Detecting anthropogenic impacts on estuarine benthic communities

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DANA CLARK

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"There's no limit to how much you'll know, depending on how far from zebra you go"

– Dr Seuss

Abstract

Our estuaries, and the benefits that we derive from them, are threatened by the cumulative effects of interacting stressors. Separating the impacts of anthropogenic stressors from natural variability in the marine environment is extremely difficult. This is particularly true for estuaries, due to their inherent complexity and the prevalence of difficult-to-manage diffuse stressors. Successful management and protection of these valuable ecosystems requires innovative monitoring approaches that can reliably detect anthropogenic stressor impacts. In this thesis, I examined approaches for detecting the effects of three diffuse land-derived stressors (sedimentation, nutrient loading, and heavy metal contamination) on estuarine benthic communities.

Using Gradient Forest analysis, I explored the relative importance of environmental factors, operating across multiple spatio-temporal scales, in influencing patterns of compositional turnover in estuarine benthic macroinvertebrate communities across New Zealand. Both land-derived stressors (sediment mud content and total sediment nitrogen and phosphorus content) and natural environmental variables (sea surface temperature, Southern Oscillation Index, and wind-wave exposure) were important predictors of compositional turnover, reflecting a matrix of processes interacting across space and time. Generalized linear models were used to link these turnover values to measures of benthic macroinvertebrate diversity, which are commonly used as indicators of ecological health. Based on compositional turnover, I could disentangle the negative effects of land-derived stressors from natural environmental variability. Critical stressor levels associated with high rates of compositional turnover were identified, providing a useful contribution to the current knowledge on land-derived stressor effects.

Once I had determined that anthropogenic impacts could be disentangled from natural variability, I developed indicators (Benthic Health Models; BHMs) to assess estuary health in response to two dominant coastal stressors (sedimentation and heavy metal contamination). Benthic macroinvertebrate community data were used in separate canonical analyses of principal coordinates to create multivariate models of community responses to these stressors. Both models performed well ($R^2 = 0.81, 0.71$), and were unaffected by regional and estuarine typology differences. They offer a sensitive and standardised approach to assessing estuarine health that allows separation of the two stressors.

I also examined the potential for emerging molecular approaches to inform estuary health assessment. Recent advances in environmental genomics allow characterization of less visible forms of benthic biodiversity, offering a more holistic view of the ecosystem and potentially providing early warning signals of disturbance. A manipulative nutrient enrichment experiment was conducted in two estuaries and environmental DNA (eDNA) metabarcoding was used to examine the response of eukaryotic (18S rRNA), diatom only (rbcL) and bacterial (16S rRNA) communities. Multivariate analyses demonstrated differential changes in examined communities between sites, suggesting a context dependent response to nutrient enrichment. These patterns aligned with changes in morphologically identified macroinvertebrate communities, confirming concordance between eDNA-based and current monitoring approaches. This work represents a first step towards the development of molecular estuary monitoring tools, which could transform current approaches to ecosystem health assessment.

This thesis demonstrates that the detection of anthropogenic impacts on estuarine benthic communities requires an understanding of the response of communities to stressors and how this response is modified by natural environmental processes operating at different spatio-temporal scales. My research contributes to the management and protection of estuaries by improving knowledge on the processes generating broad scale patterns in benthic macroinvertebrate communities, developing indicators that can be used to assess estuary health and demonstrating the potential of eDNA metabarcoding as a new tool for estuary health assessment.

Preface

This thesis comprises three research chapters (Chapters 2-4), which have been published in peer-reviewed international scientific journals. I was responsible for all field and laboratory work, data analysis and writing for this thesis. Unless referenced, the ideas in this thesis are my own and this work was carried out under the supervision of Professor Conrad Pilditch (University of Waikato), Dr Joanne Ellis (University of Waikato) and Dr Anastasija Zaiko (Cawthron Institute and the University of Auckland).

Chapter 2 has been published in Marine Ecology Progress Series under the title, "The influence of land-derived stressors and environmental variability on the compositional turnover and diversity of estuarine benthic communities" by **DE Clark**, F Stephenson, JE Hewitt, JI Ellis, A Zaiko, A Berthelsen, RH Bulmer, and CA Pilditch. DOI:10.3354/meps13714.

Chapter 3 has been published in Marine Pollution Bulletin Volume 150 (2020) under the title, "The development of a national approach to monitoring estuarine health based on multivariate analysis" by **DE Clark**, JE Hewitt, CA Pilditch, and JI Ellis. DOI: 10.1016/j.marpolbul.2019.110602.

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Chapter 1: General introduction

1.1 Stressors in estuaries

Estuaries are transitional environments that link terrestrial, freshwater, and marine ecosystems. Although they represent less than 1% of the marine environment, estuaries are among the most valuable of the world's ecosystems in terms of the goods and services they generate (Costanza et al. 1997). Ecosystem services provided by these complex coastal habitats range from food provision and recreational opportunities (Barbier et al. 2011), to coastal protection (Shepard et al. 2011, Duarte et al. 2013) and intangible physical, emotional, and mental benefits to human well-being (Martin et al. 2016). These benefits extend beyond the estuary margins and include processing contaminants from land (Dame et al. 1984, Herbert 1999), fuelling coastal productivity (Odum 2000, Savage et al. 2012), carbon sequestration (Nellemann et al. 2009) and providing juvenile nurseries for commercial offshore fisheries (Beck et al. 2001).

Many of these services are generated by the benthic communities that live within the seafloor sediments. Through their activities (e.g., feeding, bioturbation, burrow construction, sediment irrigation) these organisms affect a variety of ecological and environmental processes, including nutrient and sediment fluxes, primary and secondary productivity, and the transport and transformation of pollutants (Snelgrove 1997, Levin et al. 2001, Welsh 2003, Lohrer et al. 2012). They also connect sedimentary processes to the overlying water column and provide a critical link with higher trophic levels (Griffiths et al. 2017). Community structure underpins these ecosystem functions as certain taxa, such the large bivalves *Austrovenus stutchburyi* and *Macomona liliana*, contribute disproportionately to many of these processes (Thrush et al. 2006). Thus, the health and functioning of estuaries, and the services they provide, are fundamentally linked to the structure of the benthic communities that live within them.

Many of the ecosystem services estuaries deliver arise from their close connection to human populations. As a result, estuaries are one of the most heavily used and threatened ecosystems globally (Agardy et al. 2005, Lotze et al. 2006, Barbier et al. 2011). Estuaries are exposed to multiple stressors operating across local (e.g., coastal reclamation, anchoring) to global (e.g., climate change, invasive species) scales (Figure 1.1). Their coastal location makes them particularly vulnerable to land-derived stressors such as sedimentation, nutrient loading, and contaminants. These land-derived stressors often represent natural processes that begin to have negative effects when their rate of delivery exceeds the assimilative capacity of the system, often because of human activities. Inputs of land-derived sediments, nutrients and contaminants are frequently implicated in long-term degradative changes in estuaries (Thrush et al. 2003a). These stressors are generally diffuse, generating gradual accumulative changes over broad scales, although they can originate from point-source discharges (e.g., stormwater outfalls) and localised events (e.g., landslides, storms). As diffuse stressors, their incremental but pervasive effects can be subtle, compared with abrupt changes observed in response to point-source stressors (e.g., wastewater outfalls) or physical disturbance (e.g., dredging), making management of these stressors challenging. However, cumulatively, across large spatio-temporal scales, diffuse land-derived stressors can drive substantial disruptions to ecosystem functioning. Abrupt shifts in ecosystem functioning can also occur if a tipping point is reached (Hewitt & Thrush 2019) or in response to extreme pulse events (e.g., Thrush et al. 2003a).

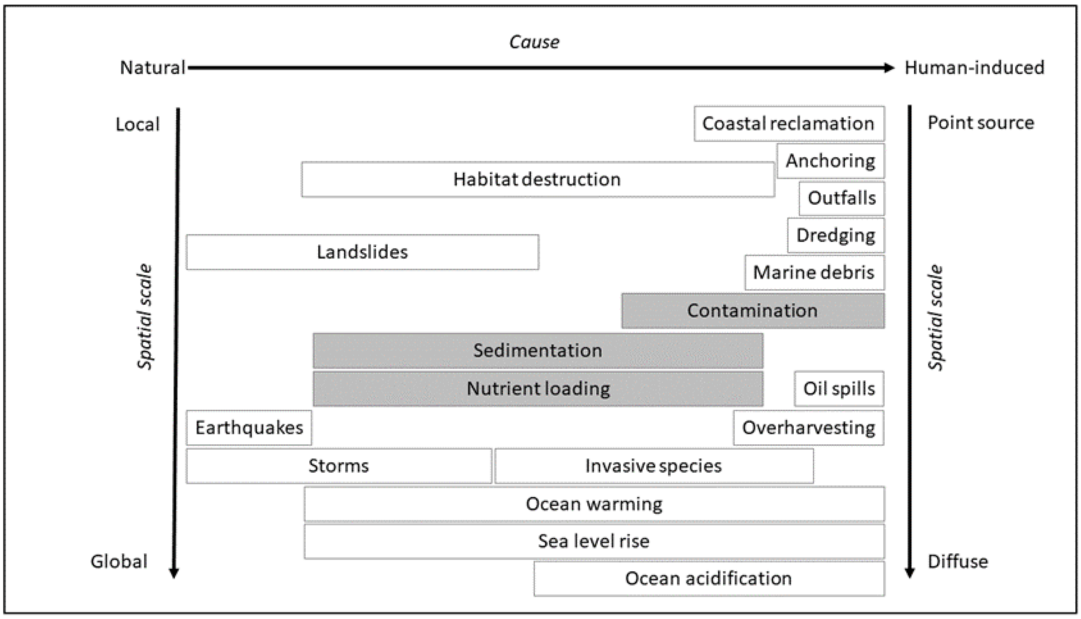

Figure 1.1 Selection of stressors and disturbance events/activities affecting estuaries, arranged along gradients representing the degree of anthropogenic influence and the spatial scales across which they operate. This thesis focuses on contamination, sedimentation, and nutrient loading (shaded in grey).

1.1.1 Key land-derived stressors

Although estuaries are natural reservoirs for terrestrial sediments changing land-use practices associated with forestry, urbanization, and agriculture have caused sedimentation to become a serious threat to estuarine and coastal systems worldwide (GESAMP 1993, Thrush et al. 2004, Magris & Ban 2019). Erosion rates are estimated to have increased by a factor of 2-10 (Saunders & Young 1983), greatly accelerating sediment delivery to our coasts. Sedimentation rates in Chesapeake Bay, for example, have increased by an order of magnitude since land clearance began in 1760 (Cooper & Brush 1993). Sediment inputs can occur through pulse disturbances associated with major events (e.g., storms, earthquakes) or more gradually over time (e.g., with typical river input). Fine sediments (‘mud’; grain-size < 63 µm) modify the physical environment by increasing water turbidity and changing the median sediment grain-size, which limits light penetration and affects biogeochemical fluxes and the porosity and stability of seafloor sediments (Norkko et al. 2002b). These changes can have adverse effects on estuarine communities by directly smothering organisms (Norkko et al. 2002b), altering food quality (Cummings et al. 2003), clogging filter-feeding structures (Ellis et al. 2002), affecting larval settlement (Rhoads & Young 1970) and reducing benthic primary production (Pratt et al. 2013). Ultimately, long-term accumulation of fine sediments results in a loss of biodiversity and a reduction in functioning as species are lost and communities and habitats are homogenised (Thrush et al. 2003b).

Human activities, such as agriculture and domestic sewage discharges, are also generating major changes in the amount of nutrients received by estuaries. Increased nutrient loads can initially stimulate production in a beneficial way but if concentrations surpass the assimilation capacity of the estuary, serious adverse effects can result via eutrophication. Eutrophication occurs when excess nutrients promote the growth of phytoplankton and opportunistic algae (e.g., sea lettuce), increasing organic inputs to the seabed. Decomposition of this additional organic material consumes oxygen, resulting in oxygen depletion in benthic habitats and the overlying water column. This process can cause declines in water quality, shifts in species diversity and functioning, loss of submerged aquatic vegetation, occurrences of harmful algal blooms, and mass mortalities of fish and benthic organisms (Smith 2003, Bricker et al. 2014). Eutrophication is now recognised as one of the greatest threats to coastal ecosystem health globally (Howarth & Marino 2006), with two thirds of estuaries in the United States moderately to highly eutrophic (Bricker et al. 2008) and a third of European estuaries affected by nutrient enrichment (EEA 2012).

Contamination, arising from catchment run-off or ocean-based activities, is another key estuarine stressor, particularly in urban areas. Contaminants include metals, oils, and persistent organic pollutants (e.g., dichlorodiphenyltrichloroethane (DDT), polycyclic aromatic hydrocarbons (PAHs) and personal care products). Many of these pollutants have long half-lives so persist in the marine environment, where their concentrations and effects continue to increase. Some chemicals bioaccumulate through food webs, becoming more concentrated and detrimental at higher trophic levels (Bryan et al. 1979). Due to the differing sensitivities of organisms to various contaminants (Ellis et al. 2017), exposure can lead to shifts in community composition. Ecosystem functioning can also be altered through behaviour changes, reproductive failures, cancers, deformations, immune suppression, and local species extinction (see Johnston et al. 2015 for a review).

1.1.2 Interactions between multiple stressors

Coastal stressors do not act in isolation, with estuaries inevitably exposed to an increasing number of potential interacting stressors, the mechanisms and cumulative effects of which are poorly understood (Cairns et al. 1993, Crain et al. 2008, Darling & Côté 2008). Interactions between overlapping stressors may increase (synergistic) or dampen (antagonistic) individual stressor effects. For example, a study examining the combined effects of sedimentation, nutrients and metal loading on estuarine macroinvertebrate diversity found that metal loading may exacerbate the impact of fine sediments (synergistic effect) but that low levels of nutrient enrichment may help to offset the negative effects of increasing mud and contaminants (antagonistic effect; Ellis et al. 2017). In addition to interacting with each other, stressors can also interact with natural temporal and spatial environmental variations (e.g., El Niño; Hewitt & Thrush 2010), further complicating both the assessment of specific effects and management decisions. Predicting the outcomes of multiple cumulative stressors is challenging given the range of possible stressors and sources of natural variation, and their potential to interact in a variety of ways. Hence, most estuarine studies (93%) still focus on single-stressor effects (O'Brien et al. 2019) and very few consider these effects within the context of natural processes acting across broader geographic and time scales (but see Hewitt & Thrush 2009, de Juan & Hewitt 2011, Denis-Roy et al. 2020).

1.2 Estuarine health assessment

1.2.1 Estuarine health

Due to its human-centric nature, estuarine ecosystem health can be defined in many ways depending on the values that people hold (Costanza 1992, O'Brien et al. 2016). In this thesis, a healthy estuary is defined as one that is able to deliver the ecosystem services that humans value, even under changing environmental conditions. This definition of health is characterised by three key elements of ecological communities: (i) structure, (ii) function, and (iii) resilience. Community structure refers to the abundance and diversity of organisms living within the ecosystem and how they relate to each other (Adey & Loveland 2007). These organisms carry out the functions that underpin the ecosystem services that humans rely on. Functions are combinations of biological, geochemical and physical processes that transform and translocate energy or materials in an ecosystem (Naeem 1998, Gladstone-Gallagher et al. 2019b), such as nutrient cycling (Lohrer et al. 2004), production (Thrush et al. 2006) and decomposition (Levin et al. 2001). Resilience refers to the ability of an ecosystem to maintain community structure and function over time, even in the presence of environmental perturbations or stress (Holling 1973, Mageau et al. 1995, Costanza & Mageau 1999, Gladstone-Gallagher et al. 2019b). These three components are tightly interwoven with highly diverse communities likely to deliver higher and more efficient functioning and have greater capacity to resist change or recover from disturbance. Thus, estuarine ecosystem health can be assessed by characterising changes in community structure, function, and resilience. It should be acknowledged that this definition represents only one view of estuarine health and other definitions are possible, such as those based on the knowledge systems of indigenous people (e.g., Tipa & Teirney 2006, Akins et al. 2013).

1.2.2 Current approaches for assessing estuarine health

Environmental assessment is increasingly required by management regulations (e.g., the Clean Water Act or Oceans Act in USA, Australia or Canada; Water Framework Directive or Marine Strategy Framework Directive in Europe, and National Water Act in South Africa; Borja et al. 2008) to assess ecological health, monitor trends over time, diagnose causes of degradation, assess the efficacy of management actions, and provide warning signals for impending ecological shifts. In New Zealand, regional councils are responsible for managing environmental effects in the coastal marine area under the

Resource Management Act (RMA 1991) and the New Zealand Coastal Policy Statement (NZCPS 2010). Historically, environmental policy and management goals centred on reducing the level of pollution entering natural systems (Cairns et al. 1993). Accordingly, estuarine environmental assessment focused on the measurement of physical-chemical variables that were expected to be influenced by human activities, such as sediment grain-size and water column nutrient concentrations. These indicators are in use today because they are generally quick and inexpensive to measure. However, consideration of physical-chemical variables on their own is of limited value because changes in these variables do not directly translate to changes in ecosystem health and functioning (Cairns et al. 1993). In addition, such measurements provide only a snapshot of environmental conditions as it is impossible to measure all anthropogenically-influenced variables, and many of those that can be measured vary highly in space and time (i.e., water quality variables; Tay et al. 2012).

In order to understand ecosystem change and avoid ecological surprises, indicators based on ecosystem responses are required (i.e., biotic indicators; Thrush et al. 2016). Two approaches exist for assessing ecosystem responses to anthropogenic stress (Cairns et al. 1993). 'Bottom-down' methods use simple systems in laboratories to predict changes in complex natural systems. Water quality criteria based on single-species laboratory dose-response experiments (e.g., Chapman 1995) are an example of this approach. These methods have limited ability to predict ecosystem change due to the difficulties of inferring the response of complex natural ecosystems from simple biological test systems (Cairns 1995, Underwood 1995). Additionally, these controlled laboratory studies rarely consider natural variation (e.g., temperature fluctuations, El Niño effects) and typically focus on single-stressor effects (O'Brien et al. 2019).

Limitations of 'bottom-down' approaches to environmental assessment has seen a shift to 'top-down' methods that directly assess ecological community change in the natural environment, and subsequently diagnose the causative agents of problems (Cairns et al. 1993). This ecosystem response data can be collected from correlative environmental surveys (e.g., Ysebaert et al. 2002, Sánchez-Moyano et al. 2010) or manipulative field experiments (e.g., Olsgard 1999, Fukunaga & Anderson 2011). Most 'top-down' environmental monitoring is in the form of environmental surveys, where periodic direct observations of communities in their natural environment are related to physical-chemical variables. These samples can be collected repeatedly from multiple locations, providing information about processes operating over broad spatio-temporal scales and the ability to generalise results. Although environmental surveys cannot be used to prove causality, they are useful for generating hypotheses about possible drivers of observed patterns that can be tested through manipulative experiments. Manipulative field studies provide information on cause and effect while accounting for the complexities of the natural environment. However, funding and logistical constraints limit the number of variables that can be manipulated, and the scales at which experiments can be conducted. These approaches can complement each other when manipulative studies are nested within a correlative framework (Hewitt et al. 2007). For example, Thrush et al. (2020) carried out a manipulative nutrient enrichment experiment in 15 estuaries, spanning a natural gradient in water turbidity, to examine the interactive effects of nutrient loading and sedimentation on estuarine functioning.

'Top-down' environmental monitoring in estuaries generally relies on benthic macroinvertebrates (i.e., animals > 0.5 mm living within the sediments) as a measure of ecosystem response because they span multiple trophic levels, are predominantly sedentary as adults, have species-specific sensitivities to stressors and integrate the effects of multiple stressors over time (Pearson & Rosenberg 1978, Gray et al. 1979, Dauer 1993). These animals are also an important component of estuarine systems, playing essential roles in ecosystem structure and function (e.g., nutrient cycling, energy transfer to higher trophic levels, sediment stabilization; Snelgrove 1997, Levin et al. 2001, Lohrer et al. 2004). Anthropogenic stressors alter the trophic and functional structure of benthic macroinvertebrate communities and cause changes in their diversity, biomass, and the relative abundance of tolerant and sensitive species (Pearson & Rosenberg 1978, Warwick 1986, Warwick & Clarke 1994, Kaiser et al. 2000). These ecosystem responses can be simplified and quantified using a variety of biotic indicators (reviewed in e.g., Diaz et al. 2004, Teixeira et al. 2016), facilitating communication with stakeholders and policy makers.

The first biotic indicators were simple community metrics such as the number of taxa or individuals, and measures of community evenness and diversity (e.g., Shannon 1948, Margalef 1958, Pielou 1966). These indicators were assessed against the Pearson and Rosenberg (1978) model of macrobenthic succession which describes how communities are expected to change along an organic enrichment gradient (i.e., moving toward a community characterised by high abundances of a few species and low diversity). Although useful for detecting large scale changes, these indicators have limited sensitivity because they do not differentiate between individual taxa responses (Cairns et al. 1993, Hewitt et al. 2005a, Ellis et al. 2015, Shade 2016).

The growing requirement to assess estuarine health over the past two decades has led to a proliferation of more complex biotic indices that integrate information on species sensitivities to stress (Diaz et al. 2004, Borja et al. 2015). These indices generally allocate taxa into pre-defined ecological groups, based on their expected response to stress, and calculate a measure of overall health using the relative proportion of taxa in each group (e.g., Grall & Glémarec 1997, Borja et al. 2000, Simboura & Zenetos 2002). More sensitive indicators of community change are provided by multivariate approaches (e.g., ordination-based techniques; Clarke 1993, Smith et al. 2001, Flåten et al. 2007) because they retain information on all taxa and their relative abundances (Gray et al. 1990, Warwick & Clarke 1991, Attayde & Bozelli 1998, Hewitt et al. 2005a, Ellis et al. 2015). With the recent shift toward more holistic marine ecosystem management objectives, there has also been increasing attention on evaluating ecosystem function as a complement to assessments based on community structure (Bremner 2008). Classifying species by their functional traits (e.g., Hewitt et al. 2008, Bon et al. 2021) allows ecosystem resilience and redundancy to be evaluated and provides a clearer mechanistic link to ecosystem services.

Indicators can be responsive to many stressors (e.g., Borja et al. 2000) or diagnostic of a particular stressor (e.g., Keeley et al. 2012, Robertson et al. 2016), but rarely both. Multi-stressor indicators are often favoured because they indicate the overall health of a system whilst accounting for interactions amongst stressors. However, management responses are unlikely to be effective or cost-efficient if degradation cannot be attributed to a specific source (Cairns et al. 1993, Niemi et al. 2004, Martinez-Crego et al. 2010).

Furthermore, multi-stressor indicators tend to rely on expert judgement due to the challenge of accurately quantifying relationships between communities and multiple stressors while accounting for interactions (Crain et al. 2008, Darling & Côté 2008) and non-linear responses (deYoung et al. 2008). In contrast, stressor-specific indices can be developed from robust empirical relationships between benthic communities and the stressor of interest, providing managers with an objective assessment of health. These single-stressor indices can be used within a broader indicator framework to assess overall changes in estuarine health (e.g., Aubry & Elliott 2006).

Biotic indicators all rely on the principle that ecological communities will exhibit characteristic changes along a gradient of stress. However, differentiating human-induced community changes from those caused by strong natural environmental gradients is difficult, particularly in estuaries which are often described as being ‘naturally stressed’ due to the high degree of variability in their physical-chemical characteristics (Elliott & Quintino 2007). Estuarine macroinvertebrate distributions can be influenced by a range of physical-chemical variables, including sediment grain-size, organic content and current dynamics (Thrush 1991). In addition to local scale changes, estuarine communities are also influenced by processes occurring over broader spatio-temporal scales, for example latitudinal temperature gradients (Engle & Summers 1999, Denis-Roy et al. 2020), climate cycles (Hewitt & Thrush 2009, Hewitt et al. 2016), differences in regional species poools (Bilton et al. 2002, Grantham et al. 2003), and estuary geomorphology (Barbone et al. 2012, Berthelsen et al. 2020a). In order to understand whether observed changes in benthic communities are indicative of degradation in ecosystem health or merely a result of natural environmental variation, this complex web of factors needs to be disentangled.

Developing site-specific indicators, or carrying out manipulative experiments on a local scale, helps to reduce this environmental noise, providing increased power to detect the stressor effect of interest. For example, Lawes et al. (2016a) investigated the combined effects of copper antifouling paint and fertiliser on the development of invertebrate assemblages at a single site within Sydney Harbour. However, results from such studies are only useful in a local context, as interactions with broad scale processes can alter the strength, and sometimes even the direction, of responses (Thrush et al. 1996, Hewitt et al. 2007). Many ecological questions, particularly those concerning diffuse human impacts, require generalisation over wider spatial and temporal scales (Thrush et al. 1997). Such questions can be answered using well designed correlative studies that encompass environmental gradients and measure potential confounding variables (Hewitt et al. 2007). This approach converts spatio-temporal variability into useful information that helps us understand why responses vary from place to place and in doing so, allows generalisation of study results (Hewitt et al. 2007).

Effective environmental management requires broadly applicable indicators that can assess estuarine health irrespective of location (Borja & Dauer 2008, Elliott 2011). Indicators that can detect change while accounting for the factors that affect communities at various spatio-temporal scales allow the health of an estuary to be placed in a wider (e.g., national or international) context. This provides managers with a more complete picture of cumulative impacts and natural variability, enabling prioritization of management actions (e.g., Heinz Center 2002, Kristensen et al. 2013, Van Niekerk et al. 2013, Schiff et al. 2015). Broadly applicable indicators also reduce the need for calibration when new sites are introduced into monitoring programmes. Although many broadly applicable biotic indices are now available for coastal assessment (see Teixeira et al. 2016 for a review), many of these were developed in Europe and the USA and may not be transferrable to other regions due to differences in species ecology and composition, stressor type or magnitude, or estuary geomorphology (Van Hoey et al. 2010, Rodil et al. 2013, Gillett et al. 2015). For example, biotic indices developed using data from New Zealand were found to outperform those developed overseas in terms of their ability to detect anthropogenic stress in New Zealand estuaries (Berthelsen et al. 2018). However, even indices that were developed specifically for New Zealand conditions were found to be strongly influenced by natural and unexplained variation, limiting their usefulness for estuary management (Berthelsen et al. 2018). Therefore, there is still a need to develop standardised and sensitive indicators of estuary health that can be applied across New Zealand.

1.2.3 Emerging approaches for assessing estuarine health

Conventional monitoring based on morphological identification of macroinvertebrates provides a reliable, time-integrated picture of recent environmental influences. However, this approach is restricted by collection and processing costs and requires taxonomic expertise that is in decline worldwide (Jones 2008, Keeley et al. 2018). Assignments to species level can be challenging, even for experienced taxonomists, due to the difficulties of identifying taxa in the absence of key body parts or morphologically distinguishing immature stages (e.g., larval, juvenile) or cryptic species (Lobo et al. 2017). The high costs, slow turnaround times, and lack of taxonomic resolution hinders effective environmental assessment and our ability to upscale biomonitoring to meet increasing demand (Cordier et al. 2020). Critically, inferring ecosystem health solely from the visible portion of communities also neglects the contribution of ubiquitous smaller organisms (e.g., bacteria, microalgae, meiofauna), which play essential roles in ecosystem functioning (Azam & Malfatti 2007, Schratzberger 2018) and are extremely responsive to environmental change (Laroche et al. 2016, Aylagas et al. 2018, Keeley et al. 2018, Pochon et al. 2020).

Recent advances in environmental genomics allow characterization of biodiversity across the tree of life, with the potential to transform biomonitoring via cost-efficient access to a wealth of biodiversity information. Environmental DNA (eDNA) metabarcoding provides information on biological communities via the analysis of genetic material present in environmental samples (e.g., water or sediment; Ruppert et al. 2019). Metabarcoding could complement the information provided by traditional benthic macroinvertebrate community composition by integrating other ecosystem components (e.g. bacteria, microalgae, meiofauna) and thereby provide a more comprehensive understanding of the connection between stressors, biodiversity and ecosystem functioning (Strong et al. 2015).

Due to their higher turnover rates, considerable shifts are expected to occur in microbial communities before noticeable stressor-induced changes occur in macroinvertebrate communities (Cairns et al. 1993). This sensitivity highlights the potential to develop indicators that provide early warning signals of approaching tipping points. Detecting community change in response to low levels of impact is a crucial step in the advancement of modern biomonitoring as it would allow for implementation of management or remediation strategies at an early stage, increasing the effectiveness of these actions (Birrer et al. 2017).

Research demonstrating the potential of eDNA for biomonitoring is rapidly growing worldwide (Pawlowski et al. 2018, Aylagas et al. 2020, Cordier et al. 2020) but the suitability of this approach is primarily derived from correlative studies (e.g., Chariton et al. 2015, Abad et al. 2017, Cordier et al. 2017, Armstrong & Verhoeven 2020, Montenegro et al. 2020) or experimental studies carried out in laboratory settings (e.g., Chariton et al. 2014, Santi et al. 2019). Manipulative field studies are rarer (although see Lawes et al. 2017, Birrer et al. 2019) but are required to prove cause and effect, characterise the response of specific taxonomic groups to selected stressors, identify potential indicator taxa for ecological status assessment and demonstrate that these effects can be consistently detected over and above natural environmental variability.

The development of eDNA-based indicators for estuarine health assessment is still in its infancy. Aylagas et al. (2014) developed a genetics-based version of the widely used AZTI Marine Biotic Index (gAMBI; Borja et al. 2000) and a similar index based on bacterial community composition (microgAMBI) has also been developed and tested globally (Aylagas et al. 2017, Borja 2018). These approaches are constrained, however, by their reliance on incomplete metabarcoding reference sequence libraries for taxonomic assignment and the use of expert judgement to assign ecological groups (Cordier et al. 2020). Judging how bacteria respond to stress is difficult due to our limited knowledge regarding the ecological roles and responses of microorganisms to anthropogenic stress.

Taxonomy free *de novo* approaches using indicator value (e.g., IndVal, Threshold Indicator Taxa Analysis, quantile regression splines; Dufrene & Legendre 1997, Anderson 2008, Baker & King 2010) or supervised machine learning (e.g., Random Forests; Breiman 2001) methods can overcome this limitation through eco-group-based profiling of communities and independently generated ecological status or known disturbance gradients (Cordier et al. 2020). For example, Lanzen et al. (2020) demonstrated the utility of two *de novo* approaches for estuary health indicator development using correlative survey data. However, like most proof-of-concept studies using *de novo* approaches, this work has not yet been validated and requires further development before it can be applied to a wider range of geographical areas. Additional empirical research examining the response of different benthic communities to selected pollutants, ideally using manipulative field studies to demonstrate causality, is required to improve our understanding of how communities respond to stress under varying natural conditions.

1.3 Thesis overview

My thesis investigates approaches for detecting anthropogenic impacts on estuarine benthic communities, to enhance management and protection of these valuable ecosystems (Figure 1.2). Broad scale degradation arising from diffuse land-derived stressors presents a particular challenge to environmental management because the gradual but pervasive effects of these stressors can be difficult to separate from background variation. Accordingly, my thesis focuses on detecting the effects of three dominant land-derived stressors impacting estuaries; sedimentation, nutrient loading, and metal contamination (Howarth & Marino 2006, Johnston et al. 2015, Magris & Ban 2019). Community responses to these anthropogenic stressors are shaped by interactions with natural environmental gradients that vary across local (e.g., wind-wave exposure, sediment grain-size), regional (e.g., regional species pools) and national (e.g., sea surface temperature, Southern Oscillation Index, estuary geomorphology) scales. Understanding how these stressors affect community responses against a background of natural variability acting at multiple scales is critical for predicting their impacts and developing broadly applicable estuarine health indicators. Although temporal variation is not explicitly examined, I make use of long-term datasets that integrate the effects of these stressors over multiple years.

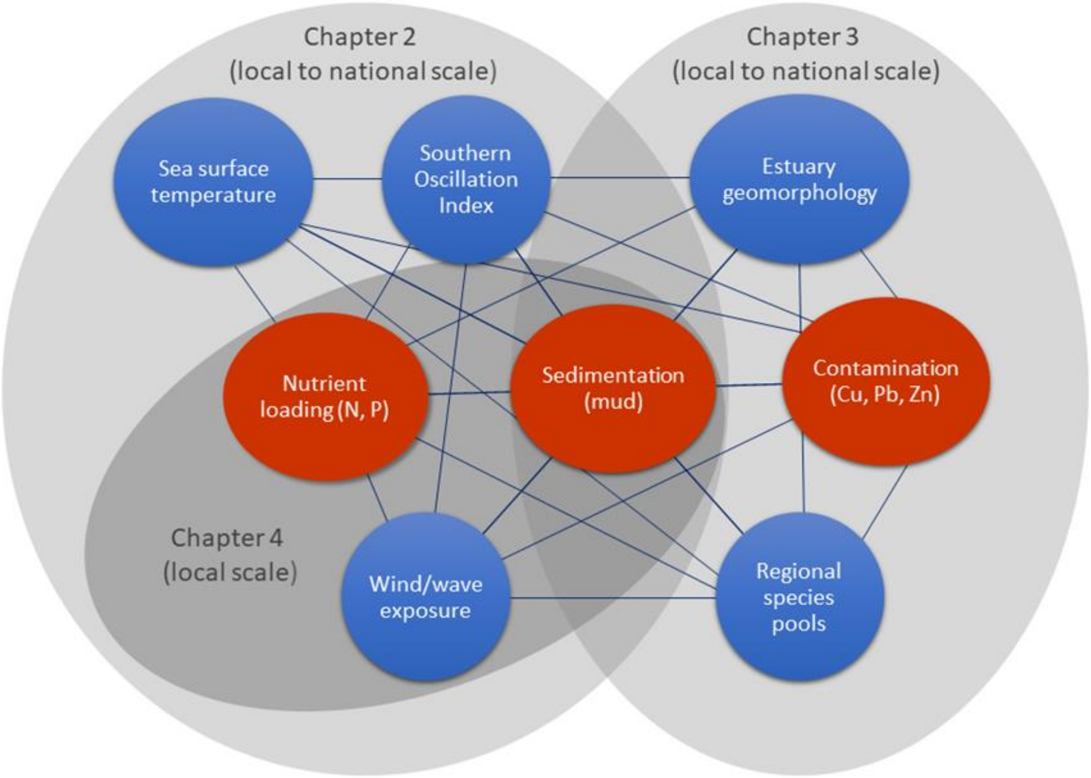

Figure 1.2 Diagram illustrating a subset of the complex web of factors affecting estuarine benthic community structure and how these are examined across different spatial scales in the chapters of this thesis. Blue indicates variables that are primarily influenced by natural variation while orange indicates important land-derived stressors and variables that act as a proxies for them (Cu = copper, Pb = lead, Zn = zinc, N = nitrogen, P = phosphorous).

Specifically, the objective of Chapter 2 was to explore the relative importance of environmental factors, operating across multiple spatio-temporal scales, in influencing patterns of compositional turnover in estuarine benthic macroinvertebrate communities across New Zealand. Gradient Forest analysis was used to separate the effects of land-derived stressors (sedimentation and nutrients) and natural environmental variables (Southern Oscillation Index, sea surface temperature, wind-wave exposure) on compositional turnover. Compositional turnover was then linked to measures of benthic macroinvertebrate diversity using generalized linear models to demonstrate how these environmental factors influence ecological health. This study enabled me to disentangle the effects of multiple land-derived stressors from natural variability across several spatio-temporal scales and identify critical stressor levels associated with high rates of compositional turnover.

In Chapter 3, I developed sensitive and standardised indicators of estuary health that can be applied at a national scale. Benthic macroinvertebrate community data were used in separate canonical analyses of principal coordinates to create multivariate models of community responses to two dominant coastal stressors (sedimentation and heavy metal contamination). To determine how broadly applicable these indicators were, I examined how natural variations in regional species pools and estuary geomorphology influenced model outputs. I also explored how spatial scale affects estuarine health assessment, by comparing outputs from these models to outputs from models developed using regional and local scale data.

The overall objective of Chapter 4 was to determine the potential of eDNA metabarcoding as a tool for estuary health assessment by conducting a manipulative nutrient enrichment experiment in two environmentally distinct estuaries. After seven months of enrichment, eDNA metabarcoding was used to examine the response of eukaryotic (18S rRNA), diatom only (rbcL) and bacterial (16S rRNA) communities. I also explored whether eDNA-derived community responses broadly align with those obtained using conventional morphological identification of benthic macroinvertebrates. This work represents a first step towards the development of molecular-based estuary monitoring tools, which could provide a more holistic and sensitive approach to ecosystem health assessment with faster turn-around times and lower costs.

My thesis concludes with a general discussion (Chapter 5), that synthesizes the findings of the three research chapters.

Chapter 2: The influence of land-derived stressors and environmental variability on the compositional turnover and diversity of estuarine benthic communities

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2.1 Introduction

Understanding the influence of human activities on coastal ecosystems requires the separation of natural and anthropogenic sources of environmental variability. Partitioning these effects is particularly difficult in estuaries, due to the inherent complexity of these ecosystems, which are highly variable in both space and time (Elliott & Quintino 2007, Dauvin & Ruellet 2009). The impact of human activities is often assessed using benthic macroinvertebrate communities because they cover numerous trophic levels, exhibit different stress-tolerances, and can integrate the effects of multiple stressors over time (Pearson & Rosenberg 1978, Dauer 1993, Borja et al. 2000). These animals are also an important component of estuarine systems, playing essential roles in ecosystem structure and function (e.g., nutrient cycling, energy transfer to higher trophic levels, sediment stabilization; Snelgrove 1997, Levin et al. 2001, Lohrer et al. 2004). However, it can be challenging, particularly at large scales, to differentiate community changes caused by stressors from the influence of strong natural environmental gradients.

Estuarine benthic community structure is influenced by a range of natural, temporally varying factors, that operate at local (e.g., wind-wave exposure, sediment grain-size, salinity, and predation; Snelgrove 2001) and broad (e.g., temperature, climate patterns; Engle & Summers 1999, Hewitt et al. 2016, Denis-Roy et al. 2020) spatial scales. Many of these natural factors can also be considered anthropogenic stressors when they exceed their natural range of variation as a result of human activities (Sanderson et al. 2002, Halpern et al. 2007). Estuarine communities are often exposed to multiple and cumulative stressors, and these commonly interact in multiplicative and non-linear ways (Crain et al. 2008, Darling & Côté 2008, deYoung et al. 2008). Many of these stressors are diffuse, operating in incremental stages and often over broad scales, particularly land-derived stressors like sedimentation and nutrient loading.

Land-derived stressors often represent natural processes that have been greatly accelerated by human activities and begin to have negative effects when their rate of delivery exceeds the assimilative capacity of the system. Sedimentation and nutrient loading are recognized as major threats to the health and functioning of estuaries globally (NRC 2000, EU Marine Strategy Framework Directive 2008, Magris & Ban 2019). For example, sedimentation rates have increased by one to two orders of magnitude in some places (Thrush et al. 2004), and 30-60% of estuaries in the United States and Europe are affected by nutrient enrichment (Bricker et al. 2008, EEA 2012). Adverse effects arising from these stressors (e.g., smothering of benthic communities, reduction in water quality; Valiela et al. 1992, Ellis et al. 2002) often manifest as reductions in species richness, evenness, and diversity, and a loss of rare taxa (Smith & Kukert 1996, Lardicci et al. 1997, Tagliapietra et al. 1998, Thrush et al. 2003b, Ellis et al. 2004, Hewitt et al. 2010). Community changes caused by land-derived stressors in the short-term are often subtle, but cumulatively, across large spatio-temporal scales, they can drive substantial disruptions to ecosystem functioning. Sometimes stressors can also cause abrupt shifts in ecosystem functioning if a tipping point is reached (Hewitt & Thrush 2019) or in response to extreme pulse disturbances (e.g., storms; Thrush et al. 2003a).

Disentangling this complex web of factors is critical for understanding whether observed changes in benthic communities are indicative of degradation in ecosystem health or merely a result of natural environmental variation. Although the need to account for natural variability has been identified in regulatory documents such as the European Water Framework Directive (2000), and is integral to ecosystem-based management (Arkema et al. 2006), it is seldom incorporated into assessment protocols due to the difficulty of teasing these factors apart and a perceived need to keep things simple (Irvine 2004). The influence of stressors and environmental variables operating on local scales needs to be considered within the context of processes acting across broader geographic and time scales within which the community is embedded (Ricklefs 1987). Such studies are uncommon in estuaries (but see Hewitt & Thrush 2009, de Juan & Hewitt 2011, Denis-Roy et al. 2020) because they require good spatio-temporal data along with methods that can quantify community response across multiple environmental gradients, while accounting for potential non-linearity and interactions among environmental variables.

New Zealand spans three water masses, 15 degrees of latitude, and a variety of estuary types, providing an ideal place to investigate estuarine community responses under a range of environmental conditions. I used Gradient Forest (GF) analysis (Ellis et al. 2012) to separate natural and anthropogenic drivers of benthic macroinvertebrate compositional turnover using a large nation-wide estuary monitoring dataset. In particular, I was interested in the community response to two pervasive land-derived stressors acting at a local (site) scale (sedimentation and nutrient loading) and three natural environmental variables representing both broad scale (national) climate fluctuations (sea surface temperature and Southern Oscillation Index) and local scale processes (wind-wave exposure). Although I have classified these variables as either land-derived stressors or natural environmental variables for the purposes of this study, I acknowledge that they could be considered as either natural components of the system or human-induced stressors, depending on values relative to background levels.

GF is one of several of statistical approaches that can be used to model constrained relationships between communities and their environments (e.g., canonical correspondence analysis (CCA), multivariate regression trees (MRT), generalized dissimilarity modelling (GDM); reviewed by Ferrier & Guisan 2006). It has been used to explore marine ecosystem response to anthropogenic and environmental pressures (e.g., Large et al. 2015, Samhouri et al. 2017, Couce et al. 2020) because it can model non-linear response shapes, deal with correlated predictors and incorporate complex interactions between multiple predictors (Ellis et al., 2012). It does this by combining information from multiple tree-based regression models (Random Forests), one for each taxon, to provide a measure of compositional turnover across environmental gradients. Compositional turnover, sometimes referred to as beta diversity, is the component of regional biodiversity that accumulates due to inter-site variation in local species assemblages (Anderson et al. 2011, Socolar et al. 2016). Examining patterns in compositional turnover is important for identifying and understanding the processes that maintain species diversity across large spatial and temporal scales (Ricklefs 1987, Soininen 2010). For example, the large natural environmental gradients in sea surface temperature and wind-wave exposure across this New Zealand-wide dataset would be expected to generate changes in turnover as community composition changes on a local scale.

Although compositional turnover provides us with a measure of change in benthic communities in response to different environmental variables, it does not provide information on whether these changes translate into positive or negative effects on benthic communities on a local scale (Socolar et al. 2016). For example, the early stages of anthropogenic impact may cause localised species loss leading to an increase in compositional turnover. However, anthropogenic impacts can also reduce compositional turnover rates, such as occurs when bottom-trawling destroys microhabitats leading to homogenization of benthic communities (Hewitt et al. 2005b). Therefore, I used generalized linear models (GLMs) to link compositional turnover along each of these environmental gradients to measures of benthic macroinvertebrate diversity (i.e., species richness, evenness, diversity, and numbers of rare taxa), which are commonly used as indicators of ecological health on a local scale.

I hypothesised that:

1) Both land-derived stressors and natural environmental variables will be important in predicting patterns of compositional turnover in estuarine benthic macroinvertebrate communities, reflecting a matrix of processes acting at different scales;

2) Compositional turnover along land-derived stressor gradients will have a negative relationship with species richness, evenness, diversity, and numbers of rare taxa.

