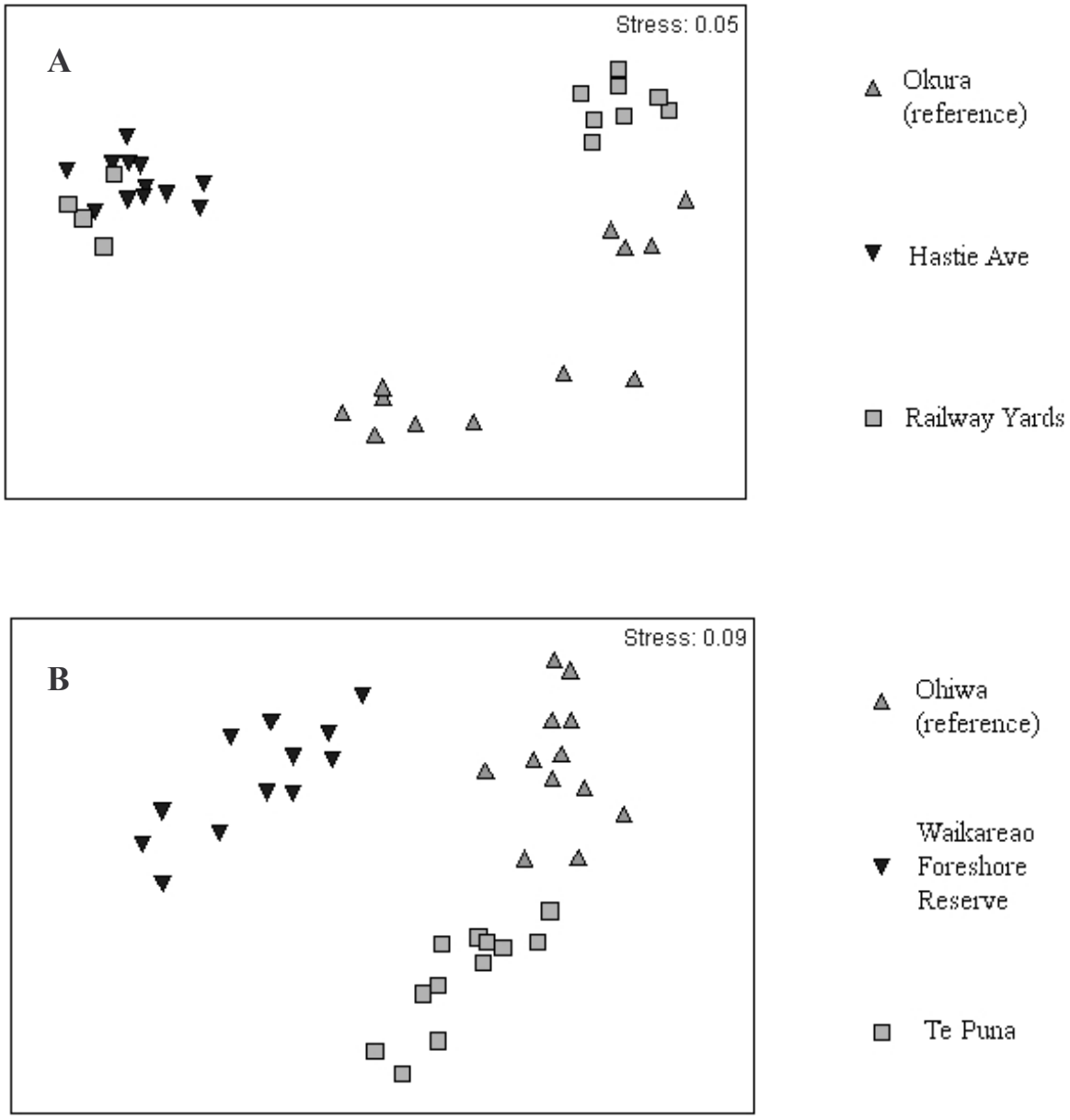

Figure 2.2. Multidimensional scaling of taxa abundances from each field site within the **A)** Auckland field region and **B)** Bay of Plenty field regions.

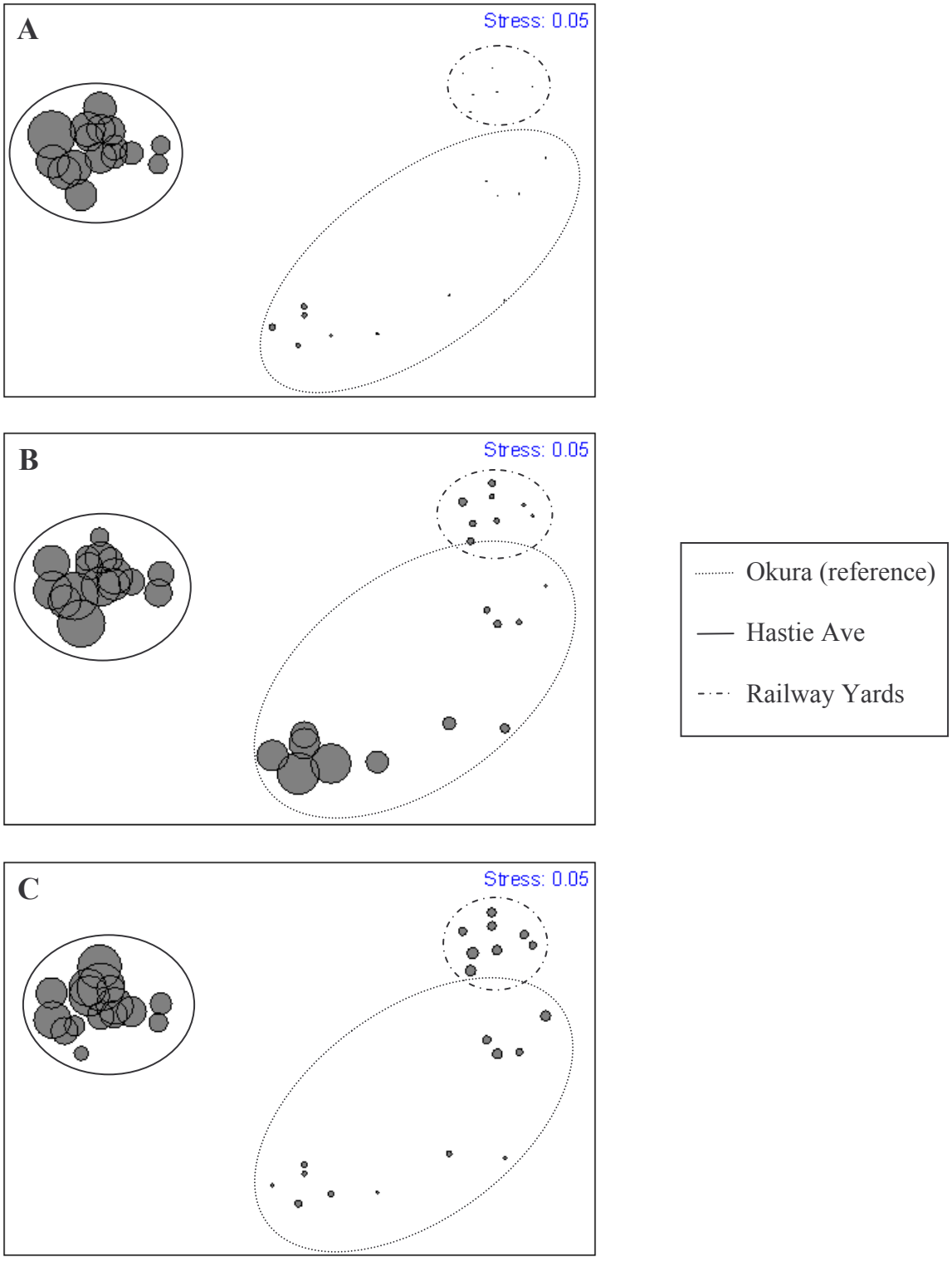

Figure 2.3. Multidimensional scaling of taxa abundances for **A)** foraminifera, **B)** nematodes and **C)** ostracods. Bubbles have been grouped relative to the Auckland field sites and the sizes represent the abundance of each taxa within each Auckland field site.

2.4.2. Redox potential discontinuity measurements

Mean redox potential discontinuity (RPD) measurements recorded in Okura (reference) were significantly different from the two polluted sites, Hastie Ave and Railway Yards (Table 2.5). This was further illustrated in Figure 2.4A where the mean RPD layer measurements were greater and therefore deeper in Okura followed by the Railway Yards and Hastie Ave field sites. This trend was not observed in the Bay of Plenty region field sites where the polluted Waikareao Foreshore Reserve site was significantly different to both Ohiwa (reference) and Te Puna (polluted) field sites (Table 2.5). This was graphically represented in Figure 2.4B whereby the mean RPD measurements were deepest in the Waikareao Foreshore Reserve site.

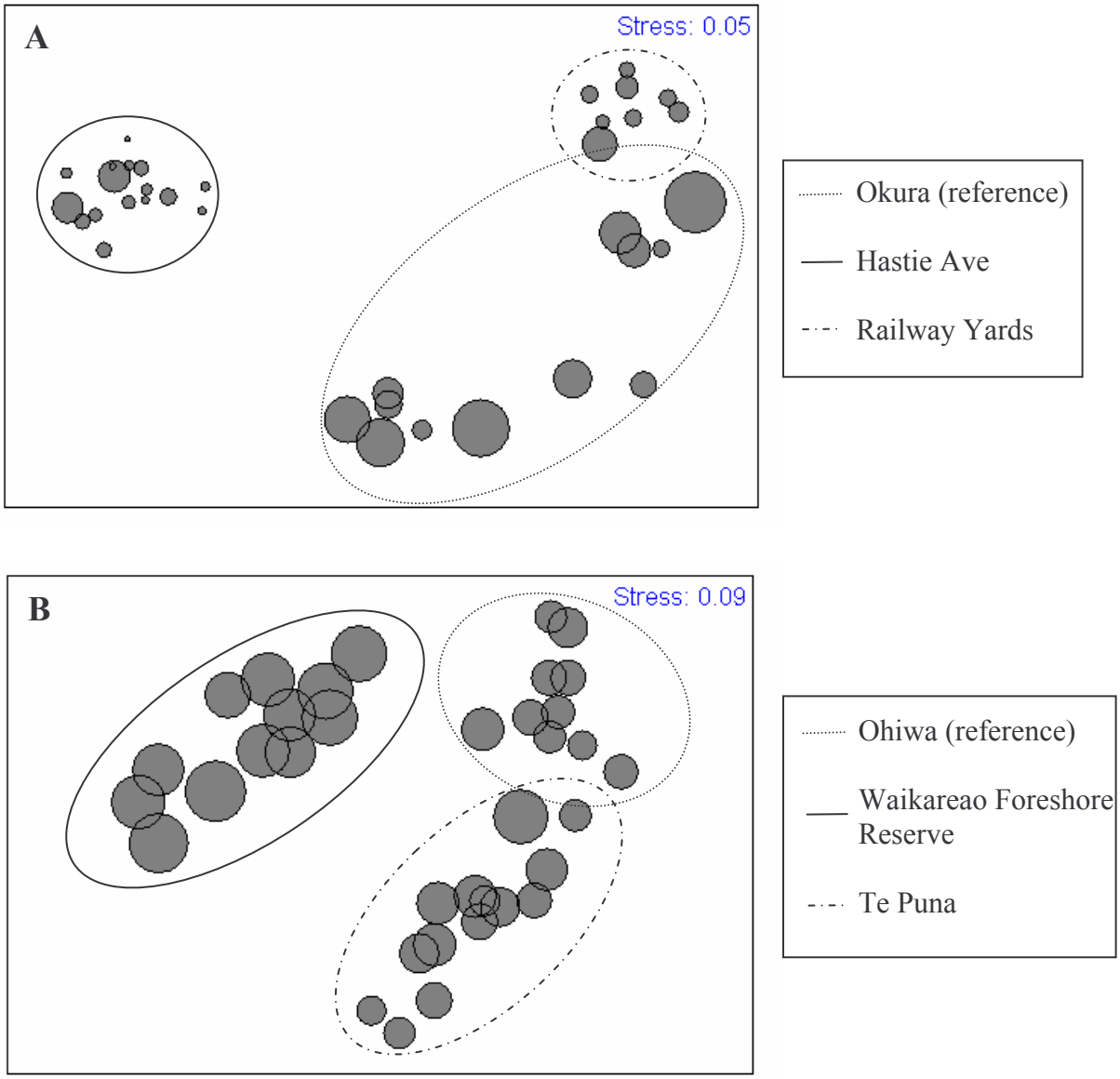

Figure 2.4. Multidimensional scaling of taxa abundances superimposed with the redox potential discontinuity (RPD) (cm) layer measurements in the **A)** Auckland and **B)** Bay of Plenty field regions. Bubbles have been grouped relative to the Auckland and Bay of Plenty field sites and the sizes represent the mean RPD values for each field site.

2.4.3. Carbon-nitrogen ratios

Mean carbon-nitrogen (C-N) ratios recorded in the polluted Railway Yard (14.6) field site were significantly different compared with the Okura (reference) (11.7) field site but no significant difference was observed compared to Hastie Ave (polluted) (12.5) (Table 2.5). This was further represented in Figure 2.5A whereby the larger mean C-N ratios occurred in the Railway Yard field site within the Auckland region. In comparison, the mean C-N ratios recorded in the Ohiwa (reference) (14.4) field site were significantly different from both the polluted Waikareao Foreshore Reserve (12.1) and Te Puna (9.8) sites. In addition, Figure 2.5B graphically represents in 2-dimensional space the larger mean C-N ratios recorded in the Ohiwa field site compared with the Waikareao Foreshore Reserve and Te Puna field sites.

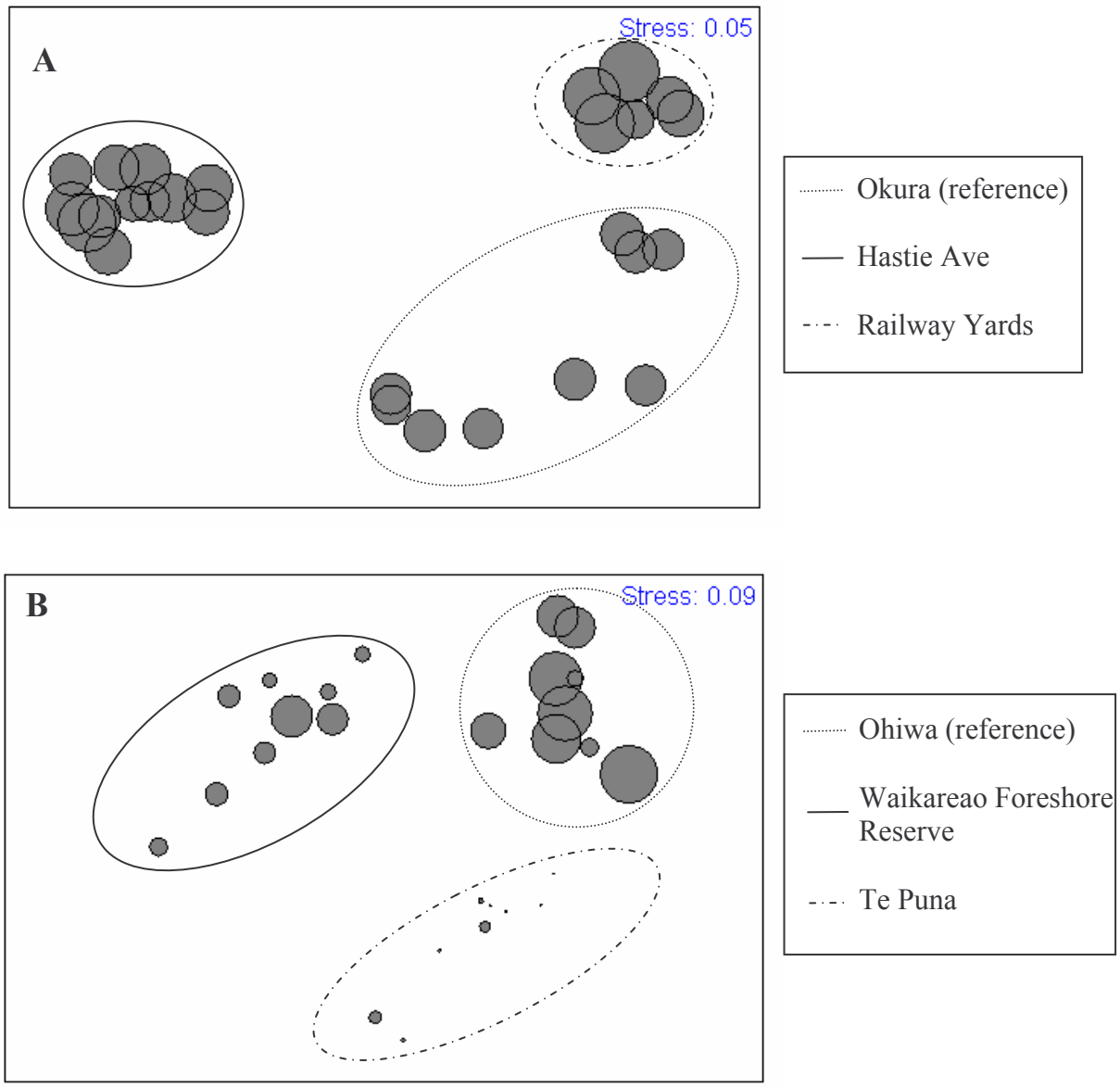

Figure 2.5. Multidimensional scaling of taxa abundances superimposed with the carbon-nitrogen ratios in the **A)** Auckland and **B)** Bay of Plenty field regions. Bubbles have been grouped relative to the Auckland and Bay of Plenty field sites and the sizes represent the mean carbon-nitrogen ratios for each field site.

2.4.4. Sediment grain size

The average sediment grain size composition in the polluted Hastie Ave and Railway Yards sites within the Auckland region was larger with an average particle size of (171.6–223.5 µm ± standard error, 46.6–31.9 µm, respectively) compared with Okura (88.7 µm ± 6.4 µm) (Table 2.5). In contrast, sediment grain size in the Ohiwa (reference) and Te Puna (polluted) field sites (217.2–218.7 µm ± 2.1–3.7 µm, respectively) were very similar and differed by only 1.5 µm and in addition, the Waikareao Foreshore Reserve (polluted) field site recorded the greatest average grain size of (252.2 µm ± 42.8 µm) (Table 2.5).

Table 2.5. Volume weighted sediment grain size (µm) averaged over distances and transects for each field site, mean redox potential discontinuity (RPD) (cm) and mean carbon-nitrogen ratios for all distances and transects for each field site within the Auckland and Bay of Plenty regions. The 95% confidence intervals (CI) or standard errors for the means are shown.

		RPD		Carbon-nitrogen		Grain size	
		mean	95% CI	mean	95% CI	mean	Standard error
Auckland	Okura (reference)	15.1	11.6-18.6	11.7	11.4-11.9	88.7	6.4
	Hastie Ave	4.8	3.9-5.7	12.5	11.5-13.4	171.6	46.6
	Railway Yards	8.8	7.0-10.6	14.6	13.0-16.2	223.5	31.9
Bay of Plenty	Ohiwa (reference)	2.6	2.3-2.9	14.4	12.9-15.8	217.2	64.5
	Waikareao Foreshore Reserve	3.8	3.7-4.0	12.1	11.3-12.9	252.2	42.8
	Te Puna	2.7	2.5-2.9	9.8	9.5-10.2	218.7	32.4

2.4.5. Shannon Weiner index of diversity

Shannon-Weiner diversity measures (H') did not indicate any significant trends with distance down the shoreline in both the Auckland and Bay of Plenty field sites as all the confidence intervals overlapped (Figure 2.6A, B). Figure 2.7A did illustrate greater variability in species diversity when averaged over all distances in the Okura and Railway Yard sites than compared with the Hastie Ave site due to the wider confidence intervals. Conversely, all diversity measures recorded within the Bay of Plenty field sites (Ohiwa (reference), Waikareao Foreshore Reserve (polluted), Te Puna (polluted)), were significantly different from each other as the confidence intervals did not overlap,

however interpretation must be done with caution as the observed differences were marginal (Figure 2.7B).

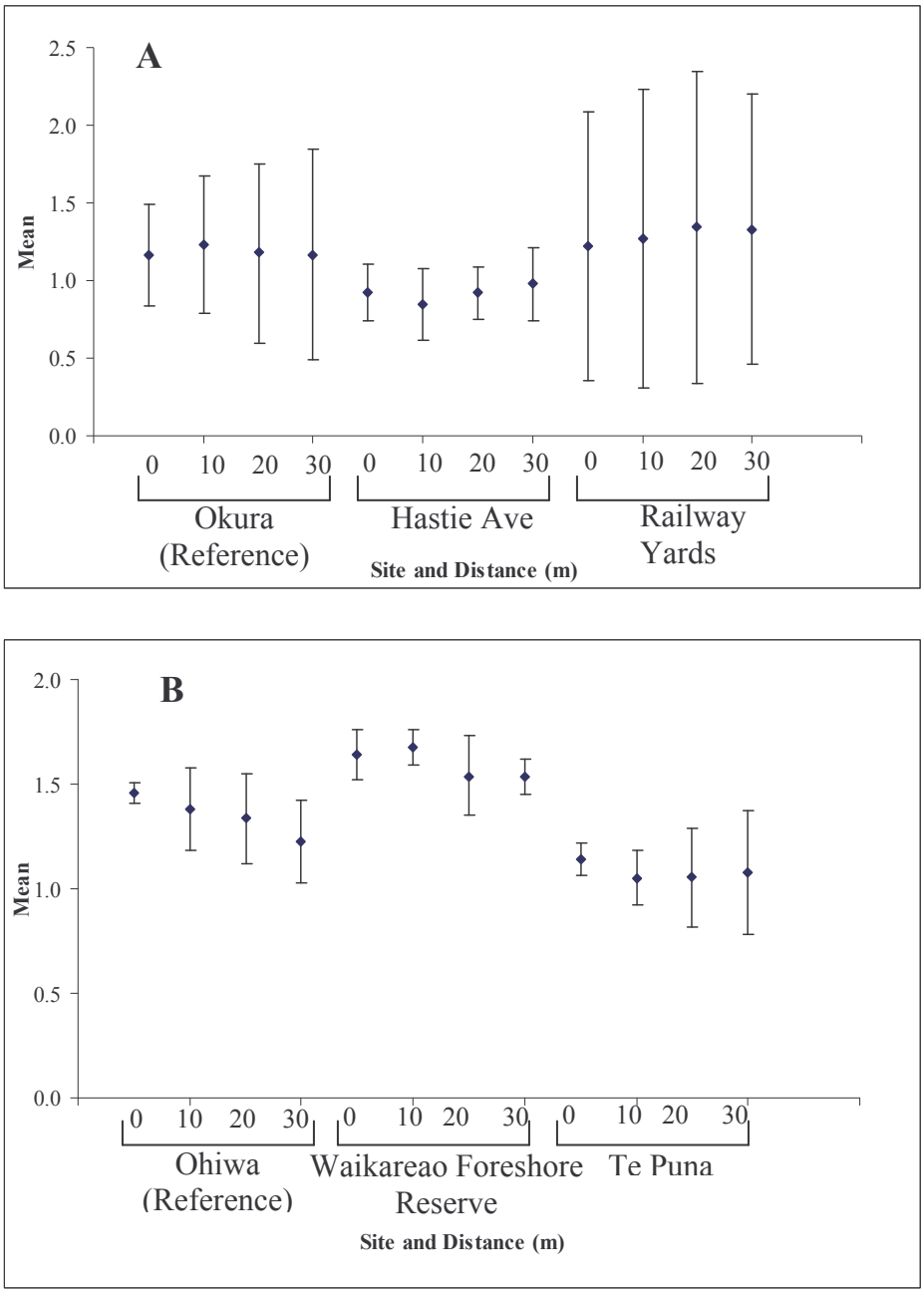

Figure 2.6. Mean Shannon Weiner index of diversity measures for each field site and distance (m) combination for the **A)** Auckland and **B)** Bay of Plenty field regions. The 95% confidence interval bars for each distance diversity measure are shown.

