

Sponge-Associated Amphipod Communities as Bioindicators for Pollution

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Abstract

Pollution negatively impacts organisms across all marine ecosystems. Coastal areas are particularly vulnerable to pollution due to their proximity to human settlements. Amphipods are commonly used as bioindicators to monitor pollution burdens, due to their high sensitivities and their ubiquity.

Pollution can reduce amphipod abundance, species richness, evenness and diversity. Community structure, proportionality of adults to juveniles and sex ratios may also be affected. Sponges often harbour high densities of amphipods, offering food, refuge and nurseries to their symbionts. Sponge-associated amphipods differ in their level of specialization on their host. This study provides first insights into the usefulness of sponge-associated amphipod communities as bioindicators. For this, it hypothesized that amphipod densities, species diversity, community structure, sex ratio and age proportionality will differ according to pollution levels.

To test this, sponges were collected from three sites with varying degrees of pollution in Wellington Harbour. The sponges were weighed, and their volume was measured. They were dissected and their amphipods were identified to species level, counted, measured (length) and their sex and life cycle stage (adult or juvenile) were recorded. From this data, amphipod densities, species richness, evenness and Shannon-Wiener diversity indices were calculated and compared among pollution levels. Community structure was also compared between sites and sponge species. Pollution level significantly affected species richness, evenness, diversity and community structure. The highest values for species richness, evenness and diversity were found in sponges from the least polluted. The lowest levels of these factors were found in sponges from the most polluted site. Sponges from the intermediate site generally harboured moderate richness, evenness and diversity compared to the other sites. Community composition was significantly affected by pollution, although effect sizes differed between sponge species. Higher pollution levels seemed to favour dominance of species that are better adapted to living in sponges. Generalists seemed to thrive in low to intermediate pollution levels. The majority of sex ratios measured had a female bias, which appeared to increase with increasing pollution although the difference was not statistically significant. The proportion of adults also showed a non-significant increase with pollution level. There was no significant difference in amphipod abundance per litre of sponge tissue between pollution levels, possibly because pollution levels may have been too low to cause a reduction in amphipod density.

These results show that sponge-associated amphipod communities are useful as bioindicators, as amphipod diversity, richness and evenness were significantly reduced by pollution and the sponge association allows for these community-scale comparisons to be made within an easily measurable framework. Species evenness in particular provided an accurate indication of different pollution levels.

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List of Abbreviations

ANOVA	Analysis of Variance
ANOSIM	Analysis of Similarities
DDTs	Dichlorodiphenyltrichloroethanes
H' diversity	Shannon-Wiener diversity
MPs	Microplastics
NPs	Nanoplastics
PBDEs	Polybrominated diphenyl ethers
PAHs	Polycyclic aromatic hydrocarbons
PCBs	Polychlorinated biphenyls
POPs	Persistent organic pollutants

Chapter I

Introduction

1.1 Introduction

Anthropogenic pollution is ubiquitous in marine ecosystems (Weber et al., 2006, 2012; Lirn et al., 2013; Dasgupta et al., 2015). The consequences of marine pollution are highly context-dependent, especially in coastal areas where terrestrial input can be both high and variable (Weber et al., 2006, 2012; Dasgupta et al., 2015). Adequate monitoring of all types of pollution therefore generally requires considerable effort and resources (Ansari et al., 2004; Weber et al., 2006, 2012; Dasgupta et al., 2015). Benthic organisms often steadily accumulate pollutants and may average out fluctuations in concentration over time. Such ‘bioindicator’ organisms can provide easily measurable estimates of time-averaged pollution levels and are therefore frequently used to survey pollution. Suitable bioindicator species are often highly abundant and sensitive to one or more pollutants (Souza et al., 2020; Collins and Fahring, 2020).

Amphipods are an extremely diverse order of malacostracan crustaceans, comprising around 10,000 species living in both aquatic and terrestrial environments (Hyne, 2011; Horton et al., 2013). They are highly influential components of their ecosystems, due to their high abundances and species diversity (Conlan, 1994; Amsler M. et al., 2009; Amsler C. et al., 2011). For example, herbivorous amphipod communities associated with macroalgal hosts along the Western Antarctic Peninsula act as ecosystem engineers, ensuring their host’s dominance by rapidly consuming filamentous algae and thus limiting the recruitment of their competitors (Amsler C. et al., 2011). The vast taxonomic diversity of amphipods is reflected in the variety of their ecological roles and feeding strategies including detritivory, omnivory, suspension feeding, necrophagy, herbivory and carnivory (Amsler M. et al., 2009). This ecological heterogeneity causes varying levels of sensitivity to different stressors in amphipods (Skilleter et al., 2005; Amsler M. et al., 2009; Reutgard and Furuhausen, 2016). Changes in amphipod community structure can give relatively precise accounts of environmental conditions, including pollution (Conlan, 1994; Hasenbein et al., 2018).

1.1.1 Factors structuring amphipod communities

Free living amphipod communities are usually organised by different levels of specialization, often caused by a variety of stressors, that progressively limit species diversity as they increase in dominance (Navarro-Barranco et al., 2013; Lacey et al., 2016). One critical factor structuring deep sea amphipod communities for example, seems to be species' sensitivity to hydrostatic pressure (Lacey et al., 2016). In oligotrophic environments, such as marine caves, carnivory tends to take priority over detritivory, while at great depths, necrophagy is a wide-spread feeding strategy (Horton et al., 2013; Navarro-Barranco et al., 2013; Duffy et al 2015; Lacey et al., 2016; Seefeldt et al., 2017). Under less extreme conditions amphipod communities are governed by a range of biotic and abiotic factors. For example, aside from pollution, estuarine amphipod assemblages are structured by suspended solids, organic carbon, dissolved oxygen, seasonality, food availability, salinity, pH and sediment texture (Navarro-Barranco et al., 2013; Lacey et al., 2016; Srinivas et al., 2019). In shallow soft-bottom environments, depth is the controlling influence (Carvalho et al., 2011). While coastal communities feature distinct configurations, they permit the survival of more generalist species, promoting higher species richness (Carvalho et al., 2011; Srinivas et al., 2019).

In contrast to free-living amphipod communities, those associated with hosts are frequently structured by the biology and habitat of their hosts and their own level of host-specificity (Poore et al., 2000; Zakhama-Sraieb et al., 2010). Amphipods most often associate with sessile hosts, such as algae or invertebrates like molluscs, cnidarians, ascidians and sponges (Thiel, 1999; Thomas and Klebba, 2007; Zakhama-Sraieb et al., 2010; Bueno et al., 2017; Martínez-Laiz et al., 2018).

Endosymbiotic amphipods often have adaptations to their host's morphology, facilitating life in the inner canals of their hosts, such as laterally compressed bodies and modified mouth parts and antennae, assisting mostly filtering or grazing feeding strategies. Interestingly, there is evidence that social organisation in these communities may have undergone adaptation, such as extended parental care (Thiel, 1999; Thomas and Klebba, 2007).

Certain amphipods associating with algae tend to be highly host-specific, such as the ectosymbiont *Caprella grandimana* (Mayer, 1882) whose preference for the alga *Jania rubens* (Linnaeus) J. V. Lamoutoux. 1816 likely originates from this species' thallus characteristics and interstitial spaces, which offer protection from predators (Martínez-Laiz et al., 2018). Others, like *Caprella takeuchii* Guerra-Garcia, Sánchez-Moyano and Garcia-Gomez, 2001 are less limited in their host choice. It is possible that the more generalist lifestyle of the latter species could allow for its advantage over a dominant competitor like *C. grandimana* during seasonal fluctuations in *J. rubens* abundance. Due to spatial limitations caused by the host's body size and abundance, such competition is common

among symbiotic amphipods and exerts another structuring influence on communities (Poore et al., 2000; Bueno et al. 2017; Martínez-Laiz et al., 2018). These periodical shifts can also be affected by other factors, including temporal differences in predation pressure which can cause variation in amphipod numbers, thus regulating competition intensity (Valério-Berardo and Flynn, 2002).

1.1.2 Amphipod-sponge associations

Amphipod associations with sponges are a well-documented phenomenon across latitudes, in fact, certain species of amphipods associate exclusively with sponges (Poore et al. 2000; Thiel, 2000). Sponges may harbour high densities of amphipods, for example over 500 amphipods per litre of sponge tissue were recorded by Amsler M. et al (2009). In this case most sponges contained 4-6 different species of amphipods without any apparent pattern of host specificity, although densities varied (Biernbaum, 1981; Abdo et al., 2007; Amsler M. et al., 2009).

The relationships amphipods form with sponges may be simple or complex, depending on the services the sponges provide. As habitat forming organisms, sponges offer physical and sometimes even chemical refuge to amphipods. The former is maintained by the sponges' three-dimensional complexity and relative rigidity, which can inhibit access of large predators to the amphipods. Chemical protection originates from the ability of certain sponge species to produce secondary metabolites to defend themselves against predatory fishes, an advantage that extends to their associated mesofauna (Skilleter et al., 2005; Huang et al., 2008; Aumack et al., 2016). These compounds may sometimes also deter spongivory by amphipods (Amsler M. et al., 2009). In contrast to species associated with algal or cnidarian hosts, suspension-feeding mesofauna can benefit from the sponges' feeding currents, which gather and concentrate organic particles from the surrounding water, providing the symbiont with a constant and relatively effortless source of food.

As many of these symbioses neither harm nor benefit the host sponge, they qualify as commensalisms. In contrast, some species of amphipods feed directly on live sponge tissue, although this seems to be relatively uncommon (Amsler M. et al., 2009).

Antarctic amphipod symbionts likely graze primarily on fouling diatoms growing on and in the sponge, rather than detritus in suspension (Amsler M. et al., 2009). Amphipod grazing is also potentially beneficial for sponges and could therefore present an instance of a mutualistic relationship between sponges and amphipods. Such a mutualism exists between certain Antarctic amphipods and their host macrophyte (Amsler C. et al., 2000; McClintock, 2005; Amsler M. et al., 2009; Aumack et al., 2016).

Nevertheless, the most common association of amphipods and sponges is a commensal provision of habitat by the sponge. Frequently, internal sponge communities are dominated by amphipods and polychaetes. Other groups include decapods, isopods, ophiuroids, gastropods and even fish species, all of which interact to form complex food webs (Amsler M. et al., 2009; Fiore and Jutte, 2010). The capacity of the sponge to sustain macrofauna is related to its growth form, and lobate species hold greater numbers than massive ones (Kourkouras et al., 1996; Neves and Omena, 2003). The more individuals a sponge harbours, the lower their species evenness and diversity (Fiore and Jutte, 2010). In some cases, host size, i.e. internal surface area of the sponge's canals correlates positively with amphipod abundance while in others, these factors are unrelated (Fiore and Jutte, 2010; Thomas and Klebba, 2007). Larger sponges may attract more commensals for several reasons: they have had more time to do so, they move larger quantities of water and thus, food, and they are easier for commensal organisms to find (Thomas and Klebba, 2007). Chemicals produced by the sponge may also play a role in host selection, especially in areas of high predation on sponges by echinoderms, such as Antarctica (McClintock, 2005; Thomas and Klebba, 2007).

Amphipod communities associating with sponges could make potentially very useful bioindicators due to the large volumes of water they come in contact within the sponge's feeding currents (Orani et al., 2018). Sponges also accumulate and retain certain types of pollution, enhancing exposure of their symbionts (Orani et al., 2018; Modica et al., 2020).

Few studies have investigated the use of host-associated species as bioindicators, though their results have been promising (Ford et al., 2004, 2006, 2007; Tian et al., 2014; Cabral et al., 2018). For instance, persistent organic pollutants (POP) reduce overall helminth parasite abundance in their host fish and changes their community composition, whereby certain helminth species are more affected than others (Vidal-Martínez et al., 2003; Pech et al., 2009; Shah et al., 2012). Limited mobility is a desirable feature for bioindicators. Thus, associations with fish are unlikely to give geographically precise accounts of environmental conditions. Amphipods and their sessile hosts may be more appropriate (de-la-Ossa-Carretero et al., 2018). A sponge-amphipod association might present an ideal system to test the utility of a localized, community-scaled, host-associated bioindicator. No literature using sponge-associated amphipod communities as bioindicators was found.

1.1.3 Coastal pollution

Since the beginning of the industrial revolution, human-driven pollution has been a problem for marine ecosystems, even in the most remote and inaccessible places (Thompson et al., 2007; Aronson et al., 2011; Polmear et al., 2015; Chiba et al. 2018). The intensity and effects of pollution on ecosystems depend on the proximity to and density of human settlements. Therefore, the worst effects of pollution occur in coastal areas, where human settlements and marine life frequently intersect and where the impacts of shipping and recreational boating are the most concentrated (Lirn et al., 2013; Bebbiano et al., 2015; Smith et al., 2016; Fowles et al., 2018). Contamination of coastal marine ecosystems can occur either through run-off from the surrounding land, or through shipping or boating-related activities. The most significant actions for sustainable harbour management are prevention and control of fuel and liquid cargo spills, management of solid waste, active preservation of coastal ecosystems and sewage treatment (Lirn et al., 2013). Marinas are sources of a variety of pollutants that harm marine organisms (Fowles et al., 2018). The types of land-based pollution experienced by harbour organisms are diverse and often vary geographically (Lirn et al., 2013; Bebbiano et al., 2015; Smith et al., 2016; Fowles et al., 2018).

1.1.3.1 Heavy metal pollution

Heavy metals occur naturally in marine sediments and have been increased by human activity such as industry, mining and household maintenance (Ansari et al., 2004; Rădulescu et al., 2019). Elements like zinc, cadmium, mercury, lead, iron, arsenic, barium, copper and manganese may accumulate in the tissues of animals (Ansari et al., 2004). Some of these, e.g. copper and zinc are especially problematic in harbours due to inputs via anti-fouling technology used in shipping (Paradas and Amado-Filho, 2007; Carić et al., 2016). Several heavy metals are important micronutrients that organisms must consume in small quantities and which only become harmful beyond a certain concentration threshold. Others have exclusively harmful effects that develop at high levels or through extended periods of exposure (Ansari et al., 2004).

In some organisms, heavy metals can accumulate in tissues faster than they can be excreted, and potentially transferred to higher trophic levels in the food web. This process is known as biomagnification and can lead to high contamination levels in top predators, which in turn are frequently consumed by humans (Escobar-Sánchez et al., 2011; Mouneyrac et al., 2014; Kothari et al., 2015). A variety of factors may affect heavy metal mobility, bioavailability and toxicity. They include organic matter, sediment texture, geology, acid-volatile sulphides, salinity, pH, season,

temperature, solubility, chemical speciation and even animal behaviour (Vasconcelos et al., 1997; Ansari et al., 2004; Copat et al., 2011).

Amphipods are among the most sensitive marine species to heavy metal toxicity (Kunz et al., 2009; Geffard et al., 2010; Arambourou et al., 2017). Unlike most larger crustaceans, they are unable to remove heavy metal contamination through moulting (Bergey and Weis, 2007; Kunz et al., 2009; Geffard et al., 2010; Arambourou et al., 2017). Feeding efficiency, reproductive and developmental processes and endocrine functioning are affected by heavy metal pollution, resulting in reduced amphipod presence when compared to unpolluted areas (Kunz et al., 2009; Reutgard and Furuhausen, 2016; Martinez-Haro et al., 2016; Jelassi et al., 2019). For example, after exposure to polluted estuaries, *Echinogammarus marinus* Leach, 1815 displayed a reduced feeding rate, lowering its overall fitness (Jelassi et al., 2019).

1.1.3.2 Persistent organic pollution

The damage caused by persistent organic pollutants (POPs) hinges on their longevity, their potential for bioaccumulation and biomagnification, and their lipophilic nature. They include a range of compounds such as PCBs (polychlorinated biphenyls), polybrominated diphenyl ethers (PBDE) and organochlorine pesticides like Mirex, chlordanes, dichlorodiphenyltrichloroethanes (DDTs) and many more (Chukmasov et al., 2019; Kalsoom and Nasreen, 2019). Burdens are typically high near industrialized sites (Jamieson et al., 2017). When consumed, POPs can impact the endocrine, reproductive, and immune system, causing neurological and developmental damage and may act as carcinogens (Kalsoom and Nasreen, 2019). The amphipod *Monoporeia affinis* (Lindström, 1855) has long been used as a bioindicator for PCB contamination, which causes embryonic deformities in this species (Löf et al., 2016). With the growing awareness of the dangers of POPs, most of them have been restricted or abolished in many countries. However, many of these compounds persist in sediments and enter the marine environment via terrestrial and atmospheric reservoirs (Morrissey et al., 2005; Lin et al., 2012; Cabrerizo et al., 2019; Chukmasov et al., 2019; Kalsoom and Nasreen, 2019; Cheney et al., 2019).