2.2 Methods

2.2.1 Study sites

Data were obtained from estuarine monitoring surveys undertaken between 2001 and 2017 by New Zealand's regional government authorities (334 site/times sampled across 208 sites in 34 estuaries; Berthelsen et al. 2020b, Berthelsen et al. 2020c). The study sites (Figure 2.1; Appendix 1) spanned 12 degrees of latitude, three geomorphological estuary types (tidal lagoons, shallow drowned valleys, deep drowned valleys; Hume et al. 2016) and a wide spectrum of land-use intensities. Samples were collected between November to May (late spring-autumn), with the majority (70%) collected during the austral summer. Surveys were generally carried out according to a standardised protocol (Robertson et al. 2002), with samples collected from sites located at mid-to-low tidal height away from point-source discharges. To standardise for salinity effects, sites suspected to be significantly influenced by freshwater, based on proximity and flow rate of nearby streams, were removed from the dataset as well as any sites located within freshwater-dominated estuaries (i.e., tidal river mouth estuaries; Hume et al. 2016).

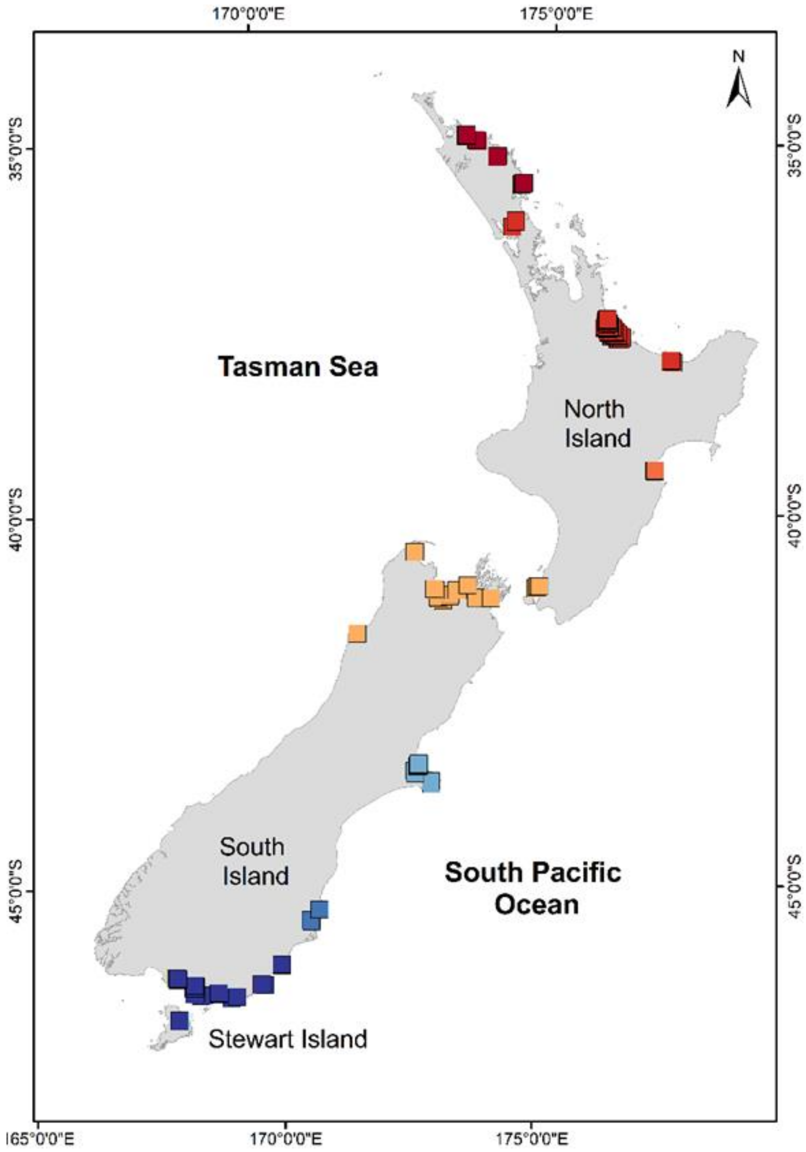


Figure 2.1 Map of New Zealand showing the location of sites used in this study, with colour providing an indication of the latitudinal gradient.

2.2.2 Macroinvertebrate data

Benthic macroinvertebrate samples (*n* = 3 to 15 replicates per site/time) were collected using a 13 cm diameter core, extending 15 cm into the sediment, and sieved to 500 µm. Experts identified organisms to the lowest practicable resolution. Taxonomic nomenclature followed the World Register of Marine Species (WoRMS Editorial Board 2017), and where differences in taxonomic resolution arose, we aggregated to higher taxonomic groups. This taxonomic aggregation may have obscured some of the true diversity; however, as taxa from all sites/times were treated the same, diversity indices are comparable on a relative scale. Some taxa were removed from the dataset before analysis, including taxa not well-represented by this sampling method (e.g., Bryozoa, meiofaunal taxa), those identified to relatively coarse taxonomic groups (e.g., Polychaeta, Annelida), larval planktonic groups (e.g., megalope, eggs), non-marine taxa (e.g., Insecta, Acari), vertebrates, plants, and bacteria. Most (74%) of the remaining 122 taxa were identified to genus or species level. Abundance data were used in all analyses, with data from replicate macroinvertebrate samples averaged by site/time.

2.2.3 Environmental data

Data for environmental variables (land-derived stressors and natural environmental variables) considered potentially important for influencing estuarine benthic macroinvertebrate turnover were collected concurrently with macroinvertebrate samples or collated from existing datasets (Table 2.1). As community responses reflect environmental processes operating over a range of scales (Thrush et al. 2005a), these variables were chosen to incorporate both local scale factors that varied by site and broad scale factors that operated at an estuary or national scale. I limited my assessment to six environmental variables, as the inclusion of many variables in regression tree approaches (such as GF) has been shown to provide only minimal improvement in predictive accuracy and to complicate interpretation of model outcomes (Leathwick et al. 2006).

Table 2.1 Summary of environmental variables (representing both land-derived stressors and natural variables), including spatial resolution and temporal correspondence with macroinvertebrate sample collection and data type. TN = sediment total nitrogen, TP = sediment total phosphorous, SST = sea surface temperature, SOI = Southern Oscillation Index.

Variable	**Spatial resolution**	**Temporal correspondence**	**Data type**	**Minimum**	**q25**	**Median**	**q75**	**Maximum**
a) Land-derived stressors								
Mud (%)	Local (site)	Concurrent	Measured	0.0	4.8	12.6	26.4	98.5
TN (mg kg^{-1})	Local (site)	Concurrent	Measured	70.3	250.0	410.0	638.3	4133.3
TP (mg kg^{-1})	Local (site)	Concurrent	Measured	51.0	211.5	340.0	473.8	1836.7
b) Natural environmental variables								
SST (°C)	Broad (estuary)	Same month/year	Modelled	11.7	15.2	18.0	19.3	22.2
SOI	Broad (national)	Same month/year	Modelled	-2.2	-0.1	0.8	2.5	2.7
Wind-wave exposure	Local (site)	Steady state	Modelled	0.5	1.0	3.5	6.6	24.0

Proxies of land-derived stressors were measured by collecting sediment samples (*n* = 1 to 12 replicates per site/time) to a depth of 2 cm concurrent with macroinvertebrate samples. Mud content is increasing in many of New Zealand's estuaries (e.g., Stevens & Robertson 2014, Davidson 2018, Urlich & Handley 2020) and this has been linked to increasing supply from land due to human activities (e.g., Gibbs 2008, Swales et al. 2015, Handley et al. 2017). Several studies (Thrush et al. 2003b, Thrush et al. 2005a, Anderson 2008, Robertson et al. 2015, Ellis et al. 2017) have used sediment mud content as an indicator of stress related to sedimentation from land-based sources. Accordingly, mud content (grain-size < 63 μm) was used as a proxy for sedimentation. Mud content was determined using either wet sieving or laser diffraction analysis. To increase comparability between different sediment grain-size analyses, sediment mud proportions were converted to a percentage of the < 2 mm sediment fraction (e.g., percentage of < 63 μm out of the < 2 mm sediment fraction) because the maximum grain-size differed between analysis methods (e.g., Malvern Mastersizer laser only analyses grains < 2 mm, while all grain-sizes are usually analysed during wet sieving). Mud concentrations at site/times used in this study covered the full spectrum (0-99% mud content), with a median of 13% (Table 2.1)

Sediment total nitrogen (TN) and total phosphorus (TP) were used as proxies for nutrient loading. Despite slight variations in methods used to analyse TN and TP at different sites, results were assumed to be generally comparable by Berthelsen et al. (2020b). Values less than the analytical detection limit (ADL) were assigned values of ADL/2. Sediment nutrient concentrations provided a wide stressor gradient, with maximum TN values (4133 mg kg^{-1}) and TP (1836 mg kg^{-1}) values comparable to highly polluted estuarine sites worldwide (Oviatt et al. 1984, Gillespie & MacKenzie 1990, Sánchez-Moyano et al. 2010, Cao et al. 2011; Table 2.1). However, higher sediment TN values have been observed in some European estuaries (e.g., up to 8600 mg kg^{-1} in Bilbao Estuary, Spain; Saiz-Salinas 1997), and median values for both these nutrients were relatively low across the study sites (410 mg kg^{-1} TN, 340 mg kg^{-1} TP). Data from replicate sediment samples (mud, TN, and TP) were averaged by site/time.

Sea surface temperature (SST), Southern Oscillation Index (SOI), and wind-wave exposure are natural environmental variables known to influence estuarine biodiversity (Engle & Summers 1999, Hewitt & Thrush 2009, Hewitt et al. 2016, Denis-Roy et al. 2020). SOI is a measure of the strength of the El Niño Southern Oscillation (ENSO), which occurs every two to seven years and is the largest source of natural variability in the global climate (Diaz 2005). Monthly estimates of SOI, corresponding with each site/time, were used as a measure of broad scale temporal variability in climate. While extreme values in this 16-year dataset were slightly less than that observed over more extended periods (-3.6 to 3.3 range since 1882), the dataset captured both El Niño and La Niña events (prolonged monthly average below -1 or above 1, respectively).

Modelled average monthly SST data were obtained from the JPL MUR MEaSUREs Project (NASA/JPL 2015) as a broad scale measure of temporal and spatial variability across the study area. Values were taken from a location near the seaward entrance of each estuary and corresponded with the month and year of macroinvertebrate and sediment sample collection. Where SST data were not available for a site/time (n = 23), median SST across other site/times and within the same estuary, or a nearby estuary, was used. Median values were calculated from site/times sampled in the same month as the missing site/time SST where possible.

Wind-wave exposure was calculated for each site following a topographical method similar to that developed by Burrows et al. (2008). Wind direction and speed data, across three years of records, were obtained from the nearest regional airport and predominant winds binned into 45° intervals to give a measure of wind-wave disturbance from eight directions. Around each site, the distance to land (fetch, measured in m) was calculated for every 1°, and each fetch value was multiplied by the total number of days when the predominant wind was from that direction and the wind speed (surface wind at 9 am, $m\ s^{-1}$) for those days. Outputs were divided by 100,000 to convert the data to a smaller scale. Where sites were too close to land to calculate exposure metrics, they were assumed to be located in a sheltered environment and assigned the minimum wind-wave exposure value. Several environmental variables showed some co-linearity (Pearson correlation r = 0.61-0.71 between mud, TN, and TP); however, this co-linearity was within limits acceptable for tree-based machine learning methods such as GF ($r < 0.9$; Elith et al. 2010, Dormann et al. 2013).

2.2.4 Relative importance of environmental variables for predicting compositional turnover

Gradient Forest (GF; Ellis et al. 2012) was used to investigate estuarine benthic macroinvertebrate turnover in response to land-derived stressors and natural environmental variables. Incidental taxa (≤ 3 occurrences across the entire dataset, n =

34) were not included in the GF models. The GF model had two components: the production of Random Forest (RF) models (Breiman 2001) for each of the 88 input taxa using the R package *extendedFores*t (Liaw & Wiener 2002) and the aggregation of the individual split points from these models to calculate species turnover along each environmental gradient using the R package *gradientForest* (Ellis et al. 2012). RF models describe the relationship between an individual taxon and environmental variables by fitting an ensemble of regression models (1000 in this study). The proportion of out-of-bag variance explained measures the predictive power of the individual RF models (R^2_f; Ellis et al. 2012), and the importance of each environmental variable (R^2) is measured by quantifying the degradation in performance when each environmental variable was randomly permuted (Pitcher et al. 2012). This R^2 value described by Pitcher et al. (2012) and Ellis et al. (2012) refers to a unitless measure of cumulative importance. It should not be confused with the more commonly used R-squared (R^2) denoting coefficient of determination.

GF aggregates the values of the tree splits from the RF models for all taxa models with positive fits ($R^2_f > 0$) to construct non-linear empirical functions of predicted compositional change along each environmental gradient for the entire assemblage (Pitcher et al. 2012), hereafter referred to as compositional turnover. The compositional turnover function is measured in dimensionless R^2 units, where species with highly predictive random forest models (high R^2_f values) have a greater influence on the turnover functions than those with low predictive power (lower R^2_f). The shapes of these monotonic turnover curves describe the predicted rate of compositional change along each environmental predictor; steep parts of the curve indicate fast assemblage turnover, and flatter parts of the curve indicate more homogenous regions (Ellis et al. 2012, Pitcher et al. 2012).

I extended the GF approach by adding a measure of uncertainty to the compositional turnover functions by bootstrapping GF models 100 times, similar to other regression tree-based methods (Leathwick et al. 2006). That is, the macroinvertebrate dataset was randomly sampled (with replacement) for each bootstrap iteration. The bootstrapping process was repeated 100 times, and at each iteration, compositional turnover functions were used to transform the environmental layers. Mean (± the standard deviation of the mean) estimates of taxa R^2_f and environmental variable importance (R^2) were calculated for each GF model from the 100 bootstrapped iterations. To examine which taxa were characterising compositional turnover along each environmental gradient, cumulative abundance changes for the five taxa that achieved the highest cumulative importance values across the entire environmental gradient were also plotted.

Compositional turnover for each environmental predictor was visualised using principal component analysis (using the function *prcomp* in the R package *stats*) to provide a multidimensional representation of variation in inferred community composition. Environmental variables were overlaid as vectors, indicating the strength and direction of the most important variables. All statistical analyses were undertaken in the software R (v 3.4.3; R Core Team 2019).

2.2.5 Relationships between compositional turnover and macroinvertebrate diversity

Generalized linear models (GLMs) were used to explore the relative importance of compositional turnover along land-derived stressor (mud, TN, TP) and natural environmental (SST, SOI, wind-wave exposure) gradients in explaining patterns in species richness (S; the number of taxa), Pielou's evenness (J': Pielou 1966), Shannon-Wiener diversity (H'; Shannon 1948), and numbers of rare taxa (those occurring only once or twice for each site/time). These four variables will be referred to collectively as diversity indices. To be consistent with the GF models, incidental taxa (≤ 3 occurrences across the entire dataset, $n = 34$) were only included when calculating rare taxa (not in calculations of S, J’, and H’). For the GLMs, the outputs of the GF model (compositional turnover values along six environmental gradients) were used as predictor variables, with compositional turnover along natural environmental gradients accounting for spatial and temporal dependency in the models. Data exploration was carried out according to the protocol developed by Zuur et al. (2010). Collinearity among predictor variables was generally low (Pearson’s $r < 0.5$), with moderate correlations found only between turnover associated with TN and TP ($r = 0.55$), mud and TP ($r = 0.66$), and mud and TN ($r = 0.74$). The lack of strong correlations, and variance inflation factor values less than 3, indicated that regressive models (including GF) should be able to separate land-based and natural variation (Zuur et al., 2010).

Models were fitted with error structures appropriate for the distribution of the data using the *stats* and *glmmTMB* (Brooks et al. 2017) packages in the software R (v 3.6.1; R Core Team 2019). A Poisson distribution with a log link function was used to model S and the number of rare taxa, a beta distribution with a logit link function was used for J,’ and a

Gaussian distribution with an identity link function was used for H’. Interactions between predictors were already accounted for in the GF analysis; therefore, no interactions were included in the GLMs. Parsimonious models were developed using backward selection based on Akaike Information Criterion (AIC) values to determine which variables were important in predicting patterns in diversity indices. As compositional turnover values were on the same scale, the relative importance of land-derived stressors and natural environmental variables in predicting patterns in diversity indices was assessed using regression coefficients, with standard errors used as a measure of uncertainty. Model assumptions were verified by plotting Pearson residuals against fitted values, against each covariate in the model and against geographical coordinates (Zuur & Ieno 2016). Final models were checked for stability by varying the order in which variables were removed.

2.3 Results

2.3.1 Relative importance of environmental variables for predicting compositional turnover

On average, across the 100 bootstrapped model runs, GF was able to effectively model species turnover for 82 (± 0.02 SD) of the 88 input taxa (mean $R^2{}_f = 0.49 \pm 0.04$ SD), based on the predictive power of the individual RF models ($R^2{}_f$). Both natural environmental variables and land derived stressors were important in predicting patterns of compositional turnover in estuarine benthic macroinvertebrate communities, with the three natural variables combined slightly more important (27% of the conditional importance) than the three land-derived stressors combined (22% of the conditional importance) overall (Figure 2.2). SST (mean $R^2 = 0.10$) and wind-wave exposure (mean $R^2 = 0.10$) had the greatest influence on compositional turnover, followed by TP (mean $R^2 = 0.08$) and mud (mean $R^2 = 0.08$), TN (mean $R^2 = 0.07$) and SOI (mean $R^2 = 0.06$).

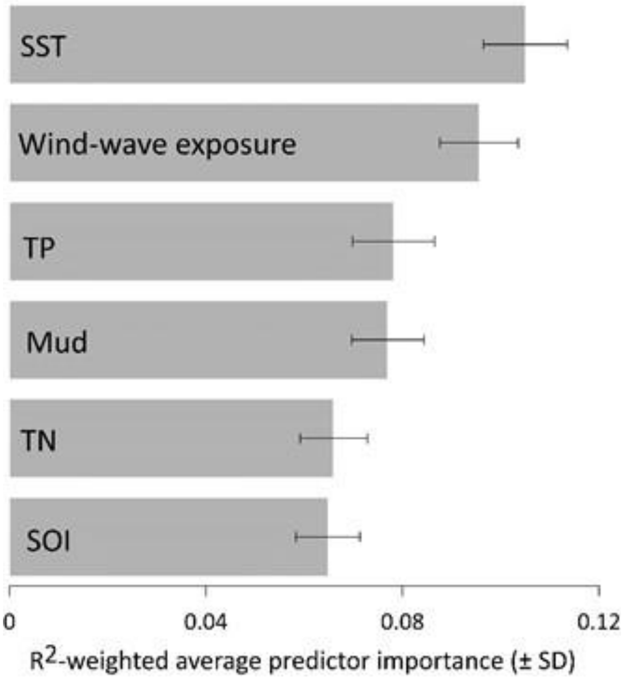


Figure 2.2 Overall importance (R^2-weighted importance across all taxa) of land-derived stressors (mud, TN, TP) and natural environmental variables (SST, SOI, wind-wave exposure) for predicting compositional turnover of estuarine benthic macroinvertebrate communities across New Zealand as assessed by bootstrapped Gradient Forest models. Bars show the mean contribution of each predictor across 100 bootstraps, and error bars show the standard deviation of the mean. SST = sea surface temperature, TP = sediment total phosphorous, TN = sediment total nitrogen, SOI = Southern Oscillation Index.

Non-linear patterns in compositional turnover were observed across all environmental gradients, except SOI, which had a relatively constant rate of turnover (Figure 2.3). Sections of rapid turnover (steep sections of the curve) were observed along the wind-wave exposure, TP, mud, and TN gradients, indicative of large changes in species abundance and composition, followed by a levelling off indicating more homogenous communities. For SST, high rates were initially followed by a slowing until 20°C and a rapid increase thereafter. The variability in mean predicted cumulative changes in composition turnover, measured by the 95% prediction intervals, was relatively low. This uncertainty differed between environmental predictors and was greatest for TP and TN and lowest for SOI. Uncertainty also varied along individual predictor gradients with greater uncertainty observed where fewer data were available to inform predictions (higher levels of mud, TN, TP, and exposure gradients).

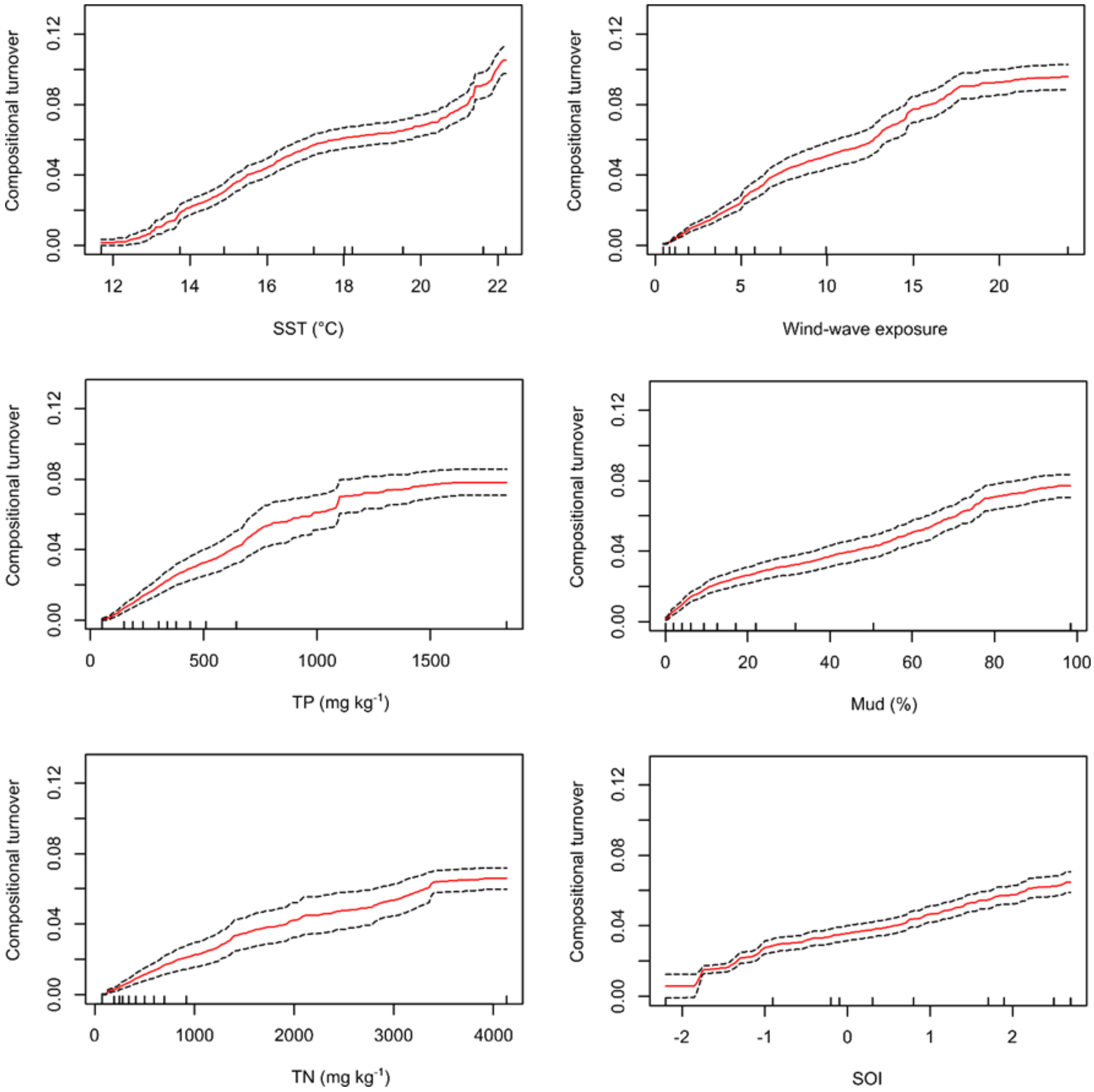


Figure 2.3 Cumulative importance curves showing the overall pattern of compositional turnover (in R^2-importance units) for all taxa across land-derived stressor (mud, TN, TP) and natural environmental (SST, SOI, wind-wave exposure) gradients. Each plot is scaled to the maximum cumulative importance to allow for direct comparison across each environmental gradient, and dashed black lines show 95% prediction intervals. Rug plots (black vertical lines) along the x-axis show deciles across each environmental gradient. SST = sea surface temperature, TP = sediment total phosphorous, TN = sediment total nitrogen, SOI = Southern Oscillation Index.

Taxa identified as being important in characterising compositional turnover differed between the environmental gradients, although some taxa were characteristic of two or three environmental variables (Figure 2.4). Many of these taxa displayed rapid changes in abundance and then plateaued to a constant level of abundance, as typified by the responses of the cockle *Austrovenus stutchburyi* and the polychaete *Aonides* along the mud gradient. Others exhibited S-shaped curves, with relatively constant changes in abundance, followed by a rapid increase and subsequent slowing down (e.g., the polychaete *Nicon aestuariensis* along the exposure gradient). Rapid changes in abundance were generally associated with low variability, measured by the 95%

prediction intervals, while higher variability was associated with flatter parts of the curves. Note that directionality of taxa response cannot be determined from these plots as they represent cumulative changes in abundance, that is, changes could be either increases or decreases in abundance at a given point along the gradient.

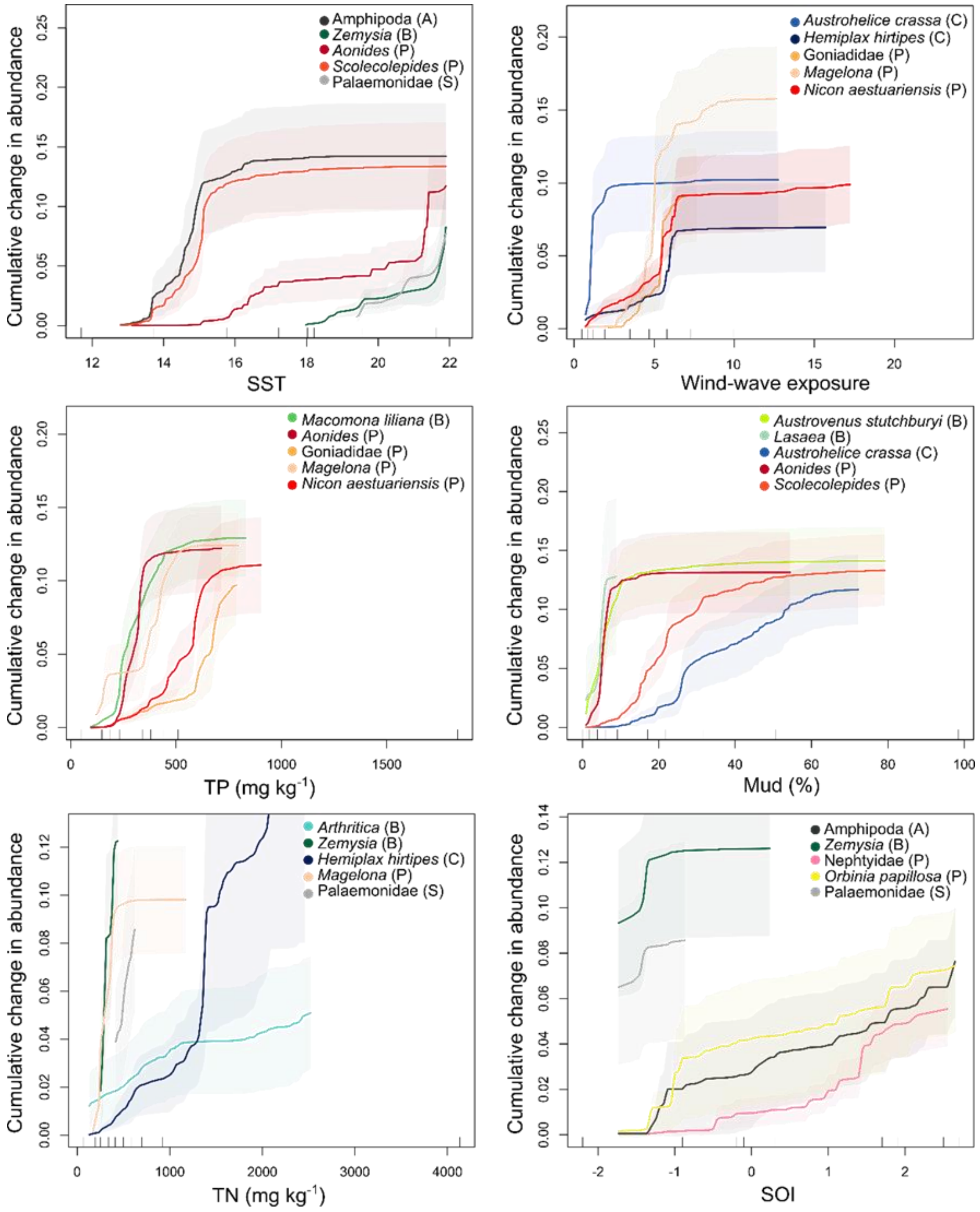


Figure 2.4 Cumulative importance curves of individual taxa (in R^2-importance units) for the five most important taxa characterising turnover along land-derived stressor (mud, TN, TP) and natural environmental (SST, SOI, wind-wave exposure) gradients. Shading indicates 95% prediction intervals and rug plots (black vertical lines) along the x-axis show deciles across each environmental gradient. Note that directionality of the change in individual taxa abundance cannot be seen in these plots. SST = sea surface temperature, TP = sediment total phosphorous, TN = sediment total nitrogen, SOI = Southern Oscillation Index. Letters in brackets after the taxa names indicate taxonomic group (A = amphipod, B = bivalve, C = crab, P = polychaete, S = shrimp).

Using these compositional turnover functions, shifts in community composition along environmental gradients were visualised in multivariate space where coordinate position represents inferred biological community composition, as associated with the environmental predictor variables (Figure 2.5). The first two axes of the ordination plot captured 68% of the total variance. This demonstrates that both natural environmental variables (SST, wind-wave exposure, and SOI) and land-derived stressors (mud, TN, TP) were important variables influencing compositional turnover. Land-derived stressors influenced compositional turnover in a similar way and, along with wind-wave exposure and SOI, were important in explaining biodiversity patterns along the first PC axis. SST also had a strong influence on compositional turnover, with site/times along the second PC axis showing high correspondence with location along the north to south gradient of New Zealand.

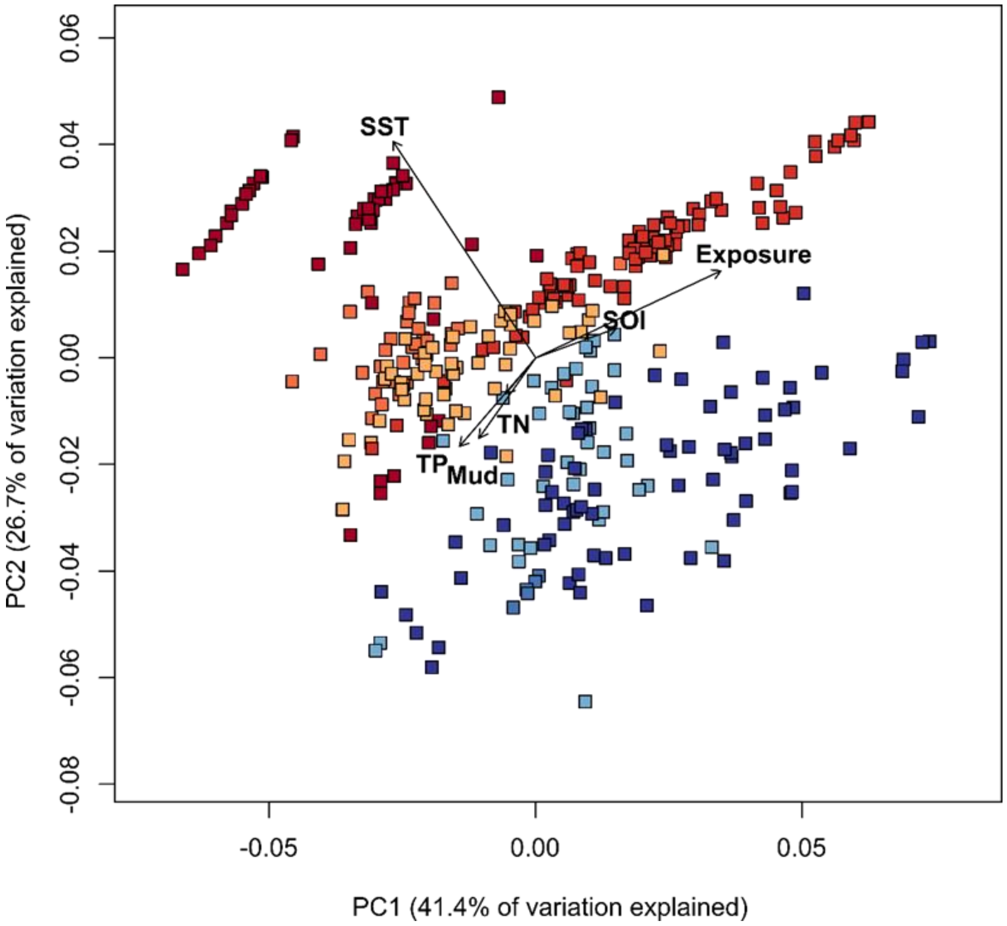


Figure 2.5 Principal Component Analysis (PCA) ordination biplot of 334 site/times using compositional turnover functions associated with land-derived stressor (mud, TN, TP) and natural environmental (SST, SOI, wind-wave exposure) predictors derived from a Gradient Forest model. Points closer together indicate similarities in inferred community composition between site/times and colour provides an indication of the latitudinal gradient (refer Figure 2.1). Vectors indicate correlations with environmental predictors used in the model, with relative importance indicated by vector length. SST = sea surface temperature, TP = sediment total phosphorous, TN = sediment total nitrogen, SOI = Southern Oscillation Index.

2.3.2 Relationships between compositional turnover and macroinvertebrate diversity

GLMs were used to determine whether compositional turnover driven by land-derived stressors and natural environmental variables resulted in positive or negative effects on macroinvertebrate diversity. The GLMs explained 7.8% to 13.4% of the variation in diversity indices, and all of the variables retained in the models were significant ($p < 0.05$), except for TP ($n = 334$, $t = -1.949$, $p = 0.052$) and wind-wave exposure ($n = 334$, $t = 1.553$, $p = 0.121$) in the model for H’ (Appendix 2). As hypothesised, compositional turnover along land-derived stressor gradients was linked to lower species richness, evenness and diversity, and fewer rare taxa (Figure 2.6). Compositional turnover along the TN gradient had a negative effect on all four diversity indices and was greater than the effect of turnover along other land-derived stressor gradients. Compositional turnover associated with increasing mud content was only important in explaining patterns in S, while turnover associated with increasing TP was only important in explaining patterns in H’. Compositional turnover along the SST and wind-wave exposure gradients had a positive effect on predicted values of J’, H’, and the number of rare taxa, with SST having a slightly stronger effect. Turnover along the SOI gradient was not important in explaining predicted patterns for any of the diversity indices. Greater uncertainty in model predictions was associated with compositional turnover along the TN and TP gradients (coefficient SE 2.0-3.5) compared with turnover along mud (coefficient SE 1.2) or natural environmental gradients (coefficient SE 0.8-1.5; Figure 2.6 and Appendix 2).

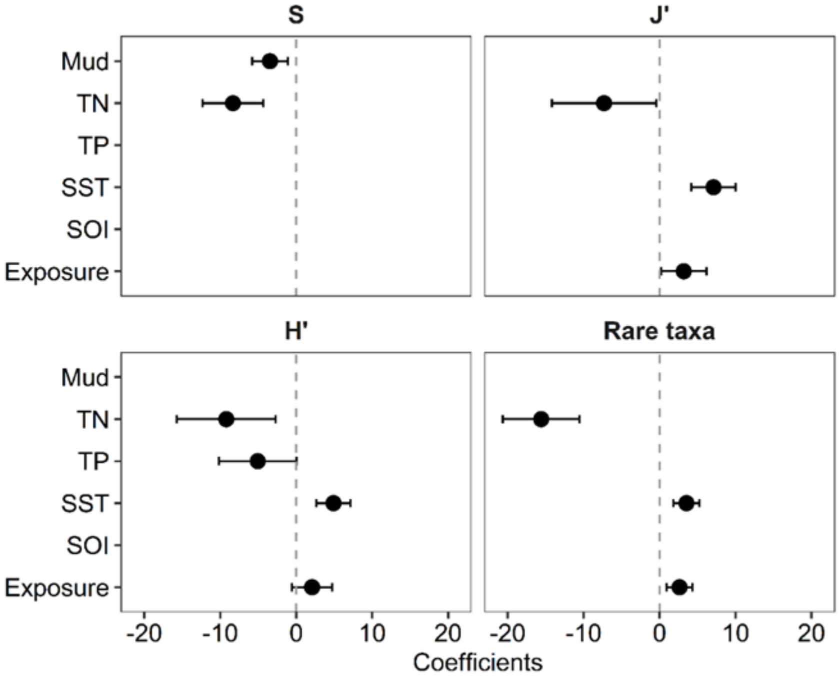


Figure 2.6 Regression coefficients (± 95% confidence intervals) of fixed effects obtained from generalized linear models for four measures of estuarine macroinvertebrate diversity (species richness (S), Pielou's evenness (J'), Shannon-Wiener diversity (H') and numbers of rare taxa) in response to compositional turnover along land-derived stressor (mud, TN, TP) and natural environmental (SST, SOI, wind-wave exposure) gradients. Coefficients are only shown for model terms selected using backwards selection on Akaike Information Criterion (AIC) values. SST = sea surface temperature, TP = sediment total phosphorous, TN = sediment total nitrogen, SOI = Southern Oscillation Index.

2.4 Discussion

Both land-derived stressors and natural environmental variables were important predictors of compositional turnover in estuarine benthic macroinvertebrate communities across New Zealand, reflecting a matrix of processes operating across multiple spatio-temporal scales. As expected, compositional turnover along land-derived stressor gradients was negatively associated with macroinvertebrate diversity indices, while turnover along natural environmental gradients (increasing SST and wind-wave exposure) generally had a positive relationship with these values.

2.4.1 Compositional turnover along natural environmental gradients

Predictably, across this large study area with its complex ocean currents influenced by both warm tropical and cold Antarctic water (Carter 2001), SST was the most important variable influencing compositional turnover. Temperature is known to be a key factor structuring communities across broad geographic scales (Tittensor et al. 2010, Denis-Roy et al. 2020), despite natural habitat characteristic such as grain-size and salinity being important on local scales (Engle & Summers 1999, Denis-Roy et al. 2020). In this study, high rates of compositional turnover occurred above 20°C SST, corresponding to samples from the far north and east of New Zealand, where ocean temperatures have been increasing over the past three to four decades (Schiel 2013, Sutton & Bowen 2019). This high turnover rate suggests that climate change could lead to large shifts in community composition as physiological temperature tolerances are reached and species distributional boundaries change (e.g., Southward et al. 1995, Sagarin et al. 1999, Johnson et al. 2011).

It is unlikely that temperature is the only driver of this compositional turnover pattern, however, with potential for it to act as a surrogate for a range of unmeasured broad scale variables operating across the latitudinal gradient (e.g., species dispersal patterns, water circulation patterns, seasonality; Hawkins 2001, Thrush et al. 2005a). Indeed, latitude was found to be an important driver of spatial patterns in fish assemblages across New Zealand (Stephenson et al. 2018) and a general latitudinal gradient in beta diversity has been observed in global scale studies (Hillebrand 2004, Soininen et al. 2007, Qian et al. 2009), with higher species turnover toward the equator. These latitudinal patterns may arise because the physical limiting factors or ecological and evolutionary processes that influence turnover are also affected by latitude (Qian et al. 2009). In this study, compositional turnover along the SST gradient had a positive relationship with J’, H’, and the number of rare taxa, but not S. The pattern suggests that compositional turnover alters the relative proportion of rare to common species along this gradient, with common species becoming rarer with increasing temperature. Thus, the number of rare species increases with turnover associated with increasing SST, but the total number of taxa does not.

Wind-wave exposure, another important driver of species distributions in estuarine environments (Warwick et al. 1991, Hewitt & Thrush 2009, Hewitt et al. 2016, Denis-Roy et al. 2020), was the next most important variable influencing compositional turnover in this study. Although exposure and mud content often co-vary, these variables were not highly correlated in this study (r = -0.2), and the GF model would have accounted for any interactions between these two variables, suggesting no confounding effect. This is further supported by the different taxa characterising turnover along the mud and exposure gradients, with the only shared species (the crab *Austrohelice crassa*) exhibiting dissimilar changes in abundance along the two gradients. Like SST, compositional turnover along the wind-wave exposure gradient had a positive relationship with J’, H’, and numbers of rare taxa. In this study, sites with high exposure were located on central sandflats of a particular estuary type (large shallow drowned valley estuaries), for which the fetch allows wind-generated circulation and mixing. These high-energy environments generally have lower rates of sediment deposition, greater potential for recovery following storm events (Norkko et al. 2002b, Thrush et al. 2003a), improved food supply (via increased organic seston flux and/or resuspension of particulate organic matter; Fréchette & Bourget 1985, de Jonge & van den Bergs 1987) and increased potential for recruitment (Commito et al. 1995), which may promote diverse benthic communities in these areas.

Of the six environmental variables considered in this study, SOI explained the least amount of variation in compositional turnover, and turnover along the SOI gradient was not found to be important in explaining patterns in estuarine benthic diversity. SOI influences a range of potentially important drivers (e.g., wind, temperature, water column productivity) that could affect population dynamics and has been shown to be an important predictor of the abundance of species and functional traits (Hewitt & Thrush 2009, Hewitt et al. 2016). Unlike the other environmental factors considered in this study, SOI is a large-scale phenomenon that predominantly varies in time rather than space. The lack of robust time-series data for many sites in my analysis may have reduced the importance of this variable in predicting patterns of turnover compared with spatially variable factors.

2.4.2 Compositional turnover along land-derived stressor gradients

In this study, land-derived stressors were less important than SST and wind-wave exposure in predicting compositional turnover patterns in estuarine benthic communities across New Zealand. This result suggests that natural environmental variables regulate species distributions, with land-derived stressors constrained to act upon these existing communities. Given the low levels of mud and nutrients across many of the study sites, which are representative of estuaries across New Zealand, I would not expect land-derived stressors to be the most important variables influencing compositional turnover on a national scale. However, it is unknown whether the relative importance of the environmental variables would change if this model was applied to a dataset where levels of land-derived stressors were consistently high.

Once mud and nutrient levels were high enough to start acting as stressors to benthic communities, they began to have a discernible effect on compositional turnover despite the influence of natural environmental variables. High rates of turnover were observed between 0-10% mud, consistent with multiple studies that have shown that functional redundancy and the abundance of sensitive taxa decline once mud content reaches 5-10% (e.g., Thrush et al. 2003b, Anderson 2008, Robertson et al. 2015, Ellis et al. 2017). For example, taxa characterising turnover along the mud gradient included the cockle *A. stutchburyi* and the polychaete *Aonides*, which showed rapid changes in abundance between 0-10% mud; species with known preferences for sandy sediments with less than 10% mud (e.g., Norkko et al. 2002a, Gibbs & Hewitt 2004, Anderson 2008, Ellis et al. 2017). In contrast, the more constant changes in the abundance of the polychaete *Scolecolepides* and the mud crab *A. crassa* may reflect the tolerance of these species for a wider range of sediment grain-sizes (e.g., Thrush et al. 2003b, Ellis et al. 2006, Anderson 2008, Robertson et al. 2015).

