Using amphipods as bioindicators of metal pollution in the marine environment

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Abstract

Heavy metals in the marine environment are a worldwide issue due to their toxicity, non-biodegradability and their ability to accumulate and magnify in organisms. Increased human activity has caused higher inputs of heavy metals, resulting in escalated pressures on delicate coastal ecosystems. A means of assessing the natural environment and how it is changing in response to pollution and other environmental degradation is through the use of biological indicator or biomonitor species. These organisms provide information on the bioavailability of metals present in the environment. In recent years amphipods, a diverse order of small crustaceans, have been increasingly used as bioindicators of disturbed aquatic communities. They are widespread and important components of many food webs, and likely to be frequently exposed to metal contamination through both sediment and seawater. The aim of this research was two-fold: 1) to use amphipods to examine variation across sites and species in concentration of 20+ trace elements and 2) to examine whether the uptake of two metals, copper (Cu) and neodymium (Nd), is mediated by the presence of the other metal or an elevated seawater temperature.

To investigate variation of trace element concentrations across sites, the amphipod *Eusiroides monoculoides* was collected from three sites in the Wellington region, approximately 5 km apart: Oriental Bay, Evans Bay and Point Halswell. To investigate differences amongst species comparisons were made between *Eusiroides monoculoides, Apohyale papanuiensis* and *Sunamphitoe mixtura* when they occurred at the same site. Analysing the trace element concentrations of 36 metals was done using an Inductively Coupled Mass Spectrometer (ICPMS). Overall, although these sites were not greatly distant from each other, there were differences among sites. Evans Bay in general had the highest concentration of trace elements. Further, there were also species-specific differences and *S. mixtura* was the species with the highest concentration of trace elements. There was also a size effect, where the average dry weight of *S. mixtura* was negatively related to the concentration of trace elements in the body.

To assess the effects of heavy metals Cu and Nd in both an ambient (14 °C) and elevated (20 °C) temperature, an experiment was run at Victoria University's Coastal Ecology Lab (VUCEL). Sand hoppers, *Bellorchestia quoyana*, were collected from a single site in Wellington (Scorching Bay) and assigned to eight treatments: ambient and warm controls in raw seawater and ambient and warm seawater doped with Cu, Nd and Cu and Nd together. Amphipods from treatments with Cu and Nd added had significantly higher concentrations of these metals from the controls, however temperature had no effect, and neither was there an interaction between the metals. Similar to

S. mixtura from the field study, dry weight of *B. quoyana* was negatively related to the concentration of trace elements in the body.

Results from this work demonstrate that when using amphipods as bioindicator species it is important to consider species and size specific effects. This thesis also provides baseline data for 20+ elements from three Wellington sites and demonstrates that there can be unexpected variation across relatively small spatial scales. The laboratory experiment did not yield results that coincided with the consensus of the literature. The experiment showed that at least in this case, temperature did not mediate the uptake of metals and there was a negative relationship between size and metal uptake.

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1 General Introduction

Metals in coastal environments

Metals occur in the environment from both natural and artificial sources and vary in concentrations across geographic regions (Pan & Wang, 2012). Natural sources include volcanism, forest fires and the release of metal enriched particles from terrestrial vegetation (Burger, 2008). Human and industrial activities can be responsible for artificial introduction of heavy metal contaminants to the marine environment via direct inputs, riverine contributions, and atmospheric deposition (Turemis et al., 2018). The term 'heavy metal' is used synonymously with 'trace metal' and includes both essential metals such as zinc (Zn) and copper (Cu), as well as non-essential heavy metals including cadmium (Cd), lead (Pb) and mercury (Hg) that occur in trace amounts within the environment or within organisms, typically <0.01% of the organism (Marsden & Rainbow, 2004). Heavy metal levels in ecosystems have become a problem worldwide due to their toxicity, non-biodegradability and their ability to accumulate and magnify in organisms (Costa-Boddeker et al., 2018; Zhuang & Gao, 2014). An example of the effects of heavy metals is from the coastal waters of southeast Korea, where there is a decreased genetic diversity of organisms in the regions polluted by heavy metals. It was estimated that a ~30% –50% reduction of species richness was caused by heavy metal contaminants (Wang et al., 2018). The extent of heavy metal contamination has become a concern for many countries and is subsequently being addressed by a number of European Union (EU) legislative measures and policies (Tornero & Hanke, 2016).

Heavy metals can be absorbed by aquatic organisms directly from the environment or ingested through food and absorbed into soft tissue or excreted (Barka et al., 2010; Ivanciuc et al., 2006). Both bioaccumulation and biomagnification of certain heavy metals occur in aquatic organisms (Lee et al., 2017). Some metals are used by organisms in biological processes such as metabolism, and thus are considered essential (Canli & Atli, 2003; Jackson et al., 2012; Rainbow, 2002). For example, Zn is present in all organisms as it is an essential trace element for metabolic processes, used as a key component for many enzymes including carbonic anhydrase (Canli & Atli, 2003; Olmedo et al., 2013; Pan & Wang, 2012). Copper is a functional part of the respiratory protein haemocyanin, found in malacostracan crustaceans (Rainbow, 2002), and both metals are actively regulated in some organisms (Feng et al., 2016; Rainbow, 2002). Nevertheless, even elements essential for basic functions can become toxic and potentially lethal if they exceed certain thresholds (Conti et al., 2016; Marsden & Rainbow, 2004; Torres et al., 2008). Non-essential metals, like Cd, Pb or Hg have no required minimum concentration and need to be detoxified or excreted immediately (Garcia et al., 2012; Mathews & Fisher, 2008; Rainbow, 2002; Ratte, 1999).

Coastal environments are delicate and provide numerous functions and services including; nurseries, filtration of large volumes of water and nutrient recycling (Conti et al., 2016; Goncalves & Marques, 2017). With 60% of the Earth's population living within 100 km of the coast, increased human activity has caused higher inputs of heavy metals resulting in elevated pressures on coastal ecosystems (Boehm et al., 2017; Pan & Wang, 2012; Wurl & Obbard, 2004). Such activities include rapid coastal urbanisation involving dredging and land reclamation, sewage runoff, as well as agricultural and industrial discharges (Chaalali et al., 2017; Karuppasamy et al., 2017; Mehanna et al., 2016). Heavy metals are a global pollutant due to their ability to be easily transported over long distances (Pan et al., 2014). Heavy metal contamination can therefore have devastating effects on the ecological balance of the environment and its inhabitants (Gentes et al., 2013; Mehanna et al., 2016; Yong et al., 2017). The effects of metal pollution on local environments and organisms can be substantial and long lasting regardless of restoration efforts (Ghrefat & Yusuf, 2006; Pan & Wang, 2012).

Marine and estuarine sediments can bind and accumulate a wide variety of trace metals, often to high concentrations (Marsden & Rainbow, 2004). Ninety percent of the metal load in aquatic systems is associated with suspended particulate matter and sediments. Sediments therefore provide better data than seawater to describe the spatial and temporal trends in metal concentrations and are the preferred medium for monitoring the concentrations of trace metals (Ungherese et al., 2010). Sediments act as records of aquatic processes and show less variation over shorter timescales than dissolved metals in the overlying water column. They also represent a sink of multiple contaminant sources (Birch, 2017; Bryan & Langston, 1992; Garcia et al., 2012; Karuppasamy et al., 2017; Rodrigues et al., 2017; Sprovieri et al., 2007). Sediment toxicity assessments also provide evidence of relationships between sediment contaminants and ecological degradation (Moran et al., 2017). Analysing sediments using core samples can provide a timeline of trace metal distribution and contamination, often with pre-industrial samples in bottom sediments and more recent samples in surface sediments (Al-Mur et al., 2017).

Understanding how trace metals interact with sediment can give insight into their bioavailability and toxicity to living organisms (Canli & Atli, 2003; Jackson et al., 2012; Rainbow, 2002). Metal accumulation in sediments is affected by sediment characteristics that vary geographically and temporally (Morillo et al., 2004). Fine grained sediments tend to have a high metal concentration mainly due to surface adsorption and ionic attraction. Organic matter on particles is dominant in fine-grained sediments, and the resulting biofilm binds a variety of trace metals (Fox et al., 2014;

Morillo et al., 2004; Zhang et al., 2007). Coastal sediments are mainly fine-grained, with higher surface area, increasing metal retention and organic matter affinity (Dos Santos et al., 2006). Trace metals vary in the way in which they bind to sediments and bind to organic matter (Yu et al., 2001).

Amphipods in the marine environment

Amphipods are an order of the diverse crustacean class Malacostraca. Order Amphipoda is part of the superorder Peracarida. Amphipods brood their young in a pouch and have no independent larval dispersal stage. Amphipods lack a carapace (hard shield over the thorax), have non-stalked eyes, have three pairs of pleopods and three pairs of uropods, setting them apart from all other malacostracan crustaceans (Horton et al., 2019).

The name Amphipoda means 'different feet' referring to the different forms of the pereopods. Amphipods are colloquially known as: scuds, shrimp or side-swimmers. Amphipods that have colonised the land and beaches are commonly referred to as land hoppers or sand hoppers and beach/sand fleas respectively (Horton et al., 2019).

The order Amphipoda currently contains 10,226 described species most of which are free-living and benthic (Gordon, 2013; Horton et al., 2019; Hurley, 1958; Hyne, 2011). Amphipods vary in size from a millimetre in length to the supergiant *Alicella gigantea* (Chevreux, 1899) measuring around 340 mm (Horton et al., 2019). Amphipods are found in all marine habitats and have also colonised freshwaters and terrestrial habitats (King et al., 2006). There is approximately 1870 amphipod species and subspecies recorded from fresh or inland waters. Amphipods are important herbivores, detritivores, micropredators, scavengers and even ectoparasites and they form an important component of both marine and freshwater ecosystems (Gordon, 2013; Hurley, 1958; Marsden & Rainbow, 2004). The large number of species and high densities of amphipods in many soft-bottom estuarine and marine benthic ecosystems render them important in food webs (Gordon, 2013; Hurley, 1958; Marsden & Rainbow, 2004). Amphipods are often the principal food for predatory fish and birds (Bousfield, 1990; Breitholtz et al., 2001; Hubbarda & Dugan, 2003; Marsden & Rainbow, 2004).

As mentioned before, coastal marine environments are vulnerable to pollution, including by heavy metals (Marsden & Rainbow, 2004). As amphipods are wide-spread and important components at the base of many food webs, they are likely to be frequently exposed to metal contamination through both sediment and seawater and are often the first organisms to disappear from contaminated sites (Al-Mur et al., 2017; Gordon, 2013; Hyne, 2011). The uptake of heavy metals in amphipods can have adverse effects on their health, such as incurring an energy cost to

the organism, disrupted reproductive processes and increased mortality (Marsden & Rainbow, 2004).

Direct impact of metal contamination on organism health

Heavy metal uptake incurs an energetic cost associated with excreting and/or detoxifying the incoming metals. Understanding when this extra energy requirement becomes significant in terms of growth and reproduction is one of the challenges faced by marine ecophysiologists (Marsden & Rainbow, 2004). It is also unknown how this extra energy requirement will translate to populations, and benthic communities as a whole (Marsden & Rainbow, 2004).

Mortality is one direct consequence of heavy metal exposure, for example amphipod mortality increased with increased concentrations of Cu in some experiments (Marsden, 2002). Significant amphipod mortality was also found by Rodrigues et al. (2017) in an area close to a major source of heavy metals from an industrial pollutant. Some benthic amphipods' survival depends on the size or life stage of the individual, with juveniles being consistently more sensitive to contaminants than adults (Marsden, 2002). Exposure to heavy metal contaminants in amphipods can have other detrimental effects as well, for example endocrine disruption which in turn impacts hormonally regulated physiological processes (Hyne, 2011). Field studies on the sediment dwelling amphipod *Monoporeia affinis* (Lindström, 1855) by Hyne (2011) showed male sexual development and the development of olfactory sensilla on the antennules was delayed or disrupted when the animals were exposed to contaminated sediment. Heavy metals such as Cd have been found to affect reproduction in amphipods. Exposure to Cd in the water flea *Daphnia magna* (Straus, 1820) delayed the onset of reproduction and reduced neonate size. Exposure of *Daphnia pulex* (Leydig, 1860) to Cd resulted in a reduction in the number of adults that produced young, the number of broods per adult, the number of young per brood, as well as reduced mean generation time (Breitholtz et al., 2001). Tests of the toxicity of Cu on four species of *Daphnia* showed different effects on brood size, which shows that closely related species can differ considerably in sensitivity to heavy metal pollutants (Breitholtz et al., 2001). The presence of trace metals in sediments also can reduce recruitment in amphipods due to behavioural avoidance of contaminated sediments. This was demonstrated through laboratory studies by Marsden & Rainbow (2004) with the uptake of Cu from sediment causing reduced recruitment in amphipods; *Hyalella azteca* (Saussure, 1858), *Corophium volutator* (Pallas, 1766) and *Paracorophium excavatum* (Thomson, 1884).

In general, rather than focusing on health effects of metal exposure, many studies focus on other aspects of the relationships between heavy metals and amphipods. For example, how amphipods reflect environmental change following heavy metal input (Abrahim & Parker, 2008; Al-Mur et al., 2017; Clason et al., 2003; Duquesne et al., 2000; Duquesne & Riddle, 2002; Zauke & Schmalenbach, 2006). Other studies consider how metal uptake by amphipods changes in the presence of other metals (Rainbow et al., 2000). Studies considering heavy metal pollution in sediments using amphipods and other invertebrates use both the sediment and organism as a means of comparison to assess environmental health (Bryan & Langston, 1992; Gust & Fleeger, 2005; Strandberg et al., 2000).

Heavy metals in organisms: bioaccumulation

Heavy metals can accumulate in an organism to the level higher than the surrounding medium, which is known as bioaccumulation (Schaefer et al., 2015). Bioaccumulation is dependent upon the concentration of metals in the water and exposure time, as well as environmental factors such as pH, salinity, and temperature (Canli & Atli, 2003; Gust & Fleeger, 2005; Muniz et al., 2004). Bioaccumulation also varies with the specific metal being uptaken. For example, under similar exposure conditions Cd does not accumulate in gammarid amphipods to the same extent as Cu (Duquesne et al., 2000). Furthermore, the uptake of one metal can affect the accumulation of another, as seen in the amphipod *C. volutator,* where Zn addition induces a reduction of Cd toxicity and accumulation (Rainbow et al., 2000).

Bioaccumulation is also affected by the biology and ecology of an organism, such as the trophic position, habitat, sex, size, moulting stage and the strategies through which it copes with metals (Canli & Atli, 2003; Pan & Wang, 2012; Ugolini et al., 2005). Bioaccumulation is also affected by weight changes, associated with growth, starvation, loss of gametes and energy reserves (Marsden & Rainbow, 2004). Different invertebrates accumulate heavy metals at varying concentrations in their bodies. Closely related aquatic invertebrate taxa living in the same habitat are likely to have different body concentrations of trace metals (Ikemoto et al., 2008; Rainbow, 2002). Differences in trace element concentrations among crustaceans can be attributed to differences in their metal accumulation and detoxification abilities (Ikemoto et al., 2008).

Within crustaceans there are differences between taxonomic groups. Some decapods have the ability to regulate their internal concentrations of essential metals (Vijayram & Geraldine, 1996). In the case of non-decapod crustaceans such as amphipods, both essential and non-essential metals

are accumulated without excretion when initially uptaken (Marsden & Rainbow, 2004). Amphipods can store accumulated trace metals in an insoluble form in the cells of the ventral caeca especially if they are uptaken from food. These granules are discharged when the cells of the ventral caeca complete the cell cycle (Marsden & Rainbow, 2004).

Amphipods as bioindicators of contamination

Biological indicators and monitor species are used as a means of assessing the natural environment and how it is changing in response to pollution and other environmental degradation. Using a living organism to monitor pollution is preferred over the traditional method of a chemical analysis of seawater as the latter does not provide information on the bioavailability of metals present in the environment (Guerra-Garcia et al., 2009; Marin, 2017; Turemis et al., 2018). A biological indicator (bioindicator) species is one that represents an ecological effect by simply being present or absent. A biological monitor (biomonitor) quantifies the degree of ecological change through the organism's response to a contaminant either behaviourally, physiologically or biochemically (Rainbow, 1995). Biomonitors are a means of establishing both geographical and temporal variations in the bioavailability of pollutants such as heavy metals in the marine environment (Clason et al., 2003; Gesteira & Dauvin, 2000; Rainbow, 1995). Ideal traits of biomonitors are that they are sedentary, easily identifiable, abundant, available for sampling year-round, and resistant to handling stress caused by laboratory studies and transportation (Birch, 2017; Rainbow, 1995). In many cases environmental monitoring studies use a single species. The concept of using only one biomonitor species rather than multiple species has been met with some criticism because using a single species cannot account for the possible effects a pollutant might have on the entire community (Sabater et al., 2007). Nevertheless, due to logistical limitations, cost, and other practical issues, many biomonitoring studies do use single taxa. Well-represented examples include bivalves, urchins and polychaetes (Gopalakrishnan et al., 2007).

In recent years amphipods have been increasingly used as bioindicators of disturbed aquatic communities (Eisenring et al., 2016; Kahle & Zauke, 2003; Marsden & Rainbow, 2004; Mouritsen et al., 2005; Ugolini et al., 2004). Amphipods are also a good choice as a biomonitor taxa as they are abundant and resistant to handling stress, as well as being the principal prey of numerous organisms (Conti et al., 2016; Marsden & Rainbow, 2004; Picone et al., 2008). Internationally, amphipods are used widely for testing contamination in sediments for the same attributes that make them suitable biomonitors (Brown et al., 1999; Marsden & Rainbow, 2004; Passarelli et al., 2017; Porri et al., 2011). The use of amphipods as bioindicators and biomonitors is further supported as crustaceans are more

sensitive to the changes produced by environmental contaminants than other organisms in their communities (Gesteira & Dauvin, 2000). Numerous international examples showcase the use of the amphipod *Gammarus pulex* (Linnaeus, 1758) for assessing water quality as it is widely distributed in Europe (Marin, 2017). The amphipods *C. volutator* and *Ampelisca brevicornis* (Costa, 1853) have been used for sediment quality characterisation in various Spanish ports (Rodriguez-Romero et al., 2013). *H. azteca* is an epibenthic amphipod also commonly used in sediment toxicity tests as it responds to contaminants in both the water and algae it feeds upon (Garcia et al., 2012; Weston et al., 2009). This species has also been used for toxicity testing in California to monitor trace elements at several prime agricultural areas (Anderson et al., 2018).

Cultural importance of the marine environment

In New Zealand, understanding degradation to the marine environment caused by pollutants such as heavy metals is exceptionally important for Māori, the indigenous people of New Zealand. Māori have a very deep and unique connection to the ocean and its inhabitants and these are essential for the spiritual, social, cultural and economic wellbeing of Māori (Waitangi Tribunal, 2002). Services and benefits provided by the marine environment are also considered precious gifts (taonga) (Freshwater Iwi Leaders Group, n.d). Taonga in relation to fisheries equates to a source of food (kai), an occupation and a source of goods for establishing and maintaining relationships. The depth of connection between Māori and the marine environment is that of self-identity (Waitangi Tribunal, 1988). Māori consider all forms of life, the ocean, landscapes and natural resources as being either family (whānau) or their ancestors (tūpuna) (Waitangi Tribunal, 1999, 2008). This view creates immutable and inseverable connections between Māori and their environment (Bess, 2001; Roberts et al., 1995). The life force (mauri) of the ocean and the people are connected, therefore maintaining the integrity of the marine environment enhances the shared life force (mauri) as well as the prestige and authority (mana) of the Māori people who associate themselves as spiritual and physical guardians of the marine environment and its inhabitants (kaitiaki) (Waitangi Tribunal, 2002).

Conclusion and research aims

Coastal environments provide numerous ecosystem functions and services. Pollutants, including heavy metals discharged into sensitive coastal environments have increased over the past decades due to increased human activities in coastal areas. Amphipods are ubiquitous and abundant in coastal environments. Heavy metals have adverse effects on amphipod health by incurring an energetic cost to the organism from either excretion or detoxification, disrupting reproductive processes and increasing mortality. Bioaccumulation is influenced by both the biology and ecology of

an organism. Both age class and size are recognised factors that affect bioaccumulation in amphipods, as well as species-specific traits. Amphipods are internationally used as a biomonitor and bioindicator species as a means of assessing the natural environment and changes in response to pollution. Amphipods are ideal organisms to use as they are abundant, resistant to handling stress and are principal prey to numerous organisms.

The aim of this thesis is to illustrate techniques used in monitoring chemical changes in the marine environment through the use of amphipods as a focal organism. New Zealand's surrounding ocean influences many elements of the economy and culture (Le Heron et al., 2016), with the state and health of the marine environment being central to societal and economic wellbeing. Of particular concern are changes to this environment brought about by anthropogenic activities. An important factor in being able to preserve our unique marine environment is understanding the chemistry of this environment and being able to monitor any changes that occur. A catalogue of marine environmental programmes in New Zealand was assembled in 2013. Of these listed programs, only two monitor heavy metals in the Tasman Bay and Marlborough (Hewitt, 2013). This highlights the opportunity for a monitoring system to detect heavy metals in the marine environment to be developed that can be implemented on a national scale.

The first chapter illustrates techniques used for assessing the state of the marine environment and examining variation across sites through the analysis of trace elements present in amphipod species across different locations around the Wellington coast. Information gathered in this chapter provides baseline data to provide comparisons of species and locations as well as changes over time (although changes over time are not addressed here). The second chapter details a laboratory experiment examining the uptake of two elements, copper (Cu) (essential) and neodymium (Nd) (non-essential), by a talitrid sand hopper species at different seawater temperatures. Information from this will contribute to the understanding of how uptake and accumulation of these metals changes with a common climate change stressor: increasing seawater temperature.

2 Trace element analysis of species from three families of amphipod around the Wellington coastline.

2.1 Introduction

In recent years, amphipods have been increasingly used as bioindicators at polluted sites as they bioaccumulate particular metals from both solution and diet (Kahle & Zauke, 2003; Pastorinho et al., 2009; Rainbow, 1995; Ugolini et al., 2004). Bioaccumulation is often a good indicator of the chemical exposures of organisms in polluted ecosystems (Luoma & Rainbow, 2005; McGeer et al., 2003). A means of analysing trace elements is through the use of an Inductively Coupled Plasma Mass Spectrometer (ICPMS) (Reis-Santos et al., 2012). An ICPMS is a common tool in environmental element research and is able to produce data for more than 20 elements within a single sample (Borga et al., 2006).

In many studies conducted on bioaccumulation within a food web, prey species are often grouped together. For example, Eagles-Smith et al. (2008) grouped insect prey species by family, whereas amphipods received no further classification. A study examining bioaccumulation in the Arctic did no further taxonomic classification of the organisms used, other than to an order level of amphipod and copepod (Borga et al., 2004; Tiano et al., 2014). Nevertheless, it is important to distinguish amphipods to a species level as element concentrations vary among species from the same area, which is related to the biological characteristics of the species and the biochemical characteristics of each element (Borga et al., 2006). Unfortunately, amphipods are not often identifed to a species level for such assessments, which may confound patterns of trace element concentrations in an individual (Borga et al., 2004; Eagles-Smith et al., 2008; Tiano et al., 2014). Other issues to consider that may also confound patterns are: age, size class effects (Breitholtz et al., 2001) and trace element uptake from seawater or associated seaweed species (Chakraborty & Owens, 2014).

Amphipods can uptake trace elements through both absorption and ingestion (Barka et al., 2010; Ivanciuc et al., 2006). Therefore, determining whether trace element concentrations occurring in algal-dwelling amphipods are a reflection of the seaweed they are associated with or the surrounding seawater can be challenging. Chakraborty and Owens (2014) analysed seawater and algae across six sites for ten heavy metals and no significant difference was found between water bodies. However, the algae showed a difference across all locations and species. Concentrations of heavy metal pollutants vary widely across algae species (Escobar et al., 2009). Studies have found

that brown algal species were found to have higher concentrations of arsenic (As) than green and red algal species (Caliceti et al., 2002). However, *Ulva* sp., a green alga, had the highest concentration of other heavy metals including: iron (Fe), Cu, Pb and chromium (Cr) (Caliceti et al., 2002). Risk assessments based only on data derived from water analyses and algae may be misleading as the data cannot provide information on patterns of contamination at the higher levels of the food chain (Torres et al., 2008).

More than 100 species of gammaridean amphipods live amongst the algae in New Zealand's intertidal zone, with more than 50% being endemic (Hurley, 1958; Barnard, 1972; Lörz et al., 2010). The species of amphipods sampled in this study belong to three different families: Ampithoidae, Hyalidae and Pontogeneiidae. Ampithoidae is a family of marine Amphipoda with approximately 230 species, belonging to 16 genera (Peart & Lörz, 2017). This family has a worldwide distribution and are typically algal dwellers, occurring commonly through tropical and temperate waters (Peart & Ahyong, 2016; Peart & Lörz, 2017). The family is one of the largest families of herbivorous amphipods in terms of number of species. Their ecological importance has encouraged many studies examining plant-herbivore interactions (Cruz-Rivera & Hay, 2001; Shin et al., 2015; Peart & Ahyong, 2016). Hyalidae is a family of exclusively marine Amphipoda with 110 species distributed into 12 genera (Jung & Yoon, 2013). Species of Hyalidae occur mainly intertidally and in littoral waters, with a high proportion of these species being benthic (Serjo, 2004; Tempestini et al., 2018). Hyalidae were among the earliest species recorded and described from the coasts of Europe, Asia and the Pacific during the 19th century (Bousfield & Hendrycks, 2002). Hyalidae species are usually associated with algae but have also been found in mussel beds (Eun et al., 2016; Spilmont et al., 2018). Pontogeneiidae occupy the marine littoral zone, but occur often in brackish and freshwater (Bousfield & Hendrycks, 1995; Hurley, 1958). Pontogeneiidae have generalist feeding mouthparts. They are almost exclusively omnivorous or detritivorous and very rarely carnivorous (Bousfield & Hendrycks, 1995).

In the Wellington region, rapid industrial and urban development between 1910 and 1970 led to increased discharges of contaminants into the harbour. Wellington harbour (Port Nicholson or Te Whanganui a Tara) is an 85 km^2 semi-enclosed natural basin located at the southern end of the North Island, New Zealand (Pilotto et al., 1998; Van Der Linden, 1967). Heavy metal pollution in the Wellington harbour sediment has historically had high concentrations of Zn and Pb (Stoffers et al., 1986). More recent surveys completed by the Greater Wellington Regional Council (GWRC) in 2011

showed heavy metal concentrations in sediment in the Wellington harbour and around Wellington's coastline (GWRC, 2014).

The aim of this chapter is to determine the concentration of a variety of trace elements not often reported on at three nearby sites along the Wellington coastline, across different amphipod species. Due to the variety of species and numbers of individuals at each site several questions were tested separately. First, the spatial variability was examined among the three sites using a single species. Due to the proximity of the sites and the mixing of the water bodies samples, a site-specific difference was not expected. Second, where pairs of species occurred at the same site, species-specific differences were examined. For elements where there were no differences across site, individuals were pooled and species-specific differences were further examined. The third aim was to examine the trace element signatures of the seaweed *Carpophyllum maschalocarpum* (Greville, 1830) that amphipods were associated with at each site, as well as sea water at each site. Lastly, for one of the larger bodied species, the presence of a relationship between body size and element concentration was examined.

2.2 Methods

2.2.1 Sampling from the field

Amphipods were collected using two methods (light trapping and bagging, described below) from three sites in Wellington harbour, ~5 km apart: Oriental Bay, Evans Bay boat ramp and Point Halswell lighthouse (Figure A.1). Collection took place across three weeks beginning May 24th through to June 17th 2018.

Light traps were used to capture amphipods as these animals can exhibit diel vertical migration (Drolet et al., 2012; Fernandez-Gonzalez et al., 2014). Light traps designed to capture fish larvae are known to catch numerous zooplanktonic invertebrates, with records of amphipods being frequently caught (Carleton et al., 2001; Chan et al., 2016; Fincham, 1974; Meekan, 2001; Michel et al., 2010). Two light trap designs were constructed and trialled. Initially a smaller trap using plastic 2 L soda bottles (Appendix A) modelled from Chan et al., (2016), Michel et al., (2010) Sponaugle, (1996) and Watson et al., (2002) was used. However, this design proved to be unreliable during the course of this project. The second light trap design was modelled from Doherty (1987) and was a three-chamber light trap following modifications suggested by others (Navarro-Barranco, 2015; Watson et al., 2002). It was used for sampling at the three sites and constructed from a plastic storage container (500L x 320W x 140H mm), with eight funnels facing inward to create entry points for specimens. The light source was provided by a battery-operated dive light attached upside-down to the lid of the storage container so that it faced towards the benthos. On the bottom surface of the storage container a collection chamber was attached, which allowed the water to drain from the trap whilst still containing the specimens. Rope was used to attach a concrete cinder block to the trap to attach it to the benthos. A buoy was attached so the trap could be easily relocated (Figure 2.1, Appendix B). Light traps were set at all sampling sites in the evening and left overnight. Each trap was set at each site in ~1.5 m depth on rocky substrate.

