Body size distributions and diet breadth: Stream food web proxies and predictions across a contaminant gradient

Justin P. F. Pomeranz

School of Biological Sciences
University of Canterbury
New Zealand

A thesis submitted for the degree of
Doctor of Philosophy in Ecology
2018

For Carlyn

General Abstract

Ecological networks, such as food webs, offer a powerful framework for understanding the structure, function, and stability of biotic communities. However, logistical constraints related to traditional food web construction methods restrict their widespread use in ecological studies. Alternative methods, such as size spectra analysis, exist which incorporate much of the variation in food web structure, but are easier to measure. Additionally, advances in mechanistic models allow for the inference of pairwise species interactions, potentially scaling up to whole-network level measures.

The aim of my thesis was to investigate the utility of several of these alternative approaches in determining food web structures in degraded ecosystems. In particular, streams impacted by Acid Mine Drainage from coal mining. Firstly, I conducted a field survey of 25 stream communities across a gradient of acid mine drainage (AMD) inputs on the West Coast of the South Island, New Zealand. Comparative size spectra analysis revealed consistent changes to the size spectra relationship across the gradient. Size spectra intercepts, or total community abundance, were significantly reduced along the gradient. The slopes of size spectra increased significantly across the gradient from ~ -1.1 to ~ -0.6, meaning that the proportion of large to small bodied individuals decreased less rapidly in effected streams. Size spectra slopes are related to trophic transfer efficiency, and shallower slopes observed in AMD impacted streams indicate a reduced transfer efficiency. Furthermore, both the largest and smallest body size classes were removed from the most heavily impacted streams, leading to a reduction in the range of body sizes present by up to two orders of magnitude. Most aquatic food webs are size-structured, with body size and trophic level generally being positively correlated. Therefore, changes in the distribution of body sizes has significant implications for food-web structure.

Another alternative to traditional food web construction is the use of models and inference techniques to predict food web structure. I developed a novel model to predict pairwise species interactions within communities. This model is mechanistic, and predicts the ability of species to interact based on observed distributions of species traits (e.g. body size). These predictions are further refined by taking into account local population densities (e.g. rare species less likely

to interact). This model successfully predicted pairwise species interactions in streams across land use types. Importantly, successfully predicting interactions between species also “scaled up” and accurately predicted the structure of the whole food web.

A further derivation of this model was used to infer the structure and stability of stream food webs using empirical data on communities across an AMD gradient. The model was modified from above in order to predict interaction probabilities, as opposed to link presence/absence. By using interaction probabilities, it is possible to assess how variable trophic interactions within a community affect estimates of stability. Generally, food webs become small and more stable in response to increasing AMD impacts. However, the distribution of the stability metric assessed appears to become bimodal, depending on how interaction strengths are estimated. This has implications on the restoration of streams impacted by AMD, suggesting that some streams may be more easily colonized by extirpated sensitive species post-restoration activities.

Overall, my findings have increased our understandings of the impacts of AMD to stream communities. Furthermore, they support the use of alternative methods, such as size spectra analysis, in biomonitoring surveys. The method developed for inferring food web structure has the potential to allow ecologists to rapidly assess likely food-web structure across large spatial or temporal scales, aiding in the ability to test ecological theories. Finally, the use of probabilistic networks to assess network structure represent an important step in taking into account the inherent variability of species interactions in ecological studies.

Table of Contents

Chapter One: Introduction

Ecological communities are under increasing stress from anthropogenic activities (Rooney and McCann 2012, Thompson et al. 2012). Climate change, land use development, resource extraction, species extinctions and invasions, all affect natural communities, possibly impacting their ability to provide ecological services, and changing their resistance and resilience to future disturbances. Although a wealth of research exists documenting the effects of anthropogenic stressors on community composition (Clements et al. 2000, Hogsden and Harding 2012a, 2012b, Byrne, Reid, and Wood 2013), there is a growing recognition that much of the ecological services and functionality of a community is dependent upon the species interactions within the community, rather than species identity *per se* (Friberg et al. 2011, Gray et al. 2014). Therefore, there is increasing awareness that ecological monitoring and biological assessments need to document interactions between species in order to understand the effects of extant disturbances, as well as predict responses to future changes (Tylianakis et al. 2010, Gray et al. 2014).

An ecological network is a simplified representation of the species and their interactions present within a community. Networks can represent a wide variety of biotic interactions including but not limited to mutualism (e.g. pollination, seed-dispersal), antagonism (e.g. predator-prey, parasite-host) and competition (e.g. soil nutrient limitation in plants; Morales-Castilla et al. 2015). The study of food webs (e.g. antagonistic trophic interactions) has long been a research focus of ecologists (Dunne 2009). However, recent advances in network theory have attempted to increase our understanding of a wide range of topics. Among these the World Wide Web and other complex networks have provided ecologists with a wealth of new tools and methods for use in the study of ecological networks (Poisot, Stouffer, and Kéfi 2016).

Improving our understanding of how ecological networks respond mechanistically to stress and disturbances can elucidate fundamental aspects of ecology, including community assembly, biogeography, coexistence, local stability, and ecosystem functioning (Tylianakis et al. 2010, Rooney and McCann 2012, Thompson et al. 2012, Morales-Castilla et al. 2015, Poisot, Stouffer, and Kéfi 2016). Likewise, it can offer insights to help develop solutions to enable adaptive management in order to maintain ecological services in the face of increasing stress (Layer et al. 2010, Tylianakis et al. 2010, Gray et al. 2014). Another practical advantage would be by understanding functional impairments that are missed by traditional monitoring methods. This would enable us to more effectively remediate sites which are currently impacted.

Food webs - Structure and stability

Understanding community assembly and stability, as well as ecosystem function and process have long been a research focus of ecologists. Community ecologists generally study relationships of biodiversity and species co-occurrences, while ecosystem ecologists focus on macro-scale function and processes. However, food webs offer a powerful framework for incorporating aspects of species richness and biodiversity, as well as the structure, function, and stability of communities (Thompson et al. 2012).

Food webs describe who eats whom by representing species as 'nodes' and the interactions (e.g. predation) as 'links' between nodes. The simplest representations of food webs are as binary networks (e.g. only the presence or absence of a link is represented). The structure of the food web can thus be described with a number of common network measures as described in Bersier, Banašek-Richter & Cattin, (2002). These include network size (S); the number of links (L) between species; linkage density (L/S): connectance, or the proportion of potential links which occur ($C = L / S^2$); generality and vulnerability, which is a measure of mean number of prey per consumer and number of consumer per prey, respectively; number of basal (consume no taxa), intermediate (both consume some taxa and are consumed by some taxa), and top taxa (not consumed by any taxa; Williams and Martinez 2000, Bersier, Banašek-Richter, and Cattin 2002, Thompson et al. 2012).

The function of food webs, and thus the ecosystem services they provide, is known to be influenced by their structure and the strength of the interactions (Strogatz 2001, Pascual and Dunne 2006, Thompson et al. 2012). Sea otters in the pacific northwest of North America are the archetypal example of this trophic cascade. Reduction in sea otters due to the fur trade reduced their top-down predation effects on sea urchins. In response, sea urchin populations increased and effectively reduced the distribution of kelp forests, and limited their formation

(Estes and Palmisano 1974). It is worth noting that these effects occurred with a significant reduction of sea otter populations, and not their complete removal (e.g. a reduction in interaction magnitude). Although this example is often used to illustrate the concept of keystone species, the mechanism underlying these responses is fundamentally that of food web structure.

In freshwater communities, prolonged drought has been observed to alter food-web structure and impact ecosystem functioning (Ledger et al. 2013). Experimental droughts in mesocosms altered the community composition of primary producers, reduced the abundance of large-bodied individuals, and biomass flows shifted to smaller-bodied macroinvertebrates. This resulted in a significant reduction in overall secondary production (Ledger et al. 2013). This can have implications for the management of fisheries which rely on *in situ* prey resources, as well as terrestrial consumers such as birds, bats and lizards which can rely heavily on the seasonal availability of aquatic-terrestrial subsidies (Baxter, Fausch, and Saunders 2005).

The relationship between food-web structure and stability has received much attention (Rooney and McCann 2012). Seminal work by May (1972) using random food web structure and randomly sampled interaction strengths, showed a negative relationship between food web size and local stability. However, Yodzis (1981) rightly criticized this work as being unrealistic, and showed that using empirical food-web structures and plausible interaction strengths led to communities with greater stability. Furthermore, Pimm and Lawton (1978) found that increasing omnivory (feeding on more than one trophic level) tended to decrease stability. These studies suggested a direct link between food-web structure and stability, but the proximal mechanisms organizing the structure of food webs remained elusive and spurred further research.

Briand (1983) studied the structure of 40 empirical food webs and found lower levels of connectance in habitats with large environmental fluctuations, suggesting that abiotic factors placed constraints on food-web structure. Building upon this dataset, Cohen and Briand (1984) found that the total number of links was roughly proportional to the total number of species within a food web, and that the proportion of links between trophic levels (i.e. basal, intermediate, and top) within a food web was proportional to the number of species within each level. Cohen and Newman (1985) proposed the cascade model which organized species hierarchically along some theoretical axis and assigned links based on this hierarchy i.e. a species can only prey upon species below it in the hierarchy. Constructing food webs using the cascade model broadly accounted for the organization of empirical webs, but the identity of the axis remained unclear. Further work by Cohen et al. (1993) found that predators are generally larger than their prey, and that larger predators have a wider diet breadth (e.g. consume a greater range of prey body sizes) than smaller predators. Body size was thus suggested as the physical interpretation of the axis on which the cascade model was ordered (Cohen et al. 1993).

While the structure of food webs has implications on their stability, it is also important to understand the strengths of these interactions (Dunne, Williams, and Martinez 2002, Montoya et al. 2006, Rooney and McCann 2012). Food webs with a few strong interactions embedded within many weak ones are generally more stable than food webs dominated by strong interactions (Neutel, Heesterbeek, and de Ruiter 2002, Tylianakis et al. 2010, Tang, Pawar, and Allesina 2014, Wootton and Stouffer 2015). Weak interaction strengths may distribute perturbations though the community, and dampen oscillations between resources and consumers (McCann, Hastings, and Huxel 1998), which increases the likelihood of the community returning to an equilibrium state (Neutel et al. 2007).

Despite the benefits that food web studies have on our understanding of the function and stability of ecological communities, they are rarely incorporated into large-scale ecological assessments through space or time. This is likely due to the high degree of sampling effort, taxonomic expertise, financial cost, and laboratory time necessary to construct detailed food webs (Thompson and Townsend 2000, Thompson et al. 2001, Gray et al. 2014). Food webs are commonly constructed either from visual identification of gut contents (Jaarsma et al. 1998) or, increasingly, a combination of this and stable isotope analysis (Post 2002). Visual gut content identification generally works well for large predators that swallow their food whole (e.g. Salmonidae). However, for taxa that consume lower trophic levels (e.g. detritus or algae) or masticate, shred, or tear their food, this technique has variable use due to the difficulty of confidently identifying food items. It often leads to the creation of large aggregate food categories such as “detritus”, or “animal”, making it difficult to define the number and structure of food web interactions, or quantify their magnitudes. In order to avoid high degrees of aggregation and construct detailed food webs, it is necessary to analyze the diets of a large number (e.g. many hundreds; Thompson et al. 2001, Layer et al. 2010) of individuals per species. This degree of sampling could impose excessive disturbance on study streams, is problematic when studying endangered or rare species, and may be impossible in impacted streams, due to the paucity of biota (Hogsden and Harding 2012a). Because of the high degree of sampling effort required, food web nodes are often aggregated to higher taxonomic or functional levels (e.g. genus, family, functional feeding groups, trophic guilds; Thompson and Townsend 2000, Dunne, Williams, and Martinez 2002). The ability of highly aggregated food webs to describe ecological function has been equivocal (Thompson and Townsend 2000, Gauzens et al. 2013), making it difficult to compare food webs constructed in this manner. However, as long as food web construction is standardized, and similar levels of aggregation are used, it is possible to compare the results across food webs (Gauzens et al. 2013). Another alternative is to construct “cumulative” or “summary” food webs (Gray et al. 2015). These are constructed by including all pairwise interactions, either directly observed or from published literature, or a combination of the two methods (Havens 1993, Piechnik, Lawler, and Martinez 2008, Layer et al. 2010). The use of summary webs assumes that if in an interaction has ever been observed between two species, that it will always occur whenever the two species co-occur (Gray et al. 2015). This method has been rightly criticized for not being sensitive to subtle changes in food web structure through space and time (Thompson and Townsend 1999, Gray et al. 2015), and conclusions drawn from the use of inferred food web links should be regarded with caution (Layer et al. 2010, Poisot, Stouffer, and Gravel 2015). However, alternative approaches, such as using the abundance and size range of individuals in a community (i.e., a size spectra approach), exist which are easier to measure and vary consistently with food web structure (Woodward et al. 2005, Trebilco et al. 2013). Likewise, recent advances in methods for inferring food web structure with a high degree of certainty (e.g. Gravel et al. 2013, Morales-Castilla et al. 2015, Bartomeus et al. 2016) make this a useful tool for studying food webs across large scales.

Food web alternative: Size spectra

The observation that an organism's density is negatively related to its size has been noted by ecologists for nearly a century (Elton 1927). This pattern holds across all size ranges of organisms observed, from bacteria to blue whales (Sheldon, Prakash, and Sutcliffe 1972, Blanchard et al. 2017), and has proven, empirically, to be one of the most universal patterns of biological organization (e.g. White et al. 2007, Petchey and Belgrano 2010). Furthermore, the relationship between body size and abundance has strong theoretical grounding in the metabolic theory of ecology (Brown et al. 2004). Because many important biological processes (e.g. metabolism, feeding rates, trophic position) correlate with body size, size-spectra relationships can be used to explain numerous aspects of biological organization, from the individual-, to population-, to whole community-levels (O'Gorman et al. 2012). Size spectra studies have aided in our understanding of community function (Dossena et al. 2012), energy transfer (Jennings and Blanchard 2004, Trebilco et al. 2013), and have been implicated in affecting local stability (Tang, Pawar, and Allesina 2014, Sentis, Binzer, and Boukal 2017).

Size spectra are constructed by measuring the body size of all individuals from a given unit of area. However, the sampling methodology used will obviously place constraints on the body sizes collected e.g. mesh size. The body sizes are then usually aggregated in $\log_2$ or $\log_{10}$ width bins (Jennings, Warr, and Mackinson 2002, Jennings and Blanchard 2004, White, Enquist, and Green 2008), and the frequency of observations in each bin is normalized by the width of each bin, respectively (White et al. 2007, White, Enquist, and Green 2008, Sprules and Barth 2015). When this relationship is plotted on log axes, the resulting curve is generally a linear or slightly parabolic dome with a slope coefficient of approximately -1. The slope is related to the efficiency of energy transfer from resources to consumers (O'Gorman et al. 2012, Trebilco et al. 2013), while the intercept generally represents the overall community abundance (O'Gorman et al. 2012, Sprules and Barth 2015). Therefore, the shape of the size spectra relationship has important implications for food web structure and ecological functions.

From an ecological management perspective changes in size spectra have been suggested as an important indicator of ecological health (Reuman et al. 2008, Petchey and Belgrano 2010). Specifically size spectra have been observed to respond to human impacts, including land use

(Martínez et al. 2016), stream warming (e.g. climate change; Dossena et al. 2012, O'Gorman et al. 2012), increased nutrients (Morin, Bourassa, and Cattaneo 2001), and commercial exploitation (Jennings and Blanchard 2004). Because size spectra are taxonomy-free descriptions of a community, they have potential as an assessment tool for novel stressors and impacts, particularly in systems which lack empirical data on taxon-specific sensitivities.

Inferring food web structure

Understanding the mechanisms that determine the ability for species to interact with one another is a fundamental question for ecologists. Knowledge of these mechanisms may allow pairwise species interactions to be scaled up to interpret the structure of whole networks. Early attempts to achieve this ambitious goal included the cascade (Cohen, Newman, and Briand 1985) and niche model (Williams and Martinez 2000) of food webs. The cascade model orders all species along a single niche axis, and assumes hierarchical feeding where each species has a probability of consuming species below it on the niche axis. This model reconstructed empirical food web data reasonably well, but failed to capture some of the natural variability observed in more realistic food webs, including trophic loops (species A eats B, B eats C, and C eats A) and cannibalism (Thompson et al. 2012). The niche model is a slight modification of the cascade model, and assigns a feeding range to each consumer along the axis. This loosens the strict hierarchy of the cascade model, by allowing consumers to predate upon species slightly larger than themselves (allowing for the formation of loops), as well as cannibalism (Williams and Martinez 2000).

The niche axis in both of these models is generally assumed to be body size. Predators are often larger than their prey, and measuring body size allows for the inference of food web structure. Gravel et al. (2013) used empirical data on predator-prey body size pairs from different habitats to develop a method for inferring parameter coefficients of the niche model for a novel community. Their method was shown to accurately recreate empirical food web structure (Gravel et al. 2013), and offers a tractable tool for inferring structure across large scales (Albouy et al. 2014, Tylianakis and Morris 2017).

Although this method performs well for inferring broad food web structure, it fails to incorporate some of the known spatial and temporal variability in species interactions. There is a growing recognition that species interactions are not static, and the observation of species interacting at a given location or time period, does not imply that they will always interact whenever they co-occur (Poisot et al. 2012). It may be possible to incorporate some of this inherent variability by taking into account the local densities of populations within a community (Poisot, Stouffer, and Gravel 2015). Neutral processes (*sensu* Canard et al. 2012, 2014) simply state that rare species are less likely to encounter one another than more locally abundant species. Indeed, empirical network structure has been accurately predicted when only looking at local population densities (Canard et al. 2012). These results prompted the authors to coin the term "neutrally forbidden links", which are simply links that are *possible* between species based on niche processes (e.g. predator-prey body size relationships), but are *unable* to occur locally due the low abundances of the species in question.

Study system: Acid mine drainage in freshwater streams

For my thesis I investigated how stream food webs respond to a specific anthropogenic impact: acid mine drainage (AMD). AMD is a global phenomenon and often the byproduct of coal extraction activities (Kelly et al. 1988), generally caused when geologic strata with high sulphur content is exposed and interacts with water and oxygen to form sulfuric acid (Hogsden and Harding 2012b, Kraus et al. 2014), causing receiving streams to become highly acidic (e.g. pH < 3; Winterbourn 1998, Greig et al. 2010, Hogsden and Harding 2012a). Ore-bearing strata often contain high levels of associated non-target elements (e.g. aluminum, iron, lead, manganese, nickel, and cadmium (Gray and Harding 2012, Kraus et al. 2014), and these can become mobilized and enter freshwater habitats. Low pH of AMD increases the solubility of the associated elements, leading to extremely high concentrations of dissolved metals in receiving waters. While fluctuations in pH caused by droughts, floods and other processes can cause different dissolved metals (e.g. Fe and Al) to precipitate onto the stream bed causing further toxic and habitat loss effects (Harding and Boothroyd 2004).

Research on the effects of AMD on stream communities has received much attention in New Zealand in the last few decades. AMD generally reduces biodiversity and extirpates sensitive taxa at all trophic levels (reviewed in Hogsden & Harding, 2012b), e.g. bacteria (Niyogi, Lewis & Mcknight, 2002), algae (Bray et al. 2008, Schowe, Harding, and Broady 2013), macroinvertebrates (Winterbourn 1998, Clements et al. 2000), and fish (Greig et al. 2010). Toxic effects can be caused by the low acidity, high metal concentrations, or a combination of both (Clements et al. 2000, Greig et al. 2010, Schmidt et al. 2010), although because metal solubility is dependent on pH, disentangling these interactive effects can be difficult. For example, fish were extirpated in naturally occurring acidic streams, but were much more sensitive to elevated metal concentrations (Greig et al. 2010). Taxa which can withstand the abiotic conditions of AMD affected streams often also display a reduction in local densities (Hogsden and Harding 2012b). This leads to small, simple communities dominated by a few tolerant taxa.

Although the effects of AMD on individual species, species diversity and community structure is well known, little work has been conducted on stream food webs affected by AMD. Pioneering work in these systems was conducted by Hogsden & Harding (2012a) and they found small, simple networks with shorter food chains, fewer links, and changes in biomass distribution. However, these results were based on summary food webs (see above) due to the difficulty in obtaining enough individuals per taxa to conduct robust visual identification of gut contents (Hogsden and Harding 2012a). The use of summary food webs does not allow pairwise species interactions to vary between sites, and is less sensitive to subtle changes in food web structure (Gray et al. 2015). Thus, there are still unanswered questions in our understanding of how AMD inputs affect stream food webs, and a need for studies to be conducted on communities with more highly-resolved taxonomic information.

AMD streams offer ideal model systems to test size spectra theory because of their relatively simple food webs and previous research on their communities.

Because of the difficulty in constructing detailed food webs through the identification of visual gut contents I explored three main research questions:

1) Do alternative approaches to assessing food webs, such as size spectra, offer a useful approach for estimating broad changes to food web structure and function in streams?

2) Can current mechanistic models of trophic interactions be expanded upon to allow for the accurate inference of stream food-web structure?

3) What can these new methods tell us about the structure and stability of AMD-impacted stream food webs?

Thesis structure and chapter outlines

The main data chapters in my thesis have been written as a series of three papers (Chapters Two to Four), which have been written as independent manuscripts for publication. As such, there is a degree of repetition of background material and methodology between chapters. Each of these three chapters will be a co-authored publication, however the majority of the work, including field and laboratory work, analysis, model development, and writing, is my own. In the final chapter (Chapter Five), I integrate my findings, discuss limitations and future research in the field.

In Chapter Two, I investigated the utility of using size spectra for assessing the impacts from anthropogenic mining activities on freshwater stream communities. To do this, I sampled stream communities from 25 sites along an AMD gradient on the West Coast of the South Island, New Zealand. All individuals at all sites were measured and their body size estimated using published length-weight regression for New Zealand fauna. Size spectra were constructed for each site and multiple linear regressions were conducted to examine the effects that mining-related stress had on the communities. This chapter consists of an accepted manuscript titled *Anthropogenic mining alters macroinvertebrate size spectra in streams. Freshwater Biology. In press*. This manuscript is co-authored by Helen Warburton and Jon Harding. As lead author of this manuscript, I conducted the field and laboratory work, analyzed the data, wrote the first and final draft of the manuscript and designed all tables and figures. Jon Harding provided advice on study design, Helen Warburton helped with analysis, and both co-authors provided feedback on the manuscript.

In Chapter Three, I used previously published data on empirical food webs from New Zealand (Thompson and Townsend 1999, 2004) which included body size and local abundances, to develop a mechanistic model that accurately inferred the structure – and, importantly, the variability – of food webs in New Zealand streams. To do this, I expanded on the method of Gravel et al. (2013), by incorporating the recommendations of (Poisot, Stouffer, and Gravel 2015). Specifically, I inferred the ability of species to interact based on niche processes, and the likelihood of species to interact based on neutral processes. I compared this model with another recently developed method, "WebBuilder" (Gray et al. 2015), which does not allow species interactions to vary based on local trait distributions or abundances. This chapter consists of an accepted manuscript titled *Inferring predator-prey interactions in food webs. Methods in Ecology and Evolution.* This manuscript is co-authored by Timothée Poisot, Ross Thompson, and Jon Harding. As lead author of this manuscript, I designed the study, analyzed the data, developed the models, wrote the first and final draft of the manuscript and designed all tables and figures. Jon Harding provided advice on study design, Ross Thompson provided data, Timothée Poisot provided critical feedback on an early version of the manuscript and suggested additional analyses, and all co-authors provided feedback on the manuscript.

In Chapter Four, I applied a modification of the food web structure inference method (developed in Chapter Three) to the empirical community data (collected in Chapter Two) to infer the probabilistic structure of food webs across an AMD gradient. In addition to inferring the structure of food webs, I also estimated community stability. I found that stability increases across the mining gradient, and discuss how these findings should be incorporated for future remediation activities. This chapter consists of a manuscript currently in preparation for submission to the journal of *Ecology*. As lead author of this manuscript, I designed the study, analyzed the data, wrote the first and final draft of the manuscript and designed all tables and figures. Jon Harding provided advice on study design and feedback on the manuscript.

In Chapter Five, I briefly outline the findings from individual chapters, and discuss their implications for future research on food webs across environmental gradients. I describe potential benefits of my findings to a wider ecological audience, as well as discuss some limitations of these methods.

References

Albouy, C., L. Velez, M. Coll, F. Colloca, F. L. Loc'h, D. Mouillot, and D. Gravel. 2014. From projected species distribution to food-web structure under climate change. Global Change Biology **20**:730–741. https://doi.org/10.1111/gcb.12467.

Bartomeus, I., D. Gravel, J. M. Tylianakis, M. A. Aizen, I. A. Dickie, and M. Bernard-Verdier. 2016. A common framework for identifying linkage rules across different types of interactions. Functional Ecology **30**:1894–1903. https://doi.org/10.1111/1365-2435.12666.

Baxter, C. V., K. D. Fausch, and C. W. Saunders. 2005. Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. Freshwater Biology **50**:201–220. https://doi.org/10.1111/j.1365-2427.2004.01328.x.

Bersier, L.-F., C. Banašek-Richter, and M.-F. Cattin. 2002. Quantitative descriptors of food-web matrices. Ecology **83**:2394–2407. https://doi.org/10.2307/3071801.

Blanchard, J. L., R. F. Heneghan, J. D. Everett, R. Trebilco, and A. J. Richardson. 2017. From bacteria to whales: using functional size spectra to model marine ecosystems. Trends in Ecology & Evolution **32**:174–186. https://doi.org/10.1016/j.tree.2016.12.003.

Bray, J. P., P. A. Broady, D. K. Niyogi, and J. S. Harding. 2008. Periphyton communities in New Zealand streams impacted by acid mine drainage. Marine and Freshwater Research **59**:1084–1091.

Briand, F. (1983), Environmental Control of Food Web Structure. Ecology, **64**: 253-263. https://doi.org/10.2307/1937073

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. Ecology **85**:1771–1789. https://doi.org/10.1890/03-9000.

Byrne, P., I. Reid, and P. J. Wood. 2013. Changes in macroinvertebrate community structure provide evidence of neutral mine drainage impacts. Environmental Science Processes & Impacts **15**:393–404. https://doi.org/10.1039/c2em30447c.

Canard, E., N. Mouquet, L. Marescot, K. J. Gaston, D. Gravel, and D. Mouillot. 2012. Emergence of structural patterns in neutral trophic networks. PLOS ONE **7**:e38295. https://doi.org/10.1371/journal.pone.0038295.

Canard, E., N. Mouquet, D. Mouillot, M. Stanko, D. Miklisova, and D. Gravel. 2014. Empirical evaluation of neutral interactions in host-parasite networks. The American Naturalist **183**:468–479. https://doi.org/10.1086/675363.

Clements, W. H., D. M. Carlisle, J. M. Lazorchak, and P. C. Johnson. 2000. Heavy metals structure benthic communities in Colorado mountain streams. Ecological Applications **10**:626–638.

Cohen, J. E., and F. Briand. 1984. Trophic links of community food webs. Proceedings of the National Academy of Sciences **81**:4105–4109.

Cohen, J. E., and C. M. Newman. 1985. A stochastic theory of community food webs. I. Models and aggregated data. Proceedings of the Royal Society B **224**:421–448.

Cohen, J. E., C. M. Newman, and F. Briand. 1985. A stochastic theory of community food webs. II. Individual webs. Proceedings of the Royal Society B **224**:449–461.

Cohen, J. E., S. L. Pimm, P. Yodzis, and J. Saldaña. 1993. Body Sizes of Animal Predators and Animal Prey in Food Webs. Journal of Animal Ecology **62**:67–78. https://doi.org/10.2307/5483.

Dossena, M., G. Yvon-Durocher, J. Grey, J. M. Montoya, D. M. Perkins, M. Trimmer, and G. Woodward. 2012. Warming alters community size structure and ecosystem functioning. Proceedings of the Royal Society B **279**:3011–3019. https://doi.org/10.1098/rspb.2012.0394.

Dunne, J. 2009. Food webs. Pages 3661–3682 *in* R. A. Meyers, editor. Encyclopedia of complexity and systems science. Springer, New York.

Dunne, J. A., R. J. Williams, and N. D. Martinez. 2002. Network structure and biodiversity loss in food webs: robustness increase with connectance. Ecology Letters **5**:558–567. https://doi.org/10.1046/j.1461-0248.2002.00354.x.

Elton, C. S. 1927. Animal Ecology. Macmillan Co., New York, NY.

Estes, J. A., and J. F. Palmisano. 1974. Sea otters: their role in structuring nearshore communities. Science **185**:1058–1060.

Friberg, N., N. Bonada, D. C. Bradley, M. J. Dunbar, F. K. Edwards, J. Grey, R. B. Hayes, A. G. Hildrew, N. Lamouroux, M. Trimmer, and G. Woodward. 2011. Biomonitoring of human impacts in freshwater ecosystems: the good, the bad and the ugly. Advances in Ecological Research **44**:1–68. https://doi.org/Friberg, Nikolai; Bonada, Nuria; Bradley, David C.; Dunbar, Michael J.; Edwards, Francois K.; Grey, Jonathan; Hayes, Richard B.; Hildrew, Alan G.; Lamouroux, Nicolas; Trimmer, Mark; Woodward, Guy. 2011 Biomonitoring of human impacts in freshwater ecosystems: the good, the bad and the ugly. Advances in Ecological Research, 44. 1-68. https://doi.org/10.1016/B978-0-12-374794-5.00001-8 <https://doi.org/10.1016/B978-0-12-374794-5.00001-8>.

Gauzens, B., S. Legendre, X. Lazzaro, and G. Lacroix. 2013. Food-web aggregation, methodological and functional issues **122**:1606–1615. https://doi.org/10.1111/j.1600-0706.2013.00266.x.

Gravel, D., T. Poisot, C. Albouy, L. Velez, and D. Mouillot. 2013. Inferring food web structure from predator–prey body size relationships. Methods in Ecology and Evolution **4**:1083–1090. https://doi.org/10.1111/2041-210X.12103.

Gray, C., D. J. Baird, S. Baumgartner, U. Jacob, G. B. Jenkins, E. J. O'Gorman, X. Lu, A. Ma, M. J. O. Pocock, N. Schuwirth, M. Thompson, and G. Woodward. 2014. Ecological networks: the missing links in biomonitoring science. Journal of Applied Ecology **51**:1444–1449. https://doi.org/10.1111/1365-2664.12300.

Gray, C., D. Figueroa, L. Hudson, A. Ma, D. Perkins, and G. Woodward. 2015. Joining the dots: an automated method for constructing food webs from compendia of published interactions. Food Webs **5**:11–20. http://dx.doi.org/10.1016/j.fooweb.2015.09.001.

Gray, D. P., and J. S. Harding. 2012. Acid Mine Drainage Index (AMDI): a benthic invertebrate biotic index for assessing coal mining impacts in New Zealand streams. New Zealand Journal of Marine and Freshwater Research **46**:335–352. https://doi.org/10.1080/00288330.2012.663764.

Greig, H. S., D. K. Niyogi, K. L. Hogsden, P. G. Jellyman, and J. S. Harding. 2010. Heavy metals: confounding factors in the response of New Zealand freshwater fish assemblages to natural and anthropogenic acidity. Science of the Total Environment **408**:3240–3250. https://doi.org/10.1016/j.scitotenv.2010.04.006.

Harding, J. S., and I. K. G. Boothroyd. 2004. Impacts of mining. Pages 36.1-36.10 *in* J. S. Harding, P. Mosley, C. Pearson, and B. Sorrell, editors. Freshwaters of New Zealand. The Caxton Press, Christchurch, New Zealand.

Havens, K. E. 1993. Predator-prey relationships in natural community food webs. Oikos **68**:117–124. https://doi.org/10.2307/3545316.

Hogsden, K. L., and J. S. Harding. 2012a. Anthropogenic and natural sources of acidity and metals and their influence on the structure of stream food webs. Environmental Pollution **162**:466–474. https://doi.org/10.1016/j.envpol.2011.10.024.

Hogsden, K. L., and J. S. Harding. 2012b. Consequences of acid mine drainage for the structure and function of benthic stream communities: a review. Freshwater Science **31**:108–120. https://doi.org/10.1899/11-091.1.

Jaarsma, N. G., S. M. de Boer, C. R. Townsend, R. M. Thompson, and E. D. Edwards. 1998. Characterising food-webs in two New Zealand streams. New Zealand Journal of Marine and Freshwater Research **32**:271–286.

Jennings, S., and J. L. Blanchard. 2004. Fish abundance with no fishing: predictions based on macroecological theory. Journal of Animal Ecology **73**:632–642.

Jennings, S., K. J. Warr, and S. Mackinson. 2002. Use of size-based production and stable isotope analyses to predict trophic transfer efficiencies and predator-prey body mass ratios in food webs. Marine Ecology Progress Series **240**:11–20.

Kelly, M., W. J. Allison, A. R. Garman, and C. J. Symon. 1988. Mining and the Freshwater Environment. Springer Netherlands, Netherlands.

Kraus, J. M., T. S. Schmidt, D. M. Walters, R. B. Wanty, R. E. Zuellig, and R. E. Wolf. 2014. Cross-ecosystem impacts of stream pollution reduce resource and contaminant flux to riparian food webs. Ecological Applications **24**:235–243.

Layer, K., J. O. Riede, A. G. Hildrew, and G. Woodward. 2010. Food web structure and stability in 20 streams across a wide pH gradient. Advances in Ecological Research **42**:265–299.

Ledger, M. E., L. E. Brown, F. K. Edwards, L. N. Hudson, A. M. Milner, and G. Woodward. 2013. Extreme climatic events alter aquatic food webs: a synthesis of evidence from a mesocosm drought experiment. Advances in Ecological Research **48**:343–395. https://doi.org/10.1016/B978-0-12-417199-2.00006-9.

Martínez, A., A. Larrañaga, A. Miguélez, G. Yvon-Durocher, and J. Pozo. 2016. Land use change affects macroinvertebrate community size spectrum in streams: the case of Pinus radiata plantations. Freshwater Biology **61**:69–79. https://doi.org/10.1111/fwb.12680.

May, R. M. 1972. Will a large complex system be stable? Nature **238**:413–414. https://doi.org/10.1038/238413a0.

Montoya, J. M., and R. V. Solé. 2002. Small world patterns in food webs. Journal of Theoretical Biology **214**:405–412. https://doi.org/10.1006/jtbi.2001.2460.

Montoya, M., S. L. Pimm, R. V. Sole, J. M. Montoya, S. L. Pimm, and R. V. Solé. 2006. Ecological networks and their fragility. Nature **442**:259–264. https://doi.org/10.1038/nature04927.

Morales-Castilla, I., M. G. Matias, D. Gravel, and M. B. Arau. 2015. Inferring biotic interactions from proxies. Trends in Ecology & Evolution **30**:347–356. https://doi.org/10.1016/j.tree.2015.03.014.

Morin, A., N. Bourassa, and A. Cattaneo. 2001. Use of size spectra and empirical models to evaluate trophic relationships in streams. Limnology and Oceanography **46**:935–940. https://doi.org/10.4319/lo.2001.46.4.0935.

Neutel, A., J. A. P. Heesterbeek, and P. C. de Ruiter. 2002. Stability in real food webs: weak links in long loops. Science **296**:1120–1124.

Niyogi, D. K., W. M. Lewis, and D. M. Mcknight. 2002. Effects of stress from mine drainage on diversity, biomass, and function of primary producers in mountain streams. Ecosystems:554–567. https://doi.org/10.1007/s10021-002-0182-9.