In this study, high rates of compositional turnover in response to nutrients were observed at 1200 mg kg^{-1}, similar to a threshold of 1000 mg kg^{-1} TN associated with a shallowing of apparent Redox Potential Discontinuity (aRPD) to near zero depths in eight Californian estuaries (Sutula et al. 2014). Shallowing of the aRPD is usually associated with hypoxic events, which can lead to reduced abundance and diversity of benthic macroinvertebrates. Rapid changes in the abundance of specific taxa were observed at lower levels of nutrients, demonstrating that management thresholds based on compositional turnover will not protect all species. For example, rapid changes in the abundance of the bivalve *Zemysia* and the polychaete *Magelona* were observed between 100-400 mg kg^{-1} TN, which is reasonably consistent with predicted distributions of these species between 200-600 and 300-550 mg kg^{-1} TN, respectively (Ellis et al. 2017). The plateauing of compositional turnover observed at 3250 mg kg^{-1} TN and 1100 mg kg^{-1} TP, nutrient levels indicative of polluted estuaries (e.g., Oviatt et al. 1984, Gillespie & MacKenzie 1990, Sánchez-Moyano et al. 2010), may reflect a loss of taxa as communities become dominated by a limited number of species tolerant of high enrichment. Indeed, the GLMs showed that compositional turnover along these nutrient gradients was associated with lower species richness and diversity. However, the wide prediction intervals associated with these TN and TP thresholds mean these values should be interpreted with caution as fewer data were available to inform the model. These values are reported as a contribution to the literature on nutrient effects and should be used in a weight of evidence approach in combination with other information, rather than relied upon as strict thresholds of community change along enrichment gradients. Additional sampling targeting locations with high levels of nutrients, as well as comparisons with thresholds identified using other approaches (e.g., Threshold Indicator Taxa ANalysis, Ecosystem Interaction Networks; Baker & King 2010, Thrush et al. 2020), would build confidence in the generality of these critical transitions.

Consistent with my second hypothesis, compositional turnover along land-derived stressor gradients was generally associated with lower S, J', H' values, and fewer rare taxa. Maintaining diversity is important for promoting stability and resistance to disturbance (Levin et al. 2001), while rare taxa can confer functional resilience and make disproportionately large contributions to community and ecosystem functioning (Ellingsen et al. 2007). The loss of rare species has been proposed as an early warning signal of ecological shifts and functional impairment associated with anthropogenic stress as more of the community becomes represented by fewer, tolerant taxa (Hewitt et al. 2010). Across the study sites, compositional turnover along the sediment TN gradient was more important in explaining patterns in diversity indices than turnover associated with mud or TP, possibly because nitrogen is often the limiting nutrient in coastal systems (Howarth & Marino 2006). For example, compositional turnover along the nitrogen gradient could be linked to eutrophication-driven species loss. However, the importance of compositional turnover driven by both TN and TP in explaining patterns in H' suggests these nutrients can affect diversity in different ways. Similarly, patterns in S were explained by both mud and TN, highlighting the influence that multiple stressors can have on benthic diversity. The distinct groups of taxa characterising each of the land-derived stressor gradients also supports the idea that these stressors affect community turnover in different ways.

Hydrodynamic controls on sedimentation rates and nutrient loading can result in upper reaches of estuaries being naturally muddier and more enriched than outer reaches. While I cannot definitively conclude that human activities were the cause of elevated mud and nutrient levels in this study, I have shown that compositional turnover along these environmental gradients results in benthic macroinvertebrate communities with lower species richness, evenness, and diversity and fewer rare taxa. I also observed rapid changes in the abundance of functionally important species, such as *A. stutchburyi* and *Macomona liliana*, along land-derived stressor gradients. These bivalves influence community structure and microphytobenthic productivity as well as a range of physical and biogeochemical processes (e.g., sediment stability, pore water oxygen concentrations, nutrient cycling; Lelieveld et al. 2004, Thrush et al. 2006, Sandwell et al. 2009, Volkenborn et al. 2012). Consequently, these are the changes likely to occur if the total area of an estuary classified as being muddy or nutrient-enriched expands, with notable follow-on effects to ecosystem functioning (e.g., macroinvertebrate-mediated nutrient cycling; Lohrer et al. 2010) and, ultimately, the ecosystem services upon which humans rely. With increasing pressure on land worldwide, these land-derived stressors are likely to become more persistent. Even without intensification of human impact, the frequency and intensity of rainfall and storms are predicted to increase with climate change, likely increasing sedimentation rates and nutrient loading in estuaries (Inman & Jenkins 1999, McLean et al. 2001).

2.4.3 Consideration of uncertainty

Failure to consider uncertainty can result in poor management decisions (Regan et al. 2005, Link et al. 2012). Accordingly, I extended the GF approach by adding a measure of uncertainty to the compositional turnover functions and the changes in the cumulative abundance of key taxa. This development allowed results to be presented as an average of what is likely, thereby reducing the influence of non-representative outcomes. Indeed, for a single model run, SOI was found to be the third most important variable explaining variation in compositional turnover but averaged across 100 model runs its relative importance decreased. Consistent with Sultana et al. (2020), who found the evenness of the environmental gradient can affect GF model performance, variability estimates associated with the compositional turnover functions and the changes in the cumulative abundance of key taxa indicated greater uncertainty where fewer data were available to inform predictions. For the key taxa, however, high rates of change were associated with low variability, providing confidence in these estimates.

Uncertainty also varied between environmental variables, with slightly less confidence associated with predictions of compositional turnover along TN and TP gradients. Although not explored explicitly in this study, greater uncertainty associated with nutrients could indicate that compositional turnover in response to nutrient loading is context dependent. For example, high turnover may occur when nutrient loading and warm temperatures coincide, fuelling primary production, but a different response may occur if the same level of nutrient loading takes place in winter. Uncertainty may also be influenced by the restricted distribution of key taxa characterising these gradients, which may reflect habitat preference or sampling bias.

The addition of uncertainty estimates into GF outputs has important implications for management, which are not fully explored in this thesis. For example, results could be spatially mapped (e.g., Pitcher et al. 2012, Stephenson et al. 2018, Couce et al. 2020), with accompanying maps of uncertainty, to show the distribution of benthic communities and the uncertainty associated with those predictions. In this study, I would expect maps to highlight greater levels of uncertainty related to predictions of communities influenced by high levels of nutrient loading. Uncertainty was considered in the GLMs by comparing the size of the standard errors. Like the GF model, there was greater uncertainty linked to compositional turnover values along the TN and TP gradients in terms of predicting patterns in diversity indices.

2.4.4 Conclusions

I have demonstrated that both land-derived stressors and natural environmental variables, operating across multiple spatio-temporal scales, shape patterns of compositional turnover in estuarine macroinvertebrate communities across New Zealand. In this study, GF enabled me to tease out the effects of land-derived stressors from natural variation and identify critical levels where compositional turnover was high. Using GLMs, these turnover values were linked to measures of benthic macroinvertebrate diversity, which indicated that turnover along land-derived stressor gradients had a negative effect on benthic communities at a local scale. Relationships identified by these exploratory models are correlative, and while they do not necessarily prove a causal link, they do identify possible drivers of patterns that could be investigated further through controlled experiments (Ellis et al. 2012). Exploratory models also allow for studies to be undertaken on much larger scales than funding for manipulated experiments would allow, providing information about processes operating over broad scales. Future work could examine other environmental variables, including biotic factors (e.g., competition for resources, predation, small-scale biological disturbance), incorporate measures of environmental variability (e.g., seasonal ranges of predictors rather than averages) and consider lag effects. GF also allows for the inclusion of abundance data from different survey methods (Ellis et al. 2012) because a dimensionless R^2 measure is used to quantity compositional turnover, meaning that other estuarine taxa, such as fish, could be included in models to provide a more holistic view of ecosystem response. This study moves towards an ecosystem-based management approach by considering how multiple land-derived stressors influence patterns of estuarine compositional turnover and diversity, against a background of natural variability operating at multiple spatio-temporal scales.

Chapter 3: The development of a national approach to monitoring estuarine health based on multivariate analysis

3.1 Introduction

Estuaries are among the most valuable of all ecosystems with regard to the services they provide to society (Costanza et al. 1997), many of which result from the high degree of connectivity with terrestrial systems and their proximity to people. However, as human populations have increased in coastal areas, so have the pressures on estuaries, which are exposed to multiple and cumulative stressors arising from adjacent catchments (e.g., increased sediment, nutrient and contaminant loads; Thrush et al. 2004, Bricker et al. 2008, Johnston et al. 2015), anthropogenic activities within the marine environment (e.g., fishing, dredging, shipping; Thrush et al. 1998, Grosholz 2002, Piló et al. 2019), and global sources (e.g., climate change; Brierley & Kingsford 2009). Such cumulative impacts have resulted in a loss of biodiversity and resilience, and an increased potential for tipping points to occur (Lotze et al. 2006). Thus, estuaries are not only one of the most heavily used, but also one of the most vulnerable natural systems worldwide (Agardy et al. 2005, Lotze et al. 2006, Barbier et al. 2011).

Environmental regulations increasingly require ecological assessment to quantify the impact of stressors on coastal ecosystem status and inform management decisions (e.g., the Clean Water Act or Oceans Act in USA, Australia or Canada; Water Framework Directive or Marine Strategy Framework Directive in Europe, and National Water Act in South Africa; Borja et al. 2008). For assessment methods to be useful they need to be (1) ecologically relevant, (2) feasible to implement, (3) linked to threshold or reference values so that users can assess the significance of an indicator value, (4) sensitive enough to measure status or trends that are relevant to policy decisions and reflect responses to management actions and ideally, (5) applicable over wide spatio-temporal scales (Borja & Dauer 2008). Benthic macroinvertebrate communities are commonly used to assess environmental status (Pearson & Rosenberg 1978, Dauer 1993, Borja et al. 2000) because they respond relatively rapidly to stressors, integrate the effects of multiple stressors over time and are composed of a diverse range of species with differing functional roles, trophic levels and sensitivities. Incorporating community information into ecosystem health assessments allows organisms to ‘tell the story’, with respect to classifying sites along a continuum from degraded to non-degraded (Diaz et al. 2004).

Historically, the first approaches to extract information from macroinvertebrate community data included the calculation of simple metrics, such as the number of taxa or individuals and measures of community evenness and diversity (e.g., Shannon 1948, Margalef 1958, Pielou 1966). These universally applicable metrics can be assessed against the Pearson and Rosenberg (1978) model of macrobenthic succession to provide an indication of environmental health, but they have limited ability to detect meaningful change because they do not differentiate amongst different types of taxa (Hewitt et al. 2005a, Ellis et al. 2015, Shade 2016). The growing requirement for assessment of marine environmental status over the last two decades has led to a proliferation of more complex biotic indices, many of which also have foundations in the Pearson-Rosenberg model (Diaz et al. 2004, Borja et al. 2015). Many of these indicators (e.g., Grall & Glémarec 1997, Borja et al. 2000, Simboura & Zenetos 2002) work by assigning taxa into previously defined ecological groups, based on their response to stressors, and examining the relative proportion of these groups in the benthic community sample. This requires predefined knowledge of how a large number of species respond to stressors, and for many species the research to determine these responses has not been carried out.

Other approaches to tracking environmental health include multivariate methods, which describe assemblage patterns of the entire community (e.g., ordination-based approaches; Clarke 1993, Smith et al. 2001, Flåten et al. 2007). Because multivariate approaches retain information on species covariance, they can detect smaller changes in community structure (Gray et al. 1990, Warwick & Clarke 1991, Attayde & Bozelli 1998, Hewitt et al. 2005a, Ellis et al. 2015). This sensitivity enables early detection of environmental deterioration, allowing management actions to be implemented before significant ecosystem damage occurs, thereby avoiding prolonged (and sometimes uncertain) recovery and/or costly remedial actions (Martinez-Crego et al. 2010). In addition, preservation of species composition information means outputs can be directly linked to changes in biodiversity and ecological functioning. This link with ecological functioning can be taken one step further by using multivariate approaches to assess changes in functional traits rather than species assemblages (e.g., Bremner et al. 2003, Hewitt et al. 2008).

Most biotic indices provide an overall measure of ecosystem health and are designed to be sensitive to a broad range of stressors. While this holistic approach can indicate the general health of a system and account for interactions amongst stressors, the inability to attribute degradation to a specific stressor makes targeted management action difficult (Niemi et al. 2004, Martinez-Crego et al. 2010). In addition, the desire to create biotic indices that track changes in ecosystem health in response to a suite of stressors has necessitated the use of expert judgement in index development. Expert opinion is often used to assign taxa to ecological groups because for many species we do not have empirical information on their response to different stressors. Furthermore, quantifying the relationships between communities and multiple stressors is complex, given the uncertainties associated with interactions (Crain et al. 2008, Darling & Côté 2008) and non-linear responses (deYoung et al. 2008). In contrast, stressor-specific indices can be developed from robust empirical relationships between benthic communities and the stressor of interest (e.g., Keeley et al. 2012, Robertson et al. 2016). In addition to providing managers with an objective assessment of health, these single-stressor indices diagnose the cause of degradation, enabling prioritization of mitigation measures. While multi-stressor indices have many merits, I advocate for the use of a suite of single-stressor indices, based on key pressures to the system, that allow managers to identify sources of degradation and interactions between stressors and apply appropriate action. These types of analyses (indices) would allow a weight of evidence approach (Magni et al. 2005) to the assessment of environmental status and methods to integrate the individual stressor scores into an overall score could be applied if required (e.g., Borja et al. 2004, Aubry & Elliott 2006).

New Zealand spans 15 degrees of latitude and three water masses and, with more than 400 estuaries (Hume et al. 2016), provides an ideal place to test the robustness of biotic indices under different conditions. Here, I developed two stressor-specific biotic indices, called Benthic Health Models (BHMs), which can be used to assess intertidal estuary health in response to increasing mud content (Mud BHM) and heavy metal contamination (Metals BHM) across New Zealand. I chose sedimentation and metal contamination as stressors of interest because they are recognised as major threats to the health and functioning of estuaries globally and are routinely monitored, both in New Zealand and elsewhere (Hewitt et al. 2005a, EU Marine Strategy Framework Directive 2008, Hewitt et al. 2009, Lohrer et al. 2012, MacDiarmid et al. 2012, Rodil et al. 2013, Hewitt et al.

2014, Magris & Ban 2019). In New Zealand, few estuaries have been unaffected by increased sediment inputs from land, increasing the total area of the estuary seafloor being classified as muddy sediments. The BHMs were developed using a constrained multivariate ordination technique that models changes in community structure along an environmental gradient. The results of the models can be simplified into a health score, which allows estuary health to be tracked over time. In this chapter, I follow Hewitt et al. (2005a) and define ‘health’ on the basis of the range of communities observed along gradients of anthropogenic impacts, rather than requiring identification of a “reference” condition or site. This definition identifies both acute effects and broader scale degradation in community structure.

The BHM approach has been successfully applied at estuary (Ellis et al. 2015) and regional scales (Hewitt et al. 2005a), however, a national model that is able to detect changes across regional species pools or estuarine types has not been tested to date. National models would provide a standardised assessment method to enable the health of an estuary to be placed in a wider context and reduce the costs required to develop separate estuary scale or regional scale models. In addition to being sensitive to changes in ecosystem health, biotic indices need to be unaffected by different species pools (Keeley et al. 2012, Gillett et al. 2015, Berthelsen et al. 2018) and natural environmental contexts (Barbone et al. 2012, Berthelsen et al. 2018). These requirements are particularly important when developing a national index for a country such as New Zealand, with a strong latitudinal gradient and estuaries open to three different water masses. To this end, I developed national BHM models and tested their ability to discriminate between effects caused by the two stressors despite differences in regional species pools and estuarine physical type (i.e., tidal lagoons and shallow river valleys).

3.2 Methods

3.2.1 Macroinvertebrate and physio-chemical dataset

Data were obtained from surveys undertaken between 2002 and 2017, by New Zealand’s regional government authorities for the purposes of estuarine monitoring (815 site/times across 70 estuaries). Where information was available for multiple years and seasons, only one sampling occasion was used, with preference given to data collected between 2010 and 2014 (66% of sites) and spring/summer (October to March; 72% of sites), the years and months when most data was collected, in order to reduce potential between-year and between-season variability. Counts of larval planktonic groups (e.g., megalope, larvae and eggs) and juvenile taxa were removed from the dataset before model development, which limits the effect of recruitment pulses on the models. The 192 sites, from 34 estuaries, spanned 12 degrees of latitude and encompassed two dominant estuary types and a range of bioregions (Figure 3.1). Surveys were carried out according to a standardised protocol (Robertson et al. 2002), with samples collected from sites located at mid-to-low tidal height away from point-source discharges. Some variations in salinity and exposure were expected to be present across site locations. However, sites suspected to be significantly influenced by freshwater, based on their location or the presence of high abundances of insects, were removed from the dataset.

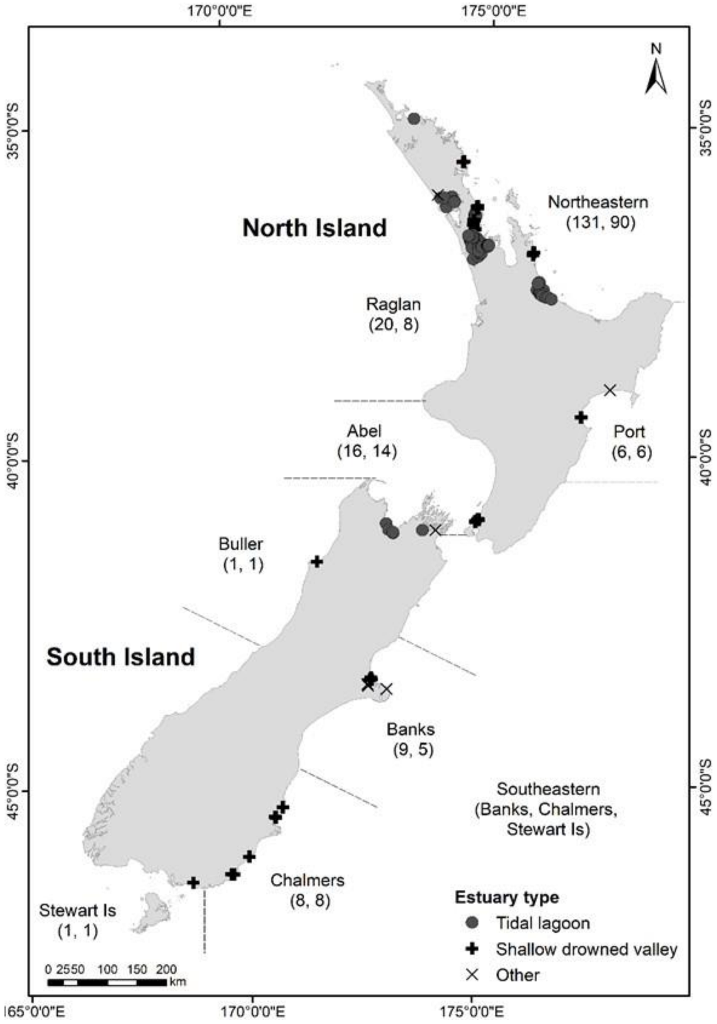


Figure 3.1 Map of New Zealand showing the location and estuary type (Hume et al. 2016) for the sites used to construct the Mud and Metals Benthic Health Models (BHMs). The number of sites for each bioregion (Northeastern, Port, Raglan, Abel, Buller, Banks, Chalmers, Stewart Island), as defined by Shears et al. (2008), is indicated in parentheses for both the Mud BHM (first number) and Metals BHMs (second number).

Macroinvertebrate samples (*n* = 3 to 15 replicates per site) were collected using a 13 cm diameter core extending 15 cm into the sediment and sieved using a 500 µm mesh. Experts identified organisms to the lowest practicable resolution. Taxonomic nomenclature followed the World Register of Marine Species (WoRMS Editorial Board 2017) and where differences in taxonomic resolution arose, I aggregated to higher taxonomic groups. Some taxa were removed from the dataset before the analysis (refer to Appendix 3 for justification). Taxonomic resolution was the same for both models and the final datasets had 125 (Mud BHM) and 109 (Metals BHM) taxa, with 80% of taxa identified to family level or lower (refer to Appendix 3 for a complete list of taxa used in the models).

Sediment samples (*n* = 1 to 12 replicates per site) were collected to a depth of 2 cm concurrent with macroinvertebrate samples. Samples were analysed for mud content (grain-size < 63 µm) using either wet sieving or laser diffraction analysis. To increase comparability between different sediment grain-size analyses, I converted sediment mud proportions to a percentage of the < 2 mm sediment fraction (e.g., percentage of < 63 µm out of the < 2 mm sediment fraction) because the maximum grain-size analysed differed between analysis methods (e.g., Malvern Mastersizer laser only analyses grains < 2 mm, while all grain-sizes are generally analysed during wet sieving). Exploratory analysis on final models showed no pattern associated with differing sediment grain-size analysis methods. At most sites (133 out of 192 from 29 of the 34 estuaries), sediment samples were also analysed for total concentrations (mg kg^{-1}) of copper (Cu), lead (Pb) and zinc (Zn), which are the key heavy metals of concern in New Zealand (ARC 2004). Despite slight variations in metal analysis methods between sites, results from the different analytical methods were assumed to be comparable by Berthelsen et al. (2020b). In general, the methods followed the US EPA 200.2 protocol of strong acid (nitric/hydrochloric) digestion followed by Inductively Couple Plasma Mass Spectrometry (US EPA 1994).

3.2.2 Model development and validation

All data were averaged to the level of site to construct the models. Differing numbers of replicates can lead to bias in multivariate analyses by underestimating species richness at sites with lower numbers of replicates and thereby overestimate dissimilarity (e.g., Chao et al. 2005). Exploratory analysis showed a slight reduction in species richness at sites where only three replicates were collected (mean number of taxa per core was 15 (*n* = 3) vs 21-23 (*n* > 3)), however, these represented only 15% of samples and no patterns were observed that would indicate the number of replicates was influencing model outputs (i.e., sites were dispersed across the health (CAP) score gradient). Previous studies have found that models based on all available information (i.e., a mixture of sample sizes) were most useful (Anderson et al. 2002, Anderson et al. 2006).

Two models were developed, one based on community response to sediment mud content (Mud BHM) and the other based on response to sediment Cu, Pb and Zn concentrations (Metals BHM). Several New Zealand studies have demonstrated that mud content can be used as an indicator of stress related to sedimentation from land-based sources (Thrush et al. 2003b, Thrush et al. 2005b, Anderson 2008, Robertson et al. 2015, Ellis et al. 2017). Exploratory analyses examining the influence of other environmental variables (nitrogen, phosphorus, organic matter, and salinity) showed mud and metals to be the key environmental stressors structuring benthic communities at the monitoring sites (data not shown). We used log-transformed percentage mud content as the environmental gradient for the mud model. Total extractable Cu, Pb and Zn were highly correlated (Pearson's *r* = 0.85-0.91) so a Principal Component Analysis (PCA) was used to derive a single variable (the first principal component axis; PC1) that would characterise a gradient corresponding to increases in the concentrations of all three metals. The PCA was performed on log-transformed Cu, Pb and Zn concentrations and the PC1 axis (PC1 metals) explained 92% of the variance. Log-transformations were chosen to render the data as close to normally distributed as possible for modelling and exploratory data analyses indicated that the choice of transformation did not affect model outputs. Zero values were assigned to metal concentrations below analytical detection limits (22% of sites for Cu, < 2% of sites for Pb and Zn). Mud concentrations at sites within the Mud BHM ranged from 0-98% and metal concentrations at sites within the Metals BHM ranged from 0-49 mg kg^{-1} for Cu, 0-70 mg kg^{-1} for Pb and 0-288 mg kg^{-1} for Zn (untransformed). Given these values represent maximum concentrations observed across 70 estuaries over the past 15 years, I believe they cover the range of values likely to be encountered in most estuaries across New Zealand.

Canonical analysis of principal coordinates (CAP; Anderson & Robinson 2003, Anderson & Willis 2003) was used to derive the model relationship between macroinvertebrate community structure and each environmental gradient (i.e., mud and metals). CAP allows a constrained ordination to be carried out on the basis of any dissimilarity or distance measure of choice and determines the axes that best discriminates an environmental gradient. All CAP analyses were performed on square-root transformed Bray-Curtis macroinvertebrate community dissimilarities (Bray & Curtis 1957) using 9999 permutations, with separate CAP models constructed for mud and metals. A square-root transformation (standard down-weighting for macrofaunal count data; Clarke & Gorley 2015a) was chosen to de-emphasis the influence of dominant taxa while still allowing differences in relative abundance to influence the results, as this was considered important for determining estuary health. Leave-one-out residual sum of squares was used to decide upon an appropriate value for the number of Principal Coordinate Analysis (PCO) axes (*m*) and diagnostics were checked to ensure this was appropriate for each model (Anderson et al. 2008).

Model CAP scores were simplified into a five-category health score system by splitting the CAP score gradient into five evenly spaced groups, which were re-scaled from 1 (least impacted) to 6 (most impacted) for ease of interpretation. One-way PERMANOVA was used to test whether the ecological health groups corresponded with significant differences in community structure. Unrestricted permutation of raw data was used, with 9999 permutations, type III sum of squares and ecological health group as a fixed factor. As a form of model validation, changes in community structure across the five ecological health groups were characterised using SIMPER to ensure that the discriminating taxa across groups were consistent with what is known about the habitat preferences and metal tolerances of organisms. Discriminating taxa that cumulatively contributed between 70-74% to the similarity of each group were assigned to one of three categories based on literature (Appendix 4). For the Mud BHM, the grain-size preference categories were sandy, intermediate/unknown, and muddy, with the intermediate/unknown group a placement for taxa that showed a preference for habitats with intermediate grain-size or for species that could not be assigned based on the literature. For the Metals BHM, the metal sensitivity categories were sensitive, mixed/unknown, and tolerant, with the mixed/unknown group a placement for taxa that showed an inconsistent response to metal contamination or for species that could not be assigned based on the literature. All statistical analyses were carried out using the statistical software PRIMER 7 (v 7.0.13) with the PERMANOVA+ add-on (Anderson et al. 2008, Clarke & Gorley 2015a).

The accuracy of each CAP model at identifying and predicting real and repeatable patterns in the data was measured by its ability to 1) correctly place validation sites onto the environmental gradient and 2) be unaffected by temporal variability that was not associated with changes in environmental drivers. The first validation is an important step because high canonical correlation does not necessarily mean good predictive power (Anderson et al. 2006). For example, high canonical correlation can be achieved by simply increasing the number of PCO axes (*m*) to be used in the CAP analysis. Validation sites were chosen to maximise spread across the environmental gradient and included a range of estuaries and regions. All validation sites were independent site/times, taken from a separate dataset from the one used to develop the models. Some of the locations of the validation sites were the same as some of the model sites but sampled in a different year, similar to the validation procedure used for the regional model (Anderson et al. 2006). Twenty-nine sites were used to validate the mud model and 20 were used for the metals model; equivalent to 15% of the number of model sites. Mud content at the Mud BHM validation sites ranged from 0.6 to 93% mud while maximum Cu, Pb and Zn concentrations at the Metals BHM validation sites were 43, 65 and 216 mg kg^{-1} respectively.

The BHMs were used to place each validation site onto the environmental gradient axes by calculating the Bray-Curtis dissimilarity between that site and the sites in the model. An option within the CAP procedure in PRIMER 7 allows the addition of new sites to the model without altering distances among other points because the dissimilarity between any two sites does not depend on the other sites in the model (Anderson et al. 2008). Physical-chemical values calculated using the BHMs were the predicted values along the environment gradient. Linear regression of sampled versus predicted physical-chemical values (either ln % mud or PC1 metals) were used to identify sites whose predicted values deviated most from their observed values and in which direction. A 1:1 line (i.e., with slope (*b*) = 1 and intercept (*a*) = 0) was drawn to help interpret the positions of the points. If prediction is exact, the points would lie precisely on this line. The slope of the linear relationship, *b*, and the strength of the relationship (coefficient of determination, R^2), between the predicted and observed values was also used to determine validation success. Models were considered good if *b* and R^2 were close to 1.

I also tested whether natural temporal variability in community composition across years resulted in a site sampled at a different time, but with similar mud or metal concentrations, having markedly different CAP scores (designated as being greater than the range of values for a single group). Nine sites (4-6 sampling occasions per site) were used to test the Mud BHM and seven sites (2-3 sampling occasions per site) were used to test the Metals BHM.

Co-variance between mud and metals can make it difficult to separate stressor effects. The potential for interactions between the Mud and Metals BHMs was examined in two ways. First the Pearson's correlation coefficient between the Mud and Metals CAP scores was calculated to examine the potential for interaction between the two models, with correlation coefficients $r \geq 0.95$ representing a strong interaction (Anderson et al. 2008). Secondly, because all the sites in the Metals BHM were also included in the Mud BHM, the variance in macroinvertebrate structure explained by each independent variable (mud and metals) could be partitioned. Following the methods of Anderson et al. (2008) and Borcard et al. (1992), sequential multiple linear regressions were conducted using the DistLM routine in PRIMER on the macroinvertebrate abundance (square root transformed) Bray-Curtis dissimilarities to partition the variance explained by mud and metals and identify the mixed effect.

3.2.3 Testing the model across different regions, estuary types and scales

In order to apply the BHM approach on a national scale, it is important that the models produce consistent results across different environmental contexts and species pools. To test whether the models were affected by such differences, sites were grouped by estuary type based on Hume et al.'s (2016) classification of New Zealand hydrosystems and region based on Shears et al.'s (2008) biogeographic classification scheme (Figure 3.1). Due to limited data availability, three bioregions (Banks, Chalmers, and Stewart Island) were combined into a single group (Southeastern) for the Metals BHM, and groups with less than five sites (Figure 3.1) were removed from the analysis of both the Metals and Mud BHMs. This resulted in two levels for the 'estuary type' factor for both models (tidal lagoons and shallow drowned valleys), six levels for the 'region' factor for the Mud BHM (Abel, Banks, Chalmers, Portland, Raglan and Northeastern) and five levels for the 'region' factor for the Metals BHM (Abel, Southeastern, Portland, Raglan and Northeastern). After initial data exploration following the protocol of Zuur et al. (2010), analysis of covariance (ANCOVA) using type III sum of squares was used to test if the relationship between the model CAP scores and the environmental gradient (either mud or metals) varied with region or estuary type using Statistical Analysis Software (SAS).

To understand how the national outputs relate to assessments carried out at finer scales of resolution, national BHM CAP scores were compared to those generated from separate BHMs developed using data from one estuary (Tauranga Harbour; Ellis et al. 2015) or one region (Auckland; Hewitt et al. 2005a) using Spearman's rank correlations. Eighteen sites in the national BHM were also in the single estuary BHM and 44 (Mud BHM) and 43 (Metals BHM) sites in the national BHM were also in the single region BHM.

3.3 Results

3.3.1 Model performance and validation

The CAP analyses underlying the Mud and Metals BHMs performed well (Figure 3.2). The CAP model ($m = 29$) based on mud content resulted in a canonical correlation of 0.90 ($R^2 = 0.81$), with the permutation test indicating that correlation between the CAP scores and the mud gradient was significantly different from zero ($n = 192$, trace test statistic = 0.81, $p < 0.0001$). CAP analysis based on metals ($m = 20$) also showed a strong (canonical correlation = 0.84, $R^2 = 0.71$) and significant ($n = 133$, trace test statistic = 0.71, $p < 0.0001$) relationship between benthic macroinvertebrate communities and sediment metal concentrations.

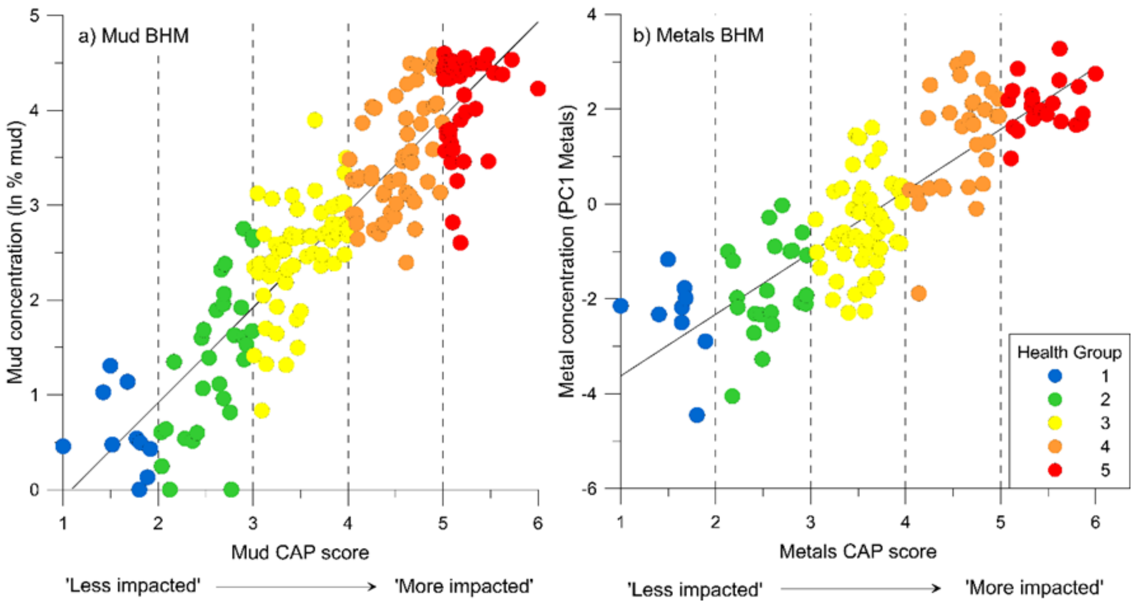


Figure 3.2 Benthic Health Models (BHMs) developed using canonical analysis of principal coordinates (CAP) constrained by either a) mud (ln % mud) or b) metals (first axis of principal component analysis based on log transformed copper, lead, and zinc). Grey dashed lines and symbol colours demarcate the ecological health categories for each model. A linear regression has been fitted for each of the models; Mud BHM $y = 1.0038x – 1.0911$, $R^2 = 0.81$, Metals BHM $y = 1.3002x – 4.9258$, $R^2 = 0.71$.

Sites were split into five ecological health groups, based on model CAP scores, and information on stressor values observed at sites within each group is provided in Appendix 4. For both models, PERMANOVA indicated a significant difference in community structure across the five groups (Mud BHM: pseudo-$F_{4,187} = 8.70$, $p < 0.0001$; Metals BHM: pseudo-$F_{4,128} = 4.66$, $p < 0.0001$). Pairwise comparisons showed these differences were significant across all groups ($p < 0.04$), apart from Group 1 and 2 for the Metals BHM, which was not significant ($t = 1.34$, $p = 0.065$). SIMPER analysis showed that community dissimilarity was 84% and 78% between Groups 1 and 5 for the Mud and Metals BHMs, respectively.

As another form of model validation, taxa characterising each ecological health group were identified using SIMPER and compared with known information related to grain-size preferences or levels of metal contamination (Appendix 4), to determine if the BHMs placed taxa in the expected ecological health groups. Unsurprisingly, taxa driving differences between Mud BHM groups have differing grain-size preferences, with most of the taxa characterising Group 1 preferring sand (e.g., the bivalve *Austrovenus stutchburyi* and *Paphies australis*, the gastropod *Notoacmea*, the polychaete *Aonides* and phoxocephalid amphipods) and many of the taxa characterising Group 5 preferring mud (e.g., the crabs *Austrohelice*, *Hemigrapsus* and *Hemiplax*, *Capitella* polychaetes, oligochaetes and corophid amphipods; Figure 3.3a). Similarly, taxa driving differences across Metals BHM groups have differing sensitivities to copper, lead, and zinc (Figure 3.3b). Many of the taxa characterising Metals BHM Group 1 have been found to be sensitive to metals (e.g., the bivalves *A. stutchburyi*, *P. australis* and *Macomona liliana*, orbinid and *Prionospio aucklandica* polychaetes, cumaceans and amphipods) while taxa more tolerant of metals (e.g., nereid and *Cossura* polychaetes, the crabs *Austrohelice*, *Hemigrapsus* and *Hemiplax* and the bivalve *Arthritica*) only begin to characterise benthic community structure in Group 3 and higher.

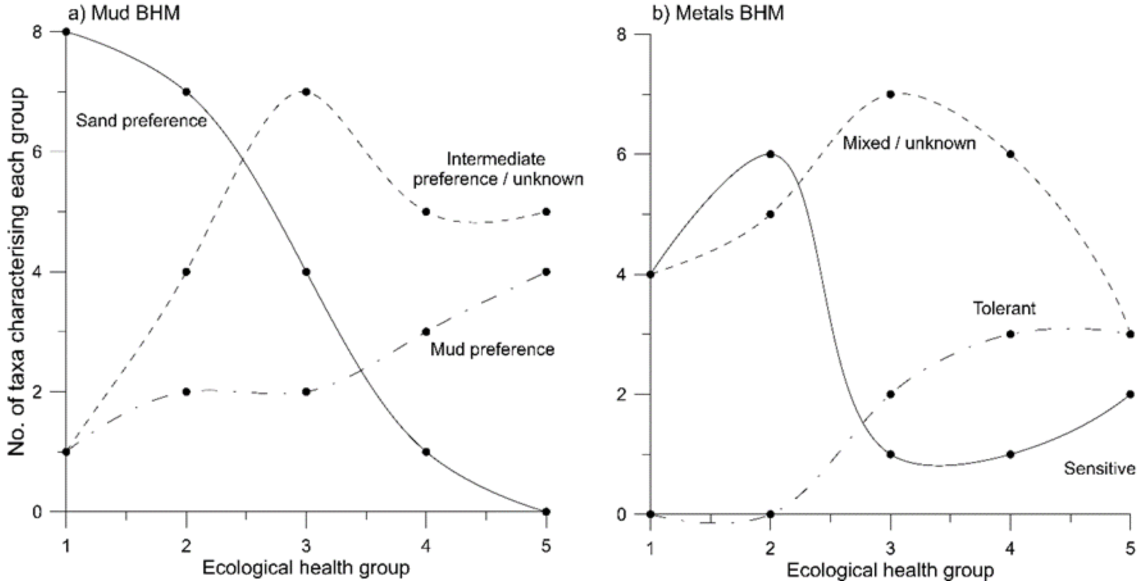


Figure 3.3 a) Number of taxa characterising Mud Benthic Health Model (BHM) ecological health groups, grouped by grain-size preference (sand, intermediate/unknown, mud), b) number of taxa characterising Metals BHM ecological health groups, grouped by metal contamination sensitivity (sensitive, mixed response/unknown, tolerant). Taxa characterising each ecological health group were identified using SIMPER (taxa that cumulatively contributed between 70-74% to the similarity of each group). Grain-size preferences and metal contamination sensitivities were assigned based on literature. Refer to Appendix 4 for further details.

Both the Mud and Metals BHMs were good at predicting the position of validation sites along the environmental gradients (R^2 = 0.90 and 0.82, respectively), with the slope of the line close to one for both models (Figure 3.4). For the temporal validation, which aimed to show that there would be no change in CAP scores if the stressor values did not change, most sites stayed within the range of an ecological health group (i.e., CAP scores within a range of 1.0), indicating that CAP scores were relatively stable and that the ecological health group boundaries are suitable (Appendix 5).

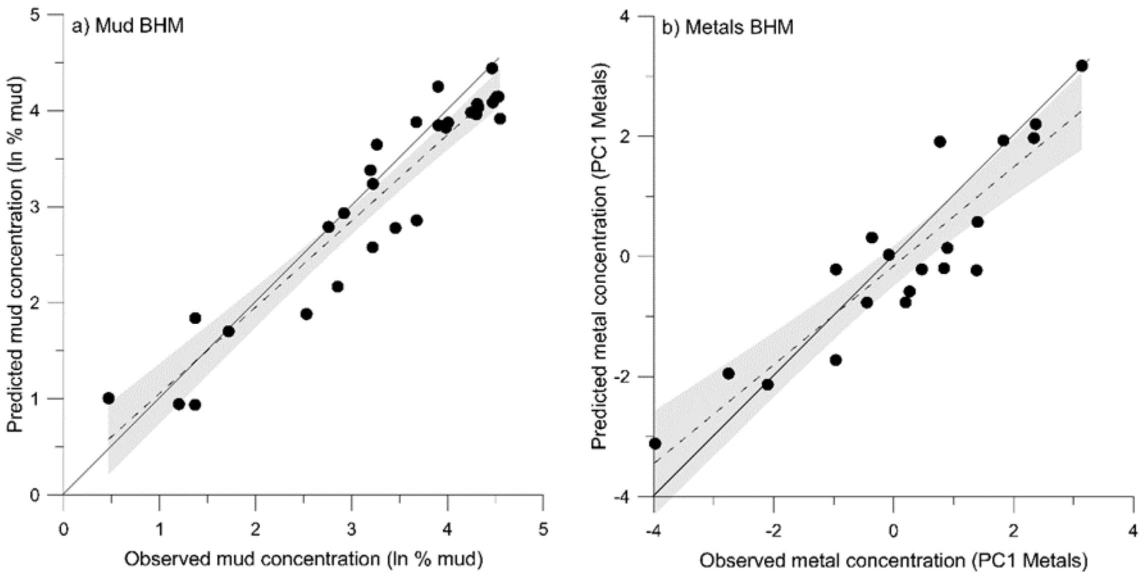


Figure 3.4 Validation of Benthic Health Models (BHMs) comparing observed a) mud (ln % mud) and b) metal (first axis of principal component analysis based on log transformed copper, lead, and zinc) concentrations with concentrations predicted by the BHMs on the basis of benthic macroinvertebrate community composition. The dashed line is the linear regression line (with 95% confidence interval indicated by grey shading) and the solid line has a slope of 1 and an intercept of zero (i.e., 1:1 line) and indicates where all points would lie if model predictions were perfect. Mud BHM $y = 0.8966x + 0.1614$, $R^2 = 0.90$. Metals BHM $y = 0.82x – 0.16$, $R^2 = 0.82$.

Moderate correlation was observed between the CAP scores from the two models ($r = 0.76$) suggesting there is potential for interaction between the two models. However, the relationship between the two models was variable (Figure 3.5) and DistLM showed that of the 13% variation in macroinvertebrate structure collectively explained by mud and metals, only 4.4% was shared between the two variables leaving 8.6% of variation that was independently explained by either mud or metals on their own. Furthermore, species shifts associated with changes in mud were not consistently the same as species shifts associated with changes in metals (Figure 3.3). The models had reduced ability to discriminate between stressors at the higher end of the range; sites with high metal concentrations always had high mud content but this was not always the case the other way around.

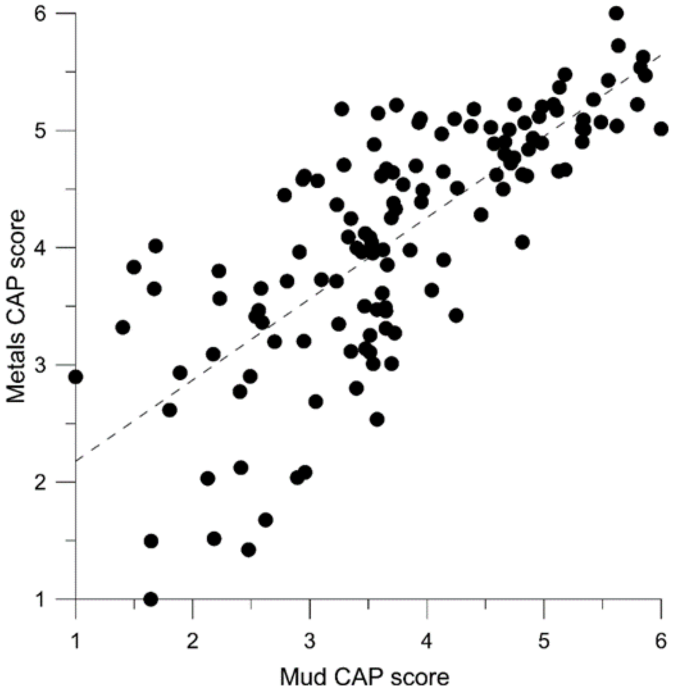


Figure 3.5 Relationship between the Mud and Metals Benthic Health Model (BHM) canonical analysis of principal coordinates (CAP) scores. A linear regression (dashed line) has been fitted ($y = 0.8376x + 0.5805$, $R^2 = 0.58$).

3.3.2 Effect of region, estuary type and scale on model results

The results of the linear regressions indicated that both the Mud and Metals BHMs could be applied across all regions and estuary types tested. The relationship between the CAP model scores and the environmental gradients did not differ across regions for either model (mud*region $F_{5,178} = 0.42$, $p = 0.802$; metals*region $F_{4,122} = 1.97$, $p = 0.103$). Similarly, there was no significant interaction between estuary type and environmental gradient for either model (mud*estuary type $F_{1,182} = 0.64$, $p = 0.647$; metals*estuary type $F_{1,127} = 3.41$, $p = 0.067$).