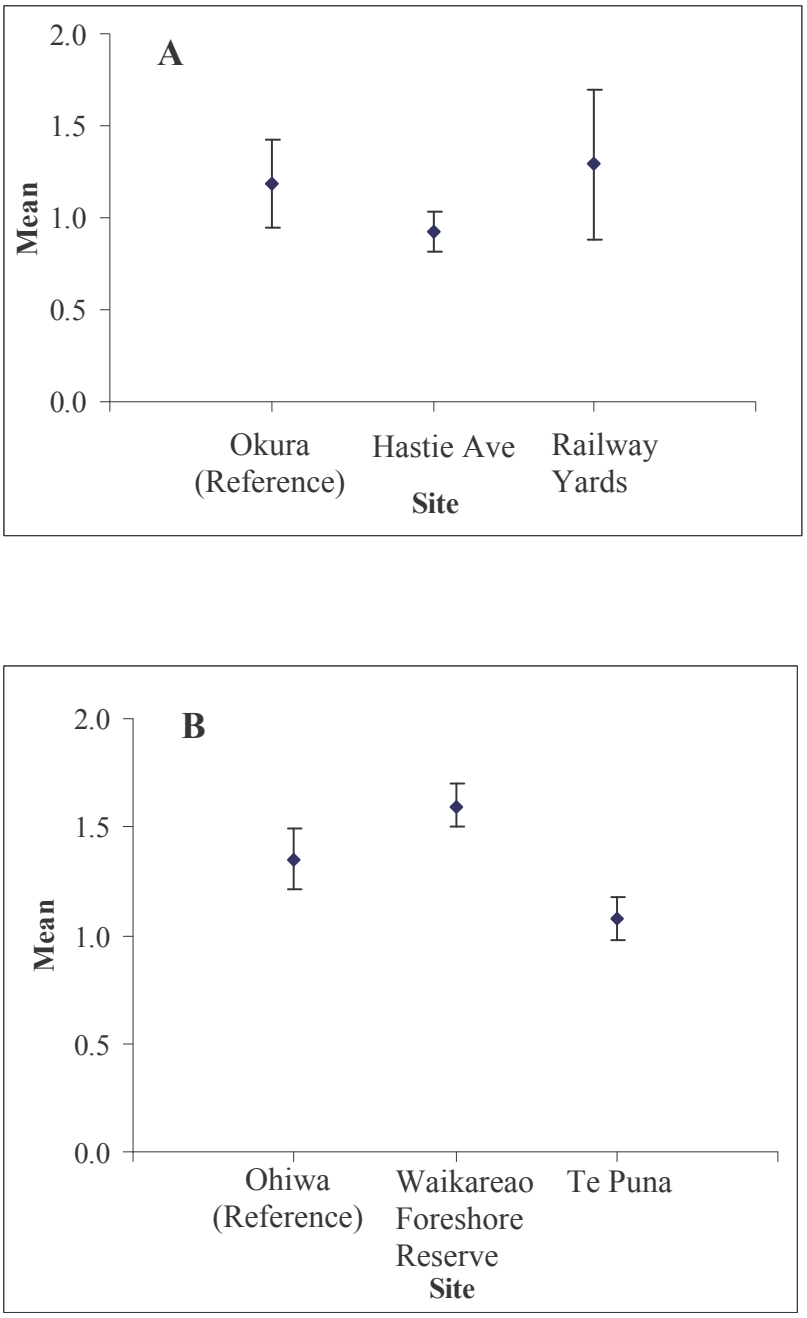

Figure 2.7. Mean Shannon Weiner index of diversity measures for each site within the **A)** Auckland and **B)** Bay of Plenty field regions. The 95% confidence interval bars for the field site diversity measures are shown.

Table 2.6. Contaminant (lead (Pb), copper (Cu) and zinc (Zn)) concentrations (mg/kg) in the 500 µm sediment fraction and polycyclic aromatic hydrocarbons (PAH) concentrations (µg/kg) in the 500 µm sediment fraction recorded in the sediments at each field site within the Auckland and Bay of Plenty field region. Contaminant data was taken from ARC (2003), Environment Bay of Plenty (2003) and Stephen Park, Environment Bay of Plenty (pers. comm.) and refer to sites as close as possible to those listed in the table below. Note: * ARC (2003) contaminant guideline values are given for the Hastie Ave site as no data was available. ARC (2003) contaminant guideline values were above the tabulated contaminant values. ND refers to no available data.

		Contaminants (mg/kg)			
Region	**Site**	**Pb**	**Cu**	**Zn**	**PAH** (µg/kg)
Auckland	Okura (Reference)	10.0	7.0	39.0	16.0
	Hastie Ave*	> 50.0	> 34.0	> 150.0	< 660.0
	Railway Yards	32.0	37.0	155.0	65.0
Bay of Plenty	Ohiwa (Reference)	3.9	4.2	22.9	3.0
	Waikareao Foreshore Reserve	4.4	1.3	40.5	32.0
	Te Puna	6.0	2.8	25.6	ND

2.5. Discussion

Extensive sampling of the benthos in the Auckland and Bay of Plenty regions in New Zealand has allowed us to provide an evaluation of the relationships between meiofauna, their sedimentary habitat and sediment contaminants. The results obtained provide a general picture of the spatial distribution of the meiofauna communities and their variability during the winter of 2004. A major limitation in this study was the collection of winter samples only and in the absence of seasonal data, could lead to an underestimation of meiofaunal distribution and abundance and associated physico-chemical parameters at each field site. The sediment physico-chemical parameters selected for investigation in this study (redox potential discontinuity layer (RPD), organic and grain size composition) represent some of the major environmental parameters known to affect the composition and diversity of meiofauna and were used to characterise the ambient situation of the meiobenthos (Higgins & Thiel 1988). Among these parameters, sediment grain size is one of the most important factors as many species of

meiofauna exploit the coarser sediments. Therefore, the greater proportion of fine sediments in a sample determines the degree of accessibility into the coarser substrate. Furthermore, it is known that meiofauna community composition decreases with increasing depth, therefore the depth of the RPD layer directly affects the proportion of oxic sediments available for meiofaunal occupation. Also, in order to understand meiofaunal community distribution and abundance, fluctuations of sediment organic content is required especially as the productivity of meiofaunal communities depends on the availability of organic matter as a food source.

The two polluted sites within the Auckland region (Hastie Ave and Railway Yards) have historically high levels of copper (Cu), lead (Pb), zinc (Zn) and polycyclic aromatic hydrocarbons (PAHs) mainly due to industrial contamination from nearby railway yard operations, steel operations and paint manufacturing (Glasby et al. 1988, Williamson et al. 1995). High levels of contaminants have also been recorded within the Bay of Plenty polluted field sites (Table 2.6). The Auckland reference site (Okura) is a relatively clean estuary but has recorded high levels of contaminants compared to the Ohiwa reference site in the Bay of Plenty (Table 2.6). Okura was selected for its relatively low impacted catchment and the low levels of sediment associated contaminants in comparison with other estuaries in the Auckland region. Furthermore, while the results of this study do not prove contaminants are affecting meiofaunal community composition, the multi-year contaminant data collected in both the Auckland and Bay of Plenty field regions provides some evidence that sediment associated contaminants may be eliciting effects on meiofauna community composition.

The differences between the Auckland field sites were reflected in the MDS plots of taxa abundances which indicated Okura had more variable taxa abundance compared with the two distinctly different polluted sites. However, the Bay of Plenty MDS plots showed clear distinctions between each field site. In addition, the abundances of the taxa, foraminifera, nematodes and ostracods that contributed to the variation between the Auckland field sites, might suggest high sediment organic content (Higgins & Thiel 1988). Furthermore, the variable taxa abundances within each field site may be an

indication of spatial and temporal environmental variability and does not necessarily indicate variation resulting from sediment contamination.

Mean sediment redox potential layer (RPD) was shallower in the polluted Auckland sites (4.8–8.8 cm) compared with a considerably deeper layer in Okura (15.1 cm) which could be attributed to the lower number of mangroves in the polluted sites able to aerate the sediment matrix via pneumatophore/sediment interactions. This result is also an indication that the dispersion of oxygen and nutrients is limited to a shallower band of sediment. This trend was not observed in the Bay of Plenty sites whereby the Ohiwa (reference) and Te Puna (polluted) field sites recorded similar but shallower RPD depth measurements (2.6–2.7 cm) compared with a deeper RPD layer in the Waikareao Foreshore Reserve (polluted) site (3.8 cm). The differences in redox potential depths between each field site may therefore be a limiting factor in the distribution and abundance of meiofauna communities in the selected field sites.

In the present study sediment organic C-N composition was greater in the Auckland region polluted field sites (Hastie Ave and Railway Yards) compared with lower levels in the reference site (Okura), however this trend was not observed in the Bay of Plenty sites. This may be related to the historical pollution loading recorded within the Auckland region and may provide further evidence to support the relationship between organically rich sediments and contaminant sequestration (Knight & Pasternack 1999, Windom 1975). Furthermore, the high sediment scour (i.e., low depositional site) resulting from wave action in Te Puna (Bay of Plenty polluted site) may provide a possible explanation for the low levels of sediment associated organics recorded in this site during the current study.

Hydrodymanic forces (e.g., sediment scouring) are a key factor in determining the sediment grain size composition (Liu et al. 2006) and is considered the most likely factor in determining the greater average grain size in the more exposed Auckland polluted field sites as compared with the smaller average grain size recorded in the more sheltered Okura (reference) site. Despite the presence of mangrove stands in the polluted areas,

wave action may still be scouring the sediments and reducing the proportion of finer sediment particles settling out of the water column in comparison with a higher depositional environment in the Okura site. Particle size differences were variable in the Bay of Plenty field sites and it appears from the current study that wave action may also be the primary factor in determining average grain size in the Te Puna field site compared with the Waikareao Foreshore Reserve and Ohiwa field sites due to the presence of coarser sediment. However, this does not appear to be related to lower levels of sediment associated contaminants as one would expect (Table 2.6) (Bryan & Langston 1992).

The Shannon Weiner diversity index provides a measure of diversity in a community and we would have expected that the unpolluted field sites, Okura and Ohiwa would have high taxa diversity due to reduced contaminant levels reflected in larger H' values and conversely, the polluted sites lower taxa diversity and lower H' values. This trend was also reported in a study by Chen et al. (2006) where the Shannon Weiner values were lower in the lower reaches of the vulnerable Tarim River environment. However the current results showed no relationship between taxa diversity and levels of field site sediment associated contaminants (Table 2.6). Interestingly, the diversity values calculated for each distance down the shore in the Okura (reference) and the Railway Yard (polluted) field sites (Auckland region) were more variable than the Hastie Ave site (polluted) (Figure 2.6A) with the possible explanation being that the meiofauna populations in these two field sites were larger than recorded in Hastie Ave resulting in greater variation.

2.5.1. Conclusions

Further investigation of the taxa and sediment characteristics utilising a wider range of polluted field sites is necessary to characterise pollution at the field sites. Additional measurements including sediment chlorophyll and phaeopigment compositions should also be recorded. To identify fine-scale changes in species distribution and abundance from sediment contamination, it is recommended that all organisms be identified to species level. Alternatively, if taxanomic expertise is limited, identification of species within a group of organisms (e.g., copepods) should be done. Furthermore, it is

recommended that long term monitoring of species composition be done in order to highlight changes in species diversity at each field site due to sediment contaminant levels and environmental variability.

CHAPTER 3. Evaluation of dietary combinations of three algal species for the rearing of the copepod *Robertsonia propinqua*

3.1. Abstract

Dietary combinations of three algal species, *Dunaliella tertiolecta* (chlorophyte), *Isochrysis galbana* (prymnesiophyte) and *Chaetoceros muelleri* (diatom) were compared for their effects on the growth and survival of the laboratory-cultured copepod *Robertsonia propinqua*. Gravid females were collected from laboratory stock cultures of *R. propinqua* and held in microplates with 74 μm mesh cup inserts and artificial seawater (pH 8.2 and salinity 32 ppt) to produce <24-h-old nauplii for the experiment. Nauplii were fed one of six algal diets: single species of *D. tertiolecta*, *I. galbana*, *C. muelleri* and paired mixtures of *D. tertiolecta:I.galbana*, *D. tertiolecta:C. muelleri*, and *I. galbana:C. muelleri*. The experiment continued to stage I copepodite. Growth was measured as developmental duration from <24-h-old nauplii to stage I copepodite and survival as the percent of the total number of nauplii per dietary treatment that survived to stage I copepodite. The developmental time of nauplii was longer when fed single algal diets (11-12 days) compared with mixed diets (10-11 days). Diet did not affect nauplii survival rates as these were the same (98%) across all treatments except the paired *I. galbana: C. muelleri* mixture which produced a 95% survival rate. The algal diets were ranked according to which promoted maximum nauplii growth, 4 being the least optimal and 1 being optimal. These were in increasing order; *I. galbana: C. muelleri* (4), *D. tertiolecta* (3), *I. galbana* (2), *C. muelleri* (2), *D. tertiolecta: C. muelleri* (2), *D. tertiolecta: I. galbana* (1).

3.2. Introduction

The gross chemical composition (total protein, carbohydrate, lipid and minerals) of microalgae varies considerably between species, even when they are grown under standard conditions. Polyunsaturated fatty acids (PUFAs), particularly 20:5(n-3) and 22:6(n-3) are essential for animal nutrition, particularly individual growth and occur in

most microalgae species, such as (prymnesiophytes (e.g., the brown alga, *Isochrysis galbana* Parke affinis *Tahiti*)) and diatoms (e.g., the brown diatom, *Chaetoceros muelleri* Lemmerman)). However, as stated by Brown et al. (1989, 1991, 1997), chlorophytes (e.g., the green alga, *Dunaliella tertiloecta* Butcher) contain very small amounts of these nutrients but are high in others, such as proteins. Therefore an algal diet containing a mixture of species may provide all the required nutrients for growth and development of cultured animals.

In the field, many of the essential dietary biochemical components such as lipids, carbohydrates and proteins cannot be produced by the copepod and are instead obtained directly from algae (Nanton & Castell 1998, 1999). Laboratory-cultured copepods depend entirely upon mass-cultured isolated strains of microalgae and these strains are typically grown in media which are enriched with nutrients and vitamins which supply the essential and non-essential dietary components required by copepods (Brown et al. 1989). Microalgae are the primary food source, but diets including bacteria (Rieper 1982, Carmen & Thistle 1985), detritus (Meyer & Bell 1989), crushed seaweed, diatoms, macroalgae and mixed cereals (Hicks & Coull 1983) have also been used. This varied diet indicates the importance of using a mixture of algae species rather than single species as a food source for the laboratory culture of copepods.

Because of the importance of diet for the nutrition of marine microcrustaceans (e.g., harpacticoid copepods), the productivity of aquatic systems may also be influenced (Breteler et al. 2004). In particular, lipids (essential for growth) in algae and other primary producers seem to determine the quality of food for higher trophic levels (Breteler et al. 2004). Laboratory studies have confirmed that copepods have better growth and survival rates when fed higher quality diets (Huntley et al. 1987, Brown et al. 1989v Rey et al. 2001). This led to there being a more nutritious food source for marine fish larvae (Alheit & Scheibel 1982), specifically sand flounder (*Rhombosolea plebei)*, yellow eyed mullet (*Aldrichetta forsteri*), plaice (*Pleuronectes platessa*) (Norsker & Støttrup 1994), and herring (*Clupea harengus*) (Kinne 1977)).

There is limited information on the optimisation of algal species mixtures for copepod development and survival. Different algal diets can lead to distinct developmental responses in copepods (Carotenuto et al. 2002, Smith & Lane 1985) and few studies have investigated the relationship between the juvenile and adult life-stages of harpacticoid copepods and the use of different algal combinations on individual growth and survival (Ustach 1982, Norsker & Støttrup 1994, Hopp & Maier 2005).

This study investigated the effects of single and mixtures of microalgae species (*D. tertiolecta* (chlorophyte), *Iso. galbana* (prymnesiophyte) and *C. muelleri* (diatom)) on growth and survival of less than 24-h-old nauplii of *R. propinqua* (T. Scott 1894) to first stage copepodite (C_1) stage. These species of algae were chosen as they are laboratory reared commercial food sources and are fed to a wide range of species larvae (e.g., sea cucumber (*Stichopus mollis*) (Morgan 2001) and brine shrimp (*Artemia salina*) (Brown et al. 1989)), throughout New Zealand and Australia mariculture operations (Brown et al. 1989). Because microalgae have varied biochemical compositions, diets based on algal species mixtures in mariculture (the cultivation of marine plants and animals in their natural environment) systems which provide animals with a more balanced mix of vitamins, minerals and nutrients for development and reproduction were tested. The aims of the pilot project were to identify which algal food types either single or paired promoted the fastest growth and development of nauplii of the cultured copepod *R. propinqua*.

3.3. Materials and Methods

3.3.1. Copepod collection

R. propinqua was obtained from clean sediment located in Tramcar Bay, Whangateau Harbour, north Auckland, New Zealand (2670240E, 6542065N). *R. propinqua* (Hamond 1972, Wells et al 1982, Higgins & Thiel 1988) was chosen as a candidate for life cycle analysis and laboratory culture as it has a ubiquitous distribution throughout New Zealand estuarine and intertidal environments and has a short generation time (Wells et al. 1982). Furthermore, to ensure the accurate identification and subsequent laboratory

culturing of *R. propinqua* Professor John Wells (Victoria University of Wellington, New Zealand) assisted with the examination of individuals collected from the Whangateau Harbour, North Island, New Zealand, the USA and from existing collections collected from the North Island of New Zealand

3.3.2. Copepod culture system

Development of the copepod culture system was based on a recirculating seawater system described in Chandler (1986). A pump-driven recirculating culture system was constructed consisting of a bulk artificial seawater (ASW) (prepared from Instant Ocean © artificial seasalts) tank, centrifugal pump with a micron canister filter and culturing tubs (Figure 3.1). An activated carbon canister filter (Aquahort Ltd, New Zealand) with centrifugal pump (Eheim 2260, Germany) and a woven cotton 5 µm filter cartridge were used to pump ASW throughout the culture system at a rate of 0.112 L/min. The activated carbon filter removed contaminants and the larger particulate matter from the seawater, and the 5µm canister filter was used to remove particulates from the liquid medium not captured by the larger activated carbon canister filter. The culture tubs were 600 mL plastic containers each with the inlet of the recirculated seawater flowing through a 55 µm mesh covering, exiting into an out-flow trough. The bulk reservoir tank was a 250 L plastic tub in which a constant volume of 220 L ASW was continuously aerated.

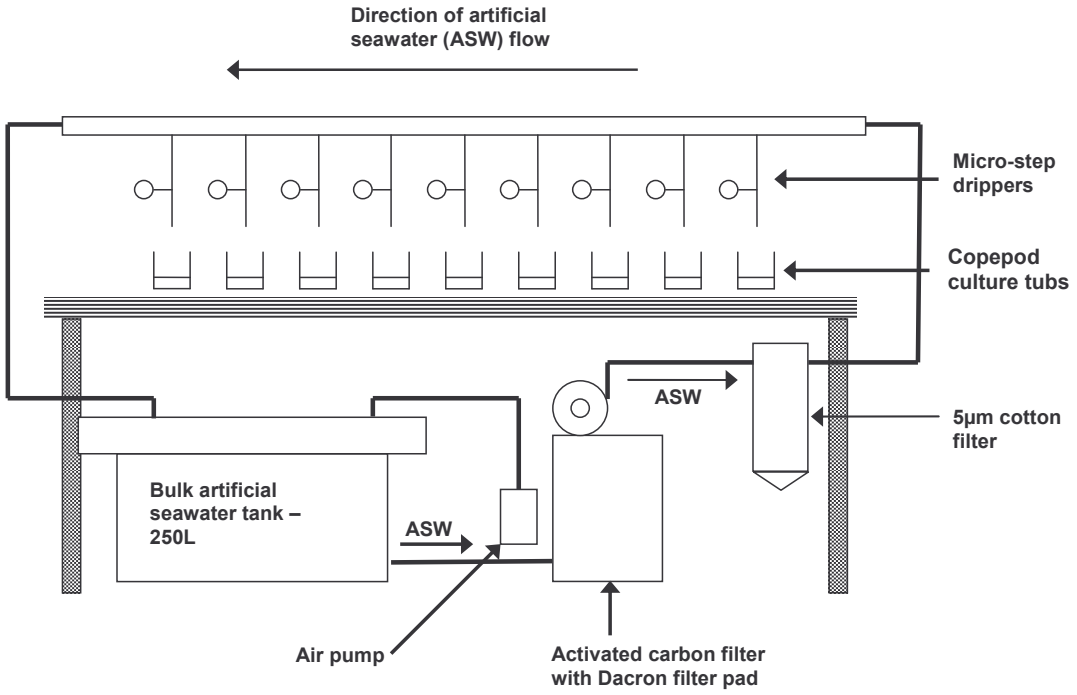

Figure 3.1. Diagram of the recirculating seawater culture system.