O'Gorman, E. J., D. E. Pichler, G. Adams, J. P. Benstead, H. Cohen, N. Craig, W. F. Cross, B. O. L. Demars, N. Friberg, G. M. Gíslason, R. Gudmundsdóttir, A. Hawczak, J. M. Hood, L. N. Hudson, L. Johansson, M. P. Johansson, J. R. Junker, A. Laurila, J. R. Manson, E. Mavromati, D. Nelson, J. S. ólafsson, D. M. Perkins, O. L. Petchey, M. Plebani, D. C. Reuman, B. C. Rall, R. Stewart, M. S. A. Thompson, and G. Woodward. 2012. Impacts of warming on the structure and functioning of aquatic communities. Individual- to ecosystem-level responses. Advances in Ecological Research **47**:81–176. https://doi.org/10.1016/B978-0-12-398315-2.00002-8.

Pascual, M., and J. A. Dunne. 2006. From small to large ecological networks in a dynamic world. Pages 3–24 *in* M. Pascual and J. Dunne, editors. Ecological Networks: Linking Structure to Dynamics in Food Webs. Oxford University Press, New York.

Petchey, O. L., and A. Belgrano. 2010. Body-size distributions and size-spectra: universal indicators of ecological status? Biology Letters **6**:434–437. https://doi.org/10.1098/rsbl.2010.0240.

Piechnik, D. A., S. P. Lawler, and N. D. Martinez. 2008. Food-web assembly during a classic biogeographic study: species' "trophic breadth" corresponds to colonization order. Oikos **117**:665–674. https://doi.org/10.1111/j.0030-1299.2008.15915.x.

Pimm, S. L., and J. H. Lawton. 1978. On feeding on more than one trophic level. Nature **275**:542–544. https://doi.org/10.1038/275542a0.

Poisot, T., E. Canard, D. Mouillot, N. Mouquet, and D. Gravel. 2012. The dissimilarity of species interaction networks. Ecology Letters **15**:1353–1361. https://doi.org/10.1111/ele.12002.

Poisot, T., D. B. Stouffer, and D. Gravel. 2015. Beyond species: why ecological interaction networks vary through space and time. Oikos **124**:243–251. https://doi.org/10.1111/oik.01719.

Poisot, T., D. B. Stouffer, and S. Kéfi. 2016. Describe, understand and predict: why do we need networks in ecology? Functional Ecology **30**:1878–1882.

Post, D. M. 2002. The long and short of food-chain length. Trends in Ecology & Evolution **17**:269–277.

Reuman, D. C., C. Mulder, D. Raffaelli, and J. E. Cohen. 2008. Three allometric relations of population density to body mass: theoretical integration and empirical tests in 149 food webs. Ecology Letters **11**:1216–1228. https://doi.org/10.1111/j.1461-0248.2008.01236.x.

Rooney, N., and K. S. McCann. 2012. Integrating food web diversity, structure and stability. Trends in Ecology & Evolution **27**:40–46. https://doi.org/10.1016/j.tree.2011.09.001.

Schmidt, T. S., W. H. Clements, K. A. Mitchell, S. E. Church, R. B. Wanty, D. L. Fey, P. L. Verplanck, and C. A. San Juan. 2010. Development of a new toxic-unit model for the bioassessment of metals in streams. Environmental Toxicology and Chemistry **29**:2432–2442. https://doi.org/10.1002/etc.302.

Schowe, K. A., J. S. Harding, and P. A. Broady. 2013. Diatom community response to an acid mine drainage gradient. Hydrobiologia **705**:147–158. https://doi.org/10.1007/s10750-012-1391-7.

Sentis, A., A. Binzer, and D. S. Boukal. 2017. Temperature-size responses alter food chain persistence across environmental gradients. Ecology Letters **20**:852–862. https://doi.org/10.1111/ele.12779.

Sheldon, R. W., A. Prakash, and W. H. Sutcliffe. 1972. The size distribution of particles in the ocean. Limnology and Oceanography **17**:327–340. https://doi.org/10.4319/lo.1972.17.3.0327.

Sprules, W. G., and L. E. Barth. 2015. Surfing the biomass size spectrum: some remarks on history, theory, and application. Canadian Journal of Fisheries and Aquatic Sciences **73**:477–495.

Strogatz, S. H. 2001. Exploring complex networks. Nature **410**:268–276. https://doi.org/10.1038/35065725.

Tang, S., S. Pawar, and S. Allesina. 2014. Correlation between interaction strengths drives stability in large ecological networks. Ecology Letters **17**:1094–1100. https://doi.org/10.1111/ele.12312.

Thompson, R. M., U. Brose, J. A. Dunne, R. O. H. Jr, S. Hladyz, R. L. Kitching, N. D. Martinez, H. Rantala, T. N. Romanuk, D. B. Stouffer, and J. M. Tylianakis. 2012. Food webs: reconciling the structure and function of biodiversity. Trends in Ecology & Evolution **27**:689–697. https://doi.org/10.1016/j.tree.2012.08.005.

Thompson, R. M., E. D. Edwards, A. R. McIntosh, and C. R. Townsend. 2001. Allocation of effort in stream food-web studies: the best compromise? Marine and Freshwater Research **52**:339–345.

Thompson, R. M., and C. R. Townsend. 1999. The effect of seasonal variation on the community structure and food-web attributes of two streams: implications for food-web science. Oikos **87**:75–88.

Thompson, R. M., and C. R. Townsend. 2000. Is resolution the solution?: the effect of taxonomic resolution on the calculated properties of three stream food webs. Freshwater Biology **44**:413–422.

Thompson, R. M., and C. R. Townsend. 2004. Land-use influences on New Zealand stream communities: Effects on species composition, functional organisation, and food-web structure. New Zealand Journal of Marine and Freshwater Research **38**:595–608. https://doi.org/10.1080/00288330.2004.9517265.

Trebilco, R., J. K. Baum, A. K. Salomon, and N. K. Dulvy. 2013. Ecosystem ecology: size-based constraints on the pyramids of life. Trends in Ecology & Evolution **28**:423–431. https://doi.org/10.1016/j.tree.2013.03.008.

Tylianakis, J. M., E. Laliberté, A. Nielsen, and J. Bascompte. 2010. Conservation of species interaction networks. Biological Conservation **143**:2270–2279. https://doi.org/10.1016/j.biocon.2009.12.004.

Tylianakis, J. M., and R. J. Morris. 2017. Ecological networks across environmental gradients. Annual Review of Ecology, Evolution, and Systematics **48**:25–48.

White, E. P., B. J. Enquist, and J. L. Green. 2008. On estimating the exponent of power-law frequency distributions. Ecology **89**:905–912.

White, E. P., S. K. M. Ernest, A. J. Kerkhoff, and B. J. Enquist. 2007. Relationships between body size and abundance in ecology. Trends in Ecology & Evolution **22**:323–330. https://doi.org/10.1016/j.tree.2007.03.007.

Williams, R. J., and N. D. Martinez. 2000. Simple rules yield complex food webs. Nature **404**:180–183. https://doi.org/10.1038/35004572.

Winterbourn, M. J. 1998. Insect faunas of acidic coal mine drainages in Westland, New Zealand. New Zealand Entomologist **21**:65–72.

Woodward, G., B. Ebenman, M. Emmerson, J. M. Montoya, J. M. Olesen, A. Valido, and P. H. Warren. 2005. Body size in ecological networks. Trends in Ecology & Evolution **7**:402–409. https://doi.org/10.1016/j.tree.2005.04.005.

Wootton, K. L., and D. B. Stouffer. 2015. Many weak interactions and few strong; food-web feasibility depends on the combination of the strength of species' interactions and their correct arrangement. Theoretical Ecology **9**:185–195. https://doi.org/10.1007/s12080-015-0279-3.

Yodzis, P. 1981. The stability of real ecoystems. Nature **289**:674–676.

Chapter Two: Anthropogenic mining alters macroinvertebrate size spectra in streams

Preface

This chapter consists of an accepted manuscript titled *Anthropogenic mining alters macroinvertebrate size spectra in streams. Freshwater Biology. In press*. This manuscript is co-authored by Helen Warburton and Jon Harding. As lead author of this manuscript, I conducted the field and laboratory work, analyzed the data, wrote the first and final draft of the manuscript and designed all tables and figures. Jon Harding funded this research, and provided advice on study design, Helen Warburton helped with analysis, and both co-authors provided feedback on the manuscript.

Abstract

Food web properties can to be used in bioassessment as indicators of ecosystem stress, although logistical constraints restrict their widespread use. Size spectra (body mass – abundance relationships) are easier to produce, still incorporate much of the variation in feeding interactions and indicate the strength of the energy transfer efficiency. Here we examined the effect of acid mine drainage on the size spectra of stream macroinvertebrate communities in 25 New-Zealand streams with a comparative survey. We predicted that the largest sized organisms would be most susceptible to acid mine drainage, leading to a reduction in their abundances and associated decrease in the range of body sizes present across the gradient, as well as a reduction in total community abundance. The largest sized organisms were more sensitive to inputs of acid mine drainage, and were absent at the most affected sites. Surprisingly, the smallest body sizes were also removed by acid mine drainage. This led to a reduction of up to two orders of magnitude in the range of body sizes present in mine impacted sites. Total community abundance decreased along the impact gradient. The changes in size spectra were also associated with changes in the proportion of functional feeding groups, suggesting concomitant changes in food web structure. Specifically, communities became dominated by collector browsers and small bodied predators across the gradient. The

simplification of the food web structure suggests that communities may be dominated by a few strong energy pathways, lowering their functionality and stability. However, the loss of large bodied predators also reduces top down pressure, likely increasing community stability. Further research is needed to elucidate the cumulative effects of these interacting processes.

Introduction

The observation that the density of organisms is negatively related to their body size was noted nearly a century ago (Elton 1927). Since that time, this concept has proven empirically to be one of the most universal aspects of biological organization (White et al. 2007, Petchey and Belgrano 2010), linking individual- and population-level traits with community structure (O'Gorman et al. 2012), function (Dossena et al. 2012), energy transfer (Jennings and Blanchard 2004, Trebilco et al. 2013), and stability (Tang, Pawar, and Allesina 2014, Sentis, Binzer, and Boukal 2017). Furthermore, size spectra relationship have strong theoretical grounding in the metabolic theory of ecology (Brown et al. 2004). When all of the individuals from a given unit of area are measured, the relationship of their log density to their log body mass is generally negative, with a linear or slightly parabolic relationship (Sprules and Barth 2015, Martínez et al. 2016). The slope of this relationship is related to the efficiency of energy transfer from resources to consumers (e.g. from small, abundant individuals to rarer large individuals in predator-prey systems), while the intercept generally represents the overall community abundance (O'Gorman et al. 2012, Sprules and Barth 2015). Therefore, the shape of the size spectra relationship at a site has important implications for food web structure and ecological functions. Changes in size spectra have been suggested as an important indicator of ecological health (Reuman et al. 2008, Petchey and Belgrano 2010) and have been observed to respond to human impacts, including land use (Martínez et al. 2016), stream warming (O'Gorman et al. 2012), increased nutrients (Morin, Bourassa, and Cattaneo 2001), and commercial exploitation (Jennings and Blanchard 2004). Because size spectra are independent of taxa identity, they have potential utility as an assessment tool for novel stressors and impacts, particularly in systems which lack empirical data on taxon-specific sensitivities. In the face of contemporary challenges, including climate change, and the increasing presence of synthetic chemicals in the environment (Bernhardt, Rosi, and Gessner 2017), understanding how size spectra respond to environmental impacts is a timely question for ecologists, and here we assess their use in acid mine drainage impacted streams.

Acid mine drainage is a multi-factor stressor (Gray 1997) affecting freshwater habitats globally (Hogsden and Harding 2012a). Acid mine drainage (hereafter AMD) is often associated with mining activities in geologic strata with high Sulphur content and high levels of associated heavy metals, including Fe, Al, Zn, and Ni (Hogsden, Webster-Brown, and Harding 2016). Excavated rock is exposed to air and water, resulting in sulphuric acid, which can significantly reduce the pH, and this increases the solubility of metal ions in the strata. Although receiving streams generally have higher pH and can neutralize the acidity in AMD, waters downstream of the source can remain very acidic ($pH < 3$) and have high levels of total dissolved metals (Hogsden and Harding 2012b). With the input of additional un-impacted tributaries, there is generally an increase in pH, which causes the solubility of the metals to decrease, resulting in metal precipitating onto the benthos causing the commonly observed “yellow boy” in AMD impacted streams. AMD has been shown to strongly affect community composition (Winterbourn 1998, Greig et al. 2010, Hogsden and Harding 2012b, Underwood, Kruse, and Bowman 2014, Gangloff et al. 2015), and simplify food web structure (Hogsden and Harding 2012a). However, to our knowledge, no study on the distribution of individual body mass has been conducted in mining impacted streams.

We assessed the impacts to size spectra in streams across an AMD gradient. We expected that the conditions of increasing AMD impacts in streams would impact size spectra following three hypotheses.
(1) The loss of individuals from environmental perturbations is rarely random, and the largest organisms in a system are generally more susceptible to local extinctions due to their increased energetic demands, larger home ranges, slower reproductive rates, and lower densities (Brown et al. 2004, Woodward et al. 2012, Brose et al. 2016). Reduction in the density of the largest individuals should lead to steeper slopes of size spectra in response to the AMD gradient.

(2) The intercept of size spectra represents the density of the average body size within the community, and is dependent on total community abundance. Previous work has shown significant reductions in benthic community abundances in response to AMD. Therefore, we predict a reduction in the intercepts across the gradient.
(3) Aquatic food webs are often size-structured, with larger individuals generally representing higher trophic levels. Additionally, because the largest taxa within a community generally occupy several body sizes and subsequent ontogenetic changes in both its predators and preys throughout its life history, these taxa are often the most well-connected (Brose et al. 2016).

Thus, the range of body sizes present at a site has implications on food-web structure and trophic height. We expect that the magnitude of the reduction in the density of the largest sized individuals (hypothesis 1) will increase across the mining gradient and will result in the complete loss of the largest size classes at the most heavily impacted sites (e.g. reduced range of sizes present). Note that this differs from (1) in that slopes are a proxy for transfer efficiency, while the range of body sizes present is a proxy for the presence of different trophic levels. If our hypotheses 1 and 2 are correct, this has strong implications in the structure and function of the food web present in these streams. As a coarse proxy for food web structure, we examined the proportion of individuals belonging to functional feeding groups (FFGs) across the AMD gradient. Different FFGs specialize on different food sources within the environment, and represent different energy pathways within the community.

Methods

Study site and stream characteristics

This study was conducted in the Buller-Grey region in the north-west of the South Island, New Zealand. The region has a long history of coalmining, and is part of the Westland Forest ecoregion, which has spatially consistent climatic conditions, geology, and freshwater biota (Harding & Winterbourn, 1997; Harding, Winterbourn & McDiffett, 1997). A total of 25 streams were sampled, thirteen streams were sampled along an AMD gradient (which we refer to as “impacted” streams) based on known and relatively constant water chemistry (e.g., pH, conductivity, dissolved Al and Fe concentrations) over time (Winterbourn et al. 2000, Greig et al. 2010, Hogsden and Harding 2012a, Kitto et al. 2015). In addition, we sampled twelve streams across a natural gradient of pH (~ 4 – 7) and low metal concentrations, in order to capture the range of natural variation. To our knowledge, these twelve streams are "un-impacted" by AMD inputs. All twenty five streams were placed into a single gradient (see below) and analyzed together. However, we occasionally discuss the differences between the AMD-impacted and un-impacted streams in order to place our results into context. No statistical analyses treated un-impacted and AMD-impacted streams as a categorical predictor. All streams were chosen to be as similar as possible with respect to other physical parameters, and were in relatively isolated catchments dominated by native vegetation. All sampling occurred during January – February 2016 (Austral summer). Stream water pH, specific conductivity, dissolved oxygen and temperature were measured in the field using standard meters (YSI 550A & YSI 63, YSI Environmental Incorporated, Ohio, USA). Random water samples (50 ml) collected for analysis of dissolved metal concentrations were filtered in the field (0.45 µm mixed cellulose ester filter) and acidified (pH < 2) using ultrapure nitric acid. Samples were analyzed for metals using inductively coupled plasma mass spectrometry (ICP-MS) at the University of Canterbury.

Mining gradient

Water chemistry variables (Supplementary Table 1) were combined into an AMD gradient using principal components analysis (PCA), using the R function *prcomp* in the stats package (R Development Core Team 2017). Dissolved metal concentrations and conductivity were $\log_{10}$-transformed (x +1) to satisfy assumptions of normality. All variables in the resemblance matrix were centered at 0 and scaled by their standard deviations before the PCA was conducted. The importance of the original variables to the PCA axes were determined using Pearson correlations. Site scores for PC axis 1 were extracted and used as a proxy for the AMD gradient in all further analyses.

Community sampling and body mass estimation

Benthic macroinvertebrates were randomly collected in three Surber samples (0.06 m^2, 0.25 mm mesh) from riffle habitats at each site (Blakely and Harding 2005). All samples were taken by the same person and with similar effort. Sample replication was a trade-off between the number of samples, the number of sites, and the processing and measurement of all individuals. Aquatic insect life histories in New Zealand are not characterized by strong seasonality (Winterbourn, Rounick, and Cowie 1981). Emergence rates for some taxa are higher in summer, but numerous studies have shown a general lack of synchronized emergence, and a wide range of size classes and cohorts of a specific species are often observed together in New Zealand streams (Devonport and Winterbourn 1976, Winterbourn, Rounick, and Cowie 1981, Drummond, Mcintosh, and Larned 2015). Therefore, we assume that there were no strong effects of emergence or life history that could affect our findings. Samples were preserved with 100% ethanol in the field and returned to the laboratory for processing. Macroinvertebrates were separated and identified to the lowest practical taxonomic level (i.e. genus for mayflies, stoneflies and caddisflies, subfamily for Chironomidae larvae and family for other true flies and beetles, order for non-insect taxa) according to (Winterbourn, Gregson, and Dolphin 2006), and unpublished keys (NIWA, Hamilton, New Zealand). Photos were taken of all individuals (~1-100 individuals per photo) using a Leica DFC295 digital camera mounted to a Leica model M125 microscope. All individuals were then measured to the nearest 0.1mm using the software package Adobe Acrobat 9 Pro (San Jose, California, USA). Measurements were conducted according to the methods of (Towers, Henderson, and Veltman 1994, Stoffels, Karbe, and Paterson 2003). Briefly, individuals were generally measured from the anterior portion of the head to the last abdominal segment (not including cerci). Caddisflies with portable cases had the maximum length of the case recorded, except Helicopsychidae which were measured at the widest portion of the case. Finally, for the snail *Potamopyrgus antipodarum*, the shell height was measured. Because accurately sampling the smallest individuals is difficult, individuals ≤ 0.5 mm were removed from the data. Body lengths were converted to dry weight estimates (grams) using published taxon-specific length-weight regressions for New Zealand invertebrate fauna (Towers, Henderson, and Veltman 1994, Stoffels, Karbe, and Paterson 2003).

Size spectra

Body-mass abundance relationships were assembled for all streams using the individual size distribution described by White et al. (2007). In each of the 25 streams, in order to get the best estimate of size spectra at each site, all individuals were combined and grouped into bins of equal logarithmic width (White, Enquist & Green, 2008). We compare the use of the two most commonly used widths, $\log_2$ and $\log_{10}$ (Jennings, Warr, and Mackinson 2002, Jennings and Blanchard 2004), in the Supplementary Table S2, and compare the number of bins for each in Fig. S1 and S2. Eighteen bins of $\log_2$ width were chosen in order to encompass the full range of body sizes present at all sites, while still allowing for robust regressions (Supplemental Material). Abundances were normalized by dividing the frequency in a bin by the linear width of the bin (White et al. 2007, White, Enquist, and Green 2008, Sprules and Barth 2015). Size spectra were plotted as $\log_{10}$ N ~ $\log_{10}$ M where $\log_{10}$ M is the midpoint of the $\log_{10}$ M class, converted from $\log_2$ (Jennings and Blanchard 2004).

The slope and y-intercept of size spectra have been shown to be correlated (Gómez-Canchong, Blanco, and Quiñones 2013). Therefore, to make these response variables independent, the data from each stream were standardized to have the size range centered at $x = 0$, and report the y-intercept of the centered data as the community height (Blanchard et al. 2005, Sprules and Barth 2015). This makes the slope and height parameters independent, with the former being more dependent on the relative abundance of small and large individuals, and the latter being more dependent on the total abundance within the community (Sprules and Barth 2015). Each stream dataset was centered by subtracting the value of the mid-point bin from all bins within that stream, respectively.

Previous New Zealand studies have documented that fish are extremely sensitive to AMD inputs (Greig et al. 2010, Hogsden and Harding 2012b) and in the present study were not found at any sites receiving AMD. Additionally, of the 12 streams that were un-impacted by AMD, 6 were naturally fishless. The presence of top predators can have impacts on biomass distributions of lower trophic levels, and can change the shape of the size spectra relationship (Brose et al. 2016). Since the present study is focused on the macroinvertebrate community size spectra response to AMD, we conducted preliminary analyses to test whether the presence of fish affected size spectra relationship of the macroinvertebrate community at the un-impacted sites. The presence of fish did not significantly impact either the slope or intercept of macroinvertebrate size spectra in un-impacted streams (Supplemental Material, Fig. S3). Because fish are orders of magnitude larger than the majority of macroinvertebrates, and their absence is not wholly dependent on AMD inputs, their inclusion in the construction of size spectra represents a potential confounding factor. Thus, only the macroinvertebrates were used to construct size spectra for all sites, in all further analyses.

Statistical analyses

Size spectra relationships are generally repeating negative parabolic domes, which only take on a linear relationship when sampling is performed across a large range of body sizes. Therefore, when focusing on a limited range of body sizes, as we do here, it is important to test both linear and quadratic models in order to maximize the amount of variation explained (Sprules and Barth 2015). We selected the best fitting model using Akaike's information criteria corrected for small sample sizes (AIC_c; Burnham and Anderson 2004). We found that the quadratic relationship best explained the variation among sites ($\Delta AIC_c << 2$). Furthermore, the conclusions reached using linear models were similar to those of the quadratic models (Supplemental Material). The results of the quadratic models are presented throughout the rest of this paper.

To test for changes in size spectra across the mining gradient, we fitted linear regressions to the global dataset testing for significant effects of the predictors (independent variables) M, M^2, PC1 and for interactions between M:PC1 and M^2:PC1. The global model was in the form of:

$$Log_{10} N = \beta_0 + \beta_1 Log_{10} M + \beta_2 Log_{10} M^2 + \beta_3 PC1 + \beta_4 PC1 Log_{10} M + \beta_5 PC1 Log_{10} M^2 + \epsilon$$

where N is the normalized abundance of individuals, M is the estimated body mass, PC1 (e.g. the mining gradient) are the site scores on the first axis determined by the PCA (see above), and ϵ is the residual error term. We then calculated the AIC_c of the global model as well as AIC_c for simplified models using the R function *dredge* in the package MuMIn (Bartoń 2016). Simplified models were obtained by systematically testing all possible terms and interactions. Models were assessed and ranked according to ΔAIC_c, and only models with $\Delta AIC_c < 2$ were retained (Table 1; Burnham and Anderson 2004). A significant coefficient for the PC1 variable (e.g. β_3 in the equation above) would indicate that the intercepts do respond to the mining gradient. Likewise, a significant coefficient for the M:PC1 and M^2:PC1 interaction term (e.g. β_4 and β_4) would indicate that the slope, and magnitude of curvature, respectively, varies in responds to the mining gradient.

To test hypothesis (3) above we assessed the range of body sizes present within communities. The range of body size were calculated as $M_{Range} = \max(\log_{10} M) - \min(\log_{10} M)$ where M is the estimated individual dry weight, in grams, within a site. Ranges were $\log_{10}$ transformed, and a linear regression was performed, with the PC1 loadings (e.g. the mining gradient) as the independent variable. In order to investigate whether changes in M_{Range} were driven by changes in the presence of the smallest or largest sized organisms, we performed quantile regression ($\tau = 0.05$ and 0.95, respectively) on the range of M present across the mining gradient. Confidence intervals for quantile slope parameters were calculated by inverting a quantile rank-score test for the two values of τ (Koenker and Machado 1999, Dunham, Cade, and Terrell 2002)

Finally, as a coarse proxy for food web structure, we qualitatively assessed the proportion of individuals belonging to different FFGs across the AMD gradient. Changes in the presence or relative proportion of FFGs can represent changes in energy pathways and feeding links within a community. FFGs were assigned as in (Winterbourn, Gregson, and Dolphin 2006, Hogsden and Harding 2012a)). All statistical analyses were conducted in R version 3.3.3 (R Development Core Team 2017). Annotated R scripts for the full analysis, including the creation of the figures, is available at https://github.com/Jpomz/mining-size-spectra-Freshwater-Biology-accepted. Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.v6g985s.

Results

Mining gradient

Streams varied markedly in pH (1.9 – 7.3), specific conductivity (15.2 – 2227 μS_{25} cm^{-1}), and concentrations of dissolved metals (Supplementary Table 1). A PCA ordination of the chemical variables showed PC axis 1 explained 78% of the variation and was highly correlated with pH, specific conductivity, and dissolved Fe, Al, Mg, Cd, Cu, Mn, Zn, Co, Ni (Pearson correlation > 0.85; Figure 1). These variables are known to vary in response to AMD, and the site scores on PC1 (Figure 1, top axis; hereafter referred to as the mining gradient) were extracted and used as a measure of the mining related stress that each community was exposed to.

Size spectra across the mining gradient

The best fitting model contained significant effects of M, M^2, PC1, and an M:PC1 interaction (β_M, $\beta_{M\char`^2}$, β_{PC1}, $\beta_{M:PC1}$, p values < 0.001, Table 2). The full equation of this model is (site specific coefficient values in Supplementary Table 3):

$$Log_{10}\, N = -5.81 - 0.077PC1 + (0.055PC1 - \ 0.94)\, Log\,_{10}\, M - \ 0.36Log\,_{10}\, M^2 + \ \epsilon$$

As expected, there was a strong, negative parabolic relationship to the number of individuals (N) within increasing body size bins (M) (β_M = -0.937, $p < 0.001$; $\beta_{M\char`^2}$ = -0.356, $p < 0.001$; adjusted $R^2 = 0.85$; $F_{4,\,284} = 405.5$; $p < 0.001$, Figure 2). The magnitude of this curvature was not affected by the mining gradient (no $\beta_{M\char`^2:PC1}$ interaction term in the best fitting model, Table 1). Because size spectra analyses are generally not performed using quadratic equations, we compare the results of both linear and quadratic equations in the Supplementary Material, and show the coefficient estimates in Supplementary Table 4. The overall conclusions reached are generally similar, despite which model was used.

Table 1. AICc evidence table for model selection of quadratic size spectra relationship. The first six columns are the estimated parameter coefficients evaluated for a candidate model, with bold values indicating significance ($p < 0.05$). Blank entries indicate that that variable was not considered in that candidate model. K is the number of estimated parameters, including the residual error term. R^2 is the coefficient of determination, logLik is the log Likelihood, AICc is Akaike's information criterion, corrected for small sample size, ΔAIC_c is the difference in AICc for a candidate model from the lowest AICc value, last column is model weight. Models are ranked by ΔAICc values, where values < 2.00 indicate equivocal support. Only the first model has a ΔAICc < 2.00 and was used in all further analyses.

Intercept	M	M^2	PC1	M:PC1	M^2:PC1	R^2	K	logLik	AICc	ΔAIC_c	weight
5.81	**-0.9366**	**-0.3560**	**-0.0766**	**0.0551**		0.85	6	-229.98	472.3	0.0	0.738
5.81	**-0.9362**	**-0.3592**	**-0.0750**	**0.0554**	-0.0018	0.85	7	-229.97	474.3	2.1	0.262
5.81	**-1.0130**	**-0.3564**	**-0.0732**			0.84	5	-241.43	493.1	20.8	0
5.81	**-1.0130**	**-0.3466**	**-0.0779**		0.0055	0.84	6	-241.32	494.9	22.7	0
5.80	**-1.0220**	**-0.3111**				0.82	4	-260.56	529.3	57.0	0
5.40	**-1.0000**		**-0.0368**	**0.0557**		0.75	5	-302.76	615.7	143.5	0
5.40	**-1.0770**		**-0.0333**			0.74	4	-309.93	628	155.7	0
5.42	**-1.0780**					0.74	3	-312.73	631.6	159.3	0
5.90		**-0.5027**	**-0.0909**			0.21	4	-471.85	951.8	479.6	0
5.91		**-0.5146**	**-0.0852**		-0.0068	0.21	5	-471.82	953.9	481.6	0
5.88		**-0.4478**				0.17	3	-478.14	962.4	490.1	0
5.33						0.00	2	-505.06	1014.2	541.9	0
5.31			-0.0348			0.01	3	-504.26	1014.6	542.4	0

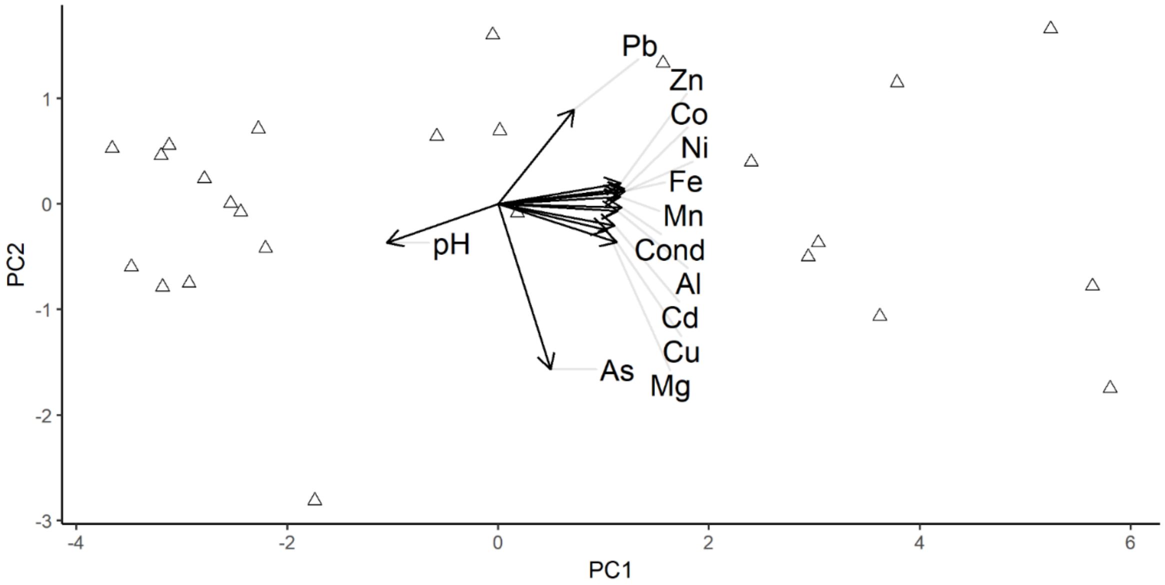


Figure 1. Principal components analysis ordination of chemical variables. The first axis, PC1, explains 77% of the variation, and is strongly correlated with pH, conductivity, and concentrations of Fe, Al, Mg, Cd, Cu, Mn, Co, Ni and can be interpreted as a mining gradient, with mining stress increasing from left to right. PC2 explains 8.6% of the variation, and was not used in further analyses. The site specific scores for PC axis 1 were extracted, and used to represent the mining gradient in all further analyses.

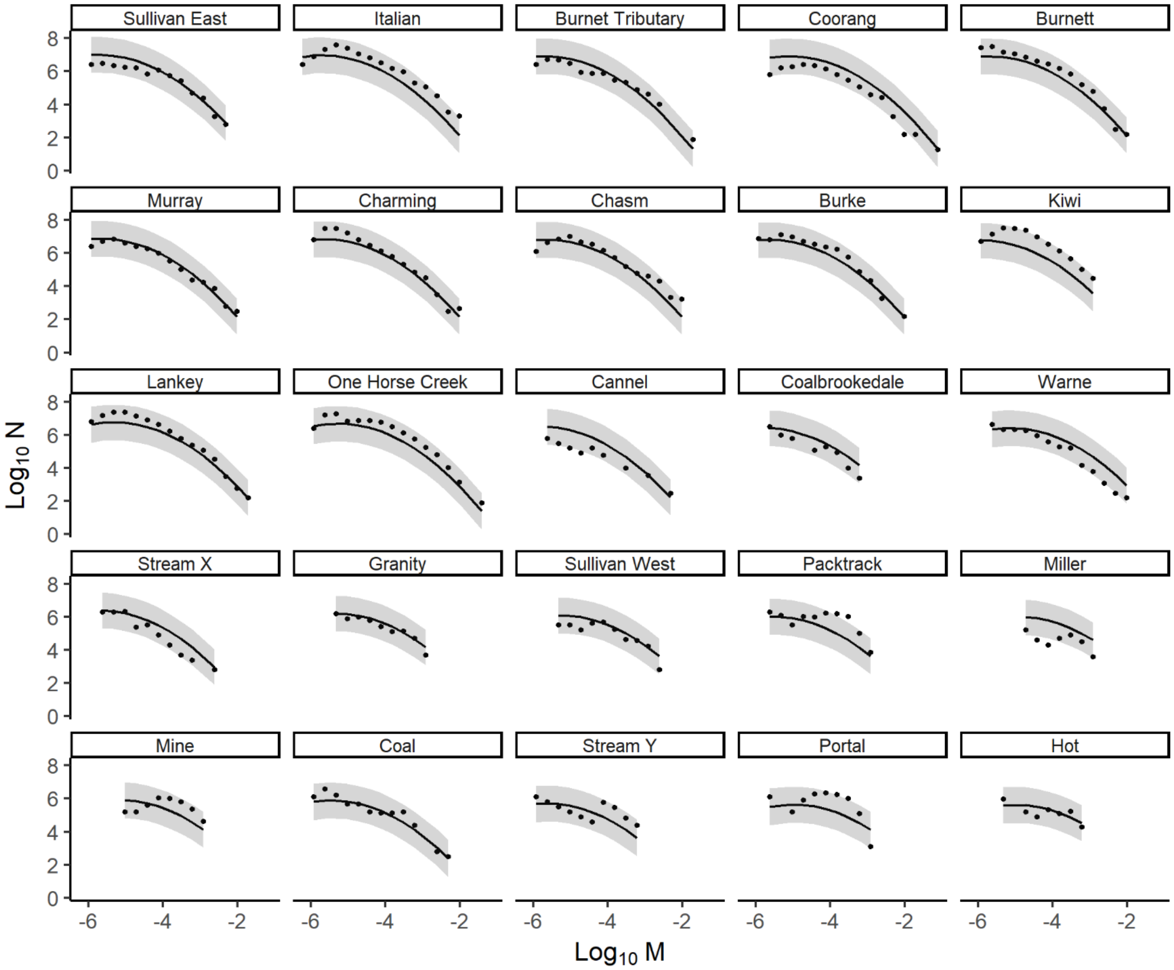


Figure 2. Body mass – abundance relationships for 25 sites across a mining gradient (impact increasing from top left to bottom right). Points are the summed abundances within each size bin from empirical data with fitted values (line) for highest ranked model (see results for specifics) with its 95% prediction interval (grey shading). Regression formula: $Log_{10} N = 5.81 - 0.94 \log_{10} M - 0.36 \log_{10} M^2 - 0.077PC1 + 0.055 \log_{10} M{:}PC1$ where N is the normalized abundance, M is the estimated dry mass in grams, and PC1 is the specific site score on PC axis 1 representing the acid mine drainage gradient (see methods).

Table 2. Results of the top least squares regression model for size spectra across the mining gradient. Estimate is the estimated coefficient value as determined by the multiple linear regression, SE is the standard error of the coefficient estimate, and P is the P-value for the coefficient.