Spearman's rank correlations between the national BHM CAP scores and the single region and single estuary BHM CAP scores showed that the national BHMs ranked sites in a similar way as the regional models (strong and moderate correlations; Mud BHM $r = 0.98$, Metals BHM $r = 0.76$) but the correlations with the single estuary models were not as high (moderate correlations; Mud BHM $r = 0.68$, Metals BHM $r = 0.42$). Refer to Appendix 6 for more details.

3.4 Discussion

In this study, I successfully developed two models that track the health of estuarine benthic communities in response to two key coastal stressors; terrestrial sedimentation and heavy metal contamination. This approach to estuary health assessment has been previously applied on a regional (Hewitt et al. 2005a) and estuary scale (Ellis et al. 2015) and here I have developed models that can be used at a national level. With the plethora of biotic indices available for monitoring (refer Diaz et al. 2004 for reviews, Borja et al. 2015), and the range of agencies responsible for coastal management, achieving consistent assessment across countries or continents can be challenging (Borja et al. 2009). Like many other countries, New Zealand does not have a standardised approach making it difficult to compare health across estuaries and set national standards. Additionally, many of the biotic indices developed overseas are not readily transferable to New Zealand due to differences in species ecology and composition, stressor type or magnitude and estuary geomorphology (Rodil et al. 2013, Berthelsen et al. 2018). The transferability of a biotic index developed in one region to another part of the world will consistently be affected by these differences, although the development of regionally specific eco-groups may improve the performance of some indices (Gillett et al. 2015). The results of this study show that the BHMs are suitable for tracking the effects of increasing mud content and metal contamination on benthic community health in estuaries across New Zealand. The models can be applied in two widespread estuary types and across most regions. Thus, I have demonstrated the utility of the BHMs as a sensitive and standardised approach to national estuary health monitoring.

In addition to being sensitive enough to detect ecologically meaningful changes, indices must also be robust across the ecological and environmental contexts over which they will be applied (Borja & Dauer 2008). I tested this by examining the response of the BHMs across different regions and estuary types. The BHMs responded to mud and metals in the same manner across all regions and estuary types tested, indicating these models were robust and suitable for application in many estuaries across New Zealand. The lack of regional differentiation suggests that local environmental drivers (e.g., anthropogenic activities, sediment grain-size, hydrodynamics) may be more important in structuring communities than regional species pools, which are driven by factors such as species dispersal and biogeographic history (Ricklefs 1987). This finding is supported by other studies, which have found macrobenthic biodiversity to be influenced more by local conditions than regional ones (Edgar et al. 1999, de Juan & Hewitt 2011). However, regional variations in benthic community structure may have also been concealed by the level of taxonomic resolution required to develop a national scale model. The BHM approach requires a common pool of taxa and higher levels of taxonomic resolution (e.g., family vs genus/species) are often required to aggregate infrequent species into common groups or correct for inconsistencies in taxonomic resolution across source data. While reducing the number of taxonomic units can help the model perform well across a range of regions, it may obscure species-specific responses to stress, decreasing model sensitivity overall. Taxonomic resolution in the dataset was primarily constrained by inconsistencies across sites and better taxonomic standardisation could have enabled more robust models, across a wider range of regions and estuary types, to be developed.

When attempting to apply biotic indices on a nationwide scale, it is important to understand how outputs relate to assessments carried out at finer scales of resolution, as these may provide a more precise estimate of environmental status for managing specific locations and their problems. This study showed that the national BHMs ranked the health of sites in a similar manner to models developed using regional data but may not have been as sensitive as models developed using data from a single estuary. As mentioned earlier, this decrease in sensitivity may have arisen from aggregation of taxa to higher levels of taxonomic resolution, potentially obscuring species-specific responses to stress. Additionally, the smaller stressor gradient in the single estuary model may allow it to discriminate over smaller changes in health. I tested this by creating a new national model that was restricted to the same stressor range as the single estuary model and observed an improvement in the correlation between the model health score rankings (Appendix 6). Reduced power caused by having fewer data points for comparison may also contribute to inconsistency between model health score rankings, and this was supported by a slight decrease in concordance between the regional and national models when comparing fewer sites (Appendix 6).

Even though the single estuary model may provide a more sensitive measure of estuary health, having a national scale model delivers clear advantages. As BHM outputs are on a relative scale, a national scale model enables the health of the estuary to be placed in a national context and provides consistency across the country. Having a national model also reduces the substantial costs that would be required to develop separate estuary scale or even regional scale models, making it possible for managers to utilise this assessment tool to evaluate any estuary for which they have appropriate macroinvertebrate data.

The outputs of the BHMs can be simplified into a five-category health score system, which allows managers to easily track the relative health of sites through time or identify thresholds for undesirable conditions, which may trigger management action (Rees et al. 2008). Monitoring directional/trend targets is a robust and reliable method and is largely independent of the concept of reference conditions because it only requires relative assessments of ecological quality status (Borja et al. 2012). It can indicate how a site is changing in response to an increasing pressure, even if the site was already impacted when monitoring began. The BHM ecological health groups provide an indication of the health of a site in the context of New Zealand, however, managers need to consider more than just the relative health category when setting management targets as the category boundaries do not necessary reflect ecological thresholds. Establishing type-specific reference conditions could help to define appropriate thresholds in different settings (e.g., upper or lower reaches of estuaries) and there are a range of methods available to estimate these (EU Water Framework Directive 2000, Stoddard et al. 2006, Barbone et al. 2012, Borja et al. 2012). However, reference conditions can be difficult to define in estuaries due to their high natural variability and the scarcity of locations remaining in an undisturbed state (Chainho et al. 2007, Barbone et al. 2012, Berthelsen et al. 2018). Further research is required to understand where community thresholds lie along different environmental gradients and in different contexts, which could inform management goals or adjustment of group boundaries in the future.

Studies have suggested estuarine sediments with less than 10-30% mud support more diverse, abundant and/or resilient benthic communities (Rodil et al. 2013, Robertson et al. 2015, Robertson et al. 2016, Ellis et al. 2017). The boundary between Mud BHM Group 3 and 4 occurs around 18% mud and transitions to Group 5 around 50% mud. Therefore, depending on management goals, aiming for Mud BHM health scores in groups less than 4 may be appropriate. However, when interpreting Mud BHM health scores, it must be acknowledged that hydrodynamic controls on sedimentation rates may naturally result in upper reaches of estuaries being muddier than outer reaches, dependent on estuary type and the magnitude of sediment inputs. The risk of natural processes affecting the use of the Mud BHM can be alleviated in three ways. First, adjustment of thresholds or reference conditions, which consider these natural variations, can be used when setting management targets (Chainho et al. 2007). Second, sites can be selected to represent both inner and outer areas of estuaries. Third, rather than relying on one-off assessments of health, I recommend examining Mud BHM health scores over time and acting if a site is progressively decreasing in ‘health’ with respect to sedimentation.

Guidelines regarding acceptable levels of metal loading in coastal sediments vary (refer Burton 2002 for a review), but many sediment quality guidelines set two threshold values, one below which effects rarely occur (threshold effects e.g., TEL, ERL, SQGV) and one above which effects are likely to occur (midrange/extreme effects e.g., PEL, ERM, SQG-High; Long et al. 1995, MacDonald et al. 1996, Simpson et al. 2013). Most threshold effect values fall within Group 4 or 5 of the Metals BHM while almost all midrange or extreme values are beyond those measured in this nationwide study (refer to Appendix 7 for more details). However, as observed in other studies (Hewitt et al. 2009, Tremblay et al. 2017), both these lower and upper thresholds may be too high to protect benthic communities, given we observed significant changes in community structure at lower metal concentrations. Many of these guidelines are developed from single-species, laboratory dose-response experiments with mortality as an endpoint (Calow 1998), which do not accurately represent the complexities of coastal systems. Indeed, guidelines derived from field-based species sensitivity distributions (Bjørgesæter & Gray 2008, Kwok et al. 2008, Hewitt et al. 2009) tend to be lower than other guidelines outlined in Appendix 7, corresponding to Metals BHM Group 3 and 4.

Although single-stressor models have advantages in terms of providing objective measures of health and diagnosing the cause of degradation, interactions between stressors can confound outputs and any strongly co-varying environmental variables should be examined to ensure the model can discriminate between them. A moderate correlation was observed between the Mud and Metals BHMs, reflective of the fact that metals commonly bind to fine sediments and/or organic matter (Power & Chapman 1992). However, consistent with previous studies (Thrush et al. 2008, Hewitt & Ellis 2010, Ellis et al. 2015), I found the collinearity between mud content and metal concentrations was not sufficient to prevent partitioning out individual effects of these stressors on macroinvertebrate communities. Only 4.4% of the explained macroinvertebrate community variation was shared by mud and metals, suggesting that both variables are important in structuring benthic communities, with neither being a replacement for the other. The differences in taxa driving changes across the two models (Figure 3.3) also supports this conclusion. However, the Metals BHM may have reduced ability to discriminate between mud and metals effects in Group 5, so I suggest the use of bivariate plots of Mud and Metals CAP scores when assessing site changes (Hewitt & Ellis 2010). If sites are moving along only one of the two axes, effects can be attributed to that stressor, but if sites are moving in both directions, a close inspection of which species are responding to the changes may be required to ascertain the environmental driver.

Multivariate approaches to assessing health have been found to be more sensitive than univariate methods because they preserve information on all taxa and their relative abundances (Warwick & Clarke 1991, Attayde & Bozelli 1998, Gray 2000, Hewitt et al. 2005a, Ellis et al. 2015). However, it is precisely for this reason that the BHMs are constrained to being applied under the same conditions as the data used to develop them. Differences in species composition restrict the application of these models to intertidal portions of estuaries within New Zealand, although this does not preclude the development of new models for other environments or regions of the world. The outputs of the models appear robust across most regions and for the two estuary types tested (which represent more than half of the estuaries in New Zealand; Hume et al. 2016), but further research is needed to determine their suitability for assessing health in other estuary types. Although the incorporation of data collected across multiple months likely reduces the influence of seasonal fluctuations in species composition on model results, it is recommended that data for new sites is collected at similar seasonal time periods (in this case October to March, i.e., the time period for which most model data was collected). These models capture the range of mud and metal concentrations likely to be encountered in most New Zealand estuaries, however, if metal values increase significantly, new sites would need to be added to the model to extend its range, affecting comparison with earlier health model scores. The BHMs provided good indicators of benthic community health at a national level in response to mud and metals, however, I advocate the use of multiple indicators to gain a more complete understanding of overall health, particularly those that represent responses to other stressors (e.g., nutrients) or the condition of other taxonomic groups (e.g., plankton, fish).

Chapter 4: Environmental DNA metabarcoding reveals estuarine benthic community response to nutrient enrichment – evidence from an *in-situ* experiment

4.1 Introduction

Cumulative impacts from anthropogenic activities occurring on land and in the ocean are resulting in a global loss of biodiversity, ecosystem resilience and the ecosystem services upon which people rely (Lotze et al. 2006, Worm et al. 2006, Barbier et al. 2011, IPBES 2019). Due to their proximity to multiple human pressures, coastal zones are among the most impacted parts of the ocean (Agardy et al. 2005). It is critical that we have good monitoring tools to detect degradation in these ecologically important and vulnerable ecosystems before a tipping point is reached. In an attempt to halt degradation of our coastal and marine environments, several national and regional initiatives have been developed (e.g., Australia’s Oceans Policy, Canada’s Oceans Act and Oceans Strategy, the USA’s Oceans Act and Europe’s Water Framework Directive and Marine Strategy Framework Directive, and South Africa’s National Water Act; Borja et al. 2008). These policies generally require an assessment of ecological integrity or status carried out at the ecosystem level, rather than relying on single species or physical-chemical variables alone.

Bottom-dwelling macroinvertebrate communities have long been used for ecological health assessment because they respond relatively rapidly to stress and integrate the effects of multiple stressors over time. These attributes arise because macroinvertebrate communities are diverse, span multiple trophic levels, are predominantly sedentary as adults and have species specific sensitivities to stressors (Pearson & Rosenberg 1978, Gray et al. 1979, Dauer 1993, Borja et al. 2000). While macroinvertebrate communities are a valuable indicator of ecosystem health, traditional visual morphological identification of these animals is time consuming, relatively expensive and requires taxonomic expertise that is in decline worldwide (Jones 2008, Keeley et al. 2018). In addition, inferring ecosystem health solely from the larger, visible portion of communities neglects the contribution of meio- and microbial taxa (e.g., bacteria, protists, microalgae, nematodes), which have been shown to be extremely diverse and often more responsive to environmental change (Kennedy & Jacoby 1999, Kemp & Aller 2004, Eiler et al. 2013, Bianchelli et al. 2016, Li et al. 2018). Communities of bacteria, microalgae, micro- and meio-eukaryotes play an essential role in ecosystem structure and functioning (e.g., carbon and nitrogen cycling, energy transfer to higher trophic levels, sediment stabilization; Azam & Malfatti 2007, Tolhurst et al. 2008, Schratzberger 2018). Inclusion of these frequently overlooked communities could offer a more comprehensive view of the ecosystem, in keeping with requirements for integrated assessments of health, provide early warning signals of disturbance (because of their higher turnover) and help us to better understand connections between biodiversity and ecosystem functioning (Strong et al. 2015).

Recent advances in environmental genomics and the emergence of high-throughput sequencing (HTS) technologies are changing our ability to evaluate community composition, including characterization of invisible biodiversity. Using a technique known as environmental DNA (eDNA) metabarcoding, species diversity can be assessed at low taxonomic resolution from genetic fragments contained in small amounts of sediment (Baird & Hajibabaei 2012, Bourlat et al. 2013, Pawlowski et al. 2018). Organisms are identified without taxonomic expertise by matching short, HTS-derived gene fragments to a reference sequence library. Although eDNA metabarcoding is rapidly expanding as a new approach to biodiversity assessment and biomonitoring, much of our understanding of the suitability of eDNA metabarcoding for environmental monitoring has relied on correlative studies (e.g., Aylagas et al. 2017, Keeley et al. 2018, Laroche et al. 2018b, Montenegro et al. 2020) or experimental research conducted in laboratory settings (e.g., Chariton et al. 2014, Santi et al. 2019). Manipulative field studies are rarer (although see Lawes et al. 2017, Birrer et al. 2019) but are required to prove cause and effect, characterise the response of specific taxonomic groups to selected stressors, identify potential indicator taxa for ecological status assessment and demonstrate that these effects can be consistently detected over and above natural environmental variability. Estuaries present a particular challenge to using eDNA metabarcoding due to high environmental variability and lack of genomic studies in these habitats (Ruppert et al. 2019).

In this study, I carried out a manipulative field experiment and used eDNA metabarcoding to characterise changes in benthic community structure in response to nutrient enrichment in two estuaries. Nutrient loading is a major threat to estuaries worldwide (CENR 2000, NRC 2000), with two-thirds of estuaries in the US assessed to have moderate-high levels of eutrophication (Bricker et al. 2008) and one-third of European estuaries affected by nutrient enrichment (EEA 2012). Using eDNA metabarcoding, I characterised how eukaryotic, diatom and bacterial community structure changed in response to nutrient loading to explore whether these communities could be a sensitive indicator of nutrient enrichment. My experiment extends previous empirical research on the response of eDNA-derived estuarine communities to sediment nutrient enrichment (Birrer et al. 2018, Birrer et al. 2019) by moving into a new habitat (intertidal sandflats), exploring the response of diatom communities in more detail and broadening the scope of the research to test these responses under differing natural conditions. To my knowledge, this study is the first field experiment providing empirical evidence that eDNA metabarcoding can detect responses to nutrient enrichment across different trophic levels of intertidal benthic biodiversity (bacteria and eukaryotes, including diatoms) in two environmentally distinct estuarine systems and is thus an important contribution toward the development of molecular tools for ecosystem health assessment.

4.2 Methods

4.2.1 Field experiment

In April 2017, manipulative nutrient enrichment experiments were set up on unvegetated mid-tide sandflats in two estuaries located 25 km apart near Nelson, New Zealand (Figure 4.1). The site in Waimea Estuary (173° 11’ 06.59 E, 41° 17’ 33.36 S) was located close to the estuary mouth on exposed sandflats while the site in Delaware Inlet (173° 27’ 39.16 E, 41° 09' 50.42 S) was positioned in a more sheltered area of the estuary. Catchments of both estuaries were dominated by native and exotic forest but modelled mean annual nitrate concentrations (Plew et al. 2015) were higher in Waimea (49.7 mg m^{-3}) than in Delaware (27.7 mg m^{-3}), likely a result of the larger catchment size of Waimea (903 km^2 vs 78 km^2) and slightly more intensive land use (more horticulture).

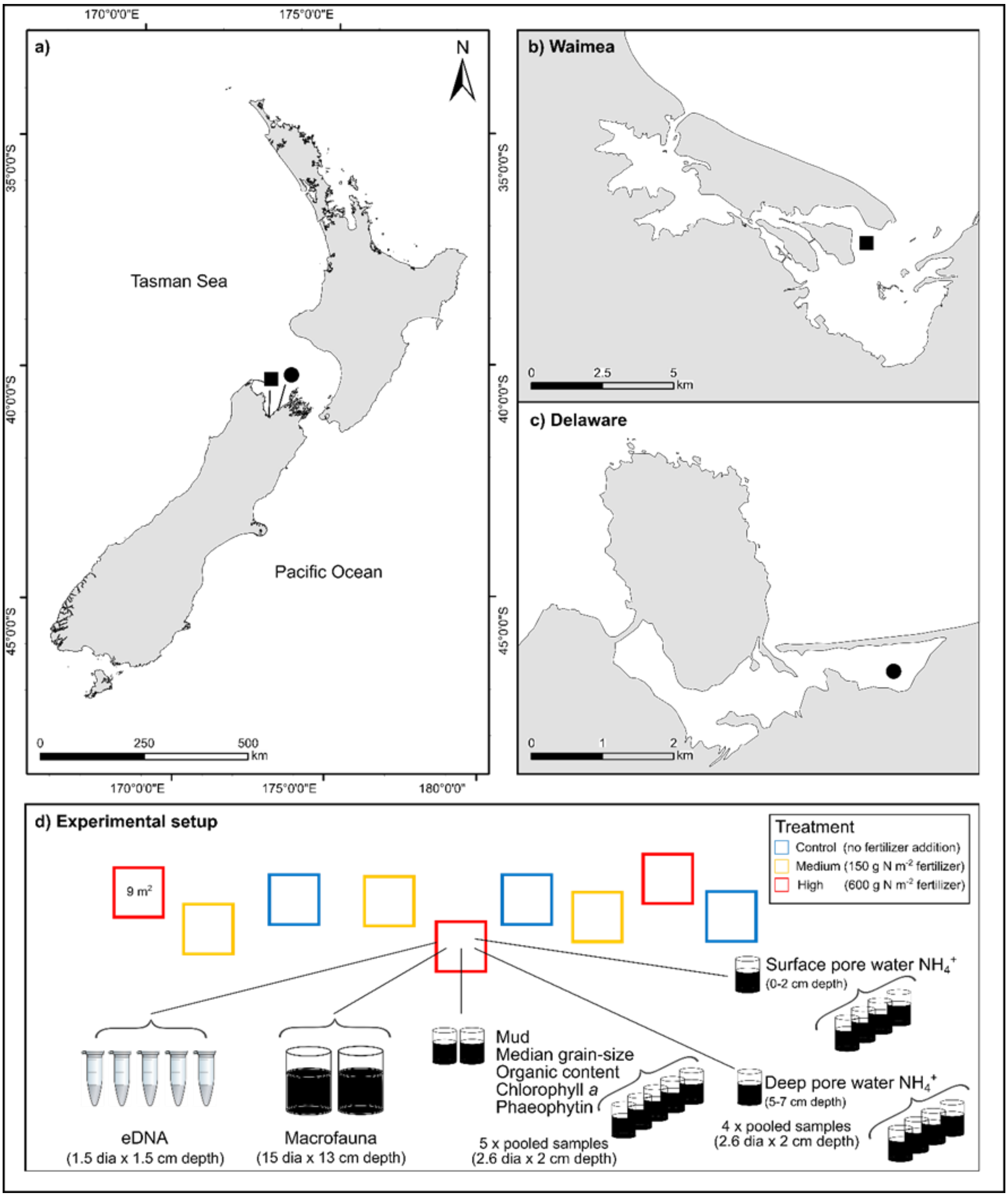


Figure 4.1 a) Map of New Zealand showing the location of Waimea Estuary (square) and Delaware Inlet (circle), b) & c) location of the study site within each estuary, d) experimental layout showing samples collected from each of the plots.

At each site, nine treatment plots arranged parallel to the incoming tide were interspersed across the sandflat, at least 3 m apart (Figure 4.1). Plots were set up by measuring a 3 x 3 m area on the surface of the sandflat and marking the corners of the plots with stakes. Plots were exposed to the elements and no attempt was made to control organism movement (e.g., with fences or cages), replicating natural conditions where organisms could respond to nutrient treatments depending on their preference and motility. The plots were randomly assigned a nutrient treatment: control (0 g N m^{-2}), medium (150 g N m^{-2} fertiliser) and high (600 g N m^{-2} fertiliser) enrichment (*n* = 3 plots per treatment). To simulate nutrient loading, I used Nutricote® slow release nitrogen (urea) fertiliser (140-200 d, 40-0-0 N:P:K) injected uniformly into the sediment at a depth of 15 cm following established methods (Douglas et al. 2016). Fertiliser granules were added to the plots by removing a sediment core (3 cm diameter x 15 cm depth), adding the fertiliser, and immediately replacing the plug to maintain sediment structure. Cores were evenly spaced (20 cores m^{-2}), with more granules added per core to achieve higher nutrient loading. This technique has been demonstrated to elevate surface (0-7 cm) sediment pore water NH_4^+ concentrations equivalent to those measured in enriched estuaries globally, with enrichment effects undetectable 0.5 m beyond the plot boundary (Douglas et al. 2016, Douglas et al. 2017, Thrush et al. 2017, Gladstone-Gallagher et al. 2020).

The plots were left undisturbed for the next seven months and sampled in November 2017. From each plot I collected two sediment samples (each consisting of five 2.6 cm diameter x 0-2 cm depth samples pooled) for grain-size, organic content, chlorophyll *a* and phaeophytin analyses, one sediment sample for pore water NH_4^+ analyses (four, 2.6 cm diameter, split into 0-2 cm and 5-7 cm depth sections and pooled) and two cores (13 cm diameter x 15 cm depth) for macroinvertebrate community composition (Figure 4.1). Chlorophyll *a* and phaeophytin concentrations were measured as a proxy for microphytobenthic biomass. Five sediment samples (1.5 cm diameter x 1.5 cm depth) were also randomly collected from each plot for eDNA metabarcoding using separate pairs of gloves and sterilized sampling vials. Field negatives for eDNA analysis were collected and consisted of three empty sampling vials handled in the same way as samples but not filled with sediment. Macroinvertebrate samples were sieved to 500 µm, preserved in 70% isopropyl alcohol and later counted and identified to the lowest possible taxonomic resolution (70% to species level). All other samples were kept in the dark, transported on ice to the laboratory and frozen (-20°C) until further processing, except for pore water, which was extracted immediately.

4.2.2 Analysis of environmental variables

Sediment grain-size was measured, after digestion in 10% hydrogen peroxide, on a Malvern Mastersizer 3000 (particle size range 0.01-3500 µm; Singer et al. 1988). Organic content was determined by drying sediment to a constant weight (60°C) and measuring weight loss on ignition of dry sediments (550°C for four hours; Parker 1983). Chlorophyll *a* and phaeophytin were extracted from sediment in 90% buffered acetone and measured fluorometrically before and after acidification (Arar & Collins 1997). Pore water was extracted by centrifugation, filtered (1.1 µm Whatman GC glass fibre filter) and frozen at -20°C. It was later analysed for NH_4^+ following the methods in Douglas et al. (2016).

4.2.3 Environmental DNA extraction, amplification, sequencing, and bioinformatics

Each step of the molecular processing (i.e., DNA extraction, polymerase chain reaction (PCR) and metabarcoding library preparation) was carried out in a separate sterile laboratory dedicated to that step with sequential workflow to ensure no cross-contamination. Each laboratory was treated with ultra-violet light for at least 15 min before use and all working surfaces wiped with 5% bleach. The PCR set-up and template addition were undertaken in laminar flow cabinets. Filter pipet tips (Axygen® and Thermo Fisher Scientific) were used throughout, and gloves changed frequently.

Environmental DNA sediment samples were homogenized via bead beating (MiniG™ 1600) for 2 min. DNA was then extracted from 2 g of sediment using the Qiagen DNeasy PowerSoil Kit, following the manufacturer's protocol. The field negative controls were processed the same way and extraction controls were added at the start of each new DNeasy PowerSoil Kit ($n = 2$). The quantity and quality of extracted DNA were measured using a NanoPhotometer (Implen). All extract products were stored frozen (-20°C) until further analysis.

Three gene markers were chosen to represent communities that were expected to respond to nutrient enrichment, either directly or indirectly (i.e., through changes in the macroinvertebrate communities that consume them). Bacterial communities were represented by short ca. 80-450 base-pair (bp) fragments of the nuclear 16S rRNA gene (V3-4 region), eukaryotic communities (including diatoms) were represented by the nuclear 18S rRNA gene (V4 region) and diatom communities were further investigated using the ribulose-1,5-bisphosphate carboxylase ⁄oxygenase large subunit (rbcL) gene (Stoof-Leichsenring et al. 2012), which provides highly resolved, complementary information to 18S (Kermarrec et al. 2013, Visco et al. 2015; Appendix 9). In this study, diatom communities assessed using the rbcL gene will be referred to separately from the eukaryotic communities assessed using the 18S gene, despite eukaryotic communities also containing diatom taxa.

Separate PCR analyses were performed on each eDNA sample for each of the three gene markers. PCR amplifications were undertaken on an Eppendorf Mastercycler in a total reaction volume of 50 µl using MyFi™ Mix (Bioline) according to the mastermix recipe and thermocycling conditions outlined in Appendix 9. One sample containing nuclease-free water (Ambion®) in place of DNA template was used as a 'no-template' negative control. PCR products were visualised on 1.5% agarose gels stained with RedSafe™ Nucleic Acid Staining Solution (Intron) to confirm the presence of 16S, 18S and rbcL fragments. Purification followed the Agencourt™ AMPureXP protocol (Beckman Coulter) using magnetic beads with products quantified using a Qubit™ 2.0 Fluorometer (Invitrogen). Purified amplicons were diluted to 3 ng ul^{-1} and sent to New Zealand Genomic Limited, University of Auckland, for library preparation following a two-step tailed PCR amplicon procedure using the Nextera XT kit and sequencing (Kozich et al. 2013). The final loading concentration of the library was 7 pM with a 15% PhiX spike and paired-end sequences (2 x 250 bp: MiSeq v2 reagents kit) were generated on a MiSeq instrument. Sequence data were automatically demultiplexed using MiSeq Reporter (v2). Raw sequence reads were deposited in the NCBI short read archive under the Project ID: PRJNA627491.

The same bioinformatics pipeline was applied for the bacterial, eukaryotic and diatom datasets, except where explicitly stated. Primers were removed using CUTADAPT (v 1.18; Martin 2011) with a single mismatch allowed and reads were subsequently processed using the DADA2 package (v 1.16; Callahan et al. 2016) within R software (v 3.6.1). Briefly, quality control of the reads was undertaken by truncating the reads (bacteria and eukaryotes forward 230 bp, reverse 228 bp; diatoms forward and reverse 110 bp), trimmed based on quality and filtered with a maxEE (maximum number of 'expected errors' allowed) of 2 for all forward reads, 4 for bacterial reverse reads and 6 for eukaryotic and diatom reverse reads. Reads were discarded if they did not match these criteria. Sequence variants for the forward and reverse reads were inferred using pseudo-pooling based on derived error profiles (first 10^8 bp in the dataset) after sequence dereplications. Using a maximum mismatch of 1 bp and a required minimum overlap of 10 bp paired-end reads were merged, discarding any reads that did not merge correctly. Chimeras were removed using the consensus method in DADA2. The resulting chimerachecked, merged amplicon sequence variants (ASVs) were taxonomically assigned using the DADA2 method, based on the rdp classifier (Wang et al. 2007) with a confidence of 50, using three distinct sequencing referencing databases. For bacteria (16S), the SILVA v 132 database (Pruesse et al. 2007) was used as a reference. For eukaryotes (including diatoms, 18S), the Protist Ribosomal Reference (PR2) database (v 4.11.1; Guillou et al. 2013) was used. For diatoms (rbcL), the reference sequences were downloaded from the National Center of Biotechnology Information (NCBI; Sayers et al. 2018) and formatted for use with DADA2. The results were then parsed into a table using the phyloseq package.

Detected contamination was negligible in negative controls, with the total number of reads in each control < 350, except for two negative controls which had 1050-2410 reads for three ASVs (a Oncholaimidae nematode and two Cylindrotheca diatoms). The number of reads for each ASV found in negative controls was subtracted across all other samples following the method described in Bell et al. (2018). Non-target taxa were also removed from the bacterial (eukaryotes, chloroplasts, and mitochondria) and eukaryotic (mammals and Actinopterygii) samples. Diatom taxa were retained in the 18S eukaryotic community dataset. ASVs with a total of < 0.005% reads across all samples were removed from the dataset. Rarefaction curves and the number of reads and ASVs remaining in each sample are presented in Appendix 10 and 11. As I was primarily interested in community structure, I retained samples with > 5000 reads and converted the number of reads to proportional abundance for the downstream statistical analyses.

4.2.4 Data analysis and statistics

Environmental data were averaged by plot and transformed, if necessary, to meet assumptions of normality (square-root was used for mud content; log was used for organic matter and pore water concentrations). Two-way analysis of variances (ANOVAs) with ‘site’ and ‘treatment’ as fixed factors were carried out in R (v 3.6.1) to test whether sediment properties, microphytobenthic biomass and pore water NH_4^+ varied significantly between sites and treatments.

Multivariate analyses were used to investigate whether eukaryotic, diatom and bacterial community structure changed across nutrient enrichment treatments. Bray-Curtis dissimilarity matrices were calculated for each dataset using fourth root transformed eDNA proportional read abundance and the results were plotted using Principal coordinates analysis (PCO). The PCO revealed four outliers (two from the control treatment at Delaware, one from the control treatment at Waimea and one from the high treatment at Waimea) and further investigation showed these samples had low DNA concentrations, numbers of ASVs or numbers of reads. These samples were removed from all three eDNA community datasets (and subsequent analyses) and the PCO was re-run.

Two-way permutational ANOVAs (PERMANOVAs) with ‘site’ and ‘treatment’ as fixed factors were used to test whether eDNA-derived community structure varied with nutrient enrichment and whether this response varied with site. Permutations of residuals under a reduced model was used, with 9999 permutations, type III sum of squares and pairwise *post-hoc* tests to identify significant differences between treatments. Differences between treatments were visualised using Canonical Analysis of Principal Coordinates (CAP; Anderson & Willis 2003), with ‘treatment’ as a factor and 9999 permutations. CAP allows a constrained ordination to be carried out based on any dissimilarity measure and determines the PCO axes that are best at discriminating among *a priori* groups. The appropriate number of axes (m) used in each CAP model was chosen by the software, which maximises a leave-one-out allocation success to groups (the proportion of samples allocated into their correct group using a leave-one-out procedure). Allocation success was also used as a measure of the sensitivity of each community at detecting nutrient enrichment effects. Allocation success was chosen in preference to canonical correlation for determining model performance because canonical correlation, and the separation between treatments on the CAP plots, increases as the number of axes in the model increases, even if the predictive capability of the underlying CAP model does not improve (Anderson et al. 2008). Based on the highly significant PERMANOVA site x treatment interaction for each community, CAP was performed on each site separately. Tests of homogeneity of dispersions (PERMDISP), which were used to quantify the variability in community structure between treatments, were also performed for each site separately, using ‘treatment’ as a group factor, 9999 permutations and calculating distances to centroids. All multivariate statistical analyses were carried out using PRIMER 7 (v 7.0.13) with the PERMANOVA + add-on (Anderson et al. 2008, Clarke & Gorley 2015b).

At each site, eukaryotic (including diatoms), diatom and bacterial taxa indicative of each nutrient enrichment treatment were identified using the *indicspecies* R package (v 1.7.8; De Caceres 2019), with Indicator Values (IndVal) measuring the strength of the association between a taxon and a treatment. ASVs present in less than three samples were discarded, taxa were then aggregated to genus-level and *indicspecies* was carried on proportional abundance read data using multipatt function with 9999 permutations and a significance level of 0.05.

Fourth root transformed macroinvertebrate community abundance data were plotted using PCO and CAP to see if patterns observed using eDNA-derived communities generally aligned with those resulting from traditional monitoring techniques. Results from other analyses (i.e., PERMANOVA, CAP, PERMDISP, *indicspecies*) carried out on macroinvertebrate abundance data can be found in Appendix 13-16.

4.3 Results

The two-way ANOVAs showed that sediment properties varied between sites but not between treatments, with Delaware having a smaller median grain-size ($F_{1,12} = 234.99$, $p < 0.0001$) and higher proportion of mud (particles < 63 µm; $F_{1,12} = 184.5$, $p < 0.0001$), organic content ($F_{1,12} = 1048.0$, $p < 0.0001$), chlorophyll *a* ($F_{1,12} = 13.1$, $p = 0.0035$) and phaeophytin ($F_{1,12} = 45.5$, $p < 0.0001$) content than Waimea (Table 4.1 and Appendix 12). The nitrogen fertiliser addition increased surface ($F_{2,12} = 25.6$, $p < 0.0001$) and deep ($F_{2,12} = 58.0$, $p < 0.0001$) pore water NH_4^+ concentrations in the medium and high treatments and this did not vary with site (Table 4.1 and Appendix 12). Pore water NH_4^+ concentrations in the medium treatment were 5-76 times greater than controls while concentrations in the high treatment were 118-760 times higher than controls (Table 4.1).

Table 4.1 Sediment properties (average ± 1 standard deviation, $n = 6$ except for pore water where $n = 3$) in experimental plots at Waimea and Delaware seven months after addition of slow-release nitrogen fertiliser (control: 0 g N m^{-2}; medium: 150 g N m^{-2}; high 600 g N m^{-2}). Full statistical results are presented in Appendix 12.

Variable	Waimea			Delaware		
	Control	Medium	High	Control	Medium	High
Sediment properties						
Mud (% < 63 µm)*	0.4 (0.7)	0.9 (0.5)	1.4 (0.9)	16.1 (4.7)	14.4 (2.4)	17.4 (5.2)
Median grain-size (µm)*	151.0 (1.7)	151.0 (1.6)	149.0 (2.3)	103.0 (8.8)	106.0 (5.9)	103.0 (9.3)
Organic content (%)*	1.3 (0.0)	1.3 (0.1)	1.3 (0.1)	2.7 (0.3)	2.7 (0.2)	2.8 (0.3)
Microphytobenthic biomass (µg g^{-1} sediment)						
Chlorophyll *a* *	2.7 (0.6)	3.7 (0.9)	2.5 (0.4)	4.2 (0.6)	4.3 (0.5)	4.4 (1.2)
Phaeophytin *	1.2 (0.2)	1.9 (1.0)	1.7 (0.2)	3.4 (0.8)	3.1 (0.3)	3.7 (0.6)
Pore water NH_4^+ (µmol N L^{-1})						
Surface sediments (0-2 cm)†	29 (28)	2,190 (2,439)	15,500 (14,240)	68.0 (16)	351 (188)	7,980 (7,104)
Deeper sediments (5-7 cm)†	40 (14)	2,900 (3,816)	28,500 (19,918)	114 (44)	2,080 (1,137)	32,500 (21,982)

*differed significantly ($p < 0.004$) between sites (see Appendix 12)
†differed significantly ($p < 0.02$) between treatments (see Appendix 12)

For all four communities (eukaryotes, diatoms, bacteria, and macroinvertebrates), the greatest variance in community structure was between sites rather than across treatments, with 30-75% of the total variance explained along the first PCO axis that seperated the two sites (Figure 4.2). PERMANOVA tests showed there was a highly significant site x treatment interaction for each community type (eukaryotes pseudo-$F_{2,79} = 2.38$, $p = 0.0001$; diatoms pseudo-$F_{2,79} = 2.40$, $p = 0.0001$; bacteria pseudo-$\mathrm{F}_{2,77} = 2.30$, $p = 0.0001$; macroinvertebrates pseudo-$F_{2,30} = 2.07$, $p = 0.0040$), which meant the response to nutrient addition varied with site (Appendix 13). Within-site *post-hoc* testing showed that there were significant differences ($p < 0.05$) in eukaryotic, diatom and bacterial community structure between all treatments, with the exception of eukaryotic communities in the medium and high treatments at Waimea ($t = 1.2$, $p = 0.0684$) and bacterial communities in the control and medium treatments at Delaware ($t = 1.1$, $p = 0.1600$; Appendix 13).

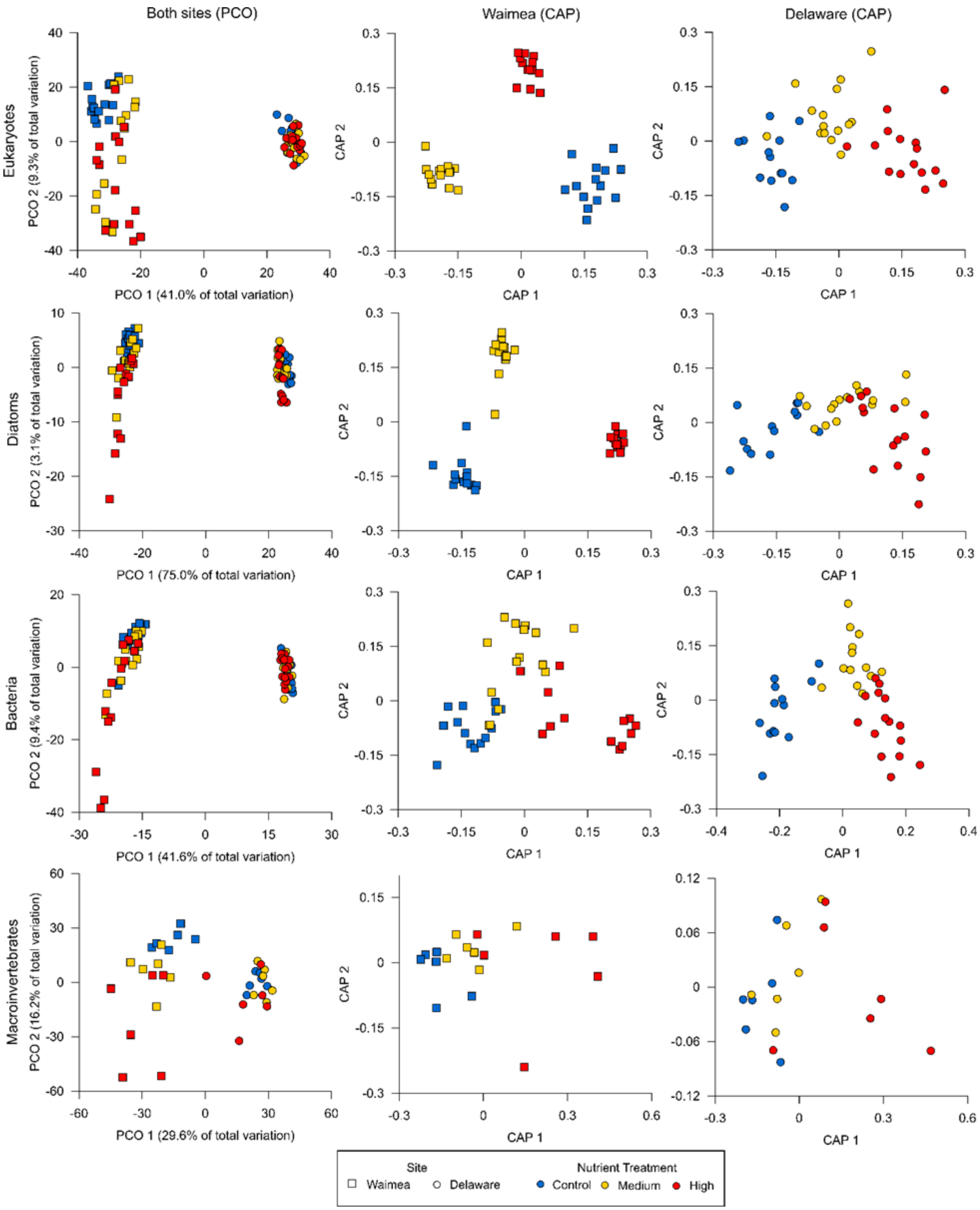


Figure 4.2 Principal coordinates analysis (PCO) plots of data from both sites (left-hand column) and canonical analysis of principal coordinates (CAP) plots of data from Waimea (middle column) and Delaware (right-hand column). Each row displays plots from different communities; eukaryotes (including diatoms), diatom only, bacteria and macroinvertebrates. Analyses were based on Bray-Curtis dissimilarities of fourth root transformed environmental DNA (eDNA) proportional read abundance data or macroinvertebrate abundance data with nutrient enrichment treatment used as a grouping factor for the CAP analyses.

The CAP ordinations (Figure 4.2) provide a visual representation of site-specific differences in community structure among nutrient enrichment treatments (canonical correlation = 0.73-0.99, *p* = 0.0001-0.0315; Table 4.2). Correlations from CAP plots based on eukaryotic, diatom and bacterial communities offer strong support for significant differences in community structure between treatments. CAP models derived from these communities were able to correctly allocate observations into the appropriate nutrient enrichment treatment 61-83% of the time, which is considerably better than the 33% success expected by chance if samples were randomly allocated into three groups. Models derived from diatom and bacterial communities performed best at Waimea (83% and 81% allocation success, respectively) followed by eukaryotic communities (71% allocation success). At Delaware, models based on eukaryotic communities performed the best (81% allocation success) followed by diatoms and bacteria (67% and 61% allocation success, respectively). The poorer performance of CAP models for eukaryotic communities at Waimea and diatom and bacterial communities at Delaware agree with the *post-hoc* PERMANOVA test results described above and the marginally significant differences between bacterial communities in the medium and high treatments at Delaware (*t* = 1.1, *p* = 0.0448; Appendix 13). For example, the PERMANOVA test showed no significant difference between bacterial communities in the control and medium treatments at Delaware (*t* = 1.1, *p* = 0.1600) and the CAP model was only able to correctly allocate samples into the control treatment 46% of the time. CAP ordinations based on macroinvertebrate communities showed the same patterns as those observed from ordinations of eDNA-derived communities (Figure 4.2).

Table 4.2 Summary of canonical analysis of principal coordinates (CAP) analyses carried out on fourth root transformed environmental DNA (eDNA) proportional read abundance data for eukaryotic (includes diatoms), diatom only and bacterial communities at two sites. *Model performance is assessed using the allocation success, with higher values indicating better performance. Details from the CAP analysis based on macroinvertebrate communities are presented in Appendix 14 because a direct comparison with eDNA-derived communities is not possible due to differing numbers of replicates.