3.3.2.1. Preparation of culture medium

The preparation of the culture medium followed methods outlined in Chandler (1986). Copepods were cultured in a sandy-mud sediment substratum prepared as follows. Two 20 L buckets of fresh, oxygenated sandy-mud surface (0-2 cm) sediments were collected by hand from intertidal sand flats in Tramcar Bay, Whangateau harbour, Northland, New Zealand and refrigerated overnight or until processing. Tramcar Bay was selected as the sediment sampling site due to it being located close to Auckland, the relatively undeveloped catchment compared with many other estuarine/intertidal areas in the Auckland region and the known presence of *R. propinqua* (Hack et al. 2007a, in press).

Sediments were washed with reverse osmosis (RO) water through a 280 µm and 180 µm sieve (30 cm diameter) stack flushed over a 20 L bucket containing 10 L of RO water. Sediment fractions retained on the 280 µm sieve formed the base layer (Base) in each of the culture tubs, while sediments passing through the 180 µm sieve formed the flocculent

layer (Floc.). The silt and fine sand fraction passing through the 180 μm sieve was captured in the bucket containing the 10 L of RO water. The bucket contents were allowed to settle for 2-h until most of the finer sand had settled out of the water column and a distinct band had formed between the heavier sand particles and the finer silt fraction. The lighter suspended silt fraction was poured off and discarded. The bucket was refilled with RO water, the sediment homogenised by hand, refrigerated overnight and then the remaining finer silt fraction again poured off. This process was repeated three times to ensure a clean sediment slurry without lighter clay and detrital particles. The sediment fraction retained on the 280 μm sieve was processed using the same methodology.

After processing, 500 mL portions of Base and Floc. sediments were spooned into 1 L glass beakers which were covered with aluminium foil and autoclaved for 1h, allowed to cool at room temperature overnight then refrigerated until required. Autoclaving semi-solidified the sediment, but it was rehydrated by adding ASW then vigorously blended by hand until a consistency similar to that of natural sediment was achieved.

3.3.2.2. Preparation of culture containers

The prepared Base sediment was added to each culture tub (nine tubs, each of which was 9 cm deep and had a surface area of 216 cm^2 (12 x 18 cm). Sediment was added to a depth of 1.5 cm then overlayed with a 1 cm Floc. layer. Aerated ASW was slowly added to each culture tub until the water level was 2-3 cm below the outflow pipe. The sediments were allowed to settle before the tubs were re-connected to the flow-through ASW system. ASW was added to the system at a rate of approximately 0.112 L/min per culture tub.

Copepods were sieved from sediment collected from Tramcar Bay one day prior to culture-tub inoculation. They were gently transferred several times into clean ASW to remove ciliates, nematodes and debris, then gently pipetted into nine culture tubs. The dripper system (see Figure 3.1) was turned off for 1h to allow the copepods to settle into the sediment substrate. Initial cultures of *R. propinqua* were started with a random

mixture of 50-100 gravid, non-gravid females and males, however, culture density increased more rapidly if the proportion of gravid females exceeded that of the non-gravid ones.

3.3.3. Algal stock culture preparation and maintenance

Stocks of *Dunaliella tertiloecta*, *Isochrysis galbana* and *Chaetoceros muelleri* were obtained from the Cawthron Institute (Nelson, New Zealand) and cultured in nutrient-enriched ASW using the methods of Guillard (1972). Algae were cultured using procedures modified from published methods (Guillard 1972, Jefferey 1980, McVey & Moore 1983, Lewis et al. 1986). In summary, 500 mL Erlenmeyer flasks containing 300 mL of ASW (salinity 32 ppt) were autoclaved and allowed to cool overnight. A prepared mixture of vitamins, nutrients and trace metals (Guillard 1972) was then added under sterile conditions. Each flask was gently sterilised under a blue flame then inoculated with enough algae or diatoms to produce an obvious colour change. All cultures were maintained at a constant temperature of 21 ± 1^{o}C and constant 24h light cycle.

3.3.4. Maintenance, feeding and harvest of copepods

After introducing copepods to the culture tubs, the system was maintained in a temperature-controlled room at 21^{o}C ± 1^{o}C and 32‰ ASW salinity with a 12h:12h light:dark photoperiod (maintained by two 18 W white florescent lights). Ammonia, nitrate, and nitrite were maintained below the minimum detection limit (0 mg/L – Aquarium pharmaceuticals (UK) Ltd test colour chart) for the assay. The presence of a buffer (bicarbonate, carbonate and borate) within the artificial sea salts (Instant Ocean © artificial seasalts) maintained the pH at 8.2. Partial ASW exchanges were made in case of elevated levels of ammonia, nitrite and nitrate. Evaporation rates equated to a salinity increase of 1‰ per week, therefore 5 L of RO water was added to the bulk seawater tank to maintain salinity at 32‰ and pH at 8.2. The temperature at which the cultures were maintained was chosen by taking haphazard samples of sediment throughout different times during all seasons and counting the approximate densities of *R. propinqua* individuals, with the intention to reduce temperature fluctuations associated with field seasonal growth rates. *R. propinqua* was observed at its lowest densities during winter.

Culture-tub dripper hosing was replaced every four months and the over-flow trough cleaned regularly of any fouling.

Harpacticoids were fed a mixed (1:1:1) diet of *D. tertiolecta*, *I. galbana* and *C. muelleri*. Early-stage cultures of 100 or fewer copepods were fed 50 mL of the mixed suspension every three days. Mature cultures were fed 50 mL every second day. Drippers were turned off for 1.5h allowing the suspension to settle.

Mature copepod cultures were harvested as follows. A 30 mL plastic syringe was used to draw 20-40% of the surface sediments and copepods into a 500 mL glass beaker. Only 20-40% of the surface sediments were removed so that sufficient numbers of copepods remained to repopulate a culture tub rapidly. Because the sediments were <180 µm in diameter, they were completely washed through a 180 µm sieve leaving only the adults and late stage copepodites to be collected into Petri dishes. The nauplii, early stage copepodites and fine sand were retained on a 70 µm sieve and were transferred back into the appropriate culture tub.

3.3.5. Life cycle and dietary combination analyses

3.3.5.1. Copepod and nauplii collection

Before experiments began, approximately 20% to 40% of the sediment surface was aspirated away from two laboratory stock culture tubs and sieved through a 180 µm sieve. All contents retained on the mesh were washed into Petri dishes and copepods sorted live under a dissection microscope at a magnification of ×40. The content which passed through the mesh was returned to the stock cultures. Artificial seawater was constantly aerated overnight using an aquatic aerator (Hagen Elite 799 air pump) before adding to the microplates. Approximately 20 gravid females/well were randomly placed, using pipettes, into 3 wells of a 12 well microplate with 74 µm mesh cup inserts (Netwell® Corning Costar, Acton, MA, USA) filled with aerated, 32‰ ASW. Microplates were maintained at a temperature of 21°C ± 1°C on a 12h:12h light:dark photoperiod until nauplii hatched. The individuals used for the experiments therefore consisted of nauplii which were <24-h-old.

3.3.5.2. Life cycle analysis

Groups of three nauplii were placed into individual wells (n = 36 wells) on a 96-well microplate and filled with aerated, 32‰ ASW. Every three days this was exchanged (>90%) with fresh ASW and 6 µL of a 1:1:1 mixture (~10^7 cells/mL) of *D. tertiolecta*, *I. galbana* and *C. muelleri* (hereafter Dun.:Iso.:Chae.) added. The endpoints recorded were stage-specific developmental rates, mean growth rate per day, mean time between molts and mean individual size at the last nauplii and copepodite stage-specific molt (µm). Body length was measured daily using a graticule on a microscope at a magnification of ×40 and measured from the tip of the rostrum to the base of the caudal rami. Caudal rami were not included in measurements as their lengths are not always consistent.

3.3.5.3. Experimental feeding regime

Aliquots (45 mL) of stock algae and diatom cultures were centrifuged and concentrated down to 15 mL. Cell densities were quantified using a haemocytometer and appropriate dilutions were made to equal treatments to ~10^7 cells/mL and treatments re-counted for verification. Microalgae treatments consisted of: copepod life cycle analysis, Dun.:Iso.:Chae., dietary analysis, *D. tertiolecta* (Dun.), *I. galbana* (Iso.), *C. muelleri* (Chae.) alone, and Iso.:Chae. 1:1, Dun.:Iso. 1:1 and Dun.:Chae. 1:1 mixtures. Three individual nauplii were placed in each of twenty wells per microplate (n = 60 nauplii per treatment, one 96-well microplate per dietary treatment) containing 200 µL of aerated, 32‰ ASW. Every three days the latter was exchanged (>90%) with fresh ASW and nauplii fed 6 µL of a 1:1:1 mixture (~10^7 cells/mL) of Dun.:Iso.:Chae.. Microplates were held at a constant temperature of 21^{o}C ± 1^{o}C and individuals monitored daily using a microscope as above. The endpoint recorded was time to successful maturation from nauplius to stage-I copepodite (F_1).

3.3.6. Data analysis

3.3.6.1. Life-cycle analysis

The growth rate per day was calculated by dividing the difference in 1) mean nauplii size at hatching and mean size at P_6 and, 2) copepodite mean size at first molt (F_1) and mean size at the final molt (F_5) by the number of days taken for each life stage to complete

development. Time between molts was calculated by dividing the developmental duration by the number of nauplii (six stages – P_1–P_6) and copepodite (F_1–F_5) life stages.

3.3.6.2. Estimation of optimal diet mixture

The mean number of days required for nauplii to molt to the copepodite stage was calculated and associated standard deviations presented. Percentage survival of individuals was calculated for each food treatment then used to provide a ranking of food type that promoted optimal growth. As the current experiment was undertaken as a pilot study no statistical analysis was done.

3.4. Results

3.4.1. Morphological comparison of *Robertsonia propinqua*

Morphological examination of *R. propinqua* individuals showed that individuals collected from the Whangateau harbour, New Zealand, the USA and from existing collections collected from the North Island of New Zealand exhibited at least one morphological difference from the published description (Hamond 1972). Small differences were discovered between the three locations but were found not to be greater than those between individuals within a sample. The specimens from the Andaman archipelago were found to be very similar to those from Sydney and nearly identical to those collected from the USA and individuals cultured for this project. These results do not indicate the specimens from the USA and New Zealand are identical genetically or physiologically therefore it is recommended that further work be done to clarify this.

3.4.2. Life-cycle analysis

The developmental duration for *R. propinqua* was recorded for both the naupliar (11 days, P_1-P_6) and copepodite (14 days, F_1-F_6, F_6 being the mature adult) life stages (Table 3.1). In contrast, the average growth rate per day was approximately 3 times higher for copepodites (21.9 days) than for the naupliar life stage (7.1 days) and there was a 155% difference in the number of days between molts for nauplii (1.8 days) and copepodites (2.8 days) (Table 3.1).

Table 3.1. Developmental duration for *Robertsonia propinqua* nauplii and copepodite life stages including growth rates and the number of days recorded between each life stage molt. Individuals (n = 108) were fed a mixed algal diet of *Dunaliella tertiolecta*, *Isochrysis galbana* and *Chaetoceros muelleri*. Standard deviations are shown in brackets.

	Development duration (days)	Growth rate per day (µm)	Time between molts (days)	Mean individual size at last life stage molt (µm)
Nauplii (P_1-P_6)	11.4 (0.70)	7.1	1.8	157.7 (1.08)
Copepodite (F_1-F_6)	14.5 (0.99)	21.9	2.8	465.5 (0.99)

3.4.3. Estimation of optimal diet mixture

The development time and percent survival for *R. propinqua* fed different combinations of algae from the hatching P_1 nauplius stage to the F_1 copepodite generation are reported in Table 3.2. From the current study, individual algal diets (Iso., Chae., Dun.) led to a marginally longer developmental time (11-12 days) respectively from P_1 to F_1, compared with the mixed algal diets (Dun.:Iso., Dun.:Chae., Iso.:Chae.) which resulted in reduced developmental time (10-11 days). Individual energy allocation to development appeared to increase as Dun. was included resulting in a shorter developmental time between the F_1 and P_1 stages. As a single algal diet Dun. promoted the slowest developmental time (12.6 days (standard deviation (SD) = 0.78), 98% survival) (Table 3.2). The algal combination Iso.:Chae promoted one of the fastest nauplii developmental time (10.2 days (SD = 1.35), although survival (95%) was lower than all other single and paired dietary food types (Table 3.2) and may be explained by the low protein value required for growth (Table 3.3). The algal food types were ranked according to which promoted maximum growth, 4 being the least optimal and 1 being optimal. These were in increasing order; Iso.:Chae. (4), Dun. (3), Chae. (2), Dun.:Chae (2), Iso. (2) and Dun.:Iso (1) (Table 3.2).

Table 3.2. Algal diet promoting maximum growth during the nauplii (P_1-P_6) developmental phase of *Robertsonia propinqua* (n = 60 individuals per treatment). Standard deviations are shown in brackets.

Food type	Total individuals	Mean developmental time (days) (standard deviation)	Survival (%)	Algal diet promoting maximum growth
Dunaliella tertiolecta	60	12.6 (0.78)	98	3
Isochrysis galbana	60	11 (0)	98	2
Chaetoceros muelleri	60	11.3 (0.95)	98	2
D.tertiolecta:I. galbana	60	10.1 (0.25)	98	1
D. tertiolecta:C. muelleri	60	11.1 (0.54)	98	2
I. galbana:C. muelleri	60	10.2 (1.35)	95	4

Table 3.3. The biochemical composition of the experimental algae/diatom species *Dunaliella tertiolecta*, *Isochrysis galbana* and *Chaetoceros muelleri* compared with other commercially available mariculture food species (from Brown et al. 1989). Note: — 22:6(n-3) and 20:5(n-3) polyunsaturated fatty acids not detectable within the algal species, + 22:6(n-3) and 20:5(n-3) polyunsaturated fatty acids are detectable within the algal species. ND refers to no available data.

	Polyunsaturated fatty acid (PUFA) analysis			Protein	Carbohydrate	Lipid	Mineral
Algae/Class	22:6(n-3)	20:5(n-3)	Other PUFA's	(%)	(%)	(%)	(%)
Chlorophyceae							
Dunaliella tertiolecta	—	—	high 16:4(n-3) high 18:3(n-3)	ND	ND	ND	ND
Dunaliella salina	ND	ND	ND	57	32	9	8
Prymnesiophyceae							
Isochrysis galbana	+	+	high 18:2(n-3) high 18:3(n-3) high 18:4(n-3)	41	5	21	13
Bracillariophyceae							
Diatom							
Chaetoceros muelleri	+	+	unknown	ND	ND	ND	ND
Chaetoceros calcitrans	ND	ND	ND	33	17	10	29

3.5. Discussion

Chlorophytes (e.g., green alga - *Dunaliella tertiolecta*) are low in the PUFAs 20:5(n-3) and 22:6(n-3) but are dominated by the 18 carbon-chain-length fatty acids which may explain their low nutritional value as a single dietary source for *R. propinqua* nauplii. This is supported by the current data as *D. tertiolecta* does not appear to be suitable as a single algal diet because of the 12 day developmental period even though percent survival (98%) was the same for all single algal diet treatments (Table 3.2). This may be explained by the lower levels of 20:5(n-3) and 22:6(n-3) polyunsaturated fatty acids (PUFAs) commonly found in high quantities in prymnesiophytes (e.g., *Isochrysis galbana*) and diatoms (e.g., *Chaetoceros muelleri*) (Table 3.3). In comparison, *I. galbana* and *C. muelleri* have high levels of the PUFAs 20:5(n-3) and 22:6(n-3) and have shown that in single and paired algal dietary combinations, growth and survival of *R. propinqua* nauplii are increased (10-11 day developmental period, Table 3.2). The current study provides some indication that growth and survival of *R. propinqua* nauplii improves when *D. tertiolecta* as a dietary constituent is combined with either *I. galbana* (prymnesiophyte) or *C. muelleri* (diatom) (Table 3.2).

In the present study, mixed algal diets, including *D. tertiolecta* with combinations of either *I. galbana* or *C. meulleri* promoted increased growth for *R. propinqua* nauplii. On the other hand, *D. tertiolecta* as a single dietary component and a combination of *I. galbana* and *C. muelleri* did not promote increased growth rates. The results for *R. propinqua* show that when fed a single diet of *D. tertiolecta*, nauplii development from <24-h-old nauplii through to C_1 stage, was approximately 12 days with a 98% survival rate (Table 3.2); the longest developmental time for all experimental feeding treatments. In comparison, a mixed algal diet including *I. galbana* and *C. muelleri* promoted a developmental time of only 10 days with a 95% survival rate, which may be related to the lower protein composition of both *I. galbana* and *C. muelleri*. However, Jónasdóttir (1994) stated that with the exception of nitrogen in a few studies (Checkley 1980, Cahoon 1981, Kiørboe 1989), the specific biochemical components (such as lipids, carbohydrates, proteins and their constituents) that affect different reproductive responses

(e.g., eggs female/day and hatching success of eggs) in copepods have not been identified.

The successful culture of meiobenthic harpacticoid copepods at levels above innoculation densities requires a varied diet as well as information on life history and sediment interactions. Chandler (1986) gave detailed methods with which to maintain continuous cultures of harpacticoid copepods at densities 4-11 times their natural maxima by feeding a mixed diet of chrysophytic (*I. galbana*) and chlorophytic (*D. tertiolecta*) algae, and one diatom species (*Thallassiosira weissflogii*) in a muddy sediment substrate. It has been found that in mixed algal diets, the high levels of protein found in *D. tertiloecta* makes this alga useful for copepod culture, provided it is fed in conjunction with algae rich in 20:5(n-3) and 22:6(n-3) since *D. tertiolecta* is deficient in these PUFAs (Brown et al. 1989, Brown & Miller 1992, Brown & Jefferey 1995). The results of the present study also suggest that *R. propinqua* nauplii (P_1-P_6) developmental rates were fastest when fed *D. tertiolecta* combined with either a prymnesiophyte or diatom species. Other meiobenthic copepod cultures require a mixed algal diet for the successful growth and development of individuals (Chandler 1986, Sun & Fleeger 1995). Kahan (1979) added a variety of common vegetables, such as lettuce and carrot to culture chambers to provide an improved method for copepod culture and to reduce the costs associated with algal culture. No analysis of the nutritional values of these different food items was done, although preliminary visual observations reported copepod densities were maintained at high levels.

3.5.1. Conclusions

In summary, these results suggest that individual growth and development of *R. propinqua* individuals appear to be fastest when fed a mixed algal diet containing *D. tertiolecta* (chlorophyte) and one species of either *I. galbana* (prymnesiophyte) or *C. muelleri* (diatom). However, as growth and development of copepods is relative to temperature, repeat testing and further evaluation using a range of temperature regimes is required to ascertain optimal growth rates. This supports the well known conclusion that a mixed algal diet provides a greater source of essential nutrients for the development of

juveniles than does a single species diet. The algal combinations used in this experiment may promote the successful laboratory cultures of *R. propinqua* for use in toxicology bioassays. However analysis of their nutritional value is now required to differentiate between growth and developmental rates of *R. propinqua* nauplii. Though we understand further detailed investigation is required to ascertain the food combination required by copepods to optimise growth and development, considering the results, growth and development of *R. propinqua* appears to be optimal when fed a mixed diet containing *D. tertiolecta* and another species of alga or diatom. The outcomes of the current experiment provide the first stage in determining a diet which will optimise laboratory reared copepod population carrying capacities.

CHAPTER 4. Range-finder tests of acute and chronic toxicity of zinc sulphate and atrazine to nauplii and adults of the marine harpacticoid copepod *Robertsonia propinqua*

This chapter, formatted differently, is accepted for publication as given below:

Hack, L. A, Tremblay, L. A., Wratten, S. D, Lister, A., Keesing, V. 2007. Acute toxicity of zinc sulphate and atrazine to nauplii and adults of the marine harpacticoid copepod *Robertsonia propinqua*. *New Zealand Journal of Marine and Freshwater Research* in press.

Co-author contribution

This chapter is in press as a paper in the *New Zealand Journal of Marine and Freshwater Research* with the exception of sections 4.3.2., 4.3.3.2., 4.4.2., 4.4.3., 4.5.2., Figures 4.1. and 4.2., as well as information relating to the chronic reproductive and developmental effects of zinc sulphate and atrazine.

4.1. Abstract

Nauplii and adults of *Robertsonia propinqua*, a marine sediment-dwelling copepod, were used in 96h static range-finder toxicity tests to assess the lethal concentration at which fifty percent of test organisms are killed (LC_{50}) by atrazine (a herbicide) and zinc sulphate. Atrazine and zinc sulphate were used at concentrations near LC_{50} values published for crustaceans. LC_{50} values for *R. propinqua* nauplii were 7.5 mg/L for atrazine and 1.7 mg/L for zinc sulphate, respectively. For *R. propinqua* adults, the corresponding values were, 31.8 mg/L for atrazine and 2.7 mg/L for zinc sulphate. *R. propinqua* nauplii were more sensitive than was the adult life stage to exposure to both compounds. This may imply the juvenile life stages of *R. propinqua* are more sensitive to pollutants than is the adult stage. This test provides some evidence that both atrazine and zinc sulphate may be affecting the reproduction of *R. propinqua*. The number of days

taken for *R. propinqua* to extrude the first egg sac appeared to decrease with increasing zinc sulphate concentrations: however the trend was reversed for the 1.3 µg/L and 13 µg/L atrazine concentrations. No trend was discernable for the number of eggs produced per egg sac extrusion at the different contaminant exposures. The data for atrazine and zinc sulphate have important implications for the development of *R. propinqua* as a bioindicator of aquatic contamination, as these range-finding LC_{50} results provide the basis for the determination of a realistic environmental concentration gradient for application in future definitive LC_{50} toxicity tests and chronic full life cycle toxicity tests.

4.2. Introduction

Monitoring the health of intertidal environments requires an understanding of the biological effects of pollutants on the organisms inhabiting the area, particularly sediment dwelling organisms. These organisms spend part of their life cycle intimately associated with the sediment matrix, using it as a source of food and habitat (Wells et al. 1982). Many aquatic habitats are contaminated with a variety of pollutants that can harm organisms because of their toxicity and persistence in the coastal environment, particularly in low energy intertidal environments (Coull & Chandler 1992, Kenaga 1979). Contamination by low concentrations of metals, particularly zinc (Zn) from anthropogenically derived sources can be toxic to many species in these environments (Coull & Chandler 1992, King et al. 2006). Herbicides are also pollutants of concern owing to the widespread application on commercial agricultural crops such as kiwifruit, grapes, corn and sorghum (Ward & Ballantine 1985). These pollutants enter the intertidal environments from a variety of sources such as industrial, urban, agricultural and horticultural runoff and aerial deposition and can persist within the water column or accumulate within the sediments (Coull & Chandler 1992, Ministry for the Environment 1997).