Coefficient	Estimate	SE	P
Intercept	5.80572	0.04427	<0.001
M	-0.93663	0.03316	<0.001
M^2	-0.35596	0.0261	<0.001
PC1	-0.07657	0.01111	<0.001
M:PC1	0.05514	0.01139	<0.001

The community height (y-intercept of the centered data) did vary significantly across the mining gradient ($\beta_{PC1} = -0.077$, $p < 0.001$), indicating a decrease in overall community abundance. To put this in context, we compared this with density estimates calculated directly from our Surber samples, and found general agreement in the conclusions reached (Supplementary Material). Additionally, there was an increase in the slope of the relationship across the mining gradient ($\beta_{M:PC1} = 0.055$, $p < 0.001$, Figure 3), leading to higher relative abundances of larger individuals in impacted streams. The M_{range} decreased significantly across the mining gradient ($p < 0.001$, adjusted $R^2 = 0.58$, $F_{1, 23} = 34.11$, intercept = 3.23, slope = -0.19, Figure 4A), and this was driven by the asymmetric loss of the largest individuals (Figure 4B). The size of the smallest organisms increases across the mining gradient (e.g. the smallest size classes were removed at impacted sites), although this is not statistically significant, as the confidence interval for the quantile regression ($\tau = 0.05$) slope estimate includes 0 (coefficient estimate = 0.03, CI = [-0.85, 0.05]). However, the size of the largest

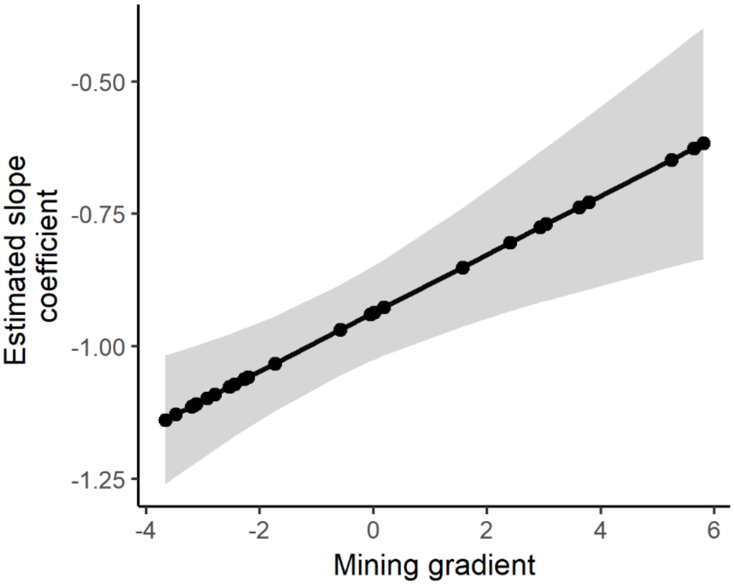


Figure 3. Estimated slope scaling coefficient for size spectra. Points are the site specific estimated slope coefficient for the best fitting model. The solid line and grey shading are the predicted slope coefficient and 95% prediction interval, respectively, across the mining gradient. Mining impacts increase left to right.

organisms significantly decreases across the mining gradient (quantile regression $\tau = 0.95$ coefficient estimate = -0.14, CI = [-0.15, -0.11]), and this was due to the removal of the largest size classes. Communities at un-impacted streams had body sizes ranging from up to 4.5 orders of magnitude, while highly impacted streams had body sizes ranging less than 3 orders of magnitude. These changes in body mass range resulted from a loss of both the smallest and largest size classes, although the larger size classes were more affected. Impacted streams lost 1 – 4 of the smallest size bins, but 5 – 8 of the largest size bins.

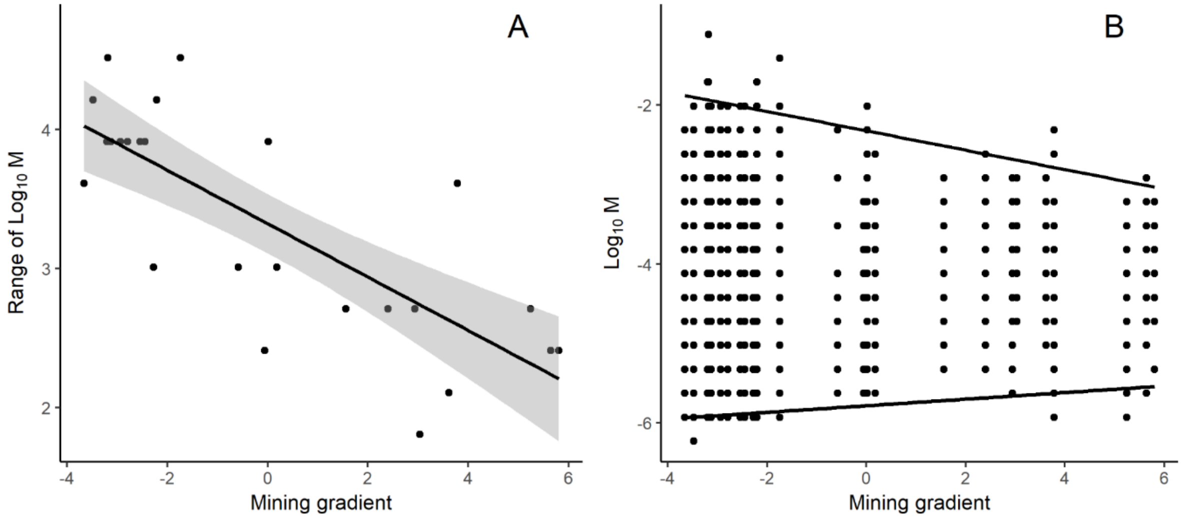


Figure 4. A) Range of body size, $\log_{10}$ [max(M) – min(M)], in grams. The range of M significantly decreases in response to the mining gradient, (black solid line, grey shading is 95% prediction interval. B) Quantile regressions of the 95th and 5th percentile (top and bottom solid lines, respectively) of body size ($\log_{10}$ M, in grams) present across the mining gradient (mine impacts increase from left to right).

Functional feeding groups

The proportion of FFGs in the un-impacted streams was remarkably similar (Fig. 5). Across the gradient, from less- to more-impacted streams, collector-browsers became the dominant group, which is entirely explained by the communities becoming dominated by the Chironomidae subfamilies Diamesinae and Orthocladiinae. Grazers were generally absent from the AMD impacted streams, which was mainly due to the loss of the New Zealand mud snail, *Potamopyrgus antipodarum*, whose shells are extremely sensitive to reduced pH. The grazers present in moderately impacted streams were dominated by the cased caddisfly *Oxyethira* spp. Although shredding taxa are generally poorly represented in New Zealand streams (Winterbourn, Rounick, and Cowie 1981), they were present in nearly all of the un-impacted sites, and completely absent in AMD impacted streams. The single exception to this was the presence of two individual Scirtidae beetles in a single stream, which is heavily impacted by AMD. Omnivores (predominantly the caddisfly *Aoteapsyche* spp.) accounted for a high proportion of the community in moderately AMD impacted streams, but were mostly excluded from the most heavily impacted sites. Filter feeders (dominated by the mayfly *Coloburiscus humeralis* and the blackfly *Austrosimulium* spp.) were present in all un-impacted streams, and generally absent in the AMD impacted sites. Predators in the un-impacted streams were dominated by the large-bodied dobsonfly *Archichauliodes diversus*, and members of the caddisfly family Hydrobiosidae. The large stonefly *Stenoperla prasina*, was also present in 8 of the 12 un-impacted sites, although they were generally found in low densities. All of the large predators were absent from the impacted streams (although a single small *S. prasina* instar was found in a moderately impacted site). Predators were completely removed in about half of the impacted streams but when they were present, their proportions were relatively high compared to the un-impacted sites. Predators in the AMD impacted streams were mostly small individuals belonging to the Empididae and Ceratopogonidae families. Although both of these families are generally classified as predators, they are most likely consuming smaller Meiofauna (or very small instars of macroinvertebrates) due to their body sizes. A table of the dominant taxa (relative proportion ≥ 0.05) by site, their relative proportions and FFG, are presented in Supplementary Table 5.

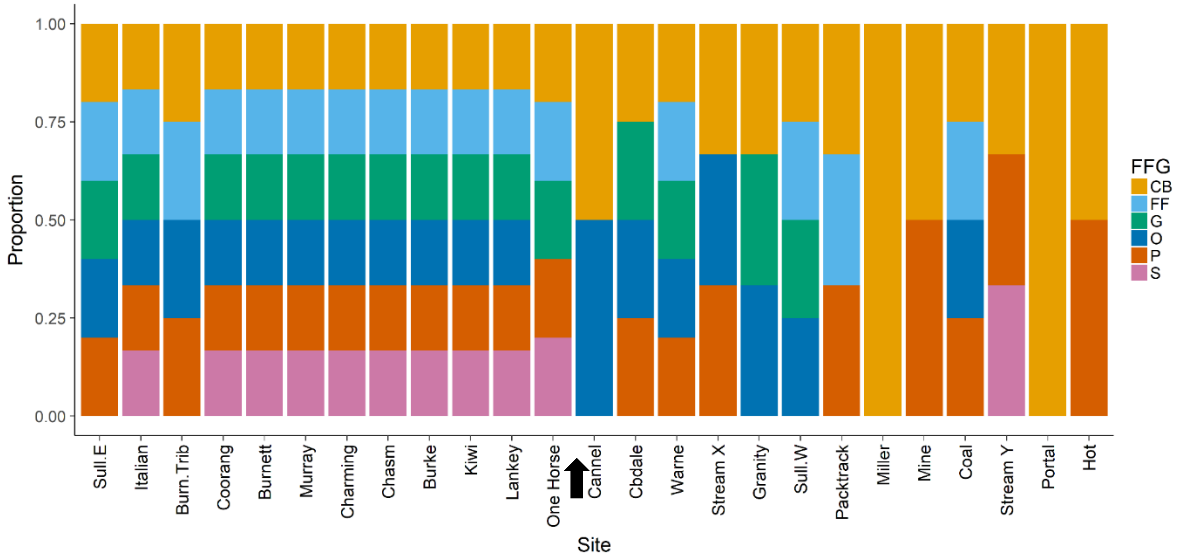


Figure 5. Proportion of individuals belonging to different functional feeding groups (FFG) at sites along an impact gradient of acid mine drainage. Site names are abbreviated where necessary for visualization, and are ordered by increasing impacts from left to right. The arrow in the axis label indicates where AMD impacts begin. All streams left of the arrow are un-impacted by acid mine drainage. CB = collector-browser; FF = filter feeder; G = grazer; O = omnivore; P = predator; S = shredder.

Discussion

The decreasing abundance of larger-sized organisms in communities is one of the most well documented allometric scaling relationships in ecology (White et al. 2007, Petchey and Belgrano 2010, Trebilco et al. 2013). Deviations from this relationship have been attributed to human impacts (Jennings and Blanchard 2004), environmental stress (e.g. drought, Woodward et al. 2012; warming, Yvon-Durocher et al. 2011, Dossena et al. 2012), and ecosystem size (Warburton 2015), and have been suggested as a potential metric for assessing ecological status (Petchey and Belgrano 2010, Gray et al. 2014). In the present study, we found systematic responses of benthic macroinvertebrate size spectra relationships to a gradient of AMD impacts. Previous research in AMD streams has noted a marked decline in taxonomic richness and subsequent change in community dominance and composition where acid and metal tolerant invertebrates survive (Winterbourn and McDiffett 1996, Winterbourn 1998, Clements et al. 2000, Hogsden and Harding 2012a). In addition to these well-documented community changes in response to AMD, our study shows changes in the distribution of biomass and size structure within the communities.

There was a significant reduction in the intercept of the centered data along the AMD gradient, which reflects an overall decrease in community abundance (e.g. mean [min; max] individuals m^{-2} across all un-impacted sites were 4,669 [1,500; 10,122], compared with 1,021 [94; 3,344] at all AMD impacted sites). This is consistent with previous studies that have shown marked declines in macroinvertebrates densities in response to AMD inputs. The reduction in community abundance could be due to an increase in metabolic demands. Low pH and elevated trace metal concentrations can both impact respiration and osmoregulation efficiencies, leading to increased respiration rates. This in turn leads to higher energy demands, potentially at the cost of secondary growth, emergence success (for taxa with an adult aerial phase), and reproductive output (Havas and Hutchinson 1983, Herrmann and Andersson 1986, Herrmann 1987, Adams et al. 2011). Predators may be at a greater risk of local extirpations due to their higher total energetic demands (due to larger body size; Brown et al. 2004) and the increased metabolic costs associated with an active foraging nature (e.g. searching, capturing, and handling prey). The higher cost of living in AMD impacted environments removes sensitive species, but also likely lowers the number of individuals of tolerant taxa which can survive. An alternative explanation for the reduction in community abundance could be lower primary production or lower quantity or quality of basal resources, which cannot support large communities. However, Hogsden, Winterbourn & Harding, (2013) showed that both the quantity and quality of basal resources was not likely a limiting factor for primary consumers across an AMD gradient, lending support to our metabolic cost hypothesis.

There was also a significant increase in the estimated slope coefficient in response to the AMD gradient. Differences in slopes indicate changes to the trophic transfer efficiency within communities. Shallow slopes, in combination with a lower y-intercept, have been attributed to a reduction in trophic transfer efficiency. However, this assumes a fixed body mass of the largest and smallest organisms within a community, respectively (O'Gorman et al. 2012). In the current study, the situation is more complex, due to the complete removal of the largest sized organisms. Generally speaking, with a few notable exceptions (e.g. parasitoids are often similar in size to their hosts, Brose et al. 2006), niche space and trophic level are highly size dependent, particularly in aquatic systems (Stouffer, Rezende, and Amaral 2011), the reduction in the range of body sizes present has strong implications for the structure of the food web, number of trophic levels, and the number of energy pathways available to the community. This is consistent with the changes to the proportion of individuals within each FFG across the gradient. Although predator FFGs are present in some of the most heavily impacted sites, they are entirely composed of small bodied predators that are likely predating upon meiofauna or extremely small macroinvertebrate instars, which are likely below the size range sampled in the present study.

Predators can also directly influence the shape of the size spectrum of their prey (Blanchard et al. 2009, Hartvig, Andersen, and Beyer 2011, Brose et al. 2016). Predator-prey interactions are largely size-structured, where a large predator generally consumes smaller individuals, which transfers energy from abundant small individuals, to fewer, larger, ones. This, in turn, causes a reduction in the abundance of the smaller individuals, leading to the theoretical and empirically observed slopes of about -1 (Jennings and Blanchard 2004, Brown et al. 2004, Trebilco et al. 2013). However, in communities feeding on non-size structured resources (e.g. detritus), size spectrum slopes are shallower (e.g. -0.56 to -0.87, Blanchard et al., 2009) than in predator prey communities. In the present study, the estimated slope coefficients for the AMD impacted streams ranged from -0.97 to -0.62, and the most heavily impacted sites had a slope coefficient shallower than -0.80. The complete removal of large predators at the most heavily impacted sites likely causes a release from top-down control on the smaller prey (Daan et al. 2005), explaining the resemblance of slopes of size spectra at impacted sites to the size spectra slopes observed in detritivore communities (Blanchard et al. 2009).

The loss of predators, coupled with changes in proportions of FFGs represents a simplification of the food web, with fewer links and energy pathways possible, and this has implications for food web stability. Food webs with many weak interactions are generally considered to be more stable compared with food webs dominated by a few strong links (Wootton and Stouffer 2015). The strength of predator-prey feeding links are often considered to be proportional to the predator-prey body size ratios (Emmerson and Raffaelli 2004, Berlow et al. 2009, Tang, Pawar, and Allesina 2014), with stronger interactions occurring between large predators and small prey. Likewise, (Tang, Pawar, and Allesina 2014) found that stability is directly influenced by the correlation of interaction strengths between predators and their prey, and that these strengths can be largely determined by body size. Our findings suggest conflicting interpretations regarding the stability of AMD impacted food webs. On the one hand, the reduction and absence of large predators means that the links present are likely weaker than in un-impacted streams, and therefore the food web should be more stable. However, the simplification of the food web and the loss of FFGs and alternative energy pathways means that there are fewer potential links, and that the links present will have stronger interaction strengths relative to un-impacted streams. This suggests that the food webs in impacted streams would be less stable than un-impacted streams. Further research is needed to tease these interacting mechanisms apart, and determine if AMD impacted food webs are more or less stable.

Changes in food web structure also have implications for the restoration of sites impacted by environmental perturbations. Biotic responses in systems which are recovering from physical or chemical impacts can be delayed or patchy in nature, are modulated by the species and interactions present, and do not follow a reverse trajectory of the response to the original impact (Scheffer et al. 2001), thereby causing the community to appear to be impacted for long after the stressor has been removed (Layer et al. 2011, Gray et al. 2014, 2016). One explanation for this is that impacted communities have found an alternative stable state, and that this state has internal ecological inertia (Layer et al. 2011), or "negative resilience" (Lake 2013), which impedes the community returning to a non-impacted state. Studies of food web stability across productivity gradients suggest that food webs alternate between stable and unstable conditions. Simple food webs in low productivity sites can be organized into stable configurations. As productivity increases, the community can support more biomass at the top of the food chain, which in turn causes stronger, destabilizing top-down interactions. When larger individuals or taxa colonize these communities, they primarily predate upon the larger species present, which decreases their abundance (and hence indirectly reducing the top-down control on smaller individuals), and ultimately rearranges the organization and strength of feeding links, leading to more stable food web configurations (Neutel et al. 2007). A similar mechanism was found in Broadstone Stream in the UK, where biotic recovery lagged chemical remediation by several years, and was characterized by the successive re-invasion of consistently larger bodied predators (Layer et al. 2011). Therefore, if the goal of a restoration project is to return the community to an un-impacted state, in addition to remediating the chemical or physical stressor, it may be necessary to assist in biotic recovery with species reintroductions, particularly when large predators have been removed.

In addition to the strong response of the communities to AMD inputs, we found a remarkable agreement in the communities at the un-impacted streams, despite covering a wide range of natural conditions (e.g. pH 4.8 to 7.3). For example, the shape of the size spectrum, and the proportion of individuals in the different FFGs were both very similar in the un-impacted sites. This adds to the growing body of literature showing that size spectra relationships are a good proxy variable for ecological status (Petchey and Belgrano 2010, Trebilco et al. 2013, Warburton 2015). This result is encouraging, because it indicates that community-level responses avoid some of the challenges that traditional, taxonomic based biomonitoring programs have faced e.g. variable species distributions, biogeographical constraints, context and pollutant specific sensitivities (Gray et al. 2014).

We found that AMD affected stream macroinvertebrate size spectra in consistent ways, represented conceptually in Figure 6. Specifically, we found that community abundance and overall body size decreased in AMD streams, resulting in smaller communities, with more homogenous distributions of biomass. The harsh environment caused by heavy AMD inputs likely increased the energetic demands on macroinvertebrates, lowering the overall community abundance (lowered y-intercept). These impacts were greater on larger individuals (asymmetric reduction in range of body sizes present). Since most predators are generally larger bodied, their removal causes a release from predation pressure, allowing the abundance of smaller individuals to decline less significantly than would be expected otherwise (shallower size spectrum slope). These findings have implications for the structure, function, and stability of food webs in impacted streams, and should be taken into account when designing remediation efforts.

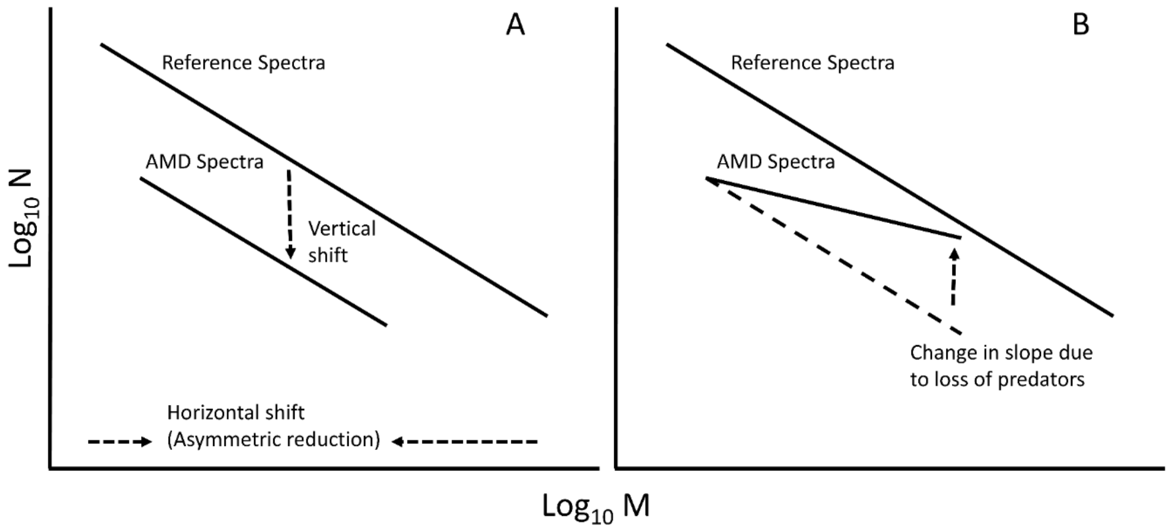


Figure 6. Conceptual figure of impacts of acid mine drainage to stream macroinvertebrate size spectra. Inputs of acid mine drainage (AMD) cause consistent changes to reference spectra. A) Spectra impacted by acid mine drainage resulted from a vertical shift (reduced community abundance) and asymmetric horizontal shift (large body sizes are more impacted). B) The loss of large predators also resulted in a reduction in top-down control of prey abundances, causing an increase in the slope (dashed arrow) of spectra impacted by acid mine drainage.

These findings add a new perspective to the impact of AMD which to our knowledge has not been previously reported, and which would not be captured by using traditional biomonitoring methods. For example, if taxonomic or functional groups were aggregated and their biomass measured directly, the relationships would be skewed as the individual-level information would be lost. Likewise, construction of food webs across a large number of streams (e.g. 25 in the present study) via visual identification of gut contents would require much greater allocations of effort. Food webs could be constructed using stable isotope methods, but again the individual level data would be lost. However, size spectra incorporate information from the individual-, population- and community-level regarding energy transfer efficiency, and the partitioning of nutrients and biomass within an ecosystem (Woodward et al. 2005, Martinez et al. 2016). Likewise, with the individual-level data retained it is easy to aggregate and to different organizational levels and analyses them separately, as needed. Therefore, we feel that the information gained from size spectra analyses far outweighs the additional labor required. Further work is needed to assess the sensitivity of size spectra across other environmental gradients, in order to further our understanding of their potential use as an indicator of ecological status.

Acknowledgements

We thank C Perovich for help in the field and the lab, and S Pohe for help with invertebrate identification. Thanks to K Hogsden, and A R McIntosh for thoughtful discussions, and 2 anonymous reviewers, whose comments greatly improved this manuscript. This research was funded by the New Zealand MBIE Endeavour Fund (Grant CLRE 1202).

References

Adams, W. J., R. Blust, U. Borgmann, K. V. Brix, D. K. DeForest, A. S. Green, J. S. Meyer, J. C. McGeer, P. R. Paquin, P. S. Rainbow, and C. M. Woody. 2011. Utility of tissue residues for predicting effects of metals on aquatic organisms. Integrated Environmental Assessment and Management **7**:75–98. https://doi.org/10.1002/ieam.108.

Bartoń, K. 2016. MuMIn: Multi-Model Inference. R Package version 1.15.6.

Berlow, E. L., J. A. Dunne, N. D. Martinez, P. B. Stark, R. J. Williams, and U. Brose. 2009. Simple prediction of interaction strengths in complex food webs. Proceedings of the National Academy of Sciences **106**:187–191.

Bernhardt, E. S., E. J. Rosi, and M. O. Gessner. 2017. Synthetic chemicals as agents of global change. Frontiers in Ecology and the Environment **15**:84–90. https://doi.org/10.1002/fee.1450.

Blakely, T. J., and J. S. Harding. 2005. Longitudinal patterns in benthic communities in an urban stream under restoration. New Zealand Journal of Marine and Freshwater Research **39**:17–28. https://doi.org/10.1080/00288330.2005.9517291.

Blanchard, J. L., N. K. Dulvy, S. Jennings, J. R. Ellis, J. K. Pinnegar, A. Tidd, and L. T. Kell. 2005. Do climate and fishing influence size-based indicators of Celtic Sea fish community structure? ICES Journal of Marine Science **62**:405–411. https://doi.org/10.1016/j.icesjms.2005.01.006.

Blanchard, J. L., S. Jennings, R. Law, M. D. Castle, P. Mccloghrie, M. Rochet, and E. Benoît. 2009. How does abundance scale with body size in coupled size-structured food webs? Journal of Animal Ecology **78**:270–280. https://doi.org/10.1111/j.1365-2656.2008.01466.x.

Brose, U., J. L. Blanchard, A. Eklöf, N. Galiana, M. Hartvig, M. R. Hirt, G. Kalinkat, M. C. Nordström, E. J. O'Gorman, B. C. Rall, F. D. Schneider, E. Thébault, and U. Jacob. 2016. Predicting the consequences of species loss using size-structured biodiversity approaches. Biological Reviews **49**. https://doi.org/10.1111/brv.12250.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. Ecology **85**:1771–1789. https://doi.org/10.1890/03-9000.

Burnham, K. P., and D. R. Anderson. 2004. Multimodel inference: understanding AIC and BIC in model selection. Sociological Methods & Research **33**:261–304. https://doi.org/10.1177/0049124104268644.

Clements, W. H., D. M. Carlisle, J. M. Lazorchak, and P. C. Johnson. 2000. Heavy metals structure benthic communities in Colorado mountain streams. Ecological Applications **10**:626–638.

Daan, N., H. Gislason, J. G. Pope, and J. C. Rice. 2005. Changes in the North Sea fish community: evidence of indirect effects of fishing? ICES Journal of Marine Science **62**:177–188. https://doi.org/10.1016/j.icesjms.2004.08.020.

Devonport, B. F., and M. J. Winterbourn. 1976. The feeding relationships of two invertebrate predators in a New Zealand river. Freshwater Biology **6**:167–176. https://doi.org/10.1111/j.1365-2427.1976.tb01601.x.

Dossena, M., G. Yvon-Durocher, J. Grey, J. M. Montoya, D. M. Perkins, M. Trimmer, and G. Woodward. 2012. Warming alters community size structure and ecosystem functioning. Proceedings of the Royal Society B: Biological Sciences **279**:3011–3019. https://doi.org/10.1098/rspb.2012.0394.

Drummond, L. R., A. R. Mcintosh, and S. T. Larned. 2015. Invertebrate community dynamics and insect emergence in response to pool drying in a temporary river. Freshwater Biology **60**:1596–1612. https://doi.org/10.1111/fwb.12591.

Dunham, J. B., B. S. Cade, and J. W. Terrell. 2002. Influences of spatial and temporal variation on fish-habitat relationships defined by regression quantiles. Transactions of the American Fisheries Society **131**:86–98. https://doi.org/10.1577/1548-8659(2002)131<0086:IOSATV>2.0.CO;2.

Elton, C. S. 1927. Animal Ecology. Macmillan Co., New York, NY.

Emmerson, M. C., and D. Raffaelli. 2004. Predator–prey body size, interaction strength and the stability of a real food web. Journal of Animal Ecology **73**:399–409.

Gaedke, U. 1992. The size distribution of plankton in a large lake and its seasonal variability. Limnology and Oceanography **37**:1202–1220.

Gangloff, M. M., M. Perkins, P. W. Blum, and C. Walker. 2015. Effects of coal mining, forestry, and road construction on southern Appalachian stream invertebrates and habitats. Environmental Management **55**:702–714. https://doi.org/10.1007/s00267-014-0429-1.

Gómez-Canchong, P., J. M. Blanco, and R. A. Quiñones. 2013. On the use of biomass size spectra linear adjustments to design ecosystem indicators. Scientia Marina **77**:257–268. https://doi.org/10.3989/scimar.03708.22A.

Gray, C., D. J. Baird, S. Baumgartner, U. Jacob, G. B. Jenkins, E. J. O'Gorman, X. Lu, A. Ma, M. J. O. Pocock, N. Schuwirth, M. Thompson, and G. Woodward. 2014. Ecological networks: the missing links in biomonitoring science. Journal of Applied Ecology **51**:1444–1449. https://doi.org/10.1111/1365-2664.12300.

Gray, C., A. G. Hildrew, X. Lu, A. Ma, D. Mcelroy, and D. Monteith. 2016. Recovery and nonrecovery of freshwater food webs from the effects of acidification. Advances in Ecological Research **55**:475–534. https://doi.org/10.1016/bs.aecr.2016.08.009.

Gray, N. F. 1997. Environmental impact and remediation of acid mine drainage: a management problem. Environmental Geology **30**:62–71.

Greig, H. S., D. K. Niyogi, K. L. Hogsden, P. G. Jellyman, and J. S. Harding. 2010. Heavy metals: confounding factors in the response of New Zealand freshwater fish assemblages to natural and anthropogenic acidity. Science of the Total Environment **408**:3240–3250. https://doi.org/10.1016/j.scitotenv.2010.04.006.

Harding, J. S., and M. J. Winterbourn. 1997. An ecoregion classification of the South Island, New Zealand. Journal of environmental management **51**:275–287.

Harding, J. S., M. J. Winterbourn, and W. F. McDiffett. 1997. Stream faunas and ecoregions of the South Island, New Zealand: do they correspond? Archiv für Hydrobiologie **140**:289–307.

Hartvig, M., K. H. Andersen, and J. E. Beyer. 2011. Food web framework for size-structured populations. Journal of Theoretical Biology **272**:113–122. https://doi.org/10.1016/j.jtbi.2010.12.006.

Havas, M., and T. C. Hutchinson. 1983. Effect of low pH on the chemical composition of aquatic invertebrates from tundra ponds at the Smoking Hills, N.W.T., Canada. Canadian Journal of Zoology **61**:241–249. https://doi.org/10.1139/z83-030.

Herrmann, J. 1987. Aluminium impact on freshwater invertebrates at low pH: A review. Pages 157–175 *in* L. Landner, editor. Speciation of Metals in Water, Sediment and Soil Systems. Springer Berlin Heidelberg.

Herrmann, J., and K. G. Andersson. 1986. Aluminium impact on respiration of lotic mayflies at low pH. Water, Air, and Soil Pollution **30**:703–709. https://doi.org/10.1007/BF00303335.

Hogsden, K. L., and J. S. Harding. 2012a. Anthropogenic and natural sources of acidity and metals and their influence on the structure of stream food webs. Environmental Pollution **162**:466–474. https://doi.org/10.1016/j.envpol.2011.10.024.

Hogsden, K. L., and J. S. Harding. 2012b. Consequences of acid mine drainage for the structure and function of benthic stream communities: a review. Freshwater Science **31**:108–120. https://doi.org/10.1899/11-091.1.

Hogsden, K. L., J. G. Webster-Brown, and J. S. Harding. 2016. Mining impacts on the water quality and ecology of New Zealand freshwaters. Pages 519–530 *in* P. G. Jellyman, T. J. A. Davie, C. P. Pearson, and J. S. Harding, editors. Advances in New Zealand Freshwater Science. Christchurch.

Hogsden, K. L., M. J. Winterbourn, and J. S. Harding. 2013. Do food quantity and quality affect food webs in streams polluted by acid mine drainage? Marine and Freshwater Research **64**:1112–1122.

Jennings, S., and J. L. Blanchard. 2004. Fish abundance with no fishing: predictions based on macroecological theory. Journal of Animal Ecology **73**:632–642.

Jennings, S., K. J. Warr, and S. Mackinson. 2002. Use of size-based production and stable isotope analyses to predict trophic transfer efficiencies and predator-prey body mass ratios in food webs. Marine Ecology Progress Series **240**:11–20.

Kitto, J. A. J., D. P. Gray, H. S. Greig, D. K. Niyogi, and J. S. Harding. 2015. Meta-community theory and stream restoration: evidence that spatial position constrains stream invertebrate communities in a mine impacted landscape. Restoration Ecology **23**:284–291. https://doi.org/10.1111/rec.12179.

Koenker, R., and J. A. F. Machado. 1999. Goodness of fit and related inference processes for quantile regression. Journal of the American Statistical Association **94**:1296–1310. https://doi.org/10.1080/01621459.1999.10473882.

Lake, P. S. 2013. Resistance, resilience and restoration. Ecological Management and Restoration **14**:20–24. https://doi.org/10.1111/emr.12016.

Layer, K., A. G. Hildrew, G. B. Jenkins, J. O. Riede, J. Stephen, C. R. Townsend, G. Woodward, L. D. Well-, K. Layer, A. G. Hildrew, and G. B. Jenkins. 2011. Long-term dynamics of a well-characterised food web: four decades of acidification and recovery in the Broadstone Stream model system. Advances in Ecological Research **44**:69–117. https://doi.org/10.1016/B978-0-12-374794-5.00002-X.

Martínez, A., A. Larrañaga, A. Miguélez, G. Yvon-Durocher, and J. Pozo. 2016. Land use change affects macroinvertebrate community size spectrum in streams: the case of Pinus radiata plantations. Freshwater Biology **61**:69–79. https://doi.org/10.1111/fwb.12680.

Morin, A., N. Bourassa, and A. Cattaneo. 2001. Use of size spectra and empirical models to evaluate trophic relationships in streams. Limnology and Oceanography **46**:935–940. https://doi.org/10.4319/lo.2001.46.4.0935.

Neutel, A. M., J. A. P. P. Heesterbeek, J. van de Koppel, G. Hoenderboom, A. Vos, C. Kaldeway, F. Berendse, P. C. de Ruiter, J. Van De Koppel, G. Hoenderboom, A. Vos, C. Kaldeway, F. Berendse, and P. C. De Ruiter. 2007. Reconciling complexity with stability in naturally assembling food webs. Nature **449**:599–602. https://doi.org/10.1038/nature06154.

O'Gorman, E. J., D. E. Pichler, G. Adams, J. P. Benstead, H. Cohen, N. Craig, W. F. Cross, B. O. L. Demars, N. Friberg, G. M. Gíslason, R. Gudmundsdóttir, A. Hawczak, J. M. Hood, L. N. Hudson, L. Johansson, M. P. Johansson, J. R. Junker, A. Laurila, J. R. Manson, E. Mavromati, D. Nelson, J. S. ólafsson, D. M. Perkins, O. L. Petchey, M. Plebani, D. C. Reuman, B. C. Rall, R. Stewart, M. S. A. Thompson, and G. Woodward. 2012. Impacts of warming on the structure and functioning of aquatic communities. Individual- to ecosystem-level responses. Advances in Ecological Research **47**:81–176. https://doi.org/10.1016/B978-0-12-398315-2.00002-8.

Petchey, O. L., and A. Belgrano. 2010. Body-size distributions and size-spectra: universal indicators of ecological status? Biology Letters **6**:434–437. https://doi.org/10.1098/rsbl.2010.0240.

R Development Core Team. 2017. R: A language and environment for statistical computing. R Core Team, Vienna, Austria.

Reuman, D. C., C. Mulder, D. Raffaelli, and J. E. Cohen. 2008. Three allometric relations of population density to body mass: theoretical integration and empirical tests in 149 food webs. Ecology Letters **11**:1216–1228. https://doi.org/10.1111/j.1461-0248.2008.01236.x.

Scheffer, M., S. Carpenter, J. A. Foley, C. Folke, and B. Walker. 2001. Catastrophic shifts in ecosystems. Nature **413**:591.

Sentis, A., A. Binzer, and D. S. Boukal. 2017. Temperature-size responses alter food chain persistence across environmental gradients. Ecology Letters **20**:852–862. https://doi.org/10.1111/ele.12779.

Sprules, W. G., and L. E. Barth. 2015. Surfing the biomass size spectrum: some remarks on history, theory, and application. Canadian Journal of Fisheries and Aquatic Sciences **73**:477–495.

Stoffels, R. J., S. Karbe, and R. A. Paterson. 2003. Length-mass models for some common New Zealand littoral-benthic macroinvertebrates, with a note on within-taxon variability in parameter values among published models. New Zealand Journal of Marine and Freshwater Research **37**:449–460. https://doi.org/10.1080/00288330.2003.9517179.