Site	**Waimea**			**Delaware**		
Community	**Eukaryotes**	**Diatoms**	**Bacteria**	**Eukaryotes**	**Diatoms**	**Bacteria**
Number of samples	42	42	42	43	43	41
Correlation	0.98	0.99	0.85	0.92	0.87	0.95
Canonical correlation	0.97	0.99	0.73	0.85	0.75	0.89
Total variation explained	99%	96%	70%	63%	63%	78%
Number of PCO axes (m)	34	30	12	11	11	23
Trace statistic	1.8908	1.9118	1.2679	1.1972	1.0065	1.3792
p	0.0040	0.0001	0.0001	0.0001	0.0006	0.0315
Allocation success* (%)	71.4	83.3	81.0	81.4	67.4	61.0
Control	85.7	100	100	76.9	61.5	46.2
Medium	64.3	73.3	66.7	86.7	73.3	69.2
High	64.3	76.3	76.9	80.0	66.7	66.7

PERMDISP results showed that at Delaware, variability in bacterial and diatom community structure was similar across all treatments (bacteria $F_{2,38} = 1.07$, $p = 0.4388$; diatom $F_{2,40} = 1.86$, $p = 0.1968$, respectively) but greater variation in eukaryotic community structure was observed in the high treatment compared to the control ($t = 3.0$, $p = 0.0085$) and medium ($t = 2.6$, $p = 0.0193$) treatments, with the average Bray-Curtis distance-to-centroid 3% greater in the high treatments (Appendix 15). At Waimea, the high nutrient treatment was associated with greater variation in community structure (eukaryotes $F_{2,39} = 23.69$, $p = 0.0001$; diatoms $F_{2,39} = 20.54$, $p = 0.0001$; bacteria $F_{2,39} = 13.62$, $p = 0.0001$) across all three eDNA-derived communities, with the average Bray-Curtis distance-to-centroid 8-16% greater than the controls and 3-8% greater than the medium treatment.

Indicator species analysis identified eukaryotic ($n = 31$), diatom ($n = 4$) and bacterial ($n = 52$) taxa significantly ($p < 0.05$) associated with different nutrient enrichment treatments. None of the eukaryotic community indicator taxa were diatoms. Eukaryotic and bacterial communities showed a clear shift in response to nutrient loading with several indicator species only present in the medium and high nutrient treatments or present in higher abundances than the control treatments (Figure 4.3). Eukaryotic and diatom indicator taxa were site-specific for all treatments and no diatom taxa were associated with high nutrient enrichment. Most bacterial indicator taxa were also site-specific, except for *Fusibacter* and *Soehngenia*, which were indicative of the medium-high treatment at Waimea and the high treatment at Delaware. Twelve bacterial indicator taxa associated with high nutrient enrichment were also shared between sites. Indicator values and abundances per treatment for each of the indicator taxa are provided in Appendix 16.

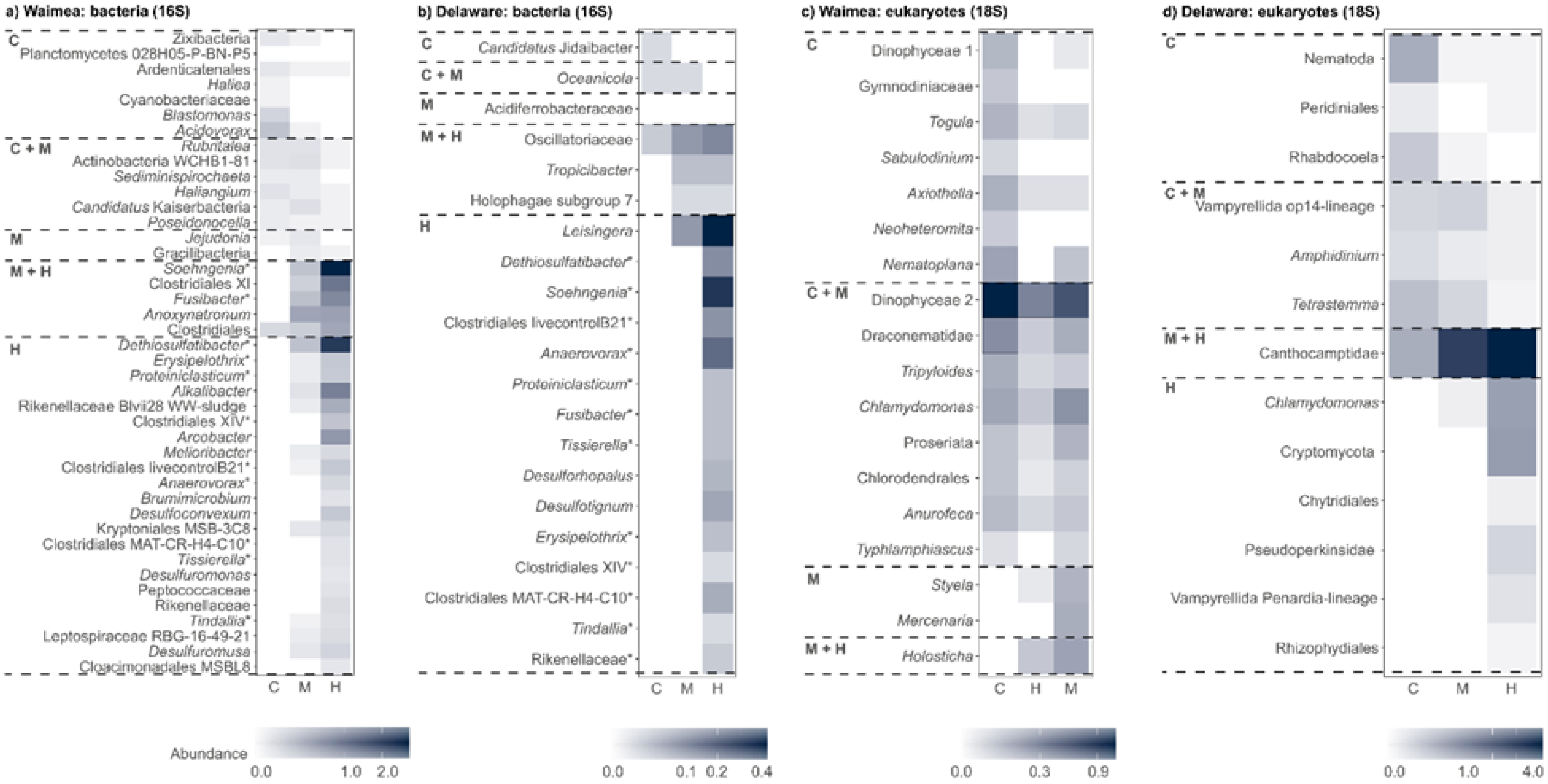


Figure 4.3 Average abundance of indicator taxa for bacterial (a, b) and eukaryotic (c, d) communities (including diatoms) across three nutrient enrichment treatments (control, medium, high) at sites in Waimea and Delaware estuaries. The nutrient enrichment treatment (or groups of treatments) that each taxon is associated with is indicated for each group (C = control, M = medium, H = high). An asterisk beside the name denotes indicator taxa shared by both sites.

4.4 Discussion

In my manipulative experimental study, the nutrient addition elevated sediment pore water NH_4^+ to levels found within eutrophic estuaries globally (Douglas et al. 2016), with clear differences in sediment pore water NH_4^+ observed as nutrient loading increased across treatments, but not between sites. Sediment properties (i.e., granulometry and organic content) were not altered by the nutrient addition, therefore, responses in benthic communities can be confidently attributed to nutrient enrichment. Nutrient enrichment is known to modify sediment and water chemistry leading to changes in the composition, biomass and diversity of benthic communities (NRC 2000). Benthic communities (eukaryotes, diatoms, bacteria, and macroinvertebrates) at the two studied estuaries were distinct from each other, most likely reflecting differing environmental conditions at each site. Regardless of the underlying differences in community structure, changes in these communities were observed at both sites in response to nutrient enrichment, demonstrating their potential use for ecosystem health assessment in response to eutrophication pressure.

Before noticeable eutrophication-related structural changes occur in macroinvertebrate assemblages, considerable shifts in the composition of microbenthos are expected in affected habitats, providing early signals of functional disturbance (Keeley et al. 2018). Diatom only and bacterial communities showed the strongest response to nutrient enrichment at Waimea while eukaryotic communities (including diatoms) were most sensitive to changes in nutrient loads at Delaware. The differing sensitivities of these communities to nutrient enrichment may reflect the differing environmental conditions at each site and suggests the development of indicators may be context dependent. For example, as nutrient loading at Waimea increased there was a reduction in diazotrophic cyanobacteria (Cyanobacteriaceae in the Order Nostocales; Blais et al. 2012) and increase in *Proteiniclasticum*, which has been found to be abundant in situations where nitrate reduction is high (Li et al. 2016). These changes may indicate that the addition of fertiliser to the sandy sediments at Waimea altered nitrogen acquisition pathways in bacterial communities (e.g., by switching from nitrogen fixation to assimilation) resulting in shifts in bacterial community composition and a strong response to enrichment. Conversely, bacterial communities in the naturally enriched muddy sediments at Delaware may already possess the ability to undertake these functional pathways, therefore, the nutrient addition may have only adjusted their rates, without major shifts in community composition. Further studies across a wider range of sites are required to understand drivers of site-specific responses. Other studies have also shown diatom (Agatz et al. 1999, Weckström & Juggins 2006, Kafouris et al. 2019, Tsikopoulou et al. 2020), bacterial (Dowle et al. 2015, Lawes et al. 2016b, Lawes et al. 2017, Keeley et al. 2018, Stoeck et al. 2018, Santi et al. 2019) and eukaryotic (Chariton et al. 2015, Santi et al. 2019) communities to be sensitive indicators of enrichment, with diatom and bacterial communities often responding more strongly than general eukaryotes (Birrer et al. 2018, Minerovic et al. 2020, Pochon et al. 2020).

Clear shifts in eukaryotic and bacterial indicator taxa were seen in response to nutrient loading but indicator taxa common to both sites were restricted to bacterial communities. These shared bacterial taxa were almost completely absent from control treatments, and often the medium treatments as well, suggesting that they were favoured once nutrients reached a certain level. Most shared indicator taxa were from the Clostridiales group, which includes a diverse range of species representing a variety of degradation pathways (Wiegel et al. 2006). Some of these taxa, such as ammonifying bacteria in the genus *Tindallia* (Kevbrin et al. 1998), identified as an indicator species in this study, can be linked to the degradation of organic matter, which is expected to increase with nutrient addition due to the stimulation of primary and secondary production. Accumulation of organic matter can lead to the formation of anaerobic sediments, which favour bacteria adapted to these environments, such as the anaerobic sulfur-reducing bacteria *Fusibacter* (Fadhlaoui et al. 2015), which was also associated with nutrient enrichment at both sites.

Many of the site-specific bacterial taxa associated with the high nutrient treatment are known to play roles in the sulfur cycle, including the anaerobic sulfate-reducing bacteria *Desulfuromonas, Desulfoconvexum, Desulfotignum*, *Desulfuromusa* and members of *Arcobacter* and the Peptococcaceae and Rhodobacteraceae families (Widdel & Pfennig 1992, Schink et al. 2002, Pujalte et al. 2014, Stackebrandt 2014). Consistent with this study, sulfate-reducing bacteria have been found to respond positively to organic carbon and nitrogen in seagrass and mangrove sediments (Sun et al. 2015, Zhu et al. 2018). Increased abundances of sulfate-reducing bacteria have also been reported from enriched sediments near fish farms (Kawahara et al. 2009, Dowle et al. 2015, Keeley et al. 2018) and changes in the gene expression of microbial nitrogen and sulfur metabolisms were observed in response to excess organic enrichment in a manipulative field experiment (Birrer et al. 2019).

For eukaryotes, unique indicator taxa were identified at each of the sites and included taxa commonly associated with estuarine sediments (e.g., dinoflagellates, nematodes, platyhelminths). Eukaryotic taxa indicative of the high nutrient treatment were only identified at Delaware, corresponding with the finding that eukaryotic communities were the most responsive to changes in nutrient loads at this site. These taxa included green algae (*Chlamydomonas* sp.) and aquatic fungi (Cryptomycota, Chytridiales, Rhizophydiales) known to infect algae (e.g., diatoms, dinoflagellates, green algae). The increase in green algae in the high nutrient treatment likely arises from nutrient loading fuelling algal metabolism (Stevenson 2014) while the increase in parasitic fungi may be indirectly linked to changes in the abundance of the aquatic algae with whom they associate.

Only four indicator taxa (all site-specific) were identified for the diatom only community at the genus level, suggesting that taxa-specific response of these communities to nutrient enrichment was more subtle than that of eukaryotic and bacterial communities. Diatom species within a genus may differ in their sensitivity to nutrient enrichment (Hillebrand & Sommer 1997) and other environmental variables (An et al. 2018), therefore, indicator taxa may not be revealed at the genus level. For example, the diatom *Melosira moniliformis* was shown respond positively to *in-situ* nitrogen enrichment in the Baltic Sea, but no response was observed for the closely related species *M. nummuloides* (Hillebrand & Sommer 1997).

My results suggest that bacterial communities, which had indicator taxa common to both sites, show the most promise for the development of benthic health assessment tools. Other studies have also shown bacterial communities to be relatively non-specific to differences in water flow regime, site, and geographic region (Keeley et al. 2018, Frühe et al. 2020), suggesting changes in these communities may be temporally consistent and regionally transferable. For index development and validation, the scale of the study will need to be expanded to ensure any patterns hold true across wider spatial and temporal scales and identify drivers of inconsistent responses. Despite the fact that nutrient enrichment did not consistently select for particular indicator diatom and eukaryotic taxa across study sites, their strong community-level structural response to nutrient enrichment shows potential for use in multivariate and multitrophic ecosystem health metrics.

Besides structural and compositional changes, response to disturbance can be manifested through other benthic community characteristics (e.g., species diversity, variation, or turnover rates). In this study, for example, benthic community variation increased with nutrient loading, supporting the idea that increased variability can act as an indicator of stress in marine communities and proximity to tipping points (Warwick & Clarke 1993, Brock & Carpenter 2006, Litzow et al. 2008, Guttal & Jayaprakash 2009). At Delaware, this pattern was only detected in eukaryotic communities while at Waimea, the trend was stronger and was observed in all eDNA-derived communities (eukaryotes, diatoms, and bacteria). Community metrics (species abundance, richness, diversity, taxonomic distinctness) and ecosystem function responses (sediment oxygen consumption, ammonium flux and gross primary production) show greater variability in sandy sediments than muddy sediments, with mud acting as a ceiling factor that limits variability, possibly explaining the weaker response at Delaware (Thrush et al. 2003b, Pratt et al. 2013).

The response of eDNA-derived communities to enrichment aligned with results from traditional morphological identification of macroinvertebrates, confirming that eDNA can provide concordant, and potentially better (Dafforn et al. 2014), information than that collected using current monitoring approaches. Macroinvertebrate communities appeared to be less responsive to enrichment effects than eDNA-derived communities, however, due to differences in the scale of sampling (number of replicates, area sampled), comparisons of quantitative results between eDNA-derived and macroinvertebrate communities should be undertaken with caution. The poorer response of macroinvertebrate communities could be a result of the fewer replicates collected in this study, the lower taxonomic resolution and limited range of taxa often associated with morphological identification, or the slower turnover rates of macroinvertebrates compared with bacteria and eukaryotes (e.g., bacteria turnover rate is minutes to days; Luna et al. 2002). eDNA sample processing has been estimated to be three times quicker and half the cost of traditional monitoring (Aylagas et al. 2018), with effort and cost decreasing as the number of samples increases. This allows more samples to be collected for an equivalent cost, while providing unprecedented volumes of biodiversity information, which can increase the power to detect change (as in this study) or expand the spatial or temporal scope of monitoring programs. Furthermore, the wide range of taxa captured by metabarcording allows for greater discrimination between ecosystem responses integrated across different temporal scales, than a dataset constrained to only macroinvertebrate responses.

Most ecological assessment methods can easily distinguish between unimpacted and impacted sites, however, it is more difficult to discriminate smaller relative differences between pristine reference sites and moderately impacted sites (Chariton et al., 2010). In my study, eDNA metabarcoding enabled eukaryotic, diatom and bacterial communities to differentiate relatively subtle changes between medium and high levels of nutrient enrichment. The community shifts and identification of eukaryotic and bacterial indicator taxa associated with medium or medium-high levels of nutrient enrichment suggests that eDNA-based biodiversity assessments could detect low-level nutrient enrichment before estuaries become too degraded. In this regard, bacteria show more potential than eukaryotes because more indicator taxa indicative of medium and medium-high nutrient enrichment were found for this group. The ability for eDNA-derived communities to distinguish between two levels of nutrient enrichment has also been demonstrated for bacterial biofilms (Lawes et al. 2017) and eukaryotic and bacterial plankton communities (Santi et al. 2019). Detecting community change in response to low levels of impact is a crucial step in the advancement of modern biomonitoring as it would allow for implementation of management or remediation strategies at an early stage, increasing the effectiveness of these actions (Birrer et al. 2017). The detectable response of eDNA-derived communities to low levels of nutrient enrichment in the field demonstrated in this study, is an important step towards developing genomic tools for ecosystem health assessment, but further work across a wider range of conditions is required to identify consistent patterns in community responses and indicator taxa.

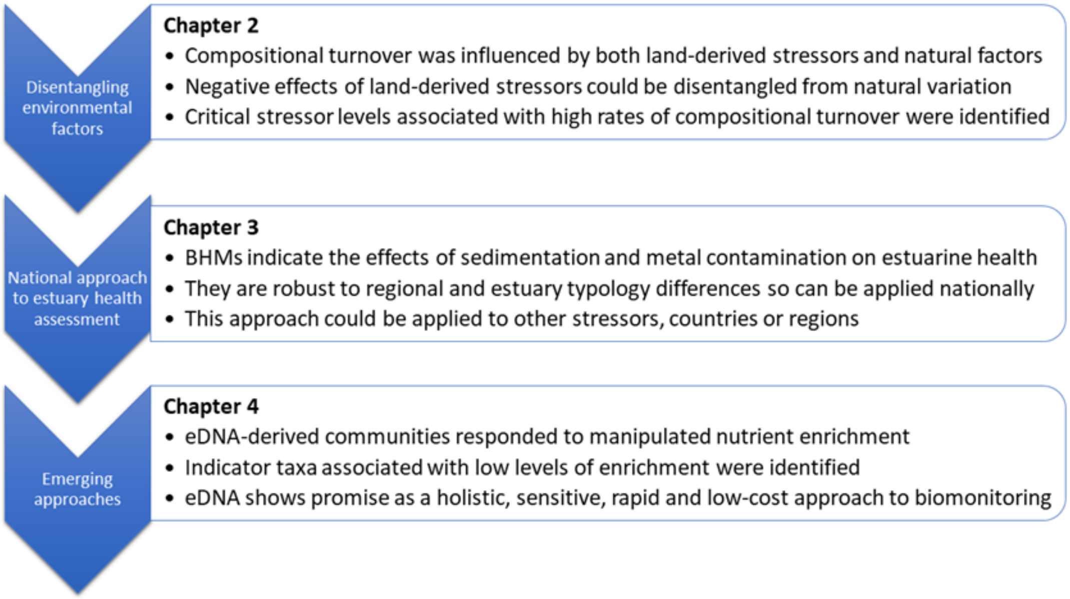
With rapid advancement of molecular technologies and constantly reducing costs of genomic sample processing, there are intensifying calls for applying omics information in environmental risk assessment and management (Leung 2018, Pawlowski et al. 2018). However, despite efforts to integrate genomic tools into monitoring programs (Bourlat et al. 2013, Valentini et al. 2016, Aylagas et al. 2018) and the development of metabarcoding-based indices (e.g., Aylagas et al. 2017, Borja 2018, Keeley et al. 2018), genomics-based monitoring of ecosystem health has yet to be implemented by regulatory frameworks (Cordier et al. 2020). In order to increase the pace of uptake and utilization of these powerful technologies, coordinated efforts to stimulate the use of omics and build up evidence from relevant case studies are imperative. In this context, the current study provides valuable insights into the applicability of eDNA-based biodiversity information for a more holistic and standardised approach to monitoring estuary health. eDNA-derived communities showed great promise for the development of monitoring tools at these two study sites but before such tools could be practically applied for ecosystem health assessment, the scale of the study needs to be expanded across wider and spatial and temporal scales to identify consistent responses. In addition, these tools would need to be tested in naturally enriched sediments to ensure responses are reliable under true conditions. Future research could also examine functional genes associated with nutrient processing (e.g., Birrer et al. 2019, Fasching et al. 2019) and the structure of biotic interactions within ecological networks (Faust & Raes 2012) to better understand the processes shaping community responses.

Chapter 5: General discussion

My thesis investigated approaches for detecting anthropogenic impacts on estuarine benthic communities, to advance the management and protection of these valuable ecosystems. Separating the effects of anthropogenic stressors from natural change is a challenge for all environmental monitoring programmes and is particularly difficult in highly complex estuarine environments. Effective indicators need to be responsive to anthropogenic stress, despite natural background variation, and broadly applicable across wide spatio-temporal scales. This General Discussion will first summarise the key findings from each chapter. The implications of these findings will then be discussed in the context of how this research informs our ability to distinguish between anthropogenic and natural drivers of change, and the importance of scale for detecting anthropogenic impacts. Finally, I will outline areas for future research.

5.1 Summary of main findings

The main findings of each of my research chapters are summarised in Figure 5.1. Using a national scale dataset, Chapter 2 demonstrated that both land-derived stressors and natural environmental variables were important predictors of compositional turnover in New Zealand estuarine benthic communities. Despite the range of factors influencing compositional turnover, the negative effects of land-derived stressors could be disentangled from natural environmental variability. Critical stressor levels associated with high rates of compositional turnover were identified, potentially providing a useful contribution to the literature on thresholds associated with land-derived stressor effects. This study moves towards an ecosystem-based management approach by considering how land-derived stressors cumulatively influence estuarine health, against a background of natural variability operating across several spatio-temporal scales. The approach could be applied to other stressors or ecosystems where appropriate data are available (i.e., ecosystem response data and concurrent environmental data across multiple spatio-temporal scales).



Chapter 2
- Compositional turnover was influenced by both land-derived stressors and natural factors
- Negative effects of land-derived stressors could be disentangled from natural variation
- Critical stressor levels associated with high rates of compositional turnover were identified

Chapter 3
- BHMs indicate the effects of sedimentation and metal contamination on estuarine health
- They are robust to regional and estuary typology differences so can be applied nationally
- This approach could be applied to other stressors, countries or regions

Chapter 4
- eDNA-derived communities responded to manipulated nutrient enrichment
- Indicator taxa associated with low levels of enrichment were identified
- eDNA shows promise as a holistic, sensitive, rapid and low-cost approach to biomonitoring

Figure 5.1 Synthesis of research chapters and their main findings.

Once I had determined that anthropogenic impacts could be disentangled from natural variability, I developed indicators (Benthic Health Models; BHMs) to assess estuary health in response to two key land-derived stressors. The BHMs developed in Chapter 3 detect changes in estuarine communities associated with increasing sediment mud content and heavy metal contamination and are unaffected by regional and estuarine typology differences. They offer a sensitive and standardised approach to assessing estuarine health that allows separation of the two stressors. Theoretically, this estuary health assessment approach could be applied to other stressors, by developing new models that are constrained by a different environment variable (e.g., sediment nutrient concentrations), or to other countries or regions (e.g., Europe) using data collected from those areas. However, the transferability of this approach will depend on influence of the anthropogenic stressor of interest relative to other factors shaping community structure.

Chapter 4 examined the potential for emerging molecular approaches to transform estuary health assessment. Research demonstrating the potential of eDNA for biomonitoring is rapidly growing worldwide but the suitability of this approach is primarily derived from correlative studies that fail to prove causality. Using a manipulative nutrient enrichment experiment, I demonstrated that eDNA metabarcoding can detect responses of benthic communities, spanning multiple trophic levels, to nutrient loading. Responses differed between estuaries, suggesting that the development of indicators may be context dependent. Response patterns aligned with changes in morphologically identified macroinvertebrate communities, confirming concordance between eDNA and conventional monitoring approaches. Bacterial communities, which had indicator taxa common to both sites, showed the most promise for the development of broadly applicable estuary health indicators. Importantly, indicator taxa associated with low levels of nutrient enrichment were identified, demonstrating the potential to develop indicators that can detect subtle changes in health before estuaries become too degraded. The results of my study provide an important contribution to the global effort to develop DNA-based indicators for biomonitoring (e.g., DNAqua-Net; Leese et al. 2016), which could provide a more holistic and sensitive approach to estuary health assessment with faster turn-around times and lower costs.

5.2 Disentangling anthropogenic impact from natural variation

Understanding the drivers of community change is critical for determining whether observed changes in benthic communities are indicative of degradation in ecosystem health or merely a result of natural environmental variation. My research demonstrated that both anthropogenic stressors and natural variation influence estuarine community turnover and structure, highlighting the need to consider natural heterogeneity when assessing anthropogenic impact. In Chapter 2, natural variables were found to be more important predictors of compositional turnover patterns than land-derived stressors, possibly reflecting the comparatively unpolluted nature of many New Zealand estuaries. Thus, including SST, wind-wave exposure and SOI as covariables when assessing estuary health should increase our ability to detect change, including impending tipping points (Hewitt & Thrush 2019). Covariables such as these can be used to partition variability, interpolate between sampling occasions, and help determine whether changes are indicative of a trend or a cyclic pattern (Hewitt & Thrush 2009). For example, inclusion of SOI as an explanatory variable enabled the detection of early warning signals of an ecological shift in a New Zealand estuary (Hewitt & Thrush 2010). Similarly, the broad scale effects of fishing were separated from other factors operating at regional spatial scales by incorporating spatial and environmental factors into the analysis (Thrush et al. 1998).

It is important to demonstrate that indicators are robust to natural spatio-temporal variation before relying upon them for environmental health assessment (Borja & Dauer 2008). The validation procedure that I undertook for the BHMs in Chapter 3 demonstrated how this could be achieved. ANCOVA was used to test whether the stressor-indicator relationship was consistent between different regions and estuary types. This analysis demonstrated that the BHMs were unaffected by these spatial variables and, therefore, suitable for use in most estuaries across New Zealand. Benthic communities can also change though time (e.g., Kröncke & Reiss 2010), as demonstrated by the importance of SOI in predicting compositional turnover in Chapter 2. Consequently, for the BHMs it was important to demonstrate that natural temporal variability in community composition across years did not result in a site sampled at a different time, but with similar mud or metal concentrations, having markedly different health scores. In addition to long-term temporal changes, community structure may vary seasonally due to recruitment pulses (e.g., Alden et al. 1997, Chainho et al. 2007). Although the influence of season was not explicitly tested, the incorporation of data collected across multiple months likely reduces the influence of seasonal fluctuations in species composition on health scores. However, when using the BHMs to assess estuary health I recommend that data be collected at similar seasonal time periods to the data used to develop the model (i.e., October to March) to avoid this seasonal effect.

The differing response of eDNA-derived communities to nutrient enrichment between the two estuaries in Chapter 4 illustrates the difficulties of detecting anthropogenic impacts in highly variable environments. Diatom and bacterial communities showed the strongest response to nutrient enrichment at Waimea while eukaryotic communities were most sensitive to changes in nutrient loads at Delaware. The differing sensitivities of these communities to enrichment likely reflects the differing environmental conditions between the two estuaries. In this study, I was able to disentangle the effects of nutrient enrichment from this natural background variability because the data were obtained from a controlled manipulative experiment. However, community response to this level of enrichment would likely have been masked by natural variability if this data had been collected from an environmental survey at only two sites. Expanding the scale of the study to encompass gradients of environmental change (e.g., sites ranging from mud to sand or sheltered to exposed) would enable this natural variation to be included as covariables, increasing our ability to detect nutrient enrichment effects and our knowledge on how these smaller organisms (e.g., bacteria, diatoms) respond to environmental change.

5.3 Importance of scale for detecting change

Separating anthropogenic impact from natural change is particularly challenging in estuaries because the processes that generate natural variability operate over different scales of space and time (Thrush et al. 2000, Barbone et al. 2012). Empirical studies in estuaries often focus on a specific ecological question that is tested on a local spatial scale and/or over a narrow temporal scale (e.g., Olsgard 1999, Fukunaga et al. 2011, Lawes et al. 2016a). Examination of effects at a fine scale minimises natural variation, providing increased power to detect the stressor effect of interest. For example, BHMs developed using data from a single estuary were found to be more sensitive than the national scale BHMs, possibly because the single estuary data was collected at a particular time from the same estuary, thus minimising background variability. However, the ability of the single estuary BHM to detect change would likely be reduced if the model was used to calculate health scores for a different year. For instance, warmer than normal temperatures or climatic fluctuations may mask the expected stressor response, decreasing the sensitivity of the model. Additionally, local scale indicators may not be able to extrapolate beyond the narrow stressor gradients that are typically used to develop them, limiting their utility for assessing future degradation. The national scale BHM, on the other hand, integrates community responses over wider spatio-temporal scales, encompassing changes in broad scale variables (e.g., SST, SOI, regional species pools, estuary geomorphology) that influence community structure. Including information from multiple sites and times increases the generality of the response, meaning the national BHMs have a greater ability to detect anthropogenic impacts against a background of natural variability.

Broadly applicable estuary health indicators, such as the national BHMs, deliver clear advantages for management. The national scale approach reduces the costs associated with the development of multiple separate small-scale models and enables the health of an estuary to be placed in a wider (e.g., national or international) context. In doing so, managers obtain a more complete picture of natural variability and cumulative impacts, enabling prioritization of management actions (e.g., Heinz Center 2002, Kristensen et al. 2013, Van Niekerk et al. 2013, Schiff et al. 2015). For example, the BHMs have been used to summarise the health of New Zealand's estuaries (Berthelsen et al. 2019) and examine relationships between the ecological health of streams and their receiving estuaries over a wide geographic scale (Berthelsen et al. 2020a). Broadly applicable indicators also support the development of environmental standards to inform policy. Specifically, the BHM approach could be used to determine ecological status under the European Water Framework Directive (2000) or develop attributes for a national objectives framework, in line with recommendations to include estuaries in the New Zealand National Policy Statement for Freshwater Management (PCE 2020).

The large spatial and temporal scales across which much of my research was carried out suggests that many of my findings will be generally applicable (Thrush et al. 1997). However, working at this scale necessitates the use of exploratory models rather than manipulative field experiments. Although the correlative relationships identified by the models in Chapter 2 (GF and GLMs) and Chapter 3 (BHMs) do not prove a causal link, they do identify possible drivers of patterns that could be further investigated through controlled experiments. For example, the manipulative enrichment experiment in Chapter 4 provided insight into the mechanisms behind the changes in compositional turnover and diversity observed in response to nutrient loading in Chapter 2. However, as community response to nutrient enrichment differed between the two estuaries, further comparative studies at multiple locations are required to understand drivers of differences and identify consistent responses that can be used as the basis for indicator development. Nesting local scale manipulative experiments within a correlative framework obtained from environmental surveys undertaken over broad scales can be an effective way of increasing the generality of findings while providing information on the mechanisms underpinning community changes (Figure 5.2; Hewitt et al. 2007). Chapter 4 was part of a larger national experiment, conducted in 12 other estuaries spanning a gradient of turbidity (Thrush et al. 2020). Results from this wider study could be used to generalise how estuarine communities respond to nutrient loading, as well as an additional stressor (turbidity).

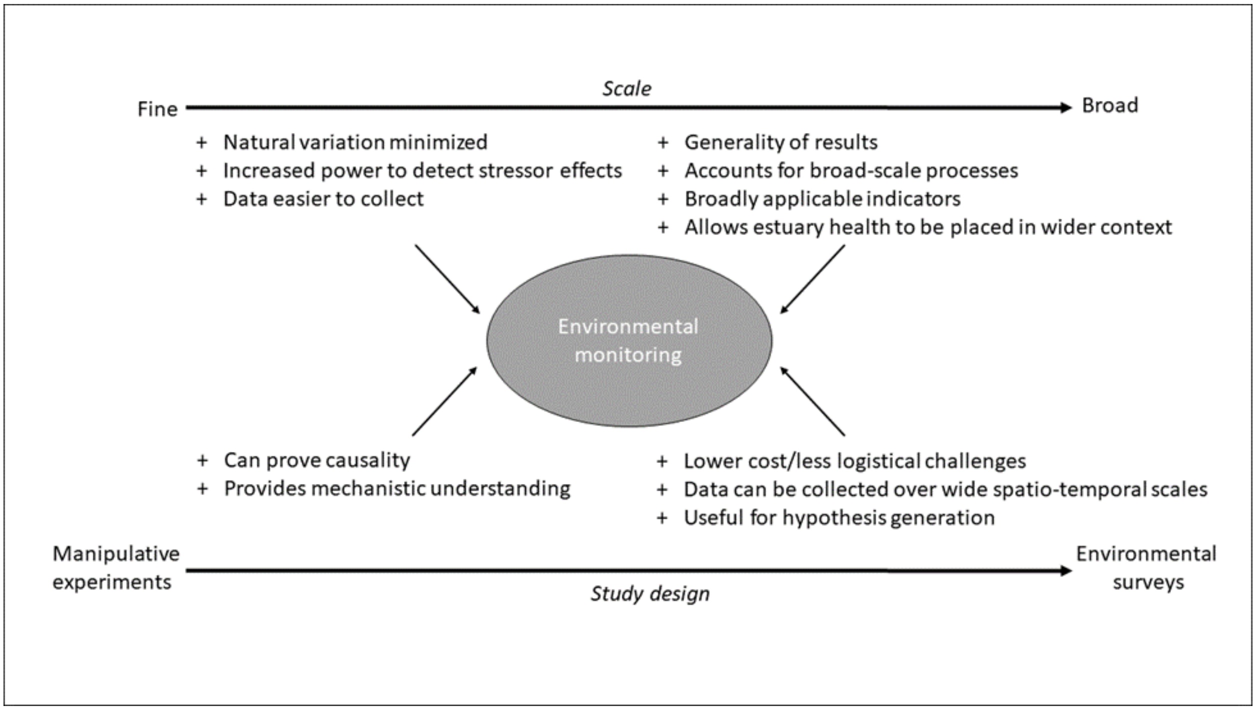


Figure 5.2 Benefits of integrating different study designs at different scales into environmental monitoring programmes.

I was fortunate in this thesis to have access to a national monitoring dataset spanning wide spatio-temporal scales. This large dataset facilitated the detection of subtle land-derived stressor effects and the development of broadly applicable indicators. However, environmental managers are frequently tasked with making decisions based on limited empirical data. The quantitative information generated in this thesis (e.g., relative importance of environmental drivers of compositional turnover, critical stressor thresholds, predictions of estuarine health in response to increasing sedimentation and contamination, mechanistic understanding of community responses to nutrient enrichment) can be used in conjunction with other tools that integrate expert opinion and/or empirical data collected at local scales. For example, McDonald et al. (2016) integrated monitoring and experimental data in a Bayesian network model to predict trophic shifts in an estuary in Australia. Such models bridge data gaps, providing a link between local scale data and ecosystem scale problems (Gladstone-Gallagher et al. 2019a). They also allow decision-makers and scientists to investigate the complex interactions regulating estuarine responses at scales relevant for efficient management of these dynamic systems (McDonald et al. 2016).

5.4 Future research

My thesis examined how estuarine community turnover and structure is affected by three land-derived stressors and several natural environmental variables. While these variables capture important drivers of spatio-temporal heterogeneity, the complexity of estuarine systems suggests that additional factors likely influence ecological communities. Biotic factors (e.g., competition for resources, predation, small-scale biological disturbance, density or biomass of key species, recruitment variation; Levine 1976, Dayton 1984, Ives 1995, Hines et al. 1997), were not explicitly considered in any of my chapters, but likely contribute to unexplained variation observed in these studies. Likewise, a range of other abiotic factors could influence species distributions including hydrodynamics (Aller 1989, Turner et al. 1997), emerging contaminants (e.g., flame retardants, pesticides; Stewart et al. 2014) and extractive activities (e.g., overharvesting, dredging; Thrush et al. 1998, Piló et al. 2019). It is unrealistic to include all these variables in a study design and indeed, increasing the complexity of models risks overfitting (i.e., inadvertently ascribing pattern to noise) and can lead to a reduction in predictive power and various interpretive constraints (Duarte et al. 2003, Merow et al. 2014). However, future research could examine these factors to improve our understanding of the role they play in shaping estuarine community turnover and structure. For example, shellfish harvesting could be included as a predictor variable in a GF analysis, BHMs could be developed to model how estuarine communities change with increasing current velocities and ecosystem interaction networks could be used to investigate interactions between eDNA-derived community data and other critical ecosystem components (e.g., large shellfish; Thrush et al. 2020).

Although Chapters 2 and 3 were carried out across broad spatio-temporal scales, further studies are required to confirm the generality of these results. For example, the BHMs were shown to be robust across most regions and for the two estuary types tested (which represent more than half of the estuaries in New Zealand; Hume et al. 2016) but further research is needed to determine their suitability for assessing health in other estuary types (e.g., tidal river mouths, fjords, deep drowned valleys). This would require collecting samples from those estuary types and testing whether the stressor-response relationship varies from that observed at the sites used in the model. Similarly, it is unknown whether the relative importance of natural versus anthropogenic factors identified in

Chapter 2 would remain the same in a study area where levels of land-derived stressors were consistently high. Repeating this study using data from different regions (e.g., Europe, North America) would help to answer this question. As mentioned earlier, results from the local scale study conducted in Chapter 4 could be generalised by replicating this experiment in different estuaries. For indicator development, it is also important to recognise how responses change over time, therefore, these experiments could be repeated seasonally at a subset of sites, to examine whether community response to nutrient loading varies through time. It would also be of interest to characterise the recovery of these communities once nutrient loading is removed as a stressor.

The BHMs developed in Chapter 3 can provide managers with information on the relative health of sites in a New Zealand context, by classifying sites into one of five categories spread equally across the gradient of impact. While this information is useful for tracking the health of sites through time, it is not clear what indicator value would trigger undesirable conditions and whether this value varies in different environment contexts. Converting relative indicator values into absolute measures of health and establishing ecologically relevant thresholds are major challenges in coastal health assessment (Muxika et al. 2007, Borja et al. 2012). The GF model developed in Chapter 2 provided insight into critical ecological thresholds along land-derived stressor gradients. This information could be used to set ecologically relevant management targets and adjust BHM group boundaries so that they align with observed community shifts. Specifically, the GF model identified high rates of compositional turnover between 0-10% mud, consistent with a range of studies that have shown that functional redundancy and the abundance of sensitive taxa decline once mud content reaches 5-10% (e.g., Thrush et al. 2003b, Anderson 2008, Rodil et al. 2013, Ellis et al. 2017). The boundary between the Mud BHM Group 2 and 3 occurs around 10% mud, suggesting an appropriate threshold for protecting these sensitive species might be a BHM score of less than three. Further research is required, however, to understand how context dependent these community thresholds are. For example, communities in small tidal creek estuaries may be naturally adapted to higher levels of sedimentation because the small fetch limits resuspension. Consequently, obtaining a BHM score of 3 or less may be unrealistic, even in the absence of human impact. Likewise, reference indicator values may vary within a given estuary (i.e., between the upper and lower reaches).

The critical stressor levels identified in Chapter 2 should be used in a weight of evidence approach in combination with other information, rather than relied upon as strict thresholds of community change. Specifically, wide predication intervals at the upper end of the stressor gradients indicate that caution should be applied when interpreting compositional turnover rates for more extreme values, as fewer data were available to inform the model. The evenness of the environmental gradient can affect the performance of GF models (Sultana et al. 2020), therefore, re-running the analysis using a more uniformly distributed dataset containing extreme values would test how robust these change-points are. The generality of these thresholds could also be examined by looking for congruence with thresholds identified using other methods (e.g., Threshold Indicator Taxa Anlaysis (TITAN); Baker & King 2010) or conducting gradient-based manipulative experiments at multiple locations encompassing a wide range of environmental conditions. Data from well-designed and scaled manipulative experiments can provide empirical evidence of thresholds by testing for changes in the architecture of ecosystem interaction networks (EINs; Thrush et al. 2014). For example, Thrush et al. (2020) identified a threshold in incident light that was related to distinct changes in the EINs that drive nutrient processing. This study also demonstrated that sediments had reduced nutrient processing capacity in turbid conditions. The interaction between nutrient processing and turbidity illustrates the risks of applying national or regional thresholds when dealing with non-linear responses to multiple stressors in naturally variable systems. Management approaches that rely on the use of generic single-stressor limits risk negative consequences (e.g., regime shifts to poorer ecosystem state; Thrush et al. 2016), therefore, it is important to continue to develop our understanding of the mechanisms linking stressors to ecosystem change.

A universal indicator of estuarine health is not feasible given the natural variability of estuaries and the increasing number of stressors affecting these systems. Instead, ecosystem health should be evaluated using a weight of evidence approach that integrates information from multiple sources. For example, the single-stressor BHMs should be considered as part of a suite of indicators encompassing multiple stressors and ecosystem components (e.g., bacteria, fish, phytoplankton, macroalgae). Statistical approaches that can combine different survey methods, such as the GF approach applied in Chapter 2, and emerging molecular approaches that characterise the non-visible portion of biodiversity, such as eDNA metabarcoding used in Chapter 4, offer new ways of incorporating multiple ecosystem components into estuary health assessment frameworks. The unprecedented volume of biodiversity information provided by eDNA metabarcoding offers huge potential for the development of indicators that can differentiate between multi-stressor impacts (e.g., Lanzen et al. 2020), which would be a significant progression in the field of biomonitoring. In addition, the sensitivity and fast execution of eDNA metabarcoding could provide much higher spatial and temporal data resolution on estuarine health than current approaches, thereby being more responsive to immediate management needs (Duarte et al. 2021).

Efforts to integrate eDNA-based tools into regulatory frameworks may be hindered by a lack of understanding of how structural changes in eDNA-derived communities (e.g., bacteria) translate into measures of ecosystem function, and thus the services we rely on. Unlike macroinvertebrates, which have a long history of studies establishing biodiversity-function relationships (Snelgrove et al. 2014), it is not immediately apparent to managers whether a shift in bacterial community structure is cause for concern or simply a natural successional change. Connecting eDNA-derived responses to shifts in ecosystem functioning could be achieved by incorporating measures of ecosystem functioning (e.g., primary production, nutrient fluxes, carbon degradation) into manipulative experiments and environmental surveys, or examining changes in functional genes using metagenomics, metatranscriptomics and metabolomics (e.g., Laroche et al. 2018a, Birrer et al. 2019, Shah et al. 2019, Zilius et al. 2020, Marshall et al. 2021). Co-occurrence networks also provide a tool to link changes in community structure to biotic interactions and potential functions (reviewed by Faust & Raes 2012). For example, anthropogenic stress has been shown to disrupt community function leading to a lower ratio of positive interactions (e.g., Laroche et al. 2018a) or a decrease in the connectivity of biological networks (e.g., Lawes et al. 2017).

5.5 Conclusion

Our estuaries, and the benefits that we derive from them, are threatened by an increasing number of interacting cumulative stressors. There is an urgent global need for innovative monitoring approaches that can detect the impacts of these stressors to assess ecological health, monitor trends over time, diagnose causes of degradation, assess the efficacy of management actions, and provide warning signals for impending ecological shifts. Understanding how stressors affect community responses against a background of natural variability acting at multiple scales is critical for predicting their impacts and developing broadly applicable estuarine health indicators. In this thesis, I have demonstrated that the detection of anthropogenic impacts on estuarine benthic communities requires an understanding of the response of communities to stressors and how this response is modified by natural environmental processes operating at different spatio-temporal scales. My research contributes to the management and protection of estuaries by improving knowledge on the processes generating broad scale patterns in benthic macroinvertebrate communities, developing indicators that can be used to assess estuary health and demonstrating the potential of eDNA metabarcoding as a new tool for estuary health assessment.

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Appendices

Appendix 1: Site information

Table A1.1 Information on the sites used in Chapter 2, with estuary type as defined by Hume et al. (2016).