Harpacticoid copepods are an important bioindicator species because they are benthic dwelling, spend their entire life cycle within the sediments, have a widespread distribution, represent a primary food source to juvenile fish, and can be cultured in the

laboratory (Wells et al. 1982, Chandler 1986, Coull & Chandler 1992). Shellfish species (e.g., cockles *Austrovenus stutchburyi*) have traditionally been used in toxicity tests in New Zealand; however, long developmental times makes it difficult to culture this group under laboratory conditions (Purchase & Fergusson 1986).

Acute and partial life-cycle toxicity tests are well developed for some species of macroinvertebrates (e.g., amphipods (Schlekat et al. 1992) and a polychaete worm (Dillon et al. 1993)) and provide inexpensive baseline information. However, these species have predominantly been used in sediment toxicity tests. Furthermore, commonly used macroinvertebrates have long generation times, are difficult to culture in the laboratory, and typically selected life stages are chosen for study, thereby limiting the biological effects range.

The present study examined the acute and chronic toxicities of zinc sulphate heptahydrate ($ZnSO_4.7H_2O$), a salt of heavy metal, and atrazine (2-chloro-4-ethylamino-6-isopropylamino-S-triazine) a herbicide. The US Environmental Protection Agency considers atrazine a priority pollutant as it is one of the most widely used weed control herbicides in US agricultural crop production (Solomon et al. 1996, Bejarano et al. 2004). Atrazine is used widely against grasses and broad-leaved weeds in a variety of vines, orchards, plantations and crops, particularly maize and sorghum (Hall et al. 1994, 1995). In Australia, atrazine has over 1600 registered uses as 30 registered products; however, its use in New Zealand is not as common (ANZECC 2000). In spite of its rarity in New Zealand estuarine environments it was included in the current chapter, as data on the reproductive and developmental effects on the USA estuarine meiobenthic copepod *Amphiascus tenuiremis* exist (Bejarano & Chandler 2003). However, comparison between the current results and the USA data was ultimately found to be difficult as high mortality was recorded for *R. propinqua*.

Zinc is one of the most problematic metals in New Zealand estuarine environments and enters these systems by either natural (e.g., weathering and erosion) or by anthropogenic

(e.g., galvanised iron (i.e., zinc coated), unpainted zinc alum and urban runoff) processes (Ministry for the Environment 1997).

The aims of the work in this chapter were to 1) assess the acute sensitivity of *R. propinqua* to both zinc sulphate and atrazine using the short-term 96-h static range-finder bioassay to establish the approximate LC_{50} values for the two copepod developmental stages; 2) assess the chronic reproductive and developmental effects of zinc sulphate and atrazine on *R. propinqua* using a full life-cycle (juvenile to reproductive adult) bioassay.

4.3. Materials and methods

R. propinqua was mono-cultured in a flow-through sediment microcosm using similar methods to those of Chandler (1986). Artificial seawater (ASW) prepared from Instant Ocean© artificial sea salts (pH 8.2 and salinity 32‰ (intertidal salinity at each field site was measured using a refractometer and determined to be 32‰, unpublished data)) and a temperature of 21°C ± 1°C was used to grow stock cultures of *R. propinqua*. These conditions enabled a generation time (egg – reproductive adult) of 25 days (Chapter 3).

R. propinqua spends its entire life cycle within the sediment, but movement into the overlying water column has been observed as a behavioural response to factors such as light and disturbance (Higgins & Thiel 1988).

4.3.1. 96h acute range-finder toxicity test

Range-finder toxicity tests were undertaken to provide preliminary LC_{50} values for atrazine and zinc sulphate. These preliminary results provide a basis from which to determine concentrations for definitive toxicity testing. However, these tests were not performed due to limited time but existing data are likely to be of value to other researchers.

Stock solutions of zinc sulphate ($ZnSO_4.7H_2O$, reagent grade) and atrazine (certified high purity crystalline standard (98%, Dr Ehrenstorfer) were made to 10 mg/mL concentration

by dissolving in reverse osmosis water and methanol (Mallinckrodt ChromAR grade anhydrous). The final atrazine stock solution contained 0.25% methanol. Stock solutions provided appropriate concentrations for subsequent serial dilutions and were kept in the dark at 0^{o}C until test initiation (Dr Grant Northcott, HortResearch, Ruakura, New Zealand, pers. comm.).

From the 10 mg/mL stock solutions of atrazine and zinc sulphate, 10× concentrations were made by adding 2 mL to 18 mL aerated (>90% dissolved oxygen (DO)) ASW (as previously indicated). Subsequent 100× (0.1 mg/mL), 1000× (0.01 mg/mL) and 10000× (0.001 mg/mL) concentrations were made from these. A control comprising a carrier solvent (methanol in seawater) and a seawater control were used in the atrazine and zinc tests, respectively.

Static (no test solution exchange) 96h acute toxicity exposures were conducted using reproductive adults of *R. propinqua* and stage I nauplii (<24-h-old). At the end of the experiment, the exposure solution concentrations made at the start of the experiment were used as an indication of the 96h copepod exposure concentrations. Methods from the American Society of Testing and Materials (ASTM) and The International Organisation for Standardisation (ISO) were used in the toxicity tests (ASTM E1192 (1997), ASTM E2317 (2004), ISO 14669:1999). Methods outlined by Stephan (1977) and Lassus et al. (1984) were used for this experiment but adjusted to use *R. propinqua* as follows: adult *R. propinqua* individuals were collected from stock cultures, sieved through a 125 µm sieve, sexed, and then placed into three 50 mL replicate crystallising dishes per test serial dilutions (n = 5 male and 5 female individuals per serial dilution) containing 20 mL of test solution. *R. propinqua* nauplii, less than 24-h-old, were collected from gravid females retained in 74 µm mesh cup insert microwells. Individuals were placed into ten 300 µL replicate wells per test serial dilution on a 96-well microplate (n = 20 nauplii per serial dilution) containing 200 µL of test solution. Each serial dilution was added to alternate rows so that all dilutions were physically separated, eliminating edge effects and cross contamination. All crystallising dishes and microplates were covered to reduce contaminant solution evaporation. The tests were carried out for 96h, at 21 ± 1^{o}C using a

12h:12h light-dark photoperiod. Dishes were examined at the same time each day for mortality via a dissection microscope and at a magnification of ×40. Animals exhibiting no swimming or appendage movements within 5 s after gentle prodding with a tungsten wire needle were considered dead. Copepods and nauplii were not fed during the test according to methods outlined in the Standards from the American Society of Testing and Materials (ASTM) and International Organisation for Standardisation (ISO) (ASTM E1192 (1997), ASTM E2317 (2004), ISO 14669:1999).

4.3.2. Chronic reproductive and developmental toxicity test

Experimental conditions followed those used in the acute range-finding toxicity test, except for the following conditions.

Test solutions of atrazine and zinc sulphate were made by adding appropriate amounts of the atrazine and zinc sulphate stock solutions to constantly aerated artificial seawater (ASW), (salinity 32‰ and pH 8.2). Experimental contaminant concentrations used included atrazine 1.3 µg/L, a concentration below the range-finder LC_{50} value recorded in the present study (Table 4.1); 13 µg/L, the ANZECC (2000) guideline trigger value; and 130 µg/L, an environmentally high concentration. Both a carrier solvent (methanol in seawater) and seawater controls were used in the atrazine tests. Zinc sulphate was applied at 1.5 µg/L, a concentration below the range-finder LC_{50} value recorded in the present study (Table 4.1); 15 µg/L, the suggested ANZECC (2000) guideline trigger value; and 150 µg/L, an environmentally high concentration.

The American Society of Testing and Materials (ASTM) standard ASTM E2317 (2004) and those methods outlined by Chandler & Green (2001) and Bejarano & Chandler (2003) were modified for this experiment. Briefly, *R. propinqua* nauplii less than 24-h-old were retained and raised in the 74 µm mesh cup insert microwells until they molted into copepodites. The <24-h-old copepodites were then collected and placed into fifty 300 µL replicate wells per test concentration on a 96-well microplate (n = 50 copepodites per contaminant concentration) containing 200 µL of test solution. Immediately after a gravid female was observed it was removed and placed into another well and 200 µL of

the same test solution added. Plates were monitored daily for development (i.e., presence/absence of molts), mating, egg sac extrusion, number of eggs, presence/absence of nauplii and mortality via a dissection microscope at a magnification of ×40. Test chambers were fed 1 mL of a 1:1:1 mixture of *Dunaliella tertiolecta* Butcher, *Isochrysis galbana* Parke affinis *Tahiti* and *Chaetoceros muelleri* Lemmerman every third day as this amount reduced fouling in each well.

4.3.3. Data analysis

4.3.3.1. Acute 96h range-finder toxicity test

Range-finder tests were conducted to establish a mortality range of 0–100% (Stephan 1977). Lethal dose values (LC_{50}) which resulted in 50% mortality of *R. propinqua* nauplii and adult individuals were established using a generalised linear model with binomial errors in the statistical package GenStat (Release 9.1 2006) (GenStat Committee 2002).

4.3.3.2. Chronic reproductive and developmental toxicity test

Means and standard errors were calculated for the number of days required for mated females to extrude successive egg sacs and the number of eggs produced at each egg sac extrusion at each contaminant exposure concentration. Unfortunately, no multi-generational information was obtained for either contaminant as no nauplii successfully hatched from the egg and developed through the six naupliar and six copepodite life stages.

4.4. Results

4.4.1. Acute 96h range-finder toxicity test

The data presented in this study represent the range-finder dose concentrations and associated 95% confidence intervals of atrazine and zinc sulphate inducing 50% mortality over 96h in a population of *R. propinqua*.

Mortality in both atrazine and zinc sulphate bioassays was 0% in methanol in seawater and in seawater controls. At a salinity of 32‰, zinc sulphate was the most toxic to *R.*

propinqua nauplii less than 24-h-old (96h LC_{50} = 1.7 mg/L, 95% confidence interval (CI) = 1.1–2.5 mg/L). Atrazine (96h LC_{50} = 7.5 mg/L, 95% CI = 2.3–21.9 mg/L) was less toxic (Table 4.1). Furthermore, the *R. propinqua* adult stage was more tolerant than was the less than 24-h-old nauplii to both zinc sulphate and atrazine with a 2.7 mg/L (95% CI = 1.7–4.2 mg/L) and 31.8 mg/L (95% CI = 20.9–49.1 mg/L) 96-h LC_{50} values, respectively (Table 4.1).

When expressing the toxicity of the two tested contaminants, the LC_{50} values for both tested life stages are above the recommended Australian and New Zealand marine and freshwater water quality trigger values (ANZECC 2000) for zinc sulphate (ANZECC marine LC_{50} = 0.015 mg/L) and atrazine (ANZECC freshwater LC_{50} = 0.013 mg/L). Although there are data on fish, crustaceans and algae, ANZECC (2000) considered it preferable to adopt the atrazine freshwater LC_{50} figure as a marine low reliability trigger value. However, this figure has been noted by the Australian and New Zealand Environment and Conservation Council (2000) as an indicative interim working level.

Table 4.1. Measured 96h lethal concentration at which fifty percent of test organisms are killed (LC_{50}) values (mg/L) and associated 95% confidence intervals for *Robertsonia propinqua* nauplius I and adult life stages. The Australian and New Zealand marine and freshwater water quality guideline trigger values are shown (ANZECC 2000).

	Nauplius		Adult copepod		ANZECC (2000) water
Chemical	LC_{50}	95 % CI	LC_{50}	95 % CI	quality trigger value (LC_{50})
Atrazine	7.5	2.3 - 21.9	31.8	20.9 - 49.1	0.013
Zinc sulphate	1.7	1.1 - 2.5	2.7	1.7 - 4.2	0.015

4.4.2. Chronic reproductive and developmental toxicity test

Complete mortality occurred in the atrazine methanol carrier control, therefore no data are presented. Mortality was also high in the artificial seawater control (ASW) with few individuals surviving to provide data analysis.

The results for individuals exposed to zinc sulphate showed a difference in the time (days) between initial male/female coupling and successive egg sac extrusion. Figure 4.1A showed that at a concentration of 150 µg/L, *R. propinqua* produced the first of five recorded paired egg sac extrusions 9 days after initial coupling followed by 15 µg/L (23 days) and 1.5 µg/L (31 days) suggesting that time required to egg sac extrusion increases with lower zinc sulphate concentrations. In addition, the number of eggs produced was variable at each concentration as seen by the variation in standard errors (Figure 4.2A).

Furthermore, atrazine appeared to have some effect on the time taken for *R. propinqua* females to extrude the first paired egg sac from 14 days (1.3 µg/L) to 22 days (13 µg/L) (Figure 4.1B). However, at a concentration of 130 µg/L, the time then appeared to reduce to 10 days, although this observation was limited to two data points (Figure 4.1B). Furthermore, the data suggest that at an atrazine concentration of 1.3 µg/L, female *R. propinqua* individuals can produce a maximum of 10 egg sac extrusions over a 57-day period (Figure 4.1B). In addition, the average number of eggs produced per female exposed to atrazine was variable, although an increase in egg numbers with each successive egg sac was recorded at an exposure of 1.3 µg/L (Figure 4.2B). All other concentrations (13 µg/L and 130 µg/L) showed variation in the average number of eggs produced (Figure 4.2B).

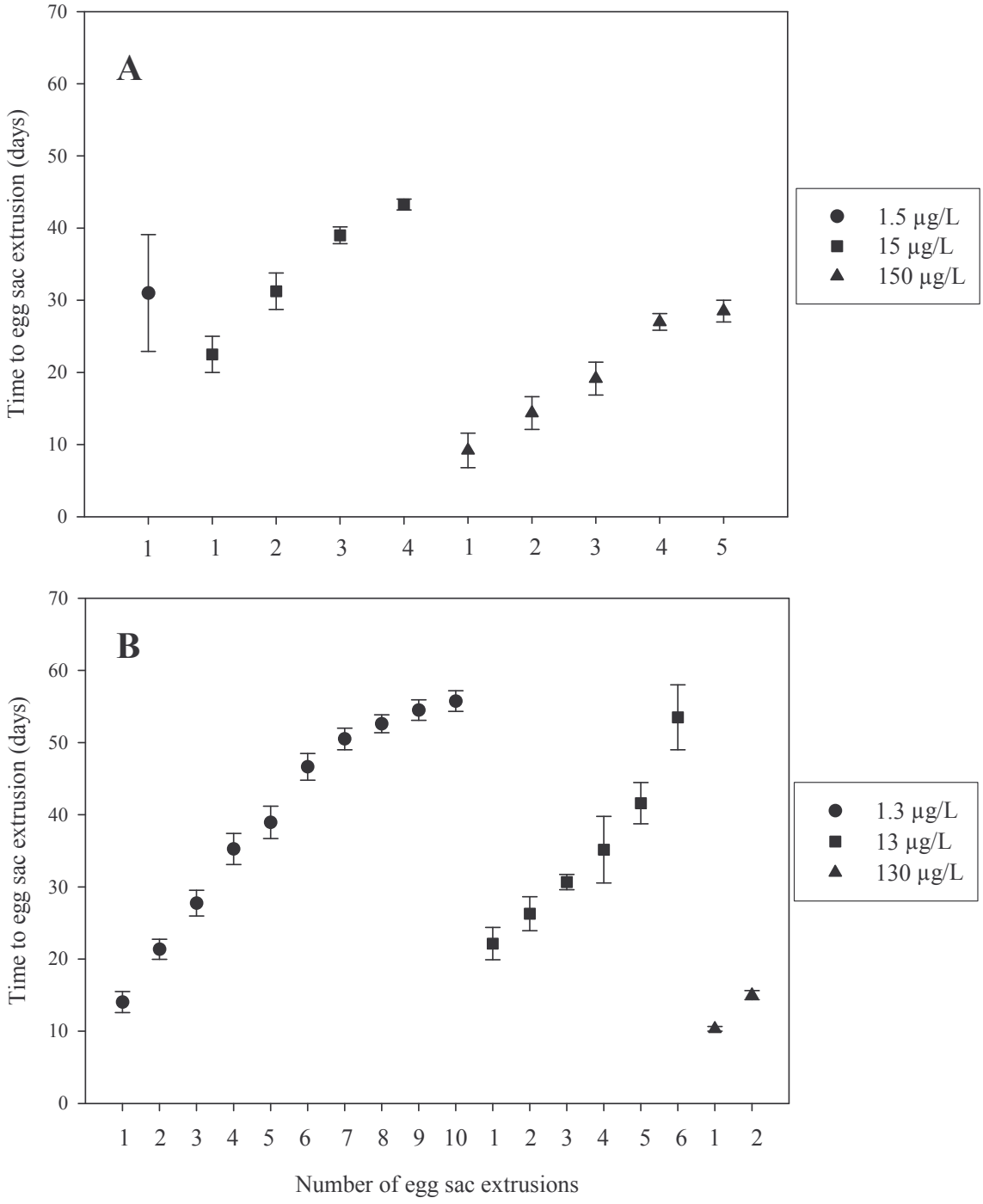

Figure 4.1. Mean time (± 1 standard error (SD)) from mating taken for individuals of *Robertsonia propinqua* to extrude successive egg sacs under the three concentration exposures (µg/L) for both **A** zinc sulphate and **B** atrazine.

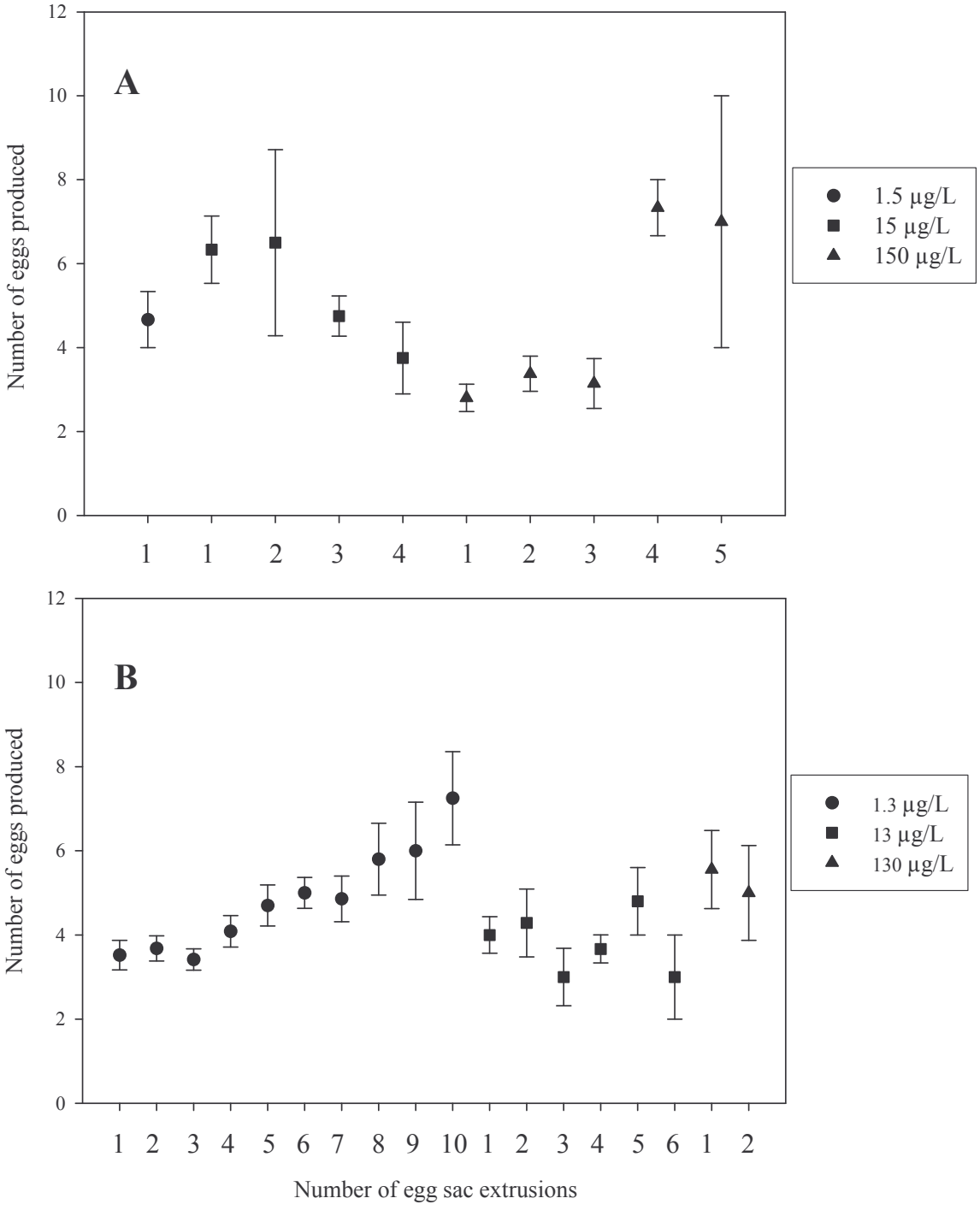

Figure 4.2. Mean number (± 1 SD) of eggs produced with each egg sac extrusion for *Robertsonia propinqua* under the three concentrations (µg/L) for both **A** zinc sulphate and **B** atrazine.

4.5. Discussion

4.5.1. Acute toxicity test

The current results provide new range-finder data on the toxicity of atrazine and zinc to *R. propinqua* and show that adult *R. propinqua* may be more tolerant to both atrazine and zinc sulphate than are the nauplii. The results show that when compared to other relevant literature, *R. propinqua* adults are more tolerant to atrazine than are the calanoid copepods, *Eurytemora affinis* Poppe (LC_{50} = 4.2–17.5 mg/L) (Ward & Ballantine 1985) and *Acartia tonsa* Dana (LC_{50} = 0.09–13.2 mg/L) (ANZECC 2000).

The greater sensitivity of *R. propinqua* nauplii to pollutants may indicate that the sensitivity of stages decreases with age (Table 4.1), a common trend observed in many invertebrates (Ringwood 1990). However, further testing using a wider range of life stages is required to confirm this. The sensitivities of nauplii may relate to the thinness of their exoskeleton leading to a higher absorption of contaminants (Forget et al. 1998) and may therefore increase bioaccumulation of toxicants within the tissues (Bryan & Langston 1992, Coull & Chandler 1992). Furthermore, increased sensitivity of nauplii may be in part due to a large surface area to volume ratio and a higher mass-specific metabolism (Miller & Harley 1996). It is also possible that starvation during the 96h test period differentially effected the tolerance of adults and nauplii, resulting in greater susceptibility of nauplii to the test concentrations.