Stouffer, D. B., E. L. Rezende, and A. N. Amaral. 2011. The role of body mass in diet contiguity and food-web structure. Journal of Animal Ecology **80**:632–639. https://doi.org/10.1111/j.1365-2656.2011.01812.x.

Tang, S., S. Pawar, and S. Allesina. 2014. Correlation between interaction strengths drives stability in large ecological networks. Ecology Letters **17**:1094–1100. https://doi.org/10.1111/ele.12312.

Towers, D. J., I. M. Henderson, and C. J. Veltman. 1994. Predicting dry weight of New Zealand aquatic macroinvertebrates from linear dimensions. New Zealand Journal of Marine and Freshwater Research **28**:159–166. https://doi.org/10.1080/00288330.1994.9516604.

Trebilco, R., J. K. Baum, A. K. Salomon, and N. K. Dulvy. 2013. Ecosystem ecology: size-based constraints on the pyramids of life. Trends in Ecology & Evolution **28**:423–431. https://doi.org/10.1016/j.tree.2013.03.008.

Underwood, B. E., N. A. Kruse, and J. R. Bowman. 2014. Long-term chemical and biological improvement in an acid mine drainage-impacted watershed. Environmental Monitoring and Assessment **186**:7539–7553. https://doi.org/10.1007/s10661-014-3946-8.

Warburton, H. J. 2015. The role of body size in predator-prey interactions and community structure. University of Canterbury.

White, E. P., B. J. Enquist, and J. L. Green. 2008. On estimating the exponent of power-law frequency distributions. Ecology **89**:905–912.

White, E. P., S. K. M. Ernest, A. J. Kerkhoff, and B. J. Enquist. 2007. Relationships between body size and abundance in ecology. Trends in Ecology & Evolution **22**:323–330. https://doi.org/10.1016/j.tree.2007.03.007.

Winterbourn, M. J. 1998. Insect faunas of acidic coal mine drainages in Westland, New Zealand. New Zealand Entomologist **21**:65–72.

Winterbourn, M. J., K. L. D. Gregson, and C. H. Dolphin. 2006. Guide to the aquatic insects of New Zealand. Bulletin of the Entomological Society of New Zealand, Auckland.

Winterbourn, M. J., and W. F. McDiffett. 1996. Benthic faunas of streams of low pH but contrasting water chemistry in New Zealand. Hydrobiologia **341**:101–111. https://doi.org/10.1007/BF00018114.

Winterbourn, M. J., W. F. Mcdiffett, S. J. Eppley, and M. Creek. 2000. Aluminium and iron burdens of aquatic biota in New Zealand streams contaminated by acid mine drainage: effects of trophic level. Science of the Total Environment **254**:45–54.

Winterbourn, M. J., J. S. Rounick, and B. Cowie. 1981. Are New Zealand stream ecosystems really different? New Zealand Journal of Marine and Freshwater Research **15**:321–328. https://doi.org/10.1080/00288330.1981.9515927.

Woodward, G., L. E. Brown, F. K. Edwards, L. N. Hudson, A. M. Milner, D. C. Reuman, and M. E. Ledger. 2012. Climate change impacts in multispecies systems: drought alters food web size structure in a field experiment. Philosophical Transactions Royal Society B **367**:2990–2997. https://doi.org/10.1098/rstb.2012.0245.

Wootton, K. L., and D. B. Stouffer. 2015. Many weak interactions and few strong; food-web feasibility depends on the combination of the strength of species' interactions and their correct arrangement. Theoretical Ecology **9**:185–195. https://doi.org/10.1007/s12080-015-0279-3.

Yvon-Durocher, G., J. M. Montoya, M. Trimmer, and G. Woodward. 2011. Warming alters the size spectrum and shifts the distribution of biomass in freshwater ecosystems. Global Change Biology **17**:1681–1694. https://doi.org/10.1111/j.1365-2486.2010.02321.x.

Supplementary Material

Table S1. Chemical variables for the 25 sites analyzed in this study. PCA was performed on pH, specific conductivity, and metal concentrations in order to determine the acid mine drainage impact gradient. PC1 are the site specific scores determined by the PCA, and are ordered by increasing impact. Stream X and Y were located on private property in the same region, and sampling access was granted on the condition that their names and locations would not be disclosed. EC is specific conductivity in µS cm-1. Metal concentrations are in mg L^{-1}.

Site	PC1	pH	EC	Mg	Al	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Pb
Sullivan East	-3.66	4.8	19	0.2482	0.1476	0.0012	0.2312	0.0000	0.0002	0.0003	0.0051	0.0002	0.0000	0.0002
Italian	-3.48	6.5	48	2.3332	0.0496	0.0068	0.1008	0.0000	0.0003	0.0004	0.0035	0.0018	0.0000	0.0001
Burnett Trib.	-3.20	5.6	16	0.3069	0.2430	0.0087	0.3879	0.0003	0.0008	0.0006	0.0086	0.0004	0.0000	0.0003
Coorang	-3.18	6.4	31	1.1870	0.2918	0.0050	0.3212	0.0001	0.0005	0.0006	0.0017	0.0034	0.0000	0.0002
Burnett	-3.12	5.5	15	0.2769	0.2390	0.0073	0.4267	0.0001	0.0004	0.0012	0.0113	0.0004	0.0000	0.0003
Murray	-2.93	7.1	73	4.9097	0.0714	0.0091	0.0805	0.0001	0.0011	0.0008	0.0049	0.0033	0.0000	0.0003
Charming	-2.79	6.5	50	0.8406	0.4030	0.0126	0.6624	0.0002	0.0016	0.0005	0.0078	0.0004	0.0000	0.0002
Chasm	-2.54	6.9	70	1.3866	0.2502	0.0125	1.1471	0.0002	0.0023	0.0007	0.0073	0.0008	0.0000	0.0002
Burke	-2.44	6.6	69	4.1808	0.2773	0.0252	0.3316	0.0005	0.0027	0.0004	0.0150	0.0005	0.0000	0.0001
Kiwi	-2.28	6.5	60	1.5435	0.0902	0.0035	0.2157	0.0000	0.0008	0.0029	0.1652	0.0005	0.0000	0.0008
Lankey	-2.21	7.3	92	7.1770	0.1301	0.0867	0.1633	0.0020	0.0039	0.0008	0.0133	0.0012	0.0000	0.0001
One Horse Creek	-1.74	6.9	42	2.5578	0.7159	0.0134	0.4738	0.0004	0.0014	0.0029	0.0172	0.0816	0.0000	0.0004
Cannel	-0.58	3.7	197	3.4499	2.6198	0.0729	1.7997	0.0031	0.0086	0.0014	0.0392	0.0002	0.0000	0.0002
Coalbrookedale	-0.05	3.5	144	1.5735	2.2036	0.0705	1.4204	0.0070	0.0127	0.0023	0.1160	0.0002	0.0000	0.0010

Warne	0.01	3.5	318	2.7762	5.2935	0.1747	1.6643	0.0040	0.0127	0.0013	0.1195	0.0003	0.0001	0.0003
Stream X	0.18	5.6	259	22.4737	0.2416	0.8487	0.8274	0.0116	0.0221	0.0098	0.0837	0.0005	0.0001	0.0001
Granity	1.56	3.3	270	2.9521	9.1341	0.1836	2.3635	0.0157	0.0353	0.0087	0.2235	0.0011	0.0002	0.0015
Sullivan West	2.40	2.7	687	5.8352	11.2588	0.3122	15.1440	0.0335	0.0666	0.0082	0.3974	0.0038	0.0001	0.0008
Packtrack	2.94	2.5	982	11.7891	21.6226	0.8518	7.7533	0.0350	0.0807	0.0131	0.5107	0.0070	0.0003	0.0005
Miller	3.03	2.6	923	13.9081	29.1945	0.8732	7.4100	0.0360	0.0845	0.0131	0.5348	0.0055	0.0003	0.0005
Mine	3.62	2.5	1285	20.1727	51.2792	1.3707	10.2336	0.0568	0.1368	0.0150	0.8447	0.0079	0.0005	0.0002
Coal	3.78	2.7	889	38.2580	17.4306	1.6715	21.6975	0.0869	0.2337	0.0089	0.8103	0.0006	0.0005	0.0009
Stream Y	5.24	3.3	1607	156.4894	9.6857	13.0668	14.5808	0.3223	0.4896	0.0116	1.3645	0.0012	0.0008	0.0024
Portal	5.64	1.9	2183	38.4871	86.2435	3.0583	23.0497	0.1237	0.3051	0.1062	1.7068	0.0109	0.0009	0.0009
Hot	5.80	2.3	2227	50.4921	99.2991	3.8870	38.3905	0.1528	0.3813	0.0730	2.0773	0.0234	0.0012	0.0005

Number of bins

The number of bins used when constructing size spectra relationships is known to affect the conclusions reached. On the one hand, wide logarithmic bins reduce the number of bins with zero observations in them (White, Enquist, and Green 2008), while broadly retaining the spectral features, whereas on the other hand wide bins can substantially mask the finer spectral details (Gaedke 1992, Sprules and Barth 2015). The two most commonly used bin widths are $\log_{10}$ and $\log_2$, which we examined here. When using $\log_2$ bins, there were a total of 18 bins across all sites (Fig. S1). Eleven of the twenty five sites had a single empty bin within the size range of their data, respectively. No sites had more than one empty bin within their data range. Additionally, the range and abundance of body sizes present in each Surber sample was similar within a site, hence, we feel that each sample per site was an adequate representation of the size spectra present at that site. Of the 18 bins used, between 6 and 16 contained values, depending on the site (Fig. S1). Missing values in bins represent missing size classes within a community, and were not included in the regression analyses.

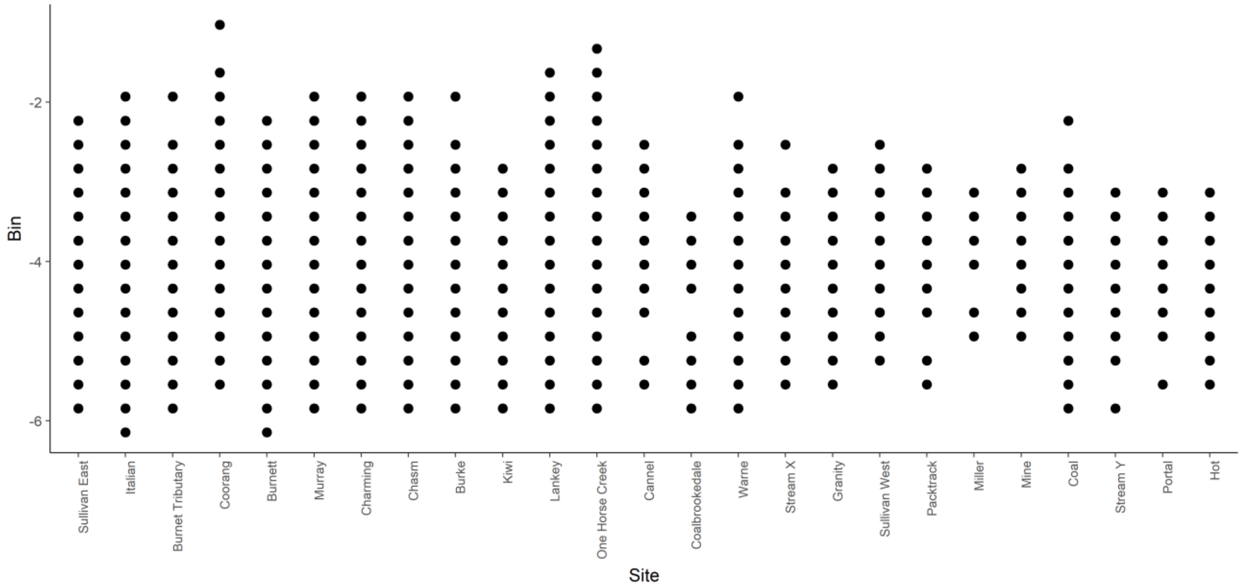


Figure S1 Log2 width bins. Points represent midpoint of bins with observed size classes present at that site. Sites are ordered by increasing AMD impacts from left to right.

While there were no empty bins across the observed size range when using $\log_{10}$ width bins, the number of bins was reduced to a total of 6 (Fig. S2). Due to the decrease in size range across the mining gradient, this resulted in only 2-6 bins per site having observed body sizes within them.

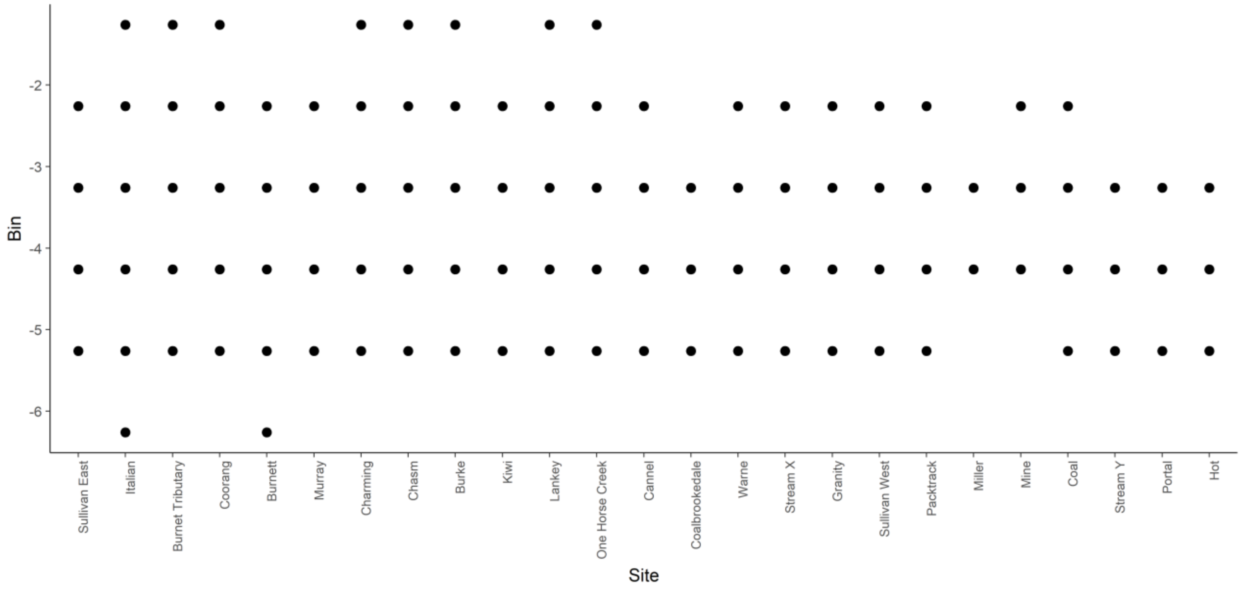


Figure S2. Log10 width bins. Points represent bins with observed size classes present at that site. Sites are ordered by increasing AMD impacts from left to right.

When using $\log_2$ width bins, our number of observations increased significantly, allowing for more robust regressions ($\log_2$ N = 289; $\log_{10}$ N = 103). Importantly, the coefficient estimates, and therefore the interpretation, are similar when using $\log_{10}$ width bins (Table S1), indicating the strength of the response of size spectra to AMD impacts. When carrying out the statistical model selection using the *dredge* function in the MuMIN package for R (Bartoń 2016) for the $\log_{10}$ width bins, two models are selected (ΔAIC <2) with approximately equal weight (0.475 and 0.362, respectively). Both of these models have significant coefficients for M, M^2, and an M:PC1 interaction. The first model contains terms for PC1 and an interaction term for M^2:PC1, although neither one of these effects are significant (p-value > 0.10). The second model contains a term for PC1, although this is barely non-significant at the $\alpha = 0.05$ level (p-value = 0.0502).

Table S2. Coefficient estimates for the top 2 models using log10 width bins, and for the top model using log2 width bins. See main text for model selection. Coefficients in bold are significant at $\alpha = 0.05$ level, while italics indicates coefficients which are likely biologically significant (p-value < 0.10). -- indicates that term was not present in the model. The ΔAIC and weight columns for the 2 log10 models can be compared directly, while the value for the log2 model is independent.

Model	Intercept	M	M^2	PC1	M:PC1	M^2:PC1	Adj R^2	ΔAIC	weight
Log10 - top model	**5.696**	**-0.902**	**-0.360**	-0.016	**0.072**	-0.035	0.832	0	0.479
Log10 - second model	**5.650**	**-0.952**	**-0.290**	*-0.050*	**0.051**	--	0.830	0.56	0.361
Log2 - top model	**5.806**	**-0.937**	**-0.356**	**-0.077**	**0.055**	--	0.851	0	0.738

Table S3. Quadratic equations for size spectra across the mining gradient. PC1 are the site specific scores determined by the PCA, and are ordered by increasing impact. Intercept and slope are the estimated coefficients taking into account the effects of the mining gradient (PC1). The quadratic term did not vary significantly across the gradient.

Site	PC1	Intercept	Slope	Quadratic term
Sullivan East	-3.66	6.086	-1.138	-0.356
Italian	-3.48	6.072	-1.128	-0.356
Burnett Trib.	-3.20	6.051	-1.113	-0.356
Coorang	-3.18	6.049	-1.112	-0.356
Burnet	-3.12	6.045	-1.109	-0.356
Murray	-2.93	6.030	-1.098	-0.356
Charming	-2.79	6.019	-1.090	-0.356
Chasm	-2.54	6.000	-1.077	-0.356
Burke	-2.44	5.993	-1.071	-0.356
Kiwi	-2.28	5.980	-1.062	-0.356
Lankey	-2.21	5.975	-1.058	-0.356
One Horse Creek	-1.74	5.939	-1.033	-0.356
Cannel	-0.58	5.850	-0.969	-0.356
Coalbrookedale	-0.05	5.810	-0.940	-0.356
Warne	0.01	5.805	-0.936	-0.356
Stream X	0.18	5.792	-0.927	-0.356
Granit	1.56	5.686	-0.850	-0.356
Sullivan West	2.40	5.622	-0.804	-0.356
Packtrack	2.94	5.581	-0.775	-0.356
Miller	3.03	5.573	-0.769	-0.356
Mine	3.62	5.529	-0.737	-0.356
Coal	3.78	5.516	-0.728	-0.356

Stream Y	5.24	5.405	-0.648	-0.356
Portal	5.64	5.374	-0.626	-0.356
Hot	5.80	5.361	-0.617	-0.356

Fish presence / absence

Twelve of the 25 sites were un-impacted by AMD inputs. However, of these 12 sites, 6 of the streams were naturally fishless. Because the presence of top predators can change the shape of the size spectra of their prey (Brose et al. 2016), we compared the relationship between un-impacted streams with and without fish. The size spectra for the un-impacted streams is shown in Fig. S1. We tested for significant effects of fish presence by conducting a linear regression with a categorical variable of fish presence (Y/N), and an interaction term between the slope and fish presence. There were no significant effects of fish presence (p-value = 0.789) or a significant interaction between fish presence and the slope of the relationship (p-value = 0.130). Only the macroinvertebrate size spectra were compared across all 25 streams.

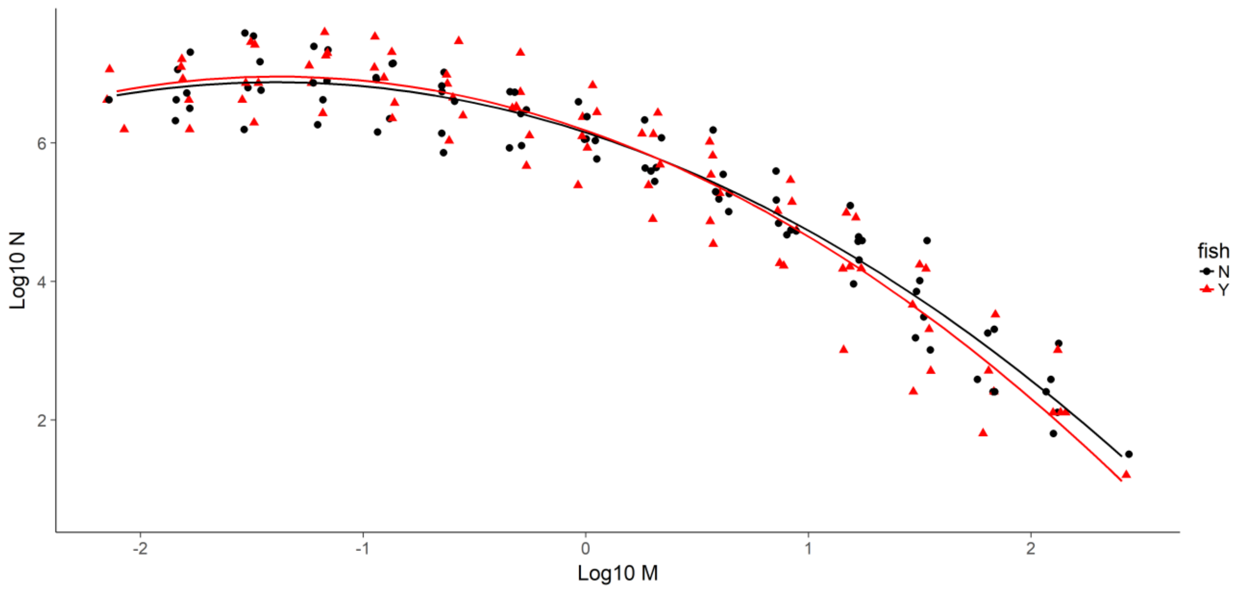


Figure S1. Size spectra for the macroinvertebrate community in un-impacted streams both with (red triangles) and without (black circles) fish present. The points are jittered for visualization.

Linear vs. quadratic model

The majority of previously published work on size spectra examines linear models of log-transformed variables. However, as Sprules and Barth (2015) note, the relationship between body size and abundance are actually a series of repeating, negative parabolic domes, which only take on a linear shape when sampling over a large enough range of body sizes. When examining size spectra across a limited range of body sizes, they recommend fitting quadratic models to account for the secondary curvature of the relationship, and using model selection (e.g. Akaike's information criterion, Burnham and Anderson 2004). We fit linear and quadratic models to the size spectra across the AMD gradient. We found that the quadratic model had far greater support than the linear model, despite the cost of extra parameters (Linear $AIC_c = 615$; Quadratic $AIC_c = 473$). However, due to the lack of quadratic relationships in previously published work, we wanted to compare the results obtained using both models, to see if there were significant differences in the conclusions reached.

Model selection was carried out for both the linear and quadratic models as described in the main text. For both models, a single top model was chosen ($\Delta AIC_c < 2.0$). Coefficient estimates are shown in Table S2. The linear model had lower estimates for the intercept, and the effect of PC1. However, all other shared coefficient estimates are similar. The major differences are in the model intercept, and the magnitude of the PC1 coefficient. This is likely due to the difference in estimated intercept (and therefore, the magnitude that PC1 changes the intercept) of a straight line compared with a parabolic curve. Additionally, the adjusted R^2 value was higher for the quadratic model, likely due to the extra explanatory power provided by accounting for the secondary curvature with the quadratic term. However, it is important to note that the overall conclusions of both models are largely in agreement. Both show a decrease in abundance (N) across increasing body mass (negative M coefficient). Likewise, both models show a decrease in intercept in response to PC1, and show highly similar changes to the slopes (M:PC1 coefficient) across the mining gradient.

Table S4 Coefficient estimates (SE) and adjusted R^2 values for both the linear and quadratic models, using $\log_2$ width bins. Significant coefficient estimates are in bold. Note the similarity of coefficient estimates.

	Intercept	M	PC1	M:PC1	M^2	ADJ R^2
Linear	**5.396**	**-1.00 (0.04)**	**-0.037 (0.01)**	**0.056 (0.01)**	--	0.7508
Quadratic	**5.806**	**-0.94 (0.03)**	**-0.077 (0.01)**	**0.055 (0.01)**	**-0.356 (0.03)**	0.8498

Comparing size spectra intercept with density estimates

To place our results in context, we compared the y-intercept results of our top model for size spectra (main text) with density estimates calculated directly from the Surber samples. The $\log_{10}$ transformed number of individuals in each Surber sample was regressed across the PC1 values. Model:

Log_{10} (No. individuals) = $\beta_0 + \beta_1\text{PC1} + \epsilon$

We found that the density of individuals significantly declines across the mining gradient (F-statistic$_{1,72}$ = 19.77, $P < 0.001$, adjusted $R^2 = 0.205$). The estimated coefficient for the effect of the mining gradient (β_{PC1}) on the $\log_{10}$ estimated density was -0.095. Although it's difficult to compare them directly, the sign and magnitude of this coefficient is similar to that found for the effect that the mining gradient had on the y-intercept of the size spectra (e.g. β_{PC1} in Table 3, main text = -0.077). Hence, we find general agreement in the decrease of the y-intercept in the size spectra model, and decreases in density calculated using more "traditional" means.

Table S5. Dominant taxa by site (listed in order of increasing impact), their relative abundance their functional feeding group (FFG), and their maximum, mean, and minimum dry weight in mg. The site scores from PC axis 1 are also listed for reference. AMD impacts start at Cannel stream. CB = collector-browser; G = grazer; FF = filter feeder; S = shredder; O = omnivore; P = predator.

Site	PC1	Taxa	Relative Abundance	FFG	Max mg	Mean mg	Min mg
Sull. East	-3.66	Deleatidium	0.378	CB	3.409	0.190	0.005
Sull. East	-3.66	Orthocladiinae	0.381	CB	1.645	0.383	0.020
Italian	-3.48	Aoteapsyche	0.116	O	8.868	0.275	0.004
Italian	-3.48	Beraeoptera	0.148	CB	0.477	0.163	0.002
Italian	-3.48	Deleatidium	0.151	CB	3.433	0.785	0.003
Italian	-3.48	Pycnocentria	0.155	CB	0.070	0.011	0.002
Burnet Trib	-3.20	Deleatidium	0.190	CB	2.291	0.641	0.028
Burnet Trib	-3.20	Oligochaete	0.145	CB	0.026	0.007	0.001
Burnet Trib	-3.20	Orthocladiinae	0.320	CB	2.351	0.672	0.019
Burnet Trib	-3.20	Zelandobius	0.130	CB	0.367	0.119	0.022
Coorang	-3.18	Austrosimulium	0.146	FF	0.575	0.207	0.003
Coorang	-3.18	Deleatidium	0.341	CB	3.291	0.340	0.007
Coorang	-3.18	Orthocladiinae	0.144	CB	2.904	0.691	0.007
Coorang	-3.18	Zelandobius	0.114	CB	1.745	0.226	0.016
Burnet	-3.12	Deleatidium	0.153	CB	2.310	0.285	0.008
Burnet	-3.12	Oligochaete	0.198	CB	0.139	0.017	0.001
Burnet	-3.12	Orthocladiinae	0.306	CB	2.154	0.287	0.006
Burnet	-3.12	Zelandobius	0.179	CB	0.461	0.182	0.027
Murray	-2.93	Deleatidium	0.314	CB	2.460	0.196	0.004
Murray	-2.93	Oligochaete	0.119	CB	0.028	0.011	0.001
Murray	-2.93	Orthocladiinae	0.166	CB	2.295	0.303	0.030
Charming	-2.79	Deleatidium	0.373	CB	2.489	0.212	0.002
Charming	-2.79	Oligochaete	0.324	CB	0.113	0.010	0.001
Charming	-2.79	Orthocladiinae	0.119	CB	1.446	0.203	0.009
Chasm	-2.54	Coloburiscus	0.107	FF	10.499	2.428	0.014
Chasm	-2.54	Deleatidium	0.285	CB	2.519	0.658	0.003
Chasm	-2.54	Orthocladiinae	0.124	CB	2.396	0.299	0.026
Chasm	-2.54	Paraleptamphopus	0.105	CB	1.852	0.161	0.001
Burke	-2.44	Chironominae	0.109	CB	1.302	0.179	0.014
Burke	-2.44	Oligochaete	0.133	CB	1.271	0.029	0.001
Burke	-2.44	Orthocladiinae	0.301	CB	1.430	0.191	0.015
Kiwi	-2.28	Deleatidium	0.505	CB	1.088	0.093	0.003
Kiwi	-2.28	Oligochaete	0.156	CB	0.062	0.010	0.001

Kiwi	-2.28	Orthocladiinae	0.125	CB	1.326	0.106	0.008
Lankey	-2.21	Coloburiscus	0.102	FF	8.660	0.267	0.010
Lankey	-2.21	Deleatidium	0.343	CB	5.046	0.321	0.003
One Horse	-1.74	Coloburiscus	0.111	FF	9.959	1.633	0.012
One Horse	-1.74	Deleatidium	0.247	CB	3.007	0.614	0.002
One Horse	-1.74	Orthocladiinae	0.339	CB	1.049	0.180	0.013
Cannel	-0.58	Aoteapsyche	0.200	O	3.762	2.200	1.291
Cannel	-0.58	Orthocladiinae	0.667	CB	0.273	0.089	0.019
Cbdale	-0.05	Oligochaete	0.161	CB	0.009	0.006	0.003
Cbdale	-0.05	Oxyethira	0.258	G	0.192	0.101	0.044
Cbdale	-0.05	Zelandobius	0.161	CB	0.440	0.145	0.042
Warne	0.01	Deleatidium	0.144	CB	3.374	0.690	0.015
Warne	0.01	Orthocladiinae	0.288	CB	0.416	0.212	0.031
Warne	0.01	Oxyethira	0.222	G	0.183	0.050	0.007
Warne	0.01	Spaniocercoides	0.111	CB	0.359	0.037	0.006
Stream X	0.18	Oligochaete	0.344	CB	0.040	0.012	0.004
Stream X	0.18	Spaniocerca	0.188	CB	0.330	0.067	0.003
Granity	1.56	Diamesinae	0.167	CB	0.990	0.424	0.041
Granity	1.56	Orthocladiinae	0.596	CB	1.009	0.235	0.016
Granity	1.56	Oxyethira	0.193	G	0.137	0.024	0.004
Sull. West	2.40	Diamesinae	0.701	CB	1.227	0.371	0.035
Sull. West	2.40	Orthocladiinae	0.175	CB	0.996	0.151	0.005
Packtrack	2.94	Orthocladiinae	0.991	CB	1.531	0.220	0.016
Miller	3.03	Diamesinae	0.195	CB	0.661	0.316	0.014
Miller	3.03	Orthocladiinae	0.756	CB	0.920	0.420	0.073
Mine	3.62	Diamesinae	0.581	CB	1.389	0.374	0.014
Mine	3.62	Orthocladiinae	0.370	CB	0.713	0.280	0.031
Coal	3.78	Orthocladiinae	0.649	CB	0.612	0.287	0.014
Stream Y	5.24	Empididae	0.576	P	0.217	0.121	0.053
Stream Y	5.24	Orthocladiinae	0.353	CB	0.734	0.274	0.045
Portal	5.64	Orthocladiinae	1.000	CB	0.877	0.196	0.012
Hot	5.80	Orthocladiinae	0.957	CB	0.651	0.245	0.029

Chapter Three:

Inferring predator-prey interactions in food webs

Preface

This chapter consists of an accepted manuscript titled *Inferring predator-prey interactions in food webs*. *Methods in Ecology and Evolution. In press.* This manuscript is co-authored by Timothée Poisot, Ross Thompson, and Jon Harding. As lead author of this manuscript, I designed the study, analyzed the data, developed the models, wrote the first and final draft of the manuscript and designed all tables and figures. Jon Harding provided funding for this project and advice on study design, Ross Thompson provided data, Timothée Poisot provided critical feedback on an early version of the manuscript and suggested additional analyses, and all co-authors provided feedback on the manuscript.

Abstract

Food webs are a powerful way to represent the diversity, structure, and function of ecological systems. However, the accurate description of food webs requires significant effort in time and resources, limiting their widespread use in ecological studies. Newly published methods allow for the inference of feeding interactions using proxy variables. Here, we compare the accuracy of two recently described methods, as well as describe a composite model of the two, for the inference of feeding interactions using a large, well-described dataset. Both niche and neutral processes are involved in determining whether or not two species will form a feeding link in communities. Three different models for determining niche constraints of feeding interactions were compared, and all three models were extended by incorporating neutral processes, based on relative abundances. The three models compared here infer niche processes through 1) phylogenetic relationships, 2) local species trait distributions (e.g. body size), and 3) a composite of phylogeny and local traits. We showed that all three methods perform well at predicting individual species interactions, and that these individual predictions scale up to the network level, resulting in food-web structure of inferred networks being similar to their

empirical counterparts. Our results indicated that inferring food-web structure using phylogenies can be an efficient way of getting summary webs with minimal data, and offers a conservative test of changes in food-web structure, particularly when there is low species turnover between sites. Inferences made using traits requires more data, but allows for greater understanding of the mechanisms underlying trophic interactions. A composite model of the two methods provides a framework for investigating the importance of how phylogeny, trait distributions, and relative abundances, affect species interactions, and network structure.

Introduction

Food webs represent networks of species' trophic interactions within an ecological community. Understanding the structure and function of food webs offers important insight into how communities change across environmental gradients (Thompson and Townsend 2004, Tylianakis, Tscharntke, and Lewis 2007, Layer et al. 2010, Pellissier et al. 2018), their potential resilience to disturbances (reviewed by Ives and Carpenter 2007), and offers a framework for integrating aspects of biodiversity and ecosystem function (reviewed by Thompson et al. 2012). The assembly of food webs in nature requires a significant commitment of sampling and laboratory effort and resources, which restricts their widespread use in ecological studies (Thompson et al. 2001, Woodward and Hildrew 2001, Gray et al. 2014, but see Jordano 2016 for a discussion of sampling ecological interactions). The lack of well-resolved (e.g. taxa identified to species level) and comparable (e.g. equal allocation of effort, consistent level of taxonomic identification) sets of food webs, particularly along environmental and stress gradients, is a significant impediment to better understanding food-web assembly and dynamics (Thompson and Townsend 2000, Dunne 2006, Thompson et al. 2012). Given this, there is a strong need for a robust method for accurately inferring species interactions from less detailed datasets, such as proxy data (Gray et al. 2014, Morales-Castilla et al. 2015).

Trophic interactions are largely determined by two processes, niche and neutral, which establish the possibility and realization of pairwise interactions, respectively (Canard et al. 2014, Poisot, Stouffer, and Gravel 2015, Bartomeus et al. 2016). We define niche and neutral processes *sensu* Poisot, Stouffer, and Gravel 2015), where niche processes are based on species traits and determine if species *can* interact, and neutral processes are based on their (local) relative abundances, and determine if they are *likely to encounter* one another. In other words, niche processes determine the probability of two species interacting based on some relevant biological characteristics such as body size (e.g. Brose et al. 2006), phenology (e.g. Hegland et al. 2009), or co-occurrence (e.g. Morales-Castilla et al. 2015). Species unable to interact based on these constraints are termed "niche forbidden links" (Olesen et al. 2008) and can be determined based on traits, phylogenetic relationships or geographic distribution (reviewed in Morales-Castilla et al. 2015).

The probability that species can interact with one another is conditional upon them being able to meet, the likelihood of which is driven by neutral processes (Canard et al. 2012, 2014). In an entirely neutral world, the probability of two species interacting is proportional to the product of their relative abundances (Canard et al. 2014, Poisot, Stouffer, and Gravel 2015), with more abundant species having a higher encounter probability and therefore more interactions (Canard et al. 2014). Interactions between rare species, below some yet undefined threshold in abundance, are termed "neutrally forbidden links" (Canard et al. 2012, Poisot, Stouffer, and Gravel 2015). Neutral processes can be accommodated by forbidding links between rare species, or by a random draw with probability proportional to the product of their relative abundances.