Estuary	Site	Year	Month	Estuary type	Latitude	Longitude
Ahuriri	a	2006	Feb	Tidal lagoon	-39.4835	176.8783
Ahuriri	a	2007	Feb	Tidal lagoon	-39.4835	176.8783
Ahuriri	a	2008	Feb	Tidal lagoon	-39.4835	176.8783
Ahuriri	a	2009	Mar	Tidal lagoon	-39.4835	176.8783
Ahuriri	a	2010	Feb	Tidal lagoon	-39.4835	176.8783
Ahuriri	a	2011	Feb	Tidal lagoon	-39.4835	176.8783
Ahuriri	a	2012	Feb	Tidal lagoon	-39.4835	176.8783
Ahuriri	a	2013	Feb	Tidal lagoon	-39.4835	176.8783
Ahuriri	a	2014	Feb	Tidal lagoon	-39.4835	176.8783
Ahuriri	a	2015	Feb	Tidal lagoon	-39.4835	176.8783
Ahuriri	b	2006	Feb	Tidal lagoon	-39.4872	176.8790
Ahuriri	b	2007	Feb	Tidal lagoon	-39.4872	176.8790
Ahuriri	b	2008	Feb	Tidal lagoon	-39.4872	176.8790
Ahuriri	b	2009	Mar	Tidal lagoon	-39.4872	176.8790
Ahuriri	b	2010	Feb	Tidal lagoon	-39.4872	176.8790
Ahuriri	b	2011	Feb	Tidal lagoon	-39.4872	176.8790
Ahuriri	b	2012	Feb	Tidal lagoon	-39.4872	176.8790
Ahuriri	b	2013	Feb	Tidal lagoon	-39.4872	176.8790
Ahuriri	b	2014	Feb	Tidal lagoon	-39.4872	176.8790
Ahuriri	b	2015	Feb	Tidal lagoon	-39.4872	176.8790
Ahuriri	c	2006	Feb	Tidal lagoon	-39.4839	176.8758
Ahuriri	c	2007	Feb	Tidal lagoon	-39.4839	176.8758
Ahuriri	c	2008	Feb	Tidal lagoon	-39.4839	176.8758
Ahuriri	d	2007	Feb	Tidal lagoon	-39.4868	176.8864
Ahuriri	d	2008	Feb	Tidal lagoon	-39.4868	176.8864
Ahuriri	d	2009	Mar	Tidal lagoon	-39.4868	176.8864
Ahuriri	d	2010	Feb	Tidal lagoon	-39.4868	176.8864
Ahuriri	d	2011	Feb	Tidal lagoon	-39.4868	176.8864
Ahuriri	d	2012	Feb	Tidal lagoon	-39.4868	176.8864
Ahuriri	d	2013	Feb	Tidal lagoon	-39.4868	176.8864
Ahuriri	d	2014	Feb	Tidal lagoon	-39.4868	176.8864
Ahuriri	d	2015	Feb	Tidal lagoon	-39.4868	176.8864
Ahuriri	e	2009	Mar	Tidal lagoon	-39.4824	176.8841
Ahuriri	e	2010	Feb	Tidal lagoon	-39.4824	176.8841
Ahuriri	e	2011	Feb	Tidal lagoon	-39.4824	176.8841
Ahuriri	e	2012	Feb	Tidal lagoon	-39.4824	176.8841
Ahuriri	e	2013	Feb	Tidal lagoon	-39.4824	176.8841
Ahuriri	e	2015	Feb	Tidal lagoon	-39.4824	176.8841
Akaroa	childrens	2009	Apr	Deep drowned valley	-43.7988	172.9643
Akaroa	childrens	2010	Mar	Deep drowned valley	-43.7988	172.9643
Akaroa	childrens	2011	Mar	Deep drowned valley	-43.7988	172.9643
Akaroa	childrens	2012	Mar	Deep drowned valley	-43.7988	172.9643
Akaroa	childrens	2013	Mar	Deep drowned valley	-43.7988	172.9643
Akaroa	childrens	2014	Mar	Deep drowned valley	-43.7988	172.9643
Akaroa	childrens	2015	Mar	Deep drowned valley	-43.7988	172.9643
Akaroa	robinsons	2009	May	Deep drowned valley	-43.7642	172.9577

Table A1.1 Continued.

Estuary	Site	Year	Month	Estuary type	Latitude	Longitude
Akaroa	robinsons	2010	Mar	Deep drowned valley	-43.7642	172.9577
Akaroa	robinsons	2011	Mar	Deep drowned valley	-43.7642	172.9577
Akaroa	robinsons	2012	Mar	Deep drowned valley	-43.7642	172.9577
Akaroa	robinsons	2013	Mar	Deep drowned valley	-43.7642	172.9577
Akaroa	robinsons	2014	Apr	Deep drowned valley	-43.7642	172.9577
Akaroa	robinsons	2015	Apr	Deep drowned valley	-43.7642	172.9577
Avonheathcote	a	2001	Feb	Tidal lagoon	-43.5492	172.7170
Avonheathcote	b	2001	Feb	Tidal lagoon	-43.5490	172.7393
Avonheathcote	c	2001	Feb	Tidal lagoon	-43.5392	172.7329
Avonheathcote	avon	2007	Apr	Tidal lagoon	-43.5206	172.7275
Avonheathcote	avon	2011	Apr	Tidal lagoon	-43.5206	172.7275
Avonheathcote	dischargepoint	2007	Apr	Tidal lagoon	-43.5408	172.7212
Avonheathcote	dischargepoint	2011	Mar	Tidal lagoon	-43.5408	172.7212
Avonheathcote	heathcote	2007	NA	Tidal lagoon	-43.5607	172.7023
Avonheathcote	heathcote	2011	Apr	Tidal lagoon	-43.5607	172.7023
Avonheathcote	humphreysdrive	2007	Apr	Tidal lagoon	-43.5543	172.7038
Avonheathcote	humphreysdrive	2011	Apr	Tidal lagoon	-43.5543	172.7038
Avonheathcote	pleasantpointjetty	2007	Apr	Tidal lagoon	-43.5314	172.7302
Avonheathcote	pleasantpointjetty	2011	Mar	Tidal lagoon	-43.5314	172.7302
Avonheathcote	ploverstreet	2007	Apr	Tidal lagoon	-43.5482	172.7431
Avonheathcote	ploverstreet	2011	Mar	Tidal lagoon	-43.5482	172.7431
Awarua	a	2005	Nov	Shallow drowned valley	-46.5760	168.4293
Awarua	b	2005	Nov	Shallow drowned valley	-46.5817	168.5090
Bluff	a	2005	Nov	Shallow drowned valley	-46.5602	168.3023
Bluff	b	2005	Nov	Shallow drowned valley	-46.5483	168.3414
Catlins	a	2016	Dec	Tidal lagoon	-46.4768	169.6997
Catlins	b	2016	Dec	Tidal lagoon	-46.4723	169.6387
Delaware	a	2009	Jan	Tidal lagoon	-41.1717	173.4369
Delaware	b	2009	Jan	Tidal lagoon	-41.1652	173.4495
Delaware	c	2009	Jan	Tidal lagoon	-41.1665	173.4218
Fortrose	a	2004	Feb	Tidal lagoon	-46.5713	168.7876
Fortrose	a	2005	Jan	Tidal lagoon	-46.5713	168.7876
Fortrose	a	2006	Jan	Tidal lagoon	-46.5713	168.7876
Fortrose	b	2004	Feb	Tidal lagoon	-46.5629	168.7880
Fortrose	b	2009	Feb	Tidal lagoon	-46.5629	168.7880
Freshwater	a	2011	Feb	Deep drowned valley	-46.9059	167.9774
Freshwater	a	2013	Feb	Deep drowned valley	-46.9059	167.9774
Freshwater	b	2011	Feb	Deep drowned valley	-46.9045	167.9888
Freshwater	b	2013	Feb	Deep drowned valley	-46.9045	167.9888
Haldane	a	2006	Feb	Tidal lagoon	-46.6412	169.0316
Haldane	a	2009	Feb	Tidal lagoon	-46.6412	169.0316
Haldane	a	2010	Feb	Tidal lagoon	-46.6412	169.0316
Haldane	a	2011	Feb	Tidal lagoon	-46.6412	169.0316
Haldane	b	2013	Feb	Tidal lagoon	-46.6427	169.0322
Havelock	a	2001	Feb	Shallow drowned valley	-41.2701	173.7690
Havelock	a	2014	Feb	Shallow drowned valley	-41.2701	173.7690
Havelock	a	2015	Mar	Shallow drowned valley	-41.2701	173.7690
Havelock	b	2001	Feb	Shallow drowned valley	-41.2706	173.7742
Havelock	b	2014	Feb	Shallow drowned valley	-41.2706	173.7742
Havelock	b	2015	Mar	Shallow drowned valley	-41.2706	173.7742
Havelock	c	2015	Mar	Shallow drowned valley	-41.2706	173.7691
Havelock	d	2015	Mar	Shallow drowned valley	-41.2707	173.7739

Table A1.1 Continued.

Estuary	Site	Year	Month	Estuary type	Latitude	Longitude
Jacobs River	a	2003	Feb	Tidal lagoon	-46.3442	168.0090
Jacobs River	a	2004	Feb	Tidal lagoon	-46.3442	168.0090
Jacobs River	a	2005	Jan	Tidal lagoon	-46.3442	168.0090
Jacobs River	a	2006	Jan	Tidal lagoon	-46.3442	168.0090
Jacobs River	a	2011	Feb	Tidal lagoon	-46.3442	168.0090
Jacobs River	b	2003	Feb	Tidal lagoon	-46.3453	167.9919
Jacobs River	b	2004	Feb	Tidal lagoon	-46.3453	167.9919
Jacobs River	b	2005	Jan	Tidal lagoon	-46.3453	167.9919
Jacobs River	b	2006	Jan	Tidal lagoon	-46.3453	167.9919
Jacobs River	b	2011	Feb	Tidal lagoon	-46.3453	167.9919
Jacobs River	c	2003	Feb	Tidal lagoon	-46.3348	167.9718
Jacobs River	c	2004	Feb	Tidal lagoon	-46.3348	167.9718
Jacobs River	c	2005	Jan	Tidal lagoon	-46.3348	167.9718
Jacobs River	c	2006	Jan	Tidal lagoon	-46.3348	167.9718
Jacobs River	c	2011	Feb	Tidal lagoon	-46.3348	167.9718
Jacobs River	d	2012	Jan	Tidal lagoon	-46.3343	167.9706
Jacobs River	d	2013	Mar	Tidal lagoon	-46.3343	167.9706
Jacobs River	e	2012	Jan	Tidal lagoon	-46.3312	168.0004
Jacobs River	e	2013	Apr	Tidal lagoon	-46.3312	168.0004
Kaipara	a	2001	Feb	Shallow drowned valley	-36.1602	174.3882
Kaipara	b	2001	Feb	Shallow drowned valley	-36.1375	174.3857
Kaipara	c	2001	Feb	Shallow drowned valley	-36.2272	174.3306
Lyttelton	governors	2011	May	Deep drowned valley	-43.6211	172.6553
Lyttelton	governors	2012	Mar	Deep drowned valley	-43.6211	172.6553
Lyttelton	governors	2013	Feb	Deep drowned valley	-43.6211	172.6553
Lyttelton	governors	2014	Mar	Deep drowned valley	-43.6211	172.6553
Lyttelton	governors	2015	Apr	Deep drowned valley	-43.6211	172.6553
Lyttelton	hob	2011	Mar	Deep drowned valley	-43.6474	172.6660
Lyttelton	hob	2012	Mar	Deep drowned valley	-43.6474	172.6660
Lyttelton	hob	2013	Mar	Deep drowned valley	-43.6474	172.6660
Lyttelton	hob	2014	Apr	Deep drowned valley	-43.6474	172.6660
Lyttelton	hob	2015	Apr	Deep drowned valley	-43.6474	172.6660
Mangonui	man-10	2016	Feb	Shallow drowned valley	-34.9970	173.5523
Mangonui	man-11	2016	Feb	Shallow drowned valley	-34.9970	173.5469
Mangonui	man-15	2016	Feb	Shallow drowned valley	-34.9759	173.5410
Mangonui	man-17	2016	Feb	Shallow drowned valley	-34.9820	173.5535
Mangonui	man-19	2016	Feb	Shallow drowned valley	-34.9897	173.5565
Mangonui	man-2	2016	Feb	Shallow drowned valley	-34.9938	173.5358
Mangonui	man-20	2016	Feb	Shallow drowned valley	-34.9957	173.5567
Mangonui	man-21	2016	Feb	Shallow drowned valley	-34.9987	173.5563
Mangonui	man-22	2016	Feb	Shallow drowned valley	-35.0034	173.5564
Mangonui	man-3	2016	Feb	Shallow drowned valley	-34.9961	173.5364
Mangonui	man-4	2016	Feb	Shallow drowned valley	-34.9994	173.5376
Mangonui	man-5	2016	Feb	Shallow drowned valley	-35.0008	173.5400
Mangonui	man-6	2016	Feb	Shallow drowned valley	-35.0032	173.5414
Mangonui	man-7	2016	Feb	Shallow drowned valley	-34.9983	173.5436
Mangonui	man-9	2016	Feb	Shallow drowned valley	-34.9928	173.5483
Moutere	a	2006	Mar	Shallow drowned valley	-41.1486	173.0175
Moutere	a	2013	Mar	Shallow drowned valley	-41.1486	173.0175
Moutere	a	2015	Mar	Shallow drowned valley	-41.1486	173.0175
Moutere	b	2006	Mar	Shallow drowned valley	-41.1696	173.0433
Moutere	b	2013	Mar	Shallow drowned valley	-41.1696	173.0433

Table A1.1 Continued.

Estuary	Site	Year	Month	Estuary type	Latitude	Longitude
Moutere	b	2015	Mar	Shallow drowned valley	-41.1696	173.0433
Nelson Haven	a	2012	Apr	Tidal lagoon	-41.2280	173.3143
Nelson Haven	b	2012	Apr	Tidal lagoon	-41.2470	173.3050
New River	a	2001	Feb	Shallow drowned valley	-46.4625	168.3426
New River	b	2001	Feb	Shallow drowned valley	-46.4769	168.3355
New River	b	2003	Feb	Shallow drowned valley	-46.4769	168.3355
New River	b	2004	Feb	Shallow drowned valley	-46.4769	168.3355
New River	b	2005	Jan	Shallow drowned valley	-46.4769	168.3355
New River	b	2010	Feb	Shallow drowned valley	-46.4769	168.3355
New River	c	2001	Feb	Shallow drowned valley	-46.4760	168.3029
New River	c	2003	Feb	Shallow drowned valley	-46.4760	168.3029
New River	c	2004	Feb	Shallow drowned valley	-46.4760	168.3029
New River	c	2005	Jan	Shallow drowned valley	-46.4760	168.3029
New River	c	2010	Feb	Shallow drowned valley	-46.4760	168.3029
New River	d	2001	Feb	Shallow drowned valley	-46.4592	168.3173
New River	d	2003	Feb	Shallow drowned valley	-46.4592	168.3173
New River	d	2004	Feb	Shallow drowned valley	-46.4592	168.3173
New River	d	2005	Jan	Shallow drowned valley	-46.4592	168.3173
New River	d	2010	Feb	Shallow drowned valley	-46.4592	168.3173
New River	e	2012	NA	Shallow drowned valley	-46.4753	168.3006
New River	e	2013	Feb	Shallow drowned valley	-46.4753	168.3006
New River	f	2012	NA	Shallow drowned valley	-46.4406	168.3288
New River	f	2013	Feb	Shallow drowned valley	-46.4406	168.3288
Ngunguru	1	2016	Mar	Tidal lagoon	-35.6447	174.4694
Ngunguru	10	2016	Mar	Tidal lagoon	-35.6394	174.4974
Ngunguru	11	2016	Mar	Tidal lagoon	-35.6354	174.5002
Ngunguru	12	2016	Mar	Tidal lagoon	-35.6421	174.4986
Ngunguru	13	2016	Mar	Tidal lagoon	-35.6398	174.4998
Ngunguru	14	2016	Mar	Tidal lagoon	-35.6350	174.5028
Ngunguru	15	2016	Mar	Tidal lagoon	-35.6396	174.5031
Ngunguru	16	2016	Mar	Tidal lagoon	-35.6309	174.5077
Ngunguru	17	2016	Mar	Tidal lagoon	-35.6295	174.5096
Ngunguru	19	2016	Mar	Tidal lagoon	-35.6334	174.5018
Ngunguru	2	2016	Mar	Tidal lagoon	-35.6458	174.4662
Ngunguru	20	2016	Mar	Tidal lagoon	-35.6305	174.5041
Ngunguru	21	2016	Mar	Tidal lagoon	-35.6277	174.5101
Ngunguru	22	2016	Mar	Tidal lagoon	-35.6282	174.5145
Ngunguru	3	2016	Mar	Tidal lagoon	-35.6421	174.4731
Ngunguru	4	2016	Mar	Tidal lagoon	-35.6429	174.4751
Ngunguru	5	2016	Mar	Tidal lagoon	-35.6450	174.4759
Ngunguru	6	2016	Mar	Tidal lagoon	-35.6429	174.4813
Ngunguru	7	2016	Mar	Tidal lagoon	-35.6392	174.4890
Ngunguru	8	2016	Mar	Tidal lagoon	-35.6424	174.4924
Ngunguru	9	2016	Mar	Tidal lagoon	-35.6380	174.4933
Ohiwa	a	2001	Feb	Deep drowned valley	-37.9976	177.0935
Ohiwa	b	2001	Feb	Deep drowned valley	-38.0068	177.1228
Ohiwa	c	2001	Feb	Deep drowned valley	-37.9911	177.0685
Ohiwa	d	2001	Feb	Deep drowned valley	-37.9825	177.0847
Orowaiti	a	2007	Jan	Tidal lagoon	-41.7452	171.6350
Orowaiti	b	2007	Jan	Tidal lagoon	-41.7529	171.6257
Porirua	pauaa	2009	Jan	Tidal lagoon	-41.0983	174.8724
Porirua	pauaa	2010	Jan	Tidal lagoon	-41.0983	174.8724

Table A1.1 Continued.

Estuary	Site	Year	Month	Estuary type	Latitude	Longitude
Porirua	pauab	2009	Jan	Tidal lagoon	-41.1004	174.9095
Porirua	pauab	2010	Jan	Tidal lagoon	-41.1004	174.9095
Porirua	poria	2009	Jan	Tidal lagoon	-41.1063	174.8633
Porirua	poria	2010	Jan	Tidal lagoon	-41.1063	174.8633
Porirua	porib	2009	Jan	Tidal lagoon	-41.1278	174.8419
Porirua	porib	2010	Jan	Tidal lagoon	-41.1278	174.8419
Ruataniwha	a	2001	Feb	Tidal lagoon	-40.6490	172.6638
Ruataniwha	b	2001	Feb	Tidal lagoon	-40.6545	172.6776
Ruataniwha	c	2001	Feb	Tidal lagoon	-40.6498	172.6672
Shag River	a	2016	Dec	Tidal lagoon	-45.4804	170.8113
Shag River	b	2016	Dec	Tidal lagoon	-45.4774	170.8080
Shakespeare	seagrass	2016	Feb	Deep drowned valley	-41.2796	173.9952
Shakespeare	unvegetated	2016	Feb	Deep drowned valley	-41.2802	173.9968
Tauranga	1	2011	Dec	Shallow drowned valley	-37.4524	175.9714
Tauranga	10	2011	Dec	Shallow drowned valley	-37.5356	175.9331
Tauranga	11	2011	Dec	Shallow drowned valley	-37.5490	175.9546
Tauranga	12	2011	Dec	Shallow drowned valley	-37.5617	175.9535
Tauranga	13	2011	Dec	Shallow drowned valley	-37.5608	175.9395
Tauranga	14	2011	Dec	Shallow drowned valley	-37.5737	175.9311
Tauranga	15	2011	Dec	Shallow drowned valley	-37.5077	175.9937
Tauranga	16	2011	Dec	Shallow drowned valley	-37.4863	175.9594
Tauranga	17	2011	Dec	Shallow drowned valley	-37.5493	176.0132
Tauranga	18	2011	Dec	Shallow drowned valley	-37.5604	176.0356
Tauranga	19	2011	Dec	Shallow drowned valley	-37.5517	176.0043
Tauranga	2	2011	Dec	Shallow drowned valley	-37.4633	175.9741
Tauranga	20	2011	Dec	Shallow drowned valley	-37.5744	176.0618
Tauranga	21	2011	Dec	Shallow drowned valley	-37.5503	175.9760
Tauranga	22	2011	Dec	Shallow drowned valley	-37.5785	175.9930
Tauranga	23	2011	Dec	Shallow drowned valley	-37.5761	175.9890
Tauranga	24	2011	Dec	Shallow drowned valley	-37.5990	176.0298
Tauranga	25	2011	Dec	Shallow drowned valley	-37.5976	176.0328
Tauranga	26	2011	Dec	Shallow drowned valley	-37.5986	175.9938
Tauranga	27	2011	Dec	Shallow drowned valley	-37.5991	175.9860
Tauranga	28	2011	Dec	Shallow drowned valley	-37.6011	175.9771
Tauranga	29	2011	Dec	Shallow drowned valley	-37.6045	176.0863
Tauranga	3	2011	Dec	Shallow drowned valley	-37.4638	175.9546
Tauranga	30	2011	Dec	Shallow drowned valley	-37.6049	176.0878
Tauranga	31	2011	Dec	Shallow drowned valley	-37.6227	176.1224
Tauranga	32	2011	Dec	Shallow drowned valley	-37.6301	176.1235
Tauranga	33	2011	Dec	Shallow drowned valley	-37.6335	176.1316
Tauranga	34	2011	Dec	Shallow drowned valley	-37.6345	176.1334
Tauranga	35	2011	Dec	Shallow drowned valley	-37.6218	176.0970
Tauranga	36	2011	Dec	Shallow drowned valley	-37.6201	176.0188
Tauranga	37	2011	Dec	Shallow drowned valley	-37.6231	175.9841
Tauranga	38	2011	Dec	Shallow drowned valley	-37.6331	175.9945
Tauranga	39	2011	Dec	Shallow drowned valley	-37.6251	176.0113
Tauranga	4	2011	Dec	Shallow drowned valley	-37.4693	175.9501
Tauranga	40	2011	Dec	Shallow drowned valley	-37.6375	176.0209
Tauranga	41	2011	Dec	Shallow drowned valley	-37.6326	176.0252
Tauranga	42	2011	Dec	Shallow drowned valley	-37.6334	176.0373
Tauranga	43	2011	Dec	Shallow drowned valley	-37.6040	176.0389
Tauranga	44	2011	Dec	Shallow drowned valley	-37.6318	176.0603

Table A1.1 Continued.

Estuary	Site	Year	Month	Estuary type	Latitude	Longitude
Tauranga	46	2011	Dec	Shallow drowned valley	-37.6504	176.0430
Tauranga	47	2011	Dec	Shallow drowned valley	-37.6592	176.0346
Tauranga	48	2011	Dec	Shallow drowned valley	-37.6795	176.0439
Tauranga	49	2011	Dec	Shallow drowned valley	-37.6589	176.0569
Tauranga	5	2011	Dec	Shallow drowned valley	-37.4681	175.9668
Tauranga	50	2011	Dec	Shallow drowned valley	-37.6619	176.0618
Tauranga	51	2011	Dec	Shallow drowned valley	-37.6477	176.1160
Tauranga	52	2011	Dec	Shallow drowned valley	-37.6411	176.0805
Tauranga	53	2011	Dec	Shallow drowned valley	-37.6567	176.0762
Tauranga	54	2011	Dec	Shallow drowned valley	-37.6639	176.0996
Tauranga	55	2011	Dec	Shallow drowned valley	-37.6739	176.1031
Tauranga	56	2011	Dec	Shallow drowned valley	-37.6794	176.1076
Tauranga	57	2011	Dec	Shallow drowned valley	-37.6747	176.1186
Tauranga	58	2011	Dec	Shallow drowned valley	-37.6585	176.1315
Tauranga	59	2011	Dec	Shallow drowned valley	-37.6642	176.1505
Tauranga	6	2011	Dec	Shallow drowned valley	-37.4805	175.9511
Tauranga	60	2011	Dec	Shallow drowned valley	-37.6630	176.1602
Tauranga	61	2011	Dec	Shallow drowned valley	-37.6680	176.1637
Tauranga	62	2011	Dec	Shallow drowned valley	-37.6816	176.1514
Tauranga	63	2011	Dec	Shallow drowned valley	-37.6824	176.1539
Tauranga	64	2011	Dec	Shallow drowned valley	-37.6850	176.1550
Tauranga	65	2011	Dec	Shallow drowned valley	-37.6820	176.1796
Tauranga	66	2011	Dec	Shallow drowned valley	-37.6755	176.1868
Tauranga	67	2011	Dec	Shallow drowned valley	-37.6832	176.2030
Tauranga	68	2011	Dec	Shallow drowned valley	-37.7052	176.1685
Tauranga	69	2011	Dec	Shallow drowned valley	-37.7228	176.1550
Tauranga	7	2011	Dec	Shallow drowned valley	-37.4943	175.9442
Tauranga	70	2011	Dec	Shallow drowned valley	-37.7224	176.1614
Tauranga	71	2011	Dec	Shallow drowned valley	-37.7071	176.1963
Tauranga	72	2011	Dec	Shallow drowned valley	-37.7066	176.2104
Tauranga	73	2011	Dec	Shallow drowned valley	-37.7077	176.2159
Tauranga	74	2011	Dec	Shallow drowned valley	-37.6980	176.2280
Tauranga	75	2011	Dec	Shallow drowned valley	-37.7195	176.1956
Tauranga	8	2011	Dec	Shallow drowned valley	-37.5022	175.9751
Tauranga	9	2011	Dec	Shallow drowned valley	-37.5244	175.9578
Tokomairiro	a	2017	Dec	Tidal lagoon	-46.2161	170.0438
Tokomairiro	b	2017	Dec	Tidal lagoon	-46.2095	170.0447
Waikawa 1	a	2016	Jan	Deep drowned valley	-41.2680	174.0398
Waikawa 2	a	2005	Jan	Tidal lagoon	-46.6224	169.1451
Waikawa 2	a	2006	Jan	Tidal lagoon	-46.6224	169.1451
Waikawa 2	a	2007	Feb	Tidal lagoon	-46.6224	169.1451
Waikawa 2	a	2008	Feb	Tidal lagoon	-46.6224	169.1451
Waikawa 2	b	2005	Jan	Tidal lagoon	-46.6285	169.1499
Waikawa 2	b	2006	Jan	Tidal lagoon	-46.6285	169.1499
Waikawa 2	b	2007	Feb	Tidal lagoon	-46.6285	169.1499
Waikawa 2	b	2008	Feb	Tidal lagoon	-46.6285	169.1499
Waikouaiti	a	2016	Dec	Tidal lagoon	-45.6350	170.6558
Waikouaiti	b	2016	Dec	Tidal lagoon	-45.6250	170.6507
Waikouaiti	c	2016	Dec	Tidal lagoon	-45.6207	170.6369
Waimea	a	2001	Feb	Shallow drowned valley	-41.3173	173.1825
Waimea	a	2006	Apr	Shallow drowned valley	-41.3173	173.1825
Waimea	a	2014	Mar	Shallow drowned valley	-41.3173	173.1825

Table A1.1 Continued.

Estuary	Site	Year	Month	Estuary type	Latitude	Longitude
Waimea	a	2015	Mar	Shallow drowned valley	-41.3173	173.1825
Waimea	b	2001	Feb	Shallow drowned valley	-41.2643	173.0878
Waimea	b	2006	Apr	Shallow drowned valley	-41.2643	173.0878
Waimea	b	2014	Mar	Shallow drowned valley	-41.2643	173.0878
Waimea	c	2001	Feb	Shallow drowned valley	-41.2996	173.1775
Waimea	c	2006	Apr	Shallow drowned valley	-41.2996	173.1775
Waimea	c	2014	Mar	Shallow drowned valley	-41.2996	173.1775
Waimea	c	2015	Mar	Shallow drowned valley	-41.2996	173.1775
Waimea	d	2001	Feb	Shallow drowned valley	-41.2809	173.1062
Waimea	d	2006	Apr	Shallow drowned valley	-41.2809	173.1062
Waimea	d	2014	Mar	Shallow drowned valley	-41.2809	173.1062
Waimea	d	2015	Mar	Shallow drowned valley	-41.2809	173.1062
Waitangi	wat10	2013	May	Deep drowned valley	-35.2681	174.0716
Waitangi	wat4	2013	May	Deep drowned valley	-35.2731	174.0758
Waitangi	wat5	2013	May	Deep drowned valley	-35.2724	174.0727
Waitangi	wat6	2013	May	Deep drowned valley	-35.2800	174.0684
Waitangi	wat7	2013	May	Deep drowned valley	-35.2758	174.0671
Waitangi	wat8	2013	May	Deep drowned valley	-35.2741	174.0601
Waitangi	wat9	2013	May	Deep drowned valley	-35.2696	174.0772
Whangarae	a	2016	Mar	Tidal lagoon	-41.0983	173.6175
Whangarae	b	2016	Mar	Tidal lagoon	-41.1017	173.6212
Whangaroa	kae	2009	Feb	Shallow drowned valley	-35.0664	173.7382
Whangaroa	kae	2010	Feb	Shallow drowned valley	-35.0664	173.7382
Whangaroa	kae	2011	Feb	Shallow drowned valley	-35.0664	173.7382
Whangaroa	kah	2009	Feb	Shallow drowned valley	-35.0491	173.7114
Whangaroa	kah	2010	Feb	Shallow drowned valley	-35.0491	173.7114
Whangaroa	kah	2011	Feb	Shallow drowned valley	-35.0491	173.7114

References Appendix 1:

Hume T, Gerbeaux P, Hart D, Kettles H, Neale D (2016) A classification of New Zealand's coastal hydrosystems. Prepared for Ministry for the Environment

Appendix 2: Generalized linear model outputs

Table A2.1 Multiple regression coefficients, standard errors, *z* values (or *t* values for H'), 95% confidence intervals (CI) and *p*-values for generalized linear models for four measures of estuary diversity (species richness (S), Pielou's evenness (J'), Shannon-Wiener diversity (H') and numbers of rare taxa) in response to compositional turnover along land-derived stressor (mud, TN, TP) and natural environmental (SST, SOI, wind-wave exposure) gradients (Chapter 2). Backward selection using Akaike Information Criterion (AIC) values was used to determine the most important variables. SST = sea surface temperature, TP = sediment total phosphorous, TN = sediment total nitrogen, SOI = Southern Oscillation Index.

Model (deviance explained)	Term	Estimate	SE	*z* or *t* value	2.5% CI	97.5% CI	*p*-value
S	Mud	-3.4566	1.20	-2.887	-5.80	-1.11	0.0039
(11.9%)	TN	-8.3245	2.03	-4.101	-12.30	-4.35	<0.0001
J'	TN	-7.3154	3.51	-2.087	-14.19	-0.44	0.0369
(7.8%)	SST	7.0988	1.49	4.770	4.18	10.02	<0.0001
	Exposure	3.1829	1.53	2.081	0.19	6.18	0.0374
H'	TN	-9.2070	3.32	-2.770	-15.72	-2.69	0.0059
(13.4%)	TP	-5.0662	2.60	-1.949	-10.16	0.03	0.0521
	SST	4.9075	1.15	4.267	2.65	7.16	<0.0001
	Exposure	2.0896	1.35	1.553	-0.55	4.73	0.1214
Rare taxa	TN	-15.589	2.58	-6.051	-20.64	-10.54	<0.0001
(11.3%)	SST	3.5401	0.87	4.053	1.83	5.25	<0.0001
	Exposure	2.6285	0.87	3.022	0.92	4.33	0.0025

Appendix 3: Application of the national Benthic Health Models

The national Benthic Health Models (BHMs) developed in this thesis (Chapter 3) are suitable for assessing estuary health in intertidal, soft-sediment habitats within New Zealand estuaries. The models have been shown to perform well in two estuary types (tidal lagoons and shallow river valleys; Hume et al. 2016) and across five to six regions (Mud BHM: Abel, Banks, Chalmers, Portland, Raglan and Northeastern; Metals BHM: Abel, Southeastern, Portland, Raglan and Northeastern; Shears et al. 2008) of New Zealand and extrapolation beyond these estuary types and regions should be undertaken with caution. It is recommended that macroinvertebrate data is collected within October to March, if possible, to reduce the influence of seasonal changes on model results. Certain taxa should be removed from the dataset before analysis (Table A3.1) and taxonomic resolution standardised following Table A3.2. Taxa that do not fit within any of the taxa categories should be removed from the analysis. Model output CAP scores need to be standardised from 1 to 6 using the following equations:

Eq. (A.1.) $\text{Mud BHM score} = 1 + (6-1) * (\text{CAP score} - -0.177114162796166) / 0.304912508295966$

Eq. (A.2) $\text{Metals BHM score} = 1 + (6-1) * (\text{CAP score} - -0.18224921053598) / 0.326788414557379$

Table A3.1 Taxa excluded from the Benthic Health Models.

Reason for exclusion	**Taxa excluded**
Insect	Chironomidae, *Chironomus*, Coleoptera, *Corynoneura scutellata*, *Dicranomyia nigrescens*, Diptera, Dolichopodidae, Elmidae, Ephydridae, Ephydridae juvenile, Ephydroidea, Formicidae, Insecta, Limnophilinae, *Limonia*, *Microvelia*, Muscidae, Orthocladiinae, *Polypedilum*, Rhyacophiloidea, Stratiomyidae, Trichoptera
Juvenile	*Amphibola crenata* juvenile, Bivalvia juvenile, *Boccardia* juvenile, *Brachyura* juvenile, Cidaridae juvenile, Gastropoda juvenile, Glyceridae juvenile, *Halicarcinus whitei* juvenile, *Hiatula* juvenile, *Lunella smaragda* juvenile, Maldanidae juvenile, Mysella juvenile, Mytilidae juvenile, Mytilus juvenile, Nereididae juvenile, Ostreidae juvenile, *Ruditapes largillierti* juvenile
Meiofauna (low likelihood of being well sampled)	Copepoda, *Copytus novaezealandiae*, *Cypridinodes concentrica*, *Cypridinodes reticulata*, *Cytherella*, *Diasterope grisea*, *Euphilomedes agilis*, Harpacticoida, *Leuroleberis zealandica*, Nematoda, Ostracoda, *Paracaudina chilensis*, *Parasterope*, *Parasterope quadrata*, *Rutiderma*, *Spio*
Not infauna (low likelihood of being well sampled)	Ascidiacea, Asteroidea, Bryozoan, Hydrozoa, Nudibranchia, *Nudibranchus*, Pantopoda, *Philine*, Porifera, Pycnogonida, Pycnogonidae, Tunicata, *Virgularia gracillima*
Not marine	Acari, Araneae, Collembola, *Daphnia*, *Daphnia carinata*, *Daphnia* juvenile, Halacaridae, Hirudinea, *Paratya curvirostris*
Aggregative species (removing improved validation success of models)	Cirripedia
Other non-target taxa	Vertebrates (e.g., fish), plants (e.g., macroalgae), bacteria, larval planktonic groups (e.g., megalope, larvae, eggs)

Table A3.2 Taxonomic resolution used in the Benthic Health Models.

Taxa name	**Group**	**Taxa included**
Amphipod other (excludes Corophiidae, Paracalliopiidae and Phoxocephalidae)	Amphipod	Amphipod other, *Aora maculata*, Caprellidae, Dexaminidae, Gammaridae, *Gammaropsis*, Ischyroceridae, *Liljeborgia*, Liljeborgiidae, Lysianassidae, *Melita awa*, Melitidae, *Methalimedon*, *Paramoera chevreuxi*, *Parawaldeckia*, Pontogeneiidae, Talitridae, Urothoidae

Table A3.2 Continued.

Taxa name	**Group**	**Taxa included**
Corophiidae	Amphipod Corophiidae (Family)	Corophiidae, *Corophium, Monocorophium, Monocorophium sextonae, Paracorophium, Paracorophium excavatum, Paracorophium lucasi*
Paracalliopiidae	Amphipod Paracalliopiidae (Family)	*Paracalliope, Paracalliope novizealandiae,* Paracalliopiidae
Phoxocephalidae	Amphipod Phoxocephalidae (Family)	Phoxocephalidae, *Torridoharpinia, Torridoharpinia hurleyi, Waitangi brevirostris*
Anemonia	Anthozoa	*Anemone*
Anthopleura hermaphroditica	Anthozoa	*Anthopleura aureoradiata*
Edwardsiidae	Anthozoa	*Edwardsia, Edwardsia, Leucomelos, Edwardsia neozelanica,* Edwardsiidae
Chaetognatha	Arrow worm	Chaetognatha
Bivalvia unid	Bivalve	Bivalvia
Carditidae	Bivalve Carditidae (Family)	Carditidae, Venericardiae
Perrierina turneri	Bivalve Cyamiidae (Family)	*Perrierina turneri*
Arthritica	Bivalve Lasaeidae (Family)	*Arthritica, Arthritica bifurca*
Lasaeidae other (excludes *Arthritica* and *Lasaea*)	Bivalve Lasaeidae (Family)	*Mysella*
Lasaea	Bivalve Lasaeidae (Family)	*Lasaea parengaensis*
Cyclomactra	Bivalve Mactridae (Family)	*Cyclomactra ovata*
Mactra	Bivalve Mactridae (Family)	*Mactra*
Paphies australis	Bivalve Mesodesmatidae (Family)	*Paphies australis*
Paphies donacina	Bivalve Mesodesmatidae (Family)	*Paphies donacina*
Myochamidae	Bivalve Myochamidae (Family)	Myadora
Arcuatula senhousia	Bivalve Mytilidae (Family)	*Arcuatula senhousia*
Mytilidae other (excludes *Arcuatula senhousia*)	Bivalve Mytilidae (Family)	Mytilidae, *Mytilus edulis, Mytilus galloprovincialis, Xenostrobus pulex*
Crassostrea gigas	Bivalve Ostreidae (Family)	*Crassostrea gigas*
Ostrea chilensis	Bivalve Ostreidae (Family)	*Ostrea chilensis*
Hiatula	Bivavle Psammobiidae (Family)	*Hiatula, Hiatula nitida, Hiatula siliquens,* Soletellina-Hiatula
Leptomya retiaria	Bivalve Semelidae (Family)	*Leptomya retiaria*
Theora lubrica	Bivalve Semelidae (Family)	*Theora lubrica*

Table A3.2 Continued.

Taxa name	**Group**	**Taxa included**
Solemya parkinsonii	Bivavle Solemyidae (Family)	*Solemya parkinsonii*
Bartschicoma edgari	Bivalve Tellinidae (Family)	*Tellina edgari*
Macomona liliana	Bivalve Tellinidae (Family)	*Macomona liliana*
Zemysina globus	Bivalve Ungulinidae (Family)	Diplodonta globus
Zemysia zelandica	Bivalve Ungulinidae (Family)	*Felaniella zelandica*, *Diplodonta zelandica*
Austrovenus stutchburyi	Bivalve Veneridae (Family)	*Austrovenus stutchburyi*
Ophiuroidea	Brittlestar	*Amphiura*, Ophionereididae, Ophiuroidea
Polyplacophora	Chiton	*Acanthochitona zelandica Chiton glaucus, Sypharochiton pelliserpentis*, chiton (common name)
Paguristes	Crab Diogenidae (Family)	*Paguristes*
Halicarcinus	Crab Hymenosomatidae (Family)	*Halicarcinus, Halicarcinus cookie, Halicarcinus varius, Halicarcinus whitei*
Paguridae	Crab Paguridae (Family)	Paguridae, *Pagurus*
Pinnotheridae	Crab Pinnotheridae (Family)	*Pinnotheres, Nepinnotheres atrinicola Nepinnotheres novaezelandiae*
Cyclograpsus lavauxi	Crab Varunidae (Family)	*Cyclograpsus lavauxi*
Austrohelice.hemigrapsus.hemiplax	Crab Varunidae (Family) and Macrophthalmidae (Family)	*Austrohelice crassa, Hemigrapsus, Hemigrapsus crenulatus, Hemigrapsus sexdentatus, Hemiplax hirtipes*, Helice-hemigrapsus-macropthalmus
Crustacea unid	Crustacean	Brachyura, Crustacea, Decapoda
Cumacea	Cumacean	*Colurostylis, Colurostylis lemurum*, Cumacea, *Cyclaspis thomsoni, Diastylopsis, Diastylopsis elongata*
Gastropoda unid	Gastropod	Gastropoda
Melanochlamys cylindrica	Gastropod Aglajidae (Family)	*Melanochlamys cylindrica*
Amphibola crenata	Gastropod Amphibolidae (Family)	*Amphibola crenata*
Pisinna zosterophila	Gastropod Anabathridae (Family)	*Pisinna zosterophila*
Amalda	Gastropod Ancillariidae (Family)	*Amalda, Amalda australis*

Table A3.2 Continued.

Taxa name	Group	Taxa included
Zeacumantus lutulentus	Gastropod Batillariidae (Family)	*Zeacumantus lutulentus*
Zeacumantus subcarinatus	Gastropod Batillariidae (Family)	*Zeacumantus subcarinatus*
Cominella adspersa	Gastropod Buccinidae (Family)	*Cominella adspersa*
Cominella glandiformis	Gastropod Buccinidae (Family)	*Cominella glandiformis*
Cominella maculosa	Gastropod Buccinidae (Family)	*Cominella maculosa*
Sigapatella novaezelandiae	Gastropod Calyptraeidae (Family)	*Sigapatella novaezelandiae*
Sigapatella tenuis	Gastropod Calyptraeidae (Family)	*Sigapatella tenuis*
Eatoniella	Gastropod Eatoniellidae (Family)	*Eatoniella*
Epitonium tenellum	Gastropod Epitoniidae (Family)	*Epitonium tenellum*
Haminoea zelandiae	Gastropod Haminoeidae (Family)	*Haminoea zelandiae*
Notoacmea	Gastropod Lottiidae (Family)	*Notoacmea, Notoacmea elongata, Notoacmea scapha*
Neoguraleus	Gastropod Mangeliidae (Family)	*Neoguraleus, Neoguraleus sinclairi*
Melanopsis	Gastropod Melanopsidae (Family)	*Melanopsis*
Xymene	Gastropod Muricidae (Family)	*Xymene, Xxymene ambiguous, Xymene plebeius*
Nassarius burchardi	Gastropod Nassariidae (Family)	*Nassarius burchardi*
Linucula hartvigiana	Gastropod Nuculidae (Family)	*Linucula hartvigiana*
Nucula	Gastropod Nuculidae (Family)	*Nucula nitidula*
Odostomia	Gastropod Pyramidellidae (Family)	*Odostomia*
Turbonilla	Gastropod Pyramidellidae (Family)	*Turbonilla*
Rissoidae	Gastropod Rissoidae (Family)	Rissoidae
Zalipais lissa	Gastropod Skeneidae (Family)	*Zalipais lissa*
Halopyrgus pupoides	Gastropod Tateidae (Family)	*Halopyrgus pupoides*
Potamopyrgus	Gastropod Tateidae (Family)	*Potamopyrgus,* *Potamopyrgus antipodarum* *Potamopyrgus estuarinus*
Euterebra tristis	Gastropod Terebridae (Family)	*Euterebra tristis*
Cantharidus.micrelenchus	Gastropod Trochidae (Family)	*Micrelenchus,* *Micrelenchus huttonii,* *Micrelenchus tenebrosus,* Cantharidus-Micrelenchus
Diloma	Gastropod Trochidae (Family)	*Diloma, Diloma nigerrimum* *Diloma subrostratum*
Lunella smaragda	Gastropod Turbinidae (Family)	*Lunella smaragda*
Taeniogyrus dendyi	Holothuroid	*Taeniogyrus dendyi*
Anthuroidea	*Isopod Anthuridae (Family)*	Anthuridae, *Anthuroidea*

Table A3.2 Continued.