For the bagging method of capturing amphipods, 1 mm mesh size drawstring bags were used to bag the common seaweed *C. maschalocarpum* at low tide at the three sampling sites. The bagging method involved turning the bag inside out and scooping up the seaweed while turning the bag in the correct way. Using a mesh bag allowed for the water to drain out while still containing any small specimens.

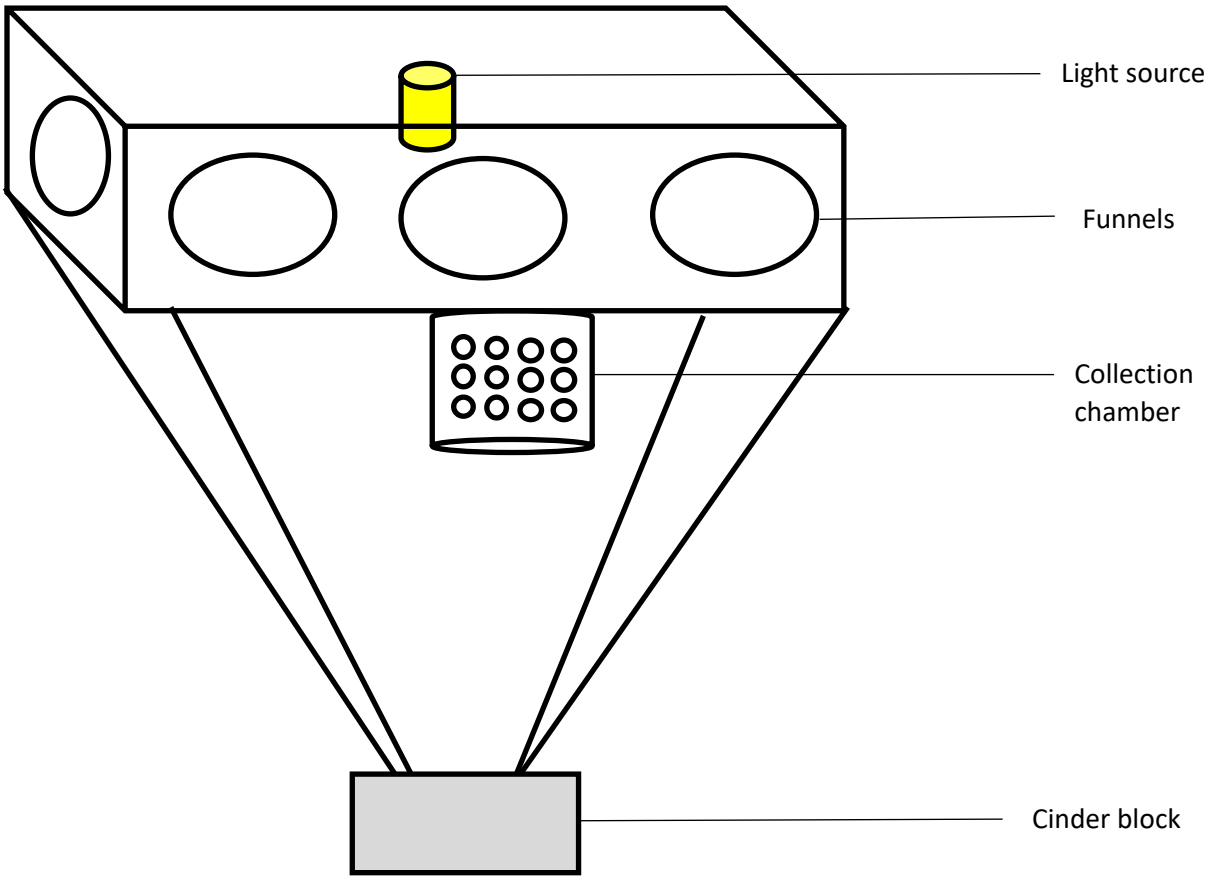


Figure 2.1 Light trap design (side view).

The traps were flushed with deionized water to dislodge specimens, which were then placed in resealable bags and labelled before being placed in a freezer at -4 °C. Similarly, to dislodge specimens from the seaweed samples, the mesh bags were frozen for 24 hours then rinsed in deionized water. The specimens collected via both methods were then transferred to glass vials with 80% ethanol and identified by Dr. Rachael Peart (National Institute of Water and Atmospheric Research, NIWA).

Seawater was collected at each sampling site once in sterile 125 mL plastic bottles. The bottle was rinsed thoroughly at the sample site with seawater before being filled. The seawater was then acidified in a laboratory using 1 mL of 3M hydrochloric acid (HCl), then refrigerated. The seawater samples were processed at the University of Otago.

Three amphipod species were selected to be processed using ICPMS to determine the trace element concentrations: *Sunamphitoe mixtura* Peart, 2017 (Ampithoidae)*, Apohyale papanuiensis* Kilgallen, 2011, (Hyalidae) and *Eusiroides monoculoides* (Haswell, 1879) (Pontogeneiidae). These species were selected because they occurred at all three sites in high enough numbers to analyse given their sizes. The amphipods were grouped into size categories to form pooled samples because

a minimum weight of 3 mg was required to process the sample for trace element analysis (Table A.1). They were measured using a dissecting scope at 1x magnification. A blade from a *C. maschalocarpum* at each sampling site was randomly selected to be analysed for trace element concentrations. The blade selected was cut to 30L x 5W mm using a ceramic scalpel on a cling-filmed bench. All samples required cleaning, drying, weighing and dissolving prior to being analysed. These pre-processing methods are described below.

2.2.2 Processing samples

Sample pre-processing

Before and between samples the work bench was wiped with 80% ethanol, followed by deionised water using kim-wipes, and then covered with cling film. Pre-cleaned teflon beakers were used to wash the amphipods. The amphipods were transferred into the beakers using plastic tweezers. The beaker was half-filled with deionised water, capped and placed into an ultrasonic bath for 10 seconds. The deionised water was pipetted out and disposed of using a clean transfer pipette. This was repeated three times, with a clean transfer pipette each time. Seaweed collected at each site was washed in the same manner as the amphipods with the modification of the washing process being repeated four times, to remove all attached particles from the seaweed. Once cleaned, the samples were transferred into aluminium foil boats using plastic tweezers.

The aluminium foil boats were placed on an aluminium foil oven tray. The oven tray was placed into a drying oven at 60 °C for one hour. Once the samples had been dried, they were transferred from the drying oven into labelled resealable bags and kept in a dissector until weighed. Weighing of all samples took place at NIWA, Greta Point Wellington using a Mettler AG245 Analytical Balance.

Before the samples were weighed, the bench was cleaned and prepared as described above. Using plastic tweezers, a 7 mL weigh boat was placed onto the microbalance and was then zeroed. A sample was then placed onto the weigh boat whilst it was still on the microbalance. After 30 seconds passed before the weight was recorded. Once the weight was recorded each sample was transferred to a plastic vial.

Dissolution procedure

The samples were digested prior to trace element abundance determination. First, they were transferred to a pre-cleaned Savillex PFA beaker, and ultrapure water was used to dislodge any of the sample remaining in the plastic vial. Optima™ trace element grade hydrogen peroxide (H_2O_2) was added to the beaker and it was then placed on a hotplate at 70 °C for 30 minutes, the temperature was increased to 80 °C, then 90 °C until the liquid had fully evaporated. Teflon distilled nitric acid (HNO_3) was added to the beakers and placed back on the hotplate at 70 °C, with the temperature increased incrementally up to 110 °C, and the samples evaporated until dry. The HNO_3 step was repeated. Teflon distilled 6M HCl was added to the beaker, capped and left on the hotplate overnight at 110 °C to reflux. The HCl was evaporated to dryness and the previous step of the addition of HNO_3 and then evaporating was repeated twice again.

Depending on the dry weight of the sample, different dilution protocols were used to prepare the dissolved samples for analysis. For samples under 5 mg in weight, 3M HNO_3 was added to the beaker which was capped and left to reflux on a hotplate overnight. The sample was then transferred to a 14 mL centrifuge tube. Ultrapure water was added to the centrifuge tube at four times the volume of the 3M HNO_3 first added to the beaker. For samples 5-10 mg in weight, 3M HNO_3 was added in the same manner as samples under 5 mg. The samples were then transferred to a clean 30 mL Nalgene bottle in place of a centrifuge tube and diluted using ultrapure water. For analysis, 10 mL of sample solution was transferred into a 14 mL centrifuge tube. For samples over 10 mg in weight, 6.3 mL of 6M HNO_3 was pipetted into the sample beaker, capped and left to reflux overnight. The sample solution was then transferred to a clean 30 mL Nalgene bottle in place of a centrifuge tube, followed by the addition of 18.8 mL of ultrapure water. A portion of this solution was then transferred to a clean centrifuge tube and brought up to 10 mL final volume using 1.5% HNO_3.

The volumes of acid and ultrapure water added to the samples were calculated so that the final solutions represented a 2500 times dilution of the original sample (based on dry weight) and 2% HNO_3 final acid molarity. The solution dilutions were weighed on a five decimal place balance to allow precise dilution factors to be calculated. Following the dilutions, all samples were centrifuged prior to being introduced to the ICPMS.

Processing samples through the ICPMS

Minor and trace element concentrations were measured using a Thermo Scientific Element2 Sector Field ICPMS in the Geochemistry Laboratory in the School of Geography, Environment and Earth Sciences, VUW. An ESI autosampler was used to introduce samples to the ICPMS. Between each solution analysed, a four-minute washout using 2% HNO_3 was undertaken. Instrument background levels were measured every third or fourth solution by analysing a 2% HNO_3 solution throughout the analytical sequence. These background counts per second (CPS) were subtracted from all analysed solutions.

Calibration of ICPMS data

The ICPMS was tuned to provide optimum signal intensity balanced with signal stability and low oxide generation. Thirty-six element masses were routinely analysed, using three different resolution modes (low, medium, high) to avoid spectral interferences (overlapping masses) that may be caused by isotopes of different elements or molecules with the same mass (Table A.2).
Data were obtained as raw CPS for each mass. These were converted to parts per million (ppm) for each element in the sample on a dry weight basis. For further details see Appendix C. Two standard reference materials (SRMs) were processed and analysed alongside samples throughout the course of this study as secondary standards to evaluate accuracy. The SRMs used were from the US National Institute for Standards and Technology, NIST1566 (oyster tissue), and Canadian National Research Council, DORM-4 (fish protein). These were used as there is no direct equivalent to the sample matrices for amphipod samples (tissue and exoskeleton). The standard reference materials were used as best available equivalents (Table A.3). For reference material used for the water samples see (Table A.4).

2.2.3 Statistical analysis

For all statistical analyses undertaken in this chapter, interpretation should be taken with care due to small sample sizes. The sample sizes in this chapter range between 3 and 5 replicates. All analyses were conducted in SAS Enterprise Guide 7.1. One-way ANOVAs were conducted to compare trace elements across *E. monoculoides* at three sites, as well as comparing amphipod species across sites. If the data did not meet the assumptions of normality or equal variance a Kruskal-Wallis test was run. Posthoc tests were run using the Tukey HSD test within SAS.
T-tests were used to compare trace elements in two amphipod species at the same site. If the data did not meet the assumptions of this test, a log of the data was used. Linear regressions were used to examine relationships between trace element concentration and dry weight of *S. mixtura*

individuals. If the data did not meet the assumptions of this test, a log of the data was used. All graphs were produced in Excel using data values imported from SAS.

2.3 Results

2.3.1 Composition of species collected

A total of 26 species of amphipods and 2505 individuals were collected across three sites, Oriental Bay, Evans Bay and Point Halswell (Table A.5). Of the species caught six were found at more than one site, and four species were found at all three study sites (Figure 2.2).

The majority of species at each site were collected from seaweed rather than the light trap (Figure 2.3).

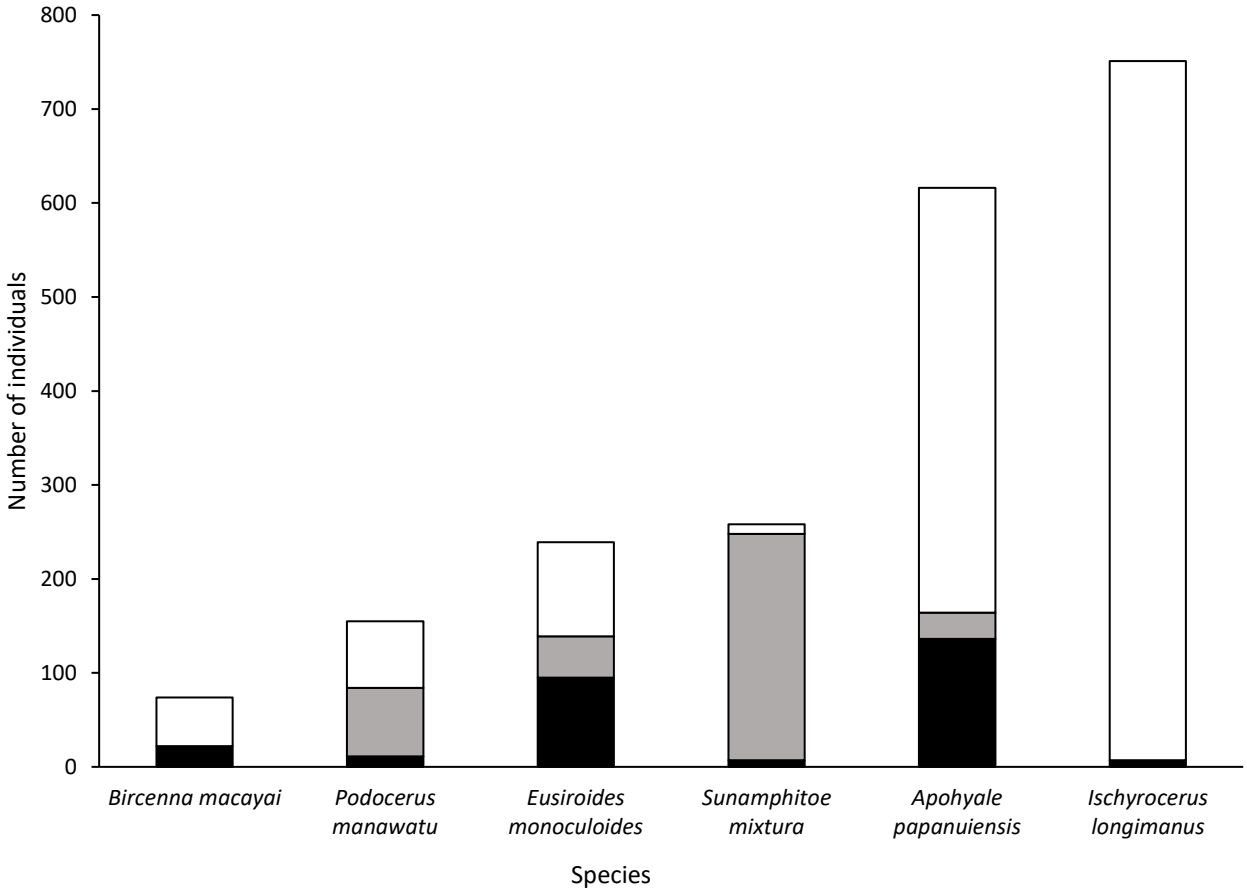


Figure 2.2 The number of individual amphipods caught at study sites, Oriental Bay (black), Evans Bay (grey) and Point Halswell (white). The amphipod species included in this graph were caught at more than one study site and totalled greater than 50. Species authorities not previously mentioned *Bircenna macayai* Lörz, Kilgallen & Thiel, 2009, *Podocerus manawatu* Barnard, 1972 and *Ischyrocerus longimanus* (Haswell, 1879).

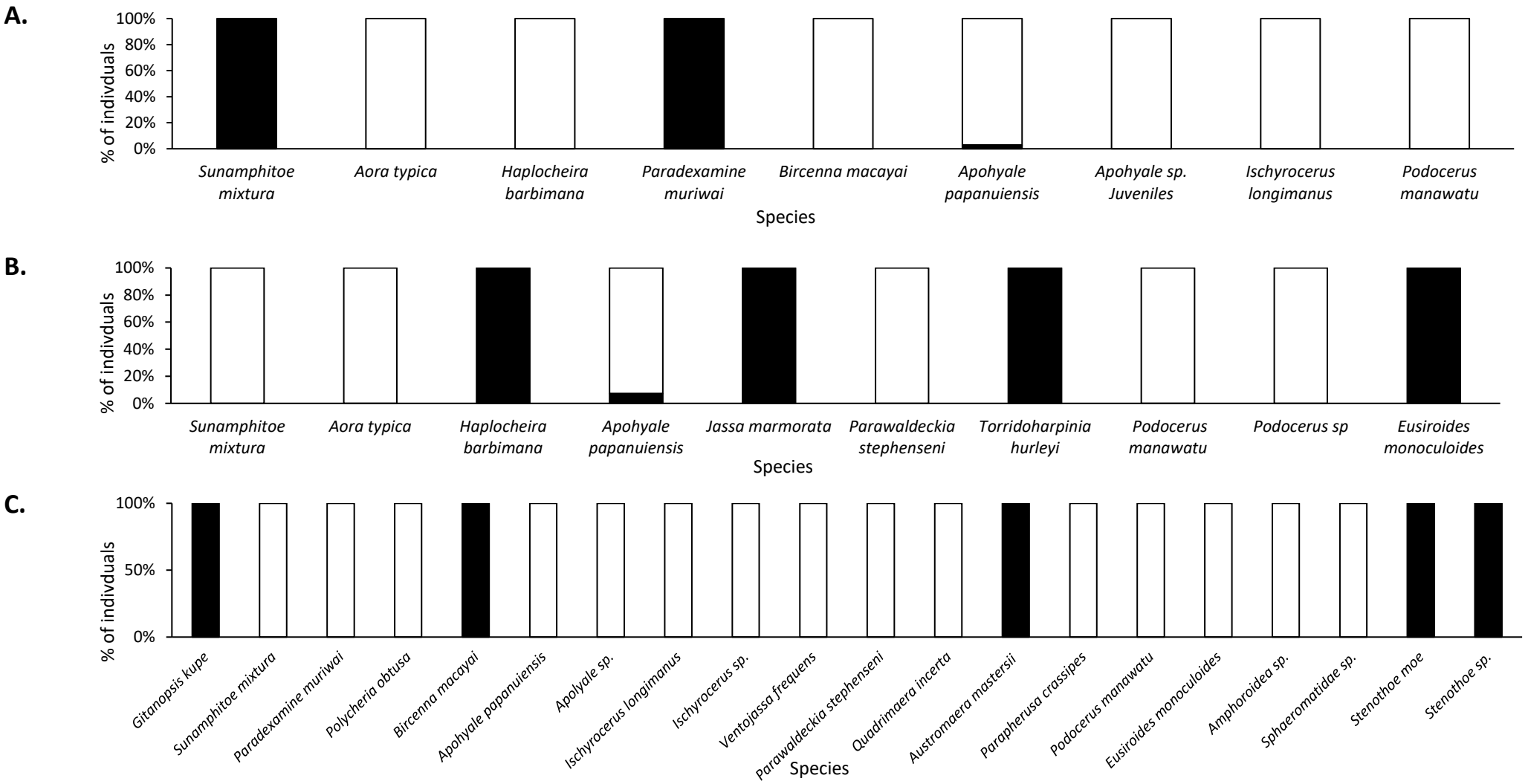


Figure 2.3 The percentage of species caught in a light trap (black) and from seaweed (open bars) at each study site. Graph A: Oriental Bay, Graph B: Evans Bay and Graph C: Point Halswell. Species authorities not previously mentioned *Aora typical* Krøyer, 1845, *Haplocheira barbimana* (Thomson, 1879), *Paradexamine muriwai* Barnard, 1972, *Jassa marmorata* Holmes, 1905, *Parawaldeckia stephensen*i Hurley & Cooper, 1974, *Torridoharpinia hurleyi* (Barnard, 1958), *Gitanopsis kupe* Barnard, 1972, *Polycheria obtusa* Thomson, 1882, *Ventojassa frequens* (Chilton, 1883), *Quadrimaera incerta* (Chilton, 1883) *Austromaera mastersii* (Haswell, 1879), *Parapherusa crassipes* (Haswell, 1879) and *Stenothoe moe* Barnard, 1972.

2.3.2 Trace elements analysed

The ICPMS analysed 36 trace elements for each sample that was processed (Table 2.1). For some samples, data for all 36 trace elements could not be generated because values were too low compared to the procedural blank. This is mentioned in text when this has occurred.

Table 2.1 Trace element symbols and the corresponding trace element name.

Trace element symbol	Trace element
Al	Aluminium
As	Arsenic
Ba	Barium
Bi	Bismuth
Ca	Calcium
Cd	Cadmium
Ce	Cerium
Co	Cobalt
Cr	Chromium
Cs	Caesium
Cu	Copper
Fe	Iron
Ga	Gallium
La	Lanthanum
Li	Lithium
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
Nb	Niobium
Nd	Neodymium
Ni	Nickel
Pb	Lead
Rb	Rubidium
Sc	Scandium
Sm	Samarium
Sn	Tin
Sr	Strontium
Th	Thorium
Ti	Titanium
Tl	Thallium
U	Uranium
V	Vanadium
Y	Yttrium
Yb	Ytterbium
Zn	Zinc
Zr	Zirconium

The average concentration (ppm) of 36 trace elements from lowest concentration to highest in three amphipod species from around Wellington's coastline was produced (Figure 2.4). *Eusiroides monoculoides* and *A. papanuiensis* follow a very similar pattern across all 36 trace elements except for Ca. *Apohyale papanuiensis* had a higher concentration then *E. monoculoides* for Ca. *Sunamphitoe mixtura* has a higher average concentration for nearly all trace elements compared to *E. monoculoides* and *A. papanuiensis*. The average concentration of 36 trace elements from lowest concentration to highest in the amphipod found at three sites: Oriental Bay, Evans Bay and Point Halswell around the Wellington's coastline is shown in Figure 2.5. All three sites show a similar average concentration in all 36 trace elements analysed.

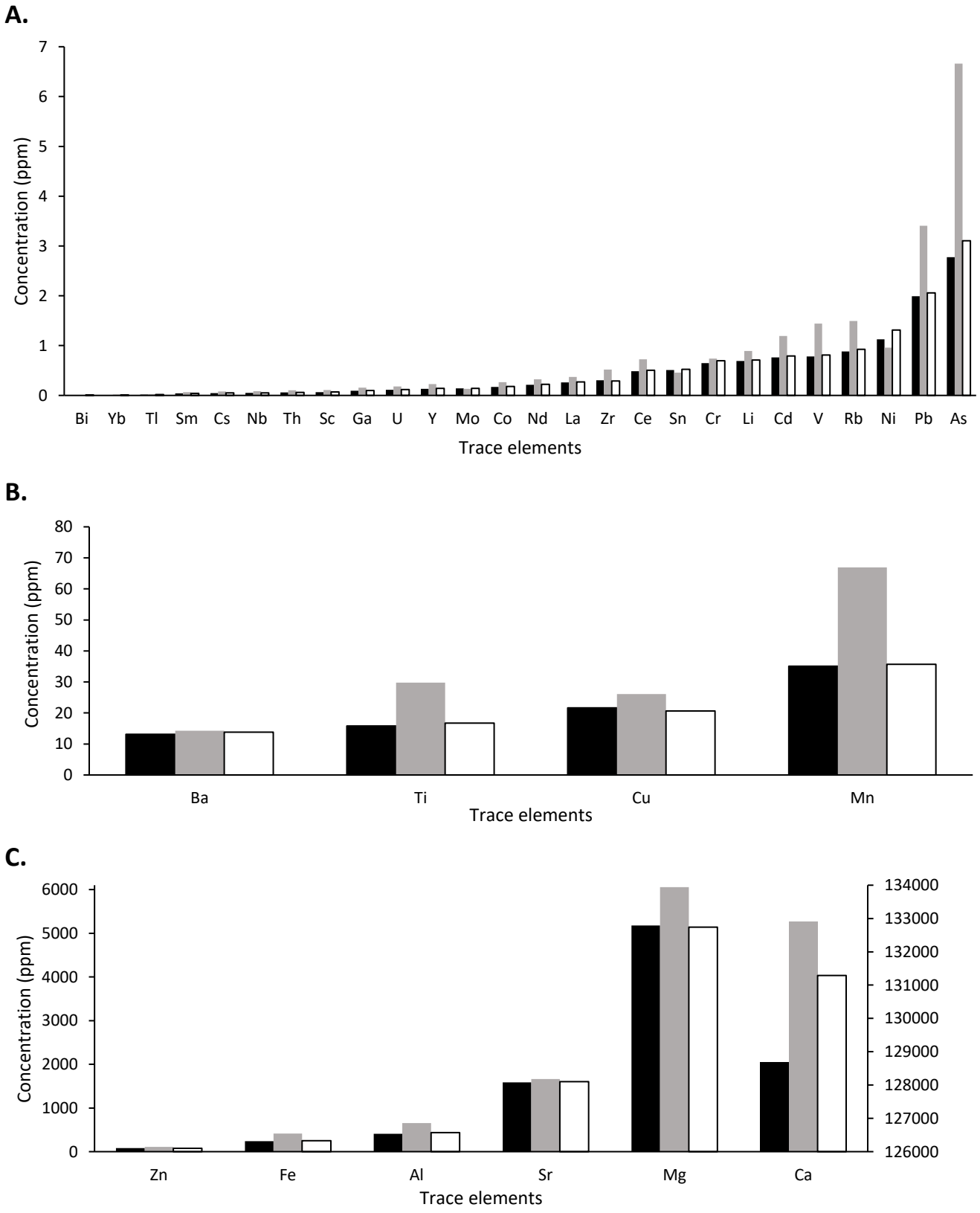


Figure 2.4 The average concentration of trace elements (ppm) across Wellington coast, in three amphipod species: *E. monoculoides* (black)*, S.mixtura* (grey) and *A. papanuiensis* (open bars). Graph A: trace elements with concentrations under 7 ppm. Graph B: trace elements with concentrations under 80 ppm. Graph C: higher trace element concentrations, Ca concentrations are recorded on the right-hand Y axis.

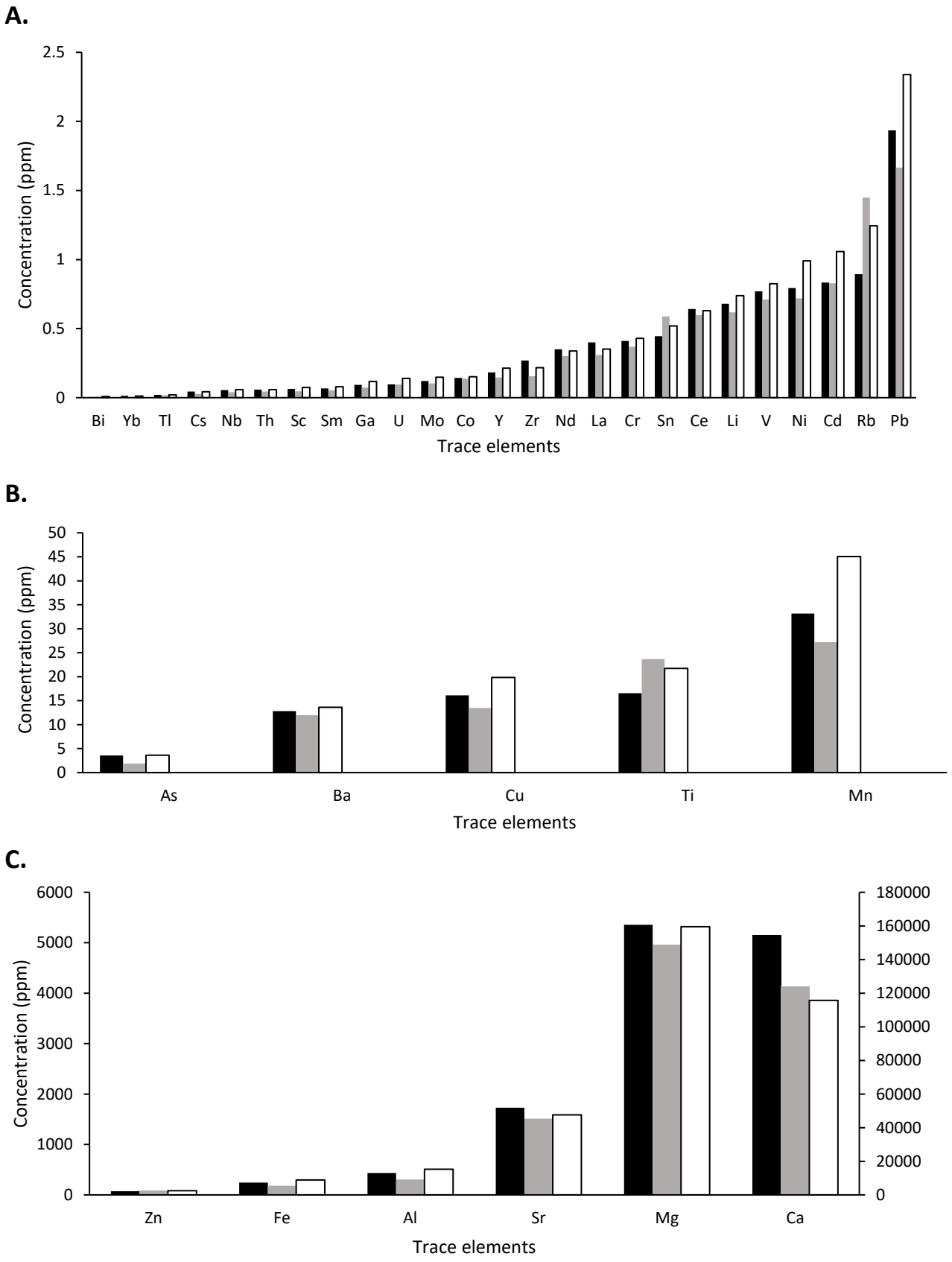


Figure 2.5 The average concentration of trace elements (ppm) in amphipod tissue across the Wellington coast, at three sites: Oriental Bay (black), Evans Bay (grey) and Point Halswell (open bars). The amphipod species used have been pooled together, species are: *E. monoculoides, S. mixtura* and *A. papanuiensis.* Graph A: trace elements with concentrations under 2.5 ppm. Graph B: trace elements with concentrations under 50 ppm. Graph C: higher trace element concentrations, Ca concentrations are recorded on the right-hand Y axis.