1.1.3.3 Oil pollution

Polycyclic aromatic hydrocarbons (PAHs) are POPs but are often treated separately. These compounds are produced during the incomplete combustion of materials like coal, wood and fuels and are present in crude oil (Net et al., 2015). Unlike other POPs they are not internationally restricted and inputs into the marine environment are comparatively common. PAHs may be introduced to the oceans through atmospheric deposition, terrestrial run-off, natural oil seepages or spills (Moroni et al., 2019). Small-scale spills of fuel and oil occur frequently in commercial and recreational shipping and boating, especially in harbours (Lirn et al., 2013). Even such small-scale contamination can have profound and lasting consequences for local biodiversity. For example, sediments contaminated with different types of lubricant oils were placed on the sea floor in O'Brien Bay in Antarctica. Their chemical fate and infaunal recruitment at the sites were monitored over a year. There were significant differences in community structure, even for the type of lubricant marketed as "biodegradable" (Thompson et al., 2007). This may be because oil also contains other toxins such as alkynaphthalenes, phenols and diphenylamine, which increase mortality across biotic assemblages (Thompson et al., 2007). Amphipods are highly sensitive to oil contamination, which significantly reduces their abundance and recruitment through mortality, decreased reproduction and avoidance, immediately and long-term (Thompson et al., 2007; Szczybelski et al. 2018).

1.1.3.4 Plastic pollution

Harm caused by heavy metals, PAHs and other POPs can be inflated by the presence of plastic particles that may act as a vector (Qui et al., 2015; Akhbarizadeh et al., 2017; Ferreira et al., 2019; Yazdani-Foshtomi et al., 2019). This was tested in the amphipod *Allorchestes compressa* Dana, 1852, which accumulated PBDEs associated with microplastics (Chua et al., 2014). Microplastics (MPs, <5mm) and Nanoplastics (NPs, <100 nm) are now unfortunately ubiquitous in marine environments. (Naji et al., 2017; Hahladakis et al., 2018). Although dumping of plastic waste into the ocean has been abolished, it continues to enter marine systems through mismanagement i.e. littering or ineffectively contained landfills (Jambeck et al., 2015).

Composition and concentration of MPs in sediments and the water-column correspond with the degree of urbanization and land-use (Yazdani-Foshtomi et al., 2019; Qiu, et al., 2015; Jang et al., 2020). Aquaculture and fisheries are important sources of plastic pollution in the ocean. Lost equipment can lead to entanglement of marine organisms, resulting in drowning and starvation (Parton, et al, 2019; Jang et al., 2020; Farias et al., 2018, Anderson and Alford, 2014). Plastic waste

can smother filter-feeding organisms and can be mistaken for food. This can lead to suffocation and starvation if the particles are too large to be excreted (Murray and Cowie, 2011, Watts et al., 2015). MP ingestion can also lower the overall energy budget of organisms. Freshwater and terrestrial amphipods show reduced food consumption, assimilation efficiency and growth when ingesting MP fibres (Au et al., 2015; Straub et al., 2017). Studies have shown that food consumption in one terrestrial amphipod was lowered with increasing MP concentration whereas another species seemed to accumulate MPs in their tissue, leading to decreased jump height, increased weight and higher mortality over time (Tosetto et al., 2016; Carrasco et al., 2019). Negative effects of MP ingestion have not been extensively studied in marine amphipods. Due to the physiological similarity to their freshwater and terrestrial counterparts and established consumption of plastic MPs, they are nevertheless likely to exist (Jamieson et al., 2019).

1.1.3.5 Eutrophication

A common form of pollution in harbours is nitrogen and phosphorous from agricultural and urban runoff (Rădulescu et al., 2019; Taylor et al., 2019). Nutrient enrichment can have an array of negative consequences as it can stimulate algal blooms, and subsequent bacterial decomposition on the ocean floor can consume oxygen. The results are dead-zones that can cover large areas (Taylor et al., 2019). Other sources of nutrients include ship-based food waste and untreated sewage. The severity of eutrophication depends, among other factors, on local wastewater processing, topography and currents (Rabalaias et al., 2002; Malagó et al., 2019; Rădulescu et al., 2019; Taylor et al., 2019; Raudsepp et al. 2019; Virtanen et al., 2019; Vaneckhaute and Fazli, 2020). While anaerobic metabolic pathways make some amphipod species more resilient to hypoxia, they cannot be sustained over longer periods and anoxic events eventually lead to amphipod decline due to mortality and avoidance (De Robertis, et al 2001; Spicer et al., 2002; Šidagytė and Arbačiauskas, 2016; Coffin et al., 2017; 2018).

1.1.3.6 Sedimentation

Solid particles in urban run-off or natural water ways can restrict light availability for photosynthesis through increased turbidity. Sediments may also smother sessile, filter-feeding organisms, e.g. sponges and corals (Churchill and Wood-Jones, 1991; Weber et al, 2006, 2012; Bartley et al., 2014; Pineda et al., 2017). Models suggest that agricultural practises are the primary source of sedimentation (Bartley et al., 2014). Natural processes include aerial dust transport, coastal erosion, and particle transport by glaciers, which are intensified by anthropogenic climate change and the resulting sea level rise (Bartley et al., 2014; Achete et al., 2017;). An indirect harmful effect of sedimentation is the necessity of regular dredging of harbours. Not only does this cause extensive physical disturbance to marine organisms, it increases turbidity further (Pollock et al., 2014, 2016; Sharaan et al, 2018). Turbidity reduces the diversity of algae-associated amphipods, while not affecting host abundance (Ali et al., 2018). Amphipod abundance and species richness and composition are also affected at sites with heightened turbidity (Ali et al., 2018). Sensitivity to turbidity varies among amphipods, often resulting in changes in community structure (Rigolet et al., 2011; Zaabar et al., 2015; Ali et al., 2018).

1.1.4 Study Site

Wellington Harbour is a typical coastal environment featuring all the usual forms of pollution in different, easily accessible locations. Thanks to circular current patterns within the harbour, and a busy international port there are varying pollution burdens (Brodie, 1958; Oliver and Conwell, 2017). Several of these sites have been carefully monitored for pollution loadings for 30 years (Stephenson et al., 2008; Oliver and Conwell, 2014; Elliot, 2016; Oliver and Conwell, 2017). Wellington Harbour was therefore chosen as the study site for this research.

1.2 Thesis purpose and structure

This thesis hypothesizes that amphipod communities associating with sponges will differ significantly between pristine environments and polluted ones. In particular, it predicts that:

1. Sites with high shipping traffic and proximity to highly urbanized and industrialized areas in Wellington Harbour will have reduced overall amphipod density and diversity.
2. High levels of anthropogenic pollution will change species composition and facilitate dominance by more tolerant species.
3. Adult:juvenile and female:male ratios of amphipods will be influenced by pollution.

Chapter 2 gives account of the amphipod communities extracted from sponges collected from three sites with differing pollution burdens in Wellington Harbour. It discusses the effects of pollution on amphipod density, species richness, evenness, Shannon-Wiener (H') diversity and community composition. It elaborates on sex ratio and age structure trends observed within these communities.

Chapter 3 provides a synthesis of the findings, gives recommendations for future research and concluding comments.

Chapter II

Sponge-associated amphipod communities as bioindicators

2.1 Introduction

Sponges often harbour complex food webs that tend to be dominated by polychaetes and amphipods (Poore et al. 2000; Thiel, 2000; Amsler M. et al., 2009; Fiore and Jutte, 2010). Amphipods are a very diverse order of malacostracan crustaceans, and some species have adapted to endosymbiosis with invertebrate hosts to varying degrees (Poore et al. 2000; Amsler M. et al., 2009; Fiore and Jutte 2010).

Sponges can provide amphipods with refuge by limiting predator access through physical and sometimes chemical barriers. Some sponges produce chemicals that repel certain predators of amphipods, although they can also discourage spongivory by the amphipods themselves (Riberio et al., 2003; Skilleter et al., 2005; Huang et al., 2008; Amsler M. et al., 2009). Food particles may be concentrated by the sponges feeding currents, benefitting filter-feeding amphipods, while grazing amphipods consume bio-fouling organisms growing in and on the sponges (Koukouras et al., 1996; Amsler C. et al., 2000; Riberio et al., 2003; Skilleter et al., 2005; Huang et al., 2008; Amsler M. et al., 2009). High proportions of juveniles and breeding females could indicate that some amphipods use sponges as breeding grounds or nurseries (Riberio et al., 2003).

Typical sponge-associated amphipod communities feature both generalists and specialist species (Koukouras et al., 1996; Amsler M. et al., 2009). Sponge morphology plays an important role in driving amphipod abundance. Lobate sponges tend to harbour the greatest density of amphipods (Neves and Omena, 2003; Amsler M. et al., 2009). Morphological features of sponges may result in adaptations in amphipods, such as filtering or grazing feeding strategies and small, compressed bodies to facilitate endosymbiosis (Thiel, 1999; Thomas and Klebba, 2007). Internal sponge morphology can also predict species density and diversity (Kourkouras et al., 1996; Fiore and Jutte, 2010). In some cases, amphipod density increases with sponge size, likely due to host accessibility, visibility and longevity (Thomas and Klebba, 2007; Fiore and Jutte, 2010). Some evidence supports adaptation in amphipod social organisation and reproductive behaviour (Thiel, 1999; Thomas and Klebba, 2007).

Amphipods are sensitive to most common forms of pollution (Thompson et al., 2007; Tosetto et al., 2016, Szczybelski et al. 2018, Coffin et al., 2018). Whether contamination leads to mortality,

sublethal effects or avoidance behaviour often depends on the longevity of the pollution source, the concentration of pollutants and their ecotoxicity (Thompson et al., 2007; Tosetto et al., 2016; Ali et al., 2018, Findlay et al., 2018, Szczybelski et al. 2018). This impact is influenced by a range of environmental conditions, including sediment texture, pH, salinity organic matter and animal behaviour as well as the type and composition of the pollutant (Thompson et al., 2007; Hastie et al., 2017; Dasgupta et al., 2017; Findlay et al., 2018; Kastelein et al., 2019).

Amphipods are most commonly used as bioindicators for heavy metals and persistent organic pollutants (POPs; Kunz et al., 2009; Geffard et al., 2010; Löf et al., 2016; Arambourou et al., 2017). Amphipod sensitivity to polycyclic aromatic hydrocarbons (PAHs) and oils is similarly well recorded (Thompson et al., 2007; Szczybelski et al. 2018). Increased turbidity has variable effects on amphipod community structure (Rigolet et al., 2011; Ali et al., 2018). Although crustaceans are relatively resilient to eutrophication-induced anoxia, they are vulnerable if exposure is prolonged (De Robertis, et al 2001; Spicer et al., 2002; Šidagytė and Arbačiauskas, 2016; Coffin et al., 2017; 2018).

Community structure can also be altered as herbivorous species benefit from intermediate levels of eutrophication due to increased primary production (Kraufvelin, 2007). Other pollutants also have the potential to affect amphipod abundance and community structure (Fox et al., 2014; Tosetto et al., 2016; Carrasco et al., 2019; Jamieson et al., 2019; Garrat et al., 2019). Both sex ratio and age distribution can be affected by pollution (Correia et al., 2003; Ford et al., 2004; 2006; 2007; Löf et al., 2016; Peschke et al., 2019). These factors add to the appeal of sponge-associated amphipods as bioindicators as pollution impacts may be seen on an intraspecific level. Not only are they exposed to large quantities of sea water, they have limited geographic range due to their sessile host, are easily collected and offer geographically precise insights into environmental conditions. These can help in pinpointing potential sources of pollution (Orani et al., 2018).

Three sites in the Wellington Harbour (Aotea Quay, Burnham Wharf and Point Halswell) were chosen to test the hypothesis that high pollution loadings will affect amphipod density, diversity, community structure, sex ratio and age structure.

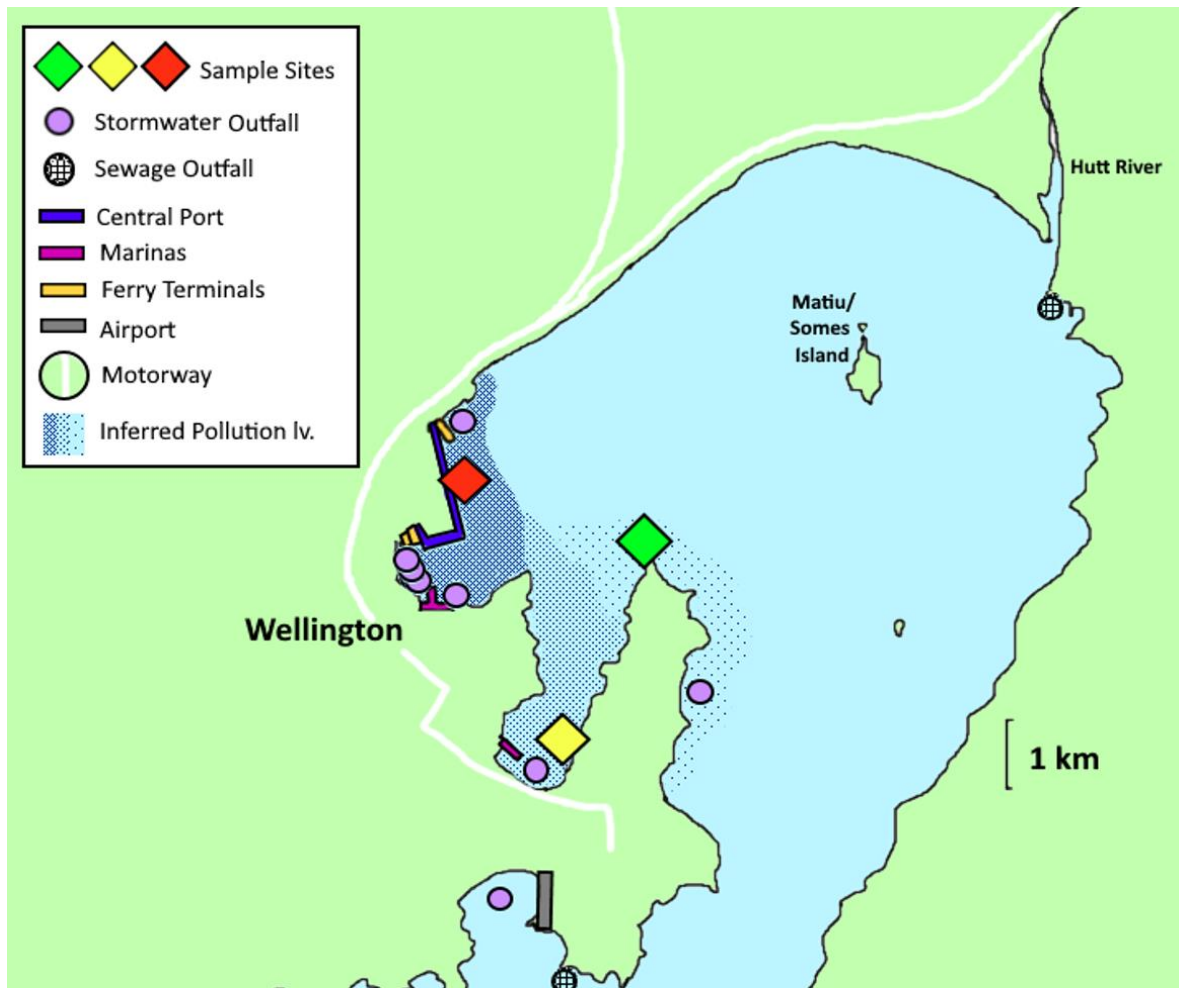


Figure 2.1. Wellington Harbour sampling sites. Point Halswell (green diamond, least polluted), Burnham Wharf (yellow diamond, more polluted), Aotea Quay (red diamond, most polluted), likely sources of pollution and inferred pollution levels are illustrated (5% shading=least polluted, 25%=intermediate, 30%=most polluted).

Aotea Quay is positioned between the two main ports for ship and ferry traffic and accommodates over 3000 arrivals annually (Fig. 2.1; Hughes et al., 2018). Cargo ships, tankers and cruises frequently berth at Aotea Quay (Hughes et al., 2018). It contains the harbour's highest concentration of stormwater outfalls, the motorway, large industrial sites and a stadium. Due to the high frequency of shipping activity, outfalls and proximity to human industry and dense settlement at Aotea Quay it was treated as the most polluted site for the purpose of this research.

Burnham Wharf is one of three ports in the Wellington Harbour capable of receiving tanker cargo (Fig. 2.1). The wharf is visited by more than 20 tankers annually. It lies near a popular recreational marina and a stormwater outfall. Particle transport into the bay is likely due to frequent strong winds (Hughes et al., 2018). Both Aotea Quay and Burnham Wharf have high levels of legacy contamination of copper, mercury, lead, PAHs and DDT in their sediments (Oliver and Conwell,

2017). Due to the much higher frequency of ship traffic, mooring and land-based human activity in Aotea Quay it was considered the most polluted site in this study. At both Aotea Quay and Burnham Wharf sponges were collected directly from the wharf piles and not from the sediment. Point Halswell lies on a peninsula containing a park and no residential or commercial buildings or landing sites for vessels. Samples were collected from a tall, submarine outcrop to mimic the vertical distribution of the wharf piles in a natural setting. As this site receives strong currents of new seawater from Cook Strait during the incoming tide and is positioned further toward the center of the harbour, it was considered the least polluted.