Taxa name	**Group**	**Taxa included**
Cirolanidae	Isopod Cirolanidae (Family)	*Cirolana*, Cirolanidae, *Eurylana, Eurylana arcuata, Eurylana cookie, Natatolana*
Exosphaeroma	Isopod Sphaeromatidae (Family)	*Exosphaeroma, Exosphaeroma chilensis, Exosphaeroma falcatum, Exosphaeroma obtusum, Exosphaeroma planulum, Exosphaeroma waitemata*
Isopod other (excludes *Anthuroidea*, Cirolanidae and *Exosphaeroma*)	Isopod	Isopod other, *Cassidina typa, Isocladus, Isocladus armatus, Munnidae, Paravireia, Sphaeroma quoianum*
Stomatopoda	Mantis shrimp	*Heterosquilla*, Stomatopoda, mantis shrimp (common name)
Nemertea	Nemertean	Nemertea
Phoronida	Phoronid	Phoronida
Platyhelminthes	Platyhelminth	Platyhelminthes, Stylochidae
Polychaeta unid	Polychaete	Polychaeta
Ampharetidae	Polychaete Ampharetidae (Family)	Ampharetidae
Heteromastus filiformis.baranatolla lepte	Polychaete Capitellidae (Family)	*Heteromastus filiformis, Barantolla lepte*
Capitella.oligochaete	Polychaete Capitellidae (Family) and Oligochaete	*Capitella, Capitella capitata,* Oligochaeta, Capitella-Oligochaeta
Notomastus	Polychaete Capitellidae (Family)	*Capitellethus zeylanicus, Notomastus, Notomastus zeylanicus*
Phyllochaetopterus socialis	Polychaete Chaetopteridae (Family	*Phyllochaetopterus socialis*
Cirratulidae	Polychaete Cirratulidae (Family)	Cirratulidae, *Aphelochaeta*
Cossura	Polychaete Cossuridae (Family)	*Cossura, Cossura consimilis*
Dorvilleidae	Polychaete Dorvilleidae (Family)	Dorvilleidae, *Dorvillea*
Eunicidae	Polychaeta Eunicidae (Family)	*Eunice, Eunice vittata, Lysidice*
Manayunkia	Polychaete Fabriciidae (Family)	*Manayunkia*
Flabelligeridae	Polychaete Flabelligeridae (Family)	Flabelligeridae

Table A3.2 Continued.

Taxa name	Group	Taxa included
Glyceridae	Polychaete Glyceridae (Family)	*Glycera, Glycera Americana, Glycera lamelliformis, Glycera ovigera,* Glyceridae, *Hemipodia simplex*
Goniadidae	Polychaete Goniadidae (Family)	*Glycinde, Glycinde dorsalis, Glycinde trifida, Goniada graham,* Goniadidae
Hesionidae	Polychaete Hesionidae (Family)	Hesionidae, *Micropodarke, Oxydromus angustifrons*
Lumbrineridae	Polychaete Lumbrineridae (Family)	Lumbrinereidae, *Scoletoma brevicirra*
Magelona	Polychaete Magelonidae (Family)	*Magelona, Magelona dakini, Magelona papillicornis*
Maldanidae	Polychaete Maldanidae (Family)	*Asychis amphiglypta, Asychis Axiothella serrata, Macroclymenella stewartensis,* Maldanidae
Aglaophamus	Polychaete Nephtyidae (Family)	*Aglaophamus, Aglaophamus macroura*
Nereididae	Polychaete Nereididae (Family)	*Ceratonereis, Neanthes,* Nereididae, *Nereis, Nicon aestuariensis, Perinereis, Perinereis brevicirris, Perinereis nuntia brevicirris, Perinereis vallata, Platynereis australis*
Armandia maculata	Polychaete Opheliidae (Family)	*Armandia maculata*
Onuphidae	Polychaete Onuphidae (Family)	Onuphidae, *Diopatra akarana*
Orbiniidae	Polychaete Orbiniidae (Family)	*Naineris, Orbinia papillosa,* Orbiniidae, *Scoloplos cylindrifer*
Owenia fusiformis	Polychaete Oweniidae (Family)	*Owenia fusiformis*
Owenia petersenae	Polychaete Oweniidae (Family)	*Owenia petersenae*
Aricidea	Polychaete Paraonidae (Family)	*Aricidea*
Paradonidae other (excludes *Aricidea*)	Polychaete Paraonidae (Family)	*Levinsenia gracilis, Paradoneis, Paradoneis lyra*
Pectinariidae	Polychaete Pectinariidae (Family)	*Pectinaria australis, Pectinaria,* Pectinariidae

Table A3.2 Continued.

Taxa name	Group	Taxa included
Polynoidae	Polychaete Polynoidae (Family)	*Disconatis accolus*, *Frennia*, *Lepidastheniella comma*, Lepidonotinae, *Lepidonotus*, *Lepidonotus polychromus*, *Paralepidonotus ampulliferus*, Polynoidae
Phyllodocidae	Polychaete Phyllodocidae (Family)	Phyllodocidae, *Eteone*
Sabellidae	Polychaete Sabellidae (Family)	*Euchone*, *Euchone pallida*, *Neosabellaria kaiparaensis*, *Pseudopotamilla*, Sabellidae
Scalibregmatidae	Polychaete Scalibregmatidae (Family)	*Hyboscolex longiseta*, Scalibregmatidae
Serpulidae	Polychaete Serpulidae (Family)	Serpulidae, *Spirobranchus cariniferus*
Sigalionidae	Polychaete Sigalionidae (Family)	Sigalionidae
Sphaerodoridae	Polychaete Sphaerodoridae (Family)	Sphaerodoridae, *Sphaerodoropsis*
Polydorid complex	Polychaete Spionidae (Family)	*Boccardia*, *Boccardia acus*, *Boccardia knoxi*, *Boccardia polybranchia*, *Boccardia syrtis*, *Polydora*, *Polydora cornuta*, *Pseudopolydora*, *Pseudopolydora paucibranchiata*, Polydorid complex
Aonides	Polychaete Spionidae (Family)	*Aonides*, *Aonides oxycephala*, *Aonides trifida*
Microspio	Polychaete Spionidae (Family)	*Microspio maori*
Paraprionospio	Polychaete Spionidae (Family)	*Paraprionospio*, *Paraprionospio coora*
Prionospio aucklandica	Polychaete Spionidae (Family)	*Prionospio aucklandica*
Prionospio other (excludes *Prionospio aucklandica*)	Polychaete Spionidae (Family)	*Prionospio cirrifera*, *Prionospio ehlersi*, *Prionospio yuriel*
Scolecolepides	Polychaete Spionidae (Family)	*Scolecolepides*, *Scolecolepides benhami*
Scolelepis	Polychaete Spionidae (Family)	*Scolelepis*
Syllidae	Polychaete Syllidae (Family)	*Exogone*, *Exogoninae*, *Sphaerosyllis*, *Sphaerosyllis semiverrucosa*, Syllidae, Syllinae, *Syllis*

Table A3.2 Continued.

Taxa name	Group	Taxa included
Terebellidae	Polychaete Terebellidae (Family)	Terebellidae
Travisia olens	Polychaete Travisiidae (Family)	*Travisia olens, Travisia olens novaezealandiae*
Trichobranchidae	Polychaete Trichobranchidae (Family)	*Terebellides stroemii*, Trichobranchidae
Fellaster zelandiae	Sand dollar	*Fellaster zelandiae*
Patiriella regularis	Seastar Asterinidae (Family)	*Patiriella regularis*
Alpheus	Shrimp Alpheidae (Family)	*Alpheus, Alpheus socialis*
Biffarius filholi	Shrimp Callianassidae (Family)	*Biffarius filholi*
Philocheras australis	Shrimp Crangonidae (Family)	*Philocheras australis*
Mysida	Shrimp Mysida (Order)	Mysidacea, Mysida, Mysidae
Palaemon	Shrimp Palaemonidae (Family)	*Palaemon, Palaemon affinis*
Nebaliacea	Shrimp-like Leptostraca (Order)	Nebaliacea
Sipuncula	Sipunculid	Sipuncula, Sipunculidae
Tanaidacea	Tanaids	Tanaidacea

References Appendix 3:

Hume T, Gerbeaux P, Hart D, Kettles H, Neale D (2016) A classification of New Zealand's coastal hydrosystems. Prepared for Ministry for the Environment

Shears NT, Smith F, Babcock RC, Duffy CAJ, Villouta E (2008) Evaluation of Biogeographic Classification Schemes for Conservation Planning: Application to New Zealand's Coastal Marine Environment. Conservation Biology 22:467-481

Appendix 4: Stressor values and taxa characterising ecological health groups

Information on stressor values (Table A4.1) and taxa characterising each ecological health group (Tables A4.2 and A4.3) are provided to put the Benthic Health Model (BHM; Chapter 3) results into wider context.

Table A4.1 Stressor values (average and range) for model sites within each ecological health group for the Mud and Metals Benthic Health Models (BHMs).

	Mud BHM	Metals BHM		
Group	**Mud (%)**	**Cu (mg kg^{-1})**	**Pb (mg kg^{-1})**	**Zn (mg kg^{-1})**
1	1 (0-3)	0.3 (0-2.2)	2.6 (0-4.5)	15 (0-26)
2	4 (0-15)	1.6 (0-6.8)	3.1 (0-9.1)	21 (0-42)
3	13 (1-48)	4.9 (0-26)	7.5 (1.3-27)	42 (11-158)
4	39 (10-97)	16 (0-49)	26 (5.3-65)	110 (18-281)
5	65 (13-98)	25 (9-47)	31 (13-70)	143 (83-288)

As a form of model validation, SIMPER was used to identify taxa characterising each of the ecological health groups to ensure that the discriminating taxa across groups were consistent with what is known about the habitat preferences and metal tolerances of organisms. Discriminating taxa that cumulatively contributed between 70-74% to the similarity of each group were assigned to one of three categories based on literature (Table A4.2 and A4.3). For the Mud BHM, the grain-size preference categories were sandy, intermediate/unknown, and muddy, with the intermediate/unknown group a placement for taxa that showed a preference for habitats with intermediate grain-size or for species that could not be assigned based on the literature. For the Metals BHM, the metal sensitivity categories were sensitive, mixed/unknown, and tolerant, with the mixed/unknown group a placement for taxa that showed an inconsistent response to metal contamination or for species that could not be assigned based on the literature.

Most of the taxa characterising Mud BHM Group 1 prefer sand, such as such as the shellfish *Austrovenus stutchburyi* and *Paphies australis*, the gastropod *Notoacmea*, the polychaete *Aonides* and phoxocephalid amphipods (Norkko et al. 2002a, Thrush et al. 2003b, Gibbs & Hewitt 2004, Ellis et al. 2006, Anderson 2008, Thrush et al. 2008, Robertson et al. 2015). Although responses of broader taxonomic groups can be variable, many amphipods, cumaceans and orbinids also prefer sandier sediments (Norkko et al. 2002a, Thrush et al. 2003b, Gibbs & Hewitt 2004, Anderson 2008, Thrush et al. 2008, Robertson et al. 2015). Conversely, many of the taxa characterising Mud BHM Group 5 prefer mud, such as the crabs *Austrohelice*, *Hemigrapsus* and *Hemiplax*, *Capitella* polychaetes, oligochaetes and corophid amphipods (Norkko et al. 2002a, Thrush et al. 2003b, Gibbs & Hewitt 2004, Ellis et al. 2006, Anderson 2008, Thrush et al. 2008, Robertson et al. 2015).

Table A4.2 Taxa characterising Mud Benthic Health Model (BHM) ecological health groups ranging from 1 (less impacted by mud) to 5 (more impacted by mud). Grain-size preferences are based on information from: (Norkko et al. 2002a, Thrush et al. 2003b, Gibbs & Hewitt 2004, Ellis et al. 2006, Anderson 2008, Thrush et al. 2008, Robertson et al. 2015).

		Mud BHM Group				
Grain-size preference	**Taxa**	**1**	**2**	**3**	**4**	**5**
Sandy	Amphipoda other [a]	X				
	Anthopleura hermaphroditica		X			
	Aonides sp.	X				
	Austrovenus stutchburyi	X	X	X	X	
	Cumacea	X	X			
	Linucula hartvigiana		X	X		
	Macomona liliana		X	X		
	Notoacmea sp.	X	X			
	Orbiniidae	X		X		
	Paphies australis	X				
	Phoxocephalidae	X	X			
Intermediate/ unknown	*Arthritica* sp.			X	X	X
	Heteromastus filiformis/Barantolla lepte		X	X	X	X
	Nereididae		X	X	X	X
	Nermertea		X	X		X
	Polydorid complex [b]			X	X	X
	Prionospio aucklandica	X	X	X		
	Scolecolepides sp.			X	X	
Muddy	*Capitella* sp.		X	X	X	X
	Corophiidae					X
	Exosphaeroma sp.	X				
	Oligochaetes		X	X	X	X
	Austrohelice/Hemigrapsus/Hemiplax sp.				X	X

[a] Includes all amphipod taxa except Corophiidae, Paracalliopiidae and Phoxocephalidae

[b] Includes *Boccardia, Polydora* and *Pseudopolydora* species

Many of the taxa with increased abundances in Group 1 are sensitive to metals, including the shellfish *A. stutchburyi, P. australis* and *Macomona liliana*, orbinid and *Prionospio aucklandica* polychaetes, cumaceans and amphipods (Roper & Hickey 1994, Morrisey et al. 1996, De Luca-Abbott 2001, Anderson et al. 2002, Anderson et al. 2006, Thrush et al. 2008, Townsend et al. 2009, Fukunaga et al. 2010, Fukunaga & Anderson 2011, Fukunaga et al. 2011, Tremblay et al. 2017, AZTI Marine Biotic Index 2018, Podlesińska & Dąbrowska 2018). Taxa with higher abundances in Group 5 include the taxa which have shown to be tolerant to metals, such as nereid and *Cossura* polychaetes, the crabs *Austrohelice*, *Hemigrapsus* and *Hemiplax* and the bivalve *Arthritica* (Anderson et al. 2002, Morrisey et al. 2003, Anderson et al. 2006, Thrush et al. 2008, Hewitt et al. 2009, Fukunaga et al. 2011).

Table A4.2 Taxa characterising Metal Benthic Health Model (BHM) ecological health groups ranging from 1 (less impacted by metals) to 5 (more impacted by metals). Metal sensitivities are based on information from: (Roper & Hickey 1994, Morrisey et al. 1996, De Luca-Abbott 2001, Anderson et al. 2002, Anderson et al. 2006, Thrush et al. 2008, Townsend et al. 2009, Fukunaga et al. 2010, Fukunaga & Anderson 2011, Fukunaga et al. 2011, Tremblay et al. 2017, AZTI Marine Biotic Index 2018, Podlesińska & Dąbrowska 2018).

		Metals BHM Group				
Metal sensitivity	**Taxa**	**1**	**2**	**3**	**4**	**5**
Sensitive	*Anthopleura hermaphroditica*		X			
	Cumacea	X				
	Macomona liliana	X	X			
	Nermertea		X		X	X
	Notoacmea sp.		X			
	Orbiniidae	X				
	Phoxocephalidae		X			X
	Prionospio aucklandica	X	X	X		
Mixed/ unknown	*Austrovenus stutchburyi*		X	X	X	
	Capitella sp./Oligochaeta	X	X	X	X	
	Corophiidae			X	X	
	Heteromastus filiformis/Barantolla lepte	X	X	X	X	X
	Linucula hartvigiana		X			
	Nereididae		X	X	X	X
	Polydorid complex [a]			X	X	X
	Scolecolepides sp.	X		X		
	Scolelepis sp.	X				
Tolerant	*Arthritica*			X	X	X
	Austrohelice/Hemigrapsus/Hemiplax sp.			X	X	X
	Cossura sp.					X

[a] Includes Boccardia, Polydora and Pseudopolydora species

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Appendix 5: Temporal validation of the Benthic Health Models

In addition to testing whether the Benthic Health Models (BHMs; Chapter 3) can predict the stressor values of sites across a range of mud/metal concentrations, I also tested whether they were unaffected by temporal variability that was not associated with changes in environmental drivers. To do this, I investigated whether natural temporal variability in community composition across years resulted in a site sampled at a different time, but with similar mud or metal concentrations, having markedly different CAP scores (designated as being greater than whatever the range of values for a single group is). Nine sites (4-6 sampling occasions per site) were used to test the Mud BHM and seven sites (2-3 sampling occasions per site) were used to test the Metals BHM. The range in CAP scores was only greater than the group range (1.0) for the sites *luc* and *okains* (Mud BHM) and *io1* (Metals BHM; Table A5.1). The *luc* site had the greatest range in mud, possibly explaining the greater range in CAP scores at this site (Figure A5.1). The *io1* and *ip5* sites are located adjacent to a motorway development project, which may explain the greater range in metal CAP scores at these sites (Figure A5.1). The decrease in the Metals BHM CAP score at *io1* was associated with a large increase in corophids, *Capitella* sp. and Oligochaetes, indicative of a decline in ecological health.

Table A5.1 Range in Benthic Health Model (BHM) canonical analysis of principal coordinates (CAP) scores for sites with similar A) mud or B) metals (PC1) concentrations over time. The number of times a site was sampled (n) and the number of years over which that sampling took place (year range) is also provided. Red text indicates where the range in CAP scores was greater than the group range (1.0). Refer to Appendix 8 for site locations.

A) Mud BHM				
Site	**Year range**	***n***	**Mud range (%)**	**CAP score range**
whau	5	5	0.5	0.39
pepe	2	4	1.8	0.65
pleasant point	9	6	7.7	0.59
rng	5	4	3.6	0.57
brig	10	4	3.9	0.71
hbv	10	6	3.6	0.71
oturu	2	5	6.8	0.79
okains	6	5	5.7	1.13
luc	10	5	8.7	1.21

B) Metals BHM				
Site	**Year range**	***n***	**PC1 range**	**CAP score range**
whau	3	2	0.73	0.47
hell	7	2	0.25	0.07
brig	7	2	0.01	0.47
ahuriri b	4	2	0.06	0.56
luc	7	2	0.15	0.93
ip5	2	3	0.31	0.96
io1	2	3	0.31	1.54

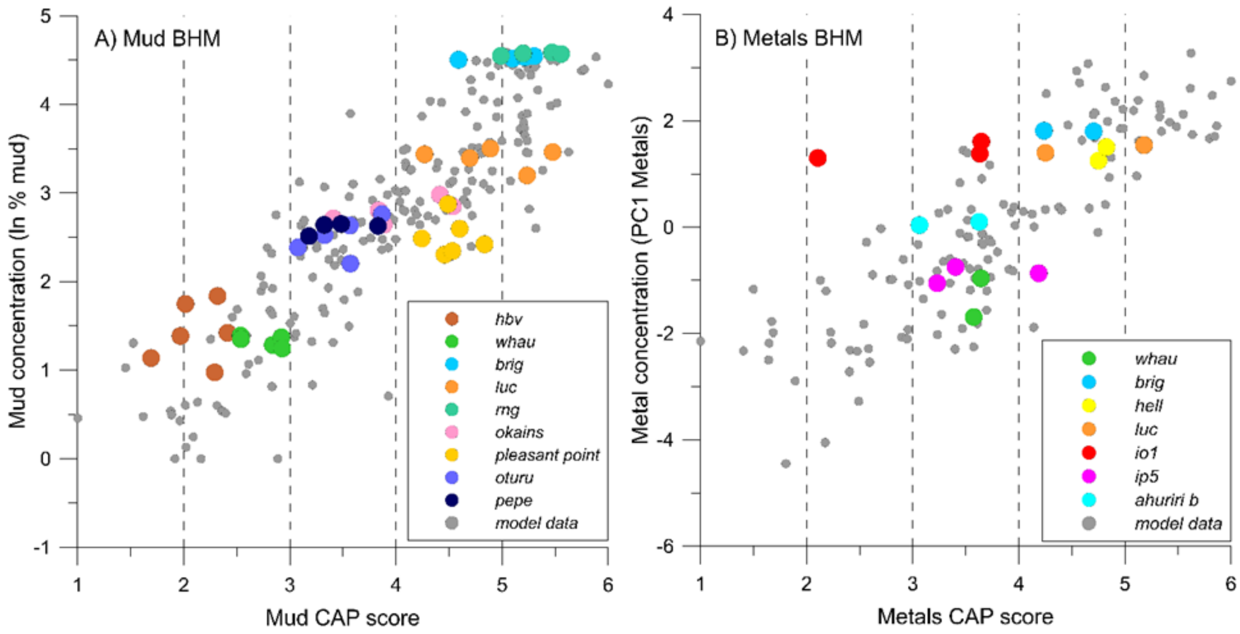

Figure A5.1 A) Mud Benthic Health Model (BHM) canonical analysis of principal coordinates (CAP) scores vs mud concentrations (ln % mud) for sites with similar mud concentrations over time. B) Metals BHM CAP scores vs mud concentrations (PC1 metals) for sites with similar metals concentrations over time. Grey dashed lines demarcate the ecological health categories for each model and colours indicate different sites, with grey circles indicating the underlying model data. Refer to Appendix 8 for site locations.

Appendix 6: Benthic Health Models over different scales

When attempting to apply biotic indices on a nationwide scale, it is important to understand how outputs relate to assessments carried out at finer scales of resolution, as these may provide a more precise estimate of environmental status for managing specific locations. The national and regional Benthic Health Models (BHMs; Chapter 3) ranked the health of sites in a similar manner (strong and moderate correlations; Mud BHM $r = 0.98$, Metals BHM $r = 0.76$) but there was some discrepancy between the national and single estuary rankings (moderate correlations; Mud BHM $r = 0.68$, Metals BHM $r = 0.42$; Figure A6.1). The difference between the national and single-estuary health ranks may occur because of 1) differences in taxonomic resolution between models, 2) differences in the size of the stressor gradient between models or because 3) fewer data points reduce the power of the national/estuary scale comparison relative to the comparison with the regional scale model.

The smaller stressor gradient in the single estuary model may allow it to discriminate over smaller changes in health. I tested this by creating a new national model that was restricted to the same stressor range of the single estuary model. The correlation between the national and estuary scale models improved when using the national model with the smaller stressor gradient (from $r = 0.68$ to $r = 0.83$ for the Mud BHM; Figure A6.1) suggesting the reduced gradient helps to discriminate differences between sites. The smaller gradient was not tested on the Metals BHM because there were too few independent sites for the comparison.

To test whether having fewer data points for comparison may have also contributed to inconsistency between model health score rankings, I reduced the number of sites in the national vs. regional model comparison. Only the first 18 sites were used, which is equivalent to the number of sites available for the national vs. estuary comparison. Correlation between the national and estuary scale models was reduced (from $r = 0.98$ to $r = 0.97$ for the Mud BHM and from $r = 0.76$ to $r = 0.60$ for the Metals BHM; Figure A6.1) suggesting that having fewer data points did reduce power slightly.

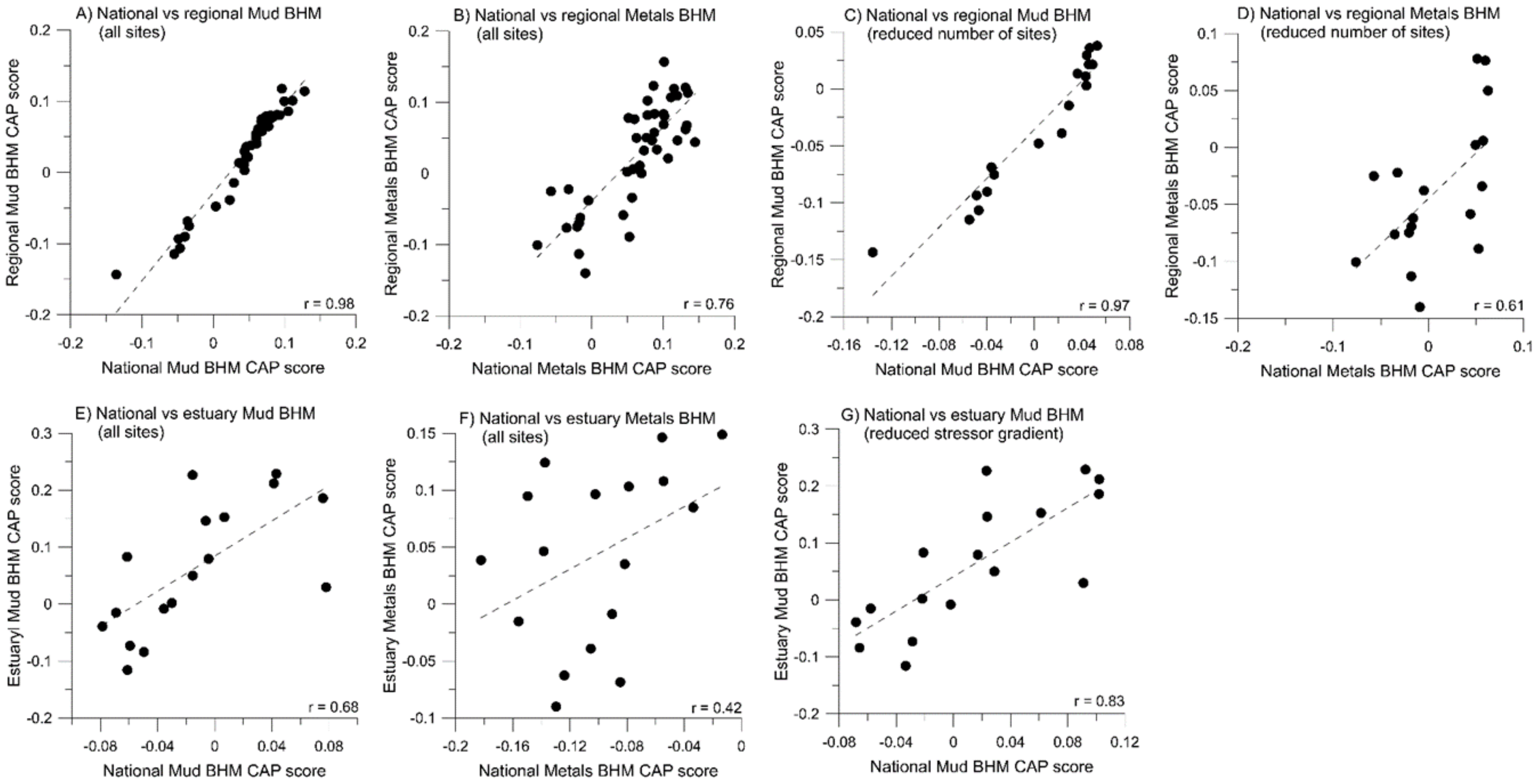

Figure A6.1 Comparison between canonical analysis of principal coordinates (CAP) scores generated using national, regional or estuary scale data for two models; the Mud Benthic Health Model (BHM) and the Metals BHM. The effect of reducing the number of sites for comparison (C & D) and using a smaller stressor gradient (G) was also examined. A linear model has been fitted (black dashed line) and *r* values indicate the Spearman's rank correlation coefficient.

Appendix 7: Sediment quality guidelines and Benthic Health Model groups

Existing sediment quality guideline values were converted to determine their position along the PC1 metals gradient and facilitate comparison with corresponding Metal Benthic Health Models (BHM; Chapter 3) CAP scores. The PC1 metals gradient is a linear combination of copper, zinc, and lead concentration. In principal component analysis (PCA), the eigenvector weights provide coefficients for a linear combination of the original variables that will yield the principal component scores. The following equation was used to determine the position of existing sediment quality guidelines along the PC1 metals axis:

$$\text{PC1 metals} = 0.653 * (X_{\text{Cu}}) + 0.536 * (X_{\text{Pb}}) + 0.535 * (X_{\text{Zn}})$$

where X equals the log (x + 1) concentration of that metal (copper, lead or zinc) in the sample minus the mean log (x + 1) concentration of that metal across the full set of model sites (mg kg^{-1}). The mean log (x + 1) concentrations used were 1.80 for copper, 2.28 for lead and 3.83 for zinc.

Table A7.1 Existing sediment quality guidelines from various sources, along with their equivalent Metals Benthic Health Model (BHM) ecological health group. Values for metals are in mg kg^{-1}.

Guideline	Location	Cu	Pb	Zn	PC1Met	CAP score	Group	Source
Threshold effect								
SQGV	NZ, Australia	65	50	200	3.232	5.6	5	a
TEL	Canada, USA	18.7	30.2	124	1.925	4.8	4	b
ERL	USA	34	46.7	150	2.629	5.2	5	c
ERC-Green	NZ	<19	<30	<124	1.931	4.8	4	d
cHC5 (TEL)	Hong Kong	23.5	29.9	57.2	1.653	4.7	4	e
SQO Target	Netherlands	36	85	140	2.944	5.4	5	f
ISQV-Low	Hong Kong	65	75	200	3.445	5.7	5	g
T_{20}	USA	32	30	94	2.711	5.3	5	h
SLG-Low	Canada	16	31	120	2.929	5.4	5	i
FEC	NZ	9.3	19.4	118	1.247	4.5	4	j
Mean Effect	Norway	3	17	20	-0.366	3.6	3	k
Midrange effect								
PEL	Canada, USA	108	112	271	4.147	6.1	>5	b
ERM	USA	270	218	410	5.318	6.7	>5	c
cHC10 (PEL)	Hong Kong	33.9	34.6	78.3	2.125	5.0	5	e
AET	USA	390	450	410	2.669	5.3	5	l
SQO PEC	Netherlands	73	530	620	5.166	6.6	>5	f
ISQV-High	Hong Kong	270	218	410	5.318	6.7	>5	g
T_{50}	USA	94	94	245	4.524	6.3	>5	h
Extreme effect								
SQG-High	NZ/Australia	270	220	410	5.323	6.7	>5	a
ERC-Red	NZ	>34	>50	>150	2.664	5.3	5	d
T_{80}	USA	280	297	636	4.545	6.3	>5	h
SLG-Severe	Canada	110	250	820	5.492	6.8	>5	i

SQGV, Sediment Quality Guideline Value; TEL, Threshold Effect Level; ERL, Effects Range Level; ERC, Environmental Response Criteria; cHC5, adjusted community Hazardous Concentration 5%; SQO, Sediment Quality Objective; ISQV, Interim Sediment Quality Value; T_{20}, 20% probability of observing sediment toxicity; SLG, Screening Level Guideline; FEC, effect concentrations; PEL, probable effects level; ERM, effects range median; cHC10, cHC5, adjusted community Hazardous Concentration 10%; AET, Apparent Effects Thresholds; SQO PEC, Sediment Quality Objective Maximum Permissible Concentration; SQG, Sediment Quality Guideline; T_{50}, 50% probability of observing sediment toxicity; T_{80}, 80% probability of observing sediment toxicity.

a Simpson (2013), b MacDonald et al. (1996), c Long et al. (1995), d ARC (2004), e Kwok et al. (2008), f ANZECC (2000), g Chapman et al. (1999), h Field et al. (2002), i Persaud et al. (1993), j Hewitt et al. (2009), k Bjørgesæter and Gray (2008), l Department of Ecology (2013).

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Appendix 8: Information on sites within the Benthic Health Models

Table A8.1 Information on sites within the Mud and Metals Benthic Health Models (BHMs; Chapter 3). Councils are Auckland Council (AC), Bay of Plenty Regional Council (BOPRC), Environment Canterbury (ECAN), Environment Southland (ES), Greater Wellington Regional Council (GWRC), Hawke's Bay Regional Council (HBRC), Marlborough District Council (MDC), Northland Regional Council (NRC), Otago Regional Council (ORC), Tasman District Council (TDC), Waikato Regional Council (WRC), West Coast Regional Council (WCRC). Estuary types are defined by Hume et al. (2016) and include tidal lagoons (7) and shallow drowned valleys (8). Regions are defined by Shears et al. 2008 and include Abel (ABL), Banks (BNK), Buller (BUL), Chalmers (CHA), Northeastern (NE), Portland (PRT), Raglan (RAG), Stewart Island (STW). M/V refers to whether a site was used in model development (M) or validation(V).

Estuary	Site	Year	Council	Estuary type	Region	Latitude	Longitude	Mud BHM	Metals BHM	M/V
Ahuriri	a	2014	HBRC	7	PRT	-39.48354	176.87834	X	X	M
Ahuriri	b	2012	HBRC	7	PRT	-39.48717	176.87898	X	X	M
Ahuriri	c	2007	HBRC	7	PRT	-39.48392	176.87579	X	X	M
Ahuriri	d	2007	HBRC	7	PRT	-39.48679	176.88644	X	X	M
Ahuriri	e	2014	HBRC	7	PRT	-39.48238	176.88408	X	X	M
Avon Heathcote	avon	2007	ECAN	7	BNK	-43.52055	172.72750	X	X	M
Avon Heathcote	avon	2015	ECAN	7	BNK	-43.52055	172.72750	X		V
Avon Heathcote	discharge point	2011	ECAN	7	BNK	-43.54084	172.72122	X	X	M
Avon Heathcote	heathcote	2007	ECAN	7	BNK	-43.56065	172.70229	X	X	M
Avon Heathcote	heathcote	2009	ECAN	7	BNK	-43.56065	172.70229	X		V
Avon Heathcote	heathcote	2011	ECAN	7	BNK	-43.56065	172.70229		X	V
Avon Heathcote	humphreys drive	2011	ECAN	7	BNK	-43.55430	172.70385	X	X	M
Avon Heathcote	humphreysdrive	2010	ECAN	7	BNK	-43.55430	172.70385	X		V
Avon Heathcote	pleasant point jetty	2011	ECAN	7	BNK	-43.53138	172.73017	X	X	M
Avon Heathcote	plover street	2012	ECAN	7	BNK	-43.54825	172.74312	X		M
Catlins	a	2016	ORC	7	CHA	-46.47684	169.69975	X	X	M
Catlins	b	2016	ORC	7	CHA	-46.47231	169.63873	X	X	M
Central Waitemata	chelsea	2004	AC	8	NE	-36.82021	174.72844	X	X	M
Central Waitemata	coxes waitemata	2004	AC	8	NE	-36.84868	174.72146	X	X	M
Central Waitemata	hbv	2005	AC	8	NE	-36.79922	174.67758	X	X	M
Central Waitemata	henderson lower	2004	AC	8	NE	-36.82798	174.64036	X	X	M

Table A8.1 Continued.

Estuary	Site	Year	Council	Estuary type	Region	Latitude	Longitude	Mud BHM	Metals BHM	M/V
Central Waitemata	henderson upper	2005	AC	8	NE	-36.83858	174.63285	X	X	M
Central Waitemata	hobson purewa bridge	2005	AC	8	NE	-36.86528	174.82283	X	X	M
Central Waitemata	los	2013	AC	8	NE	-36.81396	174.76614	X		M
Central Waitemata	lower shoal bay	2005	AC	8	NE	-36.81020	174.76942	X	X	M
Central Waitemata	meola inner	2002	AC	8	NE	-36.85699	174.70920	X	X	M
Central Waitemata	ngataringa bay	2005	AC	8	NE	NA	NA	X	X	M
Central Waitemata	oakley	2005	AC	8	NE	-36.87266	174.69555	X	X	M
Central Waitemata	purewa	2004	AC	8	NE	-36.86528	174.82283	X	X	M
Central Waitemata	shoal bay hillcrest	2004	AC	8	NE	-36.80105	174.76407	X	X	M
Central Waitemata	ups	2014	AC	8	NE	NA	NA	X		M
Central Waitemata	whakataka	2002	AC	8	NE	-36.85738	174.80731	X	X	M
Central Waitemata	whau	2010	AC	8	NE	-36.84707	174.67091	X	X	M
Central Waitemata	whau	2012	AC	8	NE	-36.84707	174.67091	X	X	V
Central Waitemata	whau east	2005	AC	8	NE	NA	NA	X	X	M
Central Waitemata	whau entrance	2004	AC	8	NE	-36.85140	174.66098	X	X	M
Central Waitemata	whau lower	2005	AC	8	NE	-36.87687	174.66336	X	X	M
Central Waitemata	whau upper	2004	AC	8	NE	-36.89862	174.67486	X	X	M
Central Waitemata	whau wairau	2002	AC	8	NE	-36.89255	174.66216	X	X	M
Central Waitemata	whau wairau	2005	AC	8	NE	-36.89255	174.66216		X	V
Central Waitemata	whau west	2005	AC	8	NE	NA	NA	X	X	M
Fortrose	a	2006	ES	7	STW	-46.57134	168.78764	X	X	M
Havelock	d	2015	MDC	8	ABL	-41.27074	173.77391	X	X	M
Kaipara	k03	2014	NRC	8	RAG	-36.17575	174.27820	X		M
Kaipara	k18	2014	NRC	8	RAG	-36.32467	174.17537	X		M
Kaipara	k19	2014	NRC	8	RAG	-36.33722	174.17828	X		M
Kaipara	k20	2014	NRC	8	RAG	-36.19953	174.06314	X		M
Kaipara	k31	2014	NRC	8	RAG	-36.19301	174.13659	X		M
Kaipara	k36	2014	NRC	6	RAG	-36.15422	174.01524	X		M
Kaipara	k43	2014	NRC	8	RAG	-36.24763	174.32941	X		M

Table A8.1 Continued.

Estuary	Site	Year	Council	Estuary type	Region	Latitude	Longitude	Mud BHM	Metals BHM	M/V
Kaipara	k44	2014	NRC	8	RAG	-36.25905	174.32443	X		M
Lyttelton	governors	2012	ECAN	9	BNK	-43.62109	172.65528	X		M
Lyttelton	hob	2011	ECAN	9	BNK	-43.64735	172.66598	X		V
Lyttelton	hob	2013	ECAN	9	BNK	-43.64735	172.66598	X		M
Mahurangi	cb	2010	AC	8	NE	-36.44981	174.71234	X	X	M
Mahurangi	dc	2010	AC	8	NE	-36.45929	174.70866	X	X	M
Mahurangi	hl	2010	AC	8	NE	-36.43488	174.71584	X	X	M
Mahurangi	hl	2011	AC	8	NE	-36.43488	174.71584	X		V
Mahurangi	jb	2010	AC	8	NE	-36.49365	174.71563	X	X	M
Mahurangi	mh	2010	AC	8	NE	-36.45219	174.72929	X	X	M
Mahurangi	tk	2012	AC	8	NE	-36.47644	174.73657	X		M
Mangemangeroa	2	2010	AC	8	NE	-36.91013	174.95652	X		M
Mangemangeroa	3	2010	AC	8	NE	-36.91095	174.95552	X	X	M
Mangemangeroa	3	2014	AC	8	NE	-36.91095	174.95552	X		V
Mangemangeroa	5	2010	AC	8	NE	-36.91095	174.95388	X		M
Mangemangeroa	6	2010	AC	8	NE	-36.91132	174.95342	X	X	M
Mangemangeroa	7	2010	AC	8	NE	-36.91312	174.94995	X		M
Mangemangeroa	9	2010	AC	8	NE	-36.91435	174.94848	X	X	M
Mangemangeroa	10	2010	AC	8	NE	-36.91562	174.94650	X		M
Mangonui	man-19	2016	NRC	8	NE	-34.98970	173.55654	X	X	M
Manukau	anns creek	2002	AC	8	RAG	-36.92983	174.82211	X	X	M
Manukau	annscreek	2005	AC	8	RAG	-36.92983	174.82211	X	X	V
Manukau	cb	2002	AC	8	RAG	-37.12254	174.70270	X	X	M
Manukau	ch	2014	AC	8	RAG	-36.95729	174.67392	X		M
Manukau	eb	2013	AC	8	RAG	-37.08223	174.79477	X		M
Manukau	kp	2014	AC	8	RAG	-37.04648	174.83301	X		M
Manukau	mangere cemetery	2005	AC	8	RAG	-36.93149	174.79573	X	X	M
Manukau	mangere inlet harania creek	2005	AC	8	RAG	-36.94438	174.81869	X	X	M

Table A8.1 Continued.

Estuary	Site	Year	Council	Estuary type	Region	Latitude	Longitude	Mud BHM	Metals BHM	M/V
Manukau	mangere inlet kiwi esplanade	2005	AC	8	RAG	NA	NA	X	X	M
Manukau	mangere inlet tararata creek	2005	AC	8	RAG	-36.94503	174.80277	X	X	M
Manukau	ps	2014	AC	8	RAG	-37.03544	174.84171	X		M
Manukau	puhinui entrance	2002	AC	8	RAG	-37.02728	174.85554	X	X	M
Manukau	pukaki	2005	AC	8	RAG	-37.00051	174.80564	X	X	M
Moutere	b	2013	TDC	8	ABL	-41.16956	173.04330	X	X	M
Ngunguru	4	2016	NRC	7	NE	-35.64290	174.47509	X	X	M
Ngunguru	6	2016	NRC	7	NE	-35.64286	174.48131	X	X	V
Ngunguru	10	2016	NRC	7	NE	-35.63944	174.49738	X	X	M
Ngunguru	14	2016	NRC	7	NE	-35.63498	174.50279	X	X	M
Okains Bay	okains	2012	ECAN	11	BNK	-43.69507	173.05414	X		M
Okains Bay	okains	2015	ECAN	11	BNK	-43.69507	173.05414	X		V
Okura	1	2010	AC	7	NE	-36.66540	174.73445	X	X	M
Okura	3	2014	AC	7	NE	-36.66770	174.72972	X		M
Okura	4	2014	AC	7	NE	-36.66833	174.72738	X		M
Okura	5	2014	AC	7	NE	-36.66805	174.72673	X		M
Okura	7	2010	AC	7	NE	-36.67097	174.72188	X	X	M
Okura	8	2014	AC	7	NE	-36.67157	174.71995	X		M
Okura	9	2010	AC	7	NE	-36.67367	174.71768	X	X	M
Okura	10	2014	AC	7	NE	-36.67458	174.71570	X		V
Orewa	1	2010	AC	7	NE	-36.59918	174.69693	X	X	M
Orewa	2	2010	AC	7	NE	-36.59808	174.69513	X		M
Orewa	3	2014	AC	7	NE	-36.59905	174.69377	X		M
Orewa	4	2010	AC	7	NE	-36.59987	174.69038	X	X	M
Orewa	5	2010	AC	7	NE	-36.59777	174.68587	X		M
Orewa	6	2010	AC	7	NE	-36.60032	174.68588	X		M
Orewa	8	2010	AC	7	NE	-36.59758	174.68298	X	X	M
Orowaiti	a	2007	WCRC	7	BUL	-41.74518	171.63497	X	X	M
Porirua	io1	2013	GWRC	7	ABL	-41.13007	174.84198	X	X	M

Table A8.1 Continued.

Estuary	Site	Year	Council	Estuary type	Region	Latitude	Longitude	Mud BHM	Metals BHM	M/V
Porirua	io1	2014	GWRC	7	ABL	-41.13007	174.84198		X	V
Porirua	io2	2013	GWRC	7	ABL	-41.12605	174.84092		X	V
Porirua	io2	2014	GWRC	7	ABL	-41.12605	174.84092	X	X	M
Porirua	io3	Feb 2013	GWRC	7	ABL	-41.12202	174.83830	X	X	M
Porirua	io3	Jun 2013	GWRC	7	ABL	-41.12202	174.83830		X	V
Porirua	ip1	2013	GWRC	7	ABL	-41.08880	174.89136	X	X	M
Porirua	ip2	2013	GWRC	7	ABL	-41.08876	174.89394	X		V
Porirua	ip2	2014	GWRC	7	ABL	-41.08876	174.89394	X	X	M
Porirua	ip3	2013	GWRC	7	ABL	-41.09521	174.90421	X	X	M
Porirua	ip4	2013	GWRC	7	ABL	-41.09943	174.91192		X	V
Porirua	ip4	2014	GWRC	7	ABL	-41.09943	174.91192	X	X	M
Porirua	ip5	2013	GWRC	7	ABL	-41.10232	174.90988	X	X	M
Porirua	ip6	2014	GWRC	7	ABL	-41.10532	174.90119	X	X	M
Porirua	ip7	Feb 2013	GWRC	7	ABL	-41.10522	174.88212		X	V
Porirua	ip7	Jun 2013	GWRC	7	ABL	-41.10522	174.88212	X	X	M
Puhoi	1	2010	AC	7	NE	-36.52687	174.71002	X	X	M
Puhoi	2	2010	AC	7	NE	-36.53133	174.70958	X		M
Puhoi	3	2010	AC	7	NE	-36.52693	174.70870	X		M
Puhoi	4	2010	AC	7	NE	-36.53027	174.70733	X	X	M
Puhoi	6	2010	AC	7	NE	-36.53087	174.70502	X		M
Puhoi	7	2010	AC	7	NE	-36.52772	174.70015	X		V
Puhoi	7	2014	AC	7	NE	-36.52772	174.70015	X		M
Puhoi	8	2004	AC	7	NE	NA	NA	X	X	M
Puhoi	9	2010	AC	7	NE	-36.52558	174.69447	X	X	M
Shag River	a	2016	ORC	7	CHA	-45.48044	170.81131	X	X	M
Shag River	b	2016	ORC	7	CHA	-45.47744	170.80796	X	X	M
Tairua	gum digger gully	2013	WRC	7	NE	-37.04046	175.83827	X		M
Tairua	manaia road	Feb 2014	WRC	7	NE	-37.00247	175.85630	X		V
Tairua	manaia road	Aug 2014	WRC	7	NE	-37.00247	175.85630	X		M

Table A8.1 Continued.