The bioindicators used in New Zealand (e.g., cockles *Austrovenus stutchburyi*, (Purchase & Fergusson 1986), pacific oyster *Crassostrea gigas* (Perera 2004)) currently provide information on the effects of aqueous phase contaminants. The use of *R. propinqua* to assess estuarine contamination will further enable the development of bioassays investigating sediment-associated contaminants and associated multi-generational effects due to its short generation time and its ability to be cultured in the laboratory (Coull & Chandler 1992). *R. propinqua* may then be used as an 'early-warning' signal of long-term population and/or community structure changes. Although the current range-finder results are not definitive values, they may provide an indication of whether a particular site is

showing signs of contamination (i.e., if a specific LC_{50} value is lower than the guideline trigger value). Moreover, as the LC_{50} values of all life stages for *R. propinqua* exposed to atrazine and zinc are above the Australian and New Zealand marine and freshwater recommended guideline trigger values (zinc = 0.015 mg/L, atrazine = 0.013 mg/L), it appears that these marine organisms may not be severely affected by pollution from these contaminants (ANZECC 2000).

There is strong evidence supporting bioavailability and trophic transfer of contaminants within aquatic food chains (Chang & Reinfelder 2002, Coull & Chandler 1992, DeLorenzo et al. 1999, Hickey et al. 1995, Moore & Waring 1998). As benthic copepods are in direct association with sediments and represent a major food source for benthic feeding fish, *R. propinqua* may be an appropriate candidate species to provide an 'early-warning' of potential trophic level effects. The benthic feeding estuarine mummichog, *Fundulus heteroclitus* Robins and the spot croaker, *Leiostomus xanthurus* Lacepede fish, have been recorded to be more tolerant to zinc and atrazine (7 day no observable effect concentration (NOEC) LC_{50} = 10.4 mg/L; LC_{50} = 8.5 mg/L, respectively) than the copepod *R. propinqua* (ANZECC 2000, Ward & Ballantine 1985, respectively). Consequently, *R. propinqua* may be a good 'early-warning' indicator of minimal lethal contaminant concentrations for marine fish. This is important as copepods are one of the main sources of food for juvenile and adult fish (Bryan & Langston 1992, Coull & Chandler 1992).

4.5.2. Chronic toxicity test

In this study, the reproductive and developmental effects of chronic exposures of aqueous zinc sulphate and atrazine using the cultured copepod *R. propinqua* were assessed across the copepod's generations. The data presented here provide an indication that both contaminants may be affecting the reproduction of the test animals as no juveniles (nauplii) successfully hatched and developed, even though the copepods continued to produce eggs under each contaminant exposure concentration. Using a larger number of test animals and better randomisation of experimental treatments per microplate will provide results that are more reliable.

Due to the complete mortality in the methanol-carrier control and partial mortality in the artificial seawater controls, it cannot be certain that the effects shown in the present study are due to contaminant exposure. However, previous data have suggested that in non-contaminated artificial seawater, reproductive output of *R. propinqua* is higher than is shown in the present contaminant exposures (Hack 2002). Therefore, it is possible that in the present study, reproduction and development may have been affected by concentrations of zinc sulphate and atrazine.

As no nauplii successfully hatched and developed in any contaminant concentrations, no chronic reproductive information could be gathered. Repeat testing using a wider range of contaminant concentrations should be done as the current concentrations prevented the successful hatching and development of nauplii. It is also interesting to note that while no nauplii successfully hatched in either zinc sulphate or atrazine exposures, some virgin female copepods regularly produced egg sacs, although all of the latter were non-viable.

The data presented here provide an indication of the potential effects generated by long-term exposure to aqueous zinc sulphate and atrazine on the life cycle of *R. propinqua*, but without further testing this cannot be certain. The current results also suggest that reproductive and developmental effects may be occurring below the ANZECC (2000) marine water quality guideline trigger values for zinc (13µg/L) and atrazine (15µg/L). If further experiments were to support the current results, this may provide an indication that the ANZECC (2000) guideline trigger values may not provide adequate protection for chronic contaminant exposure to benthic organisms (e.g., copepods).

4.5.3. Conclusions

R. propinqua may be an appropriate candidate species to assess the effects of sediment-associated contaminants in New Zealand estuarine environments. However, additional definitive toxicity testing under a wider range of salinities is needed. No definitive toxicity tests were done due to limited time. The data obtained in this study may contribute to the development of new guidelines or trigger values for both atrazine and

zinc sulphate, but further work is required to assess the definitive LC_{50} values for *R. propinqua* exposed to both contaminants, as well as to ascertain the chronic effects of contaminant exposure compared with the ANZECC (2000) guideline values.

4.5.4. Recommendations for future research

Definitive tests for each toxicant to determine the level of toxicity reduction during the 96-h acute bioassay are desirable. In addition, increasing the concentration of atrazine relative to the carrier solvent (methanol) used here may overcome the high control mortality as recorded in the current study. This may also assist in the assessment of growth and development of juveniles exposed to chronic atrazine exposures. Furthermore, spiked sediment single-pollutant and mixed pollutant bioassays using the full life cycle of *R. propinqua* are needed to quantify the environmental effects on other benthic copepods. Of relevance to this, the development of a field-contaminated sediment bioassay using the full life cycle of *R. propinqua* has been undertaken to estimate the sub-lethal effects of pollutants (Hack et al. 2007c, in press). Future studies should investigate the effects of algae on chemical speciation in each concentration as the presence of algae as a food source may reduce the toxicity of the contaminant.

CHAPTER 5. Toxicity of estuarine sediments using a full life-cycle bioassay with the marine copepod *Robertsonia propinqua*

This chapter, formatted differently, is accepted for publication as given below:

Hack, L. A., Tremblay, L. A., Wratten, S. D., Forrester, G., Keesing, V. 2007. Toxicity of estuarine sediments using a full life-cycle bioassay with the marine copepod *Robertsonia propinqua*. *Journal of Ecotoxicology and Environmental Safety* in press.

Co-author contribution

This chapter is in press in the *Journal of Ecotoxicology and Environmental Safety* excluding sections 5.3.1 (Auckland region), 5.3.1.2 (contaminant information relating to the Auckland region), 5.3.2.1., 5.3.3.1., and Table 5.6. Anne Austin is thanked for editorial services.

5.1. Abstract

Estuarine sediment contamination is a growing significant ecological issue in New Zealand and methods of assessing toxicity and ecological impacts are currently limited. Further to that is a need to develop bioassays that generate data quickly and have ecological relevance to the wider community. A chronic full life cycle bioassay to assess the toxicity of New Zealand estuarine sediments using the marine harpacticoid copepod *Robertsonia propinqua* has been investigated. Sediment samples were collected from the Bay of Plenty region and included two polluted and one reference site. Sources of pollutants in the contaminated field sites originated from a variety of sources and generally include nutrients, pesticides and herbicides and the pollutants zinc, copper, lead and polycyclic aromatic hydrocarbons (PAHs). Conversely, the reference site was exposed to low levels of contaminants due to the relatively undeveloped catchment. Adult male and female copepods were exposed to field collected sediments for 24 days under flow-through conditions at 21^{o}C and a 12h:12h light:dark photoperiod. Five endpoints were recorded: male and female survival, fecundity (number of gravid females

per replicate at the end of the test), clutch size per female, number of eggs per sample and juvenile survival (number of nauplii and copepodites per replicate at the end of the test). Adult mortality was observed in all sediment samples but the number of males/females, gravid females, clutch size per female and number of eggs produced were not affected by either the contaminated or reference sediment samples. However, the contaminated sediments did reduce reproductive output (i.e., nauplii and copepodite production). Therefore, we conclude that reproductive endpoints provide a good measure of sediment-associated contaminant effects compared with adult *R. propinqua* survivorship. It may be that a change in focus from chemical thresholds without ecological relevance or lethal dose threshold methods, to more subtle but ecologically significant elements of faunal life, such as reproductive success, are a more sensitive and a long term ecologically informative method.

5.2. Introduction

The coastal marine environment of New Zealand is mainly made up of river-fed estuaries that cover a total area of at least 100 000 hectares, providing nurseries for commercially valuable fisheries as well as nesting, feeding and resting areas for migratory birds (Ministry for the Environment 1997). Over the last 100 years these areas have become sinks for human-related contaminants. Many of New Zealand's main cities are situated within estuarine catchments (i.e., Auckland, Whangarei, Tauranga, Napier, Nelson, Christchurch and Invercargill). Over the last 40 years the pressure on the coastal environment has dramatically increased as a result of rapidly increasing urbanisation as people choose the coastal life. With urbanisation comes an increase in impervious surfaces (e.g., roads and housing developments) causing increased stormwater runoff containing contaminants from non-point sources that discharge largely untreated into coastal and estuarine environments. The primary stormwater contaminants of concern in New Zealand estuaries are zinc (e.g., from galvanised steel, tyres), copper (vehicle wiring), lead (street runoff, industrial and municipal wastewater discharges), and polycyclic aromatic hydrocarbons (PAHs) (also originating from vehicle emissions). These have accumulated in estuarine sediments over the past decades (Williamson &

Wilcock 1994, ANZECC 2000, ARC 2003). Studies are beginning to indicate that these contaminants are affecting the fauna, particularly sediment-dwelling organisms (infauna), by reducing species abundance, increasing contaminant accumulation in shellfish and crustaceans, and causing changes in growth and reproductive rates (Ministry for the Environment 1997, and references therein).

Sediments are an integral component of all aquatic ecosystems, providing food and habitat to many infaunal organisms. Contaminants typically bind to the finer silty material of estuarine sediments, creating a reservoir of contaminants that become a source of pollution to the water column and organisms (Oberholster et al. 2005).

Although chronic (long term–full life cycle of an organism) sediment toxicity bioassays have been used in a number of studies to investigate the effects of contaminated sediment on a variety of benthic dwelling organisms (Chandler & Green 1996, Kovatch et al. 1999, Stronkhorst et al. 2003, Bejarano et al. 2004, Oberholster et al. 2005, Castro et al. 2006), they are not used here. Organisms such as amphipods (Costa et al. 2005, King et al. 2006, Manyin & Rowe 2006), midge (Soares et al. 2005), water louse (De Lange et al. 2005), mayfly nymph (De Lange et al. 2005), heart urchin (Stronkhorst et al. 1999), copepods (Willis 1999) and oysters (Geffard et al. 2001, 2004) have been used in such tests and have proven successful in identifying sediments with potential toxicological effects to the organisms inhabiting the test sediment. While harpacticoids of the genera *Tisbe*, *Euterpina*, *Nitocra* and *Tigriopus* have been cultured and used in toxicity testing (Bengtsson 1978), these species are not commonly found in New Zealand therefore the native marine benthic copepod *R. propinqua* was selected as a potential regional toxicity test organism. The aim of this study was to develop a test using *R. propinqua* to assess reproductive, survival and developmental effects of chemical pollution present within field-collected sediments in the Bay of Plenty region, New Zealand. The use of *R. propinqua* is relevant as it is found throughout New Zealand estuarine and intertidal environments and is ecologically important as a food source to juvenile benthic feeding fish. In addition, it is sensitive to sediment-associated contaminants, can be cultured in the laboratory and can complete and entire life cycle from juvenile to reproductive adult

in 24 days (Chapter 3). The full life cycle of *R. propinqua* was investigated to ascertain the acute effects of sediment-associated contaminants and identify potential chronic effects resulting from continued exposure to contaminated sediment.

5.3. Materials and methods

5.3.1. Field site locations

5.3.1.1. Auckland region (Figure 5.1)

The Manukau harbour is the second largest coastal harbour on the west coast of the North island of New Zealand, adjacent to the city of Auckland and is the sixth largest harbour in the world. Tidal influences range from a mean high water spring (MHWS) value of 4.17 m to a mean low water spring (MLWS) value of 0.45 m (LINZ 2006). The harbour covers an area of approximately 350 km^2 and is surrounded with extensive urban, industrial and rural land uses. The area supports a population of around 1.3 million (Statistics New Zealand 2006). Previous studies have shown that parts of the harbour close to urban and industrial areas are moderately to heavily polluted by metals (Williamson et al. 1992) and organic pollutants (Fox et al. 1988, Holland et al. 1993, Williamson & Wilcock 1994, Hickey et al. 1995). Okura estuary is located within the Long Bay-Okura Marine reserve near the northern edge of the North Shore area of Auckland, New Zealand and is under increasing pressure from urbanisation (Ford et al. 2003). Tidal influences in this area range from MHWS 3.26 m to MLWS 0.45 m (LINZ 2006).

5.3.1.2. Bay of Plenty region (Figure 5.1)

The Tauranga harbour is located on New Zealand's northeast coast in the Bay of Plenty and experiences a MHWS level of 1.86 m and a MLWS level of 0.16 m (LINZ 2006). The harbour catchment covers an area of approximately 200 km^2 and is well developed with extensive horticultural, agricultural, urban and commercial uses. Tauranga city supports a population of around 110 000 (Statistics New Zealand 2006). Ohiwa harbour (2872800E, 6346725N, MHWS 1.9 m, MLWS 0.3 m) is located in the Whakatane region, south of Tauranga city with a population around 33 000 (LINZ 2006, Statistics

New Zealand 2006). The harbour catchment covers an area of approximately 27 km^2 and is surrounded by rural pastures and low density housing, mainly in the Ohope Beach area.

5.3.1.3. Field site classification (Figure 5.1)

Contaminant information for each field site detailed in Table 5.5 was taken from available Regional Council publications and does not represent contaminant analysis undertaken as part of this study. However, Auckland Regional Council as part of a long-term monitoring programme for chemical contaminants in marine sediments of the Auckland urban area has collected data since 1998 which enabled changes in contaminant concentrations to be recorded (ARC 2002, 2003). The present Auckland field sites were selected based on the published reports and ensured the multi-year contaminant data could be used to determine changes in community composition in the present study. Sites within the large metropolitan Auckland region included two polluted sites, Hastie Ave (2670560E, 6471585N, Manukau harbour) and the Railway Yards (2673345E, 6472140N, Manukau harbour) and one reference site within the Okura estuary (2663765E, 6501480N). Both sites within the Manukau harbour have been classified as having 'unhealthy' benthic communities and the area surrounding the Okura estuary as having 'healthy' benthic communities (ARC 2003). Okura estuary is surrounded by a small urban area and rural pastures and levels of contaminants have been recorded at or below the ANZECC (2000) water quality guideline values (ARC 2003). The primary contaminants of concern for the urbanised Auckland region are zinc, lead, copper and polycyclic aromatic hydrocarbons (PAHs) (ARC 2003). The selected sites within the harbour have recorded contaminants above the ARC (2003) high contaminant guideline values for zinc, lead and copper, which are; >150 mg/kg, >50 mg/kg and >34 mg/kg respectively, but below the PAH guideline value (i.e., <0.66 mg/kg). Lead concentrations in the Railway Yard field site have recorded levels below the guideline value (Table 5.5). Conversely, contaminant levels recorded within the Okura estuary have been recorded at levels below the low contaminant guideline values which are, <125 mg/kg, <30 mg/kg, <19 mg/kg and <0.66 mg/kg, respectively (ARC 2003). Table 5.5 outlines the contaminant values recorded by the appropriate authorities.

Sites within the smaller metropolitan Tauranga region included two polluted sites, Waikareao Foreshore Reserve (2788350E, 6384875N, Tauranga harbour) and Te Puna estuary (2779155E, 6388855N, Tauranga harbour), and one reference site within the Ohiwa harbour (2866425E, 6347630N, Whakatane). These sites were selected based on areas investigated as part of a three year marine sediment contaminant survey undertaken by Environment Bay of Plenty (2003). As for the Auckland field sites, the multi-year contaminant data could be used to determine changes in community composition in the present Bay of Plenty field sites. Both polluted sites have lower levels of sediment-associated contaminants compared with those recorded in the Auckland field sites, namely, zinc, lead and copper (25-40 mg/kg, 4-6 mg/kg, 1-3 mg/kg, respectively), but these contaminants and their respective values are nevertheless a cause of concern for the region. Polycyclic aromatic hydrocarbons (PAHs) compounds exceeded the analytical detection limits (2 μg/kg) and the ARC (2003) high contaminant guideline value (i.e., <0.66 mg/kg) within the Waikareao Foreshore reserve field site (32 μg/kg, Environment Bay of Plenty 2003), indicating a low to moderate environmental impact. No data for PAH levels were available for Te Puna. Contaminants in the Ohiwa harbour (Whakatane) have been recorded well below the low contaminant guideline values: zinc <125 mg/kg, lead <30 mg/kg, copper <19 mg/kg and PAH <0.66 mg/kg, respectively (ARC 2003) (Table 5.5).

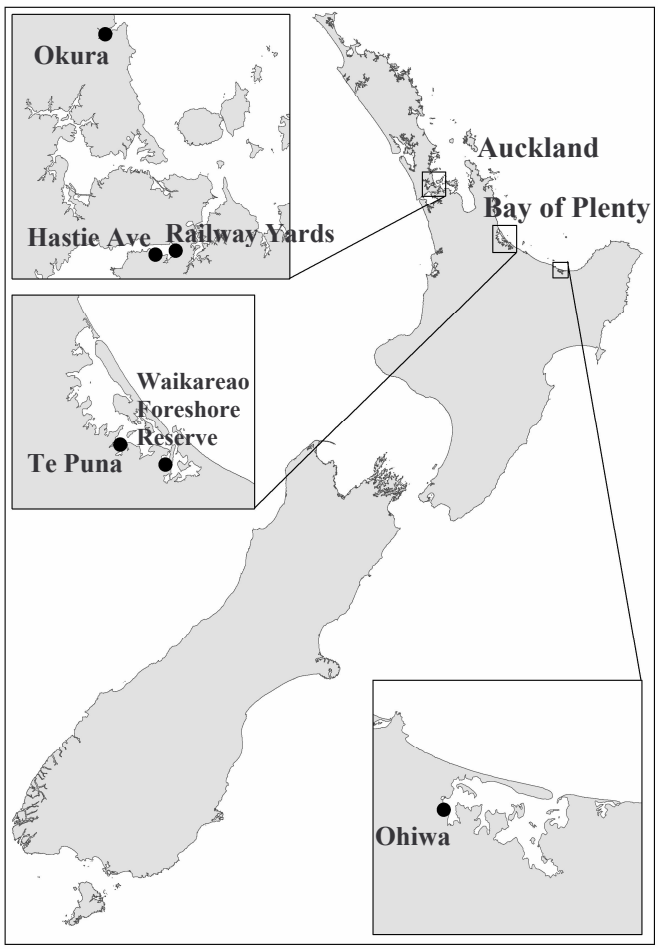

Figure 5.1. Map of New Zealand indicating location of field sites within the Auckland and Bay of Plenty field regions.

5.3.2. Experimental procedure

5.3.2.1. Preliminary study – Auckland and Bay of Plenty regions

A preliminary experiment was carried out to investigate the effects of sediment-associated contaminants on the full life-cycle of *R. propinqua*. The Auckland field region, including field sites at Okura (reference), Hastie Ave (polluted) and the Railway Yards (polluted) as well as the Bay of Plenty field region, including sites at Ohiwa (reference), Te Puna (polluted) and Waikareao Foreshore Reserve (polluted) were investigated.

Sediment samples were collected, processed and the test chambers maintained as described in section 5.3.2.2 below. Test chambers were inoculated with 5 male and 5 non-gravid female laboratory cultured copepods for the Auckland field site test sediments, and 10 male and 10 non-gravid females for the Bay of Plenty sites. Only results for the Bay of Plenty region are presented as the number of copepods used in the Auckland bioassay was too low due to low numbers available in the laboratory stock cultures.

Means and standard errors/confidence intervals of percent male and female survival, gravid female production, nauplii and copepodite production, average clutch size and potential and realised offspring production were calculated and are presented to provide some indication of the life-cycle differences between the Auckland and Bay of Plenty field region (Table 5.6).

5.3.2.2. Modified bioassay - Bay of Plenty field region

General bioassay procedures followed those described in Chandler and Green (1996) and Kovatch et al. (1999). Briefly, test sediments were collected from three field sites within the Auckland region: Okura, Hastie Ave and Railway Yards and three sites within the Bay of Plenty region: Ohiwa, Te Puna and Waikareao Foreshore Reserve, North Island of New Zealand. Three 2 L sediment samples were collected from each field site (total of 9 samples for the Bay of Plenty regions) by scraping the upper 1-2 cm of exposed oxic intertidal sediment. The sample was then frozen upon return to the laboratory. Freezing overnight rather than refrigerating sediment was found to be appropriate as refrigerating does not kill all sediment dwelling meiofauna (Kovatch et al. 1999). Sediment-associated contaminant data was sourced from Auckland Regional Council (2003) and Environment Bay of Plenty (2003) publications (Table 5.5).

Each 2 L sediment sample was press sieved through a 180 µm stainless steel sieve before adding to each test chamber. Two sediment replicates were removed from each of the processed samples (total of 18 test chambers for the Bay of Plenty region) and added to a depth of 1 cm. Test chambers consisted of 50 mL glass Erlenmeyer flasks with two

opposing 1.5 cm diameter holes covered with Nylon 55 µm monomesh (Filtercorp International Ltd ©). Each test chamber received 10 mL of press sieved sediment prior to adding 20 mL of artificial seawater (ASW) (prepared from Instant Ocean © artificial seasalts, pH 8.2 and salinity 32 ppt). Sediments were allowed to settle for approximately 1h before the test chambers were inoculated with 10 male and 10 non-gravid female laboratory cultured copepods (Hack unpublished data). Copepods were added using glass pipettes then each test chamber placed into a temperature controlled room at 21°C and 12h:12h. light:dark photoperiod. Water quality parameters were maintained at a salinity of 32 ppt, pH 8.2, ammonia 0 ppm, nitrite 0 ppm and nitrate 0 ppm. Maintenance of these parameters was done as described in Chapter 3, section 4.3.4. A recirculating seawater system was used for this bioassay to ensure the copepods were exposed to contaminants already associated with the sediments and not from aqueous contaminants from other test chambers.

Water quality tests were performed at the start of the experiment and at weekly intervals during the 24 day experimental period. Salinity was measured using a refractometer and pH, ammonia, nitrite and nitrate were measured using water quality test kits (Aquarium pharmaceuticals (UK) Ltd). Test chambers were fed 1 mL of a 1:1:1 mixture of *Dunaliella tertiolecta* Butcher, *Isochrysis galbana* Parke affinis *Tahiti* and *Chaetoceros muelleri* Lemmerman every third day as this amount reduced fouling of the mesh coverings (Hack unpublished data).