Two methods have recently been proposed for the inference of species trophic interactions as a result of niche processes; the ‘WebBuilder method’, which is based on phylogenetic relationships (Gray et al. 2015), and a ‘trait-matching method’ based on body size (Gravel et al. 2013). The WebBuilder method (Gray et al. 2015) is a function in the R programming language, which uses a registry of previously published feeding interactions which includes phylogenetic classifications. Feeding interactions are often determined by some combination of traits, which can be phylogenetically conserved, and evolutionary relationships have been used to accurately re-create empirical food-web structure (e.g. Eklöf et al. 2013). WebBuilder allows altering the level of relationship per taxa individually, and independently for their role as consumers and resources. This method allows for the transparent, reproducible construction of food webs, and only requires a list of taxa for a given site together with the registry of feeding interactions (Gray et al. 2015, 2016). A potential limitation of the WebBuilder method is that it assumes that if species have ever been documented to interact, they will always interact whenever they co-occur (Gray et al. 2015). This can lead to the over-representation of rare links (Poisot et al. 2016a), and insensitivity to changes in food-web structure in the absence of species turnover (Poisot et al. 2012, Gray et al. 2015).

The trait-matching method proposed by Gravel et al. (2013) also relies on previously published feeding interactions, but uses the body sizes of interacting partners to determine trophic links. The relationship between predator and prey body size in food webs is well documented (Brose et al. 2006, Riede et al. 2011, Eklöf et al. 2013), and is the basis of the niche food web model (*sensu* Williams and Martinez 2000). The trait-matching method uses $\log_{10}$-transformed body sizes of predators and their prey, with logistic regressions (e.g. 0.01 and 0.97 quantiles) to parameterize the niche food-web model of Williams and Martinez (2000). The trait-matching method has been shown to be remarkably robust to sampling effort, successfully predicting links even when up to 80% of the calibration data was withheld (Gravel et al. 2013). When measuring populations at a given location, this method allows for the variability of species interactions through space (assuming that the relationship is conserved) by taking into account local body-size distributions (Poisot, Stouffer, and Gravel 2015).

Here we inferred the pairwise species interactions for 17 previously published communities and food webs from streams in New Zealand (Thompson and Townsend 1999, 2004) using the WebBuilder and trait-matching methods separately, as well as a composite model combining both methods (Fig. 1). We assessed whether species pairs were capable of interacting (niche processes) and then examined interaction realization, by forbidding links between species whose relative abundance products were below arbitrarily defined thresholds (neutral processes). We hypothesized that both niche and neutral processes would be important in predicting pairwise species interactions, and that these processes would scale up to higher-level network properties, resulting in similar structure and food-web measures as those of the previously published empirical webs (Thompson and Townsend 1999, 2004)

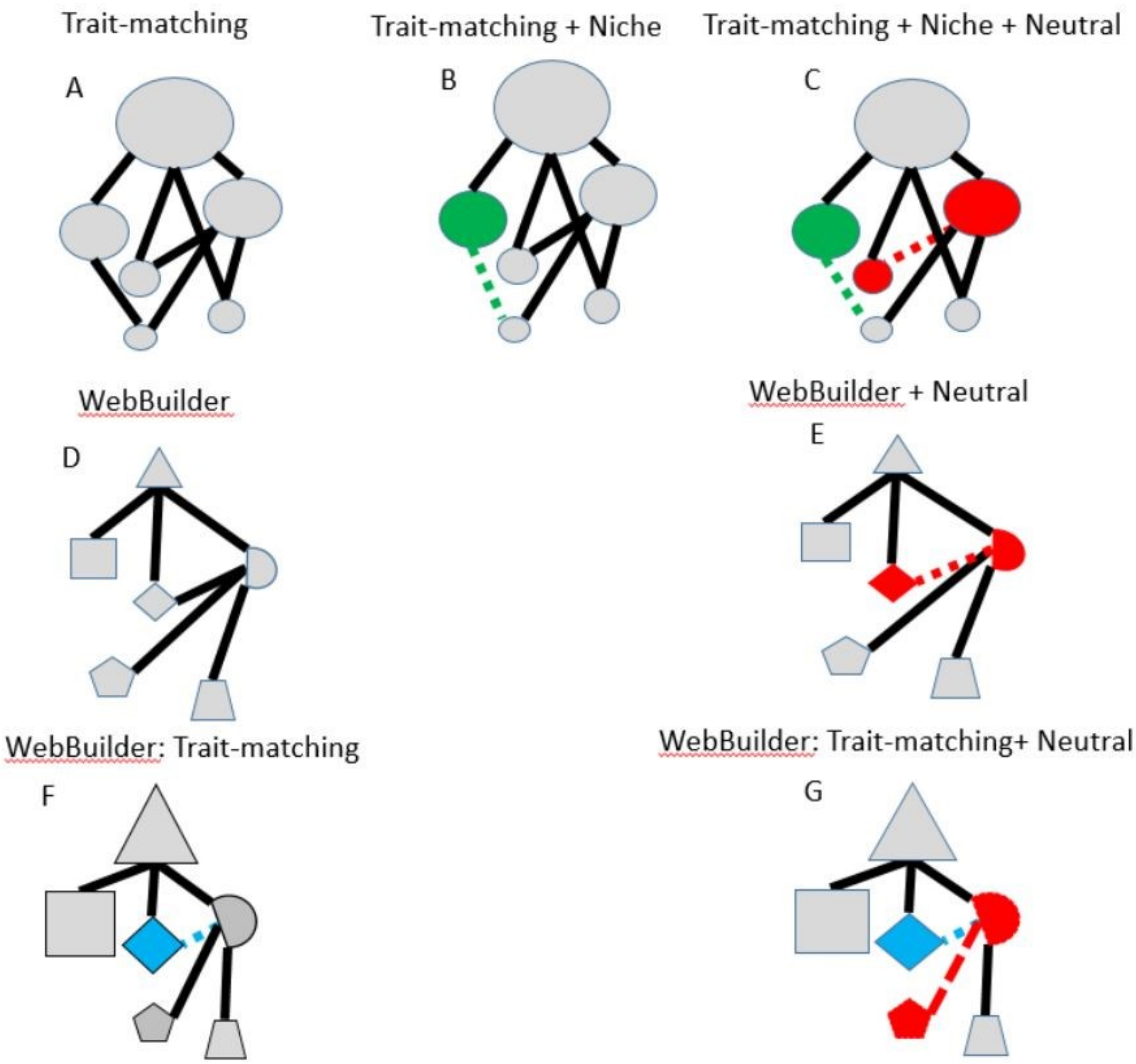


Figure 1. Conceptual illustration of the trait matching (A-C), WebBuilder (D-E) and composite (F-G) models tested. The nodes are represented by shapes, and feeding interactions are represented by solid black lines. The size of the shape corresponds to the average biomass of the node, while the shape corresponds to the taxonomic identity. Dashed lines represent links that were removed based on niche (green), or neutral (red) processes, or where trait distributions differ from expected (blue). A) Trait-matching biomass inference. B) Trait-matching + niche pruned (e.g. green circle is an herbivore). C) Neutrally forbidden links are removed. D) Nodes are assigned by taxonomic identity (shape). E) Neutrally forbidden links are removed. F) Links are assigned by taxonomic identity (shape) and body size (size of shape). The blue diamond is too large to be a prey of the half circle, and this link is removed. G) Neutrally forbidden links are removed.

Methods

Dataset description and preparation

The data used for evaluating these two methods were a series of previously published freshwater stream food webs from New Zealand (Thompson and Townsend 1999, 2004).The goal of the original studies was not to document every link within a food web, but to sample food webs using standardized allocations of effort and taxonomic resolution across streams in differing land-use types. Therefore, the observed links represent a "true" interaction, while un-observed links could represent either an actual lack of interaction, or could be a false negative (e.g. the species do interact but it was not detected by the original sampling; Jordano 2016, Stock et al. 2017). The original food webs included basal resources, invertebrate consumers and predators, and predatory fish. All fish in these streams are predatory. Only the predator-prey interactions (fish – invertebrate, and invertebrate – invertebrate) were used, as the relationship between basal resources (detritus and primary producers) and consumer body size follows different mathematical relationships (Riede et al. 2011). The animal communities were dominated at all sites by invertebrate taxa (>90%), and contained up to 4 fish species, with a majority of communities only containing a single fish species. In order to resolve naming discrepancies between food webs and associated metadata, original food webs were modified, and taxa were aggregated at the genus level for most groups (subfamily for members of Chironomidae, family for all other Diptera, Order for non-insect taxa).

The original studies included a total of 22 food webs from sites along 18 streams. Two of the streams (Dempsters and Sutton) were sampled seasonally (spring, summer and autumn), although only the spring and summer food webs from Sutton were available for the present analysis. Therefore, a total of 21 food webs were available for the WebBuilder registry (see below for details).

Average body lengths for invertebrate taxa were only available for 17 of the streams sampled during the summer season (all except Akatore A). This subset of food webs were available to parameterize coefficients for the niche model in the trait-matching method (see below for details). The body lengths were converted to dry mass estimates using published length-weight regressions for New Zealand fauna (Towers, Henderson, and Veltman 1994, Stoffels, Karbe, and Paterson 2003). The original data set included empirical estimates for all invertebrate taxa densities (Thompson and Townsend 1999, 2004), and these were used when considering neutral processes (see below for details).

Only data on fish presence/absence were available for these sites. We used the average minimum length per fish species for the Taieri watershed (the catchment that contained all empirical food webs) in the New Zealand Freshwater Fish database (https://www.niwa.co.nz/our-services/online-services/freshwater-fish-database). These lengths were used to estimate fish dry weights with length-weight regressions for New Zealand fish (Jellyman et al. 2013). Fish abundances were estimated using allometric scaling equations between body mass and abundance: $N = M^b$. Where N is the numerical abundance, and M is body mass. The scaling exponent b is theorized to equal -1 (Brown et al. 2004), and has been shown to vary from -0.1 to -1.5 in empirical data (Reuman et al. 2009). During preliminary analyses, we varied the exponent from -0.7 to -1.2. Although the estimated abundance of fish varied based on the value of the exponent (e.g. [min, max] no. m^{-2} for *Anguilla* = [0.010, 0.066] and *Galaxias* = [3.62, 9.11], respectively), this variation paled in comparison to the total number of macroinvertebrates per m^2 (e.g. 1,500 – 22,000). Furthermore, to estimate neutrally forbidden links, absolute abundances are converted to relative abundances (see below), and the degree of variation observed in response to varying the scaling exponent did not greatly alter the results of these models. Here we only present the results using an exponent of -1.

Only 17 of the stream food webs had all available information, so only these empirical food webs were inferred using the two methods. Of these 17 food webs, species average lengths were not available for all taxa present at a site. When a taxa did not have a length estimate, it was removed from the observed food webs, and the WebBuilder, and trait-matching inferences, in order to make the comparisons equitable (e.g. to ensure that the same number and identity of taxa were present for all comparisons). Additionally, when inferring feeding interactions for a given empirical food web, the local feeding interactions for that food web were withheld from the global WebBuilder registry and from the global trait-matching parameterization dataset, respectively, in order to avoid circularities. For example when inferring the pairwise interactions for Blackrock Stream, the WebBuilder registry only contained feeding interactions from the other 20 streams, while the trait-matching dataset to parameterize the niche model only contained predator-prey body sizes from the other 16 streams.

The food webs used in this analysis were modified from the originals in order to resolve discrepancies between the food web and associated metadata. All data manipulation and analyses were conducted in R version 3.3.3 (R Development Core Team 2017), and all of the data used in this analysis, along with R scripts for reproducibility, are available at [Dryad data DOI here upon article acceptance].

Trait-matching

The trait-matching method (Gravel et al. 2013) was implemented using parameter coefficients of quantile regressions (0.01, 0.5, 0.99) through Log_{10} transformed body sizes of interacting taxa to estimate the range (minimum, optimum, and maximum) of body sizes that a taxa can consume. Estimated dry weights of predator-prey pairwise interactions were compiled from all 17 food webs. Separate quantile regressions were performed for the parameterization of the niche model for each food web (with the local food-web data withheld from the global parameterization dataset).

After the models were parameterized, potential feeding links were inferred where the body size of a resource falls within the niche range of a consumer. Feeding links were transformed into adjacency matrices ($\mathbf{A}_{trait}$, Fig. 2A), which are square matrices (S x S, where S = the number of taxa present) in which the rows contain all taxa as a resource, and the columns contain all taxa as a consumer. Both rows and columns are ordered by increasing body size. $\mathbf{A}_{ij} = 1$ where taxa j can consume taxa i based on their relative body sizes, and 0 otherwise.

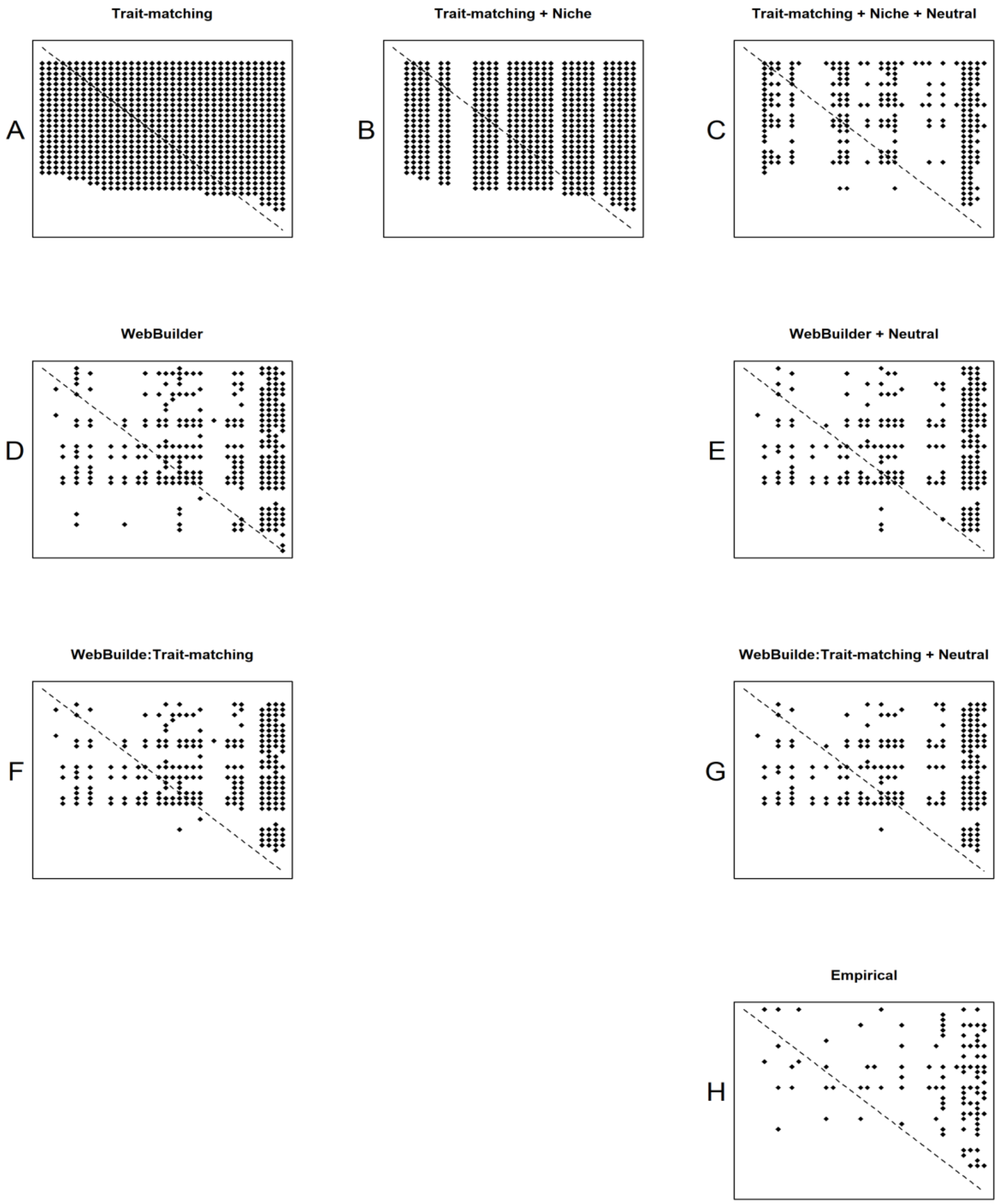


Figure 2. Example of predation matrices for Dempsters Creek for the trait-matching (top panel; A) initial inference, B) Niche pruned, C) Niche + Neutral pruned), WebBuilder (second panel; D) initial inference, E) Neutral pruned) and WebBuilder: trait-matching (third panel; F) initial inference, G) Neutral pruned) inferences. The empirical network (H) is also shown. All matrices are ordered by increasing body size from left to right and top to bottom.

Trait-matching inferred food webs were then pruned to remove niche forbidden links (Morales-Castilla et al. 2015). Niche forbidden links are defined here as links that are predicted to occur based on body size, but are impossible due to morphology. Links were pruned conservatively, and based on functional feeding groups as defined by morphology. For example, mayflies in the Leptophlebiidae family which have mouthparts specialized for "brushing" diatoms from benthic surfaces, and filter feeding taxa which have specialized morphological features, such as the cephalic fan of the blackfly genus *Austrosimulium*, were considered to be incapable of consuming animal prey. However, all filter feeders were not considered to be niche forbidden. For example, filter feeders which construct nets to collect food and have generalized mouthparts, such as the caddisfly genus *Aoteapsyche*, did not have their inferred links pruned. A list of all forbidden taxa present in the food web database is presented in Supplementary Table S1. Taxa considered to be forbidden had their column values in **A** set to zero (e.g. $\mathbf{A}_{i\bullet} = 0$, Fig. 2B).

Neutrally forbidden links were also pruned from the inferred food webs based on relative abundances (Canard et al. 2014). Relative abundances were used because they account for variable encounter probabilities based on total community abundance. Relative abundances were calculated using empirical estimates of invertebrate densities, along with fish densities calculated using allometric size density relationships. The relative abundance for a given species, $\mathbf{n}_i$, was calculated by dividing their abundance by the sum of all abundances at a site, respectively. Neutral abundance matrices (**N**) were constructed as $\mathbf{N}_{ij} = \mathbf{n}_i * \mathbf{n}_j$, where **n** is a vector of all species relative abundances. The product of all species pair relative abundances below a given threshold, $\mathbf{n}'$, were set to 0, and were considered to be neutrally forbidden (e.g. the probability of the two species meeting and interacting was so low as to be considered negligible). The two matrices were then multiplied together element-wise to calculate the adjacency matrices ($\mathbf{A}_{trait}' = \mathbf{N} * \mathbf{A}_{trait}$, Fig. 2C). $\mathbf{A}_{trait}'$ were the final inferred networks, and these were compared to results from the WebBuilder function (see below) and the observed networks ($\mathbf{A}_{obs}$, Fig. 2H).

As the probabilities of encounter are *proportional* to $\mathbf{N}_{ij}$, and it was unknown at what relative abundance a link was less likely to occur, a sensitivity analysis was conducted by setting the elements of $\mathbf{N}_{ij}$ below a given threshold of relative abundances equal to 0 (absent), and all elements greater than the threshold equal to 1 (present). This was done before multiplying them with the adjacency matrices ($\mathbf{A}_{trait}$), so that inferred links were retained *only* if they were present (e.g. equal to 1) in both the $\mathbf{A}_{trait}$ and the **N** matrices. Neutral thresholds were tested ranging from 1×10^{-8} to 0.1. Model performance was evaluated by calculating the area under the curve (AUC) of the receiver operating characteristic for each food web at each neutral abundance threshold. First, we fit a logistic regression using the glm() function in R, (family = binomial), where $\mathbf{A}_{trait}'$ was the single, binary, predictor variable, and $\mathbf{A}_{obs}$ was the response variable. We then used the prediction and performance functions from the ROCR package in R to calculate the AUC for each food web: neutral abundance threshold combination. AUC values were averaged across all webs for each neutral abundance threshold, and the threshold with the highest AUC average was chosen as our best model. Annotated R scripts for the full analysis presented here are available at https://github.com/Jpomz/honestly-you-eat-this. AUC values can be interpreted as the probability that a randomly chosen positive interaction receives a higher score than a randomly chosen negative interaction (e.g. AUC ≥ 0.5 has a ≥ 50% chance of being correct).

Pruning neutrally forbidden links performed well in predicting which invertebrate taxa interacted, however it performed poorly at predicting fish-invertebrate interactions. Abundances used in this analysis were calculated on a per m^2 basis, which likely encompasses the scale of interactions of an individual macroinvertebrate. However, fish have greater energetic requirements due to their body size, and have subsequently greater search and consumption rates (Pawar, Dell, and Savage 2012), necessitating larger foraging areas (Jetz et al. 2004). Ingestion rates were estimated based on body size using allometric relationships (Pawar, Dell, and Savage 2012), and found to be 3-4 orders of magnitude greater for fish than for invertebrate taxa. In order to take this into account, we multiplied the cross product of the fish's relative abundances by a correction factor. This artificially increases the relative abundance of fish, while still taking into account variable prey abundances. We examined increasing fish relative abundances from 1 to 4 orders of magnitude. All correction factors improved prediction outcomes, with larger correction factors resulting in greater average AUC values (Supplementary Fig. S1). Because ingestion rates for fish were approximately 3-4 order of magnitude larger than invertebrates, and a correction factor of 10^4 only slightly increased the AUC compared with a correction factor of 10^3 (Supplementary Fig. S1), despite being an order of magnitude larger, we used a correction factor of 10^3 in all models for all further analyses.

WebBuilder

WebBuilder is an R function originally presented in Appendix B in (Gray et al. 2015). The function was not modified from the original, and its full description and use is described elsewhere (Gray et al. 2015). Briefly, to infer feeding interactions at a site, a taxa list from that site is run through a registry of previously published feeding interactions. The registry contains the phylogenetic relationships of both interacting species (e.g. Order, Family, Genus), and interactions can be inferred based on varying levels of phylogenetic relationship. To implement the WebBuilder function in the present study, (Gray et al. 2015) all 21 webs in the Thompson and Townsend data set were compiled to make the registry of trophic links. Empirical links for a local food web were withheld in the construction of the registry for that web inference, in order to avoid circularities. Additional feeding interactions for New Zealand streams were compiled from published (sources in Supplementary Table S2) and unpublished data (K. Hogsden, R. Gregersen and P. Jellyman, unpublished data). Feeding links were inferred with the WebBuilder function and transformed into adjacency matrices ($\mathbf{A}_{wb}$ Fig. 2D). Neutrally forbidden links were removed (Fig. 2E), and a sensitivity analysis was carried out exactly as described above, after correcting the relative abundances of fish.

WebBuilder: trait-matching composite

In addition to testing the ability of the trait-matching and WebBuilder methods to predict feeding interactions, we examined a composite model of the two. The initial inferred matrices from both methods were combined, in order to infer links based on traits and phylogeny ($\mathbf{A}_{composite} = \mathbf{A}_{trait} * \mathbf{A}_{wb}$; Fig. 2F). Therefore, inferred links were only retained when both models predicted them. Neutrally forbidden links were pruned (Fig. 2G) after correcting for fish relative abundances, and a sensitivity analysis was carried out as above.

Comparing inferred food webs

We examined the predictive potential of a total of 7 models ($TM_{initial}$, TM_{niche}, $TM_{neutral+niche}$, $WB_{initial}$, $WB_{neutral}$, $TM{:}WB_{initial}$, $TM{:}WB_{neutral}$, where TM = trait-matching, WB = WebBuilder, and TM:WB = composite models; Fig. 1). All neutral models were assessed after correcting for fish relative abundances (see above). The performance of all of the models were evaluated by calculating the AUC, and the true skill statistic (TSS). The TSS is an overall measure of model performance, and is calculated as:

$$TSS = (ad - bc)/\big((a + c)(b + d)\big)$$

Where *a* is the proportion of true positives (link both predicted and observed), *b* is the proportion of false positives (link predicted but not observed), *c* is the proportion of false negatives (link not predicted, but observed), and *d* is the proportion of true negatives (link not predicted and not observed; Allouche, Tsoar, and Kadmon 2006). TSS scores range from 1 (perfect prediction) to -1 (inverted prediction).

In addition to total model performance, we examined the proportion of false positives (*b*) and false negatives (*c*) for each inference model. False positive rates are difficult to interpret, since it is extremely difficult to document all links in a food web (but see Stock et al. 2017) for evaluating likelihood of negative interactions in ecological network data). False positives may indicate links that do occur but were not recorded, or may falsely predict interactions that do not occur. False negatives, however, provide clear indications of a models' shortcomings (Morales-Castilla et al. 2015), by failing to predict a link that does occur empirically.

Food-web measures

A suite of common food-web measures were calculated for the networks inferred using the trait matching and WebBuilder methods, and these were compared to those of the observed networks. Seven measures were considered, including the number of links (L), connectance ($C = L/S^2$), proportion of basal (B), intermediate (I) and top (T) species, and the standard deviations of normalized generality (resources per consumer; G_i) and normalized vulnerability (consumers per resource; V_i):

$$G_i = \frac{1}{L/S}\sum_{j=1}^{S} a_{ji} \qquad V_i = \frac{1}{L/S}\sum_{j=1}^{S} a_{ij}$$

Normalizing with L/S makes these measures comparable across webs with different numbers of species (Williams and Martinez 2000). A principal components analysis (Legendre and Legendre 2012) was performed on food-web measures, and the 7 inference models were compared by measuring the Euclidean distance between them and the observed networks in multi-dimensional (n = 7) space.

Table 1. Comparison of model inferences and observed networks. Cross products of relative abundances below the neutral threshold were removed from inferences. False positive and negative values are proportions of links in the two categories. Euclidean distance is between the food-web measures calculated for model inferences and observed networks in multidimensional space.

Model	Inference step	Average AUC	Average TSS	Neutral threshold	False positive		False negative		Euclidean distance
					mean	(sd)	mean	(sd)	
Trait-matching	Initial	0.5457	0.0915	NA	0.8325	0.0986	0.0013	0.0019	24.5027
Trait-matching	Niche pruned	0.6513	0.3026	NA	0.6339	0.1100	0.0013	0.0019	5.7767
Trait-matching	Niche + Neutral pruned*	0.7680	0.5361	3.00E-04	0.2663	0.0893	0.0114	0.0099	4.2088
WebBuilder	Initial	0.8286	0.6573	NA	0.2042	0.0359	0.0059	0.0055	3.3091
WebBuilder	Neutral pruned*	0.8419	0.6837	1.50E-05	0.1724	0.0356	0.0065	0.0068	3.1795
WebBuilder: Trait-matching	Initial	0.8225	0.6449	NA	0.1966	0.0341	0.0069	0.0059	3.3365
WebBuilder: Trait-matching	Neutral pruned*	0.8342	0.6684	1.50E-05	0.1676	0.0342	0.0075	0.0074	3.1276

* Networks were pruned for neutrally forbidden links after multiplying relative fish abundances by a correction factor of 10^3.

Results

The number of pairwise interactions used to parameterize the trait matching model ranged from 379 to 469, depending on which stream was withheld from the training data. Approximately 14% of the pairwise interactions had resource body masses which were greater than consumer body masses. However, in these cases the consumer body size was between 70-100% of the body size of the resource (mean = 90%). The initial trait matching inferences were a poor predictor of feeding interactions, with an average AUC of 0.546, e.g. as accurate as a coin toss. However, predictions improved when pruning niche forbidden links, as well as neutrally forbidden links, after correcting for fish relative abundances (neutral threshold = 3 x 10^{-4}, Table 1) resulting in an average AUC of 0.768 (Supplementary Fig. S2). Average TSS also improved with each additional step (final TSS = 0.536 ± 0.112). Proportion of false positive links decreased from 0.833 (± 0.099) to 0.266 (± 0.090), while false negative links was low (e.g. < 0.02, Table 1) at all inference steps. The Euclidean distance in 7-dimensional space of the food web measures decreased with each additional step, from 24.50 to 4.21.

The registries used in the WebBuilder function varied from 1356 to 1488 pairwise interactions, depending on which stream was withheld. More than 50% of the links in the registries were from the studies originally published by Thompson and Townsend (1999, 2004). WebBuilder generally performed very well, with a high average AUC of 0.829, and a high mean TSS of 0.657 ± 0.100 (± SD, Table 1). Performance improved slightly when pruning neutrally forbidden links at a relative abundance threshold of 1 x 10^{-5} (Table 1, Supplementary Fig. S3). The average proportion of false positive links decreased slightly, while the proportion of false negatives remained essentially unchanged (Table 1). Euclidean distance of WebBuilder inferences were closer to the observed measures than the trait matching inferences, and decreased slightly when pruning neutrally forbidden links, from 3.31 to 3.18. Composite model performance was similar to WebBuilder models (Table 1).

Discussion

Understanding the structure and function of ecological networks is of both fundamental and applied interest (Memmott 2009, Thompson et al. 2012, McDonald-Madden et al. 2016). Knowledge of food webs' assembly rules and functioning can inform diverse management questions in conservation biology (McDonald-Madden et al. 2016), restoration ecology (Fraser et al. 2015), fisheries management (Eddy et al. 2017), pest management (Gómez-Marco et al. 2015) and in human-ecological systems (Dee et al. 2017). However the description of food webs requires considerable sampling and laboratory effort, limiting their widespread use (Thompson et al. 2001, Woodward and Hildrew 2001, Gray et al. 2014). Developing robust and repeatable approaches to inferring ecological network structure from community composition data would provide abundant new data to test basic food-web theory and develop management-relevant insights (Gray et al. 2014, Morales-Castilla et al. 2015). Here, we used two recently described methods for the inference of food-web structure (Gravel et al. 2013, Gray et al. 2015), and applied them to a dataset of existing stream food-webs.

All inferred networks had more links than their empirical counterparts. This result is not surprising, as it is extremely difficult to document all realized links at a site (Woodward, Speirs, and Hildrew 2005), even when considerable effort is expended, it is likely that observed links are significantly lower than those realized (Goldwasser and Roughgarden 1997, Martinez et al. 1999, Woodward, Speirs, and Hildrew 2005). Indeed, the original Thompson and Townsend (1999, 2004) studies clearly stated that the goal was not to describe every link, but to standardize the sampling effort and taxonomic resolution.

Models compared here significantly reduce the number of potential links within a site. The number of possible links at a site ranged from 225 to 1296, depending on the number of taxa present. The average proportion of realized links in the empirical networks was approximately 5%, while the models predicted approximately 25%. However, the reduction in false positive rates and consistently low false negative rate, means that these models are effective at discriminating against links which do not occur empirically, while retaining those that do. This is an encouraging result. First, links which are predicted but have not been observed empirically can inform future research by focusing observations, sampling effort, or designing experiments between species pairs which are predicted to interact (Stock et al. 2017). These new observations can be used to refine our predictive models, aiding in our ability to infer feeding interactions. Second, it suggests that these models are good at eliminating links which are trivially absent. Low false negative rates indicate that the models are accurately restricting links which are not observed empirically. Studying the difference, in ecological terms, between false and true positives will refine our understanding of the mechanisms involved in interactions.

The WebBuilder method infers feeding interactions based on phylogenetic identity and performed well. Phylogenetic identity can incorporate information on both niche and neutral processes. Closely related species share an evolutionary history, and are often similar in important traits that determine interactions, including body size, morphology, and behaviour (Eklöf et al. 2013). Traits of closely-related species may also lead to similar abundance hierarchies (e.g. all sites dominated by a common core of taxa; Thompson and Townsend 2004). Knowing the phylogenetic identity of a taxa can essentially account for much of the total variation in potential interactions by accounting for the full variability of species' trait distributions and relative numerical dominance. When traits and relative abundances within the populations are approximately as expected under "normal" conditions (e.g. local mean body size and numerical dominance similar to observations at most other locations), phylogenetic identity will likely be a good predictor of interactions. There was little variation in the average body size of a taxa across sites. Similar taxa were numerically dominant across sites, but their local abundances varied between sites. Therefore, phylogenetic identity alone was a good proxy variable for the traits which determine interactions. Forbidding links between locally rare species pairs did improve model predictions slightly, and will likely be an important mechanism to consider when inferring interactions across sites with a greater variability in local abundances. Likewise, further work is needed to explore the performance of phylogenetic inferences when inferring interactions across a large spatial scale where the trait distributions of local populations diverge (e.g. body size varies due to mean annual temperature changes across latitudinal or elevational gradients).

The performance of the WebBuilder function is highly dependent on the size and sources used for the registry. The predictive ability decreases when restricting the registry database size (Gray et al. 2015), and the predictions are entirely dependent on having sufficient observations between the taxa of interest, or at least closely related taxa. In the present study, there are a number of taxa that only occur in the Southern Hemisphere, so using a registry of feeding interactions compiled from Northern latitudes would do a poor job of predicting trophic interactions (e.g. see Poisot et al. 2016).

One limitation of the WebBuilder function is the inability to allow species interactions to vary. Currently, WebBuilder is likely to only be capable of detecting changes in food-web structure between sites when there is a large concomitant species turnover rate. This method has been used to infer changes in food-web structure across a changing pH gradient through space and time in the United Kingdom (Gray et al. 2016). However in that case, community composition altered through time with increasing pH due to recolonization by large bodied, acid intolerant predators (Layer et al. 2011, Gray et al. 2016). Since large taxa are generally more well connected due to their wide diet breadth (Brose et al. 2017), inferring food-web responses where they have recolonized may be an ideal use of WebBuilder. This may be particularly relevant to situations where large predators are being re-introduced (e.g. wolves in Yellowstone National Park; Smith, Peterson, and Houston 2003) and where large-bodied species go locally extinct (e.g. Christmas Island land crab; O'Dowd, Green, and Lake 2003) or are extirpated as a consequence of pest control (e.g. cat removal from Macquarie Island; Bergstrom et al. 2009).

WebBuilder appears to be very effective at inferring all potential feeding interactions in a community. Diets can change seasonally (Thompson and Townsend 1999), and results of food-web studies are generally only comparable if they were collected at the same time. Food webs constructed using gut content analysis only provides a snapshot in time, and is unlikely to illustrate all feeding links present (Thompson et al. 2001). WebBuilder describes "summary webs" that may be useful for controlling effects of sample effort, and allow for conservative comparisons of food webs across large spatial scales (Warren 1990, Thompson and Townsend 2003) along environmental gradients (Post, Pace, and Hairston 2000, McHugh, McIntosh, and Jellyman 2010) or to seek fundamental ecological patterns (Martinez 1992, Strong 1992).

The trait-matching method requires more data and inference steps, but was able to accurately infer food-web structure. This adds to the growing body of literature which supports the importance of both neutral and niche processes in determining interactions (Canard et al. 2014, Poisot, Stouffer, and Gravel 2015). Furthermore, the structure of many food webs is strongly determined by body size relationships (Brose et al. 2006, Eklöf et al. 2013, Laigle et al. 2018), and we feel that the methods outlined here would apply to a range of habitats and ecosystems. Because biomass, trait values, and abundances of taxa can vary based on location, and the trait-matching method is a function of these variables, it may provide a more site-specific inference of interactions. Thus, this method may be more appropriate for addressing questions that look at local changes in food-web structure at smaller scales (Nakano, Miyasaka, and Kuhara 1999, Thompson and Townsend 2005, Layer et al. 2011), or for predicting interactions among novel species pairs (e.g. invasive species, range shifts due to climate change; Van der Putten, Macel, and Visser 2010), where information on interactions may be lacking. Paradoxically, it may also perform better at predicting interactions across large spatial scales, particularly at the edges of a species geographic distribution, where trait values may differ more significantly.

Another potential strength of the trait matching method is the ability to infer the probability of a link occurring, rather than as a simple binary network. Bartomeus et al. (2016) extended the framework of Gravel et al. (2013) to estimate probabilities of a link occurring between two species based on traits. Newly developed tools allow for the probabilistic analysis of ecological networks, which allows for researchers to incorporate the inherit dynamic variability in species interactions within a network (Poisot et al. 2016a). At present, the WebBuilder function only provides presence/absence data on interactions. It is possible to modify WebBuilder by using species average traits, but this would negate the use of site-specific data.