2.3.3 Comparison of trace elements analysed for *E. monoculoides* at three sites

The concentration of 36 trace elements was analysed for 11 samples of the species *E. monoculoides* at Oriental Bay, Evans Bay and Point Halswell. Thirteen elements showed a significant difference among the three sites (Table 2.2).

Table 2.2 Results depicting the trace elements that were significantly different in *E. monoculoides* amongst study sites. Trace elements Co, Cu and Nd results were obtained from a one-way ANOVA (df,2). The remaining trace elements values were determined using a Kruskal-Wallis test (df,2).

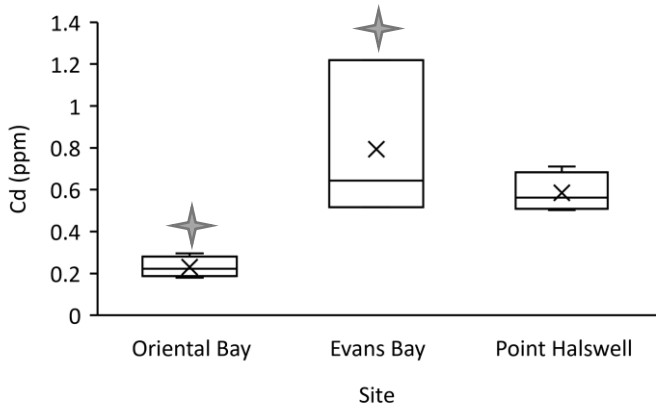
Trace Element	F-statistic	p-value
Cd	7.2121	0.009
Co	7.98	0.0124
Cu	53.17	<0.0001
Fe	6.3939	0.0248
Mn	6.1818	0.0296
Mo	6.1946	0.0452
Nb	9.51	0.0077
Rb	6.3939	0.0248
Sn	5.9621	0.0414
Ti	6.7273	0.0184
V	6.4091	0.0234

Evans Bay had the highest average concentration in *E. monoculoides* samples between the three sites for each trace element in Table 2.2.

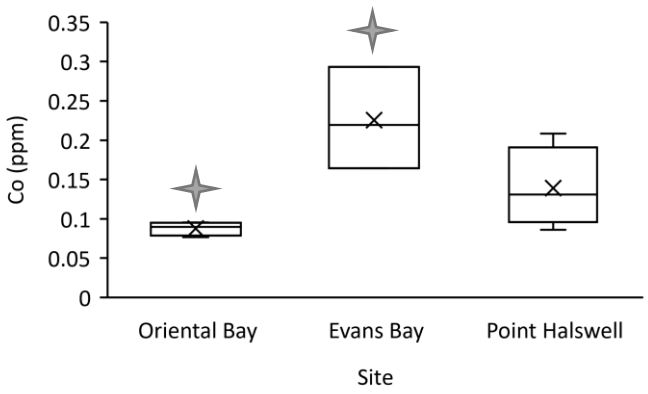
For five elements (Cd, Co, Fe, Rb and Sn) amphipods from Oriental Bay had the lowest concentration, with Point Halswell intermediate. For Mo, Point Halswell had the lowest and Oriental Bay was intermediate. For Mn, Nb, Ti and V Oriental Bay and Point Halswell were similar to each other (and both less than Evans Bay), while for Cu there was a significant difference amongst all three sites in the *E. monoculoides* samples (Figure 2.6).

Twenty-five trace elements were not different among sites (Table A.6), however, even for those there was a trend where samples from Evans Bay had the highest average concentration for 20 of these elements.

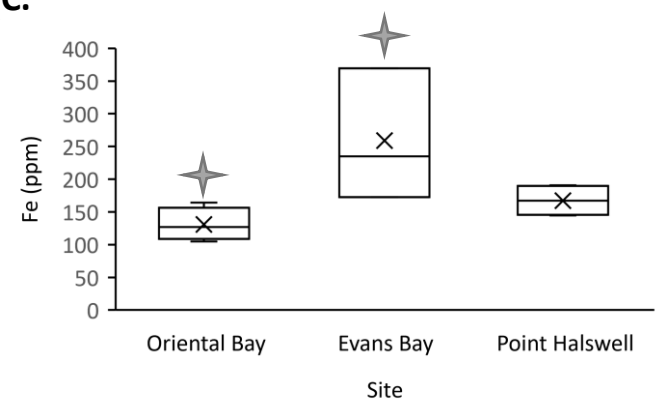
A.


Cd (ppm)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Oriental Bay
Evans Bay
Point Halswell
Site

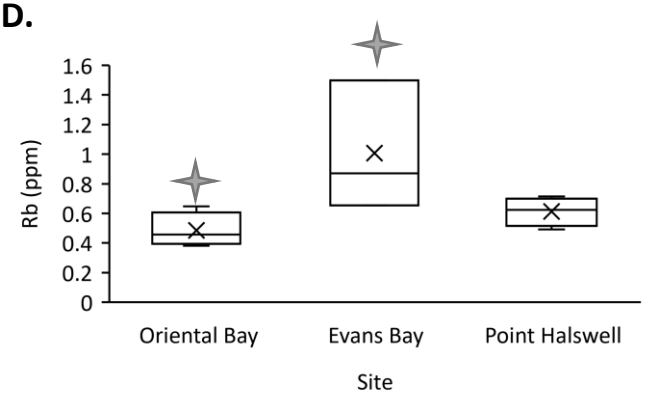
B.


Co (ppm)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Oriental Bay
Evans Bay
Point Halswell
Site

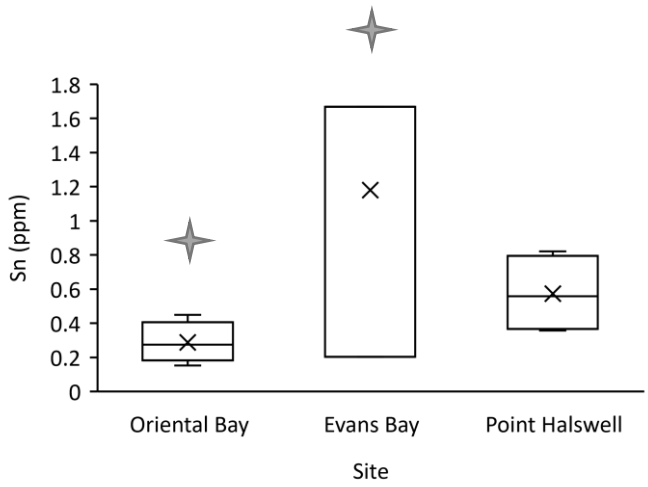
C.


Fe (ppm)
400
350
300
250
200
150
100
50
0
Oriental Bay
Evans Bay
Point Halswell
Site

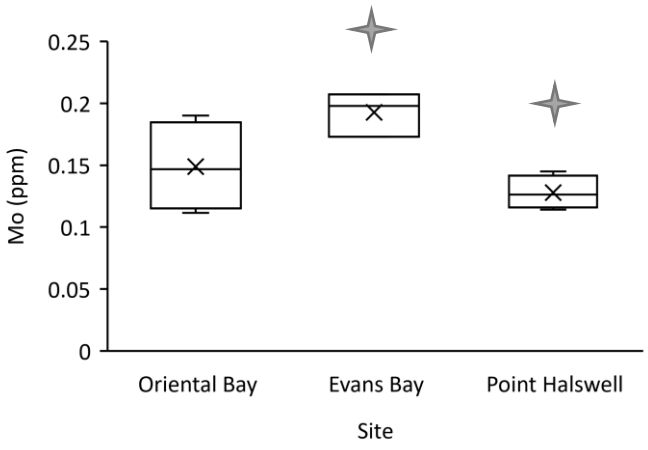
D.


Rb (ppm)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Oriental Bay
Evans Bay
Point Halswell
Site

E.


Sn (ppm)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Oriental Bay
Evans Bay
Point Halswell
Site

F.


Mo (ppm)
0.25
0.2
0.15
0.1
0.05
0
Oriental Bay
Evans Bay
Point Halswell
Site

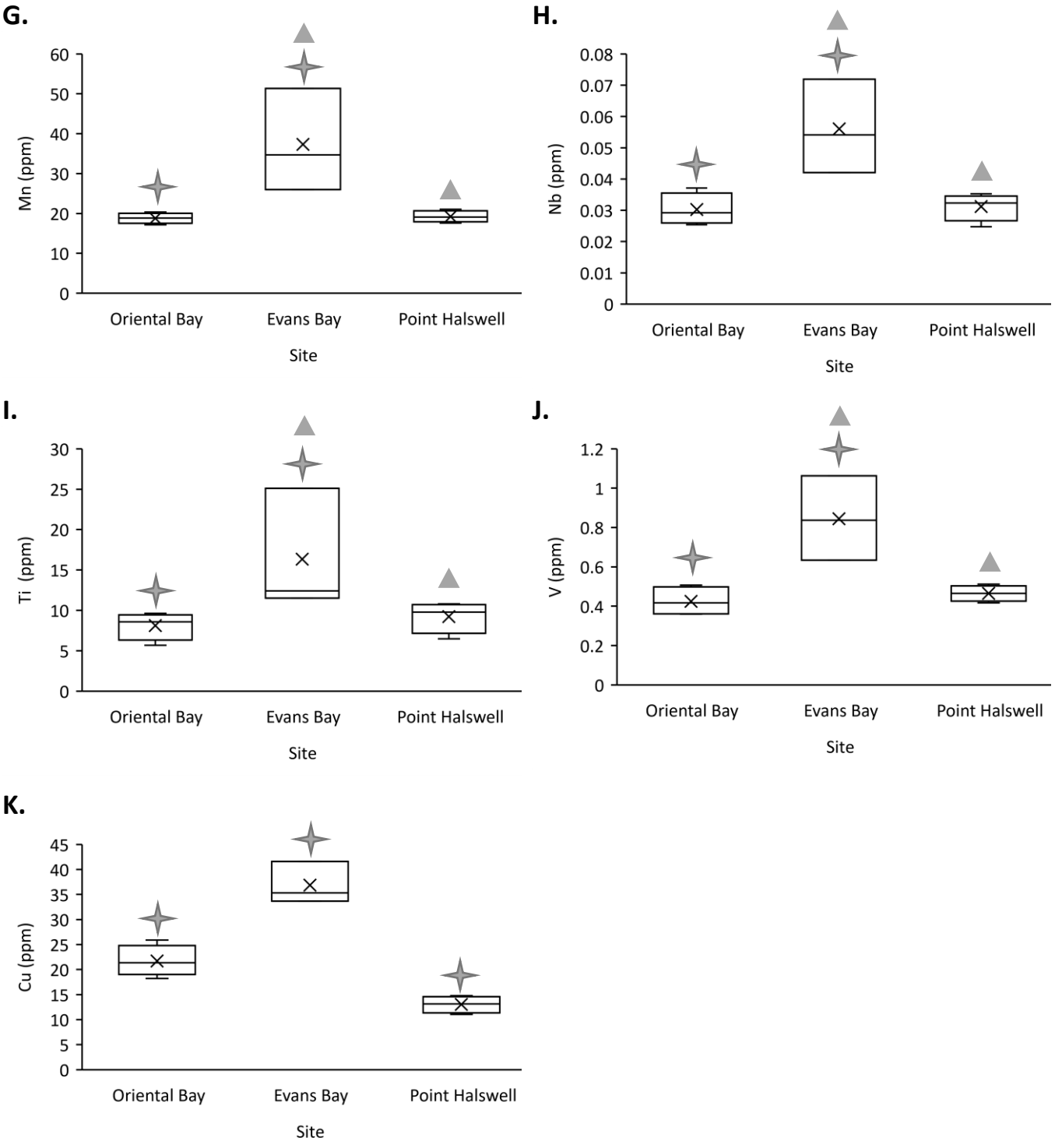


Figure 2.6 Concentration of trace elements (ppm) across three sites in *E. monoculoides.* There is a significant difference between sites for (A) Cd, (B) Co, (C) Fe, (D) R, (E) Sn, (F) Mo, (G) Mn, (H) Nb, (I) Ti, (J) V and (K) Cu. Grey shapes above the box plot for each species represent where the significant difference lies. When the symbol is the same there is a significant difference.

2.3.4 Comparison of species occurring at the same site

Amphipod species that occurred at the same site were analysed to compare species specific differences for numerous trace elements.

Evans Bay

At Evans Bay there was a significant difference in six trace element concentrations between *E. monoculoides* and *S. mixtura* (Table 2.3). *Sunamphitoe mixtura*, on average had higher concentrations than *E. monoculoides* for trace elements As, Nd, Sm, Y and Yb, while *E. monoculoides* had a higher average concentration than *S. mixtura* for Mo (Figure 2.7).

Table 2.3 Results of t-test with a significant outcome comparing trace elements in species *E. monoculoides* and *S. mixtura* at Evans Bay. Mo, Nd, Sm and Yb data were transformed to meet test assumptions (df, 6).

Trace element	t-value	p-value
As	-7.69	0.0003
Mo	2.58	0.0420
Nd	-2.45	0.0497
Sm	-2.78	0.0321
Y	-3.16	0.0195
Yb	-3.87	0.0083

Thirty trace elements analysed showed no significant difference between *E. monoculoides* and *S. mixtur*a from Evans Bay (Table A.7). *Sunamphitoe mixtura* had a higher average concentration than *E. monoculoides* for 25 of the non-significant trace elements.

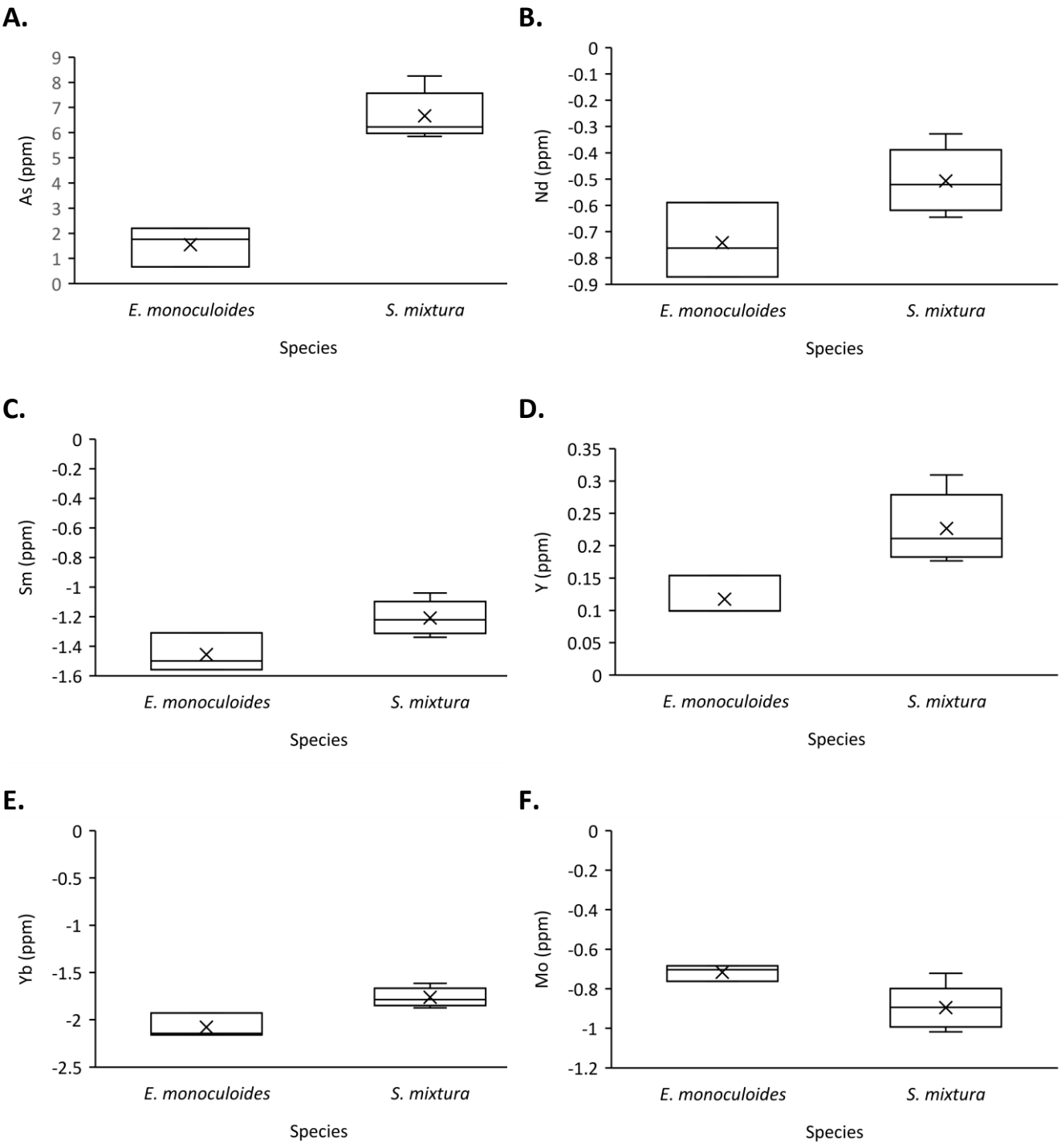


Figure 2.7 Concentration of trace elements (ppm) in 2 species *E. monoculoides* and *S. mixtura* occurring at Evans Bay. (A) As, (B) Nd, (C) Sm, (D) Y, (E) Yb and (F) Mo. Data for trace elements: Nd, Mo, Sm and Yb has been transformed to meet t-test assumptions.

Point Halswell

There was a significant difference in concentrations of four trace elements in *E. monoculoides* and *A. papanuiensis* occurring at Point Halswell (Table 2.4). *Apohyale papanuiensis* had a higher average concentration than *E. monoculoides* for As, Cd and Ni, while *E. monoculoides* had a higher average concentration than *A. papanuiensis* for Sn (Figure 2.8).

Table 2.4 Results of t-test with a significant outcome comparing trace elements in species *E. monoculoides* and *A. papanuiensis* at Point Halswell (df, 6). Ni data was transformed to meet test assumptions.

Trace Element	t-value	p-value
As	3.33	0.0158
Cd	3.45	0.0137
Ni	2.97	0.0248
Sn	-3.53	0.0124

Thirty-two trace elements analysed showed no significant difference between *E. monoculoides* and *A. papanuiensis* (Table A.8). *Apohyale papanuiensis* had a higher average concentration than *E. monoculoides* for 19 of the non-significant trace elements.

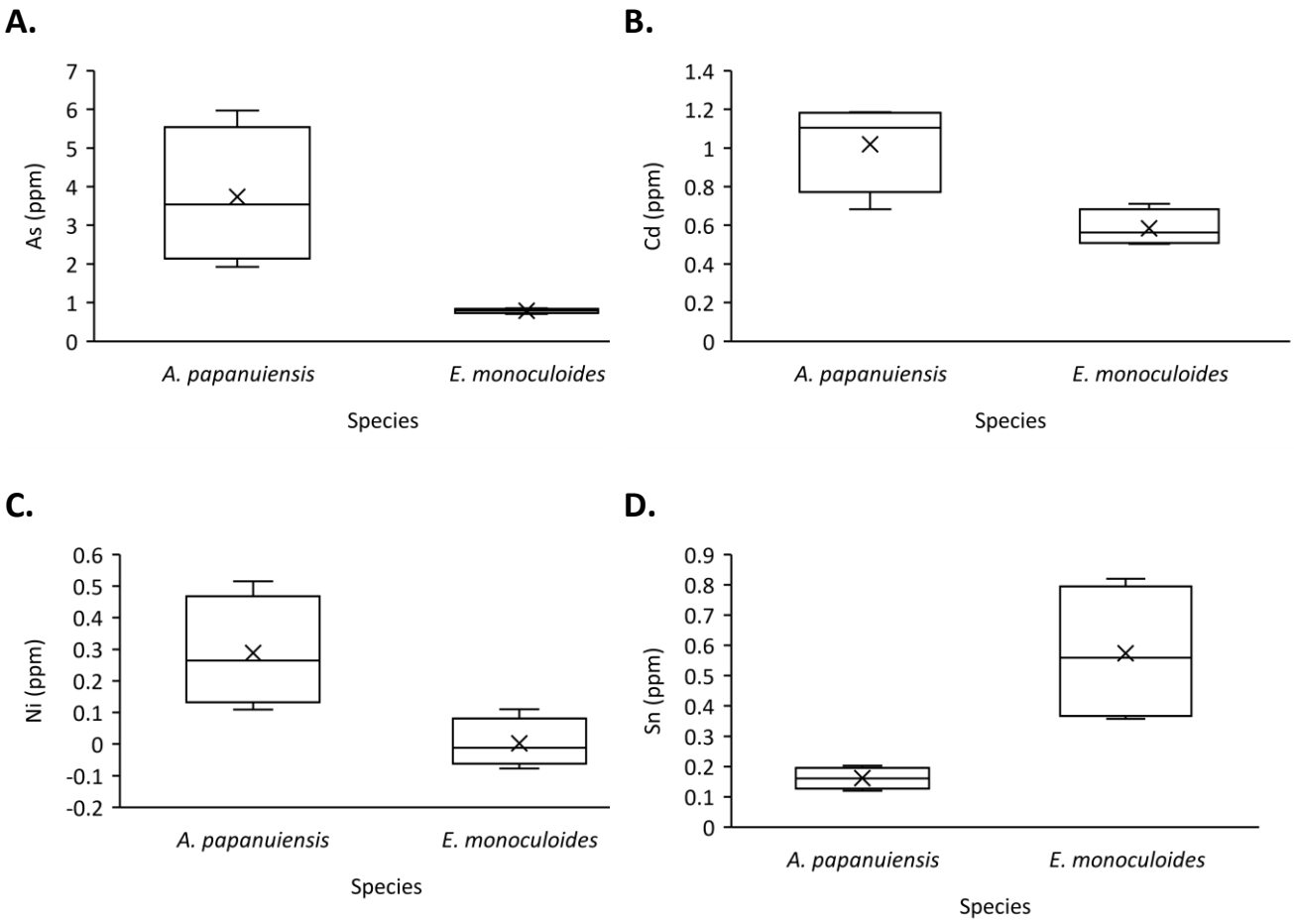


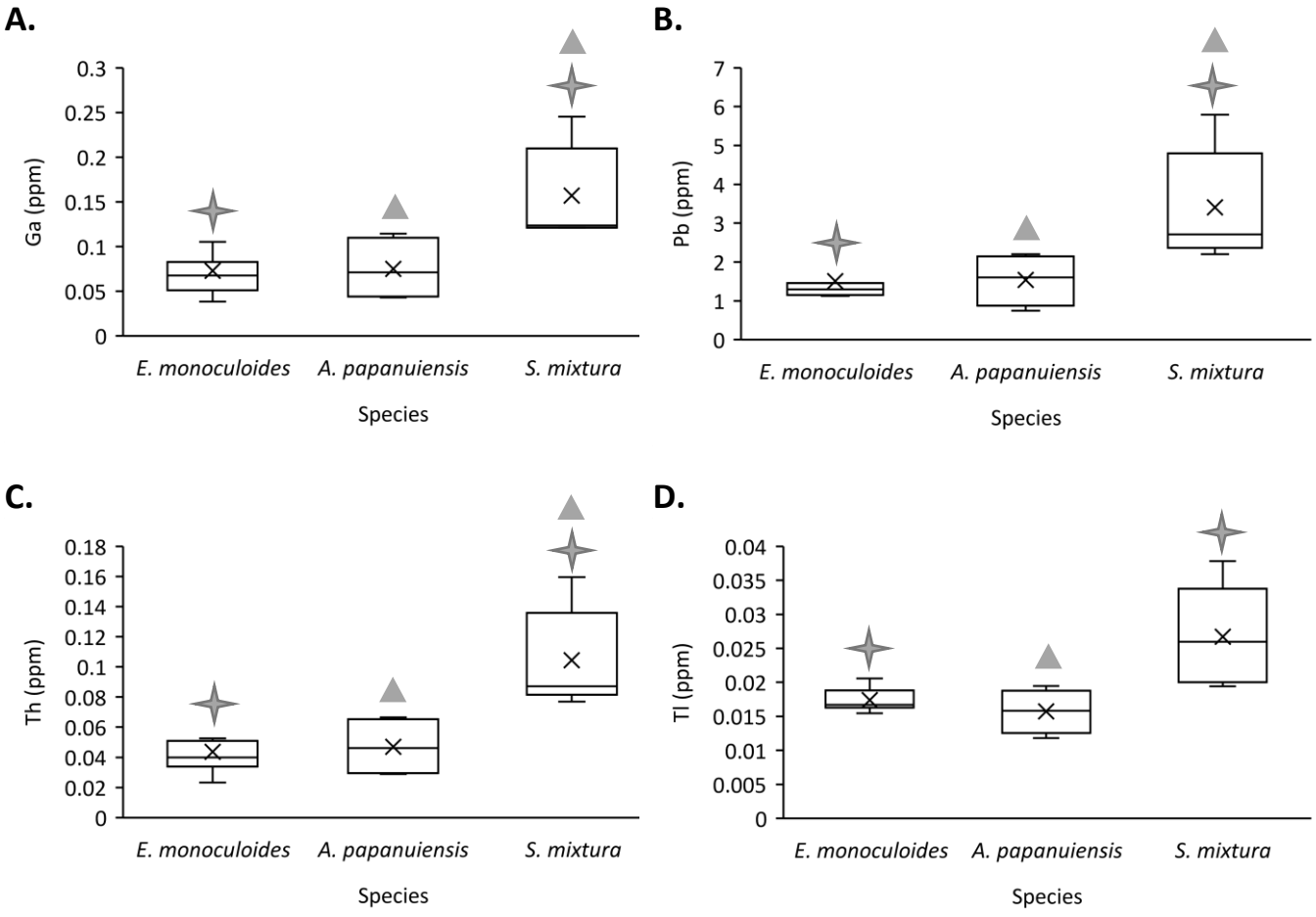
Figure 2.8 Concentration of trace elements (ppm) in 2 species *A. papanuiensis* and *E. monoculoides* occurring at Point Halswell (A) As, (B) Cd, (C) Ni and (D) Sn. Data for trace element Ni has been transformed to meet t-test assumptions.

2.3.5 Comparison of trace elements across three species.

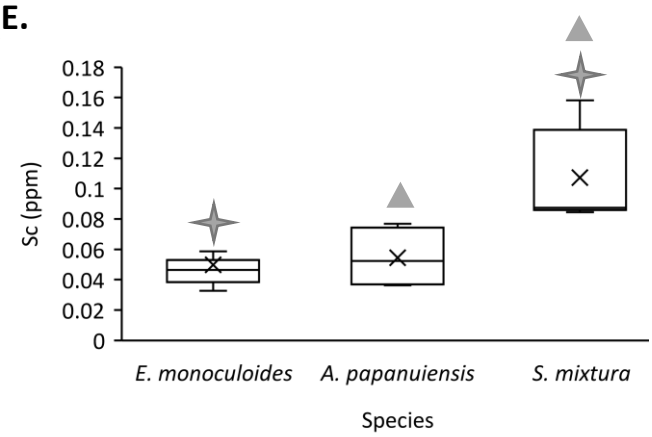
For trace elements where site was not a significant factor, all sites were pooled together. Twenty-three trace elements were compared amongst three species: *E. monoculoides, A. papanuiensis* and *S. mixtura.* For 13 of the 23 trace elements analysed there was a significant difference amongst the three species (Table 2.5). *Sunamphitoe mixtura* had the highest average concentration between the three species for each trace element that showed a significant difference amongst species (Figure 2.9). Eleven trace elements showed no significant difference among species (Table A.9).