At the end of the test period, experimental flasks were individually poured through a 55 µm stainless steel sieve, rinsed with ASW into a Petri dish and enumerated live for females, males, gravid females, nauplii, copepodites and clutch sizes of gravid females.

5.3.3. Data analysis

5.3.3.1. Preliminary study – Auckland field region

Means and standard deviations were calculated for each test endpoint (Table 5.6). Two additional parameters were calculated to analyse copepod reproductive data, potential offspring production per female and realised offspring production per female (Chandler

& Green 1996). As stated in Kovatch et al. (1999) and Chandler and Green (1996), these two calculations provide an estimate of the number of offspring capable of being produced per female based on the number of mature females and offspring that survive the 24 day test period. Potential offspring and realised offspring production per female were calculated as follows:

$$\text{Potential offspring production} = \frac{(\text{eggs} + \text{nauplii} + \text{copepodites})}{\text{surviving females}}$$

$$\text{Realised offspring production} = \frac{(\text{nauplii} + \text{copepodites})}{\text{surviving females}}$$

5.3.3.2. Modified bioassay - Bay of Plenty field region

Data from the Bay of Plenty field sites were analysed (except eggs per clutch) using the Generalised Linear Mixed Model (GLMM) procedure in the statistical package GenStat with the appropriate error structure (binomial for proportion data and Poisson for count data) (GenStat Committee 2002). Flasks were considered to be random effects and sites were considered fixed effects in the models.

To analyse the number of eggs per clutch, the average number of eggs per clutch was used as the response variable. The number of clutched eggs used to calculate the means was used as a weight in a Linear Mixed Effects model (using the (REML) procedure in GenStat). Again, flasks were considered a random effect and sites were considered the fixed effect. Furthermore, potential and realised offspring production per female was not calculated in the modified bioassay as the number of copepods was too small to provide reliable results.

5.4. Results

Modified bioassay - Bay of Plenty region

5.4.1. Chemistry

Bioassay water quality measurements remained consistent throughout the 24 day bioassay period and did not differ from initial measurements. Test parameters and measurements are listed in Table 5.1.

Table 5.1. Water quality test parameters for the system artificial seawater (ASW) at test termination.

	System ASW
Salinity (ppt)	32
pH	8.2
Ammonia (ppm)	0
Nitrite (ppm)	0
Nitrate (ppm)	0
Temperature (^{o}C)	21

5.4.2. Proportion of female and male survival and gravid female production

Within the Bay of Plenty region survival means were 100% (female) and 87% (male) for Ohiwa (reference site), 57% and 63% for Te Puna, and 64% and 62% for Waikareao (polluted sites) (Figure 5.2), however there were no significant differences in the proportion of females and males surviving in the samples from the different sites (χ^2_2 = 0.24, 2.39, p = 0.89, 0.30, respectively, Table 5.2, Figure 5.2). Furthermore, while there appeared to be a trend related to contaminant loading in the polluted sites (Te Puna and Waikareao Foreshore Reserve), there was no significant difference in the proportion of gravid females produced in samples from the different sites (χ^2_2 = 3.37, p = 0.18, Table 5.2, Figure 5.2).

Table 5.2. Mean survival of copepods (back transformed from logit scale) recorded in the Bay of Plenty field sites over a 24 day bioassay period including the 95% confidence intervals (CI). χ^2_2 figures are Wald tests from a Generalised Linear Mixed Model.

Variable	Site	Mean ± 95% CI	χ^2_2	p
Female survival	Te Puna	56.7 (33.0 - 77.7)	0.24	0.89
	Waikareao Foreshore Reserve	64.3 (40.4 - 82.7)		
	Ohiwa (reference)	100 (-)		
Male survival	Te Puna	63.3 (30.5 - 87.2)	2.39	0.3
	Waikareao Foreshore Reserve	61.7 (29.0 - 86.3)		
	Ohiwa (reference)	86.7 (62.3 - 96.2)		
Proportion gravid	Te Puna	46.7 (28.6 - 65.7)	3.37	0.18
	Waikareao Foreshore Reserve	38.3 (22.1 - 57.6)		
	Ohiwa (reference)	61.7 (42.4 - 77.9)		

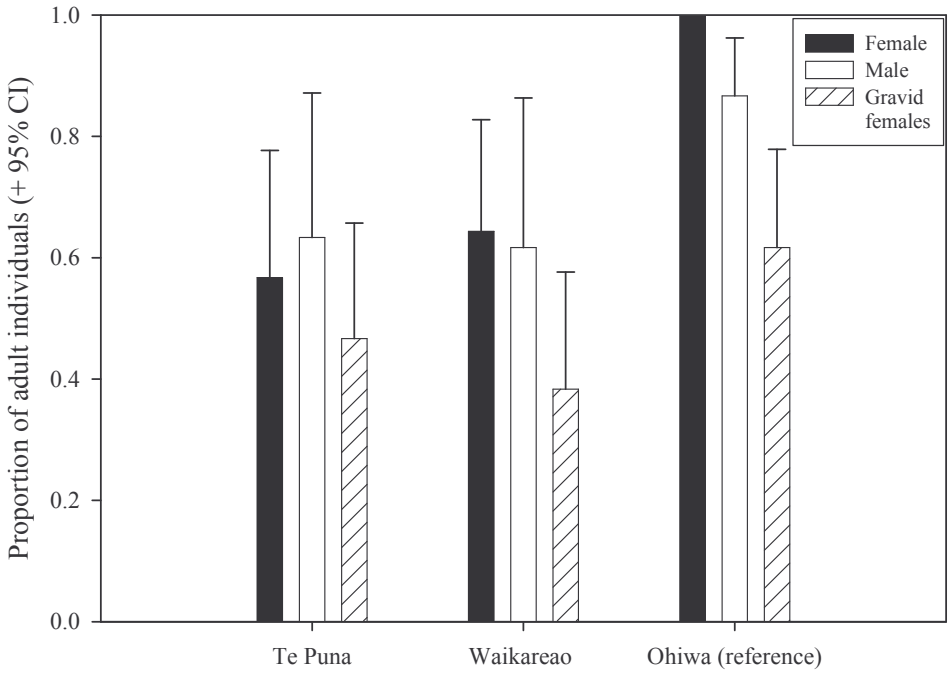

Figure 5.2. Proportion of adult female, male and gravid female copepods within the Bay of Plenty field sites over a 24 day bioassay period. The upper 95% confidence interval limit is shown.

5.4.3. Production of nauplii, copepodites and number of eggs

There were highly significant differences in the mean number of nauplii and copepodites in samples from the different sites (χ^2_2 = 69.87, 16.31, p = <0.001, <0.001, Table 5.3, Figure 5.3), with the highest numbers of nauplii and copepodites recorded in Ohiwa (reference) followed by the polluted sites (Te Puna and Waikareao Foreshore Reserve). Conversely, there were no significant differences in the number of eggs produced per egg sac in samples from the different sites (χ^2_2 = 2.58, p = <0.28, Table 5.3, Figure 5.3).

Table 5.3. Mean numbers of nauplii, copepodites and eggs (back transformed from log scale) recorded in the Bay of Plenty field sites over a 24 day bioassay period including the 95% confidence intervals (CI). χ^2_2 figures are Wald tests from a Generalised Linear Mixed Model.

Variable	Site	Mean ± 95% CI	χ^2_2	p
Numbers of nauplii	Te Puna	40.5 (26.5 - 61.9)	69.9	< 0.001
	Waikareao Foreshore Reserve	17.7 (11.6 - 27.0)		
	Ohiwa (reference)	145.8 (95.54 - 222.8)		
Numbers of copepodites	Te Puna	22.3 (9.7 - 51.6)	16.31	< 0.001
	Waikareao Foreshore Reserve	8.3 (3.6 - 19.1)		
	Ohiwa (reference)	75.1 (32.5 - 173.6)		
Number of eggs	Te Puna	72.0 (31.1 - 166.3)	2.58	0.28
	Waikareao Foreshore Reserve	40.6 (17.6 - 93.8)		
	Ohiwa (reference)	66.6 (28.8 - 154.0)		

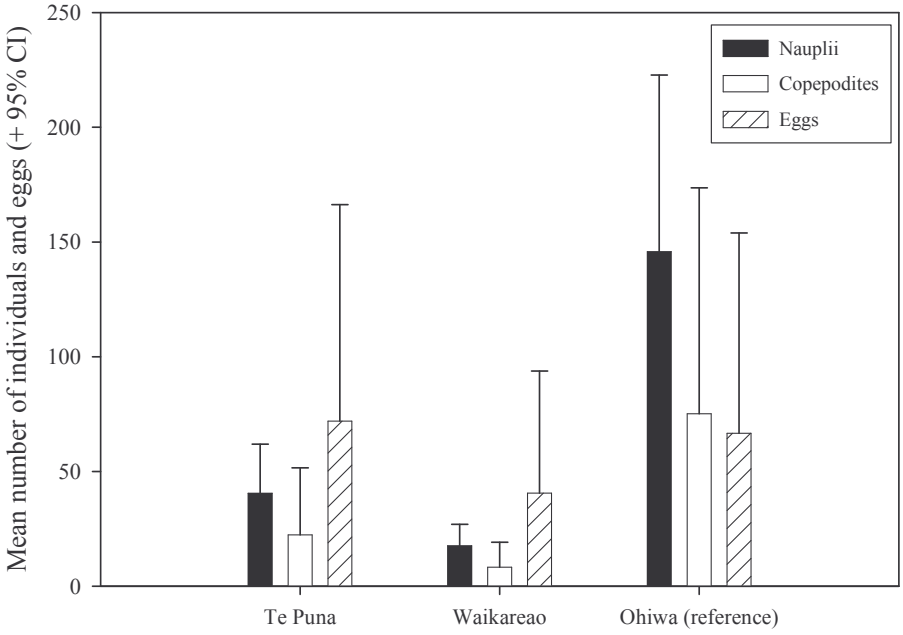

Figure 5.3. Mean numbers of nauplii, copepodites and numbers of eggs produced within the Bay of Plenty field sites over a 24 day bioassay period. The upper 95% confidence interval limit is shown.

5.4.4. Average clutch size

Furthermore, there was no significant difference in the number of eggs produced per clutch from samples from the different sites ($\chi^2_2 = 0.15$, p = <0.93, Table 5.4, Figure 5.4).

Table 5.4. Mean numbers of eggs per clutch recorded in the Bay of Plenty field sites over a 24 day bioassay period including the 95% confidence intervals (CI). χ^2_2 figures are Wald tests from a Generalised Linear Mixed Model.

Variable	Site	Mean ± 95% CI	χ^2_2	p
Mean number of eggs	Te Puna	11.0 ± 1.31	0.15	0.93
	Waikareao Foreshore Reserve	10.7 ± 1.31		
	Ohiwa (reference)	10.8 ± 1.31		

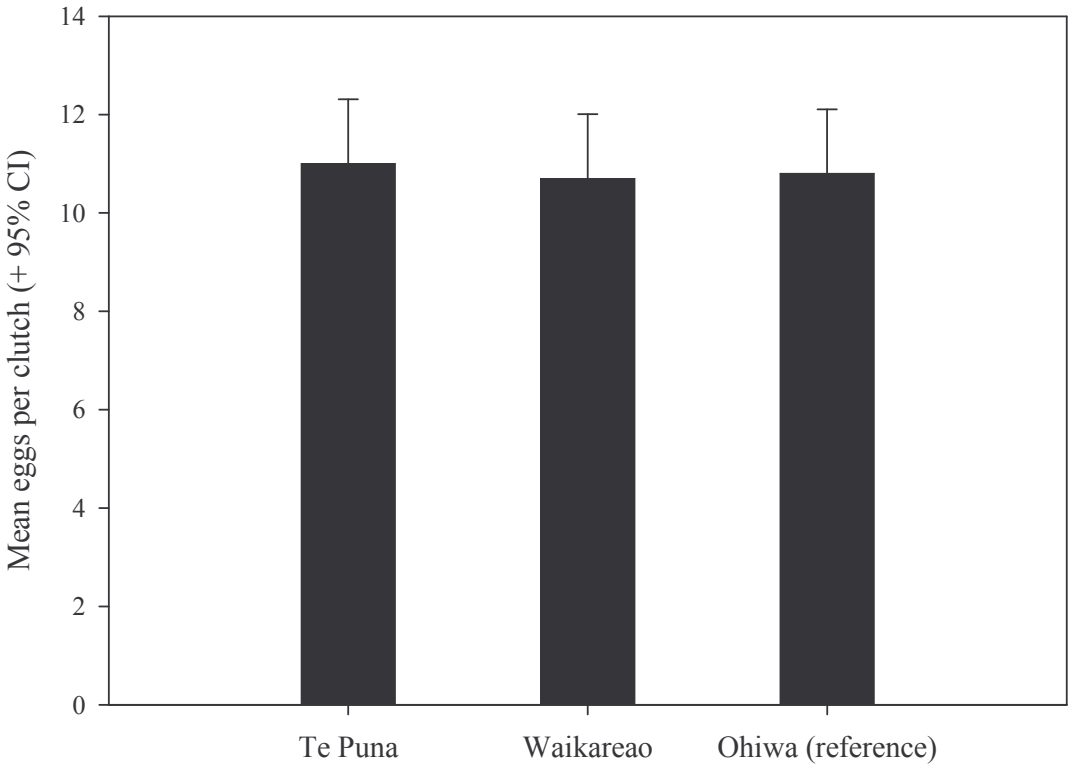

Figure 5.4. Mean number of eggs produced per clutch within the Bay of Plenty field sites within a 24 day bioassay period. The upper 95% confidence interval limit is shown.

Table 5.5. Contaminant (lead (Pb), copper (Cu) and zinc (Zn)) concentrations (mg/kg) in the 500 µm sediment fraction and polycyclic aromatic hydrocarbons (PAH) concentrations (µg/kg) in the 500 µm sediment fraction recorded in the sediments at each field site within the Auckland and Bay of Plenty field region. Contaminant data was taken from ARC (2003), Environment Bay of Plenty (2003) and Stephen Park, Environment Bay of Plenty (pers. comm.) and refer to sites as close as possible to those listed in the table below. Note: * ARC (2003) contaminant guideline values are given for the Hastie Ave site as no data was available. ARC (2003) contaminant guideline values were above the tabulated contaminant values. ND refers to no available data.

		Contaminants (mg/kg)			
Region	**Site**	**Pb**	**Cu**	**Zn**	**PAH** (µg/kg)
Auckland	Okura (Reference)	10.0	7.0	39.0	16.0
	Hastie Ave*	> 50.0	> 34.0	> 150.0	< 660.0
	Railway Yards	32.0	37.0	155.0	65.0
Bay of Plenty	Ohiwa (Reference)	3.9	4.2	22.9	3.0
	Waikareao Foreshore Reserve	4.4	1.3	40.5	32.0
	Te Puna	6.0	2.8	25.6	ND

Table 5.6. Percent male and female survival, mean gravid female production, mean nauplii and copepodite production, mean clutch size and potential and realised offspring production in reference and polluted sediments of the Auckland region over a 24 day bioassay period. Each measured parameter is ± 1 standard error (SD).

	Field region	Field site	mean	SD
Percent female survival	Auckland	Okura (reference site)	76.67	8.82
		Hastie Ave	66.67	8.82
		Railway Yards	40.00	0.00
	Bay of Plenty	Ohiwa (reference site)	100.00	0.00
		Te Puna	56.67	4.41
		Waikareao Foreshore Reserve	63.33	13.64
Percent male survival	Auckland	Okura (reference site)	46.67	8.82
		Hastie Ave	46.67	12.02
		Railway Yards	50.00	11.55
	Bay of Plenty	Ohiwa (reference site)	86.67	13.33
		Te Puna	63.33	8.33
		Waikareao Foreshore Reserve	61.67	4.41
Percent gravid female production	Auckland	Okura (reference site)	23.33	8.82
		Hastie Ave	20.00	10.00
		Railway Yards	13.33	8.82
	Bay of Plenty	Ohiwa (reference site)	61.67	3.33
		Te Puna	46.67	4.41
		Waikareao Foreshore Reserve	38.33	10.14
Nauplii production	Auckland	Okura (reference site)	54.33	13.84
		Hastie Ave	0.33	0.33
		Railway Yards	0.33	0.33
	Bay of Plenty	Ohiwa (reference site)	145.83	12.91
		Te Puna	40.50	9.84
		Waikareao Foreshore Reserve	17.67	3.09

Table 5.6. Continued.

	Field region	Field site	mean	SD
Copepodite production	Auckland	Okura (reference site)	7.33	0.88
		Hastie Ave	0.33	0.33
		Railway Yards	0.00	0.00
	Bay of Plenty	Ohiwa (reference site)	79.67	21.39
		Te Puna	24.00	10.75
		Waikareao Foreshore Reserve	8.33	1.88
Average clutch size	Auckland	Okura (reference site)	14.30	0.73
		Hastie Ave	11.70	0.53
		Railway Yards	9.50	0.29
	Bay of Plenty	Ohiwa (reference site)	10.90	0.57
		Te Puna	10.90	0.60
		Waikareao Foreshore Reserve	10.80	0.27
Potential offspring production	Auckland	Okura (reference site)	12.48	3.06
		Hastie Ave	2.91	2.00
		Railway Yards	3.25	2.12
	Bay of Plenty	Ohiwa (reference site)	29.22	2.70
		Te Puna	25.76	9.23
		Waikareao Foreshore Reserve	10.72	1.88
Realised offspring production	Auckland	Okura (reference site)	7.95	1.46
		Hastie Ave	0.11	0.06
		Railway Yards	0.08	0.08
	Bay of Plenty	Ohiwa (reference site)	22.55	2.96
		Te Puna	11.89	4.08
		Waikareao Foreshore Reserve	4.39	0.77

5.5. Discussion

The Bay of Plenty full life-cycle bioassay using the copepod *R. propinqua* indicated that the reproductive endpoints (nauplii and copepodite production) were more sensitive indicators than adult survivorship of chronic effects following exposure to sediments associated with varied land uses. Production of nauplii and copepodites was depressed in the contaminated sites (Te Puna and Waikareao Foreshore Reserve), although Ohiwa (reference) showed a greater naupliar production compared with copepodite production. The decline in survivorship between the juvenile life stages from natural causes is often very high even in optimal conditions (Carmen & Todaro 1996, Green et al. 1995, Lotufo & Fleeger 1997). Morris and Coull (1992) calculated *Microarthridion littorale* Poppe nauplii mortality to be 98% in the field and attributed it to predation and other natural causes. Life stage specific work has shown nauplii mortality to be greater than 50% in optimal conditions within 21 days for *Amphiascus tenuiremis* (Green et al. 1995). Our work here shows between 1% and 55% maturation of nauplii as measured by the number of nauplii progressing to copepodite – progression that does not appear to be related to contaminant loading, as it was often high in the contaminated sediments. Rather, contamination appears to have a measurable differential effect between the juvenile stages. The current results may provide some evidence that the nauplii life stages may be particularly sensitive indicators of environmental perturbations.

The results of this experiment provide information on the biological effects arising from exposure of *R. propinqua* to contaminated sediments and suggest a contaminant gradient (least contaminated to most contaminated) of the selected field sites within the Bay of Plenty region (i.e., Ohiwa < Te Puna < Waikareao Foreshore Reserve) was discernable using copepod assays and matched chemical data of ARC (2003), Environment Bay of Plenty (2003) and Stephen Park, Environment Bay of Plenty (pers. comm.). The results from this experiment suggest the contaminated sediments may be eliciting effects resulting in a reduction in all measured biological endpoints, particularly the reproductive potential of *R. propinqua* females; however, without repeat testing and a re-examination of contaminants at selected field sites we cannot say so with certainty. Water quality

guidelines and (ANZECC 2000) environmental response criteria trigger values (ARC 2002, 2003) provide values at which levels of contaminants can occur in the environment before they begin causing effects on the fauna of aquatic habitats. In the available literature, the analysed contaminant levels from the Te Puna and Waikareao Foreshore Reserve field sites were below the ANZECC (2000) trigger values. However *R. propinqua* nauplii and copepodite production were significantly depressed in those sediments, suggesting that the Te Puna and Waikareao Foreshore Reserve sites may not support long-term populations of *R. propinqua* given the magnitude of nauplii and copepodite reductions.

5.5.1. Conclusion

In conclusion, based on the present experimental findings, *R. propinqua* could be used effectively in full life-cycle toxicity testing. These tests appear to provide a simple measure to determine whether a site is affected by contaminants in New Zealand estuarine environments, however, further investigation is required. It may be that a change in focus from chemical thresholds without ecological relevance or lethal dose threshold methods, to more subtle but ecologically significant elements of faunal life, such as reproductive success, are a more sensitive and a long term ecologically informative method. The results from additional experiments including repeat testing of the Auckland region will help to solidify relationships between the bioassay outcomes (reproductive and developmental effects) and sediment contaminant levels. Long-term population effects of *R. propinqua* may be determined by using the production of nauplii and copepodites as an indicator of sediment contamination. Additional research with contaminant-spiked sediments is required to assess adequately the sensitivity of *R. propinqua* to contaminants frequently found in the New Zealand environment. This live animal test may eventually lead the way to better assessment of the impacts of contaminants on the food web and wider community impacts in New Zealand estuarine/intertidal environments.

CHAPTER 6. General Discussion

Estuarine sediment contamination is a growing ecological issue in New Zealand. Efficient and cost effective methods for assessing toxicity and ecological impacts in marine environments are currently limited. Research in the USA has demonstrated the utility of a full life-cycle copepods bioassay to assess estuarine sediment. The lower population density in New Zealand compared to the USA is likely to result in lower concentrations of contaminants in the marine environment. Therefore, this study aimed to select a New Zealand native species of copepod as a bioindicator of estuarine/intertidal contamination and develop an efficient and cost effective bioassay to assess environmental health.

The first step in selecting a species of copepod as a bioindicator of environmental health was to investigate and evaluate the relationships between meiofauna, their sedimentary habitat and sediment contaminants in polluted and reference sites. The Auckland and Bay of Plenty regions in the North Island of New Zealand were selected as these regions are heavily populated (Statistics New Zealand 2006) and have undergone extensive urbanisation which has consequently increased the amount of anthropogenic contaminants discharged into estuarine environments. The results obtained provide a general picture of the spatial distribution of the meiofauna communities within each field region however there were no taxa which effectively characterised pollution at any of the investigated field sites *in situ* (Chapter 2).