The composite model performed similarly to WebBuilder on its own. This is an interesting finding, because it shows that both models essentially predicted the same interactions. This offers the potential to explore the relative importance of phylogenetic identity, local trait distributions, and neutral processes all in a single model. For example, it allows for the understanding of food webs in environments where species trait distributions are being skewed in a consistent manner, such as commercial fishing, which preferentially removes the largest individuals (Jennings and Blanchard 2004) shifting the distribution of biomass within a species, without necessarily removing them completely.

The need to artificially increase fish relative abundances to account for neutrally forbidden links was an interesting, and unexpected, result. When present in empirical webs, fish consumed approximately half of all prey species present. The large body size of fish, as compared with invertebrate consumers, offers a number of potential explanations for this observation. Firstly, energetic demands scale with body size which results in greater consumption rates. This in turn can lead to larger foraging areas (Jetz et al. 2004), or segregation of individuals into size-based hierarchies and optimal foraging locations to maximize net energetic intakes (Gowan and Fausch 2002). Whether fish have larger foraging areas, segregate into optimal foraging locations, or both, at the population scale they are sampling a range of habitats with varying hydrologic conditions and productivity levels. Therefore, fish diet samples are a representation of the food web at the reach scale (10^1 - 10^2 m^2) whereas invertebrate diet samples are at the channel unit scale (e.g. invertebrates generally sampled from riffles; 10^{-1} to 10^{-2} m^2). An alternative, but not mutually exclusive, explanation may be an increased detection probability in fish diets. Fish stomachs have a greater volume, and their foraging habits of swallowing prey whole lead to a higher likelihood of detecting identifiable body parts. When inferring interactions, it is important to consider the different sampling scales and detection probabilities, and which compartments of the meta-food web these samples represent.

All approaches offer powerful ways to infer food-web structure in the absence of well-quantified empirical data on trophic interactions, but they can also be used to aid in our understanding of both theoretical and applied issues. Predicted links that have not been observed empirically can inform sampling to be verified in the field (Stock et al. 2017). Likewise, links which are predicted to occur but only observed variably can aid our understanding of how interactions change through space and time (Poisot et al. 2012), and identify other important traits or ecological variables that affect link presence. However, future work is needed to explore food web inferences where species trait distributions and abundances vary more significantly between sites, and the effects that sampling scale has on food web structure. Additionally, application of these inference methods needs to recognize their relative strengths, and direct the appropriate method to the spatial and temporal scale of the research question being considered.

Acknowledgements:

We thank C Perovich for help with data analysis and thoughtful discussion, and P Jellyman and K Hogsden for helpful comments on an early draft.

References

Allouche, O., A. Tsoar, and R. Kadmon. 2006. Assessing the accuracy of species distribution models: prevalence, kappa and the true skill statistic (TSS). Journal of Applied Ecology **43**:1223–1232. https://doi.org/10.1111/j.1365-2664.2006.01214.x.

Bartomeus, I., D. Gravel, J. M. Tylianakis, M. A. Aizen, I. A. Dickie, and M. Bernard-Verdier. 2016. A common framework for identifying linkage rules across different types of interactions. Functional Ecology **30**:1894–1903. https://doi.org/10.1111/1365-2435.12666.

Bergstrom, D. M., A. Lucieer, K. Kiefer, J. Wasley, L. Belbin, T. K. Pedersen, and S. L. Chown. 2009. Indirect effects of invasive species removal devastate World Heritage Island. Journal of Applied Ecology **46**:73–81. https://doi.org/10.1111/j.1365-2664.2008.01601.x.

Brose, U., J. L. Blanchard, A. Eklöf, N. Galiana, M. Hartvig, M. R. Hirt, G. Kalinkat, M. C. Nordström, E. J. O'Gorman, B. C. Rall, F. D. Schneider, E. Thébault, and U. Jacob. 2017. Predicting the consequences of species loss using size-structured biodiversity approaches. Biological Reviews **92**:684–697. https://doi.org/10.1111/brv.12250.

Brose, U., T. Jonsson, E. L. Berlow, P. Warren, C. Banasek-Richter, L.-F. Bersier, J. L. Blanchard, T. Brey, S. R. Carpenter, M.-F. C. Blandenier, L. Cushing, H. A. Dawah, T. Dell, F. Edwards, S. Harper-Smith, U. Jacob, M. E. Ledger, N. D. Martinez, J. Memmott, K. Mintenbeck, J. K. Pinnegar, B. C. Rall, T. S. Rayner, D. C. Reuman, L. Ruess, W. Ulrich, R. J. Williams, G. Woodward, and J. E. Cohen. 2006. Consumer-resource body-size relationships in natural food webs. Ecology **87**:2411–2417. https://doi.org/10.1890/0012-9658(2006)87[2411:CBRINF]2.0.CO;2.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. Ecology **85**:1771–1789. https://doi.org/10.1890/03-9000.

Canard, E., N. Mouquet, L. Marescot, K. J. Gaston, D. Gravel, and D. Mouillot. 2012. Emergence of structural patterns in neutral trophic networks. PLOS ONE **7**:e38295. https://doi.org/10.1371/journal.pone.0038295.

Canard, E., N. Mouquet, D. Mouillot, M. Stanko, D. Miklisova, and D. Gravel. 2014. Empirical evaluation of neutral interactions in host-parasite networks. The American Naturalist **183**:468–479. https://doi.org/10.1086/675363.

Dee, L. E., S. Allesina, A. Bonn, A. Eklöf, S. D. Gaines, J. Hines, U. Jacob, E. McDonald-Madden, H. Possingham, M. Schröter, and R. M. Thompson. 2017. Operationalizing network theory for ecosystem service assessments. Trends in Ecology and Evolution **32**:118–130. https://doi.org/10.1016/j.tree.2016.10.011.

Dunne, J. A. 2006. The Network Structure of Food Webs. Pages 27–86 *in* M. Pascual and J. A. Dunne, editors. Ecological Networks: Linking Structure to Dynamics in Food Webs. Oxford University Press, New York.

Eddy, T. D., H. K. Lotze, E. A. Fulton, M. Coll, C. H. Ainsworth, J. N. de Araújo, C. M. Bulman, A. Bundy, V. Christensen, J. C. Field, N. A. Gribble, M. Hasan, S. Mackinson, and H. Townsend. 2017. Ecosystem effects of invertebrate fisheries. Fish and Fisheries **18**:40–53. https://doi.org/10.1111/faf.12165.

Eklöf, A., U. Jacob, J. Kopp, J. Bosch, R. Castro-Urgal, N. P. Chacoff, B. Dalsgaard, C. de Sassi, M. Galetti, P. R. Guimarães, S. B. Lomáscolo, A. M. Martín González, M. A. Pizo, R. Rader, A. Rodrigo, J. M. Tylianakis, D. P. Vázquez, S. Allesina, and E. Letters. 2013. The dimensionality of ecological networks. Ecology Letters **16**:577–583. https://doi.org/10.1111/ele.12081.

Fraser, L. H., W. L. Harrower, H. W. Garris, S. Davidson, P. D. N. Hebert, R. Howie, A. Moody, D. Polster, O. J. Schmitz, A. R. E. Sinclair, B. M. Starzomski, T. P. Sullivan, R. Turkington, and D. Wilson. 2015. A call for applying trophic structure in ecological restoration. Restoration Ecology **23**:503–507. https://doi.org/10.1111/rec.12225.

Goldwasser, L., and J. Roughgarden. 1997. Sampling effects and the estimation of food-web properties. Ecology **78**:41–54. https://doi.org/10.1890/0012-9658(1997)078[0041:SEATEO]2.0.CO;2.

Gómez-Marco, F., A. Urbaneja, J. A. Jaques, P. F. Rugman-Jones, R. Stouthamer, and A. Tena. 2015. Untangling the aphid-parasitoid food web in citrus: can hyperparasitoids disrupt biological control? Biological Control **81**:111–121. https://doi.org/10.1016/j.biocontrol.2014.11.015.

Gowan, C., and K. D. Fausch. 2002. Why do foraging stream salmonids move during summer? Environmental Biology of Fishes **64**:139–153.

Gravel, D., T. Poisot, C. Albouy, L. Velez, and D. Mouillot. 2013. Inferring food web structure from predator–prey body size relationships. Methods in Ecology and Evolution **4**:1083–1090. https://doi.org/10.1111/2041-210X.12103.

Gray, C., D. J. Baird, S. Baumgartner, U. Jacob, G. B. Jenkins, E. J. O'Gorman, X. Lu, A. Ma, M. J. O. Pocock, N. Schuwirth, M. Thompson, and G. Woodward. 2014. Ecological networks: the missing links in biomonitoring science. Journal of Applied Ecology **51**:1444–1449. https://doi.org/10.1111/1365-2664.12300.

Gray, C., D. Figueroa, L. Hudson, A. Ma, D. Perkins, and G. Woodward. 2015. Joining the dots: an automated method for constructing food webs from compendia of published interactions. Food Webs **5**:11–20. http://dx.doi.org/10.1016/j.fooweb.2015.09.001.

Gray, C., A. G. Hildrew, X. Lu, A. Ma, D. Mcelroy, and D. Monteith. 2016. Recovery and nonrecovery of freshwater food webs from the effects of acidification. Advances in Ecological Research **55**:475–534. https://doi.org/10.1016/bs.aecr.2016.08.009.

Hegland, S. J., A. Nielsen, A. Lázaro, A.-L. Bjerknes, and Ø. Totland. 2009. How does climate warming affect plant-pollinator interactions? Ecology Letters **12**:184–195. https://doi.org/10.1111/j.1461-0248.2008.01269.x.

Ives, A. R., and S. R. Carpenter. 2007. Stability and diversity of ecosystems. Science **317**:58–62. https://doi.org/10.1126/science.1133258.

Jellyman, P. G., D. J. Booker, S. K. Crow, M. L. Bonnett, and D. J. Jellyman. 2013. Does one size fit all? An evaluation of length-weight relationships for New Zealand's freshwater fish species. New Zealand Journal of Marine and Freshwater Research **47**:450–468. https://doi.org/10.1080/00288330.2013.781510.

Jennings, S., and J. L. Blanchard. 2004. Fish abundance with no fishing: predictions based on macroecological theory. Journal of Animal Ecology **73**:632–642.

Jetz, W., C. Carbone, J. Fulford, and J. H. Brown. 2004. The scale of animal space use. Science **356**:266–268. https://doi.org/10.1126/science.1102138.

Jordano, P. 2016. Sampling networks of ecological interactions. Functional Ecology **30**:1883–1893. https://doi.org/10.1111/1365-2435.12763.

Laigle, I., I. Aubin, C. Digel, U. Brose, I. Boulangeta, and D. Gravel. 2018. Species traits as drivers of food web structure. Oikos **127**:316–326. https://doi.org/10.1111/oik.04712.

Layer, K., A. G. Hildrew, G. B. Jenkins, J. O. Riede, J. Stephen, C. R. Townsend, G. Woodward, L. D. Well-, K. Layer, A. G. Hildrew, and G. B. Jenkins. 2011. Long-term dynamics of a well-characterised food web: four decades of acidification and recovery in the Broadstone Stream model system. Advances in Ecological Research **44**:69–117. https://doi.org/10.1016/B978-0-12-374794-5.00002-X.

Layer, K., J. O. Riede, A. G. Hildrew, and G. Woodward. 2010. Food web structure and stability in 20 streams across a wide pH gradient. Advances in Ecological Research **42**:265–299.

Legendre, P., and L. Legendre. 2012. Chapter 9 - Ordination in reduced space. Pages 425–520 *in* P. Legendre and L. Legendre, editors. Developments in Environmental Modelling. Elsevier, Amsterdam. https://doi.org/10.1016/B978-0-444-53868-0.50009-5.

Martinez, N. D. 1992. Constant connectance in community food webs. The American Naturalist **139**:1208–1218.

Martinez, N. D., B. A. Hawkins, H. A. Dawah, and B. P. Feifarek. 1999. Effects of sampling effort on characterization of food-web structure. Ecology **80**:1044–1055. https://doi.org/10.2307/177037.

McDonald-Madden, E., R. Sabbadin, E. T. Game, P. W. J. Baxter, I. Chadès, and H. P. Possingham. 2016. Using food-web theory to conserve ecosystems. Nature Communications **7**:10245. https://doi.org/10.1038/ncomms10245.

McHugh, P. A., A. R. McIntosh, and P. G. Jellyman. 2010. Dual influences of ecosystem size and disturbance on food chain length in streams. Ecology Letters **13**:881–890. https://doi.org/10.1111/j.1461-0248.2010.01484.x.

Memmott, J. 2009. Food webs: a ladder for picking strawberries or a practical tool for practical problems? Philosophical Transactions of the Royal Society B: Biological Sciences **364**:1693 LP – 1699.

Morales-Castilla, I., M. G. Matias, D. Gravel, and M. B. Arau. 2015. Inferring biotic interactions from proxies. Trends in Ecology & Evolution **30**:347–356. https://doi.org/10.1016/j.tree.2015.03.014.

Nakano, S., H. Miyasaka, and N. Kuhara. 1999. Terrestrial-aquatic linkages: riparian arthropod inputs alter trophic cascades in a stream food web. Ecology **80**:2435–2441.

O'Dowd, D. J., P. T. Green, and P. S. Lake. 2003. Invasional 'meltdown' on an oceanic island. Ecology Letters **6**:812–817. https://doi.org/10.1046/j.1461-0248.2003.00512.x.

Olesen, J. M., J. Bascompte, H. Elberling, and P. Jordano. 2008. Temporal dynamics in a pollination network. Ecology **89**:1573–1582. https://doi.org/10.1890/07-0451.1.

Pawar, S., A. I. Dell, and V. M. Savage. 2012. Dimensionality of consumer search space drives trophic interaction strengths. Nature **486**:485–489. https://doi.org/10.1038/nature11131.

Pellissier, L., C. Albouy, J. Bascompte, N. Farwig, C. Graham, M. Loreau, M. A. Maglianesi, C. J. Melián, C. Pitteloud, T. Roslin, R. Rohr, S. Saavedra, W. Thuiller, G. Woodward, N. E. Zimmermann, and D. Gravel. 2018. Comparing species interaction networks along environmental gradients. Biological Reviews **93**:785–800. https://doi.org/10.1111/brv.12366.

Poisot, T., E. Canard, D. Mouillot, N. Mouquet, and D. Gravel. 2012. The dissimilarity of species interaction networks. Ecology Letters **15**:1353–1361. https://doi.org/10.1111/ele.12002.

Poisot, T., A. R. Cirtwill, K. Cazelles, D. Gravel, M.-J. Fortin, and D. B. Stouffer. 2016a. The structure of probabilistic networks. Methods in Ecology and Evolution **7**:303–312. https://doi.org/10.1111/2041-210X.12468.

Poisot, T., D. Gravel, S. Leroux, S. A. Wood, M. J. Fortin, B. Baiser, A. R. Cirtwill, M. B. Araújo, and D. B. Stouffer. 2016b. Synthetic datasets and community tools for the rapid testing of ecological hypotheses. Ecography **39**:402–408. https://doi.org/10.1111/ecog.01941.

Poisot, T., D. B. Stouffer, and D. Gravel. 2015. Beyond species: why ecological interaction networks vary through space and time. Oikos **124**:243–251. https://doi.org/10.1111/oik.01719.

Post, D. M., M. L. Pace, and N. G. Hairston. 2000. Ecosystem size determines food-chain length in lakes. Nature **405**:1047–1049.

R Development Core Team. 2017. R: A language and environment for statistical computing. R Core Team, Vienna, Austria.

Reuman, D. C., C. Mulder, C. Banašek-Richter, M. F. Cattin Blandenier, A. M. Breure, H. D. Hollander, J. M. Kneitel, D. Raffaelli, G. Woodward, and J. E. Cohen. 2009. Allometry of body size and abundance in 166 food webs. Advances in Ecological Research **41**:1–44. https://doi.org/10.1016/S0065-2504(09)00401-2.

Riede, J. O., U. Brose, B. Ebenman, U. Jacob, C. R. Townsend, R. M. Thompson, C. R. Townsend, and T. Jonsson. 2011. Stepping in Elton's footprints: a general scaling model for body masses and trophic levels across ecosystems. Ecology Letters **14**:169–178. https://doi.org/10.1111/j.1461-0248.2010.01568.x.

Smith, D. W., R. O. Peterson, and D. B. Houston. 2003. Yellowstone after Wolves. BioScience **53**:330–340.

Stock, M., T. Poisot, W. Waegeman, and B. D. Baets. 2017. Linear filtering reveals false negatives in species interaction data. Scientific Reports **7**:1–8. https://doi.org/10.1038/srep45908.

Stoffels, R. J., S. Karbe, and R. A. Paterson. 2003. Length-mass models for some common New Zealand littoral-benthic macroinvertebrates, with a note on within-taxon variability in parameter values among published models. New Zealand Journal of Marine and Freshwater Research **37**:449–460. https://doi.org/10.1080/00288330.2003.9517179.

Strong, D. R. 1992. Are trophic cascades all wet? Differentiation and donor-control in speciose ecosystems. Ecology **73**:747–754. https://doi.org/10.2307/1940154.

Thompson, R. M., U. Brose, J. A. Dunne, R. O. H. Jr, S. Hladyz, R. L. Kitching, N. D. Martinez, H. Rantala, T. N. Romanuk, D. B. Stouffer, and J. M. Tylianakis. 2012. Food webs:

reconciling the structure and function of biodiversity. Trends in Ecology & Evolution **27**:689–697. https://doi.org/10.1016/j.tree.2012.08.005.

Thompson, R. M., E. D. Edwards, A. R. McIntosh, and C. R. Townsend. 2001. Allocation of effort in stream food-web studies: the best compromise? Marine and Freshwater Research **52**:339–345.

Thompson, R. M., and C. R. Townsend. 1999. The effect of seasonal variation on the community structure and food-web attributes of two streams: implications for food-web science. Oikos **87**:75–88.

Thompson, R. M., and C. R. Townsend. 2000. Is resolution the solution?: the effect of taxonomic resolution on the calculated properties of three stream food webs. Freshwater Biology **44**:413–422.

Thompson, R. M., and C. R. Townsend. 2003. Impacts on stream food webs of native and exotic forest: an intercontinental comparison. Ecology **84**:145–161.

Thompson, R. M., and C. R. Townsend. 2004. Land-use influences on New Zealand stream communities: Effects on species composition, functional organisation, and food-web structure. New Zealand Journal of Marine and Freshwater Research **38**:595–608. https://doi.org/10.1080/00288330.2004.9517265.

Thompson, R. M., and C. R. Townsend. 2005. Food-web topology varies with spatial scale in a patchy environment. Ecology **86**:1916–1925.

Towers, D. J., I. M. Henderson, and C. J. Veltman. 1994. Predicting dry weight of New Zealand aquatic macroinvertebrates from linear dimensions. New Zealand Journal of Marine and Freshwater Research **28**:159–166. https://doi.org/10.1080/00288330.1994.9516604.

Tylianakis, J. M., T. Tscharntke, and O. T. Lewis. 2007. Habitat modification alters the structure of tropical host – parasitoid food webs. Nature **445**. https://doi.org/10.1038/nature05429.

Van der Putten, W. H., M. Macel, and M. E. Visser. 2010. Predicting species distribution and abundance responses to climate change: why it is essential to include biotic interactions across trophic levels. Philosophical Transactions Royal Society B **365**:2025–2034. https://doi.org/10.1098/rstb.2010.0037.

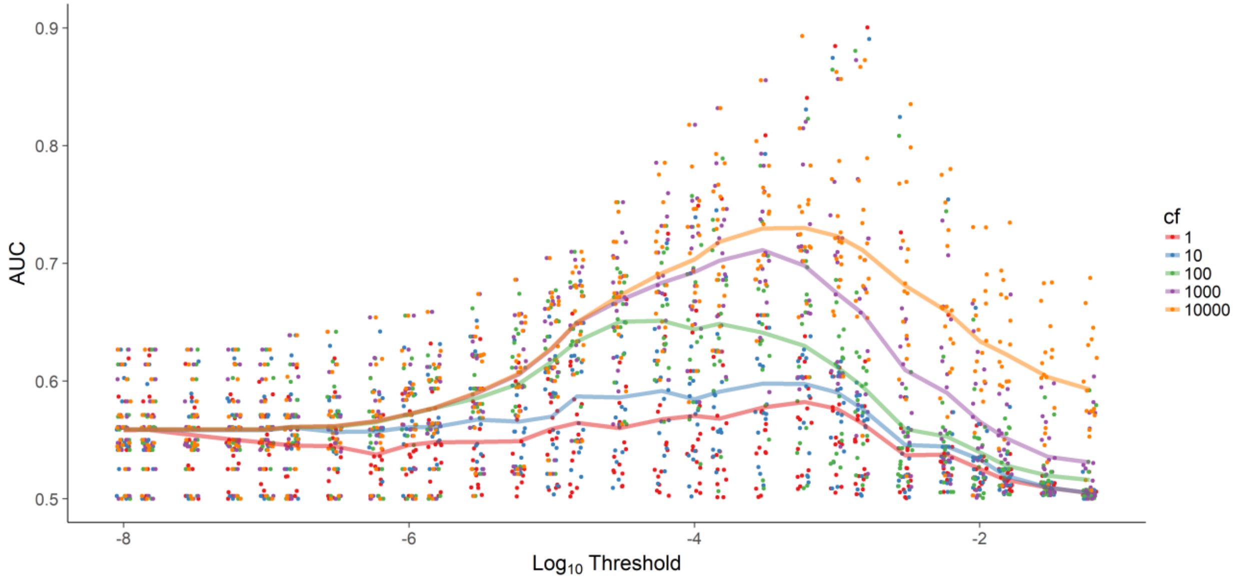
Warren, P. H. 1990. Variation in wood-web structure: the determinants of connectance. The American Naturalist **136**:689–700. https://doi.org/10.1086/285123.

Williams, R. J., and N. D. Martinez. 2000. Simple rules yield complex food webs. Nature **404**:180–183. https://doi.org/10.1038/35004572.

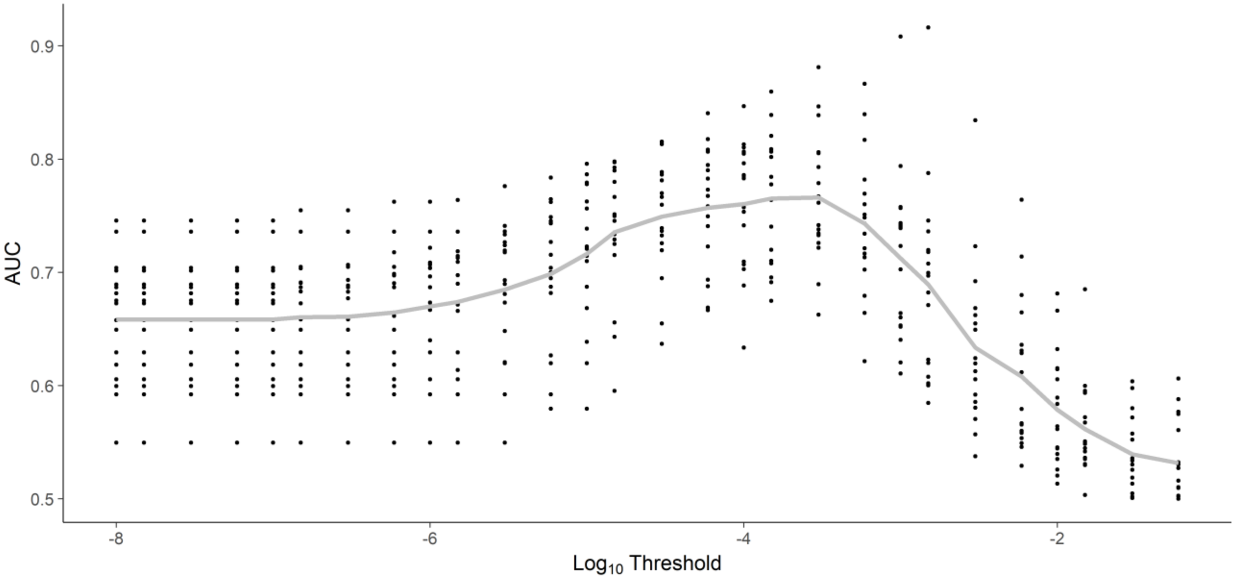
Woodward, G., and A. G. Hildrew. 2001. Invasion of a stream food web by a new top predator. Journal of Animal Ecology **70**:273–288.

Woodward, G., D. C. Speirs, and A. G. Hildrew. 2005. Quantification and resolution of a complex, size-structured food web. Advances in Ecological Research **36**:85–135. https://doi.org/10.1016/S0065-2504(05)36002-8.

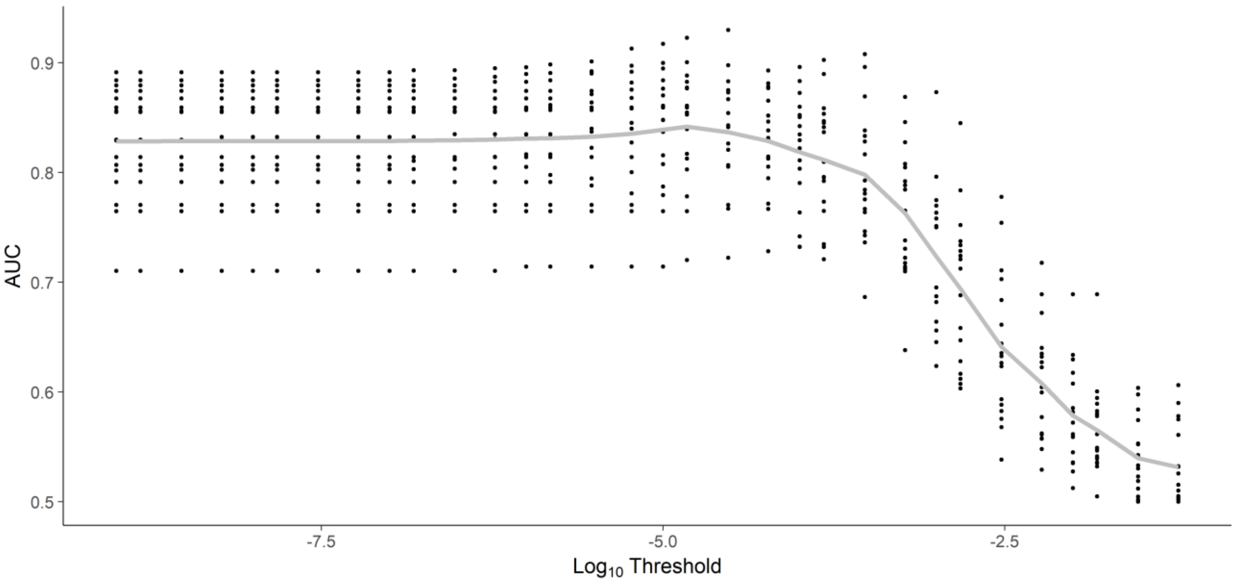
Supplementary Material



Supplemental Figure S1. AUC for the trait-matching inference varies in response to neutral abundance threshold **n'** and correction factor used. Five correction factors tested, red = 1 (no correction); blue = 10; green = 100, purple = 1000, yellow = 10 000. Each point is the AUC value for a single site at the neutral abundance threshold (x-axis), with colors matching the correction factor used (e.g. each threshold + color combination has 17 points). Points are jittered for visualization. Lines represent average AUC values for a given threshold. Consumption rates of fish were calculated to be ~3-4 orders of magnitude greater than invertebrates, and the highest average AUC values were at correction factor of 10^3 to 10^4.However, the correction factor of 10^4 increases the AUC values only slightly more than a correction factor of 10^3, despite being an order of magnitude larger. Therefore, a correction factor of 10^3 was used in all further analyses.



Supplemental Figure S2. AUC values for trait-matching inferences vary in response to neutral abundance threshold **n’**. Points are AUC values for individual food webs after pruning niche forbidden links, and multiplying fish relative abundances by 10^3, grey line is average AUC value at that threshold.



Supplemental Figure S3. AUC values for WebBuilder inferences vary in response to neutral abundance threshold **n’**. Points are AUC values for individual food webs after multiplying fish relative abundances by 10^3, grey line is average AUC value at that threshold.

Supplementary Table S1. Niche-forbidden taxa. Taxa considered to be non-predatory based on morphological features.

Non-predatory taxa	Justification
Amphipoda	Filter feeder
Atalophlebioides sp.	“Brushing” mouthparts
Austroclima sp.	“Brushing” mouthparts
Austrosimulium australense	Filter feeder
Coloburiscus humeralis	Filter feeder
Deleatidium spp.	“Brushing” mouthparts
Nesameletus ornatus	Biofilm “Grazer”
Oligochaetae	Detritivore / deposit feeder
Oxyethira albiceps	Biofilm “Grazer”
Potamopyrgus antipodarum	Biofilm “Grazer”
Zephlebia spp.	“Brushing” mouthparts

Supplementary Table S2. Additional sources of published New Zealand freshwater feeding interactions added to the WebBuilder registry.

Bonnett M.L. & Lambert P.W. (2002) Diet of giant kokopu, Galaxias argenteus. New Zealand Journal of Marine and Freshwater Research **36**, 361–369.
Collier K.J., Wright-Stow A.E. & Smith B.J. (2004) Trophic basis of production for a mayfly in a North Island, New Zealand, forest stream: Contributions of benthic versus hyporheic habitats and implications for restoration. New Zealand Journal of Marine and Freshwater Research **38**, 301–314.
Cowley D.R. (1978) Studies on the larvae of New Zealand Trichoptera. New Zealand Journal of Zoology **5**, 639–750.
Crosby T.K. (1975) Food of the New Zealand trichopterans Hydrobiosis parumbripennis McFarlane and Hydropsyche colonica McLachlan. Freshwater Biology **5**, 105–114.
Devonport B.F. & Winterbourn M.J. (1976) The feeding relationships of two invertebrate predators in a New Zealand river. Freshwater Biology **6**, 167–176.
Hogsden K.L. & Harding J.S. (2012) Anthropogenic and natural sources of acidity and metals and their influence on the structure of stream food webs. Environmental Pollution **162**, 466–474.
Hollows J.W., Townsend C.R. & Collier K.J. (2002) Diet of the crayfish Paranephrops zealandicus in bush and pasture streams: Insights from stable isotopes and stomach analysis. New Zealand Journal of Marine and Freshwater Research **36**, 129–142.
Jellyman D.J. (1996) Diet of longfinned eels, Anguilla dieffenbachii, in Lake Rotoiti, Nelson Lakes, New Zealand. New Zealand Journal of Marine and Freshwater Research **30**, 365–369.
Jellyman D.J. (1989) Diet of two species of freshwater eel (Anguilla spp.) in Lake Pounui, New Zealand. New Zealand Journal of Marine and Freshwater Research **23**, 1–10.
Jellyman P.G. & Harding J.S. (2016) Disentangling the stream community impacts of Didymosphenia geminata: How are higher trophic levels affected? Biological Invasions **18**, 3419–3435.
McDowall R.M., Main M.R., West D.W. & Lyon G.L. (1996) Terrestrial and benthic foods in the diet of the shortjawed kokopu, Galaxias postvectis Clarke (Teleostei: Galaxiidae). New Zealand Journal of Marine and Freshwater Research **30**, 257–269.
Rounick J.S. & Hicks B.J. (1985) The stable carbon isotope ratios of fish and their invertebrate prey in four New Zealand rivers. Freshwater Biology **15**, 207–214.
Winterbourn M.J. (1996) Life history, production and food of Aphrophila neozelandica (Diptera: Tipulidae) in a New Zealand stream. Aquatic Insects **18**, 45–53.
Winterbourn M.J., Cowie B. & Rounick R.S. (1984) Food resources and ingestion patterns of insects along a west coast, south island, river system. New Zealand Journal of Marine and Freshwater Research **18**, 379–388.

Chapter Four: Inferring food web structure and stability across an anthropogenic stress gradient

Preface

This chapter consists of a manuscript in preparation titled "*Inferring food web structure and stability across an anthropogenic stress gradient"*. The target journal for this manuscript is *Ecology.* This manuscript is co-authored with Jon Harding. As lead author of this manuscript, I designed the study, analyzed the data, developed the models, wrote the first and final draft of the manuscript and designed all tables and figures. Jon Harding provided funding and advice on study design and provided feedback on drafts of the manuscript.

Abstract

Understanding what makes food webs stable has long been a goal of ecologists. Topological structure and the distribution and magnitude of interaction strengths in food webs have been shown to confer important stabilizing properties. However, little work has been conducted investigating how variable food web structure affects the stability of food webs. Here, I applied mechanistic models to infer pairwise species interaction probabilities in communities across an anthropogenic stress gradient. Multiple food webs were iterated for each community based on these probabilities, in order to incorporate the variability of realistic food web structure. Interaction strengths were estimated in a number of ways, and stability analyses were conducted. I found that realistic food web structure increases stability compared to randomly assembled food webs. Additionally, I found that impacted (e.g. small) communities are generally more stable than reference communities. However, the stability of some impacted communities becomes bimodal depending on how interaction strengths are estimated. Management and restoration of impacted sites needs to consider their increased stability, as this has important implications for the re-colonization of desirable species. Furthermore, active species introductions may be required to overcome the internal ecological inertia of affected communities.

Introduction

The relationship between food web diversity and stability has had a long and contentious debate in ecology (May 1972, Yodzis 1981, Pimm 1984, McCann, Hastings, and Huxel 1998, Montoya et al. 2006, Christianou and Kokkoris 2008, Wootton and Stouffer 2015, Brose et al. 2017). Early studies of large, randomly connected food webs found a negative relationship between stability and complexity (May 1972). However, since that time, ecologists have discovered a number of features that stabilize real food webs. For example, the topological structure of natural food webs is decidedly non-random (Yodzis 1981, Williams and Martinez 2000), and this non-random structure provides stability to communities (Neutel, Heesterbeek, and de Ruiter 2002, Allesina and Tang 2012). Likewise, the distribution of interaction strengths in large, stable food webs is skewed towards many weak interactions with relatively few strong ones (de Ruiter et al. 1995, McCann, Hastings, and Huxel 1998, Neutel, Heesterbeek, and de Ruiter 2002, Wootton and Stouffer 2015). Low magnitude interactions likely distribute any perturbations weakly though out the community, and dampen oscillations between resources and consumers (McCann, Hastings, and Huxel 1998), increasing the likelihood of the community returning to an equilibrium state (Neutel et al. 2007).

Body size is a strong organizing principle in food webs, particularly in aquatic habitats (Cohen, Jonsson, and Carpenter 2003, Brose et al. 2006, Petchey et al. 2008). Body size can determine who interacts with whom, as well as the strength of the interactions. Predators are generally larger than their prey (Brose et al. 2006) and diet breadth also correlates with body size, resulting in the largest-sized predators also having the widest diet breadth (Brose et al. 2017). Interaction strengths also correlates with predator: prey body size ratios (Emmerson and Raffaelli 2004, Berlow et al. 2009) and these allometries contribute to local food-web stability (Brose, Williams, and Martinez 2006, Tang, Pawar, and Allesina 2014). Finally, a strong, negative correlation between the positive and negative interaction magnitudes (e.g. effect of resource on consumer, and consumer on resource, respectively) has also been shown to drive stability in natural food webs (Tang, Pawar, and Allesina 2014).

Stability is generally considered a positive attribute of a community, but this is not always the case (Tylianakis et al. 2010). Stable communities are also often resistant to the introduction of new species which has implications for remediation activities aimed at returning the communities to non-impacted conditions (Monteith et al. 2005, Hildrew 2009, Tylianakis et al. 2010). This may explain the commonly observed lag in biotic recovery of sites, even after the restoration of the original physical or chemical disturbance (Hildrew 2009, Gray et al. 2014). For example, small, stable communities have been observed in acidified streams, and high stability has been suggested as a potentially general feature of acid-impacted streams (Layer et al. 2010).