Table 2.5 Results depicting the trace elements that were significantly different amongst species *E. monoculoides, A. papanuiensis and S. mixtura*. Results were obtained from a one-way ANOVA (df,2) for all trace elements except Tl. The trace element, Tl values were determined using a Kruskal-Wallis test (df,2).

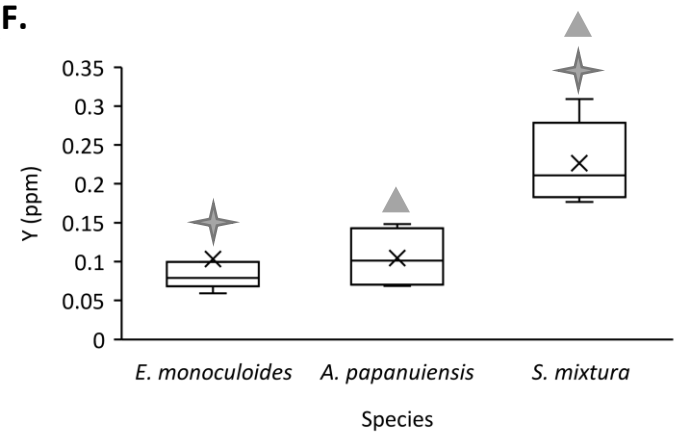
Trace Element	F-statistic	p-value
Al	7.35	0.005
As	14.7512	<0.0001
Bi	4.49	0.0273
Ga	8.72	0.0025
Mg	4.05	0.0365
Pb	8.54	0.0027
Sc	10.99	0.0009
Th	13.28	0.0003
Tl	9.9001	0.002
Y	9.56	0.0017
Yb	12.53	0.0005
Zr	4.21	0.0326



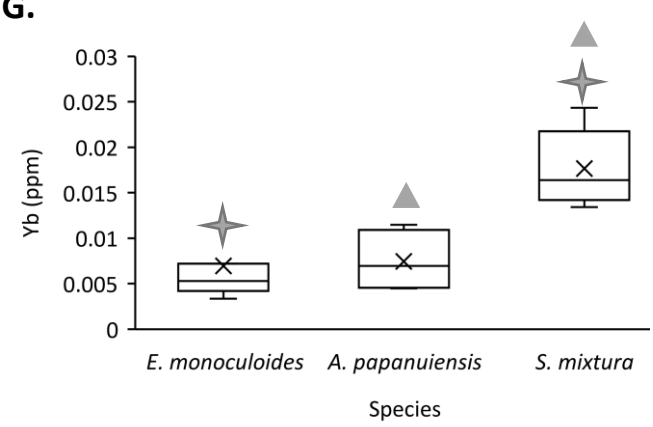
E.


Sc (ppm)
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
E. monoculoides
A. papanuiensis
S. mixtura
Species

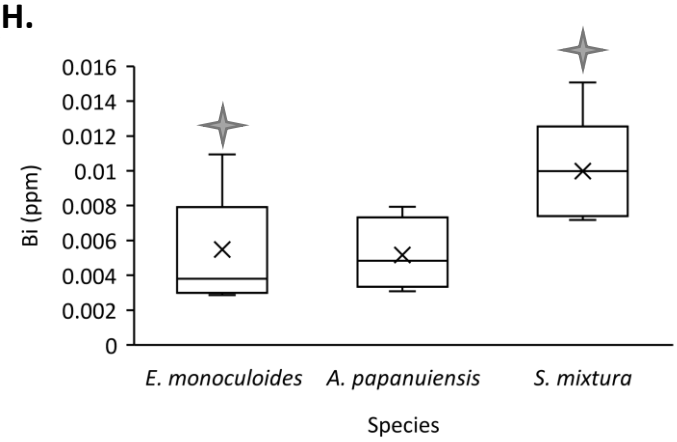
F.


Y (ppm)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
E. monoculoides
A. papanuiensis
S. mixtura
Species

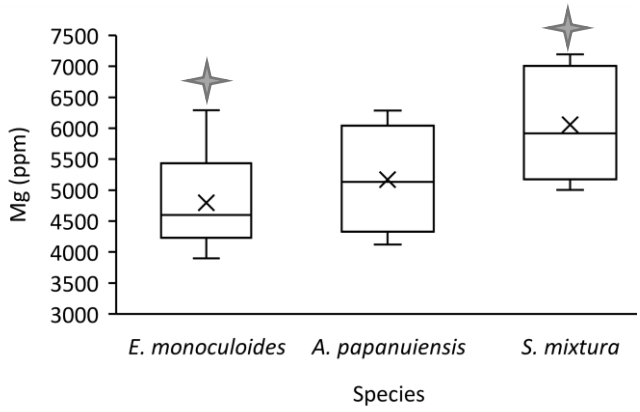
G.


Yb (ppm)
0.03
0.025
0.02
0.015
0.01
0.005
0
E. monoculoides
A. papanuiensis
S. mixtura
Species

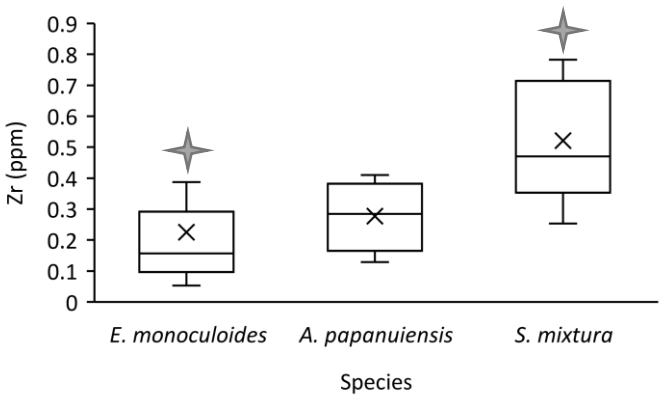
H.


Bi (ppm)
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
E. monoculoides
A. papanuiensis
S. mixtura
Species

I.


Mg (ppm)
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
E. monoculoides
A. papanuiensis
S. mixtura
Species

J.


Zr (ppm)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
E. monoculoides
A. papanuiensis
S. mixtura
Species

K.

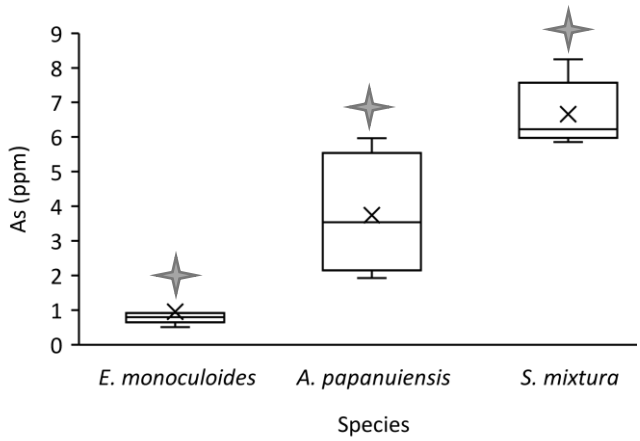


Figure 2.9 Concentration of trace elements (ppm) across three species of amphipod. (A) Ga, (B) Pb, (C) Th, (D) Tl, (E) Sc, (F) Y, (G) Yb, (H) Bi, (I) Mg, (J) Zr and (K) As. Grey shapes above the box plot for each species represent where the significant difference lies. When the symbol is the same there is a significant difference.

2.3.6 Seawater and seaweed trace element analyses

Across all three sites, the concentration of the trace elements analysed in a seawater sample was reasonably consistent (Figure 2.10). The concentration of all trace elements was much higher in seaweed, *C. maschalocarpum* than seawater, and even nine times greater for the trace element Sc. This was also reflected in the amphipod species analysis. The concentration of trace elements in seaweed across all sites was commonly highest at Evans Bay (Figure 2.11).

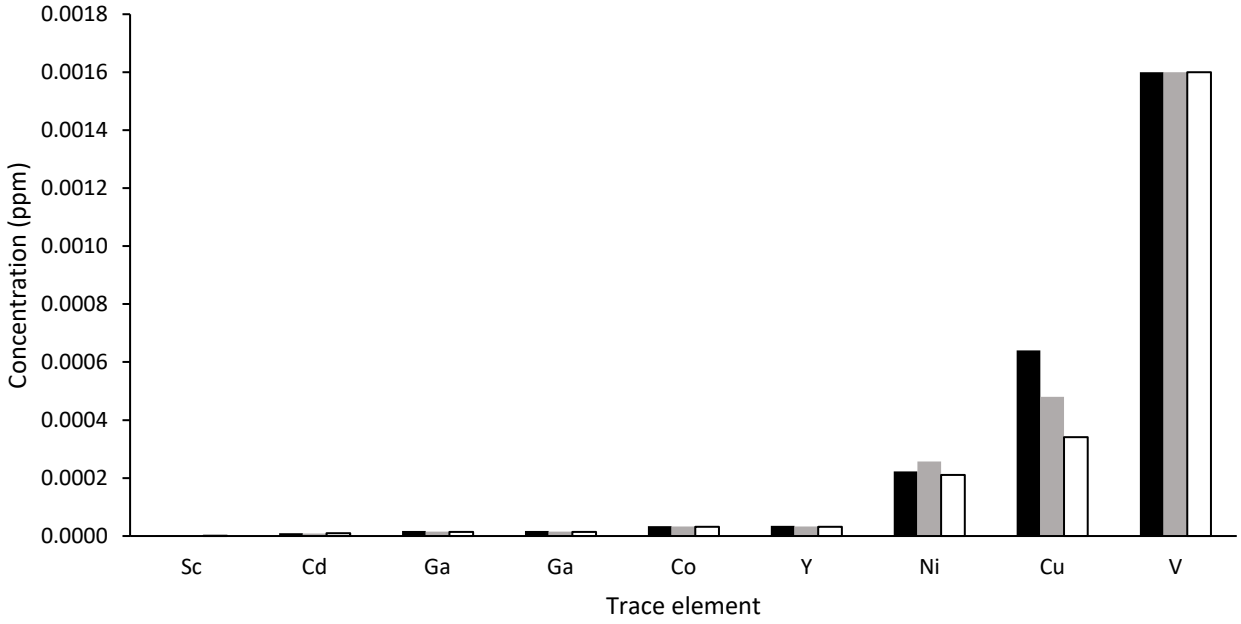


Figure 2.10 The concentration of trace elements (ppm) in a seawater sample taken at Oriental Bay (black), Evans Bay (grey) and Point Halswell (open bars).

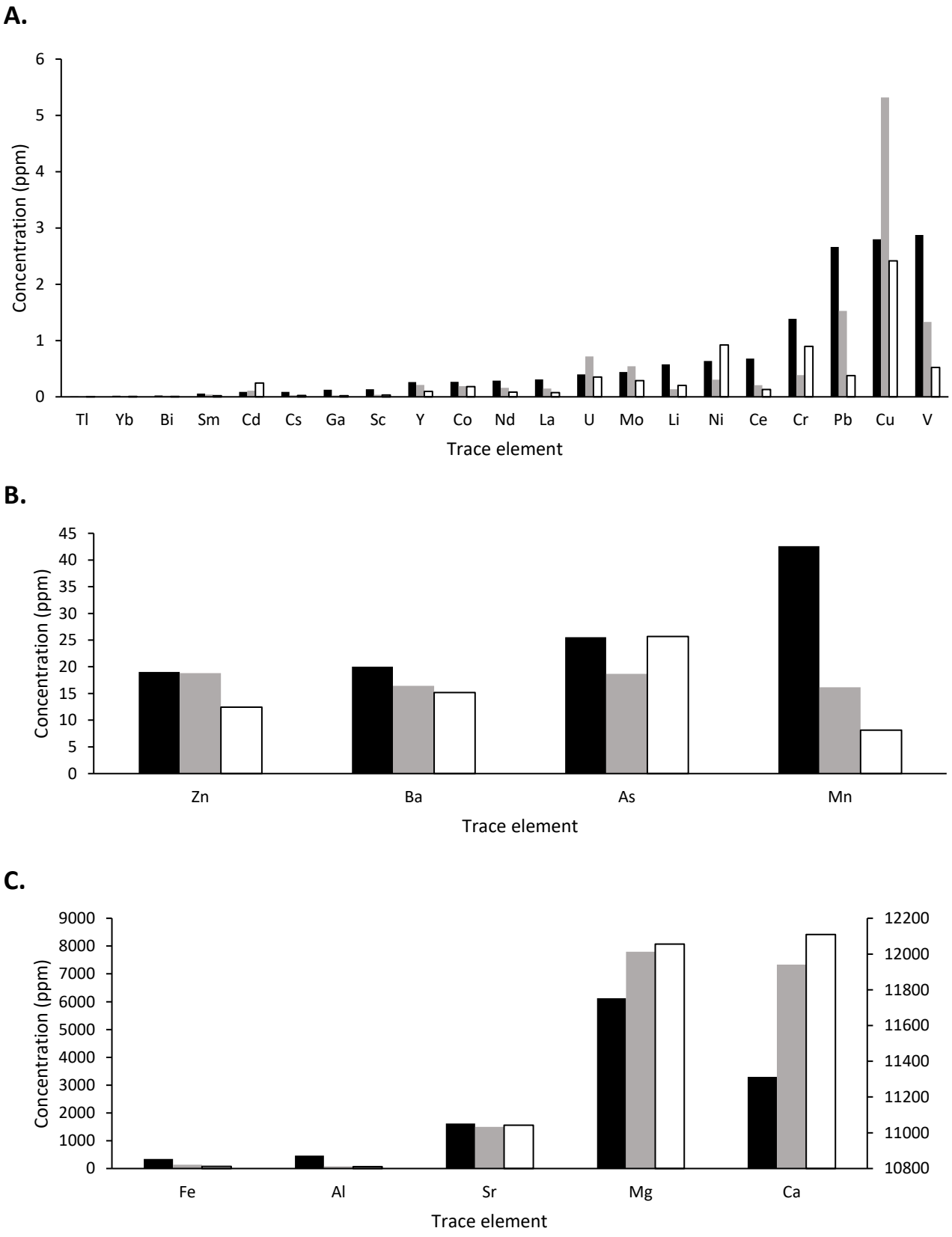


Figure 2.11 The concentration of trace elements (ppm) in *C. maschalocarpum* taken at Oriental Bay (black), Evans Bay (grey) and Point Halswell (open bars). Graph A: trace elements with concentrations under 6 ppm. Graph B: trace elements with concentrations under 45 ppm. Graph C: higher trace element concentrations, Ca concentrations are recorded on the right-hand Y axis.

2.3.7 Comparison between amphipods and seaweed collected across study sites

Trace element concentrations were compared for seaweed, *C. maschalocarpum* and amphipod species that had been collected at the same site. Thirty trace elements were compared between *C. maschalocarpum* and amphipod species as data for six trace elements: Nb, Rb, Sn, Th, Ti and Zr was unreliable for the seaweed samples.

Oriental Bay

The average concentration of *E. monoculoides* and the concentration of *C. maschalocarpum* for 30 trace elements at Oriental Bay is shown in Figure 2.12. For 23 trace elements, the concentration in *C. maschalocarpum* was greater than the average concentration for *E. monoculoides.* For seven trace elements (Ca, Cu, Ni, Sr, Tl, Yb and Zn) the average concentration in *E. monoculoides* was greater than *C. maschalocarpum.*

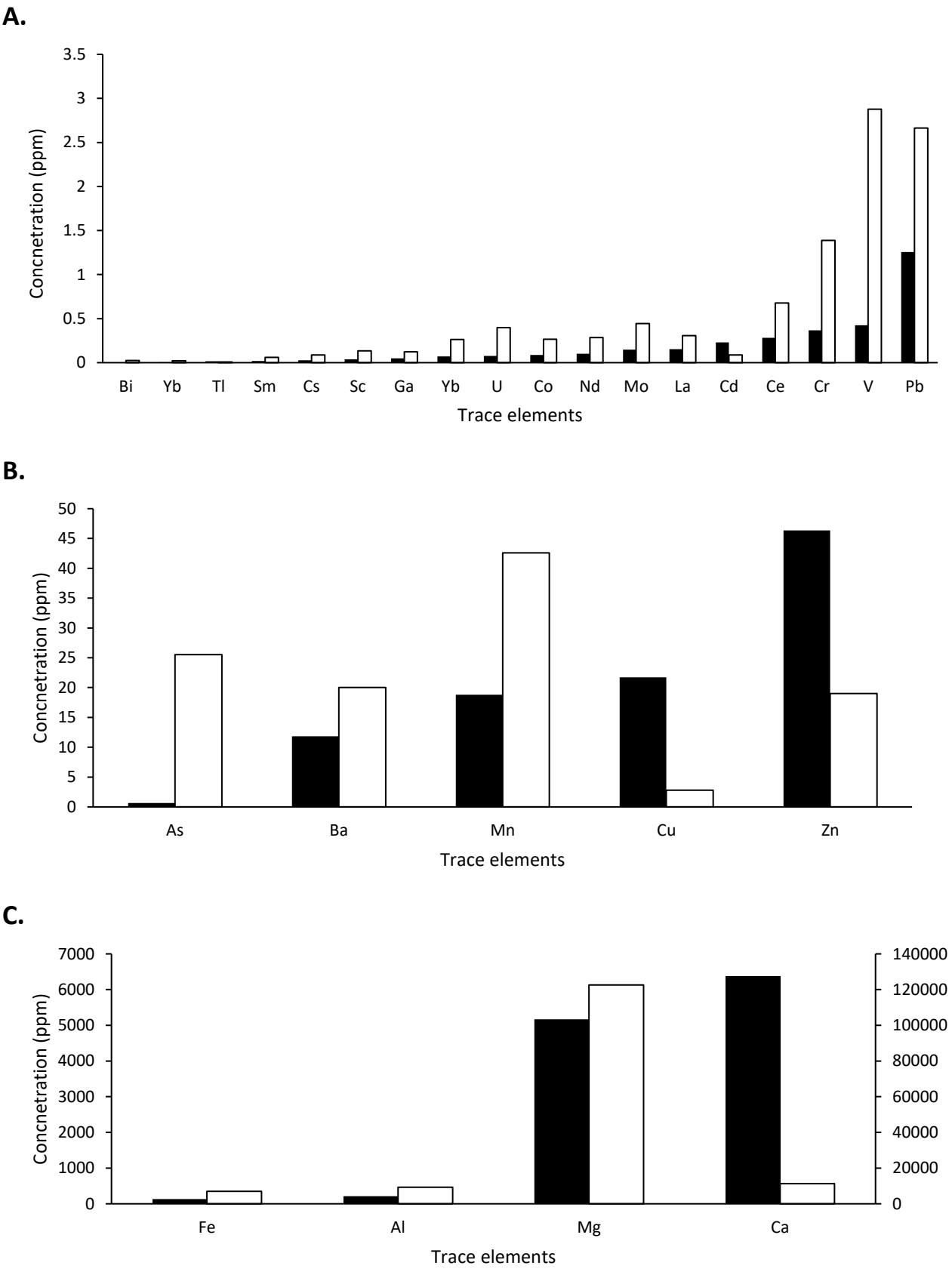


Figure 2.12 The average concentration of trace elements (ppm) at Oriental Bay in *E. monoculoides* (black) and *C. maschalocarpum* (open bars). Graph A: trace elements with concentrations under 3.5 ppm. Graph B: trace elements with concentrations under 50 ppm. Graph C: higher trace element concentrations, Ca concentrations are recorded on the right-hand Y axis.

Evans Bay

The average concentration of the amphipods *E. monoculoides* and *S. mixtura* and the concentration of the seaweed *C. maschalocarpum* for 30 trace elements at Evans Bay is shown in Figure 2.13. For 19 trace elements, the average concentration in *E. monoculoides* was greater than in *C. maschalocarpum.* For 11 trace elements (As, Ba, Bi, Mg, Mo, Sm, Sr, U, V, Y and Yb) the concentration in *C. maschalocarpum* was greater than the average concentration for *E. monoculoides*.

For 23 trace elements, the average concentration in *S. mixtura* was greater than *C. maschalocarpum.* For seven trace elements (Bi, Cd, Mo, Sr, Ga, Y and Yb), the concentration in *C. maschalocarpum* was greater than the average concentration for *S. mixture.*

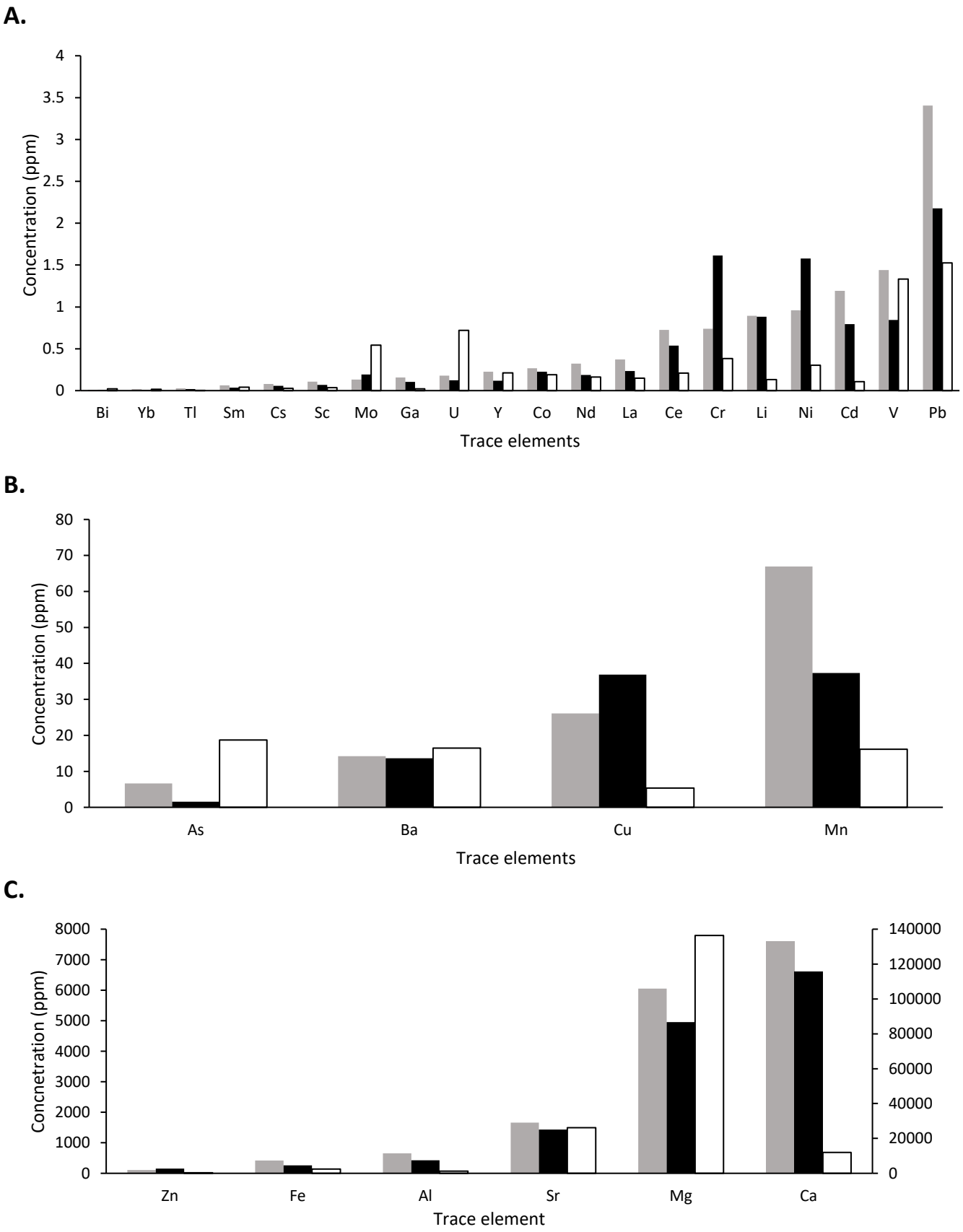


Figure 2.13 The average concentration of trace elements (ppm) at Evans Bay in *E. monoculoides* (black), *S. mixtura* (grey) and *C. maschalocarpum* (open bars). Graph A: trace elements with concentrations under 4 ppm. Graph B: trace elements with concentrations under 80 ppm. Graph C: higher trace element concentrations, Ca concentrations are recorded on the right-hand Y axis.

Point Halswell

The average concentration of the amphipods *E. monoculoides* and A. *papanuiensis* alongside the concentration of the seaweed *C. maschalocarpum* for 30 trace elements at Point Halswell is shown in Figure 2.14.

For 18 trace elements, the average concentration for *E. monoculoides* was greater than *C. maschalocarpum.* For 12 trace elements (As, Ba, Bi, Co, Cr, Mg. Mo, Sr, U, V, Y and Yb) the concentration in *C. maschalocarpum* was greater than the average concentration for *E. monoculoides.* For 21 trace elements, the average concentration for *A. papanuiensis* was greater than *C. maschalocarpum.* For nine trace elements (As, Ba, Bi, Co, Cr, Mg, Mo, U, Yb), the concentration in *C. maschalocarpum* was greater than the average concentration for *A. papanuiensis.* Both Evans Bay and Point Halswell showed a greater average concentration of trace elements in the amphipod species than the seaweed at their location.

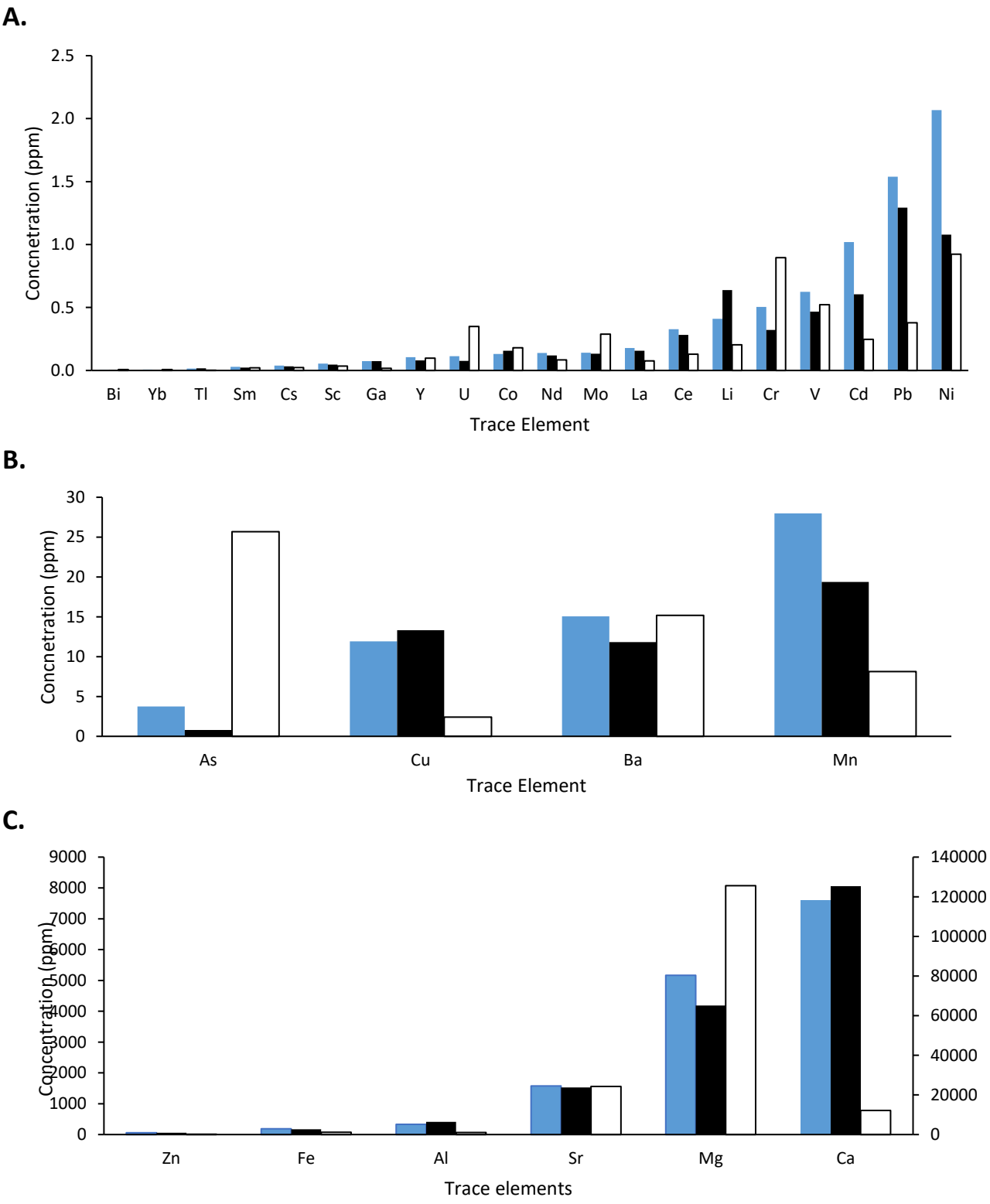


Figure 2.14 The average concentration of trace elements (ppm) at Oriental Bay in *A. papanuiensis* (blue), *E. monoculoides* (black), and *C. maschalocarpum* (open bars). Graph A: trace elements with concentrations under 2.5 ppm. Graph B: trace elements with concentrations under 30 ppm. Graph C: higher trace element concentrations, Ca concentrations are recorded on the right-hand Y axis.