2.2 Methods

2.2.1 Sampling

Sponges of different morphologies were collected by NIWA divers from three sites in Wellington Harbour, New Zealand, which were chosen with respect to their inferred pollution levels (Fig. 2.1 and Table 2.1). Based on the Greater Wellington Regional Council's Coastal Water Quality and Ecology monitoring programme annual data report 2016/17, sediments at Aotea Quay and Burnham Wharf can be considered highly polluted. Point Halswell was not monitored in the programme. Burnham Wharf was expected to have intermediate pollution burdens as it is subject to relatively few potential sources of pollution. Air and water temperatures were recorded (using online data and an on-board Lowrance plotter¹, respectively) and a water sample was taken separately to measure pH in the laboratory (SevenCompact pH/Ion meter¹). The depth at which sponge samples were collected was recorded using a Shearwater Perdix¹ depth gauge. At all sites, samples were immediately chilled, transported to the laboratory, and frozen. Sponges were identified using a photographic guide (Kelly, 2018).

Table 2.1: Times and conditions of sites and samples collected in Wellington Harbour

Site	Point Halswell	Burnham Wharf	Aotea Quay
Lat Long	41°17'02.4"S 174°49'31.5"E	41°18'47.1"S 174°48'39.2"E	41°16'04.6"S 174°47'13.1"E
Date (Time)	19/06/2019 (12:25 pm)	17/06/2019 (11:55 am)	26/08/2019 (11:35 am)
Tide	Low	Low / rising	Low / rising
Sponge substrate	Rock	Wharf piles	Wharf piles
Water Temp	12.5 °C	11 °C	14 °C
Air Temp	7 °C	12 °C	11 °C
Sponges (n, Depth)	<i>Crella</i> sp. (n=8, 7m) <i>Crella</i> sp. (n=8, 9.2m) Unid. sp. 2, white (n=8, 8.6m)	<i>Crella</i> sp. (n=8, 2 – 4m) <i>Callyspongia ramosa</i> (n=8, 5 – 7m) Unid. sp. 1, purple (n=8, 3 – 5m)	<i>Crella</i> sp. (n=6, 4m) <i>Callyspongia ramosa</i> (n=6, 6 – 7.5m)
pH	8.14	8.08	7.81

¹ <https://www.metservice.com/towns-cities/locations/wellington>; Manufacturing details were inaccessible due to COVID-19 lockdown

2.2.2 Sponge processing

Four individuals of each sponge were randomly chosen from the individuals collected at each sampling station, thawed and photographed. Large sponges were divided into vertical subsample sections of roughly equal size (25-50g), which were morphologically representative of the rest of the sponge. This was done to save time and to standardize sizes. Small sponges were treated the same as these subsamples (Tables 2.2-2.4).

Table 2.2: Sponges collected at Burnham Wharf

Species	Individual #	Mass (g)	Volume (ml)	Number of sub-samples cut	Number of sub-samples used
<i>Crella</i> sp.	1	304.4	287	10	2
<i>Crella</i> sp.	2	155.8	160	5	3
<i>Crella</i> sp.	3	105.2	118	6	2
<i>Crella</i> sp.	4	235.4	232	8	2
<i>Callyspongia ramosa</i>	1	313.3	323	6	2
<i>Callyspongia ramosa</i>	2	597.6	600	3	2
<i>Callyspongia ramosa</i>	3	305.6	315	4	2
<i>Callyspongia ramosa</i>	4	747.3	745	3	2
Unidentified sp. 1	1	55.8	65	2	2
Unidentified sp. 1	2	23.3	25	1	1
Unidentified sp. 1	3	64.1	70	2	2
Unidentified sp. 1	4	43.1	45	1	1

Table 2.3: Sponges collected at Point Halswell

Species	Individual #	Mass (g)	Volume (ml)	Number of sub-samples cut	Number of sub-samples used
<i>Crella sp.</i>	1	12.3	15	1	1
<i>Crella sp.</i>	2	15.3	15	1	1
<i>Crella sp.</i>	3	61.1	60	1	1
<i>Crella sp.</i>	4	17.9	17	1	1
Unidentified sp. 2	1	51.9	30	2	2
Unidentified sp. 2	2	63.6	65	1	1
Unidentified sp. 2	3	145.5	150	1	1
Unidentified sp. 2	4	159.3	155	1	1

Table 2.4: Sponges collected at Aotea Quay

Species	Individual #	Mass (g)	Volume (ml)	Number of sub-samples cut	Number of sub-samples used
<i>Crella sp.</i>	1	60.9	60	2	2
<i>Crella sp.</i>	2	98.7	105	3	2
<i>Crella sp.</i>	3	50.8	50	1	1
<i>Crella sp.</i>	4	37.3	40	1	1
<i>Callyspongia ramosa</i>	1	281.1	290	6	2
<i>Callyspongia ramosa</i>	2	65.9	80	3	2
<i>Callyspongia ramosa</i>	3	238.5	320	4	2
<i>Callyspongia ramosa</i>	4	47.7	45	1	1

Each subsample was drained for 30 seconds before being weighed. The volume of the subsample or small sponge was measured by recording water displacement in a 500mL measuring cylinder with increments of 5mL, and then frozen. The volume of water collected with the sponges was also measured and frozen in separate bags.

The sponges were later thawed, and two to three subsamples were randomly chosen for each individual sponge. Each subsample was further divided into smaller portions, about 3-5cm in diameter. Using forceps, each of these was separated into small fragments (ca. 125mm³) and transferred into 50mL plastic containers, to which 45mL distilled water were added. The covered containers were shaken for 10 seconds before the water was poured through a 5mm plastic mesh into a 100µm mesh net. The sponge tissue was then spread out and pressure-rinsed on the mesh with distilled water. Shaking and rinsing was repeated 5 times for all portions, before the sponge tissue was discarded. The contents of the net were then transferred into black plastic weigh boats and observed under a dissecting microscope (OLYMPUS SZX7, Tokyo, Japan). The water collected with the sponges was defrosted, passed through the net and viewed separately.

Amphipods found in sponges and water were grouped by their morphological features and preserved in ethanol. Amphipod body length was measured from the end of the rostrum to the tip of the telson using a graticule. Their sex was determined by the development of sexual organs, as well as obvious sexually dimorphic characteristics, e.g. enlarged or setose gnathopods 2 in *Colomastix* spp or *Podocerus cristatus* Thomson, 1879 respectively. If these were absent and the individual's length was sufficiently small, it was recorded as a juvenile. Broken amphipods were counted if they could be identified, otherwise they were excluded from all analyses.

2.2.3 Analysis

Species richness and evenness were assessed using standard formulae (Krebs, 1999). Two replicates (AM6 and PE4) were excluded in calculating evenness because they only contained a single amphipod species. To adjust for differing sponge size, amphipod counts were expressed as “amphipods per litre of sponge tissue”. Shannon-Wiener (H') diversity indices were calculated for amphipod communities from each site for all sponges and for the cumulative H' diversity at each site. There were three sponge species collected from Burnham Wharf and only two from the other sites, therefore three sets of two sponge species from Burnham Wharf were compared to the other sites. Shannon-Wiener indices were calculated in Excel using the formula:

$$H' = - \sum_{i=1}^s p_i \ln p_i$$

Here, s is the number of species, p_i describes the ratio of individuals per L of sponge tissue from species i divided by the total frequency of all individuals per L of sponge tissue from all species. The H' diversity index usually ranges between 1.5 and 3.5 (Gaines et al., 1999). All further analyses were calculated using RStudio. Bray Curtis dissimilarity between communities from different sites was calculated for all sponges, for different combinations of sponges from Burnham Wharf, for each sponge species (*Crella sp.* and *Callyspongia ramosa*) and for their pooled amphipod communities using the formula (Ricotta and Podani, 2017):

$$BC_{UV} = \frac{\sum_{j=1}^S |x_{Uj} - x_{Vj}|}{\sum_{j=1}^S (x_{Uj} + x_{Vj})}$$

Where x_{Uj} and x_{Vj} are amphipod abundances per litre of sponge tissue of species j in sponges from sites U and V, respectively. S is the total number of species recorded at the two sites.

2.2.3.1 Statistical analyses

The significance of differences in species richness and evenness between sites was assessed using two Analyses of Variance (ANOVA). Amphipod density was compared between sites for all sponges and *Crella sp.* only, using linear regressions. Effect of adult amphipod length on host choice was tested using linear regression. Five Analyses of Similarities (ANOSIM) were calculated using the VEGAN package in R to compare species compositions between sites for all sponges, different combinations of species from Burnham Wharf and *Crella sp.* only at all sites (Dixon, 2013). Another two ANOSIMs compared community structure between sponge species (*Crella sp.* and *Callyspongia ramosa*) at Aotea Quay and Burnham Wharf. Linear regressions were made comparing female:male ratio and juvenile:adult ratio between sites.

2.3 Results

2.3.1 Sites

At Aotea Quay and Burnham Wharf sponges were collected from wharf piles (Fig 2.2) while at Point Halswell the samples came from a natural outcrop (Fig. 2.3). Most sponges were not collected in their entirety and instead, representative pieces were taken.

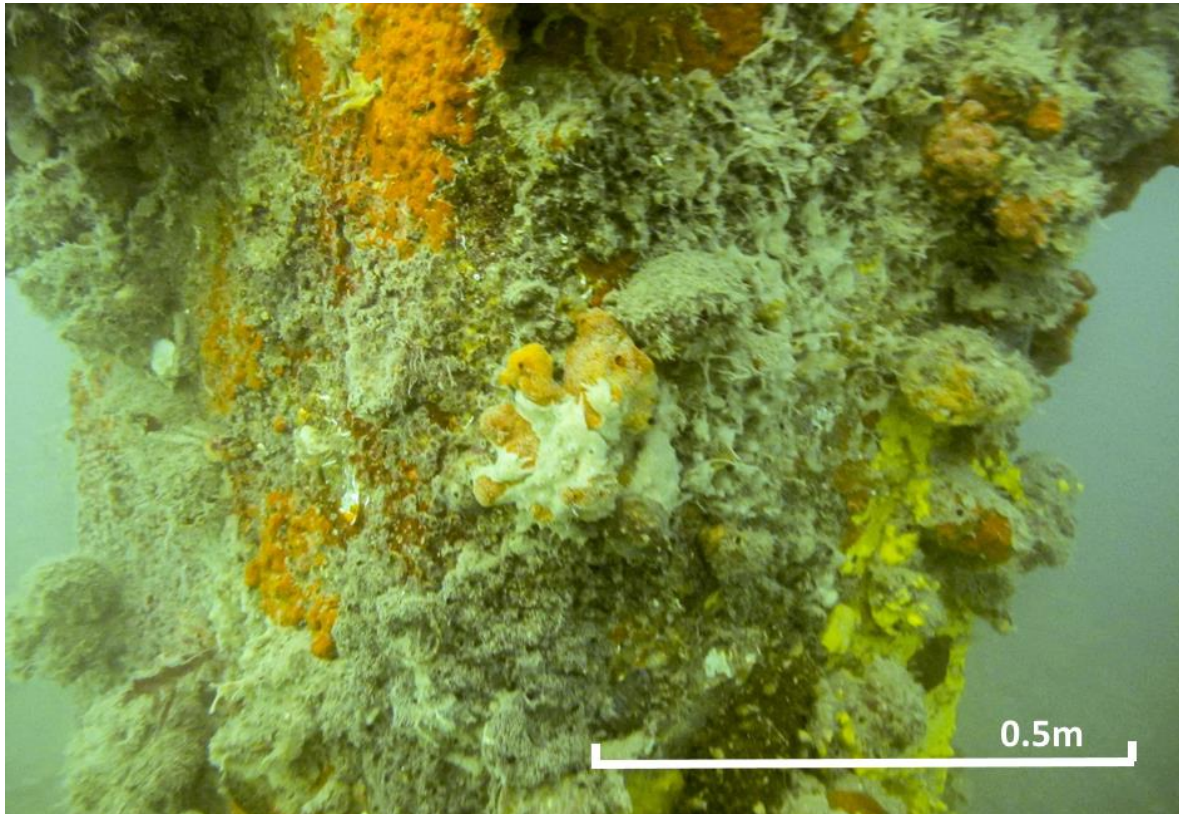


Figure 2.2. *Crella sp.* sponges growing on wharf piles at Burnham Wharf. Photograph: Serena Cox

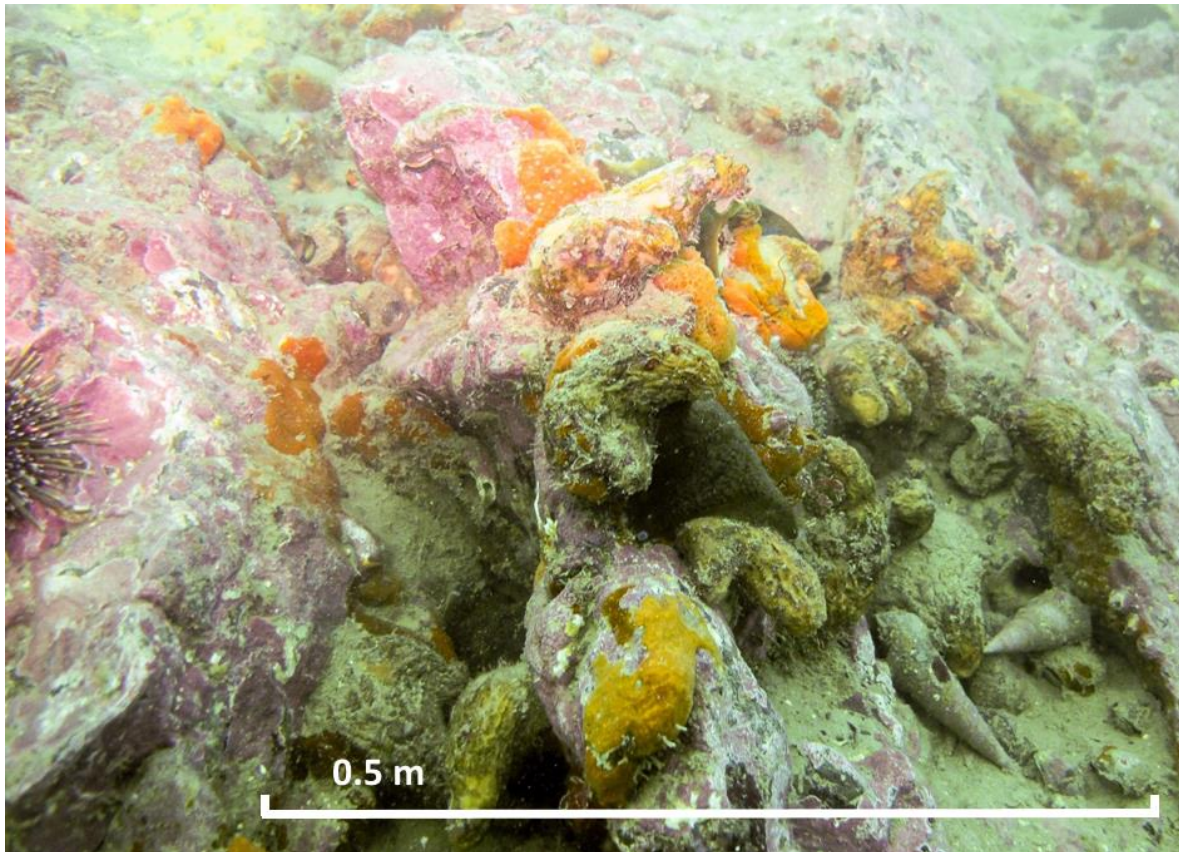
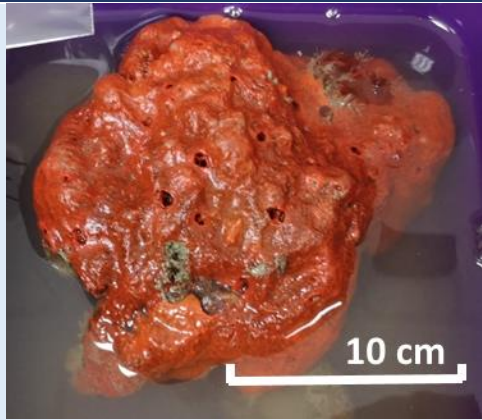

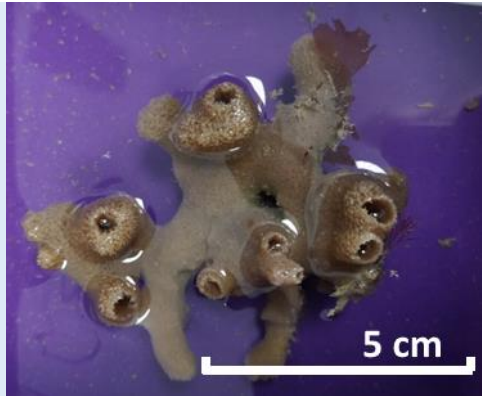



Figure 2.3. *Crella* sp. sponges growing on outcrop at Point Halswell. Photograph: Serena Cox

2.3.2 Sponge species

Four species of sponges were collected from Wellington Harbour (Table 2.5). Two species were collected at Aotea Quay and Point Halswell, and three from Burnham Wharf. *Crella* sp. was found at all three sites, *Callyspongia ramosa* at Aotea Quay and Burnham Wharf and unidentified species 1 and 2 were found at Burnham wharf and Point Halswell, respectively. Specimen volume was highly variable and for large individuals, subsamples were therefore dissected, and findings standardized to dissected volume. *Callyspongia ramosa* samples from Burnham Wharf were particularly large, possibly because the individuals growing there were especially tall, and divers aimed to sample entire lengths of sponge to represent the whole sponge (Fig. 2.4). One *Crella* sp. sample was excluded from analysis as it was attached to a large piece of substrate (139.8g) and yielded highly unusual results.