The second step involved culturing the copepod *Robertsonia propinqua*, and then determine the algal dietary combinations promoting maximum nauplii developmental duration in laboratory experiments. This was demonstrated in Chapter 3, where the algal combinations *D. tertiolecta: I. galbana* and *D. tertiolecta: C. muelleri* promoted a maximum nauplii growth rate from nauplii to stage I copepodite of 10 days with 98% survival. This was an important finding as these dietary combinations provide the first stage in determining a diet which will optimise laboratory reared copepod population carrying capacities.

R. propinqua nauplii experienced 50% mortality when exposed to atrazine and zinc sulphate at 7.5 mg/L and 1.7 mg/L, respectively. This was compared with an adult tolerance of 31.8 mg/L and 2.7 mg/L, respectively over a 96h period. This marked difference is a clear indication of the toxic nature of zinc sulphate to *R. propinqua* nauplii; implying juveniles are more sensitive to pollutants than the adult stage. These results compared well with other estimates of acute toxicity of contaminants to different life stages of copepods (Chapter 4). It is comforting to see that the LC_{50} values for both nauplii and adults are above the ANZECC (2000) recommended guideline trigger values. While the LC_{50} values provide an indication of lethal exposure effects, chronic long-term effects were not clearly determined and require further detailed investigation.

Furthermore, long-term population effects of *R. propinqua* may be determined by using the full life-cycle contaminated sediment bioassay as demonstrated in Chapter 5. Additional research with contaminant-spiked sediments is required to adequately assess the sensitivity of *R. propinqua* to contaminants frequently found in the New Zealand environment.

6.1. Conclusions

In conclusion, the short-generation and culturable copepod *R. propinqua* is an appropriate bioindicator species to assess and provide 'early-warning' signals of potential biological effects resulting from chemical pollutants in intertidal/estuarine environments, as indicated by short-term acute aqueous bioassays and the long-term full life-cycle contaminated sediment bioassay. This suggests that *R. propinqua* would therefore be a good bioindicator of minimal lethal concentrations for other intertidal organisms. However, additional research is needed to assess the level of sediment-associated contaminants at the selected field sites and further work should be undertaken to identify the species collected in each field sample. Fine-scale taxanomic information will assist in identifying changes in distribution and abundance at contaminated and reference field sites. Furthermore, additional research is needed using contaminant-'spiked' sediments to adequately assess the sensitivity of *R. propinqua* to contaminants frequently found in the

New Zealand environment by undertaking further lethal concentration (LC_{50}) and long-term contaminant exposure experiments.

With regards to the initial hypotheses:

- Species composition of benthic meiofauna communities did not differ according to sediment contaminant levels (Chapter 2) as no taxon effectively characterized pollution at any of the investigated field sites. The nematode-copepod ratio (Appendix 1) was investigated as a tool with which to differentiate between polluted and non-polluted sites but this was not achieved.

- Of all the microalgal diets examined, a mixed diet promoted the fastest developmental time of *R. propinqua* compared with a single algal species diet (Chapter 3).

- Exposure of juvenile and adult *R. propinqua* to aqueous atrazine and zinc sulphate produced a lethal dose (LC_{50}) of 7.5 mg/mL and 1.7 mg/mL for juveniles, respectively and 31.8 mg/mL and 2.7 mg/mL for adults, respectively (Chapter 4). Preliminary investigation showed that chronic atrazine and zinc sulphate exposures reduced reproductive and developmental output of *R. propinqua* (Chapter 4).

- *R. propinqua* exposed to field contaminated sediments provided a predictive measure with which to assess potential long-term population changes as indicated by the significant reduction in the number of juveniles produced and developing to reproductive adults (Chapter 5).

6.2. Future work

The principle aim of the current research was to develop a tool to evaluate the health of estuarine sediments. From the results of the thesis, a proper bioassay to determine sediment toxicity using *R. propinqua* has not been fully developed, however the current

research provides a solid foundation from which to develop *R. propinqua* as a whole organism bioindicator for estuarine environmental health.

Future research would require short-term definitive testing of a wider range of concentrations of both atrazine and zinc sulphate as well as other environmentally relevant contaminants such as copper, lead, polycyclic aromatic hydrocarbons (PAHs) and selected nutrients/pesticides on the mortality of *R. propinqua*. However, definitive tests do not provide information on the chronic long-term (egg–reproductive adult) effects of contaminant exposure. To investigate chronic toxicity, *R. propinqua* should be exposed to a range of concentrations based on the findings of the definitive tests and non-contaminated seawater, then effects on parameters such as number of eggs produced, number of juveniles successfully hatching and developing through to the adult stage, number of egg sac extrusions, sex ratio and developmental duration investigated. Results may indicate whether contaminants are eliciting effects below the ANZECC (2000) standard water quality guidelines values and assist in the review of these values.

The sensitivity of *R. propinqua* nauplii and adults to atrazine and zinc sulphate, indicates this species may contribute to and complement those outlined in the Australian and New Zealand marine and freshwater quality guidelines (ANZECC 2000) for the response of higher trophic organisms to contaminants in New Zealand estuarine environments. Although single-contaminant aqueous bioassays are useful for estimating the reproductive and developmental effects of contaminants on benthic copepods quickly and cost effectively under laboratory conditions, they cannot provide a realistic environmental assessment of effects on benthic dwelling copepods. Spiked 'clean' sediment single-pollutant and mixed pollutant bioassays using the full life cycle of the selected bioindicator species are needed to quantify these effects as studies have shown that copepod populations are sensitive to mixtures of pollutants in the field (Coull & Chandler 1992, Hagopian-Schlekat et al. 2001). Validation of the above described bioassays would be undertaken by exposing *R. propinqua* to field collected sediments and the effects of exposure to the full life-cycle recorded. Chemical analysis of these sediments may then enable the identification of individual contaminants eliciting

reproductive and developmental effects. Validation experiments would be laboratory based due to the difficulty (e.g., mesh fouling) in undertaking these experiments *in situ*.

The bioassays developed during the current research (Chapters 4 and 5) may be used to 1) test the short-term acute effects of aqueous phase contaminants discharged into the marine environment, 2) identify the long-term reproduction and developmental effects of aqueous phase contaminants and 3) better assess food web and wider ecological community impacts of contaminants in estuarine/intertidal ecosystems.

Furthermore, chemical pollution from urban and rural land uses is having significant impacts on human and wildlife populations, with widespread problems such as soil contamination, aquatic algal blooms, and shellfish poisoning. Therefore, further investigation of a wider range of intertidal/estuarine areas throughout New Zealand is necessary in order to determine the extent of environmental pollution with particular respect to biological community composition. Categorisation of estuarine/ intertidal sites within New Zealand has not currently been undertaken and to date there are no standard methods with which this can be done.

With regards to the laboratory culture and the microalgal diet required for the rearing of *R. propinqua*, further testing is required using a range of temperature and salinity regimes to ascertain optimal growth rates. A detailed knowledge of these factors will facilitate the successful maintenance of stock cultures for use in additional toxicity bioassays.

Based on the present experimental findings, *R. propinqua* can be used effectively in full life-cycle toxicity testing. These tests appear to provide a simple measure to determine whether a site is affected by contaminants in New Zealand estuarine environments. However, the reliability and actual sensitivity requires further study. The results from additional experiments will help to solidify a relationship between the bioassay outcomes and the contaminant levels. Increasing the number of individuals used in the toxicity tests will strengthen the statistical differences in the present study. Additional research with contaminant-spiked sediments is required to adequately assess the sensitivity of *R.*

propinqua to contaminants frequently found in the New Zealand environment. Moreover, this live animal test may eventually lead the way in better assessing food web and wider ecological community impacts of contaminants in New Zealand estuarine/intertidal ecosystems.

7 APPENDIX. Assessment of the nematode-copepod ratio as an indicator of pollution in New Zealand intertidal environments

7.1. Abstract

Raffaelli and Mason (1981) and Raffaelli (1982) proposed that the ratio of free living nematodes to benthic copepods could be used as a bioindicator of organic pollution in sandy habitats and that the greater the ratio the more polluted the site and conversely for non-polluted sites. To date this ratio has been used once to determine whether it could be an effective tool with which to assess metal pollution in intertidal environments (Lee et al. 2001). The nematode-copepod ratio for metal pollution biomonitoring is assumed to increase as pollution increases and sediment grain size decreases. The practical application of this method to characterise polluted and non-polluted sites within the Auckland and Bay of Plenty regions, New Zealand was undertaken. These regions are heavily populated and have undergone extensive urbanisation which has consequently increased the amount of anthropogenic contaminants discharged into estuarine environments. The data collected showed that the nematode-copepod ratios did not differentiate between non-polluted and polluted sites. Sediment grain size and total organic composition did not correlate with the ratio within any region. The results presented in this study support the conclusion that the nematode-copepod ratio is not an appropriate indicator in environmental health assessment in suspected polluted areas in New Zealand intertidal environments.

7.2. Introduction

Raffaelli and Mason (1981) were the first to suggest the use of the nematode-copepod ratio for biomonitoring. Ratios are assumed to increase as pollution increases and sediment grain size decreases. This suggestion generated considerable controversy and discussion among meiobenthologists (Coull et al. 1981, Raffaelli 1981, Raffaelli & Mason 1981, Warwick 1981, Amjad & Gray 1983, Lambshead 1984, Platt et al. 1984, Shiells & Anderson 1985, Lee et al. 2001) and in a rebuttal by Coull et al. (1981) it was

argued that the nematode-copepod ratio was not a valid pollution biomonitoring tool and that the ratio was an over simplification of very complex meiofaunal community interactions. This was further supported by Coull et al. (1981) and Lambshead (1984). However, others (Warwick 1981) suggested the addition of nematode and copepod metabolic body sizes and the relative rates of metabolism to add precision to the ratio.

Although the ratio has been suggested as an attractive biomonitoring tool, few studies exploring the potential of this ratio have been performed (Raffaelli & Mason 1981, Raffaelli 1982, Warwick 1981, Amjad & Gray 1983, Lambshead 1984, Shiells & Anderson 1985, Lee et al. 2001, Bejarano et al. 2005). To date the ratio has been used on organic enrichment studies (Raffaelli & Mason 1981, Raffaelli 1982, Amjad & Gray 1983, Shiells & Anderson 1985) and the ratios from sites considered polluted were always higher than from unpolluted sites. Lee et al. (2001) investigated the potential use of the ratio to determine the extent of metal pollution in the Chañaral area of Northern Chile but ruled out the applicability in biomonitoring of metal enrichment. The use of the nematode-copepod ratio in both organic and metal enrichment studies has failed to obtain universal acceptance.

Studies of the distribution and abundance of a meiofauna community in polluted and reference locations in the Auckland and Bay of Plenty regions, New Zealand, were carried out during the winter of 2004. The aim of this study was to identify whether the nematode-copepod ratio could be applied to New Zealand intertidal environments and provide a tool with which to categorise contaminated sites.

7.3. Material and Methods

Samples for meiofauna composition were collected from the Auckland region (Okura estuary (reference), Hastie Ave, Railway Yards, and the Bay of Plenty region (Ohiwa harbour (reference), Waikareao Foreshore Reserve and Te Puna) during winter (July-August) 2004. The sites can be broadly ranked from most to least polluted. The Railway Yards (Manukau harbour), Hastie Ave (Manukau harbour) and Waikareao Foreshore

Reserve (Tauranga harbour), Te Puna (Tauranga harbour) represented the polluted sites within the Auckland and Bay of Plenty regions, respectively. Okura and Ohiwa represented the reference sites respectively. See Chapter 2 for a full description of field sites and sampling methodologies.

Briefly, three sediment samples for meiofauna composition were collected from within two 1 m^2 quadrats at 10 m intervals (0, 10, 20, 30 m) from three 30 m transects at each site, preserved in 70% ethanol and analysed for total meiofauna composition. Samples were gently sieved through 180 μm and 75 μm stacked stainless steel sieves. All nematodes and copepods were enumerated and recorded. Physico-chemical parameters including grain size composition and percent organic composition were also collected alongside samples for meiofauna analysis. One sediment sample for grain size analysis and total organic content was collected from within a single quadrat from each transect at 15 m and 0, 10, 20 m respectively. Grain size composition was undertaken by the University of Auckland, New Zealand and mean particle size (μm) analysed using Malvern laser diffraction (Low Angle Laser Light Scattering (LALL)) (Rathbone et. al. 1990). Total organic carbon (g/100g) and nitrogen (g/100g) analysis was undertaken by Landcare Research Ltd (Palmerston North) using methods adapted from those described by Blakemore et al. (1987). Contaminant analyses were not done due to the high cost involved in analysis. An assessment of the relative amounts of pollution at each site was based on the available literature.

7.3.1. Field site classification

Sites within the large metropolitan Auckland region included two polluted sites, Hastie Ave (Manukau harbour) and the Railway Yards (Manukau harbour) and one reference site within the Okura estuary. Both sites within the Manukau harbour have been classified as having 'unhealthy' benthic communities and the area surrounding the Okura estuary as having 'healthy' benthic communities (ARC 2003). The benthic communities included the taxa amphipoda, bivalvia, crustacean, cnidaria, echinodermata, gastropoda, isopoda, polychaeta, nemertina and oligochaeta. The primary contaminants of concern for the urbanised Auckland region are zinc, lead, copper and polycyclic aromatic hydrocarbons

(PAHs) (ARC 2003). The selected sites within the harbour have recorded contaminants above the ARC (2003) high contaminant guideline values for zinc, lead and copper, which are; >150 mg/kg, >50 mg/kg and >34 mg/kg respectively, but below the PAH guideline value (i.e., <0.66 mg/kg) (Table 7.3). Conversely, contaminant levels recorded within the Okura estuary have been recorded at levels at or below the low contaminant guideline values; <125 mg/kg, <30 mg/kg, <19 mg/kg and <0.66 mg/kg, respectively (ARC 2003).

Sites within the smaller metropolitan Tauranga region included two polluted sites, Waikareao Foreshore Reserve (Tauranga harbour) and Te Puna estuary (Tauranga harbour) and one reference site within the Ohiwa harbour (Whakatane). Both polluted sites have lower levels of sediment-associated contaminants compared with those recorded in the Auckland field sites, namely, zinc, lead and copper (25-40 mg/kg, 4-6 mg/kg, 1-3 mg/kg, respectively), but these contaminants and their respective values are nevertheless a cause of concern for the region (Table 7.3). PAH compounds exceeded the analytical detection limits (2 µg/kg) within the Waikareao Foreshore reserve field site (32 µg/kg, Environment Bay of Plenty 2003) indicating a low to moderate environmental impact (Table 7.3). No data for PAH levels was available for Te Puna. Contaminants in the Ohiwa harbour (Whakatane) have been recorded well below the low contaminant guideline values: zinc <125 mg/kg, lead <30 mg/kg, copper <19 mg/kg and PAH <0.66 mg/kg, respectively (ARC 2003) (Table 7.2).

7.3.2. Statistical analysis

The nematode/copepod ratio was calculated using the methods outlined in Lee et al. (2001). Briefly, the ratio was calculated for quadrat as (total number of nematodes from 3 cores)/(total number of copepods from 3 cores) values for the two quadrats then averaged to give the ratio for each distance (0, 10, 20, 30 m) position along each transect. As stated in Lee et al. (2001), ‘where there were no copepods present in a sediment sample the number of nematodes present was used to represent the ratio. Where nematodes were absent and copepods present the ratio was zero, and zero was also used where both groups were absent. Pearson product-moment correlation coefficients were performed

using nematode-copepod ratios versus percent sediment organics, sediment grain size (μm) and sediment redox potential discontinuity depth (cm) and coefficients calculated for all field sites within the Auckland and Bay of Plenty regions.

7.4. Results

The total number of nematodes and copepods fluctuated from site to site within both the Auckland and Bay of Plenty field regions (Table 7.1, Figure 7.1A, B). The total number of nematodes and copepods were variable within the Auckland region field sites and showed no clear relationships, although the Okura (reference) site did show a paired increase in both nematodes and copepods (Figure 7.1A). Conversely, Hastie Ave (contaminated site) showed consistently high numbers of both nematodes and copepods. In the Railway Yard field site there appeared to be some distance effect down the shore (high tide – low tide) or across the site (Transect 1 – 3) as seen by the change in taxa dominance (Figure 7.1A). This was further supported by an increase in nematode and copepod total numbers with increasing distance down transects 1 and 2 (Figure 7.5). This particular trend was not seen in transect 3 where nematode numbers were comparatively high but showed a reduction in total numbers with increasing distance down the transect (Figure 7.5). In comparison, total numbers of copepods remained constant along the transect. There also appeared to be an across site effect occurring in the Railway Yard due to the observable increase in nematode numbers in transect 3 from an approximate average of 75 individuals (transect 1 and 2) to approximately 450 individuals (transect 3) (Figure 7.5).

The total number of copepods was variable within the Ohiwa (reference) and Waikareao Foreshore Reserve field sites but this was not observed for nematodes (Table 7.1, Figure 7.1B). The Te Puna field site showed a reduction in total copepod numbers but nematode numbers remained high (Figure 7.1B). In the Ohiwa (reference site), an observable shift in taxa dominance occurred whereby the total numbers of copepods increased as the numbers of nematodes decreased (Figure 7.1B), indicative of an unpolluted site. In comparison, total numbers of nematodes and copepods were greater in the Waikareao

Foreshore Reserve (contaminated site) and showed greater data spread (Figure 7.1B). Te Puna (contaminated site) showed high numbers of nematodes and correspondingly low numbers of copepods as indicated by the close clustering of points indicative of a contamination effect.

Nematode-copepod (N-C) ratios compared with percent organic carbon in all field sites within the Auckland region were variable. There was no positive association between the N-C ratio and percent sediment organic content in the Okura (reference) site and polluted sites (Hastie Ave and Railway Yards) as seen by the negative correlation coefficients (Table 7.2). A similar trend was also observed in the Bay of Plenty field sites, however, Te Puna showed increases in both sediment organics and N-C ratios. This trend was not linear as seen by the correlation coefficient ($r = 0.07$) and an observed increase in variability (Table 7.2, Figure 7.2B).

There was a trend between large N-C ratios and increased sediment grain size in the Auckland field sites ($r = 0.205$), however interpretation of this is cautioned due to the outlying Railway Yard value (Table 7.2, Figure 7.3A). As the N-C ratio increased sediment grain size also increased and this trend was observed in a gradient of field sites from least polluted to polluted (Okura < Hastie Ave < Railway Yards) (Figure 7.3A). A strong negative correlation ($r = -0.99$) was calculated for Okura which indicated no relationship between sediment grain size and N-C ratio, however interpretation must be cautioned as there is little variation in grain size between the samples (Table 7.2). Furthermore, a positive relationship was observed between sediment grain size and N-C ratio was recorded for the Auckland contaminated field sites ($r = 0.205$, Table 7.2, Figure 7.3A). The Ohiwa (reference) and both contaminated Bay of Plenty field sites showed no association between N-C ratios and sediment grain size as seen by the negative correlation coefficients (Table 7.2, Figure 7.3B).

Nematode-copepod (N-C) ratios and redox potential discontinuity (RPD) depth measurements were variable throughout all Auckland field sites (Figure 7.4A). A negative association ($r = -0.457$) and high variability was observed between RPD depth

and N-C ratios for the Okura (reference) field site (Table 7.2, Figure 7.4A). Conversely, the positive association observed in the Auckland contaminated field sites may indicate a relationship between N-C ratios and RPD depth, however the correlation coefficient indicates a weak relationship (r = 0.218, Table 7.2, Figure 7.4A). In comparison, there was no positive association between N-C ratios and RPD depth in either the Bay of Plenty reference (r = -0.241), although the relationship was marginally stronger in the contaminated sites (r = -0.756) (Table 7.2, Figure 7.4B).

Table 7.1. Minimum and maximum nematode copepod (N-C) ratios calculated for each of the Auckland and Bay of Plenty field regions relative to site and transect number.

		Auckland	
Site	Transect	Min.	Max.
Okura estuary	1	4.33	37.63
(reference)	2	4.57	19.07
	3	18.17	29.54
Hastie Ave	1	19.46	30.84
(polluted)	2	15.08	53.55
	3	12.77	163.83
Railway Yards	1	1.84	22.71
(polluted)	2	2.11	3.25
	3	59.20	451.00
		Bay of Plenty	
Site	Transect	Min.	Max.
Ohiwa harbour	1	1.63	11.84
(reference)	2	1.46	14.58
	3	0.71	4.87
Te Puna	1	4.67	20.21
(polluted)	2	2.70	5.61
	3	2.44	2.90
Waikareao Foreshore	1	16.00	66.50
Reserve	2	72.00	90.50
(polluted)	3	36.75	79.75

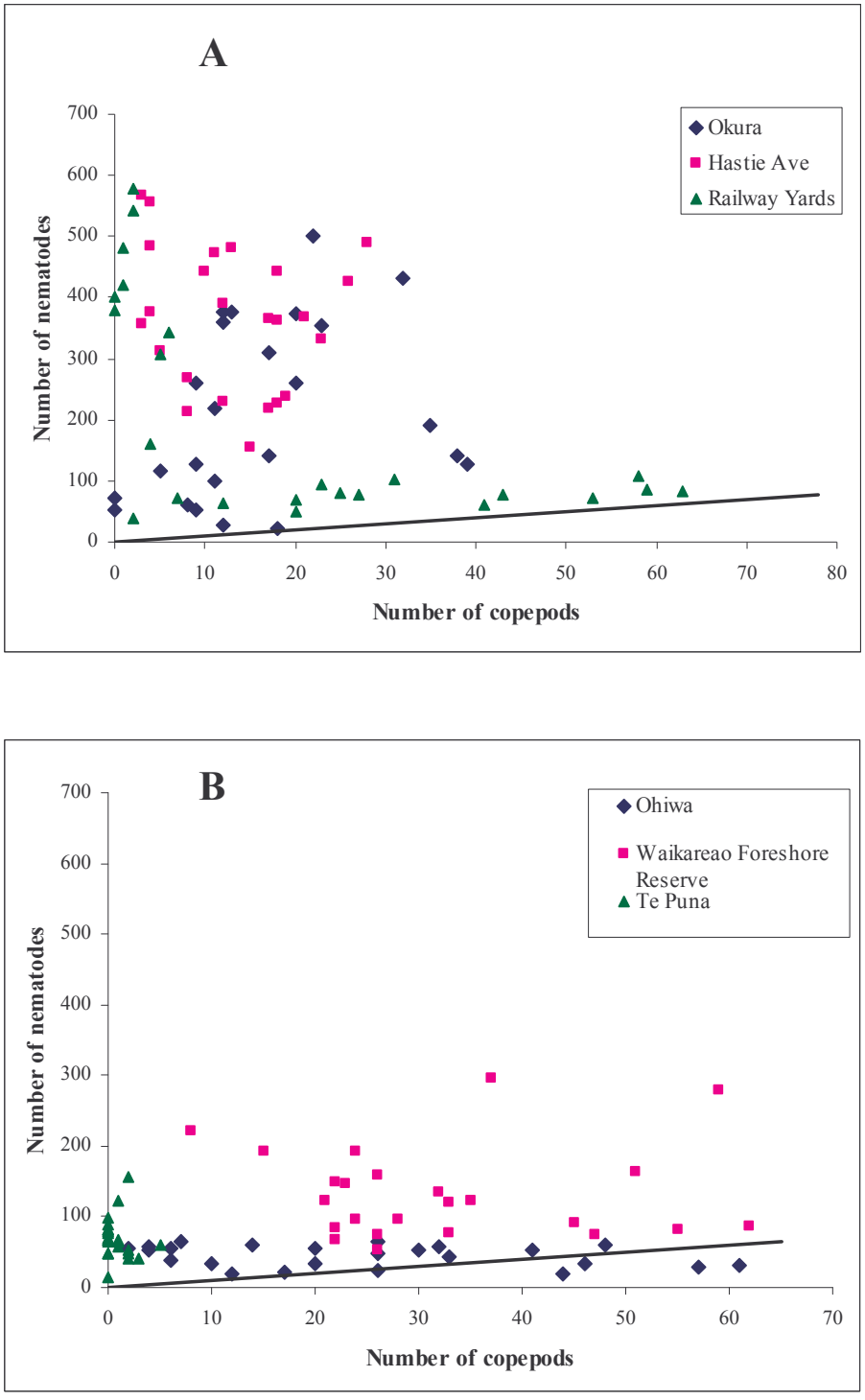

Figure 7.1. The total number of nematodes (totalled over three cores) plotted against the total number of copepods totalled over the same cores for each field site in both **A** the Auckland region and **B** the Bay of Plenty region. The line Y = X corresponds to equal numbers of nematodes and copepods.