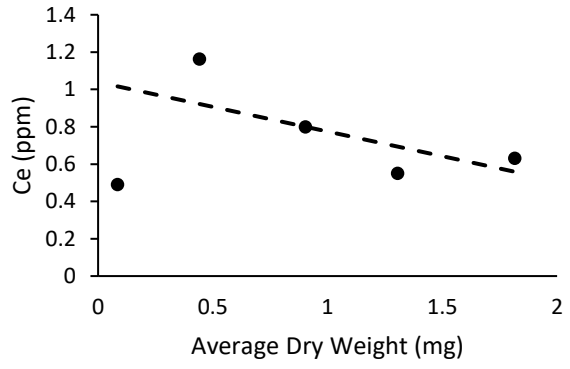
2.3.8 Size relationship

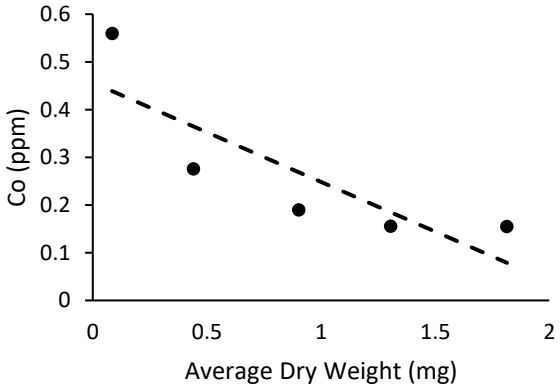
To examine the effects of body size on the trace element concentrations, the relationship between the concentration of a given trace element (ppm) and the average dry weight (mg) of *S. mixtura* individuals from Evans Bay was determined. Fourteen of the 36 trace elements had a significant negative relationship between the trace element concentration and dry weight (Table 2.6 and Figure 2.15). Of the 22 non-significant elements (Table A.10), 19 showed a negative linear relationship between concentration and dry weight, with only three trace elements: As, Ca and Sr showing a positive relationship.

Table 2.6 Results from a linear relationship between the concentration of a given trace element (ppm) and the average dry weight (mg) of an *S. mixtura* individual from Evans Bay. All data was transformed to meet test assumptions with the exception of trace elements, Co and Zn.

Trace element	F-statistic	p-value	R square	Line Equation
Ce	11.90	0.0410	0.7986	y=0.0160-0.1944*x*
Co	12.43	0.0388	0.8056	y=-0.350-0.3075*x*
Cs	15.58	0.0290	0.8385	y=-0.9439-0.1908*x*
Cu	86.88	0.0026	0.9666	y=1.6419-02899*x*
Mn	51.84	0.005	0.9453	y=2.1376-0.4313*x*
Nd	12.6	0.0381	0.8076	y=-0.3582-0.1635*x*
Rb	18.62	0.0229	0.8612	y=0.3061-0.1599*x*
Sm	12.36	0.039	0.8047	y=-1.0701-0.1531*x*
Tl	55.39	0.005	0.9486	y=-1.4335-0.1684*x*
U	19.96	0.0209	0.8693	y=-0.5436-0.2585*x*
V	53.91	0.0052	0.9473	y=0.3403-0.2261*x*
Y	14.4	0.0321	0.8276	y=-0.5353-0.1300*x*
Yb	10.73	0.0466	0.7815	y=-1.644-0.1305*x*
Zn	21.8	0.0185	0.8791	y=2.2591-0.2944*x*

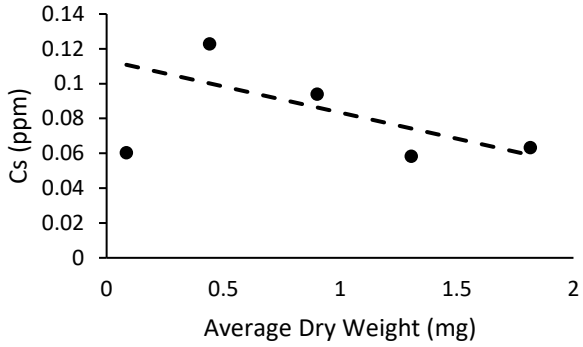
A.



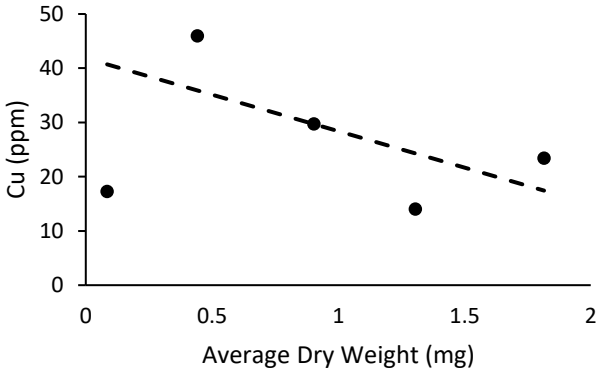


B.

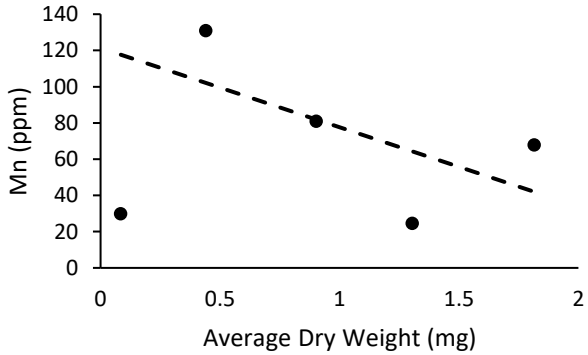
C.


Cs (ppm)
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
0.5
1
1.5
2
Average Dry Weight (mg)

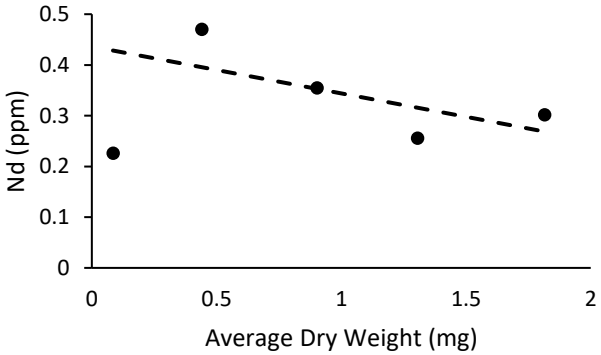
D.


Cu (ppm)
50
40
30
20
10
0
0
0.5
1
1.5
2
Average Dry Weight (mg)

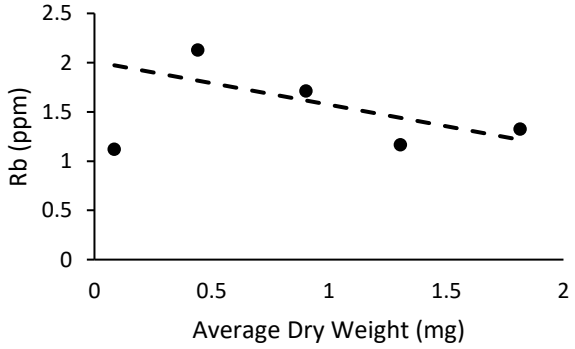
E.


Mn (ppm)
140
120
100
80
60
40
20
0
0
0.5
1
1.5
2
Average Dry Weight (mg)

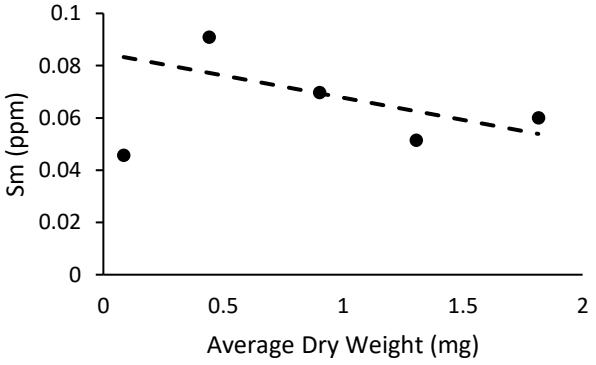
F.


Nd (ppm)
0.5
0.4
0.3
0.2
0.1
0
0
0.5
1
1.5
2
Average Dry Weight (mg)

G.


Rb (ppm)
2.5
2
1.5
1
0.5
0
0
0.5
1
1.5
2
Average Dry Weight (mg)

H.


Sm (ppm)
0.1
0.08
0.06
0.04
0.02
0
0
0.5
1
1.5
2
Average Dry Weight (mg)

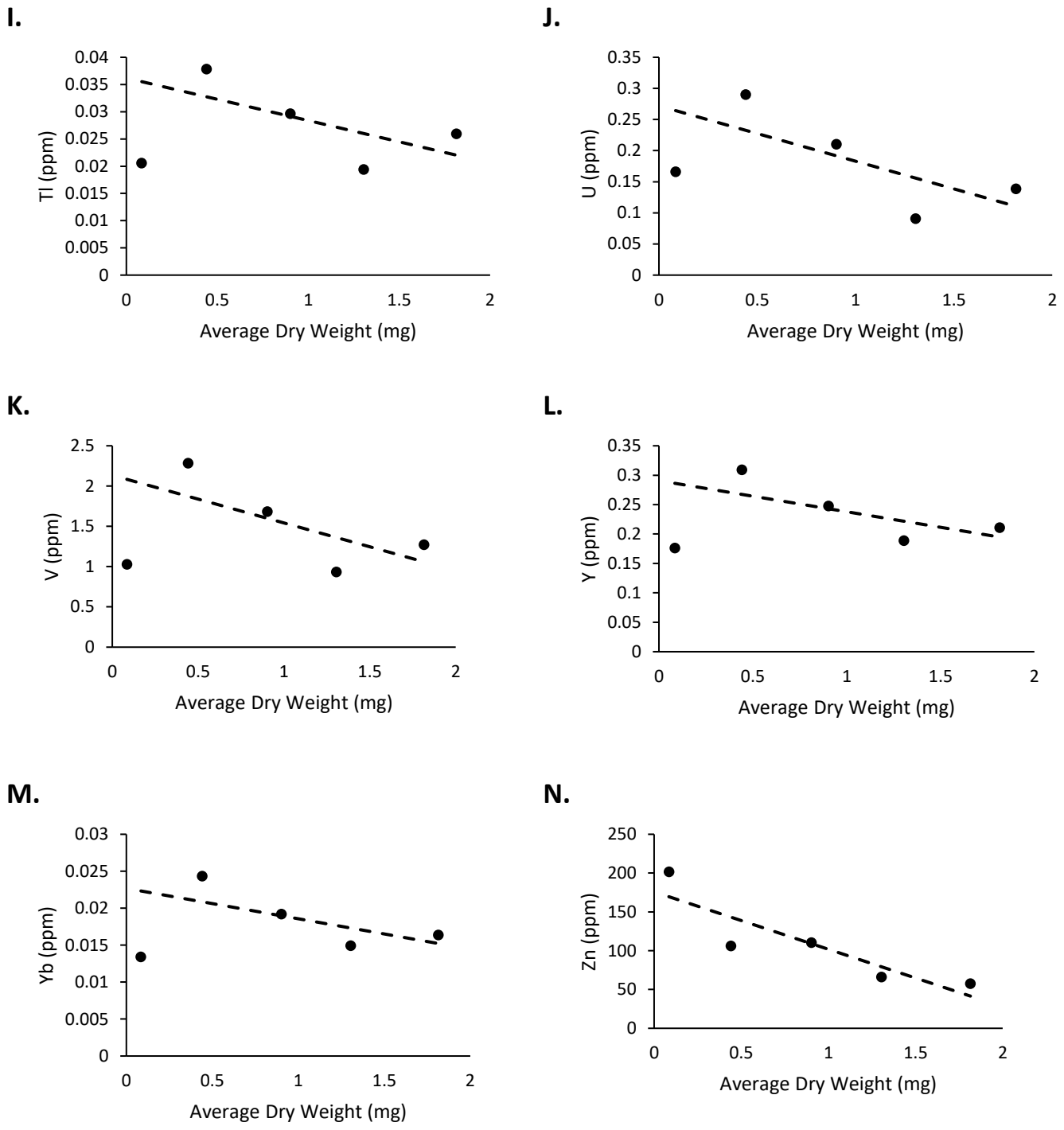


Figure 2.15 Relationship between concentration of trace elements (ppm) and average dry weigh of an *S. mixtura* individual from Evans Bay. Raw data was used for each graph, (n=5). (A) Ce, (B) Co, (C) Cs, (D) Cu, (E) Mn, (F) Nd, (G) Rb, (H) Sm, (I) Tl, (J), U, (K) V, (L) Y, (M) Yb and (N) Zn.

2.4 Discussion

Both spatial and species-specific differences in trace element concentrations of amphipods were found in this study. *Eusiroides monoculoides,* found at all three sites, had the highest average concentration in Evans Bay for the majority of the trace elements analysed (23 trace elements out of 36). This was surprising because Evans Bay is between the other two sites, and the distance between each is relatively small (~5 km). A common means of determining differences in water bodies has been to use fish otoliths, a crystalline structure in the inner ear of teleost fish. Otoliths provide trace metal environmental signatures of the bodies of water the fish has resided in (Swearer et al., 1999). Warner et al. (2006) found that there was a difference between otolith signatures along sites only ten km apart in the waters off Santa Barbara, California. This shows that chemical differences found along an open coast are strong enough to show readable differences among otoliths (Warner et al., 2006). This study, which in some ways is similar to Warner et al., (2006) shows that the analysis of an amphipod can also detect chemical differences in seawater on a small spatial scale.

Two collection methods were used in this chapter, light traps and off seaweed. Amphipods caught from different methods might suggest different feeding behaviours. For example, amphipods caught in light traps may be benthic feeders, where as amphipods off seaweed may be herbivorous. All amphipods analysed in this chapter were generalist feeders so no comparisons were made between collection methods.

As heavy metals can be absorbed or ingested by amphipods; sediment, seawater and seaweed (food source) were investigated as possible contamination sources (Barka et al., 2010; Ivanciuc et al., 2006). Sediments are considered a sink for heavy metals in the marine environment, which often leads to them having elevated concentrations. These metals can therefore remain a toxic risk in coastal benthic systems (Watson et al., 2018). Sediment data was not analysed in this study, so comparisons between sediments at each site were made using the “Wellington harbour subtidal sediment quality monitoring” survey generated by the GWRC. This survey found the sites in the Lambton Basin (comparable to the study site, Oriental Bay) and Evans Bay had higher concentrations of both Cu and Zn than any other site in the Wellington harbour (GWRC, 2014). The survey also found hydrocarbons were highest in Evans Bay and Oriental Bay (GWRC, 2014). Trace elements analysed in seawater were very similar across all sites. A noticeable difference was seen in the concentration of Cu, with highest concentrations at Oriental Bay and lowest at Point Halswell. Trace elements analysed in seaweed at each site were more variable than the seawater. Evans Bay had noticeably higher concentrations for Cu and U in the seaweed samples. Concentrations of trace

elements were much greater in seaweed than seawater samples. Little indication is given from either the seawater or seaweed data as to why the highest concentrations of trace elements were seen at Evans Bay, with the exception of Cu. However, as the seawater and seaweed data were all from a single sampling event, they were only able to provide a snapshot of possible sources of trace element contamination.

A suggested reason for why Evans Bay had the highest average concentration of trace elements is its proximity to the Evans Bay Marina. Marinas are often associated with high levels of pollution due to concentrated human activity (Megina et al., 2016) and have high concentrations of heavy metals such as Pb and Cu in sediment (An & Kampbell, 2003; Egardt et al., 2018; Kenworthy et al., 2018; Rivero et al., 2013). Heavy metals in sediment can become resuspended between the sediments and the water column. The resuspension of pollutants, occurs either naturally by physical or biological processes or by human activity such as dredging (Al-Mur et al., 2017; Garcia et al., 2012; Marsden & Rainbow, 2004; Pan & Wang, 2012; Rodrigues et al., 2017; Sprovieri et al., 2007; Yong et al., 2017). However, Marcus et al. (1988), found marinas were not always a significant input of heavy metals into surrounding sediment, although hydrocarbon levels were influenced by the presence of marinas. To understand why Evans Bay had a greater concentration of trace elements than the other sampled sites, more background data collection would be necessary.

Sunamphitoe mixtura most frequently had the highest average concentration of trace elements amongst the three species examined. Often bioaccumulation studies focus on one species so species comparisons are uncommon (Sabater et al., 2007). However, other studies that have examined heavy metal concentrations in more than one amphipod species have also found significant species differences (King et al., 2006; Olgunoglu, 2015; Strode & Balode, 2013; Wilkund et al., 2003), including amongst amphipod and other crustacean species collected at the same site (Olgunoglu, 2015; Ugolini et al., 2005). These findings align with the outcomes of this study. Interspecific variations amongst heavy metal concentrations has been suggested to be due to feeding habits and trophic level (Alam et al., 2012; Jakimska et al., 2011; Pourang, 1994). However, it's not clear whether that is a factor here because all of the amphipod species examined belong to either herbivorous or omnivorous families.

Sex-specific differences can account for differences within single and multiple species comparisons. For example, differences amongst Cu concentrations in two blue crabs *Callinectes sapidu* (Rathbun, 1896) and *Callinectes bocourti* (Milne-Edwards, 1879) were attributed in part to differences in sex (Sastre et al., 1999). The higher accumulation of Cu in females than males was attributed to their

moult cycle, as males moult more frequently than females (Sastre et al., 1999). Differences in Cd concentrations between sexes have also been reported in the coral prawn *Metapenaeopsis crassissima* (Racek & Dall, 1965) from Australia (Sastre et al., 1999). Although Turoczy et al. (2001) found sex to not be a significant factor in bioaccumulation for the king crab *Pseudocarcinus gigas* (Lamarck, 1818). Other components that could influence differences in heavy metal concentrations in some species include: body size, moult cycle and reproductive cycle (Gherardi et al., 2002). Without having recorded the sex of the amphipods analysed in this chapter it is impossible to determine if sex was a factor among differences within each species as well as across species. However, because samples pooled individuals this seems unlikely to be important here. Interspecific differences may also be related to each species' physiological requirements for essential trace metals as well as their detoxification and excretion ability (Gherardi et al., 2010; Guerra-Garcia et al., 2009). Bioaccumulation capacity may also be influenced by the ability of a species to cope with other stresses such as salinity stress resistance (Ugolini et al., 2005). Species suggested as bioindicators of marine pollution are ones with the greatest sensitivity (Alam et al., 2012).

There was a negative relationship between the dry weight of *S. mixtura* and heavy metal concentrations. This result coincides with the findings of other studies. Body size is often measured as dry weight and is amongst the most important factors likely to influence the tolerance and bioaccumulation of heavy metals in amphipods (Manciocco et al., 2014; Marsden & Rainbow, 2004; Marsden & Wong, 2001). Numerous studies have found smaller amphipods are more sensitive to heavy metal contaminants than larger individuals (Borgmann et al., 1996). Generally, in toxicity tests juvenile amphipods are less tolerant than adults (Marsden & Wong, 2001). For example, *Echinogammarus marinus* (Leach, 1815) neonates accumulated higher amounts of some metals than juveniles (three-fold for Cd and two-fold for Zn) or adults (five-fold for Cd and two and a half times for Zn) (Pastorinho et al., 2009). *Gammarus locusta* (Linnaeus, 1758) juveniles accumulated higher levels of Zn and Cd than adults. Young amphipod stages moult more frequently than older individuals. When there is a thinner body covering there is the potential for even greater metal uptake in young stages (Pastorinho et al., 2009). Copper concentration of juvenile *Epimeria macrodonta* (Walker, 1906) in the brood pouch is higher than that found in adult females (Keil et al., 2008). This may indicate that the enzymatic requirements and demand for Cu as a component of haemocyanin in early life history stages is not met without bioaccumulation of this essential metal after hatching (Keil et al., 2008). During this early life history stage of *E. macrodonta* Cd levels were also elevated. This could be the consequence of the inability of uptake mechanisms to distinguish

between trace metals. However, an increase in heavy metal concentration from smaller to larger individuals is seen in amphipods *Themisto libellula* (Lichtenstein in Mandt, 1822) from the Greenland Sea and *Eusirus propeperdentatus* (Andres, 1979) from Antarctica (Zauke & Schmalenbach, 2006).

The findings on body size and accumulation concentrations are not consistent within the literature. Differences in the relationship between body size and accumulated concentration occur between metals and closely related species. For example, concentrations of Zn decrease with dry weight in *Palaemon elegans* (Rathke, 1837) and *Parapenaeus longirostris*, (Lucas, 1846) but not in *Palaemonetes varians* (Leach, 1814) (Marsden & Rainbow, 2004). For *G. pulex* and *Pontagammarus robustoides* (Sars, 1894) highest sensitivity to Zn was seen in juveniles, however for Cd and Cu they had similar sensitives for both juveniles and sub-adults (Strode & Balode, 2013). Some studies have found body size is not a significant factor in heavy metal accumulation (Maclean et al., 1996). Adult *Hyalella curvispina* (Shoemaker, 1942) were significantly more sensitive to Cd than juveniles (Garcia et al., 2012) and for *M. affinis* there was no significant difference amongst juveniles and subadults for Cd, Cu and Zn in sensitivities (Strode & Balode, 2013). Cd concentrations in *H. azteca* from three lakes in central Ontario were independent of body size (Maclean et al., 1996). This was also the case for Cu and Zn concentrations for both control and metal-exposed amphipods, even over a 100-fold range in body size (0.02-2 mg dry weight) (Borgmann & Norwood, 1995). Notably, bioaccumulation of heavy metals was not significantly affected by body size for animals between 0.1 and 0.6 mg dry weight (Borgmann et al., 1996). A portion of animals analysed in this chapter fit within this weight range (11 of the 20 samples), which could explain as to why the negative relationship was not statistically significant for 22 of the 36 trace elements analysed.

To conclude, Evans Bay had the highest concentration of trace elements out of the three study sites in the Wellington harbour. Metal concentrations in the sediment, seawater and seaweed at each site did not explain the differences amongst sites. For a clearer understanding of site-specific differences, further data would need to be collected. *Sunamphitoe mixtura* had the highest concentration of trace elements out of the three amphipod species analysed. A species-specific difference was supported by the literature. The negative relationship for *S. mixtura* between body size and trace element in concentration also followed the consensus of the literature.

3 Bioaccumulation of trace elements, copper and neodymium by sand hoppers, *Bellorchestia quoyana* (Milne-Edwards, 1840) at different seawater temperatures.

3.1 Introduction

An influential stressor in the marine environment is climate change, of which a major consequence is increased sea water temperatures (Chapman, 2017). Temperature is a critical environmental factor that influences the behaviour, physiology, phenology, and distribution of organisms (Bae et al., 2016). An increase in water temperature of up to 2 °C may lead to adverse effects on both the ecosystem and its inhabitants (Peric et al., 2018). Exposure to temperatures above an organism's thermal tolerance range alters the rates of physiological and biochemical reactions and the stability of an organism's molecules (Sokolova & Lannig, 2008; Sung et al., 2018). For example, elevated temperature promotes accelerated moulting and maturity in the crustacean *Moina micrura* (Kurz, 1875) (Chen et al., 2015). It can also impact aspects of reproduction such as total number of offspring and time to first brood. Additionally, increased temperature can increase an organism's metabolic rate (Bae et al., 2016).

Although biological processes and rates are temperature dependent, for many aquatic organisms the role of temperature on bioaccumulation of metals is not well understood (Mubiana & Blust, 2007). Temperature may have important effects on bioaccumulation, by either reducing or increasing the bioavailability of heavy metal containments (Chapman, 2017; McLusky et al., 1986). Many studies have focused on the toxicity of heavy metals in the presence of elevated temperatures. For example, studies conducted on Zebrafish found elevated temperatures increased Cd and Cu toxicity (Guo et al., 2018; Lapointe et al., 2011). The same results have been found in studies conducted in crustaceans, with the toxicity of heavy metals increasing with elevated temperatures (Blust et al., 1994; Kadiene et al., 2017; McLusky et al., 1986; Schmidlin et al., 2015; Van de Perre et al., 2018). It should be noted that examining the effects of heavy metal toxicity and temperature in crustaceans can be complicated due to moulting and even sometimes, cannibalism (McLusky et al., 1986).

Some studies have reviewed the differences in uptake between essential and non-essential heavy metals in the presence of increased temperatures. In the mussel *Mytilus edulis* (Linnaeus, 1758), a positive relationship was seen between elevated temperature and the concentration of non-essential metals, Cd and Pb. Whereas essential metals Cu and cobalt (Co) were independent and

inversely related to temperature respectively (Mubiana & Blust, 2007). Mubiana and Blust (2007) concluded that the effect of temperature on heavy metal uptake was due to the changes in solution chemistry that favour higher uptake in a high temperature. However, particularly in biologically essential metals such as Cu, complex physiological responses mean that this relationship of higher uptake in higher temperatures does not always occur (Mubiana & Blust, 2007). Another study conducted on *M. edulis* found bioaccumulation for non-essential metals silver (Ag) and Americium (Am) was the greatest in elevated temperatures compared to essential metals Co and selenium (Se) (Baines et al., 2005). Vanhattum et al. (1993) found freshwater isopods, *Asellus aquaticus* (Linnaeus, 1758) accumulated the non-essential metal, Pb at significantly higher rates in elevated temperatures, whereas accumulation of essential heavy metal Zn was unaffected by temperature. Both Pb and Zn seemed to be stored in stable body compartments (Vanhattum et al., 1993). There appears to be a gap in the literature in regards to how multiple metals interact in the presence of one another, in elevated seawater temperatures.

Sandy beaches are at risk of both increasing temperature and heavy metal contaminates. These pressures are predicted to heighten as the proportion of the human population living near the coast increases (Griffina et al., 2018; Ungherese et al., 2012). Sandy beaches are complex transitional systems between the sea and land (Bessa et al., 2017; Schlacher et al., 2017). Their transitional nature allows the input of terrestrial materials into the marine environment, such as nutrients, sediment or detritus, and anthropogenic contaminants (Del Vecchio et al., 2017; Porri et al., 2011; Ugolini et al., 2008).

Talitrid amphipods are common and often the most abundant herbivores and detritivores on exposed sandy beaches. These amphipods can achieve very high densities (>1000 individuals m^2) on some beaches. Due to their numerical abundance, they are often used as bioindicators of pollution and also as ecological indicators of disturbances (Guerra-Garcia et al., 2009; Porri et al., 2011). Studies have been carried out using the species *Talitrus saltator* (Montagu, 1808) in Poland, the United Kingdom and the Mediterranean coast because they are a main component of faunal community in the supralittoral zone of sandy beaches (Ungherese et al., 2010). These amphipods play an important role in energy flow within the ecosystem through feeding on both terrestrial and marine organic matter as well as being a food source for fishes and birds (Baring et al., 2014; Dugana et al., 2003; Lastra et al., 2008; Olabarria et al., 2010; Porri et al., 2011; Ungherese et al., 2012). *Talitrus saltator* are also easily collected and bred for laboratory studies (Conti et al., 2016).

Examples of using talitrid amphipods include ecological indicators of disturbances such as beach grooming (Griffina et al., 2018; Vieira et al., 2016) and beach trampling (Ugolini et al., 2008).

The aim of this chapter is to examine the uptake of an essential (Cu) and non-essential (neodymium, Nd) trace metal by the sand hopper *B. quoyana,* at an ambient and warm temperature using a laboratory experiment. This experiment will address the following questions: Does the presence of more than one heavy metal affect the uptake of each metal by the organism? Are heavy metals uptaken differently at different temperatures?