Table 2.5. Sponge species collected from Wellington Harbour

Picture	Species and Sites	Growthform	Description
	<i>Crella sp.</i> Aotea Quay Burnham Wharf Point Halswell	Encrusting to massive	<ul style="list-style-type: none"> • Highly variable size • Soft, fibrous • Small to medium oscula • colour 'seeps out', rinsed fibers are yellow
	<i>Callyspongia ramosa</i> Aotea Quay Burnham Wharf	Tall branching	<ul style="list-style-type: none"> • Large specimens • Very tough • Small, regular oscula • Light tan tissue throughout • Produces sticky, purple mucous
	Unidentified sp. 1 Burnham Wharf	Short branching	<ul style="list-style-type: none"> • Large oscula • Soft • Grey-ish purple colour
	Unidentified sp. 2 Point Halswell	Massive	<ul style="list-style-type: none"> • Large oscula • Very soft • Tan inner tissue • White 'crust' of spicules

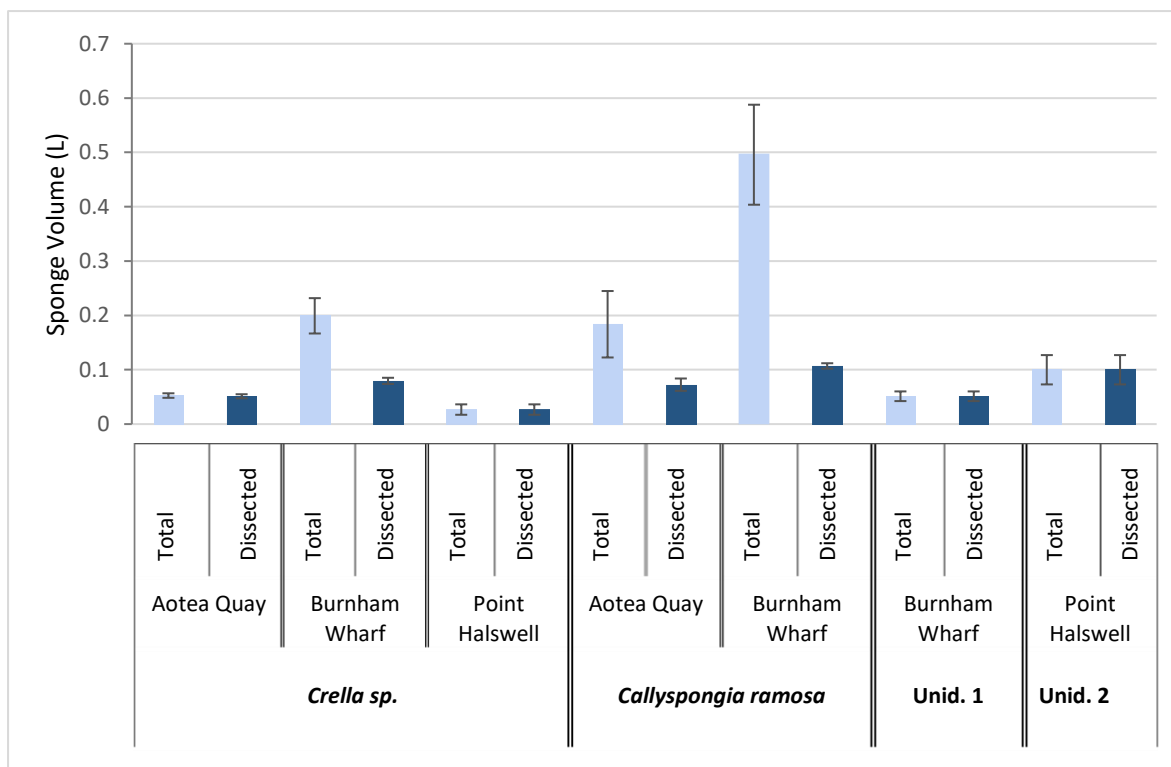


Figure 2.4. Mean total (light blue) and dissected (dark blue) sponge volume of all specimens collected from different sites. Error bars show standard error.

2.3.3 Amphipod density

A total of 2,142 individuals belonging to 13 different amphipod species were found, averaging 1044.4 amphipods per litre of sponge tissue regardless of sponge species. The highest amphipod density was found in sponges from Burnham Wharf, the lowest at Point Halswell (Fig. 2.5) A regression for the whole dataset found no significant difference in amphipod density between sites ($p=0.2$; $R^2 = 0.03$). There was no significant difference between sites for amphipod density in *Crella sp.* sponges only ($p=0.39$, $R^2=0.01$).

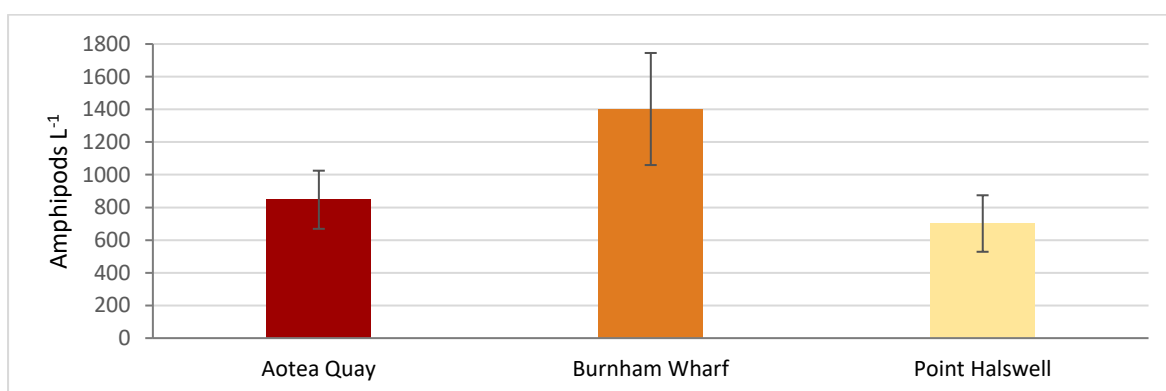


Figure 2.5. Mean amphipods L⁻¹ of sponge tissue between sites. Error bars show standard error.

Amphipod densities were highly variable among sponge species (Fig. 2.6). Unidentified sponge species 1 contained the greatest amphipod densities, *Crella sp.* from Point Halswell the least. For *Crella sp.* only, amphipod density was very similar between Aotea Quay and Burnham Wharf and lowest at Point Halswell.

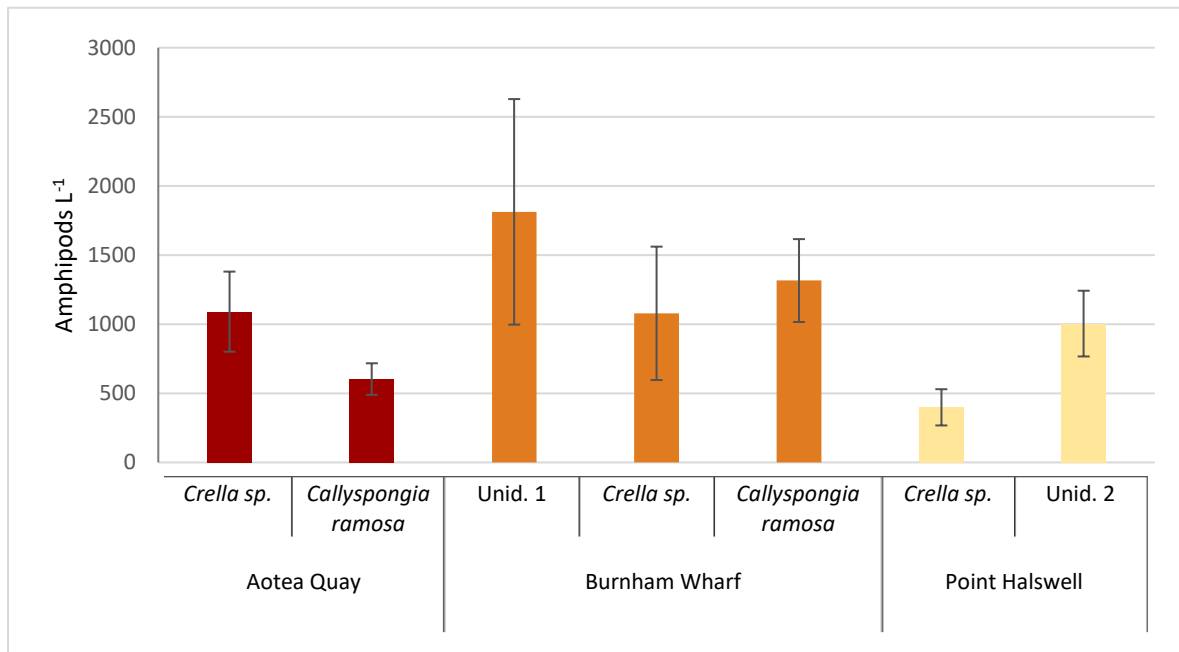


Figure 2.6. Mean amphipod density (amphipods L⁻¹ sponge tissue) between sites and sponge species. Error bars show standard error.

2.3.4 Amphipod richness, evenness and diversity

There were 13 species of amphipods in total (Table 2.6). Across all sites there was an average of 4.68 different species of amphipod per sponge. The highest species richness was found in sponges from the least polluted site (Point Halswell), averaging 5.75 species per sponge (Fig. 2.7). The mean species richness at the intermediate site (Burnham Wharf) was very similar at 5.25. In contrast, sponges from the most polluted site (Aotea Quay) contained approximately half the species as the other sites (2.75 amphipod species per sponge). Species richness differed significantly between Aotea Quay and Burnham Wharf (ANOVA, $F=20.97$, $p < 0.01$) but not between Aotea Quay and Point Halswell (ANOVA, $F = 4.38$, $p = 0.06$) or Burnham Wharf and Point Halswell (ANOVA, $F = 1.83$, $p = 0.67$).

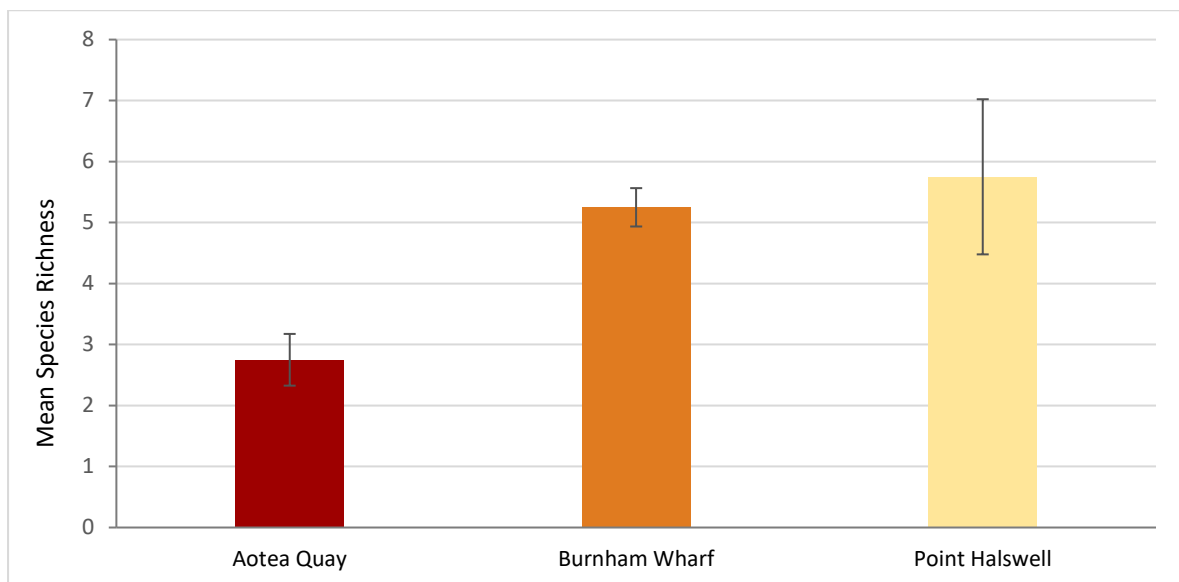


Figure 2.7. Mean species richness across sampling sites. Error bars show standard error.

Species evenness was greatest at Point Halswell and smallest at Aotea Quay (ANOVA, $F=4.65$; $p=0.02$; Fig. 2.8). There was a significant difference between Point Halswell and Aotea Quay (Post Hoc, $p < 0.01$). The differences between Aotea Quay and Burnham Wharf (Post Hoc, $p = 0.11$) and Burnham Wharf and Point Halswell (Post Hoc, $p=0.11$) were not significant.

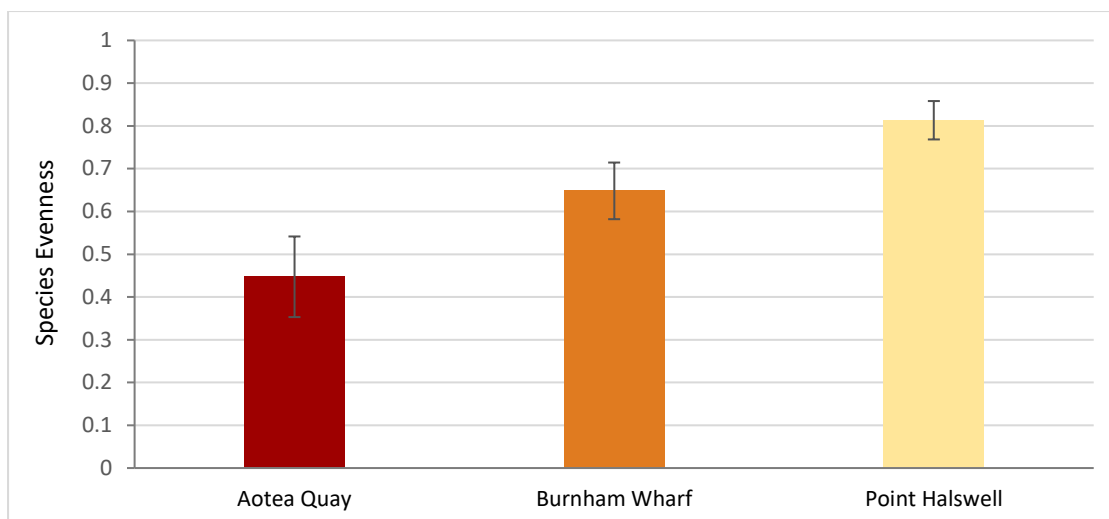
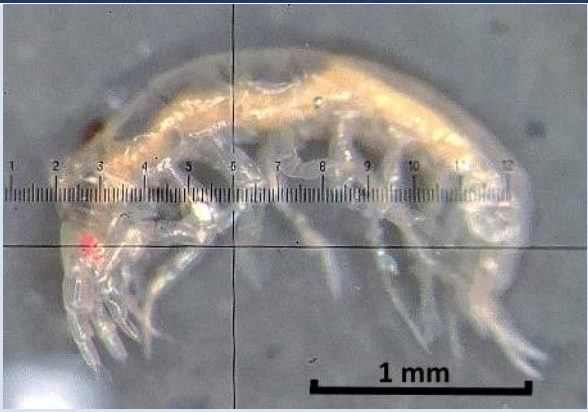




Figure 2.8. Mean species evenness across sites. Error bars show standard error.

Thirteen species of amphipods were found at the three sites and these are illustrated in Table 2.6.

The two *Colomastix* species had the shortest bodies. *Colomastix magnirama* also had the highest proportion of females. Sex ratios were not available for all species because of species absence from sites, as well as difficulties in determining sex of broken individuals.