Estuary	Site	Year	Council	Estuary type	Region	Latitude	Longitude	Mud BHM	Metals BHM	M/V
Tairua	oturu stream	Feb 2014	WRC	7	NE	-37.03063	175.83606	X		V
Tairua	oturu stream	Nov 2014	WRC	7	NE	-37.03063	175.83606	X		M
Tairua	pauanui	2013	WRC	7	NE	-37.00756	175.85765	X		V
Tairua	pepe inlet	2014	WRC	7	NE	-37.00130	175.84457	X		M
Tamaki	bengazi	2004	AC	8	NE	-36.89332	174.87185	X	X	M
Tamaki	bowden rd	2004	AC	8	NE	-36.91498	174.85510	X	X	M
Tamaki	middlemore	2005	AC	8	NE	-36.94976	174.85555	X	X	V
Tamaki	otahuhu creek	2004	AC	8	NE	-36.93206	174.85851	X	X	M
Tamaki	pakuranga	2005	AC	8	NE	NA	NA	X	X	M
Tamaki	pakuranga mid	2005	AC	8	NE	NA	NA	X	X	M
Tamaki	princes st	2004	AC	8	NE	-36.93617	174.86237	X	X	M
Tauranga	1	2011	BOPRC	8	NE	-37.45242	175.97145	X	X	M
Tauranga	4	2011	BOPRC	8	NE	-37.46932	175.95012	X	X	M
Tauranga	13	2011	BOPRC	8	NE	-37.56082	175.93954	X	X	M
Tauranga	14	2011	BOPRC	8	NE	-37.57370	175.93107	X	X	M
Tauranga	17	2011	BOPRC	8	NE	-37.54926	176.01320	X	X	M
Tauranga	18	2011	BOPRC	8	NE	-37.56042	176.03560	X	X	M
Tauranga	20	2011	BOPRC	8	NE	-37.57441	176.06183	X	X	M
Tauranga	25	2011	BOPRC	8	NE	-37.59759	176.03275			V
Tauranga	27	2011	BOPRC	8	NE	-37.59913	175.98601	X	X	M
Tauranga	28	2011	BOPRC	8	NE	-37.60107	175.97709	X	X	M
Tauranga	29	2011	BOPRC	8	NE	-37.60451	176.08629	X		V
Tauranga	37	2011	BOPRC	8	NE	-37.62310	175.98411	X	X	M
Tauranga	38	2011	BOPRC	8	NE	-37.63306	175.99453	X	X	M
Tauranga	40	2011	BOPRC	8	NE	-37.63748	176.02088	X	X	M
Tauranga	4410	2011	BOPRC	8	NE	-37.63181	176.06030	X	X	M
Tauranga	47	2011	BOPRC	8	NE	-37.65917	176.03456	X	X	M
Tauranga	56	2011	BOPRC	8	NE	-37.67943	176.10763	X	X	M
Tauranga	62	2011	BOPRC	8	NE	-37.68156	176.15141	X	X	M

Table A8.1 Continued.

Estuary	Site	Year	Council	Estuary type	Region	Latitude	Longitude	Mud BHM	Metals BHM	M/V
Tauranga	65	2011	BOPRC	8	NE	-37.68197	176.17956		X	V
Tauranga	73	2011	BOPRC	8	NE	-37.70772	176.21590	X	X	M
Tauranga	74	2011	BOPRC	8	NE	-37.69804	176.22797		X	V
Tokomairiro	a	2017	ORC	7	CHA	-46.21615	170.04382	X	X	M
Turanga	1	2014	AC	8	NE	-36.90670	174.97477	X		M
Turanga	3	2010	AC	8	NE	-36.91158	174.96332	X		M
Turanga	4	2010	AC	8	NE	-36.91568	174.96228	X	X	M
Turanga	6	2010	AC	8	NE	-36.92100	174.96358	X		M
Turanga	8	2010	AC	8	NE	-36.92900	174.97057	X	X	M
Turanga	8	2014	AC	8	NE	-36.92900	174.97057	X		V
Upper Waitemata	brig	2011	AC	8	NE	-36.77730	174.60528	X	X	M
Upper Waitemata	brig	2012	AC	8	NE	-36.77730	174.60528	X		V
Upper Waitemata	hell	2010	AC	8	NE	-36.78316	174.68372	X		V
Upper Waitemata	hell	2011	AC	8	NE	-36.78316	174.68372	X	X	M
Upper Waitemata	hin	2011	AC	8	NE	-36.77660	174.65838	X	X	M
Upper Waitemata	hiw	2005	AC	8	NE	-36.78378	174.65749		X	V
Upper Waitemata	hiw	2011	AC	8	NE	-36.78378	174.65749	X	X	M
Upper Waitemata	kaipatiki	2005	AC	8	NE	-36.78003	174.69768	X	X	M
Upper Waitemata	luc	2005	AC	8	NE	-36.76889	174.66204		X	V
Upper Waitemata	luc	2011	AC	8	NE	-36.76889	174.66204	X	X	M
Upper Waitemata	lucus te wharau	2004	AC	8	NE	-36.76193	174.67203	X	X	M
Upper Waitemata	lucus upper	2005	AC	8	NE	-36.75129	174.67675	X	X	M
Upper Waitemata	main c	2011	AC	8	NE	-36.77050	174.64237	X	X	M
Upper Waitemata	main u	2005	AC	8	NE	-36.77140	174.61250	X	X	M
Upper Waitemata	mainu	2011	AC	8	NE	-36.77140	174.61250		X	V
Upper Waitemata	mainu	2014	AC	8	NE	-36.77140	174.61250	X		V
Upper Waitemata	ohbv	2011	AC	8	NE	-36.79048	174.67900	X	X	M
Upper Waitemata	ohbv	2012	AC	8	NE	-36.79048	174.67900	X		V
Upper Waitemata	paremoremo	2005	AC	8	NE	-36.75912	174.63435	X	X	M

Table A8.1 Continued.

Estuary	Site	Year	Council	Estuary type	Region	Latitude	Longitude	Mud BHM	Metals BHM	M/V
Upper Waitemata	paremoremo upper	2005	AC	8	NE	-36.76124	174.63295	X	X	M
Upper Waitemata	rng	2010	AC	8	NE	-36.76428	174.60212	X		V
Upper Waitemata	rng	2011	AC	8	NE	-36.76428	174.60212	X	X	M
Upper Waitemata	rng old	2005	AC	8	NE	NA	NA	X	X	M
Waikawa	a	2016	MDC	9	ABL	-41.26805	174.03985	X	X	M
Waikopua	1	2010	AC	8	NE	-36.90185	174.97667	X	X	M
Waikopua	3	2010	AC	8	NE	-36.90477	174.97922	X	X	M
Waikopua	4	2010	AC	8	NE	-36.90547	174.97213	X		M
Waikopua	6	2014	AC	8	NE	-36.90495	174.98632	X		M
Waikopua	7	2010	AC	8	NE	-36.90673	174.98807	X		M
Waikopua	8	2010	AC	8	NE	-36.90735	174.99102	X		M
Waikopua	9	2010	AC	8	NE	-36.90815	174.99267	X	X	M
Waikopua	9	2014	AC	8	NE	-36.90815	174.99267	X		V
Waikouaiti	a	2016	ORC	7	CHA	-45.63501	170.65582	X	X	M
Waikouaiti	b	2016	ORC	7	CHA	-45.62498	170.65072	X	X	M
Waikouaiti	c	2016	ORC	7	CHA	-45.62065	170.63692	X	X	M
Waimea	a	2015	TDC	8	ABL	-41.31726	173.18255	X		M
Waimea	b	2014	TDC	8	ABL	-41.26429	173.08775	X	X	M
Waimea	c	2015	TDC	8	ABL	-41.29958	173.17750	X		M
Wairoa	a	2012	HBRC	6	PRT	-39.05463	177.42425	X	X	M
Wairoa	a	2013	HBRC	6	PRT	-39.05463	177.42425	X	X	V
Waiwera	1	2010	AC	7	NE	-36.54268	174.70577	X	X	M
Waiwera	1	2014	AC	7	NE	-36.54268	174.70577	X		V
Waiwera	2	2010	AC	7	NE	-36.54180	174.70613	X		M
Waiwera	3	2010	AC	7	NE	-36.54092	174.70505	X	X	M
Waiwera	5	2004	AC	7	NE	-36.53973	174.70390	X	X	M
Waiwera	6	2014	AC	7	NE	-36.54083	174.70263	X		M
Waiwera	7	2010	AC	7	NE	-36.54055	174.70153	X		M
Waiwera	8	2010	AC	7	NE	-36.54135	174.69788	X	X	M

Table A8.1 Continued.

Estuary	Site	Year	Council	Estuary type	Region	Latitude	Longitude	Mud BHM	Metals BHM	M/V
Waiwera	9	2010	AC	7	NE	-36.54060	174.69645	X		M
Waiwera	10	2004	AC	7	NE	-36.54033	174.69550	X	X	M
Whangateau	1	2010	AC	7	NE	-36.35141	174.77342	X		V
Whangateau	1	2014	AC	7	NE	-36.35141	174.77342	X		M
Whangateau	2	2010	AC	7	NE	-36.34778	174.77147	X		M
Whangateau	3	2010	AC	7	NE	-36.34262	174.76265	X		M
Whangateau	4	2014	AC	7	NE	-36.33042	174.76510	X		M
Whangateau	5	2014	AC	7	NE	-36.32262	174.75262	X		M
Whangateau	6	2010	AC	7	NE	-36.31178	174.77753	X		M
Whangateau	7	2014	AC	7	NE	-36.31666	174.76217	X		M
Whareama	b	2008	GWRC	6	COO	-41.01187	176.09282	X	X	V

References Appendix 8:

Hume T, Gerbeaux P, Hart D, Kettles H, Neale D (2016) A classification of New Zealand's coastal hydrosystems. Prepared for Ministry for the Environment

Shears NT, Smith F, Babcock RC, Duffy CAJ, Villouta E (2008) Evaluation of Biogeographic Classification Schemes for Conservation Planning: Application to New Zealand's Coastal Marine Environment. Conservation Biology 22:467-481

Appendix 9: Information on gene markers

Table A9.1 Primers, mastermix recipe and thermocycling conditions for each gene (Chapter 4).

Gene	**16S**	**18S**	**rbcL**
Primer	341F	Uni18SF	705F
	805R	Uni18SR	808R
Primer sequence	CCTACGGGNGGCWGCAG	AGGGCAAKYCTGGTGCCAG	AACAGGTGAAGTTAAAGGTTCATAYT
	TACHVGGGTATCTAATCC	CGRCGGTATCTRATCGYCTT	TTGTAACCCATAACTAAATCGATCA T
Amplicon size	~450 bp	~450 bp	80-130 bp
Reference	(Klindworth et al. 2012)	(Zhan et al. 2013)	(Stoof-Leichsenring et al. 2012)
Mastermix (µl)			
MyFi	25	25	32
Bovine Serum Albumin	-	1	-
Forward primer	1	2	2
Reverse Primer	1	2	2
Water	19	14	10
DNA	4	6	4
Thermocycling conditions			
Initial denaturation	95°C 5 min	94°C 2 min	94°C 2 min
Denaturation	94°C 30 s x 32 cycles	94°C 30 s x 36 cycles	94°C 30 s x 38 cycles
Annealing	53°C 30 s x 32 cycles	52°C 30 s x 36 cycles	43.6°C 30 s x 38 cycles
Extension	72°C 45 s x 32 cycles	72°C 45 s x 36 cycles	72°C 30 s x 38 cycles
Final extension	72°C 7 min	72°C 7 min	72°C 10 min

References Appendix 9:

Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner FO (2012) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Research 41:e1-e1

Stoof-Leichsenring KR, Epp LS, Trauth MH, Tiedemann R (2012) Hidden diversity in diatoms of Kenyan Lake Naivasha: a genetic approach detects temporal variation. 21:1918-1930

Zhan A, Hulák M, Sylvester F, Huang X, Adebayo AA, Abbott CL, Adamowicz SJ, Heath DD, Cristescu ME, MacIsaac HJ (2013) High sensitivity of 454 pyrosequencing for detection of rare species in aquatic communities. Methods in Ecology and Evolution 4:558-565

Appendix 10: Rarefaction curves

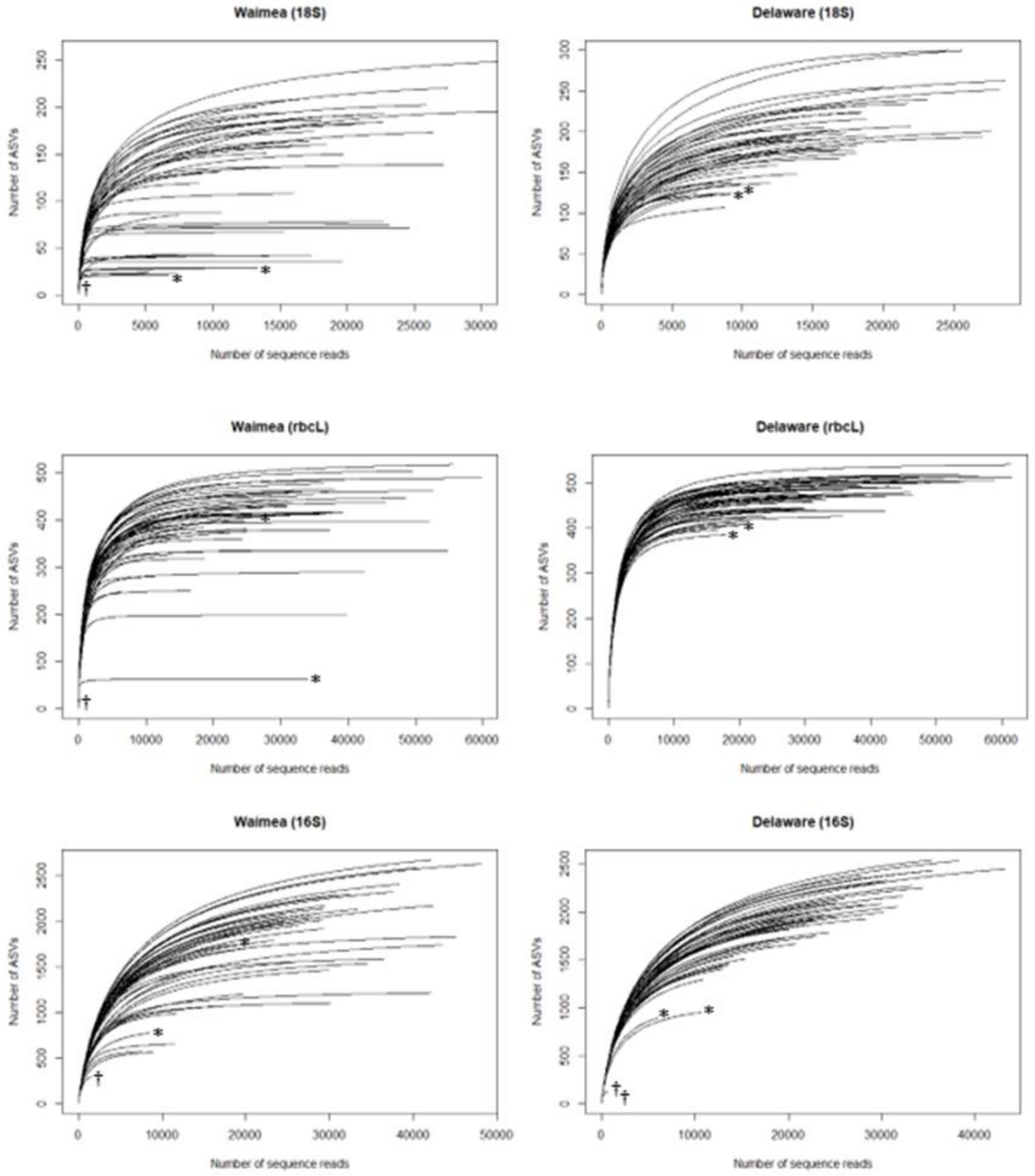

Figure A10.1 Rarefaction curves for the eukaryote (18S), diatom only (rbcL) and bacteria (16S) datasets (Chapter 4). Samples with low reads † and outliers * were removed prior to analysis.

Appendix 11: Numbers of sequence reads and amplicon sequence variants

Table A11.1 Number of sequence reads and amplicon sequence variants (ASVs) in each sample from Delaware estuary after reads associated with contamination and non-target taxa were removed (Chapter 4). Nutrient enrichment treatments: C = control, M = medium, H = high.

Delaware	**Eukaryotes (18S)**		**Diatoms only (rbcL)**		**Bacteria (16S)**	
	Reads	**ASVs**	**Reads**	**ASVs**	**Reads**	**ASVs**
C1-1	9861	88	42797	459	24265	1071
C1-2	9188	91	30350	437	30086	1173
C1-3	24604	143	40317	478	32270	1239
C1-4	20066	127	41083	471	20800	1032
C1-5	Removed*	Removed*	Removed*	Removed*	Removed*	Removed*
M1-1	12830	107	22549	438	Removed†	Removed†
M1-2	13851	99	29256	449	20104	1102
M1-3	18110	103	46455	443	31710	1158
M1-4	27692	111	33170	433	24433	1228
M1-5	13742	107	38981	456	29633	1297
H1-1	9183	78	27313	420	13084	892
H1-2	16929	105	50556	478	30524	1307
H1-3	17965	104	58912	473	29951	1174
H1-4	16085	105	56497	474	34367	1256
H1-5	27047	110	61360	494	35359	1333
C2-1	17378	124	35717	451	13708	925
C2-2	Removed*	Removed*	Removed*	Removed*	Removed*	Removed*
C2-3	18281	133	49187	468	22990	1034
C2-4	12302	113	28318	410	12810	919
C2-5	9853	94	44748	458	14143	955
M2-1	21941	123	41242	465	13372	957
M2-2	11994	99	30930	437	25220	1238
M2-3	5690	88	28643	414	10876	841
M2-4	18457	138	53564	481	23628	1185
M2-5	9915	95	51263	471	21965	1090
H2-1	21777	144	61538	479	27879	1213
H2-2	25600	147	23950	404	35080	1333
H2-3	15553	108	42141	406	25157	1138
H2-4	6765	75	20830	389	19582	1075
H2-5	16858	112	35749	407	43155	1320
C3-1	15779	108	23482	404	22784	1178
C3-2	11598	109	29682	424	22900	1112
C3-3	23096	126	38582	454	19647	1130
C3-4	14739	111	30061	419	38258	1384
C3-5	8416	81	19775	389	15301	908

* Outliers

† Low number of reads

Table A11.1 Continued.

Delaware	**Eukaryotes (18S)**		**Diatoms only (rbcL)**		**Bacteria (16S)**	
	Reads	**ASVs**	**Reads**	**ASVs**	**Reads**	**ASVs**
M3-1	18797	126	39165	445	35400	1352
M3-2	17935	117	46118	452	18472	1030
M3-3	18110	113	31709	419	Removed†	Removed†
M3-4	28628	142	53568	468	23801	1143
M3-5	28266	128	51140	467	33243	1234
H3-1	8757	79	16639	379	16431	1012
H3-2	16449	107	46004	442	28224	1129
H3-3	12453	105	31209	430	27897	1303
H3-4	18780	114	34308	440	22017	1105
H3-5	9652	86	44022	463	25946	1203

* Outliers

† Low number of reads

Table A11.2 Number of sequence reads and amplicon sequence variants (ASVs) in each sample from Waimea estuary after reads associated with contamination and non-target taxa were removed (Chapter 4). Nutrient enrichment treatments: C = control, M = medium, H = high.

Waimea	**Eukaryotes (18S)**		**Diatoms only (rbcL)**		**Bacteria (16S)**	
	Reads	**ASVs**	**Reads**	**ASVs**	**Reads**	**ASVs**
C1-1	32708	118	48520	404	29225	1205
C1-2	17140	112	22140	379	7057	664
C1-3	27456	138	59786	447	37599	1273
C1-4	22737	130	29468	413	24651	1210
C1-5	13352	129	34896	416	13802	1002
M1-1	25887	132	6350	241	24763	1221
M1-2	21309	119	31847	386	38313	1360
M1-3	5771	30	25575	319	11758	665
M1-4	20808	122	55504	470	30733	1334
M1-5	10095	32	52564	421	29318	1176
H1-1	22709	72	52039	366	36459	906
H1-2	60001	150	Removed†	Removed†	45098	1060
H1-3	14133	40	39753	186	11515	432
H1-4	Removed*	Removed*	Removed*	Removed*	Removed*	Removed*
H1-5	7368	22	54699	307	42181	725
C2-1	22641	122	27312	399	24983	1194
C2-2	Removed*	Removed*	Removed*	Removed*	Removed*	Removed*
C2-3	26363	126	33092	414	29361	1269
C2-4	18006	126	39418	430	40465	1443
C2-5	9415	88	18436	356	12180	879
M2-1	23105	46	37986	386	34504	975
M2-2	Removed†	Removed†	35748	387	19595	768
M2-3	16005	69	42344	418	25308	959
M2-4	24626	60	37241	349	43431	1049
M2-5	15291	47	36640	376	29878	920
H2-1	5227	22	16560	235	7691	385
H2-2	19629	35	42403	266	30074	670
H2-3	17323	37	11206	264	8889	355
H2-4	10612	58	24240	335	Removed†	Removed†
H2-5	27141	89	24620	371	23252	1078
C3-1	17528	117	28571	417	32353	1326
C3-2	10352	89	15073	343	26217	1142
C3-3	13941	122	36328	433	42055	1443
C3-4	14755	119	49536	452	27880	1164
C3-5	19664	99	38017	418	48162	1389
M3-1	14162	92	45593	403	42307	1228
M3-2	13263	117	31663	402	16364	979
M3-3	9008	89	13922	312	7748	673
M3-4	14854	114	24860	358	33266	1206
M3-5	16373	121	18495	357	17631	1082

* Outliers

† Low number of reads

Table A11.2 Continued.

Waimea	**Eukaryotes (18S)**		**Diatoms only (rbcL)**		**Bacteria (16S)**	
	Reads	**ASVs**	**Reads**	**ASVs**	**Reads**	**ASVs**
H3-1	9391	27	18726	303	16725	667
H3-2	18485	98	24876	348	29084	1250
H3-3	13957	104	30923	391	26984	1203
H3-4	7454	66	22615	368	19928	1072
H3-5	15895	94	39200	385	28067	1234

* Outliers

† Low number of reads

Appendix 12: ANOVA results

Table A12.1 Effect of site, nutrient enrichment treatment and their interaction on environmental variables, determined using two-way ANOVA (Chapter 4). Where necessary data were transformed (type indicated in brackets) to meet assumptions of normality. Significant *p*-values ($p < 0.05$) are shaded in gray. Results of *post-hoc* Tukey Honest Significant Differences (HSD) tests are shown for significant treatment effects ($p < 0.05$). Nutrient enrichment treatments: C = control, M = medium, H = high.

TWO-WAY ANOVA										
Source of variation	**df**	**SS**	**MS**	***F*-value**	***p***					
Mud (sqrt)										
Site	1	43.03	43.03	184.525	<0.0001					
Treatment	2	0.45	0.22	0.957	0.412					
Site x Treatment	2	0.23	0.12	0.494	0.622					
Residuals	12	2.80	0.23							
Organic matter (log)										
Site	1	2.3998	2.3998	1047.975	<0.0001					
Treatment	2	0.0038	0.0019	0.837	0.457					
Site x Treatment	2	0.0019	0.0009	0.407	0.674					
Residuals	12	0.0275	0.0023							
Median grain-size										
Site	1	9980	9980	234.992	<0.0001					
Treatment	2	32	16	0.381	0.691					
Site x Treatment	2	8	4	0.096	0.909					
Residuals	12	510	42							
Chlorophyll *a*										
Site	1	7.94	7.94	13.122	0.0035					
Treatment	2	1.114	0.557	0.92	0.4248					
Site x Treatment	2	1.469	0.734	1.213	0.3312					
Residuals	12	7.261	0.605							
Phaeophytin										
Site	1	14.951	14.951	45.54	<0.0001					
Treatment	2	0.446	0.223	0.679	0.526					
Site x Treatment	2	0.796	0.398	1.212	0.331					
Residuals	12	3.940	0.328				**TUKEY HSD TEST**			
Surface pore water NH_4^+ (log)						**Treat**	**Diff**	**Low**	**Upp**	***p***
Site	1	0.13	0.13	0.081	0.781	C/M	2.75	0.83	4.66	0.0063
Treatment	2	78.98	39.49	25.624	<0.0001	C/H	5.13	3.21	7.04	<0.0001
Site x Treatment	2	4.52	2.26	1.468	0.269	M/H	2.38	0.47	4.29	0.0156
Residuals	12	18.49	1.54							
Deep pore water NH_4^+ (log)										
Site	1	1.39	1.39	1.506	0.243	C/M	3.10	1.62	4.58	0.0003
Treatment	2	106.88	53.44	57.996	<0.0001	C/H	5.97	4.49	7.45	<0.0001
Site x Treatment	2	0.57	0.28	0.307	0.741	M/H	2.87	1.39	4.35	0.0006
Residuals	12	11.06	0.92							

Appendix 13: PERMANOVA results

Table A13.1 Effect of site, nutrient enrichment treatment and their interactions on eukaryotic (including diatoms), diatom only, bacterial and macroinvertebrate communities, determined using PERMANOVA based on Bray-Curtis dissimilarities of fourth root transformed proportional read abundance data or macroinvertebrate abundance data (Chapter 4). Significant *p*-values ($p < 0.05$) are shaded in gray and *post-hoc* pairwise tests show differences between treatments for each site with % sim indicating average similarity between groups. Nutrient enrichment treatments: C = control, M = medium, H = high.

PERMANOVA							***POST-HOC* PAIRWISE TESTS**						
								Delaware			**Waimea**		
Source of variation	**df**	**SS**	**MS**	**Pseudo-*F***	***p***	**Unique permutations**	**Treat**	***t***	***p***	**% sim**	***t***	***p***	**% sim**
Eukaryotes (including diatoms)													
Site	1	70385	70385	60.786	0.0001	9907	C/M	1.2	0.0326	60.6	1.6	0.0039	48.3
Treatment	2	6862.5	3431.2	2.9633	0.0001	9794	C/H	1.8	0.0001	56.2	2.3	0.0001	41.8
Site x Treatment	2	5515.7	2757.8	2.3817	0.0001	9844	M/H	1.4	0.0035	57.8	1.2	0.0684	38.5
Residuals	79	91474	1157.9										
Diatom only													
Site	1	51824	51824	263.59	0.0001	9917	C/M	1.3	0.0408	84.6	1.4	0.0096	79.5
Treatment	2	1206.7	603.35	3.0688	0.0001	9850	C/H	1.7	0.0006	83.2	2.2	0.0001	74.0
Site x Treatment	2	944.76	472.38	2.4026	0.0001	9833	M/H	1.3	0.0457	83.8	1.5	0.0016	73.2
Residuals	79	15532	196.61										
Bacteria													
Site	1	30203	30203	60.641	0.0001	9886	C/M	1.1	0.1600	73.9	1.5	0.0002	68.3
Treatment	2	2558.2	1279.1	2.5682	0.0001	9764	C/H	1.3	0.0002	73.6	2.0	0.0001	60.0
Site x Treatment	2	2293.7	1146.9	2.3027	0.0001	9786	M/H	1.1	0.0448	75.0	1.6	0.0024	58.9
Residuals	77	38350	498.1										
Macroinvertebrate													
Site	1	20999	20999	14.504	0.0001	9940	C/M	0.9	0.6107	65.8	1.4	0.0263	43.7
Treatment	2	7690.6	3845.3	2.656	0.0003	9904	C/H	1.9	0.0074	49.7	1.8	0.0033	20.8
Site x Treatment	2	5991.5	2995.8	2.0692	0.0040	9897	M/H	1.4	0.0730	52.2	1.3	0.0795	30.0
Residuals	30	43434	1447.8										

Appendix 14: CAP results

Table A14.1 Summary of canonical analysis of principal coordinates (CAP) analyses carried out on fourth root transformed abundance data for macroinvertebrate communities at the two sites (Chapter 4).

Site	**Waimea**	**Delaware**
Number of samples	18	18
Correlation	0.79	0.75
Canonical correlation	0.63	0.57
Number of PCO axes (m)	3	3
Trace statistic	0.7327	0.6260
p	0.0198	0.0572
Allocation success (%)	66.7	50.0
Control	83.3	50.0
Medium	66.7	50.0
High	50.0	50.0

Appendix 15: PERMDSIP results

Table A15.1 Test of homogeneity of dispersions for eukaryotic (including diatoms), diatom only, bacterial and macroinvertebrate communities using PERMDISP (Chapter 4). The analysis is based on Bray-Curtis dissimilarities of fourth root transformed proportional read abundance data or macroinvertebrate abundance data. *Post-hoc* pairwise comparisons show differences between treatments for each site. Significant *p*-values ($p < 0.05$) are shaded in gray. Nutrient enrichment treatments: C = control, M = medium, H = high.

	Delaware		**Waimea**	
Eukaryotes (including diatoms)	$F_{2,40} = 5.463$, $p = 0.0136$		$F_{2,39} = 23.686$, $p = 0.0001$	
Pairwise comparisons	*t*	*p*	*t*	*p*
C/M	0.65	0.5607	5.16	0.0002
C/H	3.01	0.0085	8.06	0.0001
M/H	2.60	0.0193	0.91	0.4417
Average Bray-Curtis distance to centroid (SE)				
C	26.0 (0.8)		26.2 (1.2)	
M	26.7 (0.7)		39.9 (2.4)	
H	29.2 (0.7)		42.5 (1.6)	
Diatom only	$F_{2,40} = 1.865$, $p = 0.1968$		$F_{2,39} = 20.544$, $p = 0.0001$	
Pairwise comparisons	*t*	*p*	*t*	*p*
C/M	0.57	0.5973	3.84	0.0011
C/H	1.74	0.1141	6.63	0.0001
M/H	1.34	0.2194	2.75	0.0223
Average Bray-Curtis distance to centroid (SE)				
C	10.1 (0.4)		11.3 (0.63)	
M	10.4 (0.4)		15.5 (0.9)	
H	11.3 (0.5)		19.2 (1.0)	
Bacteria	$F_{2,38} = 1.073$, $p = 0.4388$		$F_{2,39} = 13.623$, $p = 0.0002$	
Pairwise comparisons	*t*	*p*	*t*	*p*
C/M	1.05	0.3644	2.48	0.0453
C/H	1.50	0.1934	4.78	0.0006
M/H	0.34	0.7675	3.04	0.0226
Average Bray-Curtis distance to centroid (SE)				
C	18.1 (0.6)		18.1 (1.4)	
M	17.0 (0.8)		22.9 (1.3)	
H	16.7 (0.7)		30.4 (2.2)	
Macroinvertebrate	$F_{2,15} = 6.269$, $p = 0.0061$		$F_{2,15} = 11.818$, $p = 0.0006$	
Pairwise comparisons	*t*	*p*	*t*	*p*
C/M	1.4	0.2253	0.7	0.5314
C/H	3.8	0.0094	3.6	0.0050
M/H	3.0	0.0128	4.2	0.0029
Average Bray-Curtis distance to centroid (SE)				
C	20.5 (2.2)		35.2 (2.6)	
M	24.4 (1.6)		32.8 (2.4)	
H	33.1 (2.5)		51.2 (3.6)	

Appendix 16: Indicator taxa

Table A16.1 Indicator taxa associated with nutrient enrichment treatments (or groups of treatments) for three eDNA-derived communities (eukaryotes including diatoms, diatom only, and bacteria) and macroinvertebrate communities (Chapter 4). Indicator Values (IndV) are a measure of the strength of the association between a taxon and a treatment, and the *p*-values represent the significance of this relationship. Only taxa with *p*-values < 0.05 are displayed. Mean abundances of indicator taxa for each nutrient enrichment treatment are shown: C = control, M = medium, H = high. Shading highlights taxa absent in a given site/treatment and an asterisk beside the name denotes indicator taxa shared by both sites.

	Waimea					**Delaware**					**Taxonomy**	
	Ind V	*p*	C	M	H	Ind V	*p*	C	M	H	Order	Family
Eukaryotic indicator taxa												
Control												
Dinophyceae	0.85	<0.001	0.10	0.01	<0.01						-	-
Gymnodiniaceae	0.78	<0.001	0.06	<0.01	▨						Gymnodiniales	Gymnodiniaceae
Togula	0.73	0.006	0.11	0.03	0.02						Gymnodiniales	Gymnodiniaceae
Sabulodinium	0.70	0.001	0.03	<0.01	▨						Peridiniales	-
Axiothella	0.69	0.003	0.12	0.02	0.02						-	Maldanidae
Nematoda						0.66	0.012	0.51	0.01	0.01	-	-
Peridiniales						0.63	0.013	0.03	<0.01	0.01	Peridiniales	-
Neoheteromita	0.58	0.016	0.05	<0.01	▨						Glissomonadida	Sandonidae
Nematoplana	0.55	0.038	0.17	0.07	<0.01						Proseriata	Nematoplanidae
Rhabdocoela						0.47	0.046	0.21	0.01	▨	Rhabdocoela	-
Control + medium												
Dinophyceae	0.88	0.003	1.17	0.65	0.32						-	-
Draconematidae	0.87	0.002	0.26	0.13	0.05						Desmodorida	Draconematidae
Tripyloides	0.82	0.012	0.13	0.05	0.03						Araeolaimida	Tripyloididae
Chlamydomonas	0.79	0.013	0.16	0.24	0.06						Chlamydomonadales	-
op14-lineages						0.79	0.029	0.12	0.15	0.02	Vampyrellida	op14-lineage
Proseriata	0.78	0.005	0.07	0.11	0.02						Proseriata	-
Amphidinium						0.76	0.030	0.09	0.03	0.02	Gymnodiniales	Gymnodiniaceae
Chlorodendrales	0.75	0.003	0.07	0.04	0.01						Chlorodendrales	Chlorodendraceae
Anurofeca	0.74	0.042	0.09	0.06	0.03						Ichthyosphonida	Pseudoperkinsidae
Tetrastemma						0.67	0.013	0.30	0.12	0.01	Monostilifera	Tetrastemmatidae
Typhlamphiascus	0.60	0.038	0.02	0.03	▨						Harpacticoida	Miraciidae
Medium												
Styela	0.68	0.005	<0.01	0.11	0.01						Stolidobranchia	Styelidae
Mercenaria	0.64	0.005	<0.01	0.13	<0.01						Venerida	Veneridae

Table A16.1 Continued.

	Waimea					Delaware					Taxonomy	
	Ind V	*p*	C	M	H	Ind V	*p*	C	M	H	Order	Family
Medium + high												
Canthocamptidae						0.81	0.016	0.49	3.13	4.42	Harpacticoida	Canthocamptidae
Holosticha	0.60	0.044	▨	0.17	0.06						Hypotrichia	Holostichidae
High												
Chlamydomonas						0.80	0.001	<0.01	0.02	0.69	Chlamydomonadales	-
Cryptomycota						0.77	<0.001	▨	<0.01	0.74	-	-
Chytridiales						0.58	0.010	▨	<0.01	0.02	Chytridiales	-
Pseudoperkinsidae						0.58	0.007	▨	▨	0.13	Ichthyosphonida	Pseudoperkinsidae
Penardia-lineage						0.52	0.028	▨	▨	0.06	Vampyrellida	Penardia-lineage
Rhizophydiales						0.49	0.045	<0.01	▨	0.01	Rhizophydiales	-
Diatom indicator taxa												
Control												
Thalassiosira						0.64	0.008	0.01	<0.01	<0.01	Thalassiosirales	Thalassiosiraceae
Gyrosigma	0.54	0.014	<0.01	<0.01	▨						Naviculales	Pleurosigmataceae
Control + medium												
Extubocellulus	0.59	0.042	0.01	0.01	▨						Cymatosirales	Cymatosiraceae
Medium + high												
Pleurosigma						0.91	<0.001	0.01	0.05	0.12	Naviculales	Pleurosigmataceae
Bacterial indicator taxa												
Control											-	-
Zixibacteria	0.78	0.001	0.03	0.01	<0.01						-	-
028H05-P-BN-P5	0.73	0.027	0.02	<0.01	0.01						Ardenticatenales	-
Ardenticatenales	0.71	0.045	0.03	0.01	0.01						Cellvibrionales	Halieaceae
Haliea	0.67	0.014	0.01	<0.01	<0.01						Nostocales	Cyanobacteriaceae
Cyanobacteriaceae	0.65	0.004	0.01	<0.01	<0.01						-	-
Blastomonas	0.64	0.002	0.09	<0.01	▨						Sphingomonadales	Sphingomonadaceae
Acidovorax	0.64	0.003	0.18	0.01	▨						Betaproteobacteriales	Burkholderiaceae
Candidatus Jidaibacter						0.63	0.006	0.01	<0.01	<0.01	Rickettsiales	Midichloriaceae
Control + medium												
Rubritalea	0.89	<0.001	0.04	0.04	0.01						Verrucomicrobiales	Rubritaleaceae
WCHB1-81	0.83	0.013	0.04	0.05	0.01						-	-
Sediminispirochaeta	0.77	0.007	0.02	0.02	<0.01						Spirochaetales	Spirochaetaceae
Haliangium	0.76	0.048	0.04	0.02	0.01						Myxococcales	Haliangiaceae
Candidatus Kaiserbacteria	0.75	0.007	0.02	0.05	0.01						*Candidatus* Kaiserbacteria	-

Table A16.1 Continued.

	Waimea					Delaware					Taxonomy	
	Ind V	*p*	C	M	H	Ind V	*p*	C	M	H	Order	Family
Oceanicola						0.68	0.023	0.01	0.01	<0.01	Rhodobacterales	Rhodobacteraceae
Medium												
Jejudonia	0.74	0.009	0.01	0.03	<0.01						Flavobacteriales	Flavobacteriaceae
Gracilibacteria	0.68	0.003	<0.01	0.02	0.01						-	-
Acidiferrobacteraceae						0.52	0.032	<0.01	<0.01	▨	Acidiferrobacterales	Acidiferrobacteraceae
Medium + high												
*Soehngenia**	1.00	<0.001	<0.01	0.18	2.98						Clostridiales	Family XI
XI	0.96	<0.001	<0.01	0.11	1.01						Clostridiales	Family XI
Oscillatoriaceae						0.94	<0.001	0.02	0.08	0.11	Nostocales	Oscillatoriaceae
*Fusibacter**	0.92	<0.001	<0.01	0.20	0.73						Clostridiales	Family_XII
Anoxynatronum	0.89	0.002	<0.01	0.41	0.45						Clostridiales	Clostridiaceae 2
Clostridiales	0.85	0.037	0.07	0.12	0.39						Clostridiales	-
Tropicibacter						0.78	0.001	<0.01	0.03	0.03	Rhodobacterales	Rhodobacteraceae
Subgroup 7						0.76	0.023	<0.01	0.01	0.01	Subgroup 7	-
High												
*Soehngenia**						0.85	<0.001	▨	<0.01	0.35	Clostridiales	Family XI
*Dethiosulfatibacter**	0.96	<0.001	<0.01	0.19	2.27	0.86	<0.001	▨	▨	0.10	Clostridiales	Clostridiales *Incertae sedis*
Leisingera						0.89	<0.001	<0.01	0.08	0.44	Rhodobacterales	Rhodobacteraceae
*Erysipelothrix**	0.83	<0.001	▨	0.02	0.15	0.62	0.005	▨	<0.01	0.03	Erysipelotrichales	Erysipelotrichaceae
*Proteiniclasticum**	0.83	0.001	<0.01	0.02	0.14	0.77	<0.001	▨	▨	0.03	Clostridiales	Clostridiaceae 1
Alkalibacter	0.81	0.019	<0.01	0.04	0.86						Clostridiales	Eubacteriaceae
Blvii28 WW-sludge	0.80	<0.001	▨	0.02	0.34						Bacteroidales	Rikenellaceae
XIV*	0.78	<0.001	▨	<0.01	0.17	0.63	0.003	▨	▨	0.01	Clostridiales	Family XIV
Arcobacter	0.73	<0.001	▨	<0.01	0.55						Campylobacterales	Arcobacteraceae
Melioribacter	0.73	0.004	<0.01	0.02	0.07						Ignavibacteriales	Melioribacteraceae
Desulforhopalus						0.73	<0.001	▨	▨	0.04	Desulfobacterales	Desulfobulbaceae
livecontrolB21*	0.72	0.007	▨	0.01	0.15	0.82	<0.001	▨	▨	0.09	Clostridiales	livecontrolB21
*Anaerovorax**	0.72	0.004	<0.01	<0.01	0.08	0.82	<0.001	▨	▨	0.18	Clostridiales	Family_XIII
Brumimicrobium	0.68	0.001	▨	▨	0.04						Flavobacteriales	Crocinitomicaceae
Desulfoconvexum	0.68	0.001	▨	<0.01	0.15						Desulfobacterales	Desulfobacteraceae
Desulfotignum						0.68	<0.001	▨	▨	0.06	Desulfobacterales	Desulfobacteraceae
MSB-3C8	0.67	0.006	<0.01	0.03	0.07						Kryptoniales	MSB-3C8
*Fusibacter**						0.63	0.002	▨	▨	0.03	Clostridiales	Family_XII

Table A16.1 Continued.

	Waimea					**Delaware**					**Taxonomy**	
	Ind V	*p*	C	M	H	Ind V	*p*	C	M	H	Order	Family
MAT-CR-H4-C10*	0.62	0.001	▨	▨	0.04	0.58	0.009	▨	▨	0.05	Clostridiales	MAT-CR-H4-C10
*Tissierella**	0.62	0.001	▨	▨	0.04	0.73	<0.001	▨	▨	0.03	Clostridiales	Family XI
Desulfuromonas	0.62	0.004	<0.01	▨	0.03						Desulfuromonadales	Desulfuromonadaceae
Peptococcaceae	0.61	0.003	▨	<0.01	0.04						Clostridiales	Peptococcaceae
Rikenellaceae*	0.61	0.005	▨	<0.01	0.06	0.52	0.027	▨	▨	0.02	Bacteroidales	Rikenellaceae
*Tindallia**	0.57	0.026	▨	0.01	0.05	0.58	0.009	▨	▨	0.01	Clostridiales	Clostridiaceae 2
RBG-16-49-21	0.55	0.042	▨	0.02	0.06						Leptospirales	Leptospiraceae
Desulfuromusa	0.53	0.049	▨	0.03	0.09						Desulfuromonadales	Desulfuromonadaceae
MSBL8	0.48	0.029	▨	▨	0.03						Cloacimonadales	MSBL8
Macroinvertebrate indicator taxa												
Control												
Exosphaeroma waitemata	0.88	0.006	8.50	2.17	0.33						Isopoda	Sphaeromatidae
Control + medium												
Hiatula siliquens						0.92	0.0173	14.8	4.67	1.83	Cardiida	Psammobiidae
Heteromastus filiformis						0.91	0.0289	2.83	2.83	0.67	-	Capitellidae
Orbinia papillosa	0.87	0.016	1.00	1.83	▨						-	Orbiniidae
Macomona liliana	0.82	0.034	1.67	1.33	▨						Cardiida	Tellinidae
Capitella	0.82	0.0347	1.83	0.50	▨						-	Capitellidae
Medium + high												
Paracorophium excavatum	0.91	0.017	0.17	81.5	20.0						Amphipoda	Corophiidae