3.2 Methods

3.2.1 Laboratory experiment

A factorial experiment was used to examine the uptake of Cu and Nd by the sand hoppers *B. quoyana* at different seawater temperatures. This experiment had eight treatments, each with four replicate containers (Table 3.1). The concentration of Cu used in the experiment was based on the concentrations used by Rainbow and White (1989). The Nd concentration used was calculated at two orders of magnitude lower than the Cu. The Nd concentration used was much lower than the Cu concentration as it naturally occurs at very low levels, and its toxicity is unknown.

The seawater temperatures used for this experiment were 14 °C (ambient) and 20 °C (warm). The ambient temperature was that of the raw seawater used in the experiment from Victoria University Coastal Ecology Lab (VUCEL) and was not manipulated. The warmer temperature of 20 °C was decided upon based on seawater temperatures in the Wellington harbour during the 2017/2018 summer period documented by the GWRC (GWRC, 2019), so was considered at the upper end of the nature range for these organisms (Figure A.2).

Table 3.1 Laboratory experiment factorial design. Control refers to no metal added to the seawater. Cu refers to the addition of 40 µg/L of copper (III) sulfate pentahydrate. Nd refers to the addition of 0.4 µg/L Neodymium (III) chloride.

Treatment	Water Temperature	Metal added
1	Warm	Control
2	Warm	Cu
3	Warm	Nd
4	Warm	Cu x Nd
5	Ambient	Control
6	Ambient	Cu
7	Ambient	Nd
8	Ambient	Cu x Nd

Experimental design

Water baths were used to maintain the desired temperature inside these containers. For the ambient temperature water baths, 14 L tanks were used that allowed a hose to flow raw seawater (pumped directly to the VUCEL wet lab) into the water bath and overflow. Eight ambient water baths were required, each housing two animal containers. For the warm water bath, a 30 L tank was filled, and an aquarium heater was placed in the centre to heat the water. Water was added at a steady rate to the warm water baths to maintain an equal water volume. Four warm water baths were required, each housing four 2.5 L animal containers. See Figure A.3 for the layout of the experiment

in the VUCEL wet lab. The lids of the containers had a mesh-covered opening (400 µm mesh, 60 cm diameter opening) to allow for draining the water while retaining the specimens. Each lid also had a 6 mm hole drilled in the left-hand corner for an air tube to aerate the water.

Sand hoppers were collected over the period of a month from a single site in Wellington harbour, Scorching Bay (41°17'48.4"S 174°50'03.0"E). The sand was dug to a depth of ~50-300 mm near washed up seaweed until sand hoppers were visible. They were then captured by hand and placed into a plastic container. Sand hoppers were collected on the day the experiment began; a subset was immediately frozen to provide a baseline sample. Seawater was also sampled from Scorching Bay and VUCEL to provide a snapshot of the differences between the two locations. The process for sampling seawater is as described in chapter two.

3.2.2 Experiment process

Sand hoppers were randomly allocated into the 32 containers (15-18 per container) in addition to a fist-sized rock (~7cm in length) and 2 L of raw seawater. The rock served the purpose of providing shelter for the sand hoppers as well as anchoring the containers within their water baths. The initial salinity, temperature, dissolved oxygen and pH were recorded prior to adding the metals.

To avoid any cross contamination, the metals were added away from the experimental area. Clean pipette tips and gloves were used for each container when doping took place. Once doped, the containers were returned to the wet lab and into their appropriate water bath with an air tube added to each container. The equipment used to take the measurements of the salinity, pH, temperature and dissolved oxygen were; an ATAGO Pocket Refractometer, Digitech pH meter pen QM- 1670 and EcoSense DO200A respectively. The pH and salinity readers were recalibrated after measurements from four replicates of the same treatment were recorded. All equipment was washed thoroughly with deionized water between measuring different treatments. The rock was removed from each container and the water was drained through the mesh in the lid, five leaves of frozen spinach were fed to the sand hoppers and the rock returned to the container (Rainbow & White, 1989). The spinach was left in the container for 24 hours without seawater then removed and the containers refilled with raw seawater. The water was then doped if necessary and the measurements of pH, temperature, salinity and dissolved oxygen were recorded again. During the 24 hours without seawater, the containers remained out of their appropriate water baths. Sand hoppers were sampled on the first day of the experiment and then every following seven days, using plastic tweezers. Sand hoppers sampled were placed into the freezer at -4 °C. When sampling

occurred, either one large individual was collected (larger than 10 mm in length) or two small sand hoppers were sampled (less than 10 mm in length). This process was repeated for three weeks or until there were no sand hoppers remaining in the container.

Different replicate containers were started at different dates over the course of a month. Each container followed the same cycle of measuring, feeding and sampling just at different stages over the course of time. This was due to learning experiences to keep the sand hoppers alive. Issues that resulted in replicates being restarted included: too few amphipods to complete the projected four-week experiment, crushing the sand hoppers with the rock in the containers, and having amphipods die in hypoxic water.

3.2.3 Processing samples

Sand hoppers that had been sampled for the purpose of being analysed using the ICPMS were measured from head to tail on a clean cling filmed surface. In total 32 sand hoppers, four replicates from each of the eight treatments were sampled two weeks into the experiment. One spinach leaf was also randomly selected from each bag of spinach that was used throughout the experiment to be processed. Preparing and processing the samples, including spinach, for trace element analysis using ICPMS was undertaken as described in chapter two.

3.2.4 Statistical analysis

All analyses were conducted in SAS Enterprise Guide 7.4. Comparisons were made between baseline samples, samples that were not subject to any experimental processes and experimental controls. The purpose of this comparison was to determine if there was any difference between the analysed trace element concentrations in the experimental controls and the naturally occurring concentrations. Trace element concentrations were analysed using a two-way ANOVA, with temperature and metal treatment as fixed factors. Because there was no temperature effect, temperature treatments were pooled for analysing Cu and Nd. A type 3 model was used, as the data was unbalanced. If the assumptions for this test were not met, the data was logged. To examine the measurements of salinity, pH, dissolved oxygen and temperature, a one-way ANOVA was used. If assumptions of normality and equal variance were not met for the one-way ANOVAs, a Kruskal-Wallis test was used. To examine effects of body size, the relationship between the concentration of a given trace element and the dry weight of individual sand hoppers from Scorching Bay were compared. The sand hoppers from the experiment were pooled together for this comparison. A

linear regression was used for this analysis. If the assumptions for this test were not met, the data was logged. All graphs were produced in Excel using data values imported from SAS.

3.3 Results

The ICPMS analysed 21 trace elements for each sample processed in this chapter (Table 3.2). For some samples, data for all 21 trace elements could not be used because the values were too low compared to the procedural blank. This is mentioned in text when this has occurred.

Table 3.2 Trace element symbols and the corresponding trace element name.

Trace element symbol	Trace element
As	Arsenic
Ba	Barium
Ca	Calcium
Cd	Cadmium
Ce	Cerium
Co	Cobalt
Cs	Caesium
Cu	Copper
Fe	Iron
La	Lanthanum
Li	Lithium
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
Nd	Neodymium
Rb	Rubidium
Sm	Samarium
Sr	Strontium
U	Uranium
Y	Yttrium
Yb	Ytterbium

3.3.1 Baseline and Experimental Controls Comparison

Seven sand hoppers were randomly selected from the baseline samples to be compared to the control treatments (both ambient and warm), from weeks one and two of the experiment. Baseline samples were those collected on sampling days whereas the control samples were sand hoppers that were used in the experiment as the control treatment. There was a significant difference for Mn, Mo and U between the baseline and control treatments (Table 3.3). For trace elements Mo and U, the highest average concentration was seen in the warm control followed by the ambient control and lastly baseline. For Mn, the ambient control had the highest average concentration followed by warm control and baseline (Figure 3.1). There was no distinct trend between the average concentrations across the three treatments. For 18 of the trace elements analysed, there was no significant difference between the baseline and control treatments (Table A.11). This comparison helps to show that there were no major changes in the average

concentration of the trace elements analysed between the sand hoppers used in the experimental control and their natural variability.

Table 3.3 Results depicting the trace elements that were significantly different between baseline and control samples. Results were obtained from a one-way for trace elements Mo and U ANOVA (df, 2). Results were obtained from a Kruskal-Wallis test for trace element Mn (df, 2).

Trace Element	F-statistic	p-value
Mn	7.3821	0.0146
Mo	17.44	0.0003
U	5.18	0.0238

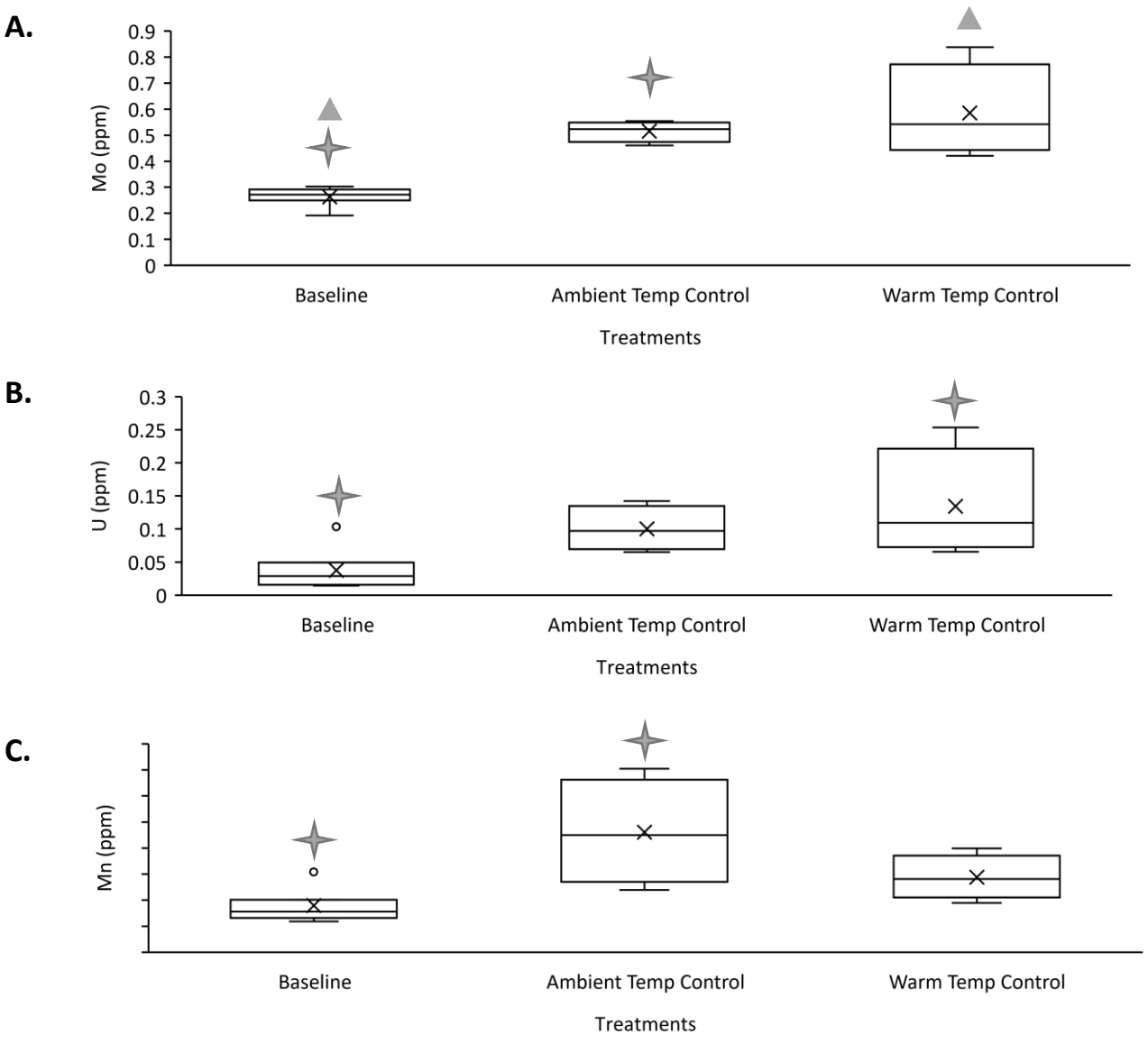


Figure 3.1 Concentration of trace elements (ppm) in sand hoppers across three treatments: baseline, ambient control and warm control for trace elements (A) Mo, (B) U and (C) Mn. Treatments with a significance between them have a grey shape above the corresponding box plot. When the symbol is the same there is a significant difference.

Water analyses

Water samples from Scorching Bay and VUCEL were analysed for concentrations of seven trace elements, as all other trace element data for these water samples were unreliable. Trace elements were similar in each water body, with the exception of Cu in the water at VUCEL having a higher concentration than Scorching Bay (Figure 3.2).

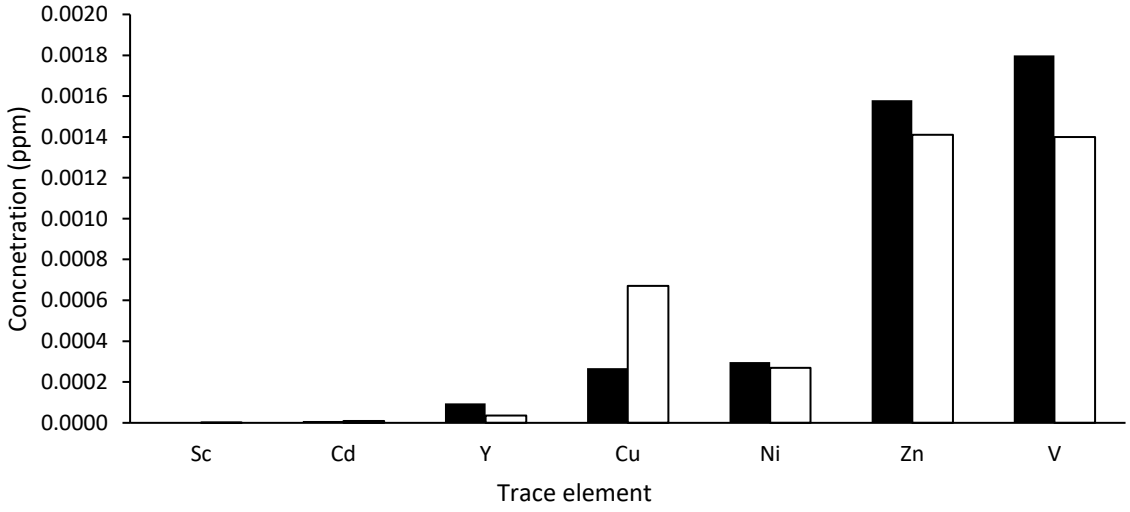


Figure 3.2 The concentration of trace elements (ppm) in Scorching Bay (black) and VUCEL (open bars) seawater.

3.3.2 Experimental data

Trace Elements: Cu and Nd

As expected, both Cu and Nd were elevated in treatments where each had been added. There was no significant difference between temperature treatments (Figure 3.3 and Figure 3.4), nor was there a significant interaction between temperature and trace element treatments (Table 3.4 and Table 3.5). For this reason, Figure 3.3 and Figure 3.4 depict Cu and Nd concentrations in the sand hoppers pooled across temperature treatments. For treatments with added Cu, there was a significant difference from the control. There was no significant difference between treatments where one metal or two metals were added. For treatments with added Nd, there was a significant difference between the control and the treatment with both metals. All other trace elements analysed showed no significant difference across any of the treatments (Table A.12).

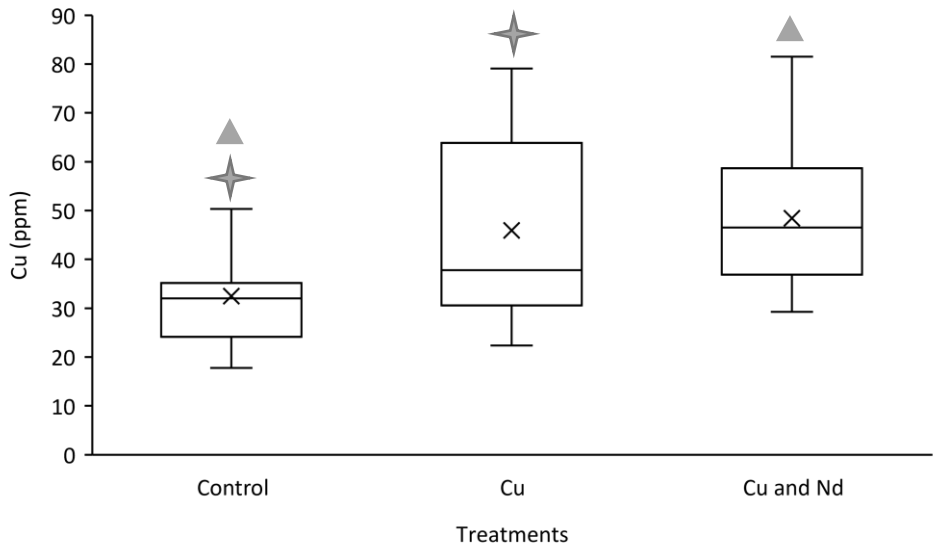


Figure 3.3 Concentration of trace element Cu (ppm) in sand hoppers across experimental treatments: control, Cu and Cu x Nd. Data was pooled across temperature treatments as there was no significant effect of temperature. Grey shapes above the box plot for each species represent where the significant difference lies.

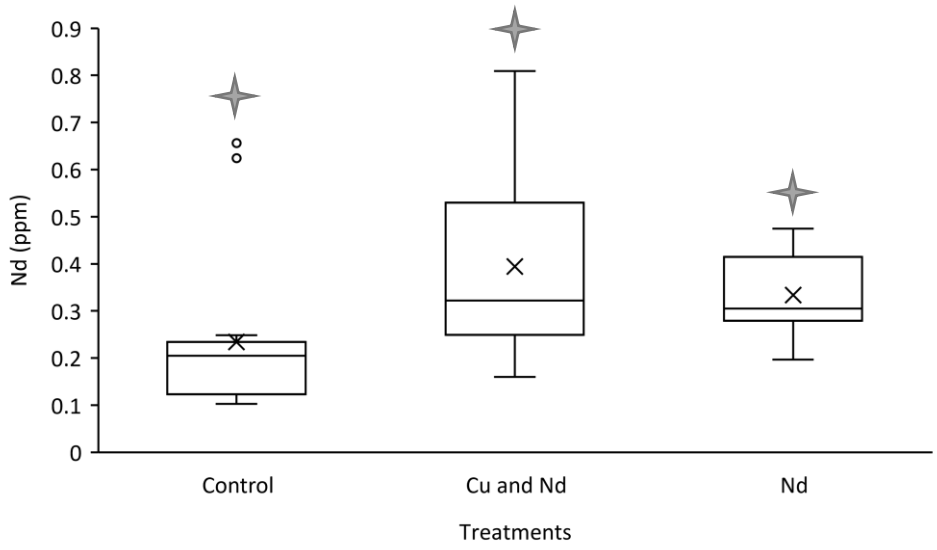


Figure 3.4 Concentration of trace element Nd (ppm) in sand hoppers across experimental treatments: control, Cu x Nd and Nd. Data was pooled across temperature treatments as there was no significant effect of temperature. All treatments were significantly different from each other.

Table 3.4 Results for two- way ANOVA for trace elements Cu concentration in experimental sand hoppers.

Factors	df	SS	F-Statistic	p-value
Temperature	1	168.7211	0.75	0.3944
Trace Element	2	1742.3785	3.84	0.0352
Temperature * Trace element	2	454.0078	1.01	0.3785

Table 3.5 Results for two- way ANOVA for trace elements Nd concentration in experimental sand hoppers. Data for Nd was logged to meet test assumptions.

Factors	df	SS	F-Statistic	p-value
Temperature	1	0.0087	0.18	0.6774
Trace Element	2	0.3951	4.03	0.0303
Temperature * Trace element	2	0.0542	0.55	0.5820

Salinity and pH did not vary across treatments (Table A.13). There was a significant difference in temperature between all ambient and warm treatments (Figure A.4). There was a significant difference between treatments for the dissolved oxygen, however all treatment averages of dissolved oxygen still fell within 3% of each other.

Trace element in food (spinach) samples

The average concentration of the spinach samples used as food during the laboratory experiment for 20 trace elements is shown in Figure 3.5.

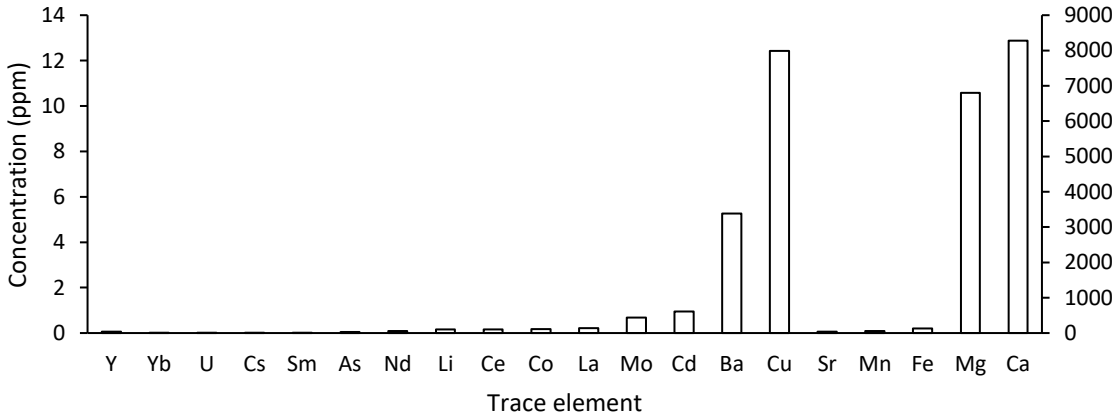


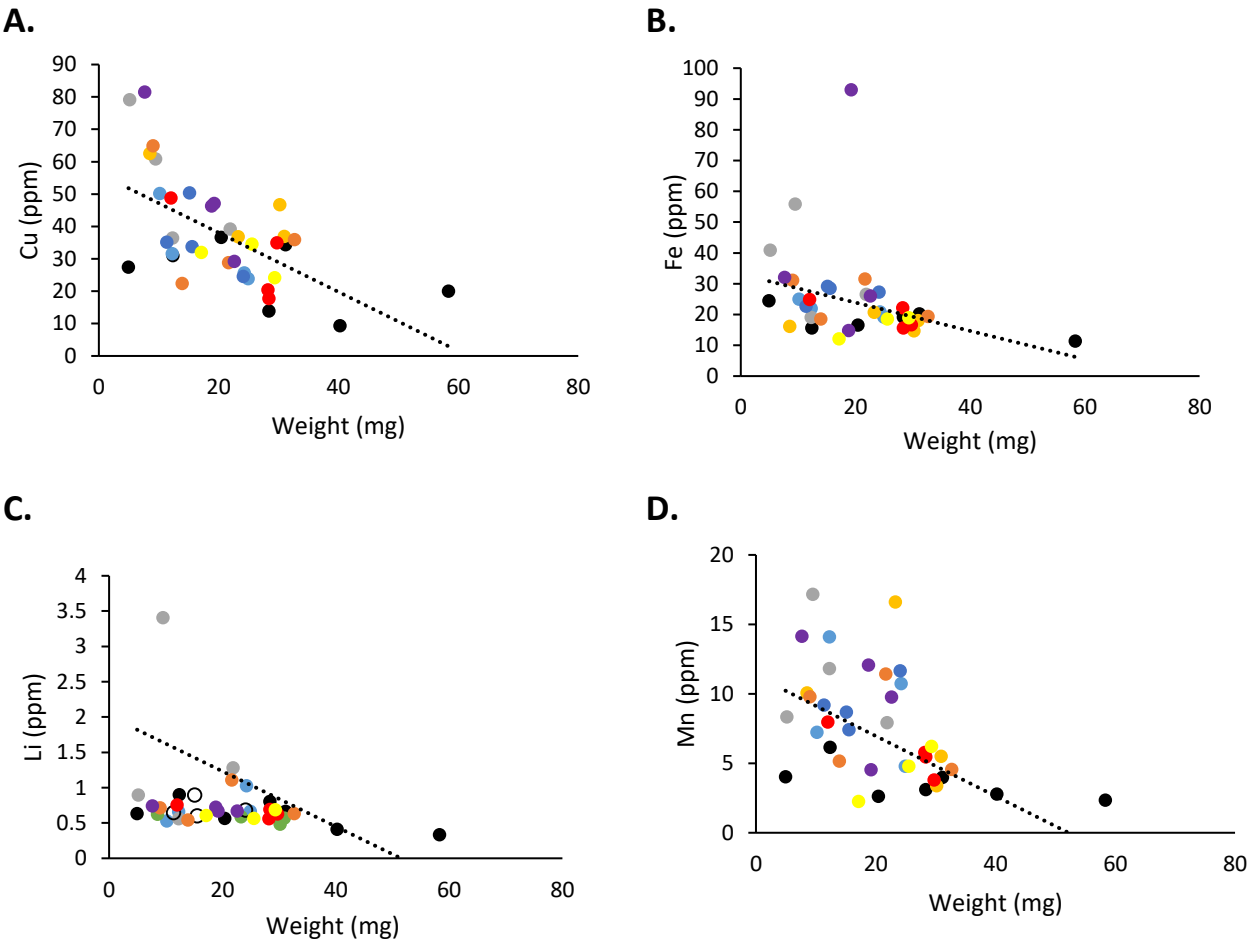
Figure 3.5 The average concentration of trace elements (ppm) in spinach samples fed to the sand hoppers throughout the laboratory experiment. Trace elements: Sr, Mn, Fe, Mg and Ca are plotted on the right-hand Y axis.

3.3.3 Size relationship

Six elements showed a significant negative relationship between dry weight and trace element concentration (Table 3.6 and Figure 3.6). Fifteen trace elements showed no significant relationship between dry weight of an individual sand hopper and trace metal concentration (Table A.14).

Table 3.6 Results from a linear relation between the concentration of a given trace element (ppm) and the average dry weight of a sand hopper. Data for trace elements: Fe and Li were transformed to meet test assumptions.

Trace element	F-statistic	p-value	R square	Line Equation
Cu	20.71	<0.0001	0.3652	y=-56.3203-0.916*x*
Fe	10.06	0.0031	0.2185	y=-33.0749-0.4210*x*
Li	7.63	0.009	0.1749	y=-1.014-0.0124*x*
Mn	10.92	0.0022	0.2328	y=-11.2934-0.179*x*
Mo	6.19	0.0176	0.1468	y=-0.7015-0.0079*x*
U	7.99	0.0076	0.1817	y=-0.1664-0.0026*x*



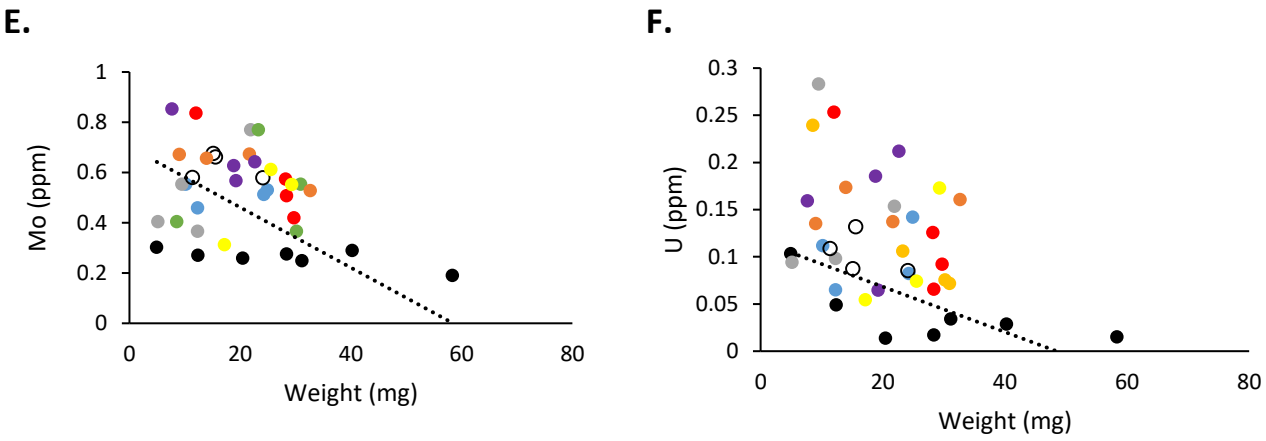


Figure 3.6 Relationship between concentration of trace elements (ppm) and dry weight of an individual sand hopper (n=9). All treatments have been pooled together for this analysis. Raw data was used for each graph, (A) Cu, (B) Fe, (C) Li, (D) Mn, (E) Mo and (F) U. Treatments: baseline (black), Ambient control (blue), Ambient x Cu (grey), Ambient x Cu x Nd (green), Ambient x Nd (open circles), Warm control (red), Warm x Cu (orange), Warm x Cu x Nd (purple) and Warm x Nd (yellow). Data for Fe and Li was transformed to meet test assumptions.