Table 2.6. Amphipod species found in sponges

Description	Picture
<p><i>Colomastix magnirama</i> Hurley, 1954</p> <p>Mean length (mm \pm s.e.):</p> <ul style="list-style-type: none"> Female : 2.4 ± 0.04 Male : 2.3 ± 0.07 <p>Mean sex ratio (Female:Male \pm s.e.):</p> <ul style="list-style-type: none"> Aotea Quay : 2.9 ± 0.8 Burnham Wharf : 0.7 ± 0 	
<p><i>Colomastix</i> sp. 1</p> <p>Mean length (mm \pm s.e.):</p> <ul style="list-style-type: none"> Female : 2.3 ± 0.03 Male : 2.2 ± 0.03 <p>Mean sex ratio (Female:Male \pm s.e.):</p> <ul style="list-style-type: none"> Aotea Quay : 2.9 ± 0.2 Burnham Wharf : 0.7 ± 0.2 Point Halswell : 2 ± 0.7 	
<p><i>Gammaropsis typica</i> (Chilton, 1884)</p> <p>Mean length (mm \pm s.e.):</p> <ul style="list-style-type: none"> Female : 3.9 ± 0.2 Male : 3.8 ± 0.2 <p>Mean sex ratio (Female:Male \pm s.e.):</p> <ul style="list-style-type: none"> Burnham Wharf : 1.6 ± 0.3 Point Halswell : 1.2 ± 0.2 	

Haplocheira barbimana barbimana

Thomson, 1879

Mean length (mm \pm s.e.):

- Female : 5.4 ± 0.3
- Male : 5.0 ± 0.2

Mean sex ratio (F:M \pm s.e.):

- Burnham Wharf : 1.9 ± 0.7
- Point Halswell : 1.2 ± 0.4



***Jassa slatteryi* Conlan 1990**

Mean length (mm \pm s.e.):

- Male : 5.5 ± 0

Mean sex ratio (F:M \pm s.e.):

- There was not enough data to calculate sex ratios



***Leucothoe* sp. 1**

Mean length (mm \pm s.e.):

- Female : 9.6 ± 0.82
- Male : 7.6 ± 0.7

Mean sex ratio (F:M \pm s.e.):

- There was not enough data to calculate sex ratios



Neocyproidea otakensis
(Chilton, 1900)

Mean length (mm \pm s.e.):

- Juvenile : 1.1 ± 0.2

Mean sex ratio (F:M \pm s.e.):

- There was not enough data to calculate sex ratios



Paradexamine pacifica
Thomson, 1879

Mean length (mm \pm s.e.):

- Female : 3.1 ± 0.17
- Male : 3.4 ± 0.18

Mean sex ratio (F:M \pm s.e.):

- There was not enough data to calculate sex ratios



***Parawaldeckia* sp. 1**

Mean length (mm \pm s.e.):

- Female : 3.1 ± 0.66
- Male : 4.2 ± 0

Mean sex ratio (F:M \pm s.e.):

- There was not enough data to calculate sex ratios



***Podocerus cristatus* Thomson, 1879**

Mean length (mm \pm s.e.):

- Female : 4.0 ± 0.2
- Male : 4.0 ± 0.23

Mean sex ratio (F:M \pm s.e.):

- Aotea Quay : 1 ± 0
- Burnham Wharf : 1.1 ± 0.3
- Point Halswell : 1.2 ± 0.3



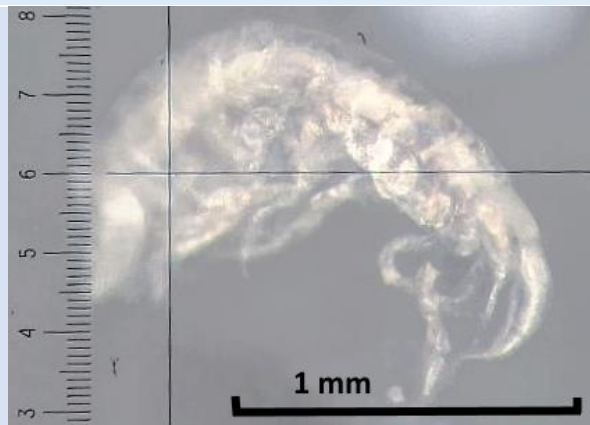
***Seba* sp. 1**

Mean length (mm \pm s.e.):

- Juvenile : 1.46 ± 0.15

Mean sex ratio (F:M \pm s.e.):

- There was not enough data to calculate sex ratios



***Stenothoe* sp. 1**

Mean length (mm \pm s.e.):

- Female : 3.0 ± 0.2
- Male : 0.3 ± 0.3

Mean sex ratio (F:M \pm s.e.):

- Burnham Wharf : 1.8 ± 0.3
- Point Halswell : 1.0 ± 0.0



***Ventojassa* sp. 1**

Mean length (mm \pm s.e.):

- Female : 3.6 \pm 0.13
- Male : 3.0 \pm 0.07

Mean sex ratio (F:M \pm s.e.):

- Burnham Wharf : 0.6 \pm 0.0
- Point Halswell : 0.6 \pm 0.0



Amphipod communities from Point Halswell were the most diverse, closely followed by Burnham Wharf, for all three combinations of the three sponge species found there. Shannon-Wiener diversity assumes equal sample sizes, which was a problem for this analysis because there were three sponge species (n=4) collected at Burnham Wharf and only two at the other sites. This would result in an inflated H' diversity index for this site. To adjust for this, Shannon-Wiener diversity indices were calculated for different pairs of sponge species from Burnham Wharf. These were then compared to those at the other sites. Amphipod H' diversity in pooled communities at Point Halswell was 73% higher than at Aotea Quay, while Burnham Wharf had an intermediate value (Fig. 2.9).

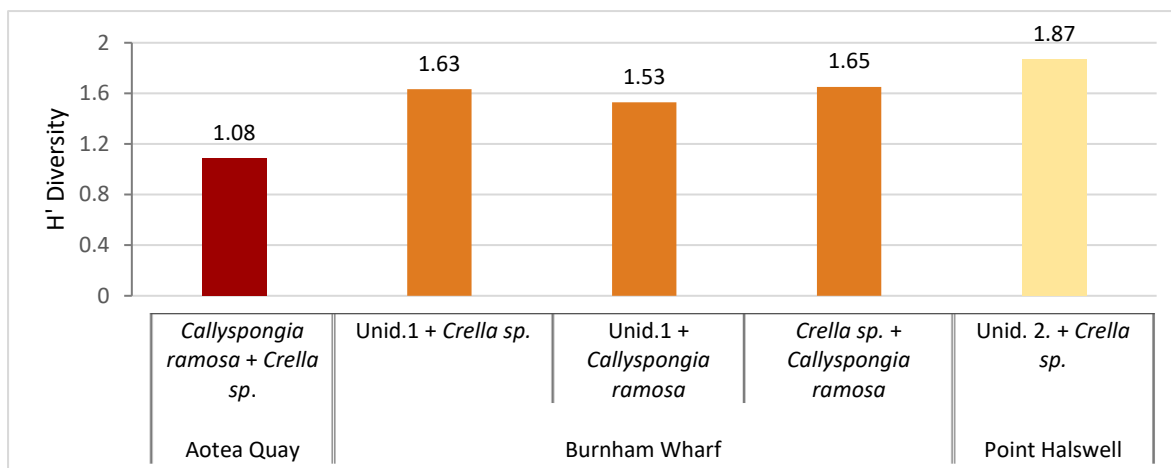


Figure 2.9. H' diversity indices for pooled communities at different sites, including different combinations of sponges from Burnham Wharf. There are no error bars as these are calculated values.

When sponge species were considered separately, the differences in H' diversity were even more pronounced (Fig. 2.10). Diversity was generally lowest in *Callyspongia ramosa* at Aotea Quay and Burnham Wharf, although the difference was much smaller than that between sites for the same species (Table 2.7). The H' diversity index for *Crella* sp. at Aotea Quay was less than half the value of Burnham Wharf. At Point Halswell H' diversity was more than three times higher than Aotea Quay. The H' diversity for Unidentified species 1 was very similar to that of *Callyspongia ramosa* at the same site. That of Unidentified species 2 was equivalent to the value calculated for *Crella* sp. at Point Halswell.

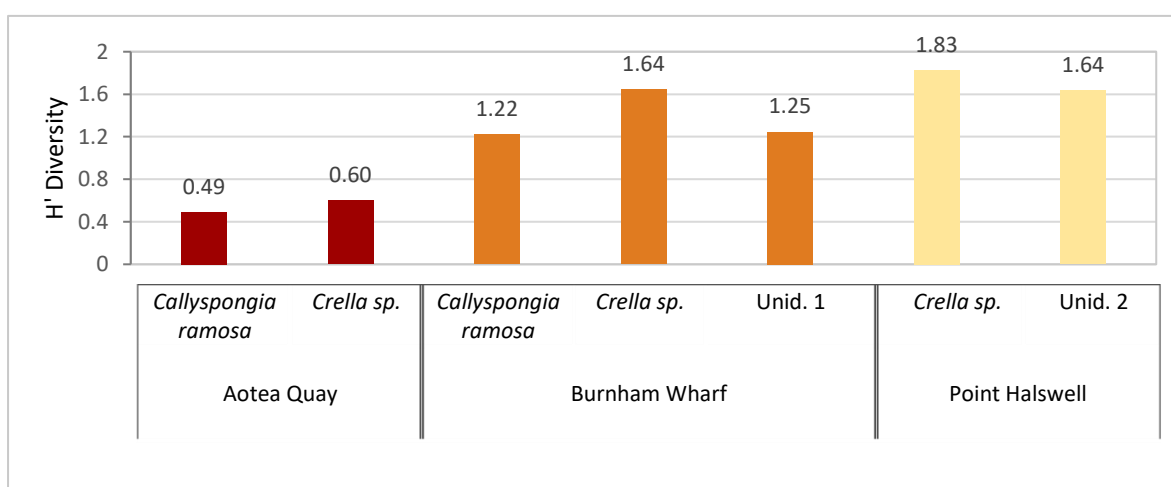


Figure 2.10. H' diversity indices for each sponge species at each site. There are no error bars as these are calculated values.

2.3.5 Species Composition

Species composition varied significantly between sites (Fig. 2.11, 2.12, 2.13).

Both sponge species from Aotea Quay were dominated by a single *Colomastix* species, with other species occurring at very low numbers. While *C. magnirama* co-occurred with *Colomastix* sp. 1 in *Callyspongia ramosa*, *Colomastix* sp. 1 was completely absent from all four *Crella* sp. sponges dominated by *C. magnirama*.

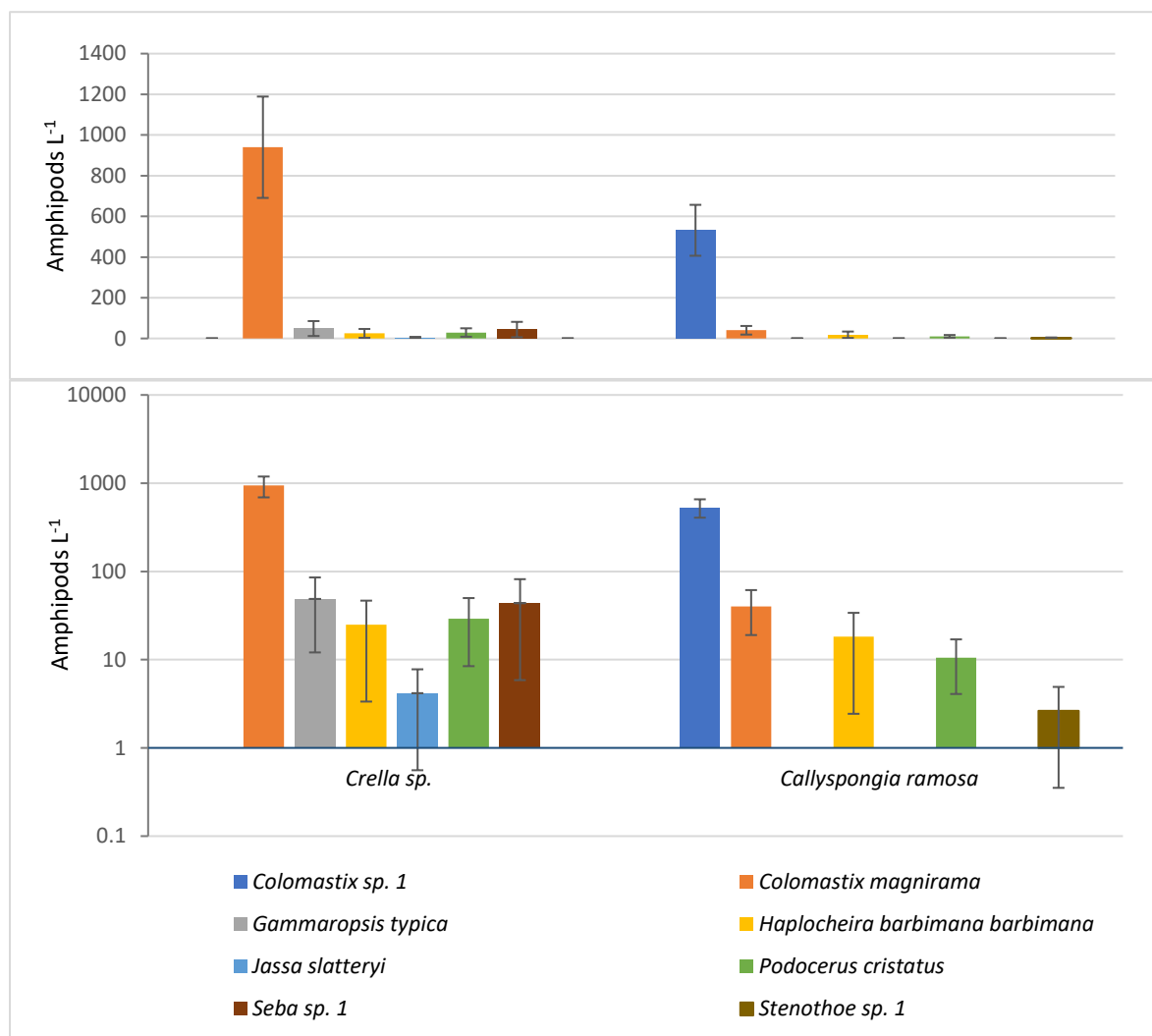


Fig.2.11. Species composition in two species of sponge at Aotea Quay. Note the y-axis of the lower plot is a logarithmic scale, while the upper plot presents the same data on a linear scale. Error bars show standard error.

Species richness and diversity were higher at Burnham Wharf (Fig. 2.12), although sponges still each contained a somewhat dominant species. At this site, variability in species abundance was often quite high. *C. ramosa* sponges from Burnham Wharf were dominated by the same amphipod species as at Aotea Quay, *Colomastix* sp. 1.

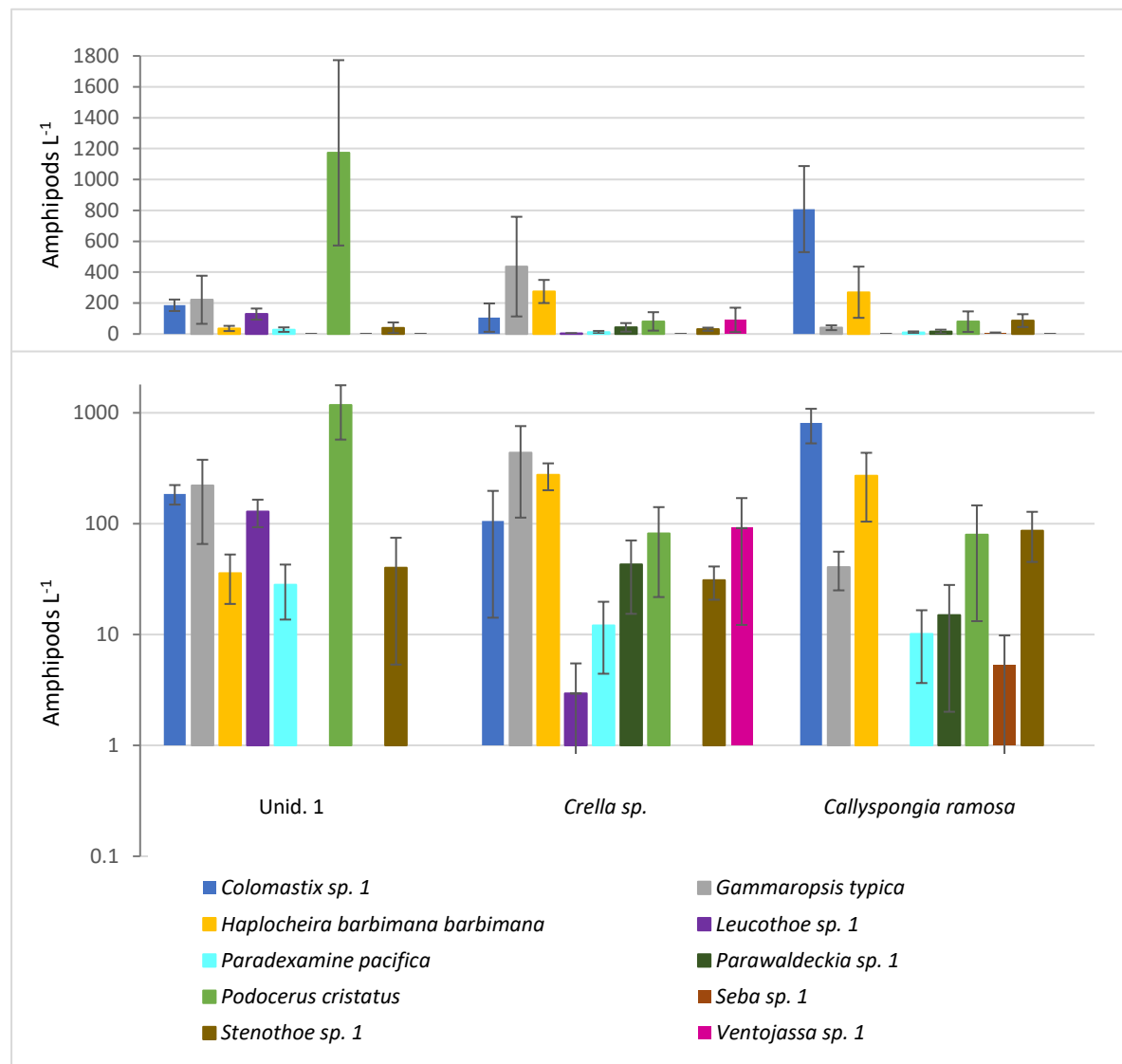


Fig. 2.12. Species composition in two species of sponge at Burnham Wharf. Note the y-axis of the lower plot is a logarithmic scale, while the upper plot is the same data presented on a linear scale. Error bars show standard error.