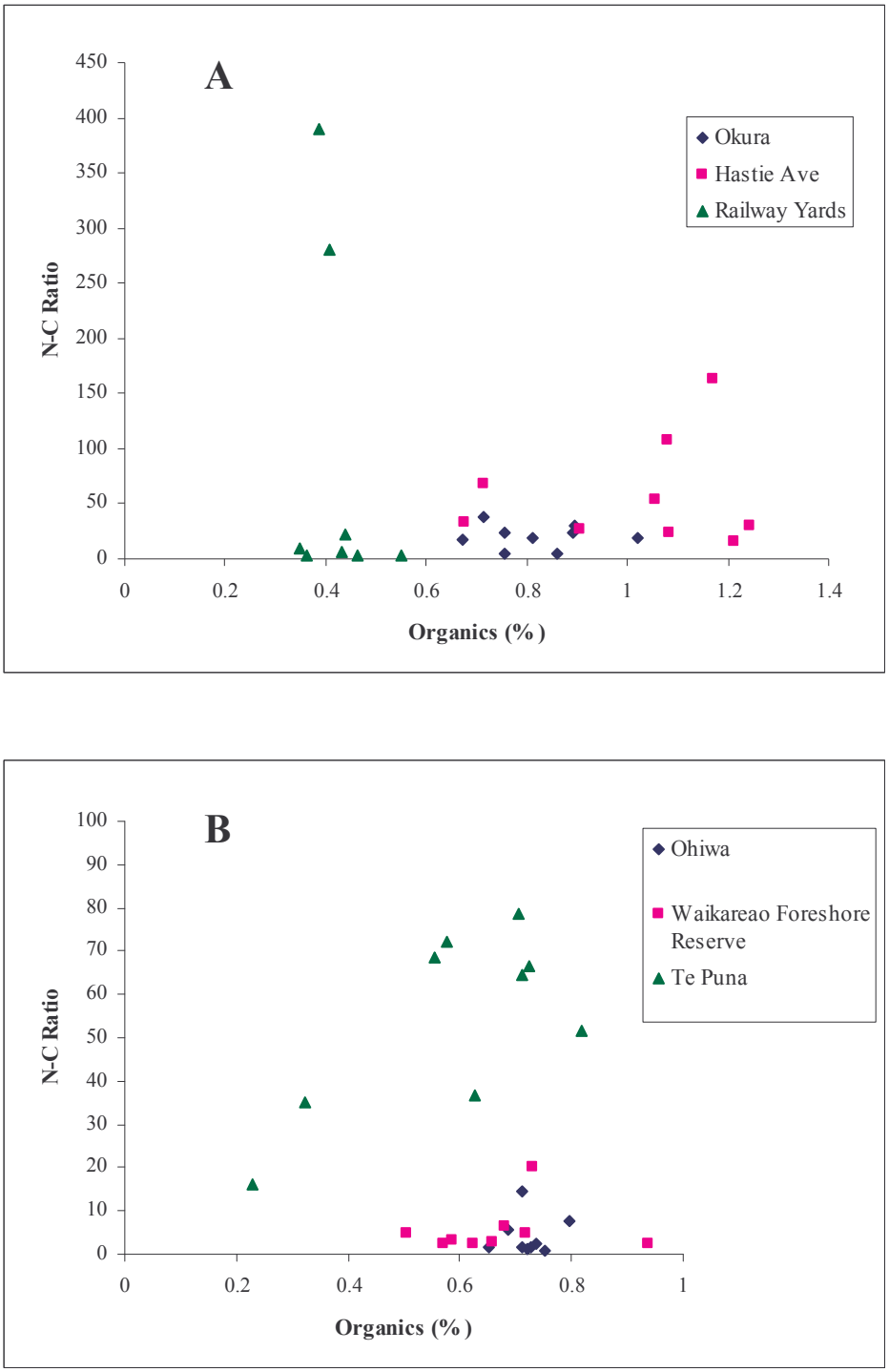

Figure 7.2. Nematode – copepod ratio plotted against sediment organic carbon content (%) at each transect, distance and field site combination in both **A** the Auckland region and **B** the Bay of Plenty region.

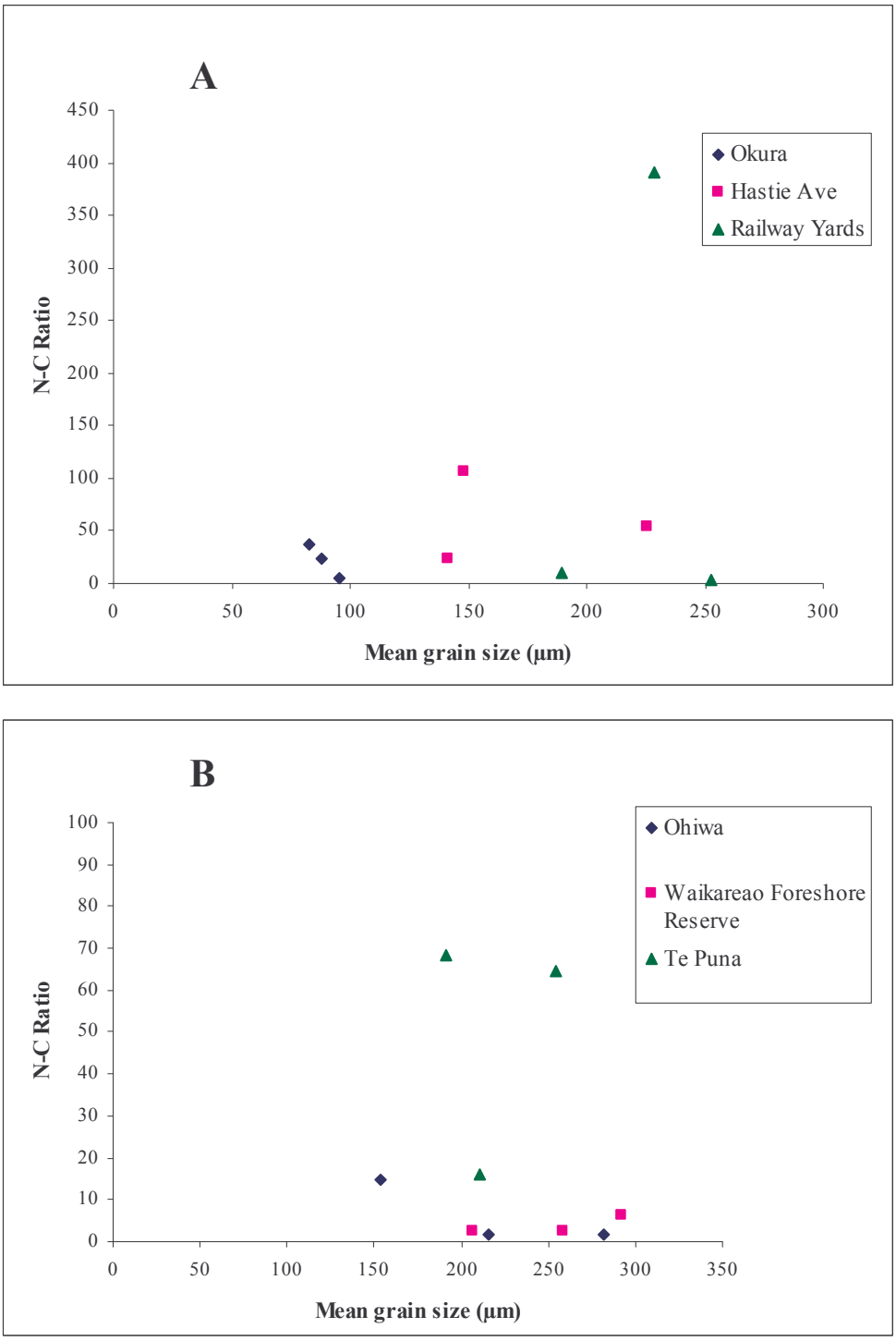

Figure 7.3. Nematode – copepod ratio at 10 m plotted against mean sediment grain size (µm) at 15 m at each field site in both **A** the Auckland region and **B** the Bay of Plenty region.

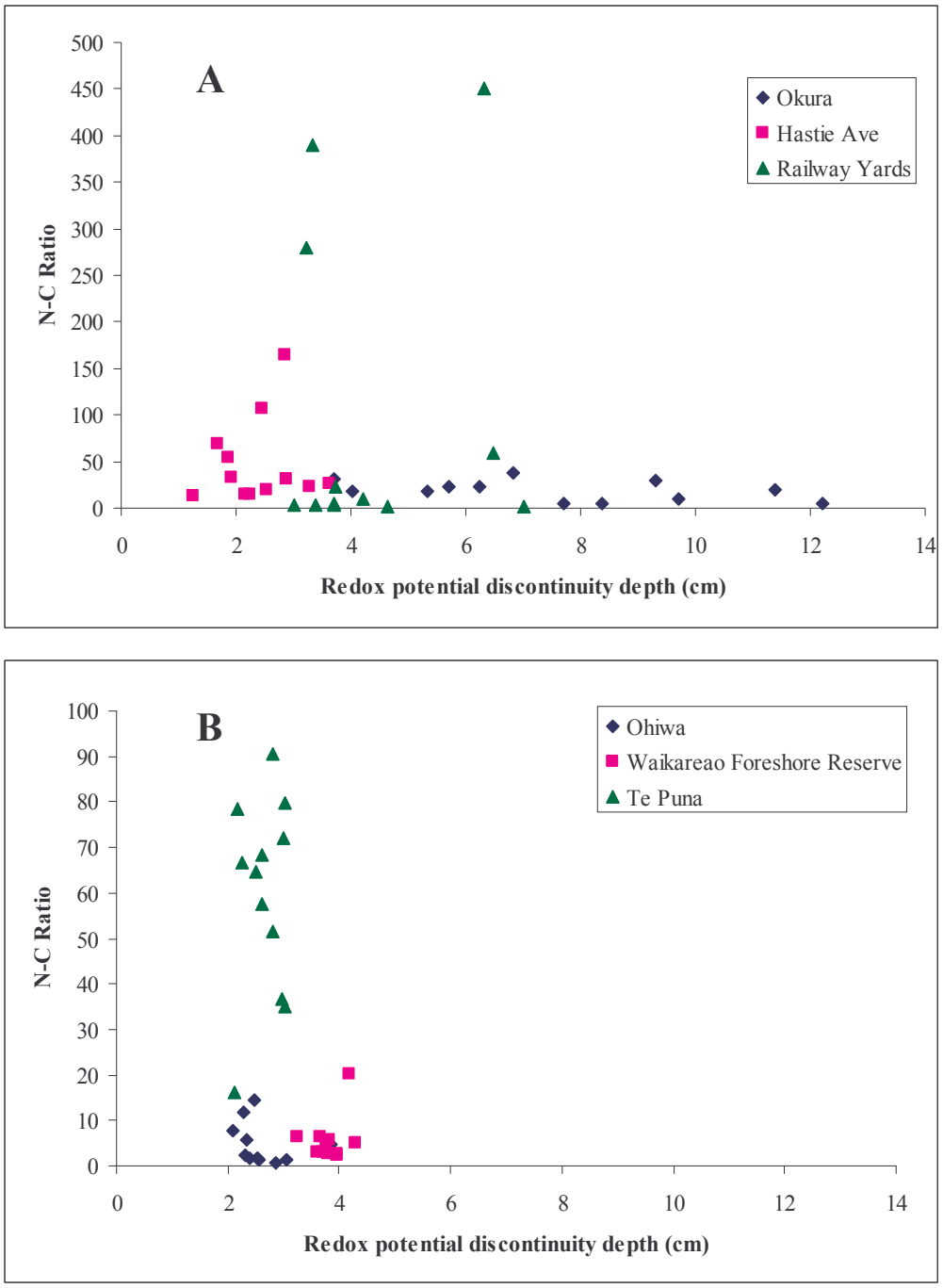

Figure 7.4. Nematode – copepod ratio plotted against redox potential discontinuity depth (cm) at each transect, distance and field site combination in both **A** the Auckland region and **B** the Bay of Plenty region.

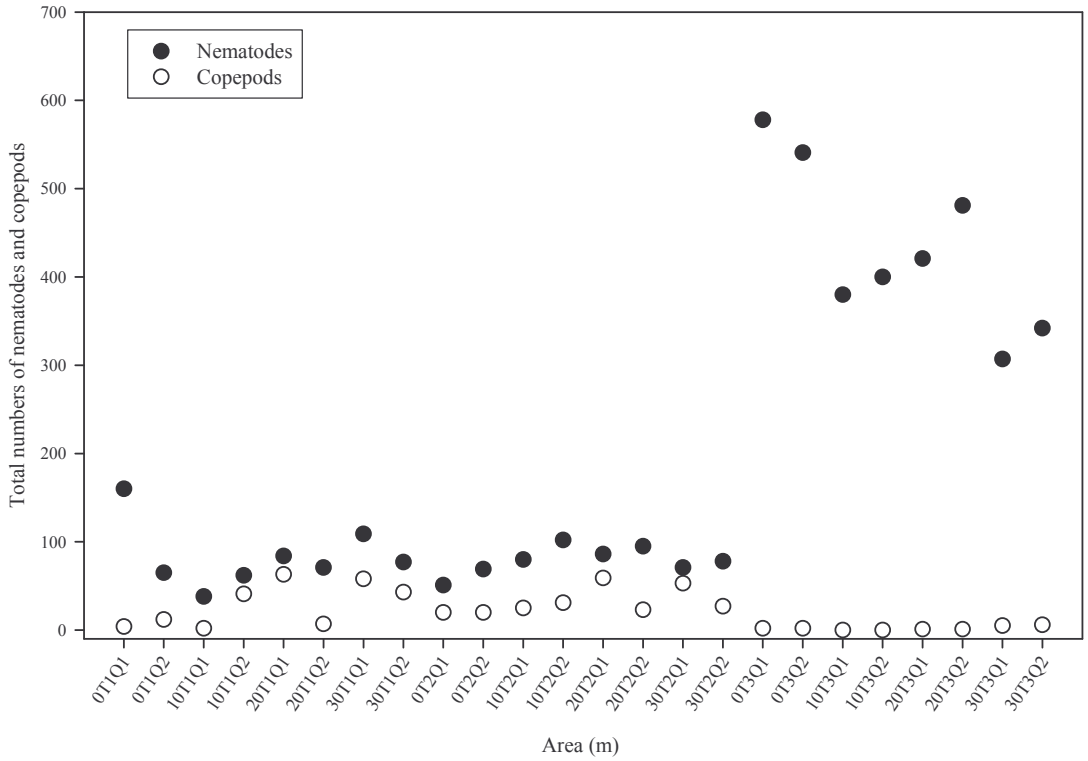

Figure 7.5. Total numbers of nematodes and copepods at each distance and transect combination in the Railway Yard field site within the Auckland region. NOTE: e.g., 20T2Q2 = 20m, Transect 2, Quadrat 2.

Table 7.2. Correlation coefficients (r) for the nematode-copepod ratio compared with organics, sediment grain size and redox potential depth for all sites within each field region.

Region	Site	Nematode-copepod ratio and organics (%)	Nematode-copepod ratio and grain size (μm)	Nematode-copepod ratio and redox potential depth (cm)
Auckland	Reference	-0.067	-0.999	-0.457
	Contaminated	-0.256	0.205	0.218
Bay of Plenty	Reference	0.118	-0.859	-0.241
	Contaminated	0.067	-0.306	-0.756

Table 7.3. Contaminant (lead (Pb), copper (Cu) and zinc (Zn) concentrations (mg/kg) in the 500 µm sediment fraction and polycyclic aromatic hydrocarbons (PAH) concentrations (µg/kg) in the 500 µm sediment fraction recorded in the sediments at each field site within the Auckland and Bay of Plenty field region. Data taken from ARC (2003), Environment Bay of Plenty (2003) and Stephen Park, Environment Bay of Plenty (pers. comm.) refer to sites as close as possible to those listed in the table below. Note: * ARC (2003) guideline values are given for the Hastie Ave site as no raw data was available. ARC (2003) stated contaminant values were above these values for the tabulated contaminants. ND refers to no available data.

		Contaminants (mg/kg)			
Region	**Site**	**Pb**	**Cu**	**Zn**	**PAH** (µg/kg)
Auckland	Okura (Reference)	10.0	7.0	39.0	16.0
	Hastie Ave*	> 50.0	> 34.0	> 150.0	< 660.0
	Railway Yards	32.0	37.0	155.0	65.0
Bay of Plenty	Ohiwa (Reference)	3.9	4.2	22.9	3.0
	Waikareao Foreshore Reserve	4.4	1.3	40.5	32.0
	Te Puna	6.0	2.8	25.6	ND

7.5. Discussion

The evidence presented in this study casts doubt on the validity of the nematode-copepod ratio as a tool to detect metal pollution in New Zealand estuarine environments. The nematode-copepod ratio proposed by Raffaelli and Mason (1981) is based on the well known detail that as sediment grain size decreases nematode numbers increase whereas copepod numbers decrease (Hicks & Coull 1983, Higgins & Thiel 1988). Coull et al. (1981) argued that the use of the ratio was an over simplification of a meiofaunal community to categorise sites based on marine pollution, that the ratio would not be universally applicable and that inexperienced workers may fail to understand other factors interacting on species abundances.

In the present study, correlations between the nematode-copepod ratios in both clean and 'contaminated' sediments did not indicate significant relationships with sediment grain

size or percent organic carbon at any of the six field sites, which would indicate other factors are affecting the distribution and abundance of nematodes and copepods at each field site. This is important as sediment grain size and total organic content are known to be important environmental factors that affect meiofaunal community composition (Hicks & Coull 1983). The absence of correlations indicates the influence of another variable and in the case of total numbers of nematodes and copepods at each field site this may be due to sediment-associated contaminants (Table 7.3). It is unclear to what extent these contaminants are affecting nematode and copepod abundances as the total numbers are more variable in the more highly polluted Auckland sites and less variable in the less polluted Bay of Plenty field sites.

The current data shows no clear differentiation between non-polluted and polluted sites when using the ratio as a measure of pollution induced environmental stress. In the Auckland field sites, including reference and polluted sites, the ratio of nematodes to copepods was greater than 1 indicating polluted sediments. From these results it would appear the reference site is being impacted by some pollutant source and these results are consistent with those recorded in a copepod full life cycle contaminated sediment bioassay (Hack unpublished data). Conversely, within the Ohiwa (reference site, Bay of Plenty) samples there was a clear shift in dominance from nematodes (indicative of contaminated sediments) to copepods which would indicate non-contaminated sediments. However, the available contaminant data does not support such a finding due to the very low levels of contaminants found in this harbour (Table 7.3). It is suggested that further laboratory analyses be undertaken to assess the sensitivities of nematodes and copepods to selected contaminants both aqueous phase and sediment-associated. Information on the feeding mechanisms and metabolic requirements of nematodes and copepods (Warwick 1981) would also assist in determining contaminant bioaccumulation rates and therefore species distribution and abundance. This additional information may help to refine the nematode-copepod ratio as a potential indicator of environmental metal contamination, similar to the widely used Macroinvertebrate Community Index (MCI). The MCI, a biotic index, was first proposed by Stark (1985) for use in stony riffles in New Zealand but has been used elsewhere (Pinel-Alloul et al. 1996). This index takes into account habitat

characteristics (e.g. current velocity, water depth and substratum), environmental factors and species compositions and biogeography and ranks New Zealand taxa between 1 (pollution tolerant) and 10 (pollution intolerant) based on their occurrence in stony riffles at sites classified as unpolluted, moderately polluted, or grossly polluted (Stark 1993, Collier 1995, Williamson et al. 1995, Hicks 2002, Wright-Stow & Winterbourn 2003).

For the nematode-copepod ratio to be a good indicator of metal pollution, values would be expected to vary according to pollution gradients. There is a clear suggestion in this study that the ratio does not provide sufficient evidence for inclusion as a tool in the assessment of contaminated sediments in New Zealand estuarine environments. However, further work is required to ascertain whether the reported trends occur throughout other contaminated New Zealand estuarine environments. Investigation into whether New Zealand environmental factors (e.g., seasonal influences) and habitat (e.g., substratum) affect the nematode-copepod ratio is required coupled with detailed information on the sensitivities between nematodes and copepods to contaminants. A hypothesis may then be developed that the nematode-copepod ratio may predict levels of contaminants in New Zealand intertidal/estuarine environments.

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