3.4 Discussion

The first question addressed in this experiment was, does the presence of more than one heavy metal affect the uptake of each metal or others by the organism? There was no significant difference between the metal Nd concentration when the metal was in the presence of Cu and when it was the only metal present. Heavy metal contamination is rarely by a single contaminant but rather a mixture of contaminants which may interact in an additive, synergistic or antagonistic manner (Uwizeyimana et al., 2017). Studies have found conflicting results for the effects of bioaccumulation when more than one metal is present for different amphipod species. Duquesne et al. (2000) found that the bioaccumulation of Cd in *Paramorea walkeri* (Stebbing, 1906) was the same both on its own and when mixed with Cu.

Many studies address the toxicity as well as differences in accumulation of metals in the presence of one another. Bat et al. (1998) found that the mixture of Cd and Zn was less toxic than Cd on its own for *C. volutator.* Cd induced mortality was reduced in the presence of Zn and the Cd levels in *C. volutator* were also reduced (Bat et al., 1998). Oakden et al. (1984) found that when Zn and Cd were mixed the mortality was less for *Rhepoxynius abronius* (Barnard, 1960) and *Eohaustorius sencillus* (Barnard, 1962) than when exposed to Cd on its own. Sundelin, (in Forbes and Forbes, 1994) similarly found that when Cd and Zn were mixed the mortality was less for *Pontoporeia affinis* (Lindström, 1855) than when exposed to Cd on its own. As well the levels of Cd in *P. affinis* tissue were less when mixed with Zn. These studies all showed significant results in heavy metal uptake when examining an essential metals (Cu and Zn) with a non-essential metal (Cd). Norwood et al. (2007) used *H. azteca* to determine if exposure to a mixture of 10 metals would effect the bioaacumualtion of each metal. Bioaccumulation of the metals As and Pb increased in the presence of the other metals, while bioaccumulation of Co, Cd and Ni decreased (Norwood et al., 2007). Another study using other crustaceans also reached these conflicting findings. Negilski et al. (1981) found for the shrimp *Callianassa australiensis* (Dana, 1852) that the combination of Cu and Cd increased toxicity compared to these metals on their own. In the same study the combination of Zn and Cd mixed was less toxic than Zn alone (Negilski et al., 1981).

The second question addressed whether heavy metals are uptaken differently at different temperatures. Although both the metals Cu and Nd were elevated in the sand hoppers in the doped treatments, temperature was a non-significant factor in the uptake of either metal. This was also the case for other trace elements examined during this experiment. Changes in temperature influence the physiological responses of marine organisms to heavy metals (Manciocco et al., 2014). Elevated temperatures increase metabolic rates, which includes uptake and accumulation rates (Bae et al.,

2016; Muyssen et al., 2010). However, Baines and Fisher (2008) found temperature did not influence accumulation of heavy metals in marine invertebrates from an aqueous solution. Mechanisms leading to metal elimination in marine invertebrates are still not well understood (Bae et al., 2016). The influence of temperature on toxicity is also dependent on the sensitivity of the test organisms (Heugens et al., 2003). Generally, there is an increase in toxicity with increased temperatures (Manciocco et al., 2014; Richards & Chaloupka, 2009), but this was not explored in this study. Exposure to Cu in brine shrimp, A*rtemia franciscana* (Kellog, 1906) in temperatures ranging from 10 °C to 35 °C showed an increased Cu uptake with increasing temperature (Blust et al., 1994). Saher & Siddiqui (2019) found in an *in situ* study conducted on the crab *Metopaulias depressus* (Rathbun, 1896), that temperature differences across study sites were one of the components responsible for different heavy metal concentrations. Cd in the presence of elevated temperatures resulted in higher tissue concentrations in daphnids (Heugens et al., 2003).

These results were not generally supported by the literature, which predicts that a difference in temperature treatments and a difference in uptake of Cu and Nd in the presence of each other would have occurred.The results here may have been due to the length of exposure time, the temperatures used in the experiment or the doped concentrations used for each metal in the experiment. The sand hopper samples analysed had been exposed for two weeks. Bioaccumulation is a time-dependent process (Duquesne et al., 2000), although, Duquesne et al., (2000) found that after two days of exposure to Cu the body concentrations of *P. walkeri* were higher than that of the control treatment. Jelassi et al. (2019) determined that 14 days was sufficient to test an animal's response to a contamination. These findings from the literature and the fact that the doped treatments were significantly different from the control treatments, suggest the experiment ran for a long enough time for bioaccumulation to take place.

An increase of just 2 °C in water temperature may have adverse effects on both the ecosystem and its inhabitants (Peric et al., 2018). This experiment used an increased water temperature of 6 °C for the warm temperature treatment. Sand hoppers have wide-ranging thermal tolerances, with a temperature of 20 °C being within their limit. The sand hopper, *Talorchestia martensii* (Weber, 1892) was able to tolerate temperatures from 5 °C to 45 °C. The optimal temperature for survival for this sand hopper was 25 °C (Lalitha et al., 1988). Using an abundance model, the highest amphipod abundance along the Uruguay coastline was predicted to be at areas with a sediment water temperature of ~20 °C (Gomez & Defeo, 2012). *Orchestia gammarellus* (Pallas, 1766) has been recorded to have a wide tolerance range of -6 °C to 37 °C (Morritt & Spicer, 1998). The tropical sand

hopper, *T. martensii* was shown to have an optimum temperature of 25 °C (Morritt & Spicer, 1998). The upper littoral to supralittoral zone of sandy beaches where sand hoppers are found is an extremely dynamic environment. The moisture content and salinity vary greatly during periods of repeated inundation and desiccation (Tsubokura et al., 1997). The results from this experiment suggest that *B. quoyana* is a good biomonitor species as there wasn't a strong temperature effect in the experiment, suggesting monitoring studies using them would not get confounding results by temperature changes in the future.

Making comparisons between the concentrations used in the experiment conducted in this chapter and other studies is difficult as using Nd as a non-essential metal was novel. King et al. (2006), Othman & Pascoe (2002) and Rainbow & White (1989) used similar concentrations for Cu toxicity testing in amphipods. Concentrations used in toxicity testing is often to a lethal dose or lethal concentration (Shubert et al., 1978). This was not the purpose of this study so the concentrations used in this chapter are much lower than has been used in much of the literature. It must still be acknowledged that the dosages used, although enough to be significantly different from the control, may not have been great enough to cause an effect on the uptake of Cu and Nd in the presence of one another. An uptake difference in Cu and Nd may have been expected, as they are both essential and non-essential metals. Whether a trace element is essential or non-essential can influence its metabolism, tissue accumulation and elimination (Duquesne et al., 2000). Copper is an essential element and may be accumulated in preference to the non-essential metals (Duquesne et al., 2000). Amphipods have a relatively high Cu requirement as oxygen-transport occurs by the copper-based, respiratory protein hemocyanin (Veltman et al., 2008). Detoxification of Cu is a characteristic seen in decapod crustaceans, but not observed in the other Crustacea orders (Guven et al., 1999). The final metal concentration in an individual is dependent on the accumulation strategy of a species for a particular metal (Strode & Balode, 2013).

A negative relationship was seen between the dry weight of *B. quoyana* and trace element concentrations. The trend between uptake of trace elements and body size is examined in the discussion of chapter two. Overall, the sand hoppers here followed the trend suggested in the literature of a decrease in accumulation in larger individuals. However, only six of the 21 trace elements showed a significant negative relationship between concentration and dry weight. Concentrations of Cu and Zn in supralittoral, semi-terrestrial and eulittoral talitrid amphipods show no significant relationship between trace metal concentration and body weight (Marsden & Rainbow, 2004).

To conclude, this experiment found no significant difference amongst metal accumulation for Cu and Nd in an elevated temperature and Cu in the presence of another metal. Findings from the literature suggested a difference might have been seen. The lack of a significant difference might be due to experimental design choices such as the length of experiment, the elevated temperature and concentration used for doping, or may be due to this species' physiological ability to tolerate a wide temperature range. The negative relationship for *B. quoyana* between body size and trace element concentration is consistent with the literature. Larger individuals have a lower concentration of trace elements than smaller sized individuals.

4 Concluding Comments

This study has reported on baseline information for 36 trace elements across three study sites in the Wellington harbour and three amphipod species. Monitoring studies are important for assessing heavy pollution in different marine environments and also compiling baseline data for future monitoring (Chakraborty et al., 2014). Studies conducted have ranged from assessing heavy metal contamination in algae (Chakraborty et al., 2014) to marine mammals (Burek et al., 2008; Rosa et al., 2008). Studies encountered in the literature offer a snapshot into the heavy metal pollution at a site, but do not offer ongoing comparisons. Baseline data can serve as a starting point for further investigations into possible environmental changes (Metcheva et al., 2010). Understanding metal pollution as an ecological disturbance in light of climate change has become an important topical issue (Chakraborty et al., 2014). Baseline data is therefore important to be able to observe future changes in marine degradation (Rainbow et al., 1998). As well as baseline data, biomonitor species are able to provide an insight into heavy metal bioavailability at a specific point in time (Fialkowski et al., 2009). There are few ongoing biomonitoring programs in New Zealand. The GWRC sediment survey mentioned in chapter two, is part of a monitoring program which takes place every four years (GWRC, 2014). This report however does not have the number of trace elements that were examined in this study.

This study identified *S. mixtura* as a potentially effective biomonitor species. However, it might be reasonable to extrapolate from a single biomonitor to draw conclusions about local metal bioavailabilities to the biota in general (Fialkowski et al., 2009). As amphipods are principal prey organisms, any increase of heavy metals will be magnified along the food chain (Laskowski et al, 2010). Conclusions could be drawn for metal availabilities to other marine organisms in higher trophic levels. This study also found the recorded concentrations of trace elements were generally greater in small individuals than large individuals for all amphipods analysed. This information is extremely useful when considering biomonitor species, as the organism size will reflect trace element availability for that age class. This should also be taken into consideration when selecting biomonitor species.

Beaches are a greatly under represented ecosystem in the literature on climate-change ecology (Schoeman et al., 2014), and given the importance of climate change, *B. quoyana* could be a good biomonitor species for sandy beaches. It is important to know how temperature affects uptake of the elements being monitored. Temperature often impacts an organism's survival, growth and reproduction (Kordas et al., 2011). Although it is well known that temperature is biologically

important, effects of environmental temperatures are being readdressed with renewed vigour as climate change is altering temperature patterns around the globe (Brierley & Kingsford 2009; Kordas et al., 2011). Heavy metals are a problematic contaminant due to their toxicity, persistence in the marine environment and their ability to accumulate and magnify in organisms (Costa-Boddeker et al., 2018; Zhuang & Gao, 2014). Understanding how elevated temperature and heavy metals interact in a laboratory scenario can help predict how these two stressors will behave *in situ*. Using *B. quoyana* as a biomonitor species gives the opportunity to examine the effects of pollutants without the confounding effects of increasing temperatures.

Limitations of this study

A key limitation of this thesis was the number of samples examined in both chapters. Due to the small size of amphipods multiple individuals were grouped together to make a single sample. This study could have accounted for variation within the samples better, by grouping sexes together and age classes. Individuals were grouped by length to form a single sample; this could have masked age class differences. All the sites surveyed in chapter two were sites with some form of human development. This was the case as the man-made structures at each site made collection easier, and the setting of light traps safer. This did not allow for a 'clean' comparison site. Although that is not essential in this study, it would have been useful to use as a reference for the level of contamination in each amphipod species at each site. More samples of amphipods, seawater and seaweed would have made for more robust statistical analysis, which would have provided more confidence in the findings of this thesis. However, processing and running the samples through the ICPMS was time consuming (~2 weeks for data output per round of sampling containing 16 samples) and expensive (~$50 a sample), so sample sizes were limited.

Advanced statistical tests were not possible in either chapter due to the sample sizes. Principal component analysis would have been run to determine if there was a relationship between the concentrations of trace elements within a sample, if possible. However, Shaukat et al., (2016) suggests either a minimum of 100 samples or a sample size of five times the number of variables. Chapter three faced similar limitations as chapter two in regards to sample sizes and therefore the statistical robustness of the findings in this chapter.

Conclusions and suggestions for further research

This thesis has contributed to information on trace element information at various sites around the Wellington Coastline for amphipod species. This information can be used as a

comparison at other locations and amongst other amphipod and marine organisms. This information provides baseline information for a large number of trace elements, which can be used as time series data. The data collected in chapter two provides an excellent opportunity to begin a biomonitoring program for amphipod species both around Wellington and within New Zealand. Chapter two identified both a site and species with elevated trace element concentrations. Understanding the impact of multiple stressors, heavy metals and elevated temperatures together was examined in this thesis. This study can provide insight into an experimental design to examine these stressors, with methods that did and did not work. Further development on the experimental design constructed in this thesis could result in a more robust experiment. From the experiment in chapter three *B. quoyana* was identified as a good biomonitor species for understanding how pollutants affect behaviour without the confounding effect of temperature.

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Appendix

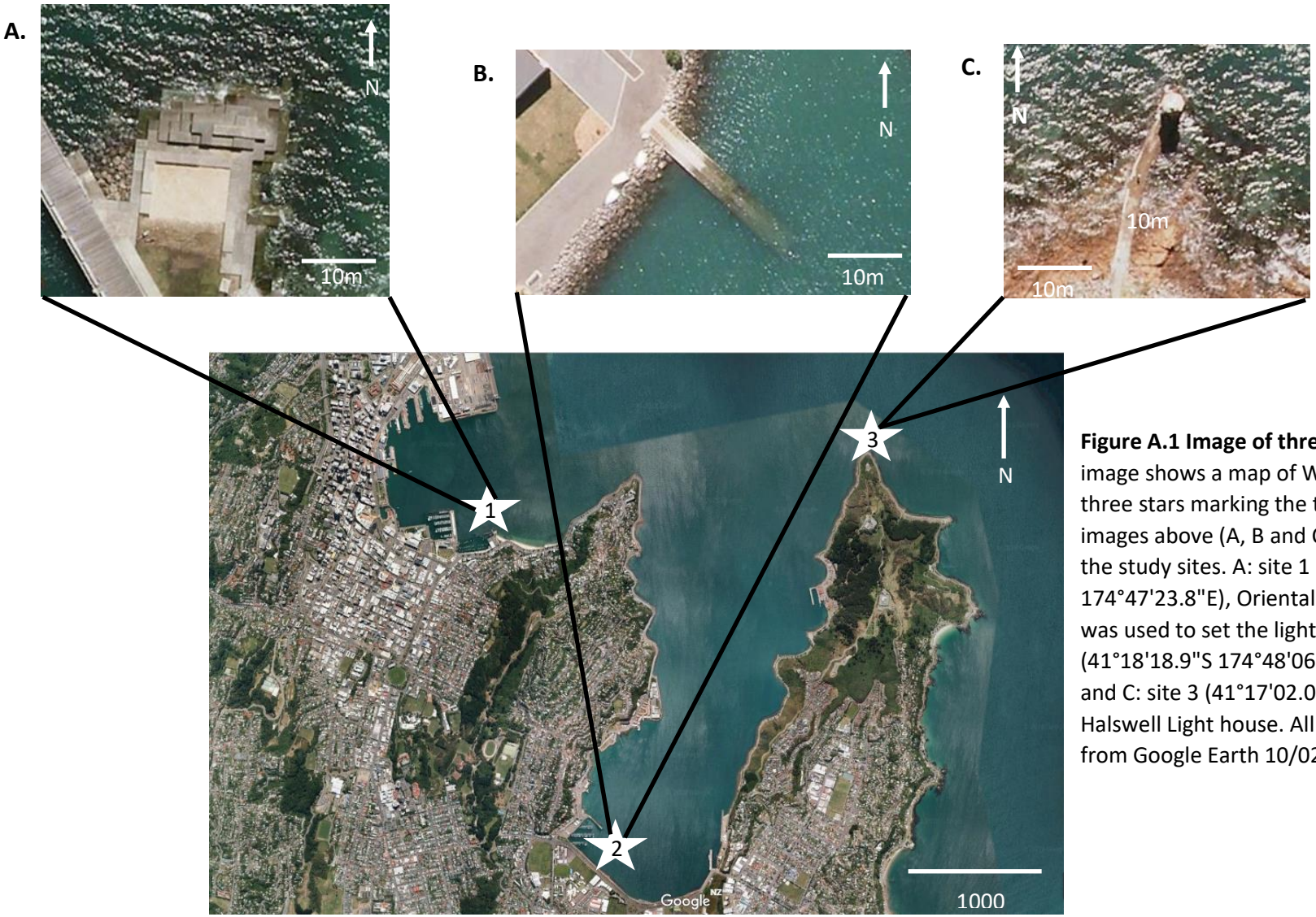
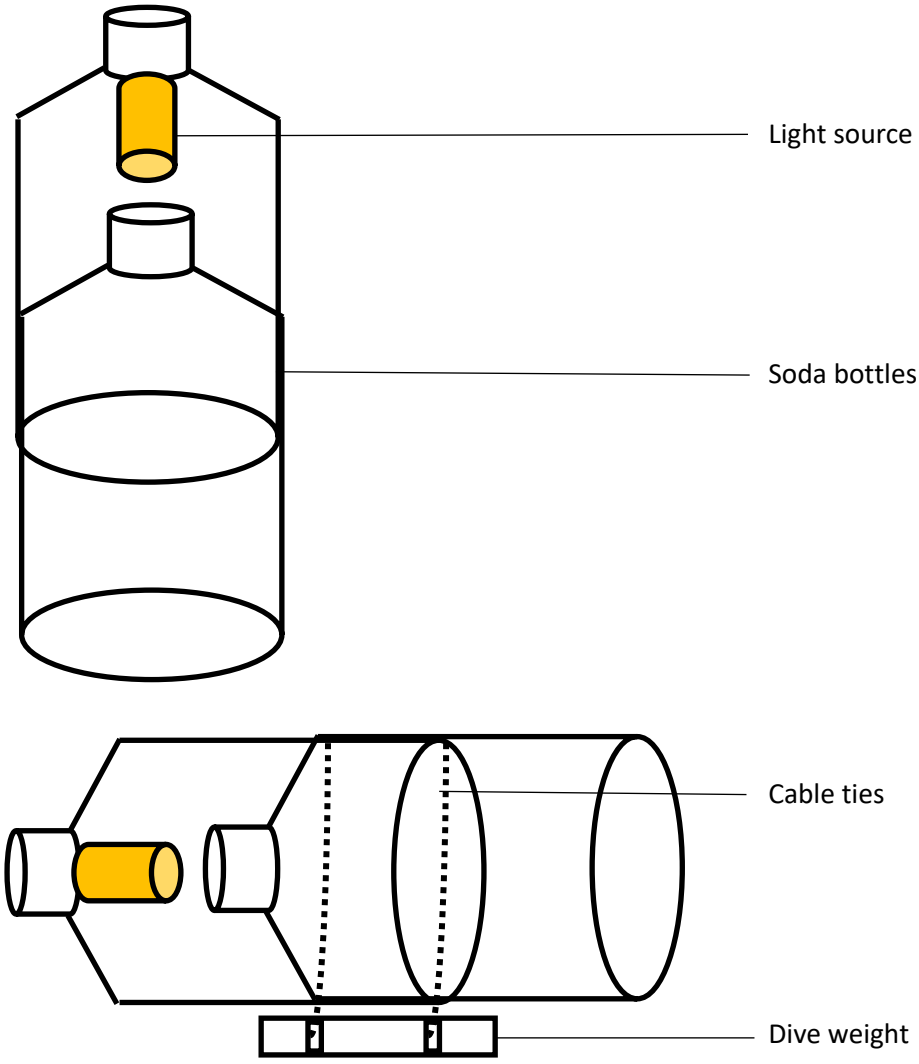


Figure A.1 Image of three study sites. The bottom image shows a map of Wellington coastlines with three stars marking the three study sites. The three images above (A, B and C) provide a closer image of the study sites. A: site 1 (41°17'23.2"S 174°47'23.8"E), Oriental Bay where a concrete jetty was used to set the light traps from. B: site 2 (41°18'18.9"S 174°48'06.1"E), Evans Bay boat ramp and C: site 3 (41°17'02.0"S 174°49'33.9"E), Point Halswell Light house. All images were retrieved from Google Earth 10/02/2019.

Appendix A. Soda bottle light trap design

The soda bottle light trap consisted of two 2.25 L soda bottles which were inserted into each other. The bottom end of the plastic bottles were cut off at the mid-point of the bottle. A battery powered dive light (Tektite Mark-Lite LED Marker) was used as the light source. This light was placed in the first soda bottle, then the second soda bottle was inserted within the first and secured with duct tape. Duct tape was used to secure the two bottles allowing them to be easily separated so the light can be powered off when the light trap was not in use. Lead 2.5 kg dive weights were attached to the soda bottle light trap to weight it in place. Two slits were made in the soda bottles once they were attached together to thread a cable tie through and then attach it to the dive weight (Appendix A Image 1).



Appendix Figure 1. Soda bottle light trap design. The diagram on the top depicts the soda bottle light trap standing upright. The diagram on the bottom depicts the position in which the soda bottle light traps were deployed.

This light trap was then placed in the water in the evening and retrieved the next day. Issues encountered with this design included; the depth at which the trap could be placed, vulnerability to interference, unreliability and potential for metal contamination. These traps needed to be placed in very shallow water, as setting them required placing them down on their side to ensure they would collect specimens (Appendix A Image 2). Collecting these light traps was a difficult task as they did

not have a collection chamber, so when lifted from the water specimens could escape. Due to the shallow positioning of these traps they were easily removed or interfered with by members of the public, reducing their effectiveness. The soda bottle light traps only proved to be effective at capturing amphipods at one site, which wasn't used as one of the study sites in this thesis. Lastly there was concern raised that the lead dive weights used could contaminate results.

Appendix Figure 2. Two soda light trap bottles set at low tide in Pauatahanui inlet to trial the effectiveness of this light trap design in August 2017.

Appendix B. Light trap design used for this project

The final light trap design used in this project was constructed from a 14 L Sistema container with two hinged clips on the ends of the container. The containers dimensions were 494L x 322W x 138H mm. Eight entry points were made in the container, three along the lengths of the container and one on either end of the container. The entry points were made with a 95 mm hole saw piece, a 4 mm pilot hole was drilled in the centre of each circle to begin the cut.

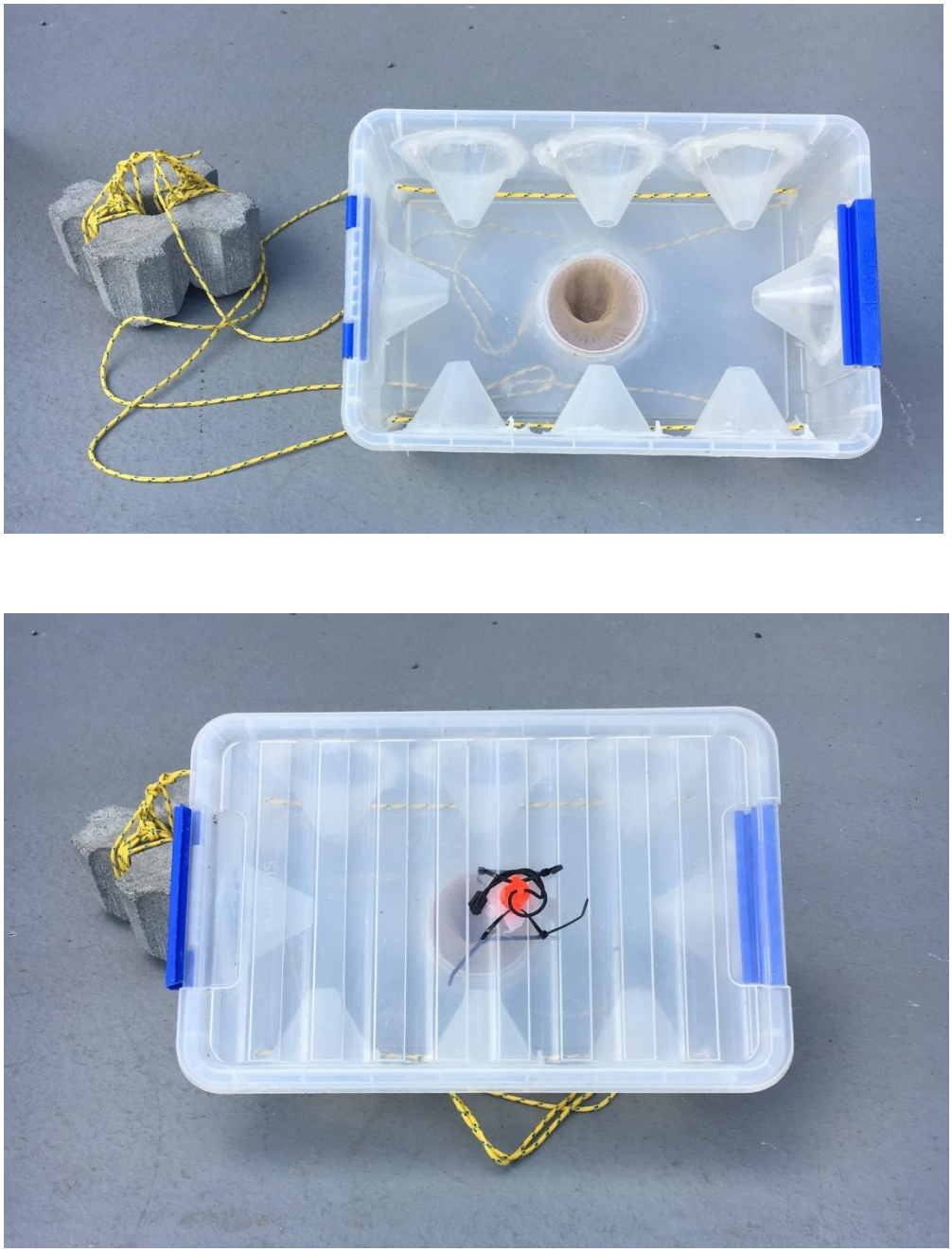
Funnels were attached to the eight entry points with the funnels smallest end facing inward. The funnels used were clear plastic, medium sized Necessity Brand and measured 110 mm in diameter including the lip of the funnel. The smaller end of the funnel was sawed off to create a larger opening of 30 mm. The funnels had a 5 mm wide lip, which was used for attaching the funnel to the Sistema container. The surface to which the funnel was attached to on the Sistema container was sanded to make a rough surface and coated with Selley's plastic glue primer. The outer edge of the lip on the funnel was sanded to make a rougher surface. It was then coated with Selley's plastic glue primer and glue then attached to the inside of the Sistema container. This was left to set for 24 hours. A plastic tag on the lip of each funnel (for the purpose of hanging the funnel on an instore display), was used to install a plastic nut and bolt through both the funnel and Sistema container. A 4 mm hole was drilled in the Sistema container 20 mm away from the 95 mm hole to allow the nut and bolt to pass through both the funnel's plastic tab and the Sistema container to provide extra strength. The funnel edges and around the area to which they were attached on the Sistema container were sanded with sandpaper to roughen the surface. Selley's clear glass silicone was then piped around the inside and outside of the funnel to finish securing the funnels to the Sistema container.

A hole was drilled in the centre of the bottom of the Sistema container to create a collection chamber. The hole in the bottom of the container was drilled following the same method as the holes on the sides of the Sistema container. A circular plastic container was then pushed into the hole. The same process was followed to attach the circular container as the funnels without the use of plastic nuts and bolts. The circular container prior to being attached to the Sistema container had 4 mm holes drilled in a circular fashion around the sides and the base of the container. These holes were for drainage when the light trap was removed from the water. The circular container came with a salad strainer which was used to attach a nylon stocking to stop specimens being lost through the drainage holes and create a collection chamber.

On the lid of the container a 20 mm hole was cut with a hole saw and a battery powered dive light (Tektite Mark-Lite LED Marker) was inserted and secured with cable ties. Around the hole and light handle silicone was applied. The light was directed towards the benthos. In the bottom four corners of the Sistema container 4.5 mm holes were drilled. Through these holes 4 mm nylon rope was threaded and a cinder block was attached as a weight. The length of rope was ~1.5 m. A buoy was attached to the light trap as a means to locate and remove the light trap from the water (Appendix B Image 1).

To set the light traps, they were placed in water at ~1.5 m depth. The study sites used, all had structures that allowed the light traps to be placed in the water at roughly the same depth. To collect the light traps, the buoy and rope attached were used as a handle to pull the light trap up out of the water. As it was being raised, the seawater drained out through the collection chamber in the bottom. Light traps were set in the evening and collected the following morning, remaining in the water for a minimum of 12 hours.

The disadvantage to light traps were weather conditions and tampering. Light traps could not be set in strong wind, as the trap moved around too much making them less effective at catching specimens. Having the traps at an accessible point meant that sometimes members of the public were able to remove the light trap from its site, which happened on two occasions.