Amphipod densities were more evenly distributed at Point Halswell than at the other sites (Fig. 2.13). Although there were some species that occurred at higher densities, i.e. *Gammaropsis typica* and *Ventojassa* sp. 1, the magnitude of their ‘dominance’ was much lower than at Burnham Wharf and especially Aotea Quay.

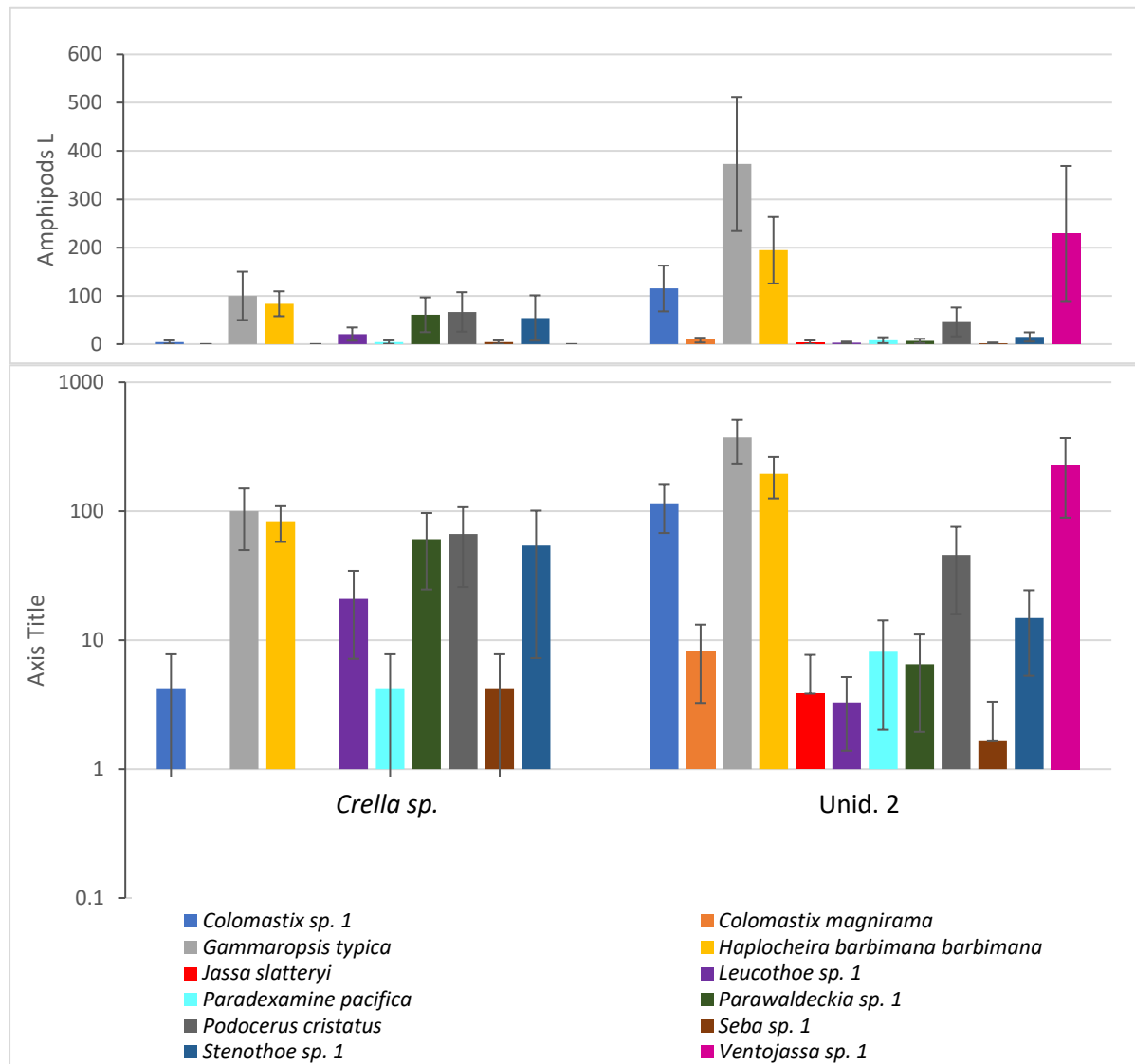


Fig.2.13. Species composition in two species of sponge at Point Halswell. Note the y-axis of the lower plot is a logarithmic scale, while the upper plot is the same data presented on a linear scale. Error bars show standard error.

Interestingly, *C. magnirama* was only found in high densities at Aotea Quay and two *Crella* sp. replicates from Point Halswell at low densities. *Colomastix* sp. 1 was present in *C. ramosa* samples from both Aotea Quay and Burnham Wharf. *Colomastix* sp. 1 abundance was higher at Burnham Wharf. *Haplocheira barbimana barbimana* was the most ubiquitous species, only two replicates

from Aotea Quay did not contain this species. The highest density of this species was present at Burnham Wharf, especially in *Crella* sp and *C. ramosa* (Fig. 2.12). *Colomastix* sp. 1 and *G. typica* were also very common amphipod species at all sites. *Colomastix* sp.1 occurred at high densities in *C. ramosa* samples from both Aotea Quay and Burnham Wharf. Across all sponges this species had the highest frequency and density in samples from Burnham wharf. *P. cristatus* density was considerably higher at Burnham Wharf than at the other sites. Most of this is due to densities in the unidentified sponge species 1, which averaged 1172.7 amphipods L⁻¹. This was the highest density of any amphipod species from any sponge species. Variance for this value was high, mostly because the species was completely absent from one replicate.

Table 2.7. Presence and density of amphipod species at different sites

	All Sites n = 28		Aotea Quay n = 8		Burnham Wharf n = 12		Point Halswell n = 8	
	Occurrence (%)	Density (in L ⁻¹)	Occurrence (%)	Density (in L ⁻¹)	Occurrence (%)	Density (in L ⁻¹)	Occurrence (%)	Density (in L ⁻¹)
<i>Colomastix</i> sp. 1	64	250.2 ± 70.6	50	265.8 ± 12.9	75	366.7 ± 113.9	63	59.7 ± 28.5
<i>Colomastix magnirama</i>	32	141.2 ± 71.2	88	490.0 ± 02.3	0		25	4.1 ± 2.6
<i>Gammaropsis typica</i>	64	174.2 ± 62.6	25	24.4 ± 20.3	83	232.6 ± 128.3	75	236.5 ± 81.1
<i>Haplocheira barbimana</i> <i>barbimana</i>	71	128.9 ± 34.5	25	21.6 ± 13.4	92	193.7 ± 68.9	88	139.1 ± 38
<i>Jassa slatteryi</i>	7	1.2 ± 0.8	13	2.08 ± 2	0		13	1.92 ± 1.8
<i>Leucothoe</i> sp. 1	32	22.3 ± 10.0	0		42	43.9 ± 21.1	50	12.1 ± 7.5
<i>Neocyproidea otakensis</i>	7	1.1 ± 0.8	0		17	2.5±1.7	0	
<i>Paradexamine pacifica</i>	32	9.0 ±3.2	0		50	16.8 ± 6.3	38	6.1 ± 3.3
<i>Parawaldeckia</i> sp. 1	29	17.9 ± 8.0	0		33	19.3 ± 11.4	50	33.6 ± 20.5
<i>Podocerus cristatus</i>	61	212.3 ± 114.4	50	19.9 ± 11.3	67	444.6 ± 250.9	63	56.3 ± 24.4
<i>Seba</i> sp. 1	14	7.8 ±6.2	13	21.88 ± 20.1	8	1.75 ± 1.7	25	2.92 ± 2
<i>Stenothoe</i> sp. 1	43	32.7 ± 11.7	13	1.32 ± 1.2	67	52.5 ± 19.6	38	34.5 ± 24.8
<i>Ventojassa</i> sp. 1	18	45.8 ± 25.7	0		8	30.4 ± 29.1	50	114.6 ± 72.9

There was a significant difference between amphipod communities from *Crella sp.* and *Callyspongia ramosa* at Aotea Quay (ANOSIM, $p=0.03$) but not at Burnham Wharf (ANOSIM, $p=0.12$, Fig. 2.14). ANOSIM between all sites was calculated for different combinations of sponge species from Burnham Wharf, to adjust for the larger sample size at this site (Table 2.8).

Table 2.8. ANOSIM analyses show significant differences in community structure between sites for all combinations of sponge species from Burnham Wharf, as well as for *Crella sp.* only at all sites.

Burnham Wharf combination	p-value	R value
Unid.1 + <i>Crella sp.</i> + <i>Callyspongia ramosa</i>	<0.01	0.31
<i>Crella sp.</i> + <i>Callyspongia ramosa</i>	<0.01	0.32
Unid. 1 + <i>Callyspongia ramosa</i>	<0.01	0.37
<i>Crella sp.</i> + Unid. 1	<0.01	0.32
<i>Crella sp.</i> (at all sites)	<0.01	0.55

When all three sponge species from Burnham Wharf were considered, amphipod community structure from the most polluted site (Aotea Quay) differed greatly from the other two, particularly from the least polluted site (Point Halswell, Fig. 2.14). Sponge species was a decisive factor in controlling Bray Curtis Dissimilarity between sites. Pairwise comparisons of the Aotea Quay and Point Halswell with three different combinations of sponge species from the Burnham Wharf resulted in considerable variation in dissimilarity. In two out of three combinations, amphipod communities from the least and most polluted sites were the most dissimilar. In the third, those from the most polluted and intermediate sites were slightly more dissimilar. The intermediate and least polluted sites were moderately dissimilar throughout all comparisons.

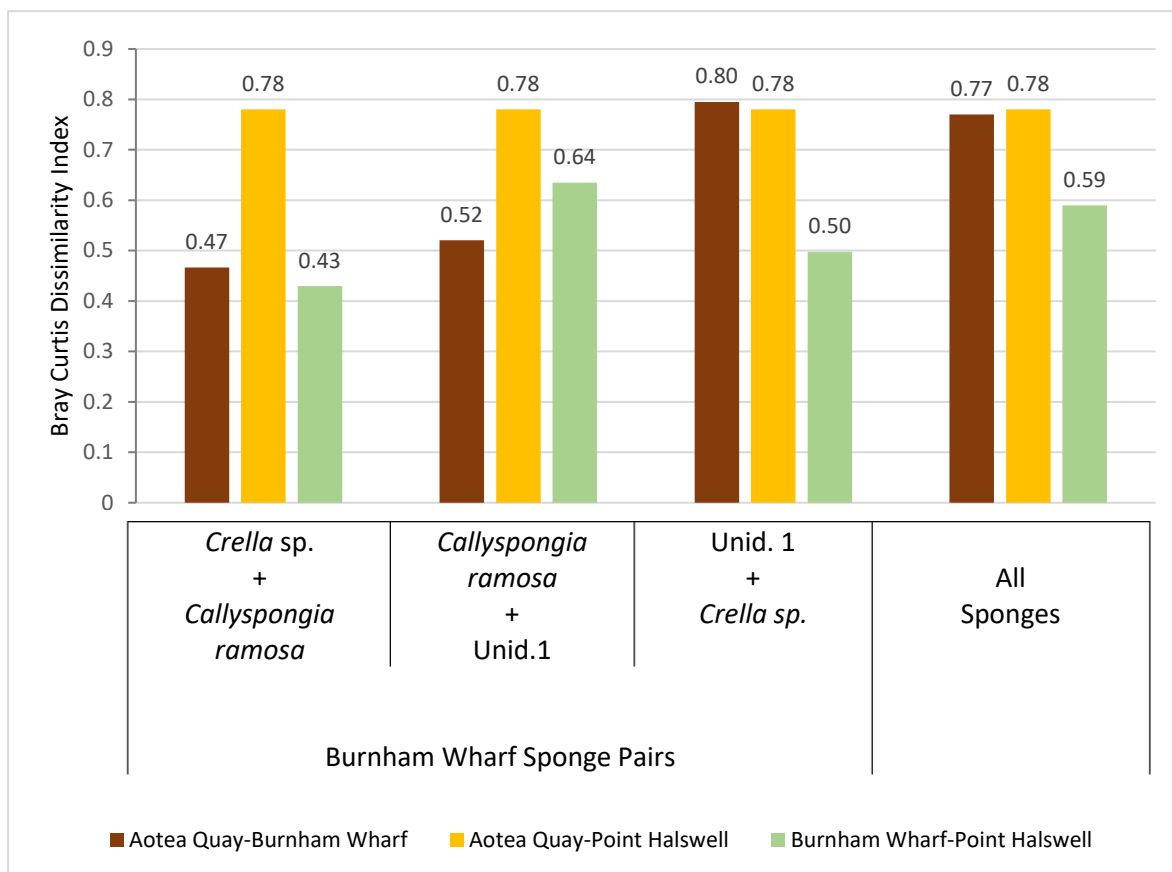


Figure 2.14. Pair-wise Bray Curtis Dissimilarity comparisons across sites for different combinations of sponges from Burnham Wharf. There are no error bars because these are calculated values.

When only *Crella* sp. was considered, community structure differed most between Aotea Quay and Burnham Wharf (Fig. 2.15). Amphipod communities from Burnham Wharf and Point Halswell were intermediately dissimilar (Ricotta and Podani, 2017). The Bray Curtis Dissimilarity index for the communities in *Crella* sp. was almost twice that of *Callyspongia ramosa* sponges, when compared between Aotea Quay and Burnham Wharf. Amphipod communities from *Crella* sp. sponges showed the highest dissimilarities for comparisons of Aotea Quay with the other sites.

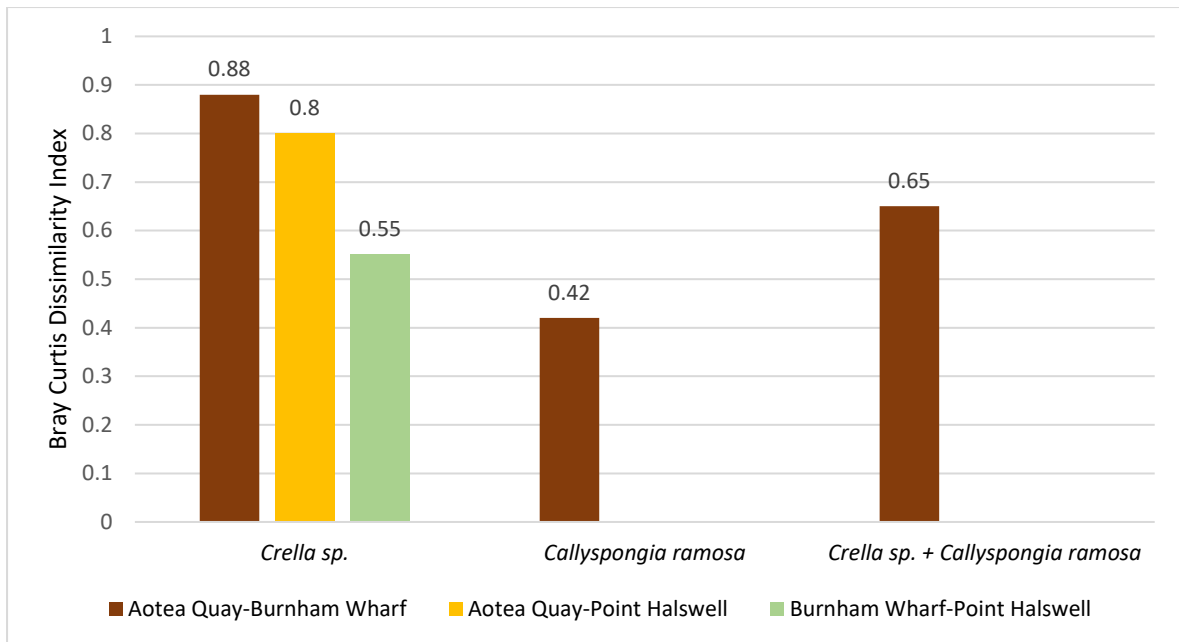


Figure 2.15. Pair-wise Bray Curtis Dissimilarity comparisons across sites for sponge species present at more than one site. There are no error bars because these are calculated values.