Broadstone Stream in the United Kingdom has been studied since the 1970's and represents one of the most well characterized freshwater food webs in the literature (Gray et al. 2014). Broadstone Stream experienced a gradual reduction of surface water pH from the 1970's to 1990's, in response to international efforts to reduce acid deposition (Woodward and Hildrew 2002, Hildrew 2009, Layer et al. 2011). As pH increased, there was an expectation of a concomitant change in community structure, becoming more similar to analogous circumneutral streams nearby. However, even as pH increased, the macroinvertebrate community composition remained relatively unchanged, and the abundance of some species even declined (Hildrew 2009, Gray et al. 2014). Biotic recovery (i.e. increasing community size and complexity and the return of acid-sensitive species) of Broadstone Stream only occurred after the reinvasion of successively larger-sized predators, culminating in the colonization of brown trout (Layer et al. 2011). As larger predators became established, their presence re-organized the food-web architecture and caused a redistribution of biomass throughout the community (Layer et al. 2011), potentially creating opportunities and opening niche space for other species to occupy. For example, the presence of acid-tolerant generalist detritivores filled the ecological role of acid-sensitive specialist grazers, and prevented their re-colonization, even after pH increased. However, the colonization of larger predators may have exerted top-down control on the generalists, allowing the successful re-colonization of the specialist grazers (Ledger and Hildrew 2005, Hildrew 2009, Layer et al. 2010, 2011).

Research in soil food webs also supports the importance of colonization by new top predators during the process of community succession. Neutel et al. (2007) sampled soil food webs across natural productivity and plant successional gradients, and found a pattern of communities alternating between stable and less stable structures. As productivity and plant successional stage increased, the biomass of the top trophic level also increased (e.g. predatory nematodes in mid-level successional stage), leading to stronger top-down interaction strengths which resulted in a reduction of community-wide local stability. However, this was counteracted by the arrival of a new top predator (e.g. predatory mites in later successional stages), which reduced the biomass of the former top trophic level, leading to an overall reduction in top-down effects, and an increase in local stability at the community level. During this process, additional intermediate consumers also successively colonized, leading to increases in community size and complexity (Neutel et al. 2007). These findings indicate that the order and/or timing of colonization is paramount in their contribution to community succession. For example, the buildup of biomass in the current top trophic level may be necessary to exert top-down control on lower consumers, opening up niche space and allowing the colonization of other intermediate consumers. Likewise, the buildup of biomass may be necessary as a prey base for the successful establishment of a higher trophic level.

While work in the last four decades has significantly improved our understanding of the mechanisms that stabilize food webs, nearly all of the previous work assumes that network structure is static. Food webs, however, are not static. Pairwise species links are variable, can change through space and time and are based on resource availability (Thompson and Townsend 1999, Poisot et al. 2012, 2016, Poisot, Stouffer, and Gravel 2015). In fact, this variability in trophic interactions has been theorized as promoting stability because consumers will allocate foraging effort differentially based on resource availability, potentially dampening environmental effects on population densities (Kondoh 2003). Likewise, larger predators are generally more mobile and can rapidly moderate their behavior in response to changing resource conditions, which can connect spatially-distant food webs and increase stability across the meta-webs (McCann, Rasmussen, and Umbanhowar 2005). Incorporating the variability in species interactions and network structure is an important next frontier in our understanding of food web stability.

The presence, structure, and strength of interactions all play important roles in conferring stability to an ecological network. Our primary aim was to determine if network theory can offer mechanistic explanations for if and why mining impacted communities are more stable. We used a dataset of stream communities across an anthropogenic stress gradient of acid mine drainage (AMD) known to affect network size (e.g. species richness), local population densities, and biomass distributions (Chapter Three, Pomeranz et al.2019). Secondly, we incorporated variable food-web structure into our assessments in order to explore the effects of variable species interactions and how the range of realistic food-web topologies affects our analytical results. We expected the structure of food webs across the AMD gradient to change in a consistent manner. Specifically, we expected webs to become small (e.g. lower species richness), simple (e.g. fewer links), and well-connected (e.g. high proportion of potential links realized) with increasing AMD impact. Furthermore, we expected these structural changes to lead to food webs with higher stability. We explored the robustness of our results by estimating interaction magnitudes in one of four ways (see methods). Finally, we compared our results to randomly-assembled null food webs in order to test whether “realistic” food web structure has a stabilizing effect. We expected randomly-assembled networks to be less stable with increasing size (May 1972) and that all randomly-assembled networks to be less stable than similarly-sized networks assembled using interaction rules.

Methods

Study site and stream characteristics

This study was conducted in the Buller-Grey region in the north-west of the South Island, New Zealand. The region has a long history of coal mining, and is part of the Westland Forest ecoregion, which has spatially-consistent climatic conditions, geology, and freshwater biota (Harding and Winterbourn 1997, Harding, Winterbourn, and McDiffett 1997). A total of twenty-five streams were sampled based on known and relatively constant water chemistry (e.g., pH, conductivity, dissolved Al and Fe concentrations) over time (Winterbourn et al. 2000, Greig et al. 2010, Hogsden and Harding 2012a, Kitto et al. 2015). Thirteen of the streams sampled were along an AMD gradient (which we refer to as "impacted" streams), and twelve of the streams represented a natural gradient of pH (~ 4 – 7) and low metal concentrations. These were sampled in order to capture the range of natural variation present. To our knowledge, these twelve streams are not impacted by AMD inputs. All twenty-five streams were placed into a single gradient (see below) and analyzed together. All streams were chosen to be as similar as possible with respect to other physical parameters, and were in relatively isolated catchments dominated by native vegetation. All sampling occurred during January – February 2016 (Austral summer). Stream water pH, specific conductivity, dissolved oxygen and temperature were measured in the field using standard meters (YSI 550A & YSI 63, YSI Environmental Incorporated, Ohio, USA). Random water samples (50 ml) collected for analysis of dissolved metal concentrations were filtered in the field (0.45 µm mixed cellulose ester filter) and acidified ($pH < 2$) using ultrapure nitric acid. Samples were analyzed for metals using inductively coupled plasma mass spectrometry (ICP-MS) at the University of Canterbury (Christchurch, New Zealand).

All water chemistry variables (Chapter Two, supplementary table 1) were analysed to generate an AMD gradient using principal components analysis (PCA) as described in Pomeranz et al. (2019, Chapter Two). Briefly, dissolved metal concentrations and conductivity were $\log_{10}$-transformed ($x + 1$) to satisfy assumptions of normality and were combined with pH in a resemblance matrix centered at 0 and scaled by their standard deviations. Site scores for PC axis 1 were extracted and used as a proxy for the AMD gradient.

Community sampling and body mass estimation

Benthic macroinvertebrates were randomly collected in three Surber samples (0.06 m^2, 0.25 mm mesh) from riffle habitats at each site (Blakely and Harding 2005). Samples were preserved with 100% ethanol in the field and returned to the laboratory for processing, as described in Pomeranz et al. (2019, Chapter Two). Macroinvertebrates were identified to the lowest practical taxonomic level and the body lengths of all individuals was measured (nearest 0.1 mm). Body lengths were converted to dry weight estimates (grams) using published taxon-specific length-weight regressions for New Zealand invertebrate fauna (Towers, Henderson, and Veltman 1994, Stoffels, Karbe, and Paterson 2003). Biomass estimates were averaged by taxa for food-web inferences (see below) for each site where they occurred. For this analysis, taxa which were rare or little was known about their ecology were removed from the dataset (e.g. non-insect taxa such as Acari, Ostracods, Platyhelminthes). After removal, a single stream only had one animal taxa remaining, and this site was removed from all further analyses (i.e. 24 sites used in all further analyses).

Fish were sampled using quantitative electrofishing techniques from a 20 m reach within each site. Stop nets were placed at the top and bottom of the reach and fish were removed during three successive passes. All fish captured had their fork length measured and were converted to dry weight estimates using length-weight regressions for New Zealand fish (Jellyman et al. 2013). Mean dry weight estimates for each fish taxa were calculated as above.

Inferring food-web structure

The probability of a link occurring between species pairs is proportional to their likelihood of encounter (neutral effects, *sensu* Canard et al., 2014, Poisot et al., 2015, Pomeranz et al., 2018, Chapter Three) multiplied by their ability to interact (niche effects, *sensu* Gravel et al., 2013, Poisot et al., 2015, Bartomeus et al., 2016, Pomeranz et al., 2018, Chapter Three). Encounter probabilities can be calculated as being proportional to the product of species relative abundances (Canard et al. 2014, Poisot, Stouffer, and Gravel 2015), and niche effects can be calculated based on relevant traits of the potentially interacting species (e.g. body size; Gravel et al., 2013, Morales-Castilla et al., 2015, Bartomeus et al., 2016). Incorporating empirical estimates of local densities and a method for inferring predator-prey interactions based on body size (Gravel et al. 2013) has been used to successfully recreate empirical food-web structure in streams from different land-use types in New Zealand (Pomeranz et al., 2018, Chapter Three).

Bartomeus et al. (2016) extended the method of Gravel et al. (2013) for inferring the probability of species interactions, rather than simply as binary (presence/absence). We used the associated Traitmatch package (Bartomeus et al. 2016) in the R statistical language (R Development Core Team 2017) to infer the probability of antagonistic interactions based on body size at all of our streams. Individual predator-prey interactions from Broadstone Stream and Tadnoll Brook, originally published in Woodward et al. (2010), were used to parameterize the model. Using these parameter coefficients, we then predicted the probability of feeding interactions for all pairwise taxa combinations at each of our sites (Bartomeus et al. 2016). Species interaction probability vectors were converted to square (S x S, where S = the number of taxa present) interaction probability matrices, **P.** Columns and rows of **P** represent species in their role as consumers and resources, respectively. Therefore, $\mathbf{P}_{ij}$ represents the probability that species *j* consumes species *i*. The matrices were ordered by increasing body size from left to right, and top to bottom.

After inferring the probabilities of all possible pairwise interactions, we further refined these possible interactions by restricting niche forbidden links (*sensu* Morales-Castilla et al., 2015, Pomeranz et al., 2018, Chapter Three). Niche forbidden links were defined as in Chapter Three and Pomeranz et al., (2018). Briefly, we restricted predatory interactions between animals which are known to be non-predatory, or which lacked morphological adaptations for the consumption of animal prey (e.g. set $P_{\bullet j}$ to 0). For example, members of the blackfly family, Simuliidae, have cephalic fans modified for filter feeding, and lack the ability to consume animal prey, so their predatory probabilities (matrix column) were set to zero. Conversely, net-spinning Hydropsychid caddisflies construct nets to filter feed, but retain chewing mouthparts and are able to consume animal prey they capture, so their predation probabilities were not modified. Niche forbidden taxa in this study are presented in Supplementary Table 1.

We also scaled interaction probabilities based on the product of species relative abundances to account for neutral effects (*sensu* Canard et al., 2014). This simply takes into account that two rare species are less likely to interact than two abundant species. The modified interaction probabilities for each site were calculated as $\mathbf{P}_{ij}' = \mathbf{P}_{ij} * \mathbf{N}_{ij}$, where $\mathbf{N}_{ij}$ is the product of relative abundances of species *i* and *j*, scaled from 0.5 to 1 respectively (e.g. abundant species pairs = 1 and are assumed to interact based on niche probabilities, while rare species are less likely to encounter one another, so their overall interaction probabilities are reduced).

Finally, the probability matrices for each stream were converted to 250 binary adjacency matrices (**A**). Adjacency matrices are square matrices with taxa in their role as predators in columns and their role as prey in rows as in the probability matrices (**P**), where $\mathbf{A}_{ij} = 1$ when taxa *j* consumes taxa *i*, and 0 otherwise. This was done by conducting Bernoulli (i.e. binomial) trials, where the probability that $\mathbf{A}_{ij} = 1 = \mathbf{P}_{ij}$. This allowed us to assess the effect of variable food-web structure on network measurements and stability (see below).

Food-web measures

We calculated a suite of standard food-web measures including the number of links (L), connectance ($C = L / S^2$, where S = the number of species), proportion of taxa which were bottom, intermediate, and top trophic levels, and the variability (standard deviation) of normalized vulnerability (mean number of consumers per resource) and normalized generality (mean number of resources per consumer) for all 6000 networks. Vulnerability and generality for each iteration were normalized by dividing them by L/S which forces their means to equal 1, and allows for comparison across networks of different size (Williams and Martinez 2000).

Interaction strength

The adjacency matrices $\mathbf{A}_{ij}$ calculated above, were transformed into Jacobian matrices, where the element $\mathbf{J}_{ij}$ quantifies the effect that species *j* has on species *i* growth rate. For antagonistic (e.g. predatory) interactions assessed here, $\mathbf{J}_{ij} > 0$ and $\mathbf{J}_{ji} < 0$. The magnitude, distribution, and correlation of interaction strengths are known to be an important component of food-web stability. In order to assess the effects of network structure (presence/absence of links), and the effects of interaction strength distributions and correlations, we estimated interaction strengths in four ways: 1) Random interaction strength to test the effects of network topology. Using the methods of Sauve et al. (2016), we estimated all non-zero elements of **J** by sampling them from a half normal distribution $|(\mu = 1, \sigma^2 = 0.1)|$ and multiplied the positive and negative interactions by 1 and -1, respectively; 2) Scaling interaction strengths by body size. Interaction strength is known to scale with predator: prey body size ratios, and this has been suggested as a key process increasing stability in natural food webs (Brose, Williams, and Martinez 2006). To examine these effects, we again sampled interaction strengths from a half normal distribution, but scaled them by predator:prey body size ratios (e.g. smallest positive and greatest negative effects between large predators and small prey); 3) Correlating the top-down (negative) and bottom-up (positive) interaction strengths. The correlation between positive and negative interactions has been shown to have important implications in local stability (Tang, Pawar, and Allesina 2014), with the magnitude of negative effects being greater than the magnitude of positive effects. For this, we sampled the negative interactions from a half normal distribution, and scaled the corresponding positive interactions by multiplying it by 0.7. This can be interpreted as a 70% conversion efficiency of prey biomass by predators from stream habitats as estimated from empirical studies (Woodward et al. 2005, Montoya et al. 2009); 4) Interaction strengths scaled by body size and positive and negative interactions correlated. Here, we sampled the negative effects as in (3), and scaled them by predator:prey body size ratios. We then calculated the corresponding positive effects by multiplying the scaled negative effect by 0.7. This takes into account the scaling of interaction strengths by body size and the correlation of positive and negative effects. For all interaction strength estimates, we used a modified version of the jacobian_binary() function available in the supplemental information from Sauve et al. (2016)

Stability

For each adjacency matrix (24 streams × 250 trials = 6,000 matrices), interaction strengths were estimated in one of four ways (see above) and a stability analysis was conducted. Here, a network is defined as stable if all of the real parts of its eigenvalues are negative. The stability metric, *s*, was defined as the minimum amount of intraspecific competition (e.g. the diagonal of the Jacobian matrix, $\mathbf{J}_{ii}$) necessary for a food-web iteration to be stable (Neutel, Heesterbeek, and de Ruiter 2002, Tang, Pawar, and Allesina 2014, Sauve et al. 2016). Smaller values of *s* are considered to be more stable (Neutel, Heesterbeek, and de Ruiter 2002, Sauve et al. 2016), however this is a continuous qualitative measurement. There is no known value or threshold of *s* which separates networks from being stable or not. Lower values of *s* simply imply that that network is *more* stable than high values of *s*. We calculated the *s* metric using the stability() function available in the supplementary information of Sauve et al. (2016). This method is equivalent to that used by Allesina & Tang, (2012) and Tang, Pawar, and Allesina (2014).

Null model

To test the stabilizing properties of "realistic" network structure (e.g. inferred from interaction rules), we conducted stability analyses on random adjacency matrices while maintaining observed values of network size (S), and connectance (C) (i.e. Erdòs-Rényi random directed graph, Tang, Pawar, and Allesina 2014). For each observed value of S in our empirical communities, we created S × S matrices, and assigned links randomly throughout the matrix with equal probability C. For each value of S, we used three values of C (mean, ± 1 SD), and generated 100 networks (24 sites × 3 values of C × 100 iterations = 7,200 networks). Some of the empirical communities had the same number of species (S) present (e.g. 3 sites each had 3 species, and 2 sites each had 9 species), resulting in a total of 19 uniquely-sized communities. However, because values of C varied even among networks of the same size, we generated null models for each community and value of C (e.g. total of 9 random matrices with 3 species). All the parameter combinations assessed are presented in Supplementary Table S2.

Statistical analyses

We fitted linear regressions for each food-web measure and the stability metric, *s*, across the AMD gradient by conducting robust linear regressions using the lmrob() function from the robustbase package in R (Maechler et al. 2018). We conducted robust linear regressions because they are less sensitive to violations of assumptions compared to Ordinary Least Squares (OLS) regressions. Specifically, they are robust to the effects of outliers and heteroscedasticity of variance. Robust linear regressions generally use M-estimators (maximum likelihood type) to iteratively converge on a solution (Yohai 1987).

All food-web measures analyzed here displayed heteroscedasticity of variance (Breusch-Pagan test p-values all < 0.001). Because the variation in response variables are not homogenous across the AMD gradient, true F-statistics are not appropriate. However, the Wald statistic offers an alternative method for model selection by comparing nested models. Here, we fit null (intercept only), linear and quadratic models with the site specific PC1 scores as the independent variable, and selected the best fitting model (Wald test p-value < 0.05 supports model with greater number of terms). The quadratic model was a better fit than the linear or null models for all food web measures (Wald test p-values < 0.001) except for the proportion of species classified as top trophic levels (Wald test p-value = 0.592), which was best explained by a linear relationship.

We also tested for changes in the number of species across the mining gradients. Because the value of S does not change between iterations, we only used one observation per site (e.g. N = 24) to avoid pseudoreplication. The variance in S was homoscedastic (Breusch-Pagan test p-value = 0.26), so we compared null, linear, and quadratic OLS regressions using Akaike's information criterion corrected for small sample size (AIC_c, Burnham & Anderson, 2004). Both the linear and quadratic models provided a better fit than the null model. AIC_c analysis indicated equal support for both linear and quadratic models ($\Delta AIC_c < 2$), however, the quadratic model explained more of the variation and was used in all further analyses.

Results

AMD gradient

The Principal component results of Pomeranz et al. (2019, Chapter Two) were used to test responses of food web structure to increasing AMD stress. Briefly, Principal component axis 1 explained 77% of the variation in chemical variables across the sites (Chapter Two, Fig 1), and the site specific scores on PC axis 1 were extracted and used as a measure of the AMD stress in all further analyses.

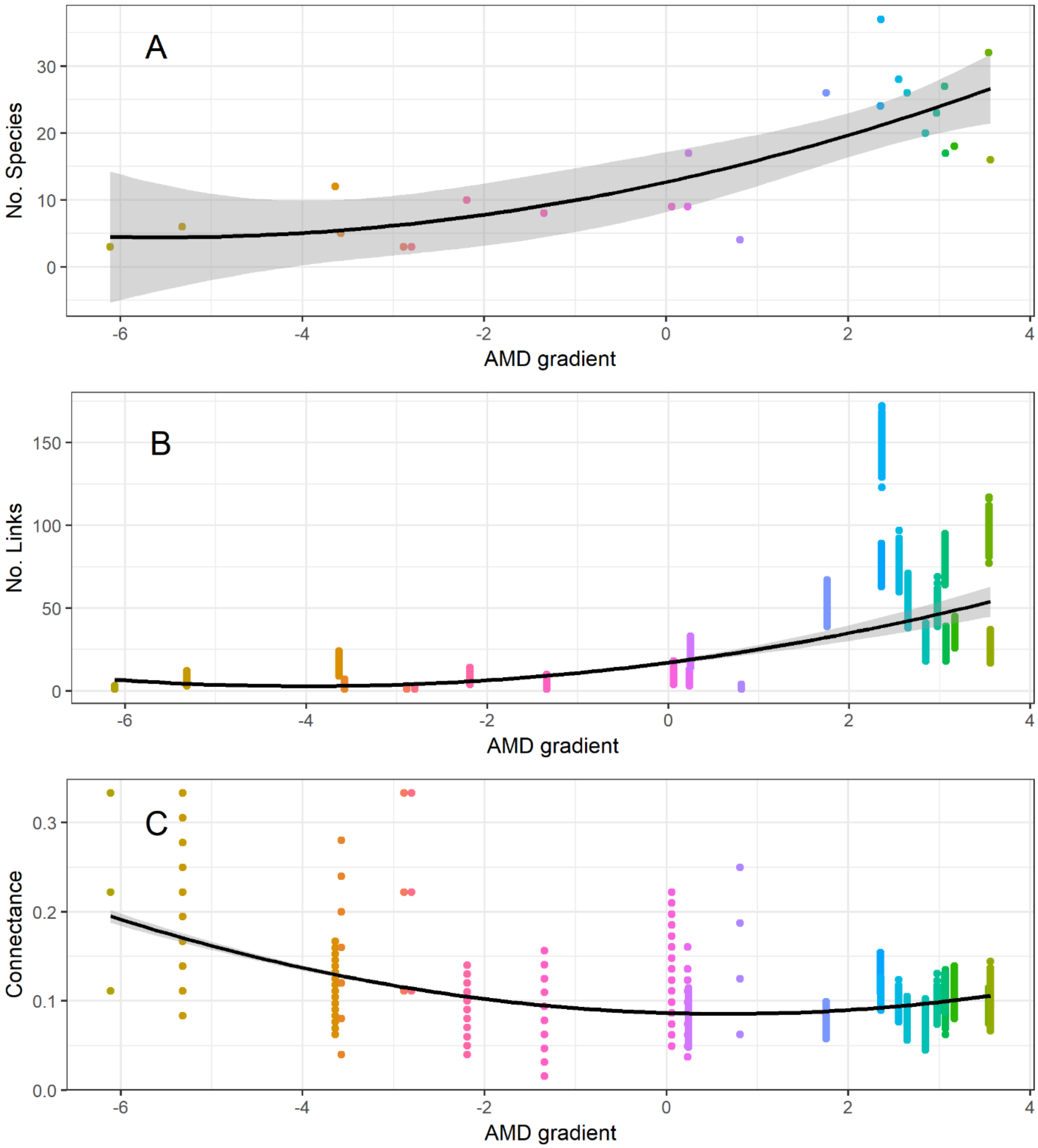


Figure 1 Number of species (A); inferred links (B); and inferred connectance (C) across the AMD gradient. Impacts increase right to left (PC1 axis reversed for visualization). Black line in panel A is OLS regression, and grey shading is 95% confidence interval. Black lines in panels B and C are robust linear regression estimate, and grey shading is 95% confidence interval.

Community composition

Generally, there was a significant decline in species richness with increasing AMD-stress (Table 1, Fig1A). Likewise, community structure was affected consistently. Fish were completely absent in AMD impacted streams, and most large-bodied invertebrate predators were also removed (Chapter Two, Pomeranz et al. 2019).

Table 1 Statistics for robust linear regressions across the mining gradient. The best fitting model (linear, quadratic) is indicated with an X. Coefficient estimates in bold have P-values < 0.001. S = network size (number of species), L = links, C = Connectance (L / S^2), B, I and T are the proportion of basal, intermediate, and top species, respectively. VulSD and GenSD are the standard deviations of normalized Vulnerability and normalized Generality, respectively.

					Coefficient estimate	
Response	Linear	Quadratic	Adjusted R2	Intercept	PC	PC^2
S		X	0.60	**15.16**	**-2.97**	0.27
L		X	0.56	**17.17**	**-7.142**	**8.92E-01**
C		X	0.38	**0.087**	**3.00E-03**	**2.00E-03**
B		X	0.03	**0.370**	**-2.00E-03**	**-1.00E-03**
I		X	0.41	**0.205**	**-5.00E-02**	**7.00E-03**
T	X		0.4	**0.229**	**3.00E-02**	NA
VulSD		X	0.84	**7.07E-03**	**3.10E-03**	**4.70E-04**
GenSD		X	0.86	**1.02E-02**	**5.00E-03**	**7.40E-04**

Food-web measures

All food-web measures varied significantly across the AMD gradient (Table 1). The number of inferred links were negatively related to the AMD gradient (Fig 1B). The variance in the number of inferred links also decreased across the gradient (Breusch-Pagan test p-value < 0.001), indicating that AMD impacted streams have fewer possible topological structures. In contrast, inferred values of connectance increase across the gradient (Fig 1C). This is due to the fact that a reduction in the number of species reduces the denominator of the connectance equation exponentially (e.g. $C = L/S^2$).

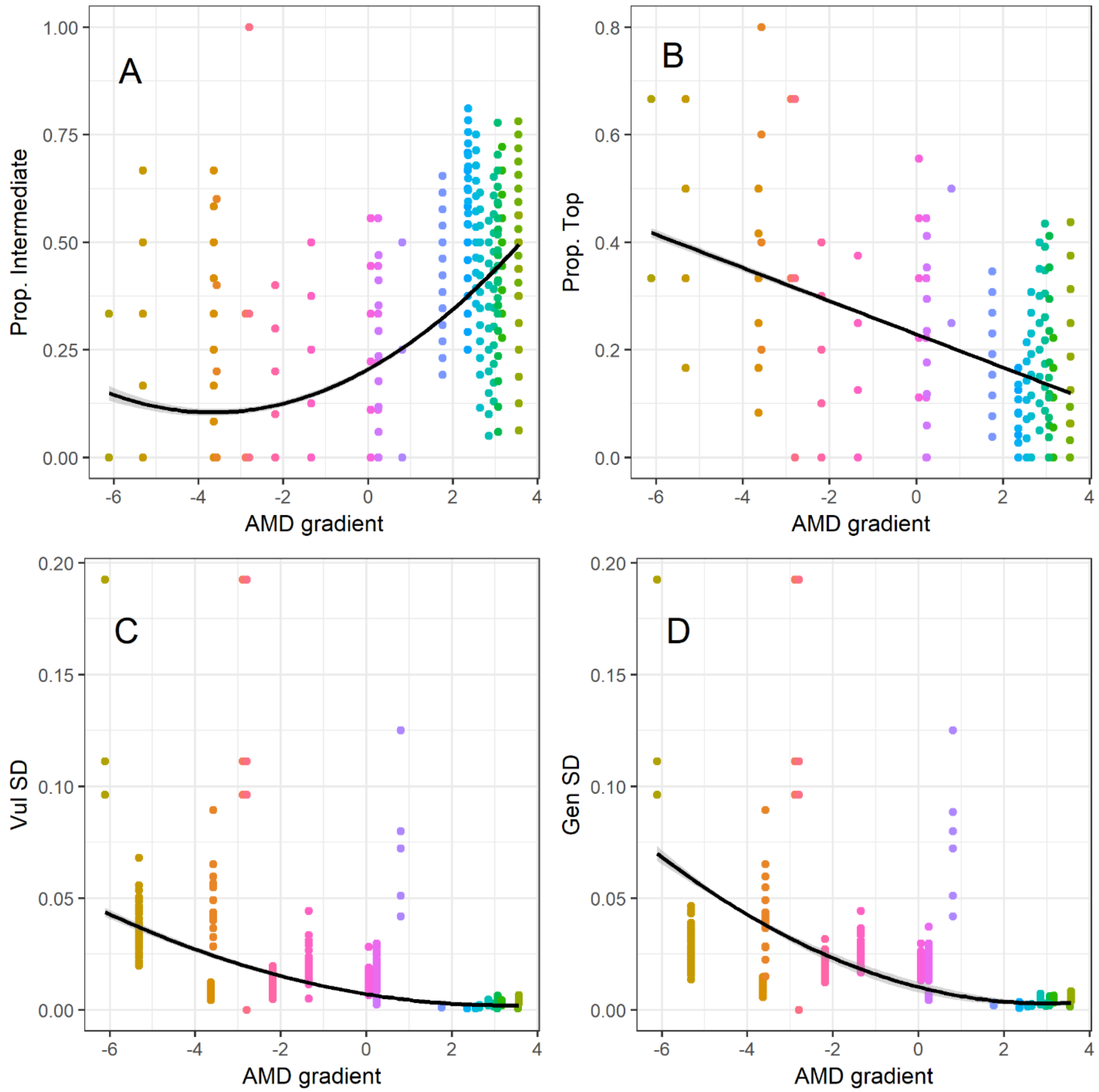


Figure 2 Proportion of inferred intermediate (A) and top (B) species; inferred standard deviations of Vulnerability (C) and Generality (D) across the AMD gradient. Impacts increase right to left (PC1 axis reversed for visualization). Black lines are robust linear regression estimates, and grey shading is 95% confidence interval.

The number of basal species slightly decreased across the AMD gradient, although the magnitude of the coefficient estimate was low, and the model poorly explained the observed variation (Table 1). However, the number of intermediate species significantly decreased and the number of top species significantly increased across the AMD gradient (Table 1, Fig 2A and B).

Vulnerability and generality (e.g. number of consumers per resource, and resources per consumer, respectively) both increased across the AMD gradient (Table 1, Fig 2B and C). This indicates a homogenization of food web structure in AMD-impacted streams. Essentially, consumers are utilizing a larger proportion of the resources available and all resources are being consumed by a larger proportion of the available consumers than in un-impacted streams.

Stability

In general, stability increased (lower *s* indicates higher stability) exponentially with increasing AMD stress (i.e. smaller network size), regardless of how interaction strengths were estimated (Table 2, Fig. 3). This finding was consistent across all methods of estimating interaction strengths (i.e. sampling interaction strengths randomly, scaling interaction strengths by body size, correlating positive and negative interaction strengths, and the combination of scaling and correlating interaction strengths). While the response of *s* across the gradient had the same general shape regardless of how interaction strengths were estimated, there are some key differences worth noting. First of all, the range of *s* when scaling the interaction strengths by body size was lower for all networks than all other interaction strength estimations. This supports the observation of stabilizing effects that predator:prey body size ratios have in natural food webs (Brose et al. 2006, Rooney and McCann 2012). Secondly, when interaction strengths were correlated (e.g. positive interactions = 0.7 × negative interactions), the distribution of *s* for some of the impacted streams becomes bimodal (Figure 3C and D) indicating that impacted sites vary between stable and unstable structures. This may also explain why the amount of variation explained decreases when correlating the interaction strengths (Table 2).

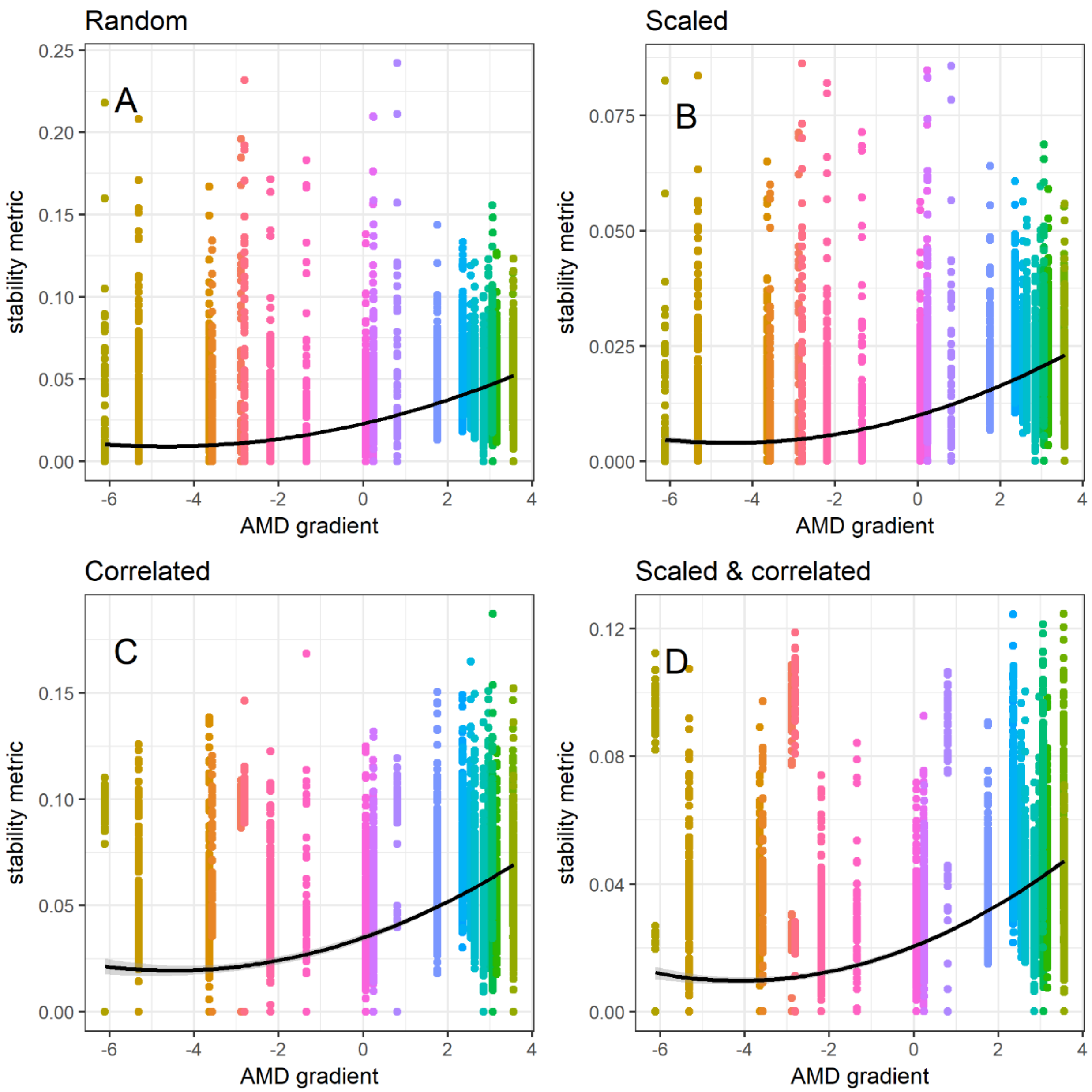


Figure 3 Inferred stability metric, s, across the AMD gradient when varying the estimate of interaction strengths (see methods). A) Random interaction strengths; B) random interaction strengths scaled by body size; C) Random interaction strengths, positive and negative interactions correlated; D) random interaction strengths, scaled by body size, and positive and negative interactions correlated. Note difference in scale of y-axis. AMD stress increases right to left. Black lines are robust linear regression estimates, and grey shading is 95% confidence interval.

The randomly-assembled networks also displayed a positive relationship between network size and *s* (slope coefficient estimate = 0.012, adjusted R^2 = 0.486, Fig. 4). Although small randomly-structured networks were more stable than large randomly-structured networks, all were less stable than networks of similar size inferred using interaction rules (note difference in scale of *y*-axis in Fig. 3 panels compared with Fig. 4).

Table 2 Statistics for stability across mining gradient. Coefficient estimates in bold have P-values < 0.001.