Appendix Figure 3. Images of the light trap design used throughput this project. The top image is of the light trap without the lid attached, the bottom image has the lid attached to the light trap.

Table A.1 Amphipods used in samples selected to be processed for trace metal analysis. Samples were made of multiple individuals grouped together by length in order to form a single sample with a minimum dry weight of 3 mg.

Sample ID	Species	Number of individuals in sample	Total number and length of each individual in sample	Average length of each individual (mm)	Total weight of sample (mg)	Average weight of individual in sample (mg)	Collection site	Collection method
186	*Sunamphitoe mixtura*	60	13 individuals @ 2 mm, 47 individuals @ 3 mm	2.78	5.08	0.09	Evans Bay	Seaweed
182b	*S.mixtura*	9	7 individuals @ 5 mm, 2 individuals @ 5.5 mm	5.11	3.97	0.44	Evans Bay	Seaweed
184a	*S.mixtura*	4	4 individuals @ 7 mm	7	3.61	0.90	Evans Bay	Seaweed
194	*S.mixtura*	4	4 individuals @ 8 mm	8	5.22	1.31	Evans Bay	Seaweed
	S.mixtura	2	2 indivduals @ 10 mm	10	3.63	1.82	Evans Bay	Seaweed
122	*Apohyale papanuiensis*	4	2 individuals @ 5 mm, 2 individuals @ 6 mm	5.50	3.12	0.78	Pt. Halswell	Light Trap
223	*A. papanuiensis*	59	12 individuals @ 1 mm, 16 individuals @ 2 mm, 31 individuals @ 3 mm	2.32	5.77	0.10	Pt. Halswell	Seaweed
222	*A. papanuiensis*	25	14 individuals @ 3 mm, 11 individuals @ 4 mm	3.44	3.29	0.13	Pt. Halswell	Seaweed
221	*A. papanuiensis*	4	2 individuals @ 5 mm, 2 individuals @ 7 mm	6	3.41	0.85	Pt. Halswell	Seaweed
101	*Eusiroides monoculoides*	8	5 individuals @ 4 mm, 3 individuals for 4.5 mm	4.19	3.04	0.38	Oriental Bay	Light Trap
131	*E. monoculoides*	8	3 individuals @ 4 mm, 5 individuals @ 5 mm	4.63	3.52	0.44	Oriental Bay	Light Trap
061	*E. monoculoides*	9	3 individuals @ 4.5 mm, 5 individuals @ 5 mm, 1 individual @ 5.5 mm	4.89	4.88	0.54	Oriental Bay	Light Trap
103	*E. monoculoides*	8	8 individuals @ 5 mm	5	5.60	0.70	Oriental Bay	Light Trap

Sample ID	Species	Number of individuals in sample	Total number and length of each individual in sample	Average length of each individual (mm)	Total weight of sample (mg)	Average weight of individual in sample (mg)	Collection site	Collection method
121	*E. monoculoides*	12	12 individuals @ 4 mm	4	3.86	0.321	Pt. Halswell	Light Trap
123b	*E. monoculoides*	13	13 individuals @ 4 mm	4	4.40	0.34	Pt. Halswell	Light Trap
123a	*E. monoculoides*	8	8 individuals @ 5 mm	5	3.92	0.49	Pt. Halswell	Light Trap
E122	*E. monoculoides*	6	6 individuals @ 6 mm	6	4.42	0.74	Pt. Halswell	Light Trap
033	*E. monoculoides*	4	4 individuals @ 6 mm	6	3.48	0.87	Evans Bay	Light Trap
032	*E. monoculoides*	1	1 individual @ 8 mm	8	3	3	Evans Bay	Light Trap
0509	*E. monoculoides*	12	7 individuals @ 4 mm, 4 individuals @ 5 mm, 1 individual @ 6 mm	4.50	3.60	0.3	Evans Bay	Light Trap

Table A.2 Elements analysed by ICPMS for amphipod samples**.** Masses were analysed using three different resolution modes (low, medium, high).

Resolution	Elements
Low Resolution (mass resolution ~ 400)	Li7, Rb85, Y89, Nb93, Mo95, Cd111, Sn116, Cs133, Ba137, La139, Ce140, Nd146, Sm147, Yb172, Tl205, Pb208, Bi209, Th232, U238
Medium Resolution (mass resolution ~ 4000)	Ca43, Sc45, Ti47, V51, Cr52, Mn55, Co59, Ni60, Cu63, Zn66, Ga69, Sr86
High Resolution (mass resolution ~ 9500 – 10,000)	Mg25, Al27, Fe56, As75.

Appendix C. Further information for calibration of data for the ICPMS

The equation used to convert the raw CPS data to ppm for each element uses the following relationship:

Concentration sample (ppm) = [(CPS sample/Calibration Factor) x dilution of sample] x 1000; where the Calibration Factor was obtained from the measured multi-element standard by dividing the measured CPS for the standard solution by the concentration of each element in the solution (in parts per billion, ppb): (CPS/ppb).

Table A. 3 Standard reference material data summary for oyster tissue and fish protein.

NIST 1566 Oyster Tissue						
Element	Measured Average *ppm*	n=17 SD *ppm*	RSD *%*	Reference certified* *ppm*	95CI *ppm*	Average/ Reference
**norm factor	*0.92*	*0.09*	*10*			
Mg	*1280*			1280	90	
Ca	1199	288	24	1500	200	0.80
Sr	10.22	0.74	7	10.36	0.56	0.99
Al	162	37	22			
Fe	194	21	11	195	34	0.99
Cu	64	4	6	63	3.5	1.01
Zn	797	56	7	852	14	0.94
Ba	3.91	0.86	22			
Mn	18.6	1.20	6	17.5	1.2	1.06
Ti	2.55	1.05	41			
Li	0.27	0.04	14			
Sc	0.06	0.01	23			
V	2.38	0.35	15	2.3	0.1	1.03
Cr	0.62	0.18	30	0.69	0.27	0.89
Co	0.33	0.02	7	*0.4*		0.81
Ni	1.09	0.17	16	1.03	0.19	1.06
Ga	0.04	0.01	29			
As	12.2	0.7	6	13.4	1.9	0.91
Rb	4.43	0.42	9	4.45	0.09	1.00
Y	0.40	0.04	9			
Nb	0.01	0.00	28			
Mo	0.19	0.02	9	*<0.2*		
Cd	3.31	0.20	6	3.5	0.4	0.95
Sn	0.97	0.42	43			
Ce	0.38	0.18	48			
Nd	0.26	0.08	31			
Sm	0.06	0.02	25			

NIST 1566 Oyster Tissue continued						
Element	Measured Average *ppm*	n=17 SD *ppm*	RSD *%*	Reference certified* *ppm*	95CI *ppm*	Average/ Reference
Yb	0.03	0.00	9			
Tl	0.01	0.00	30	*<0.005*		
Pb	0.45	0.03	7	0.48	0.04	0.95
Bi	0.01	0.00	9			
Th	0.04	0.04	91	*0.1*		0.42
U	0.11	0.01	10	0.116	0.006	0.93

DORM- 4 Fish protein						
Element	Measured Average *ppm*	n=9 SD *ppm*	RSD *%*	Reference certified* *ppm*	95CI *ppm*	Average/ Reference
**norm factor	*0.85*	*0.30*	*35*			
Mg	*910*			910	80	
Ca	1,998	536	27	2360	140	0.85
Sr	9.9	0.9	9	10.1	0.8	0.98
Al	1,471	115	8	1280	340	1.15
Fe	377	31	8	343	20	1.10
Cu	15.6	1.1	7	15.7	0.46	0.99
Zn	48.7	4.4	9	51.6	2.8	0.94
Ba	5.08	0.40	8			
Mn	3.40	0.26	8	3.17	0.26	1.07
Ti	7.03	0.58	8			
Li	1.13	0.11	10	1.21		0.93
Sc	0.11	0.00	4			
V	1.57	0.11	7	1.57	0.14	1.00
Cr	1.84	0.19	10	1.87	0.18	0.99
Co	0.26	0.02	7	0.25		1.04
Ni	1.33	0.09	7	1.34	0.14	0.99
Ga	0.35	0.02	7			
As	6.31	0.57	9	6.87	0.44	0.92
Rb	5.86	0.34	6			
Y	0.16	0.01	7			
Nb	0.02	0.00	14			
Mo	0.30	0.02	5	0.29		1.02
Cd	0.29	0.03	9	0.299	0.018	0.97
Sn	0.08	0.01	9	0.061	0.018	1.33
Ce	0.82	0.20	25			

DORM- 4 Fish protein continued						
Element	Measured Average *ppm*	n=9 SD *ppm*	RSD *%*	Reference certified* *ppm*	95CI *ppm*	Average/ Reference
Nd	0.39	0.09	24			
Sm	0.08	0.02	20			
Yb	0.01	0.00	8			
Tl	0.01	0.00	7			
Pb	0.31	0.03	10	0.404	0.062	0.77
Bi	0.01	0.00	18			
Th	0.14	0.02	11			
U	0.06	0.01	14	0.05		1.17

The SRMs are from the US National Institute for Standards and Technology, NIST1566 (oyster tissue), and Canadian National Research Council, DORM-4(fish protein). These were used as a guide for evaluating the quality of the data analysed by the ICPMS. Only a limited number of elements have certified concentrations in the SRMs, and some values are informational only. The certified values are based on a minimum of 250mg of material, whereas typically 50 – 70 mg of material was weighed and processed for each SRM analysis in this study. Consequently, a greater level of heterogeneity is expected for the analyses here, leading to larger standard deviations and variations from the reference values. For most elements, measured values were within 1 – 15% of the reference values.

* where no 95CI value is given, this is an informational (not-certified) concentration

**Norm Factor: Concentrations were typically systematically low, owing to variable amounts of water absorbed by the SRM powders prior to weighing. This was typically less than 15%, but for data evaluation, the analyses were normalised to certified mg concentrations to account for this variation. The average normalisation factor is given. This does not affect samples, as samples were dried fully and held in a desiccator immediately until weighing.

Table A.4 Standard reference material data summary for water samples. Samples were processed by Otago University.

	Mn (ug/kg)	Fe (ug/kg)	Co (ug/kg)	Ni (ug/kg)	Cu (ug/kg)	Zn (ug/kg)	Cd (ug/kg)	Pb (ug/kg)
Otago (n=4)	0.713	0.315	0.0137	0.227	0.181	0.416	0.0133	0.00232
+/- 1 SD	0.009	0.004	0.0002	0.001	0.002	0.005	0.0001	0.00005
Certified value	0.74	0.34	0.014	0.24	0.20	0.41	0.016	0.0025
+/- 1 SD	0.06	0.03	0.001	0.02	0.01	0.08	0.002	0.0008

Reported errors are 1SD; these are conservatively based on repeat measurements of the in-house.

Table A.5 Total number of amphipods species caught in light traps and collected from seaweed. Species identification is done to the species level when possible.

Amphipod species	Number of individuals collected at Oriental Bay	Number of individuals collected at Evans Bay	Number of individuals collected at Point Halswell
Amphilochidae *Gitanopsis kupe*			12
Ampithoidae *Sunamphitoe mixtura*	7	241	10
Aoridae *Aora typica*	2	44	
Corophiidae *Haplocheira barbimana*	15	1	
Dexaminidae *Paradexamine muriwai*	15		1
Dexaminidae *Polycheria obtusa*			1
Eophliantidae *Bircenna macayai*	22		52
Hyalidae *Apohyale papanuiensis*	136	28	452
Hyalidae *Apohyale sp. Juveniles*	19		
Hyalidae *Apolyale sp.*			4
Ischyroceridae *Ischyrocerus longimanus*	7		744
Ischyroceridae *Ischyrocerus sp.*			1
Ischyroceridae *Jassa marmorata*		2	
Ischyroceridae *Ventojassa frequens*			212
Lysianassidae *Parawaldeckia stephenseni*		1	2
Maeridae *Quadrimaera incerta*			42
Maeridae *Austromaera mastersii*			1
Melitidae *Parapherusa crassipes*			5
Phoxocephalidae *Torridoharpinia hurleyi*		2	
Podoceridae *Podocerus manawatu*	11	73	71
Podoceridae *Podocerus sp*		10	
Pontogeneiidae *Eusiroides monoculoides*	95	44	100
Sphaeromatidae *Amphoroidea sp.*			4
Sphaeromatidae *sp.*			2
Stenothoidae *Stenothoe moe*			6
Stenothoidae *Stenothoe sp.*			8
Total Individuals	**329**	**446**	**1730**

Table A.6 Results depicting the trace elements which were not significantly different in *E. monoculoides* across three sites. Trace elements: Al, Bi, Ca, Ce, La, Mg, Nd, Sm, Sr, Tl, Th, U, Y, Yb, Zn and Zr values were obtained from a one-way ANOVA (df,2). Trace elements: As, Ba, Cr, Cs, Ga, Li, Ni, Pb and Sc values were determined using a Kruskal-Wallis test (df,2).

Trace Element	F-statistic	p-value
Al	3.91	0.0653
As	3.4167	0.1948
Ba	0.7121	0.7307
Bi	2.65	0.1305
Ca	0.38	0.6979
Ce	0.81	0.4774
Cr	4.8939	0.0836
Cs	2.9621	0.2433
Ga	4.8939	0.0836
La	0.66	0.5449
Li	1.8	0.4443
Mg	1.65	0.2504
Nd	0.79	0.4857
Ni	2.4167	0.3152
Pb	2.7212	0.2764
Sc	5.2955	0.0632
Sm	0.82	0.4731
Sr	2.22	0.1715
Th	2.52	0.1418
Tl	1.16	0.3615
U	2.65	0.1312
Y	1.01	0.4058
Yb	1.48	0.2843
Zn	4.67	0.545
Zr	1.18	0.3543

Table A.7 Results of t-test with a non-significant outcome comparing trace elements in species *E. monoculoides* and *S. mixtura* at Evans Bay. Trace elements Ba, Co, Cr, Cs, Cu La, Mn, Nb, Pb, Rb, Ti, Tl, U, Zn, and Zr data was transformed to meet test assumptions (df, 6).

Trace element	t-value	p-value
Al	-1.48	0.1886
Ba	-0.47	0.6536
Bi	-0.78	0.4631
Ca	-0.74	0.4879
Cd	-2.11	0.0798
Ce	-0.99	0.3598
Co	-0.19	0.8559
Cr	0.81	0.4506
Cs	-1.36	0.2223
Cu	1.53	0.1777
Fe	-1.72	0.1360
Ga	-1.4	0.2097
La	-2.13	0.0767
Li	-0.04	0.9687
Mg	-1.48	0.1884
Mn	-0.99	0.3591
Nb	-2.02	0.0995
Ni	0.98	0.3637
Pb	-1.48	0.1883
Rb	-1.76	0.1286
Sc	-1.67	0.1454
Sn	1.88	0.1086
Sr	-1.54	0.1743
Th	-1.95	0.0996
Ti	-2.22	0.0681
Tl	-2.02	0.0893
U	-1.13	0.3017
V	-1.75	0.1315
Zn	0.77	0.4692
Zr	-1.28	0.2488

Table A.8 Results of t-test with a non-significant outcome comparing trace elements in species *E. monoculoides* and *A. papanuiensis* at Point Halswell (df, 6). Data for trace elements: Cu, Fe, La, Nd, Mg, Mn, Rb, Sm, Sr, Ti, Tl, V and Y was transformed to meet t-test assumptions.

Trace Element	t-value	p-value
Al	-0.81	0.45
Ba	2.32	0.0598
Bi	0.21	0.8389
Ca	-0.86	0.4218
Ce	-0.62	0.5554
Co	-0.23	0.8288
Cr	0.98	0.364
Cs	0.49	0.6392
Cu	-0.53	0.6149
Fe	0.47	0.652
Ga	-0.15	0.8887
La	-0.85	0.4258
Li	-1.98	0.0946
Mg	1.79	0.1229
Mn	1.61	0.1589
Mo	0.62	0.5611
Nb	1.62	0.1573
Nd	-0.85	0.4293
Pb	0.49	0.6417
Rb	0.69	0.5154
Sc	0.52	0.6202
Sm	-0.77	0.4685
Sr	-0.02	0.9834
Th	0.69	0.5159
Ti	1.2	0.276
Tl	-0.52	0.6226
U	1.96	0.098
V	1.22	0.2699
y	-0.42	0.6894
Yb	-0.11	0.9131
Zn	0	0.9993
Zr	2.12	0.0788

Table A.9 Results depicting the trace elements which were not significantly different amongst species E. *monoculoides, A. papanuiensis* and *S.mixture*. Results were obtained from a one-way ANOVA (df,2) for all trace elements.

Trace Element	F-statistic	p-value
Ba	2.23	0.1386
Ca	0.27	0.7635
Ce	2.97	0.0780
Cr	0.12	0.8900
La	1.83	0.1907
Li	3.34	0.0599
Nd	1.83	0.19
Ni	3.48	0.0541
Sm	2.18	0.1439
Sn	1.75	0.2042
Sr	0.53	0.5985

Table A.10 Non siginifcant results from a linear regression between the concentration of a given trace element (ppm), the average dry weight (mg) of an *S. mixtura* individual from Evans Bay (n=5). Trace elements: Ba, Bi, La, Mo, Ni, Pb, Sn, Th, and Ti data was transformed to meet test assumptions.

Trace element	F-statistic	p-value	R square	Line Equation
Al	8.46	0.0621	0.7113	y=892.2588-263.1100*x*
As	0.09	0.7839	0.0381	y=6.4114+0.2756*x*
Ba	7.52	0.0712	0.7149	y=1.2089-0.0636*x*
Bi	3.37	0.1636	0.5292	y=-1.8935-0.1356*x*
Ca	1.78	0.2747	0.3720	y=108521+26802*x*
Cd	4.45	0.1253	0.5975	y=1.3716-0.1974*x*
Cr	5.99	0.0919	0.6663	y=1.10761-0.4061 *x*
Fe	6.11	0.0899	0.6716	y=562.9034-161.7508*x*
Ga	9.05	0.0573	0.7220	y=0.2184-0.0675*x*
La	9.75	0.0524	0.7647	y=-0.3083-0.1466*x*
Li	9.74	0.0524	0.7655	y=1.1226-0.2513*x*
Mg	1.80	0.2720	0.3346	y=6774.1436-789.5679*x*
Mo	1.16	0.3602	0.2172	y=-0.8256-0.0766*x*
Nb	2.83	0.1911	0.5395	y=0.1115-0.0275*x*
Ni	4.51	0.1239	0.6003	y=0.18116-0.3050*x*
Pb	5.83	0.0945	0.0660	y=0.6865-0.2005*x*
Sc	9.50	0.0540	0.7600	y=0.1442-0.0405*x*
Sn	7.32	0.0734	0.7094	y=-0.1551-0.2456*x*
Sr	2.34	0.2235	0.4267	y=1445.8421+240.2343*x*
Th	6.73	0.0808	0.6916	y=-0.8571-0.1541*x*
Ti	4.58	0.1218	0.6044	y=1.6086-0.1726*x*
Zr	5.27	0.1055	0.6371	y=0.7348-0.2349*x*

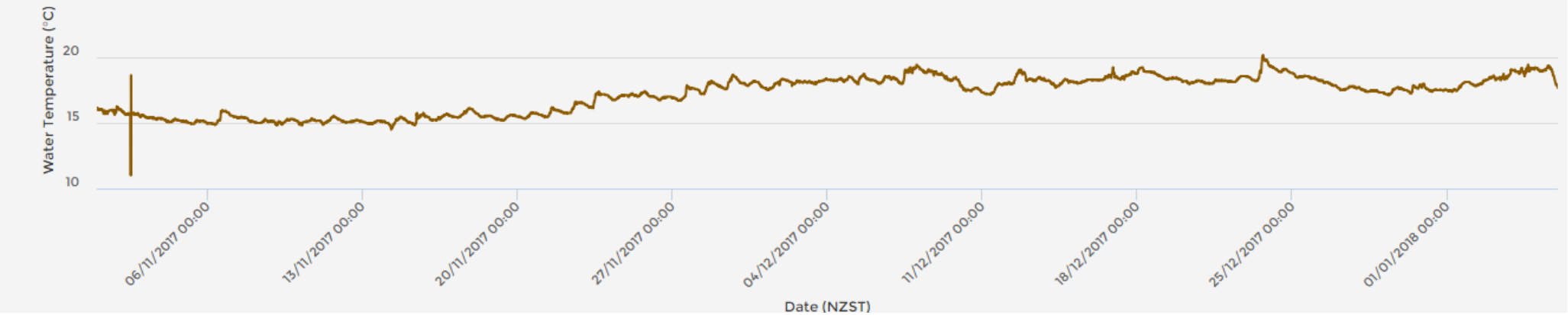


Figure A.2 Water temperature in Wellington harbour. Seawater temperature in Wellington harbour, Queens wharf recorded by the GWRC for environmental monitoring and research. Retrieved from http://graphs.gw.govt.nz/?siteName=Wellington%20Harbour%20at%20Queens%20Wharf&dataSource=Water%20Temperature 21/01/2019

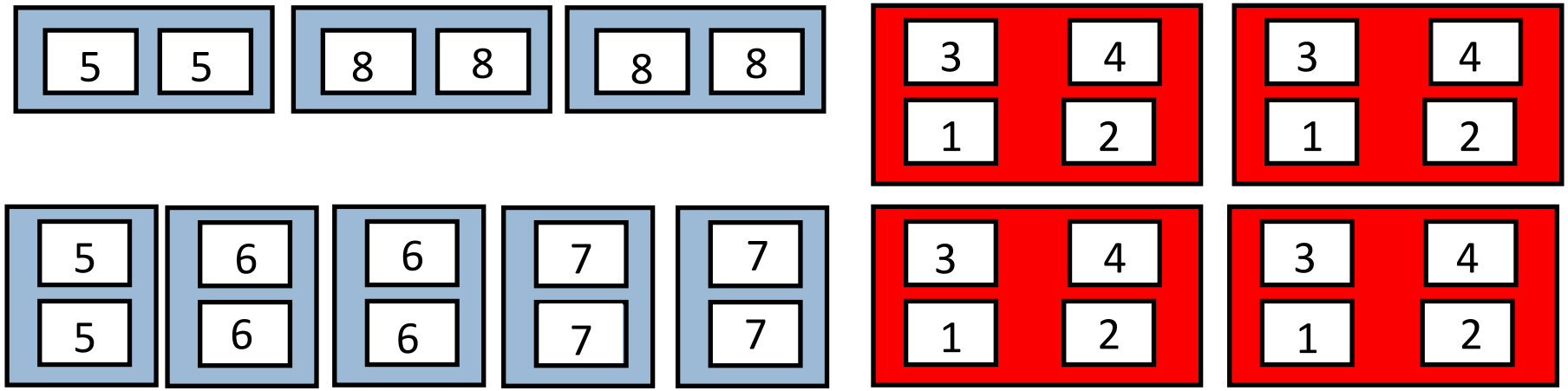


Figure A.3 Laboratory layout for experiment. All containers used in this experiment were kept in a sea tray table in the wet lab at VUCEL. The red rectangles depict the warm water baths and the blue rectangles depict the ambient water baths. The white squares represent the animal containers and each number corresponds to the treatment in each animal container.

Table A.11 Results depicting one-way ANOVA of non-significant trace elements between baseline and control sample, ambient and warm (df, 2).

Trace element	F-statistic	p-value
As	0.23	0.7946
Ba	1.39	0.2868
Ca	3.63	0.0584
Cd	0.02	0.9776
Ce	0.31	0.7411
Co	3.56	0.0612
Cs	4.53	0.0341
Cu	0.68	0.5268
Fe	1.6	0.2428
La	0.37	0.6969
Li	0.41	0.6725
Mg	0.35	0.7093
Nd	0.12	0.8837
Rb	0.12	0.8861
Sm	0.08	0.9211
Sr	2.08	0.1677
Y	0.01	0.9948
Yb	0.04	0.9563

Table A.12 Results depicting non-significant 2-way ANOVA between trace elements amongst experimental treatments (df, 7). Data for trace elements: Ba, Ce, Cs, Fe, La, Li, Sm, Y and Yb was transformed to meet assumptions.

Trace element	F-statistic	p-value
As	0.76	0.6246
Ba	0.69	0.6805
Ca	1.07	0.4151
Cd	0.47	0.8466
Ce	0.92	0.5065
Co	0.43	0.8712
Cs	0.89	0.5291
Fe	2.03	0.0943
La	0.91	0.5174
Li	1.93	0.1100
Mg	1.37	0.2640
Mn	1.33	0.2809
Mo	0.55	0.7840
Rb	0.49	0.8302
Sm	0.84	0.5682
Sr	0.75	0.6336
U	0.61	0.7388
Y	0.83	0.5742
Yb	1.39	0.2548

Table A.13 Result from comparisons between experimental treatments for laboratory measurements. A one-way ANOVA was used for pH and dissolved oxygen measurements. A Kruskal-Wallis test was used for salinity and temperature measurements (df, 7).

Lab Measurement	F-statistic	p-value
Salinity	10.2721	0.1737
pH	1.92	0.0697
Temperature	54.884	<0.001
Dissolved Oxygen	2.8	0.0092

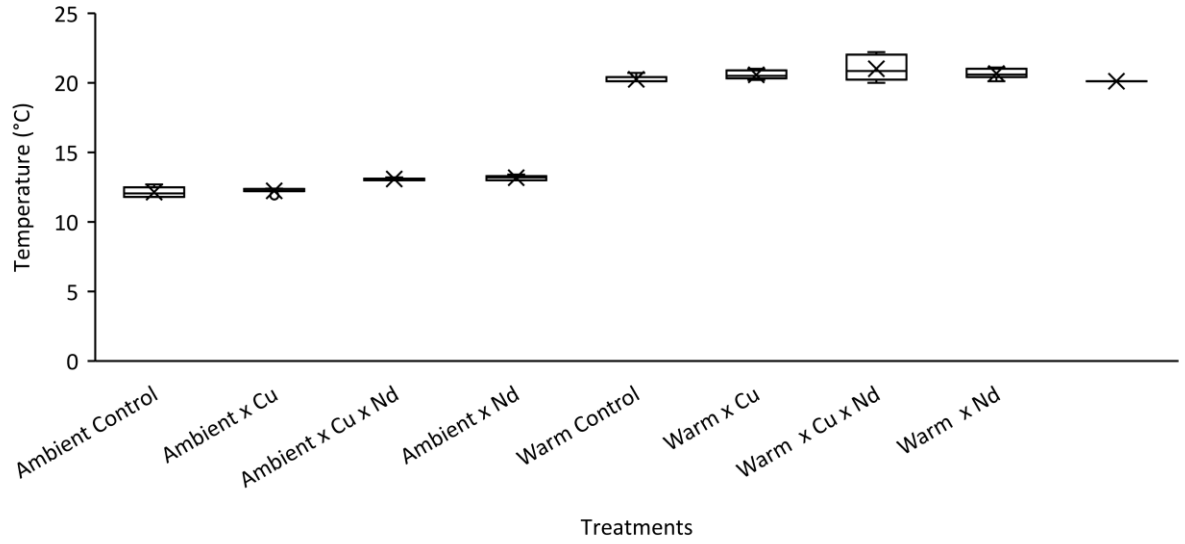


Figure A.4 Temperature (°C) across experimental treatments. There is a significant difference amongst all ambient and warm treatments

Table A.14 Non-significant results from linear regression between the given concentration of a trace element (ppm) and the average dry weight of an individual sand hopper (n=9). Data for trace elements Ba, Ce, Cs, La, Nd, Sm, Y and Yb was transformed to meet test assumptions.

Trace element	F-statistic	p-value	R square	Line Equation
As	0.88	0.3546	0.0238	y=-17.7898+0.0865*x*
Ba	0.0	0.9808	0.0	y=-0.7503+0.00008*x*
Ca	0.88	0.3534	0.0240	y=-64510-280.7376*x*
Cd	0.17	0.6799	0.0048	y=-7.4196+0.02267*x*
Ce	0.58	0.4494	0.0160	y=--0.3858+0.0022*x*
Co	1.33	0.2568	0.0356	y=-0.3064-0.002*x*
Cs	3.01	0.0911	0.0773	y=--1.8922+0.0035*x*
La	0.73	0.3977	0.0199	y=--0.4928+0.0024*x*
Mg	1.54	0.2232	0.0409	y=-8053.3613-67.4895*x*
Nd	0.0	0.9873	0.0	y=--0.6349+0.00006*x*
Rb	0.04	0.8482	0.0010	y=-2.9148+0.0017*x*
Sm	0.38	0.5423	0.0104	y=--1.7642+0.0019*x*
Sr	0.05	0.8253	0.0014	y=-1281.9724-1.4837*x*
Y	0.0	0.9800	0.0	y=--1.2358+0.00007*x*
Yb	1.27	0.2665	0.0342	y=--2.7583-0.0042*x*