Adult amphipod length had no effect on host choice when compared across all sponge species (regression, $p=0.46$ appendix Sup. Fig 1, appendix).

2.3.6 Sex ratio and age structure

Sex ratio had a female bias in 61% of amphipod species. Even though female:male ratios varied among sites, none of the differences were statistically significant (Fig. 2.16, regression, $R^2=0.03$, $p=0.11$).

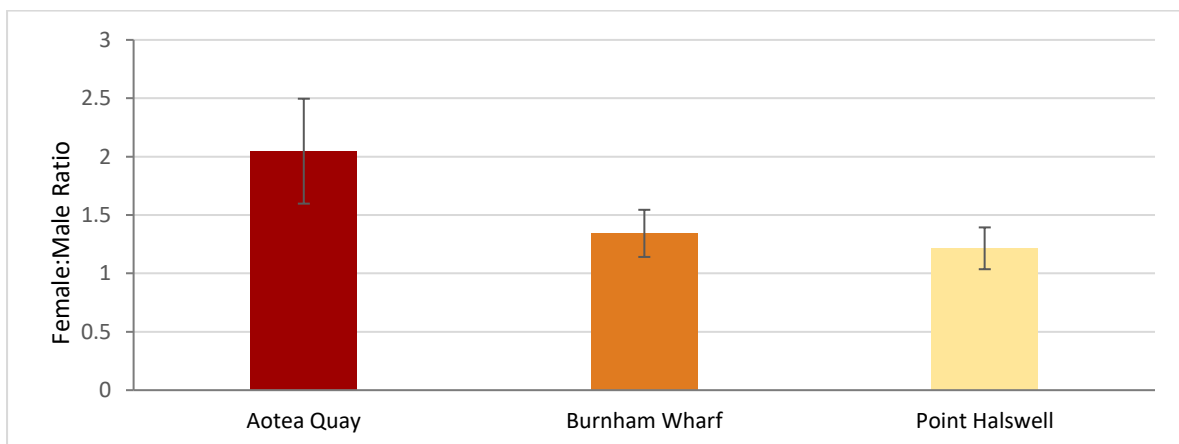


Figure 2.16. Mean female:male ratios across sites. Error bars show standard error.

Five of the seven species for which data was available had female:male ratios larger than 1 at the more polluted site (Fig. 2.17). The sex ratios of *C. magnirama* were particularly interesting due to a high female skew at Aotea Quay, where there were almost 3 times as many females as males. There was a moderate male skew at Burnham Wharf for this species, although this ratio was calculated from amphipods found in a single sponge replicate. There were not enough data points to enable comparisons between sites for *Crella sp.* only.

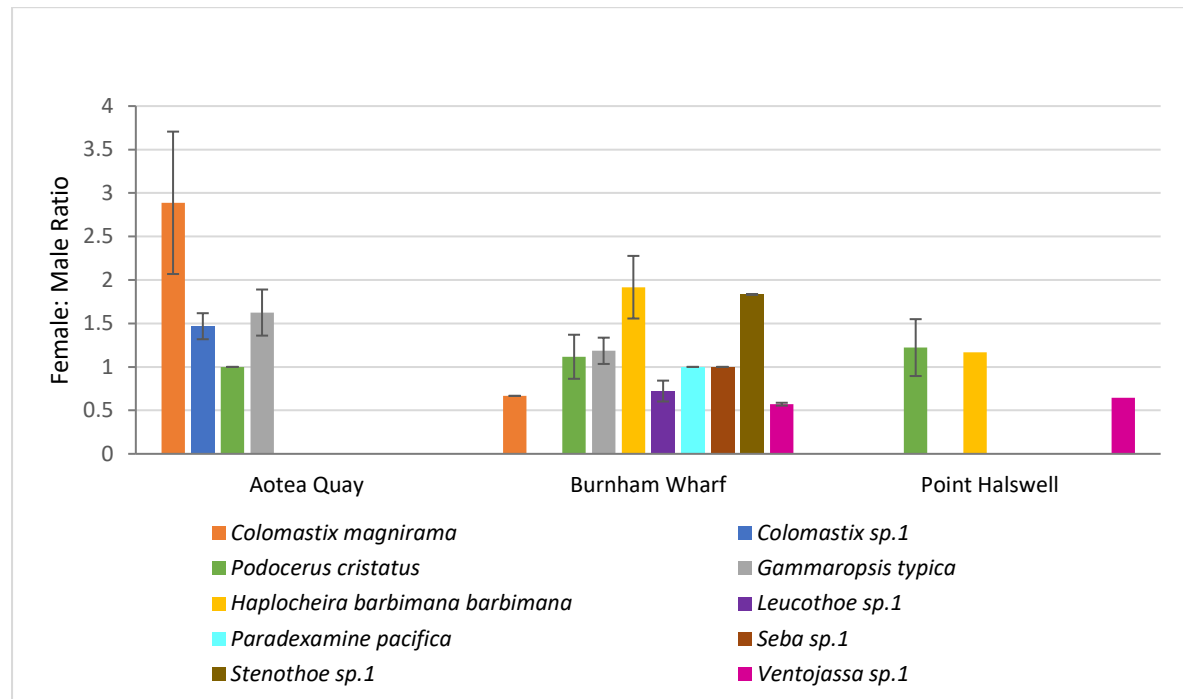


Figure 2.17. Mean female:male ratios for amphipod species across sites. Error bars show standard error.

Data for adult:juvenile ratios was also sparse. The proportion of adults decreased relative to pollution loadings (Fig. 2.18, $R = 0.08$, $p = 0.01$). At Point Halswell, adult:juvenile ratio was closer to 1 than at the other sites. At Aotea Quay, there were on average twice as many adults as there were juveniles. Burnham Wharf had 50% more adults than juveniles.

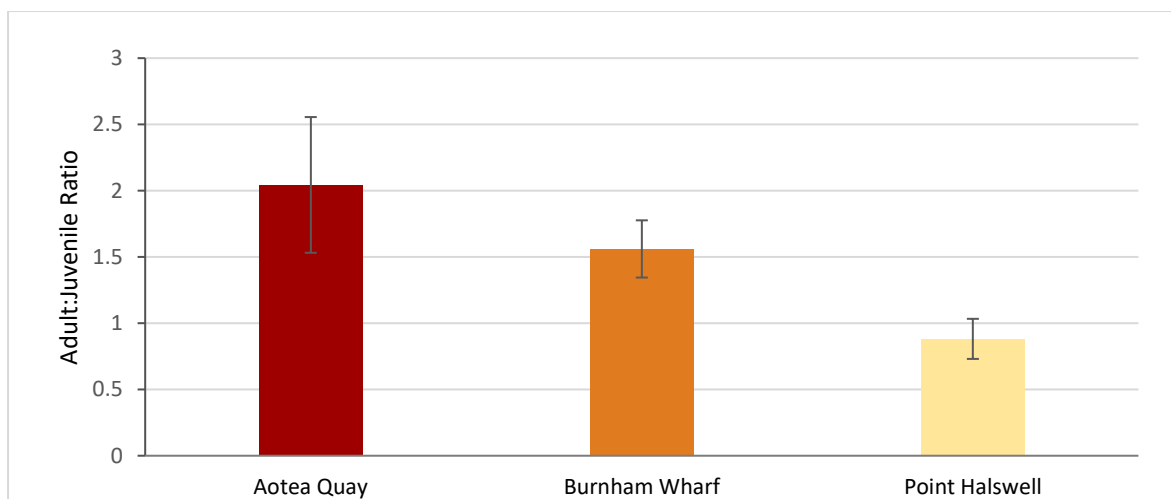


Figure 2.18. Mean adult:juvenile ratio between sites. Error bars show standard error.

The proportion of adult amphipods was especially high in *Podocerus cristatus* and *Colomastix magnirama* at Aotea Quay compared to the cleaner sites (Fig. 2.19). The slightly higher adult to juvenile proportion of *Colomastix* sp. 1 was largely consistent across all sites. Higher proportions of juvenile individuals than adults were present at all sites, but they were twice as common at Burnham Wharf and Point Halswell.

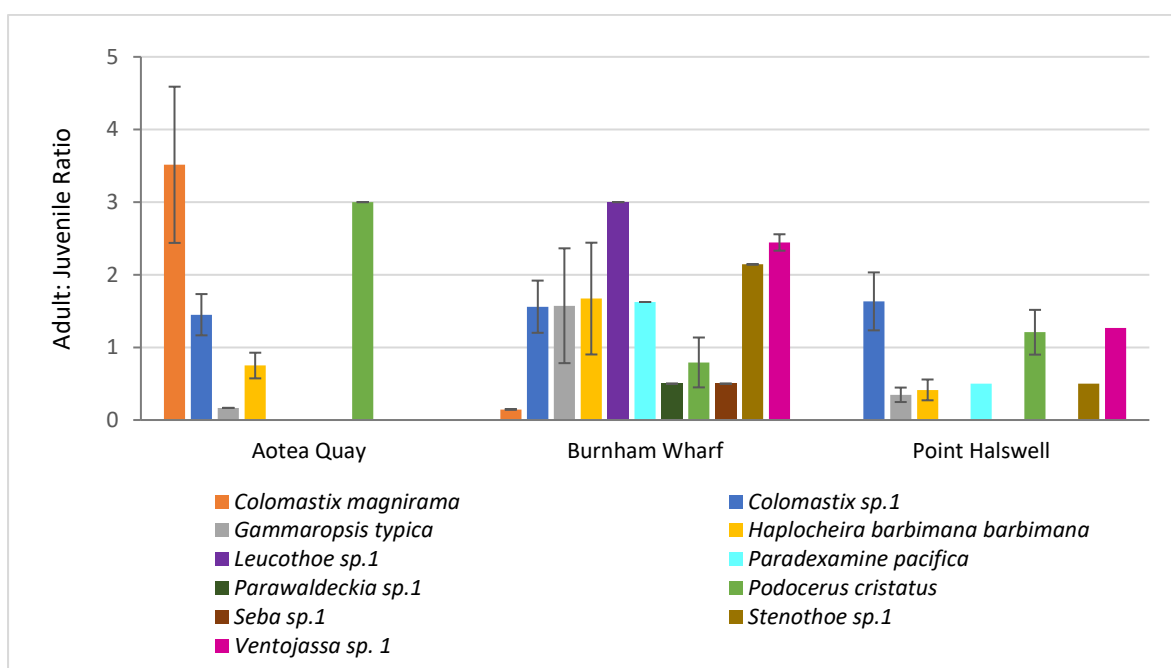


Figure 2.19. Mean adult: juvenile ratios for amphipod species across sites. Error bars show standard error.

In all analyses except species richness, amphipod density (amphipod L⁻¹) was used as a proxy for abundance, because it accounts for habitat size, i.e. sponge size, which is an important assumption in Shannon-Wiener Diversity, ANOSIM and Bray Curtis Dissimilarity analyses. This was done by Amsler M. et al. (2009) and is common practise for this kind of study. One limitation of this study was the relatively long period before the 3rd sample collection at Aotea Quay. This may have introduced bias as seasonal fluctuations are not uncommon in amphipod communities (Jeong et al., 2008; Zaabar et al., 2015).

2.4 Discussion

There was a significant decrease in species richness with increasing pollution levels. This was particularly apparent in comparing the least and most polluted sites, whereby sponges from the former harboured twice the average number of species found at the most polluted site. The intermediate site contained similar amphipod species richness to the least polluted site. On average, amphipod communities at Point Halswell contained the highest number of species and these were most balanced in density. This is likely a consequence of varying sensitivity levels among amphipods, making some more susceptible to pollution than others (Fox et al., 2014; Šidagytė and Arbačiauskas, 2016; Ali et al., 2018). These findings support the initial hypothesis as the total number of species was affected by pollution.

Species evenness showed a similar and even more dramatic trend with increasing pollution from one site to another. It was greatest at the least polluted site, and lowest at the most polluted site. At the intermediate site, evenness was almost exactly the overall average. Evenness was the lowest at Aotea Quay where amphipod communities were dominated by two *Colomastix spp.* and the highest at Point Halswell where there were no obviously dominant species. These findings confirm an effect of pollution on species richness and evenness which is broadly consistent with existing literature, although generally species richness tends to best reflect pollution levels, not evenness (Johnston and Roberts, 2009). This may be an effect of the host-association, which was not a factor in previous studies.

H' diversity was also affected by pollution level. Amphipod diversity was reduced by increased pollution which is coherent with existing literature (Ali et al., 2018). Amphipod communities had the lowest H' diversity at the most polluted site, and the highest at the least polluted. Notably, heightened habitat complexity has been linked to an increase in biodiversity in algae-associated amphipods (Eriksson et al., 2006; Carvalho et al., 2018). This could explain why diversity at the intermediate site was more similar to the least polluted site because sponges were collected from wharf piles, which appeared to offer a more suitable substrate to sessile organisms, judging by their abundance (Fig. 2.2 and 2.3). Reduced biodiversity resulting from pollution is a well-documented phenomenon (Guerra-García and García-Gómez, 2004; Johnston and Roberts, 2009; Zaabar et al., 2015). Existing literature suggests that amphipod density is negatively correlated with species evenness and diversity, due to competition (Fiore and Jutte, 2010). Amphipod densities were highest at the intermediate site, while species evenness and diversity were lowest at the most polluted site, which suggests that competition is not the structuring force for these communities. *Crella sp.* by itself also shows a disconnect between amphipod density and diversity at different pollution levels

that is inconsistent with this principle. These findings support the initial hypothesis that pollution reduces the diversity of sponge-associated amphipod communities.

Community composition was significantly affected by pollution levels. This was confirmed by both ANOSIM and Bray Curtis dissimilarity. It was the most obvious when only *Crella* sp. was considered. Because dissimilarities between sites were greatly influenced by sponge species, this was the most reliable comparison of community composition.

The distribution and densities of *C. magirama* and *Colomastix* sp. 1 were notable. *C. magirama* was uncommon at both Burnham Wharf and Point Halswell but occurred reliably in all samples from the most polluted site, particularly in *Crella* sp. with densities ranging between 220 and 1616 individuals L⁻¹. *Colomastix* sp. 1's distribution seemed to be affected by host choice. Amphipods of the family Colomastigidae typically live in sponges, although other hosts have been recorded (Winfield and Ortiz, 2010). They were the only species to occur in great densities at the most polluted site.

This suggests that higher pollution favours species that are better adapted to living in sponges. A possible mechanism behind this could be that pollution increased the influence of other selecting features in sponge-associated amphipod communities, namely the level of adaptation to the sponge host. The low densities of *Leucothoe* sp.1, another known sponge commensal at the most polluted site could be explained by the much larger body size of this species compared to the *Colomastix* species (Thomas and Klebba, 2006). This may have mostly excluded it from the sponge species collected from Aotea Quay. Of all the species documented, only those of the genera *Colomastix* and *Leucothoe* have reported associations with sponges (Thomas and Klebba, 2006; Winfield and Ortiz, 2010), all the other genera noted are generalist in nature and have been found to be opportunistic in their habitat preferences (Nelson, 1981; Takeuchi and Watanabe, 2002; Paz-Ríos and Pech, 2004; Jeong et al., 2009). Lowered generalist densities observed at polluted sites are consistent with other studies (Edward, 2014; Zaabar et al., 2015). While coastal marine communities are generally dominated by generalists, pollution can act as a selection pressure, which can lead to reduced evenness and increased species dominance by specialists, as was observed in this study (Edward, 2014; Pratiwi et al., 2020). These findings confirmed the hypothesis that pollution causes changes to community structure in sponge-associated amphipods. Due to a lack of literature on pollution sensitivities of the recorded amphipod species, hypothesis that more tolerant species would become dominant under increased pollution could not be proven or disproven. However, this study implies potential selection pressures for commensal amphipods and against generalists in sponge-associated amphipod communities subjected to pollution.

To a degree these changes were certainly influenced by differences in sampled sponge species. Nevertheless, both amphipod communities from *Crella* sp. and *Callyspongia ramosa* sponges

displayed conspicuous differences in species richness, evenness, H' diversity and community composition between sites. Considering the sum of pollution sources these sites are subjected to it is very likely that these effects were caused by pollution (Johannesson and Martin, 1955; Brodie, 1958; Stoffers et al., 1986; Dickinson et al., 1996; Pilotto et al., 1999; Barton and Foster, 2003; Oliver and Conwell, 2017; 2018).

Although not statistically significant, amphipod densities were highest at Burnham Wharf, both in all sponges and *Crella* sp. only. This data was highly variable due to the small sample size. Some amphipod species had reduced densities at Aotea Quay, i.e. *Haplocheira barbimana barbimana*, *Gammaropsis typica* and *Podocerus cristatus* while others were absent. i.e. *Stenothoe* sp. 1, *Paradexamine pacifica* and *Leucothoe* sp.1. Overall amphipod density was very similar between the intermediate and most polluted sites. A possible explanation for this could be that the aforementioned advantage offered by increased habitat complexity and host density on wharf piles may have out-weighed the negative impacts of heightened pollution at the intermediate site (Eriksson et al., 2006; Amsler M. et al., 2009). If this is the case the intermediate and most polluted sites would make for a more accurate comparison of amphipod density.

Although there was no statistically significant effect of pollution level on amphipod sex ratio between sites, the overall proportion of females was higher than that of males. A female bias in amphipod population is not uncommon, however the average proportion of females was higher at the more polluted sites (Prato and Biandolino, 2003). Certain types of pollution can lead to a higher proportion of female amphipods (Peschke et al., 2019; Ford et al., 2004). This could potentially be explained by the presence of parasitic microsporidia, which cause an increase in intersex females in affected communities and seem to interact with pollution (Ford et al. 2006). It is feasible that pollution could convey an advantage to the parasite by suppressing the host's immune response. Alternatively, the microsporidia might themselves be affected by pollution, causing the effect (Ford et al., 2004). During dissections, no intersex individuals were observed, although it is possible that intersex features may have been missed due to the small size of the amphipods. The considerable female bias of *C. magnirama* is an interesting feature of this data set. Population models suggested that the higher proportions of intersex females induced by pollution could be beneficial to the survival of affected populations (Ford et al., 2007). This could contribute not only to the high proportion of females recorded at Aotea Quay, but also to the high densities of this species, despite the high pollution levels as the increase of reproductive females could cause an overall higher fecundity. However, *C. magnirama* sex ratio at Burnham Warf was calculated from a single sponge replicate and does therefore not make for a reliable comparison. The overall sample size is relatively

low and highly variable, so it is not possible to draw a conclusion as to the effect of pollution on sex ratio. There were increasingly high proportions of adult individuals with higher pollution loadings. This was again particularly apparent in *Colomastix magnirama*, as well as *Podocerus cristatus*. For example, contamination by zinc and cadmium occurs at a higher rate in juvenile *Echinogammarus marinus* Leach 1815 than in adults, likely due to different dietary preferences and smaller size of juveniles (Pastorinho et al., 2009). This may have increased offspring mortality. Sediments in Aotea Quay and Burnham Wharf are contaminated with heavy metals, however zinc concentrations are relatively low (Oliver and Conwell, 2017). This mechanism would however be likely to lead to an overall reduction in amphipod density, which seems unlikely, considering that *Colomastix magnirama* presents both high density and high adult proportions at the most polluted site. Seasonality could also explain both the differences in age and sex ratio, as fecundity and female bias in amphipods depend on seasonality and different species have varying numbers of lifecycles per year (Prato and Bandolino, 2003; Jeong et al., 2008). While the interval between the collections at the sites with low and intermediate pollution loadings was relatively short, it may have contained a spike in amphipod fecundity. However, because adult proportions between the highly polluted and intermediate sites were so similar, this seems improbable. It seems more likely that the higher number of juveniles at Point Halswell could be due to amphipods there using sponges as nurseries, which would be a reasonable assumption in the light of their mostly generalist nature at this site. As with the sex ratio comparison, there is not enough data available to make a confident comparison of age structure in amphipod communities between sites.

Sponge species had an influence on community structure and the degree of dissimilarity between pollution levels: *C. magnirama* was highly dominant in *Crella* sp. at the most polluted site. One way this could be facilitated is via pollution-driven changes to chemicals exuded by the *Crella* sp. sponge. Metabolites produced by an unidentified species of *Crella* prevented feeding by the amphipod *Gondogeneia antarctica* (Chevreux, 1906) (Amsler M. et al., 2009). Another possibility is that *C. magnirama* competitively excludes *Colomastix* sp. 1 once it becomes dominant. It is also possible that *Callyspongia ramosa* has morphological or chemical features that repel or restrict access for *C. magnirama*, but not *Colomastix* sp. 1 due to their level of adaptation to this host. The unusually high densities of *P. cristatus* in unidentified sp. 1 sponges at Burnham Wharf were another interesting feature in this study. This is most likely another effect of host choice. *P. cristatus* is a filter feeding amphipod and could have additionally benefitted from heightened biomass and vertical distribution of organisms on the wharf piles at Burnham Wharf, as well as intermittent upheaval of detritus by tankers (Hughes, 2016). This sponge species was absent from the other sites, so it is impossible to

make an adequate comparison between sites to explain the significance of pollution to this phenomenon.

Sponge morphology was most likely influential in controlling amphipod density. Unidentified sponge species 1 harboured the greatest mean density of amphipods, which supports previous studies that demonstrated this to be true for branching and lobate species with high three-dimensional complexity (Kourkouras et al. 1996, Neves and Omena 2003; Amsler M. et al., 2009). *Callyspongia ramosa*, although branching, likely hosts reduced densities, because of the morphological and chemical characteristics of this sponge.

This study provides a first record of the use of sponge-associated amphipod communities to investigate water quality and pollution levels. There is strong evidence that amphipod evenness, H' diversity and community structure in sponge hosts may present valuable bioindicators of pollution. If sampling and processing methods were improved to increase efficiency, they could make useful models to study the effects of pollution, especially over long periods.

Chapter III

Synthesis and Conclusion

3.1 Summary

To monitor marine pollution, bioindicators are in high demand, even more so if they are cost effective, versatile and precise (Souza et al., 2020; Collins and Fahring, 2020). The use of host-associated invertebrate communities as bioindicators has recently been explored (Tian et al., 2014; Cabral et al., 2018). Amphipods are commonly used as bioindicators. Some amphipod families are associated with sponges and other hosts (Conlan, 1994; Poore et al., 2000; Zakhama-Sraieb et al., 2010; Hasenbein et al., 2018). These associations restrict their geographical range, making them easy to locate and sample. To assess the utility of sponge-associated amphipod communities as bioindicators, sponges were collected from three sites with varying levels of pollution in Wellington Harbour. They were dissected and their associated amphipod communities were identified to species level and individuals counted to calculate their densities. Amphipod length was measured, and sex determined. Amphipod density, H' diversity, community structure, age structure and sex ratio were then compared between pollution levels.

3 1.1 Were amphipod richness, evenness and H' diversity affected by pollution levels?

Species richness and evenness were both reduced by increasing pollution levels. Species evenness corresponded best with the supposed pollution increase between the sites. The occurrence of species dominance was reduced at less polluted sites. Species richness was similar between the intermediate and most polluted sites but still decreased with increasing pollution.

This study found good evidence that increased pollution levels negatively affected amphipod H' diversity, likely because of species' differing sensitivities. (Skilleter et al., 2005 ; Thompson et al., 2007; Amsler M. et al., 2009; Rigolet et al., 2011; Zaabar et al., 2015; Reutgard and Furuhaugen, 2016). While H' diversity differed between sponge species, both species present at the most polluted site could be compared between sites and showed a relevant decrease in H' diversity. The same trend was apparent when the intermediate and least polluted sites were compared although

the effect may have been attenuated by the increased habitat complexity at the intermediate site (Eriksson et al., 2006; Amsler et al., 2009).

3.1.1 Was amphipod community structure affected by pollution levels?

Amphipod community structure was significantly affected by pollution levels, as confirmed by both Bray Curtis Dissimilarity and ANOSIM. There were indications that specialized sponge commensals had an advantage at elevated pollution levels, as opposed to generalists. The most polluted site was dominated by two *Colomastix* species that are known commensals of sponges. Their small body sizes make them well suited to life in sponge canals. *Colomastix magnirama* was almost completely absent from the other sites, while *Colomastix* sp.1 was most abundant at the intermediately polluted site. These patterns could indicate competition between the two *Colomastix* species as a structuring force under high pollution. It is also possible that *Colomastix magnirama*'s high densities were facilitated by pollution interfering with chemicals produced by the sponge to repel spongivory (Amsler M. et al., 2009). Another known sponge commensal, *Leucothoe* sp. 1 was however uncommon at the most polluted site, possibly due to its large body size, which may have restricted its access to the sponges. There were increasing densities of generalists with decreasing pollution.

3.1.3 Were amphipod sex or age proportions affected by pollution levels?

In most amphipod species females were more common than males across all sites, as is common in amphipods, but particularly among the higher pollution levels (Prato and Bandolino, 2003). *Colomastix magnirama* stood out due to its particularly high female bias at the most polluted and moderate male skew at the intermediate site. In other studies sex ratio was influenced by microsporidian parasites which can cause feminisation in males (Ford et al., 2004; 2006; 2007). However, this was not further investigated. The mean proportion of adult individuals increased relative to the pollution level. Due to their diet, juveniles accumulate higher levels of heavy metals, which may have led to a reduction in offspring survival. It is also plausible that the large proportion of generalists at the cleaner sites used the sponges as nurseries, rather than adult habitats which could explain this trend. The seasonality of the lifecycle could not be assessed without repeated collections. Neither of these results were statistically significant but as the statistical tests were limited by a small sample size, the possibility of an impact of pollution on sex or age ratio should not be altogether rejected.

3.1.4 Was amphipod density affected by pollution levels?

There was no significant effect of pollution level on amphipod density, although differences were observed between sites. Amphipod densities in *Callyspongia ramosa* sponges decreased with increasing pollution. However, when only *Crella* sp. was considered, which occurred at all sites, amphipod densities at the highest and moderately polluted sites were similar, while the least polluted site harboured the lowest density. It is possible that the data may have been distorted because one of the sites differed from the others in that it was a natural structure and relatively sparsely populated by sessile organisms. This may have caused amphipod densities to be lower than they would have been at a more similar site due to reduced habitat complexity (Eriksson et al., 2006; Amsler M. et al., 2009).

3.2 Synthesis of findings

Coastal areas worldwide are subject to various forms of pollutions, especially in harbours (Smith et al., 2016; Bebbiano et al., 2015; Fowles et al., 2018; Lirn et al., 2013). Amphipods are frequently used as bioindicators to monitor pollution due to their high sensitivities, which often result in reduced densities at polluted sites (Conlan, 1994; Hasenbein et al., 2018). These sensitivities vary according to the species and the type of pollutant, therefore pollution can affect changes in amphipod community structure (Skilleter et al., 2005; Amsler M. et al., 2009; Reutgard and Furuhaugen, 2016). Amphipod communities limited in range by sessile hosts are potentially useful bioindicators. This thesis provides a first assessment of the practicality and usefulness of host-associated amphipod communities as bioindicators. This usefulness is examined by comparing amphipod communities from sponges collected at three different sites across Wellington Harbour. Pollution burdens for these sites were inferred from the sum of potential sources and proximity to human settlement.

These results indicate that amphipod communities have great potential as bioindicators. Amphipod H' diversity, richness, evenness and community structure give an accurate impression of local pollution levels. Based on this study, researchers can expect to find higher proportions of generalists and species dominance at more polluted sites. As species evenness of sponge-associated amphipod communities incorporates both aspects of species richness and dominance, it would be a particularly suitable indicator of pollution. Even though Wellington Harbour contains many sources of pollution, these levels are relatively low when compared to other harbours globally (Oliver and Conwell, 2017). As amphipod abundance is the commonly observed factor in amphipod bioindicators, it is possible that H' diversity, richness and evenness could have indicated pollution levels too low to reduce overall abundance. Unlike for most traditional bioindicators, total abundance may therefore not be the best measure for detecting pollution using sponge-associated amphipod communities in Wellington Harbour.

Other research supports the conclusions about the value of species richness, evenness and diversity as useful bioindicators (Conlan, 1994; Ali et al., 2018; Hasenbein et al., 2018). While sponge dissection is relatively time-consuming, the sponges provided a distinct framework for observations of community features which could easily be compared between sites. There are some interesting trends for female:male and adult:juvenile ratios apparent between pollution levels, although they are not useful factors to consider for a bioindicator, because they are much more time-consuming to investigate than they are telling.

3.3 Future research directions

An interesting aspect this study did not examine was the prevalence of spongivory between sites. In light of the effects of pollution on amphipod community structure and the subsequent increase of specialists, spongivory may be more commonplace at higher pollution levels. This could be tested by dissecting amphipod gut content to detect spicules. As this can be very difficult for smaller individuals, Hertwig's solution is sometimes used to make invertebrates transparent so that their gut contents can be viewed without having to dissect them (Navarro-Barranco et al., 2013). Another option would be stable isotope analysis which can give a more long-term account of the prevalence of sponge tissue in amphipod diets (Farlin et al., 2010).

In order to achieve rigorous statistical power, future studies should investigate the effect of pollution level on amphipod communities using at least 7 replicates per sponge species for each site. This number was calculated using the G* Power software tool for multiple regression at the 5% significance level (Bruin, 2006). Researchers should ensure that sample sites are as analogous as possible, all horizontal or vertical and covered in roughly comparable densities of sessile invertebrates. Ideally, future studies should include measurements of actual pollution levels at the time of collection. Due to natural variation and a plethora of factors affecting organisms *in situ*, the results of this study should be confirmed experimentally under controlled conditions. For this, sea-water tanks could be dosed with different levels of harbour typical loads of pollutants and sponges could be stocked with highly diverse amphipod communities. Over time, these amphipods communities should become similar to those sampled in this study.

3.4 Conclusion

This thesis provides a first account of the use of sponge-associated amphipod communities as bioindicators. Amphipod richness, evenness, H' diversity and community composition reliably reflected pollution levels at different sites. Particularly evenness provided good evidence for the effects of pollution. Increased pollution levels appeared to intensify the selection pressure of host specialization in amphipods, stepwise reducing the proportion of generalists between sites. This study found some evidence for intraspecific changes in amphipods subjected to high pollution levels as proportions of females and adults appeared to increase relative to pollution levels, although these trends were not statistically significant. These results suggest that sponge associated amphipod communities could be valuable bioindicators to help researchers in monitoring pollution levels.

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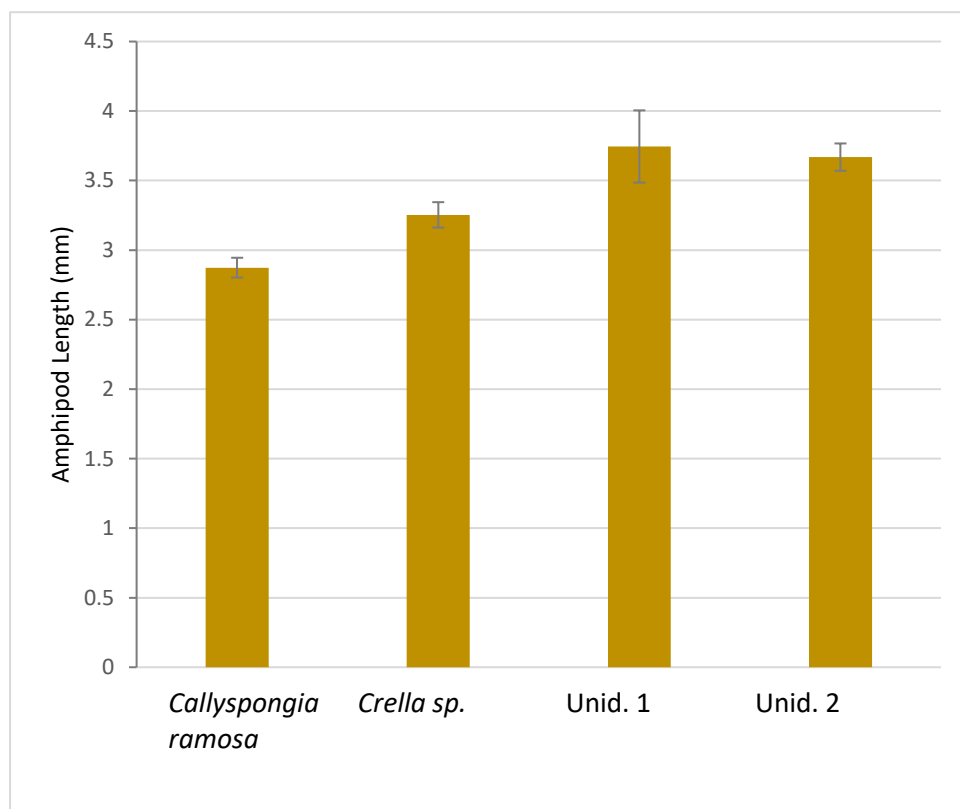
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Appendix



Supplementary figure 1: Mean amphipod length in different sponge species. Error bars show standard error.