			Coefficient Estimate	
Interaction Strength Estimate	Adjusted R2	Intercept	PC	PC^2
Random	0.38	**2.29E-02**	**-5.94E-03**	**6.35E-04**
Scaled	0.38	**9.96E-03**	**-2.64E-03**	**2.91E-04**
Correlated	0.27	**3.49E-02**	**-6.90E-03**	**7.67E-04**
S & C	0.34	**2.05E-02**	**-5.24E-03**	**6.36E-04**

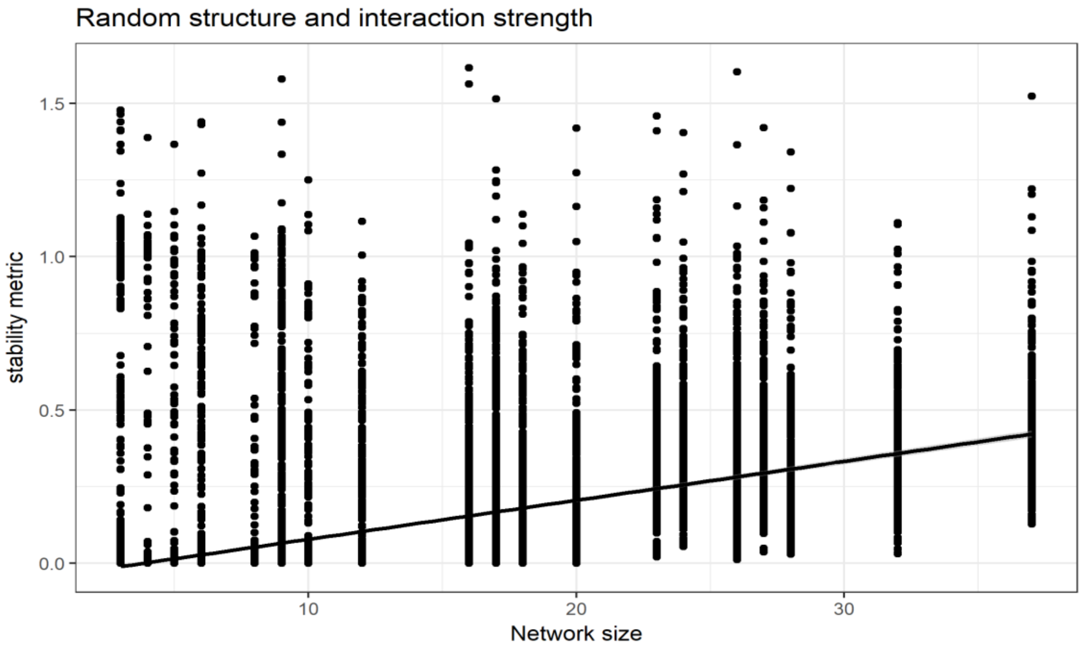


Figure 4 Stability metric for the randomly structured networks when sampling interaction strengths from a half normal distribution. Black line is robust linear regression estimate and grey shading is 95% confidence interval. Note difference in scale of y-axis compared with Figure 3.

Discussion

We inferred the structure and stability of food webs across an anthropogenic stress gradient based on interaction probabilities determined by the local distribution of macroinvertebrate and fish body sizes and species densities. Our results show that AMD impacts lead to small, simple, and stable food webs. Furthermore, this study adds to our understanding of the stabilizing attributes of networks, including size (e.g. species number), topological structure, distribution of body sizes, and interaction strengths.

Inferred network structure

Species richness decreased significantly across the AMD gradient. Likewise, AMD-impacted communities had simplified structure, namely the loss of the largest sized taxa (e.g. fish, large-bodied invertebrate predators). This is consistent with the findings of several studies showing a decline in species richness and trophic levels in response to AMD inputs (Chapter Two, Pomeranz et al*.,* 2019, reviewed in Hogsden & Harding, 2012b). The number of pairwise interactions (e.g. feeding links) also decreased across the AMD gradient. A reduction in links may translate to fewer energy pathways available (Hogsden and Harding 2013), reducing ecological efficiency or functional diversity (Petchey and Gaston 2002). Likewise, the interaction magnitude in food webs with fewer links may increase relative to webs with many links. Having a few strong links is generally considered to be destabilizing (Wootton and Stouffer 2015). On the other hand, because interaction strengths are related to body size and AMD inputs cause the loss of the largest-sized predators (Chapter Two, Pomeranz et al., 2019), the links present in impacted streams are likely to be weak, possibly increasing stability. Indeed, when interaction strengths were scaled based on body size (see below) stability increased across all networks.

Connectance increased significantly across the AMD gradient, which means that a high proportion of the possible links in the food web were realized. This is in agreement with previous work which has shown a negative relationship between network size and connectance (Schmid-Araya et al. 2002). Generality and vulnerability also increased across the AMD gradient, meaning that each resource taxa was exploited by a high proportion of the consumer taxa present, and also that each of the consumers was exploiting a high proportion of the resources available. Additionally, the proportion of intermediate species declined, while the proportion of top species increased. Previous work has also indicated a positive relationship in the proportion of intermediate species with network size (Schmid-Araya et al. 2002). This indicates that top predators were lost, and the intermediate consumers replaced them at the top of the food chains. These results were as expected, because trophic position (Riede et al. 2011) and diet breadth (Brose et al. 2017) generally increase with body size and the AMD gradient is known to remove the largest body sizes from the community (Chapter Two, Pomeranz et al. 2019). All of these results support findings of previous studies on food webs in AMD impacted streams (Hogsden and Harding 2012a), and indicate a re-organizing of food web structure resulting in small, simple, and well-connected communities.

Effects of network size and structure on stability

Our results indicate that stability decreases with increasing network size, whether networks are constructed randomly or using mechanistic-based interaction rules. This supports May's (1972) theoretical results finding a negative relationship with stability and network size, as well as empirical results from Layer et al. (2010) which showed small, simple stream food webs to be more stable than large ones. Although stability generally decreased with increasing network size, realistically structured networks were more stable than randomly assembled networks of similar size. This supports the idea that the non-random structure of food webs confers stabilizing properties to the community (Williams and Martinez 2000, Neutel, Heesterbeek, and de Ruiter 2002, Tang, Pawar, and Allesina 2014).

Distribution of interaction strengths

Scaling interaction strengths based on body size increased stability for all streams across the AMD gradient compared with sampling interactions strengths randomly which is in agreement with previous studies (Emmerson and Raffaelli 2004, Otto, Rall, and Brose 2007). When positive and negative interaction strengths are correlated, the distribution of the stability metric across all sites is similar to that observed when sampling interaction strengths randomly. However, in some impacted streams, the distribution appears to become bimodal (e.g. some impacted streams more stable, some less). The streams which are "less" stable have stability metric distributions similar to un-impacted streams, potentially making them good candidates for restoration. For example, stable communities generally have high resistance to species introductions, but a typical goal of restoration is often the re-establishment of the pre-disturbance community composition, or the return of sensitive species (Lockwood and Pimm 1999). Therefore, focusing restoration actions on impacted communities which are less stable may provide a higher likelihood of recolonization by desirable species. Furthermore, when interaction strengths are both scaled by body size and positive and negative interactions are correlated, the general pattern of the stability metric across the gradient is similar to the pattern when estimating random interaction strengths. However, the "bimodal" distribution of some impacted streams is retained.

Conclusions

Our results indicate that AMD inputs consistently alter food-web structure, and that some AMD-impacted streams may be more receptive to restoration than others. When inferring realistic food web structure, along with more biologically-relevant estimations of interaction strengths (e.g. scaling and correlating magnitude) some of the impacted streams have high food-web stability. For successful restoration of all streams, the chemical conditions need to be returned to a pre-disturbance state. Streams with lower stability may lack internal inertia and have low resistance to species invasions and only require chemical remediation to place them on a trajectory of community succession. However, in impacted streams with high food web stability, beneficial disturbances (e.g. scouring flood, riparian plantings) or active species reintroductions may need to occur to overcome the internal ecological inertia of these communities. This is because small, stable communities have high resistance to changes in community composition and may inhibit the successful colonization of desirable species. However, because of their lower stability, it may also be necessary to actively monitor the sites to ensure that non-desirable (e.g. exotic invasive) species don't colonize the site.

Further work is needed to understand the effect of species introductions. If the goal of a restoration activity is for community composition to be similar to a pre-disturbance state, or the return of species with commercial value (e.g. fisheries), it may be necessary to set the community on a trajectory of community assembly, rather than introduce the desired species at the outset (i.e. the "myth of fast-forwarding" *sensu* Hilderbrand, Watts & Randle, 2005). For example, it may be necessary to introduce primary or secondary consumers (e.g. grazers, filter-feeders) in order to increase ecological efficiency and make more energy available for the successful establishment of higher trophic levels (Pimm 1982, Thompson and Townsend 2005). Likewise, it may be necessary to introduce medium sized predators (e.g. as occurred naturally in Broadstone Stream, Layer et al., 2011) in order to restructure the food web architecture before larger predators (e.g. fish) can successfully colonize the site.

References

Allesina, S., and S. Tang. 2012. Stability criteria for complex ecosystems. Nature **483**:205–208. https://doi.org/10.1038/nature10832.

Bartomeus, I., D. Gravel, J. M. Tylianakis, M. A. Aizen, I. A. Dickie, and M. Bernard-Verdier. 2016. A common framework for identifying linkage rules across different types of interactions. Functional Ecology **30**:1894–1903. https://doi.org/10.1111/1365-2435.12666.

Berlow, E. L., J. A. Dunne, N. D. Martinez, P. B. Stark, R. J. Williams, and U. Brose. 2009. Simple prediction of interaction strengths in complex food webs. Proceedings of the National Academy of Sciences **106**:187–191.

Blakely, T. J., and J. S. Harding. 2005. Longitudinal patterns in benthic communities in an urban stream under restoration. New Zealand Journal of Marine and Freshwater Research **39**:17–28. https://doi.org/10.1080/00288330.2005.9517291.

Brose, U., J. L. Blanchard, A. Eklöf, N. Galiana, M. Hartvig, M. R. Hirt, G. Kalinkat, M. C. Nordström, E. J. O'Gorman, B. C. Rall, F. D. Schneider, E. Thébault, and U. Jacob. 2017. Predicting the consequences of species loss using size-structured biodiversity approaches. Biological Reviews **92**:684–697. https://doi.org/10.1111/brv.12250.

Brose, U., T. Jonsson, E. L. Berlow, P. Warren, C. Banasek-Richter, L.-F. Bersier, J. L. Blanchard, T. Brey, S. R. Carpenter, M.-F. C. Blandenier, L. Cushing, H. A. Dawah, T. Dell, F. Edwards, S. Harper-Smith, U. Jacob, M. E. Ledger, N. D. Martinez, J. Memmott, K. Mintenbeck, J. K. Pinnegar, B. C. Rall, T. S. Rayner, D. C. Reuman, L. Ruess, W. Ulrich, R. J. Williams, G. Woodward, and J. E. Cohen. 2006. Consumer-resource body-size relationships in natural food webs. Ecology **87**:2411–2417. https://doi.org/10.1890/0012-9658(2006)87[2411:CBRINF]2.0.CO;2.

Brose, U., R. J. Williams, and N. D. Martinez. 2006. Allometric scaling enhances stability in complex food webs. Ecology Letters **9**:1228–1236. https://doi.org/10.1111/j.1461-0248.2006.00978.x.

Burnham, K. P., and D. R. Anderson. 2004. Multimodel inference: understanding AIC and BIC in model selection. Sociological Methods & Research **33**:261–304. https://doi.org/10.1177/0049124104268644.

Canard, E., N. Mouquet, D. Mouillot, M. Stanko, D. Miklisova, and D. Gravel. 2014. Empirical evaluation of neutral interactions in host-parasite networks. The American Naturalist **183**:468–479. https://doi.org/10.1086/675363.

Christianou, M., and G. D. Kokkoris. 2008. Complexity does not affect stability in feasible model communities. Journal of Theoretical Biology **253**:162–169. https://doi.org/10.1016/j.jtbi.2008.03.001.

Cohen, J. E., T. Jonsson, and S. R. Carpenter. 2003. Ecological community description using the food web, species abundance, and body size. Proceedings of the National Academy of Sciences **100**:1781–1786.

Emmerson, M. C., and D. Raffaelli. 2004. Predator–prey body size, interaction strength and the stability of a real food web. Journal of Animal Ecology **73**:399–409.

Gravel, D., T. Poisot, C. Albouy, L. Velez, and D. Mouillot. 2013. Inferring food web structure from predator–prey body size relationships. Methods in Ecology and Evolution **4**:1083–1090. https://doi.org/10.1111/2041-210X.12103.

Gray, C., D. J. Baird, S. Baumgartner, U. Jacob, G. B. Jenkins, E. J. O'Gorman, X. Lu, A. Ma, M. J. O. Pocock, N. Schuwirth, M. Thompson, and G. Woodward. 2014. Ecological networks: the missing links in biomonitoring science. Journal of Applied Ecology **51**:1444–1449. https://doi.org/10.1111/1365-2664.12300.

Greig, H. S., D. K. Niyogi, K. L. Hogsden, P. G. Jellyman, and J. S. Harding. 2010. Heavy metals: confounding factors in the response of New Zealand freshwater fish assemblages to natural and anthropogenic acidity. Science of the Total Environment **408**:3240–3250. https://doi.org/10.1016/j.scitotenv.2010.04.006.

Harding, J. S., and M. J. Winterbourn. 1997. An ecoregion classification of the South Island, New Zealand. Journal of environmental management **51**:275–287.

Harding, J. S., M. J. Winterbourn, and W. F. McDiffett. 1997. Stream faunas and ecoregions of the South Island, New Zealand: do they correspond? Archiv für Hydrobiologie **140**:289–307.

Hilderbrand, R. H., A. C. Watts, and A. M. Randle. 2005. The myths of restoration ecology. Ecology and Society **10**. https://doi.org/10.5751/ES-01277-100119.

Hildrew, A. G. 2009. Sustained research on stream communities: a model system and the comparative approach. Advances in Ecological Research **41**:175–312. https://doi.org/10.1016/S0065-2504(09)00404-8.

Hogsden, K. L., and J. S. Harding. 2012a. Anthropogenic and natural sources of acidity and metals and their influence on the structure of stream food webs. Environmental Pollution **162**:466–474. https://doi.org/10.1016/j.envpol.2011.10.024.

Hogsden, K. L., and J. S. Harding. 2012b. Consequences of acid mine drainage for the structure and function of benthic stream communities: a review. Freshwater Science **31**:108–120. https://doi.org/10.1899/11-091.1.

Hogsden, K. L., and J. S. Harding. 2013. Leaf breakdown, detrital resources, and food webs in streams affected by mine drainage. Hydrobiologia **716**:59–73. https://doi.org/10.1007/s10750-013-1544-3.

Kitto, J. A. J., D. P. Gray, H. S. Greig, D. K. Niyogi, and J. S. Harding. 2015. Meta-community theory and stream restoration: evidence that spatial position constrains stream invertebrate communities in a mine impacted landscape. Restoration Ecology **23**:284–291. https://doi.org/10.1111/rec.12179.

Kondoh, M. 2003. Foraging adaptation and the relationship between food-web complexity and stability. Science **299**:1388–1391. https://doi.org/10.1126/science.1079154.

Layer, K., A. G. Hildrew, G. B. Jenkins, J. O. Riede, J. Stephen, C. R. Townsend, G. Woodward, L. D. Well-, K. Layer, A. G. Hildrew, and G. B. Jenkins. 2011. Long-term dynamics of a well-characterised food web: four decades of acidification and recovery in the Broadstone Stream model system. Advances in Ecological Research **44**:69–117. https://doi.org/10.1016/B978-0-12-374794-5.00002-X.

Layer, K., J. O. Riede, A. G. Hildrew, and G. Woodward. 2010. Food web structure and stability in 20 streams across a wide pH gradient. Advances in Ecological Research **42**:265–299.

Ledger, M. E., and A. G. Hildrew. 2005. The ecology of acidification and recovery: changes in herbivore-algal food web linkages across a stream pH gradient. Environmental Pollution **137**:103–118. https://doi.org/10.1016/j.envpol.2004.12.024.

Lockwood, J. L., and S. L. Pimm. 1999. When does restoration succeed? Pages 363–392 *in* E. Weiher and P. A. Keddy, editors. Ecological assembly rules: perspectives, advances and retreats. Cambridge University Press, Cambridge, UK.

Maechler, M., P. Rousseeuw, C. Croux, V. Todorov, A. Ruckstuhl, M. Salibian-Barrera, T. Verbeke, M. Koller, E. L. T. Conceicao, and M. A. di Palma. 2018. robustbase: Basic Robust Statistics R package version 0.93-2.

May, R. M. 1972. Will a large complex system be stable? Nature **238**:413–414. https://doi.org/10.1038/238413a0.

McCann, K., A. Hastings, and G. R. Huxel. 1998. Weak trophic interactions and the balance of nature. Nature **395**:794–798. https://doi.org/10.1038/27427.

McCann, K. S., J. B. Rasmussen, and J. Umbanhowar. 2005. The dynamics of spatially coupled food webs. Ecology Letters **8**:513–523. https://doi.org/10.1111/j.1461-0248.2005.00742.x.

Monteith, D. T., A. G. Hildrew, R. J. Flower, P. J. Raven, W. R. B. Beaumont, P. Collen, A. M. Kreiser, E. M. Shilland, and J. H. Winterbottom. 2005. Biological responses to the chemical recovery of acidified fresh waters in the UK. Environmental Pollution **137**:83–101. https://doi.org/10.1016/j.envpol.2004.12.026.

Montoya, J. M., G. Woodward, M. C. Emmerson, and R. V. Solé. 2009. Press perturbations and indirect effects in real food webs. Ecology **90**:2426–2433. https://doi.org/10.1890/08-0657.1.

Montoya, M., S. L. Pimm, R. V. Sole, J. M. Montoya, S. L. Pimm, and R. V. Solé. 2006. Ecological networks and their fragility. Nature **442**:259–264. https://doi.org/10.1038/nature04927.

Morales-Castilla, I., M. G. Matias, D. Gravel, and M. B. Arau. 2015. Inferring biotic interactions from proxies. Trends in Ecology & Evolution **30**:347–356. https://doi.org/10.1016/j.tree.2015.03.014.

Neutel, A., J. A. P. Heesterbeek, and P. C. de Ruiter. 2002. Stability in real food webs: weak links in long loops. Science **296**:1120–1124.

Neutel, A. M., J. A. P. Heesterbeek, J. van de Koppel, G. Hoenderboom, A. Vos, C. Kaldeway, F. Berendse, P. C. de Ruiter, J. V. D. Koppel, G. Hoenderboom, A. Vos, C. Kaldeway, F. Berendse, and P. C. D. Ruiter. 2007. Reconciling complexity with stability in naturally assembling food webs. Nature **449**:599–602. https://doi.org/10.1038/nature06154.

Otto, S. B., B. C. Rall, and U. Brose. 2007. Allometric degree distributions facilitate food-web stability. Nature **450**:1226–1229. https://doi.org/10.1038/nature06359.

Petchey, O. L., A. P. Beckerman, J. O. Riede, and P. H. Warren. 2008. Size, foraging, and food web structure. Proceedings of the National Academy of Sciences **105**:4191–4196.

Petchey, O. L., and K. J. Gaston. 2002. Extinction and the loss of functional diversity. Proceedings of the Royal Society B **269**:1721–1727. https://doi.org/10.1098/rspb.2002.2073.

Pimm, S. L. 1982. Food webs. University of Chicago Press, Chicago.

Pimm, S. L. 1984. The complexity and stability of ecosystems. Nature **307**:321–326. https://doi.org/10.1038/307321a0.

Poisot, T., E. Canard, D. Mouillot, N. Mouquet, and D. Gravel. 2012. The dissimilarity of species interaction networks. Ecology Letters **15**:1353–1361. https://doi.org/10.1111/ele.12002.

Poisot, T., A. R. Cirtwill, K. Cazelles, D. Gravel, M.-J. Fortin, and D. B. Stouffer. 2016. The structure of probabilistic networks. Methods in Ecology and Evolution **7**:303–312. https://doi.org/10.1111/2041-210X.12468.

Poisot, T., D. B. Stouffer, and D. Gravel. 2015. Beyond species: why ecological interaction networks vary through space and time. Oikos **124**:243–251. https://doi.org/10.1111/oik.01719.

Pomeranz, J. P. F., R. M. Thompson, T. Poisot, J. S. Harding. 2018. Inferring predator–prey interactions in food webs. Methods in Ecology and Evolution **00**:1–12. https://doi.org/10.1111/2041-210X.13125

Pomeranz, J. P. F., H. J. Warburton, J. S. Harding. 2019. Anthropogenic mining alters macroinvertebrate size spectra in streams. Freshwater Biology. **64**:81–92. https://doi.org/10.1111/fwb.13196

R Development Core Team. 2017. R: A language and environment for statistical computing. R Core Team, Vienna, Austria.

Riede, J. O., U. Brose, B. Ebenman, U. Jacob, C. R. Townsend, R. M. Thompson, C. R. Townsend, and T. Jonsson. 2011. Stepping in Elton's footprints: a general scaling model for body masses and trophic levels across ecosystems. Ecology Letters **14**:169–178. https://doi.org/10.1111/j.1461-0248.2010.01568.x.

de Ruiter, P. C., A. Neutel, J. C. Moore, P. C. D. Ruiter, A. Neutel, and J. C. Moore. 1995. Energetics, patterns of interaction strengths, and stability in real ecosystems. Science **269**:1257–1260.

Sauve, A. M. C., E. Thébault, M. J. O. Pocock, and C. Fontaine. 2016. How plants connect pollination and herbivory networks and their contribution to community stability. Ecology **97**:908–917.

Schmid-Araya, J. M., P. E. Schmid, A. Robertson, J. Winterbottom, C. Gjerløv, and A. G. Hildrew. 2002. Connectance in stream food webs. Journal of Animal Ecology **71**:1056–1062. https://doi.org/10.1046/j.1365-2656.2002.00668.x.

Stoffels, R. J., S. Karbe, and R. A. Paterson. 2003. Length-mass models for some common New Zealand littoral-benthic macroinvertebrates, with a note on within-taxon variability in parameter values among published models. New Zealand Journal of Marine and Freshwater Research **37**:449–460. https://doi.org/10.1080/00288330.2003.9517179.

Tang, S., S. Pawar, and S. Allesina. 2014. Correlation between interaction strengths drives stability in large ecological networks. Ecology Letters **17**:1094–1100. https://doi.org/10.1111/ele.12312.

Thompson, R. M., and C. R. Townsend. 1999. The effect of seasonal variation on the community structure and food-web attributes of two streams: implications for food-web science. Oikos **87**:75–88.

Thompson, R. M., and C. R. Townsend. 2005. Food-web topology varies with spatial scale in a patchy environment. Ecology **86**:1916–1925.

Towers, D. J., I. M. Henderson, and C. J. Veltman. 1994. Predicting dry weight of New Zealand aquatic macroinvertebrates from linear dimensions. New Zealand Journal of Marine and Freshwater Research **28**:159–166. https://doi.org/10.1080/00288330.1994.9516604.

Tylianakis, J. M., E. Laliberté, A. Nielsen, and J. Bascompte. 2010. Conservation of species interaction networks. Biological Conservation **143**:2270–2279. https://doi.org/10.1016/j.biocon.2009.12.004.

Williams, R. J., and N. D. Martinez. 2000. Simple rules yield complex food webs. Nature **404**:180–183. https://doi.org/10.1038/35004572.

Winterbourn, M. J., W. F. Mcdiffett, S. J. Eppley, and M. Creek. 2000. Aluminium and iron burdens of aquatic biota in New Zealand streams contaminated by acid mine drainage: effects of trophic level. Science of the Total Environment **254**:45–54.

Woodward, G., B. Ebenman, M. Emmerson, J. M. Montoya, J. M. Olesen, A. Valido, and P. H. Warren. 2005. Body size in ecological networks. Trends in Ecology & Evolution **7**:402–409. https://doi.org/10.1016/j.tree.2005.04.005.

Woodward, G., and A. G. Hildrew. 2002. Body-size determinants of niche overlap and intraguild predation within a complex food web. Journal of Animal Ecology **71**:1063–1074.

Wootton, K. L., and D. B. Stouffer. 2015. Many weak interactions and few strong; food-web feasibility depends on the combination of the strength of species' interactions and their correct arrangement. Theoretical Ecology **9**:185–195. https://doi.org/10.1007/s12080-015-0279-3.

Yodzis, P. 1981. The stability of real ecoystems. Nature **289**:674–676.

Yohai, V. J. 1987. High Breakdown-Point and High Efficiency Robust Estimates for Regression. The Annals of Statistics **15**:642–656.

Supplementary Material

Table S1 Non-predatory taxa considered to be niche forbidden. Note: some “Non-predatory” taxa may predate upon microfauna, but because their prey are not adequately sampled using the methods here, they are considered facultative non-predatory.

Taxa	Justification
Austroclima	Scraper
Austrosimulium	Morphology
Blephariceridae	Scraper
Coloburiscus	Non-predatory
Deleatidium	Scraper
Elmidae	Non-predatory
Helicopsyche	Scraper
Hydraenidae	Non-predatory
Nesameletus	Non-predatory
Oligochaetae	Non-predatory
Olinga	Non-predatory
Oxyethira	Non-predatory
Potamopyrgus	Scraper
Rakiura	Scraper
Scirtidae	Shredder
Spaniocerca	Non-predatory
Spaniocercoides	Non-predatory
Zelandobius	Non-predatory
Zelolessica	Non-predatory
Zephlebia	Scraper

Table S2. All combinations of species (S) and connectance (C) values used in the generation of random adjacency matrices.

S	Mean C	High C	Low C
3	0.208889	0.289296	0.128481
3	0.230667	0.293472	0.167862
3	0.276444	0.338817	0.214072
4	0.12275	0.17201	0.07349
5	0.14128	0.182642	0.099918
6	0.173889	0.215176	0.132601
8	0.06975	0.091129	0.048371
9	0.090864	0.114718	0.06701
9	0.136049	0.161023	0.111076
10	0.0898	0.109789	0.069811
12	0.113306	0.133531	0.093081
16	0.102609	0.117033	0.088185
17	0.101702	0.115332	0.088072
17	0.084	0.095624	0.072376
18	0.106383	0.118306	0.094459
20	0.07139	0.081477	0.061303
23	0.096711	0.107116	0.086306
24	0.136035	0.145266	0.126804
26	0.081124	0.089561	0.072687
26	0.077213	0.085063	0.069363
27	0.110074	0.118208	0.101941
28	0.095577	0.103839	0.087314
32	0.093223	0.100233	0.086212
37	0.107947	0.114358	0.101537

Chapter Five: Discussion

Ecological networks, such as food webs, are simplified representations of the species and their interactions within a community. The importance of incorporating species interactions in understanding the structure, function and stability of ecological communities has gained momentum in recent years. This is one reason why it has been recommended that ecological networks be included in standard biomonitoring activities (Gray et al. 2014). Likewise, There is increasing interest in the field of conservation biology about the importance of conserving networks, rather than individual species (Tylianakis et al. 2010).

Despite their importance, ecological networks are rarely incorporated into biomonitoring or ecological studies across gradients (but see Thompson and Townsend 2004, Layer et al. 2010). This is primarily due to the considerable logistical constraints in constructing reasonable estimations of food webs. The quality of empirically constructed food webs is dependent on sampling effort with many hundreds of observations per taxa generally needed (Thompson et al. 2001, Layer et al. 2010). In impacted communities with low population densities, in can be difficult or overly destructive to collect enough individuals of each taxa to construct reasonable estimations of food webs (Hogsden and Harding 2012). One method to counter this limitation is the use of easy to measure alternative variables that can be used as a proxy for food web attributes, one such approach is the use of size spectra. Another method to avoid the constraints of traditional food web construction is the use of summary food webs (Gray et al. 2015). Summary food webs simply assume that whenever a link has been observed between two species, it always occurs whenever those species co-occur within a community. However, there is growing evidence that links between species are dependent on other factors, including phenology, resource availability, and trait distributions (Morales-Castilla et al. 2015, Poisot, Stouffer, and Gravel 2015). However, summary webs do not account for species interactions varying among sites, and may be less sensitive to subtle changes in structure (Gray et al. 2015).

I tested the utility of food web alternatives (e.g. size spectra), developed a novel, mechanistic method for inferring empirical food-web structure, and I applied this method using field survey and modelling approaches in stream communities across an AMD gradient. I suggest that this work has increased our understanding of how AMD impacts food-web structure and stability.

Size-spectra relationships are consistently affected by AMD inputs

Size-spectra capture much of the variability in food-web structure, but require less effort to construct. In addition, size spectra have been shown to respond to environmental impacts, and have been suggested as a "universal" indicator of ecological conditions (Petchey and Belgrano 2010).

In my study, which used data from a wide range of AMD impacted streams, size-spectra responded consistently to increasing AMD inputs (Chapter Two). Shallower slopes of size spectra relationships in impacted streams indicated a reduction in energy transfer efficiency, and lower intercepts indicated a reduction in total community abundance. A decrease in the range of sizes, particularly the loss of largest individuals, indicated the loss of top predators and hence shorter food chain lengths. However, not all of the largest body sizes are predators. For example, the stonefly genus *Austroperla* is relatively large-bodied (compared to other New Zealand stream invertebrates) but is classified as non-predatory (Chadderton 1988). However, a decrease in their abundance still leads to reduced energy transfer, because larger animals have greater total energy demands than smaller ones.

While my results clearly indicate changes in food-web structure, they are equivocal on the implications for food-web stability. Specifically, a reduction in energy pathways and transfer efficiency suggests a decrease in stability due to a loss of ecological redundancy and reliance on only a few energy pathways. Alternatively, because interaction strengths are related to body size (Emmerson and Raffaelli 2004), and the largest size classes are lost (Chapter Two), this suggests that the magnitude of the remaining food web links in AMD impacted streams are weak, a network structure which is generally associated with stability (Neutel, Heesterbeek, and de Ruiter 2002). Therefore, my results suggest that size spectra analysis may not be appropriate for studies on food-web stability. However, size spectra analyses still provided useful approximations of food-web structure, and can be used for the rapid assessment of sites.

Size spectra analyses offers the ability to understand how novel stressors affect communities. Because this method is independent of taxa identity, it foregoes the necessity of having prior knowledge of species-specific responses to contaminants, or knowledge of sensitive and tolerant taxa. With the growing presence of synthetic chemicals in the environment (Bernhardt, Rosi, and Gessner 2017), increasing impacts of climate change (Dossena et al. 2012), and species introductions and extinctions (Pereira et al. 2010), the ability to rapidly assess food-web structure and function without having vast *a priori* knowledge of a specific system or species within the community will offer valuable insights for future management and mitigation of anthropogenic impacts on ecosystems. While my study supports the use of size-spectra analysis as an ecological indicator, further research is needed in different habitats and in response to additional stressors before a robust theory of size-spectra responses to environmental disturbances can be confidently formulated.

These results support the use of size-spectra as an indicator of ecological health; however; this method has some limitations. For example, it is necessary to measure the size of all individuals (e.g. many hundreds to thousands) from a sample. If length-weight relationships are available, only linear measurements are necessary (Martínez et al. 2016). Otherwise, all individuals would need to be weighed, greatly increasing the laboratory processing time. However, when length-weight relationships are known, size-spectra analyses are arguably faster than traditional food-web methods, which require the visual identification of gut contents from a similar number of individuals. Furthermore, advances in analytic techniques such as machine learning may alleviate some of the logistical constraints of this process. For example, computer models have been trained to identify animal species from digital images captured from stationary camera traps (Willi et al. 2018, Norouzzadeh et al. 2018). Using similar techniques, it may be possible to train a computer model to identify and measure the longest axis of all objects in a digital image, greatly reducing the time necessary for these analyses

While the shape of size spectra relationships are correlated with changes in food-web structure, there is evidence that other factors can also affect this shape. For example, the exploitation of allochthonous resources in the form of terrestrial invertebrates by trout leads to shallower slopes (e.g. higher abundance of large body sizes) than would be expected based on allometric scaling predictions (Perkins et al. 2018). This makes it difficult to attribute changes in size spectra to changes in food-web structure with complete certainty. However, my research findings suggest that further studies of size spectra relationships across multiple habitats and under varying environmental conditions will increase our ability to use them as ecological indicators.

Novel method accurately infers food-web structure

The inference of food-web structure in order to avoid logistical constraints of sample size, laboratory time, expertise, and financial burdens is an important goal for contemporary ecologists (Morales-Castilla et al. 2015). In recent years, improvements in mechanistic understanding of what drives pairwise-species interactions, as well as improvements in computing and the widespread adoption of open-source programming languages such as R have led to the development of methods which can be used to infer biotic interactions (Gravel et al. 2013, Morales-Castilla et al. 2015, Bartomeus et al. 2016, Poisot et al. 2016). Methods exist for the inference of interaction backbones within networks (Morales-Castilla et al. 2015), as well as identification of links which are likely to occur, even in the absence of direct empirical evidence (Stock et al. 2017).

I have developed a novel method of inferring food-web structure which accurately reproduces that observed empirically in streams under different land use types (Chapter Three). This novel method retains the ability of species-interactions to vary based on the local distributions of traits (e.g. body size) and population densities (Poisot, Stouffer, and Gravel 2015). This differs from methods using summary webs, which retain all pairwise species interactions, whenever the two species co-occur (Chapter Three, Gray et al. 2015). With data on species-average body sizes and densities, this method allows for the rapid assessment of potential food-web structure across spatial and temporal scales, and across environmental gradients (e.g. Poisot et al. 2016, Tylianakis and Morris 2017). Understanding changes in food-web structure can aid in our ability to test ecological theory across broad scales (e.g. How do so many species coexist? What are the processes driving community assembly? How will communities respond to climate change?).

One critical assumption of these models is that the relationship between predator and prey body sizes does not vary in response to changes in habitat or abiotic conditions. However, the strength of the allometric relationships between predator and prey body size has been shown to vary within and between habitats (Brose et al. 2006, Gravel et al. 2013), and there is evidence that the relationship can change in response to prey limitation (Costa-Pereira et al. 2018). However, these shortcomings may be alleviated by the use of individual interaction data as opposed to species averages (Nakazawa 2017). Furthermore, this method greatly reduces the number of possible links to sample (e.g. Morales-Castilla et al. 2015) allowing researchers to focus their sampling efforts where they will be most beneficial (Stock et al. 2017).

Inferring trophic interactions across an AMD gradient reveals changes to structure and stability

In Chapter Four, I applied a modification of the method developed in Chapter Three in order to infer the food-web structure of 25 streams across an AMD gradient. I inferred link probability and generated 250 iterations of food webs for each stream in order to assess the effects of variable food-web structure. My results were in general agreement with a previous study of food webs in AMD impacted streams (Hogsden and Harding 2012). Namely, AMD impacted food webs were small, simple, and well-connected. Furthermore, the inferred reduction in links in AMD impacted streams indicates a reduction in energy pathways and lower trophic transfer efficiency to higher trophic levels. Reduced energy pathways and trophic transfer efficiency was also suggested by an experimental leaf breakdown study of AMD impacted streams in New Zealand (Hogsden and Harding 2013). This is in agreement with the conclusions reached in Chapter Two, where a shallower size-spectra slope also indicates reduction of trophic transfer efficiency.

My results demonstrate that AMD impacted streams are generally more stable than non-impacted streams. This supports the notion that stability may not always be desirable (Tylianakis et al. 2010, Lake 2013), which has implications for the restoration of AMD impacted streams. For example, macroinvertebrate community composition in restored streams was not significantly different from untreated sites after 11 years of passive AMD treatment (DeNicola and Stapleton 2016). Impacted streams with a high degree of food-web stability may require other restoration activities in addition to the amelioration of abiotic conditions (Lake 2013). These may include physical disturbances (e.g. scouring flood) or active species reintroductions (Jourdan et al. 2018) in order to reorganize the food-web structure and allow the successful recolonization of extirpated sensitive species. However, some of the impacted streams had stability values similar to the un-impacted streams. While the mechanisms driving lower stability of some impacted streams remains unclear (Chapter Four), these streams may be better candidates for restoration than the more stable AMD impacted streams. Future work is needed to investigate the responses of impacted streams with varying levels of stability to restoration projects.

Conclusions

My research supports the use of size-spectra analysis as a tractable method for biomonitoring and assessing ecological condition (Chapter Two). Size spectra analysis may offer an important middle ground in biomonitoring, which incorporates some of the structural and functional changes in food-web structure (Chapters Two and Four), without the logistical constraints of constructing food webs through visual identification of gut contents. Furthermore, my work has expanded our understanding of how AMD inputs affect the structure, function, and stability of stream food webs by inferring links (Chapter Four). Finally, the method I developed for inferring food-web structure (Chapter Three) allows for the rapid assessment of potential food-web structure with reduced logistical burden compared to traditional empirical food web construction. Although my work focuses on the effects of AMD stress on stream communities, these results have much broader ecological implications, and support the use of alternative metrics in biomonitoring surveys. As size spectra analysis are taxonomy-free descriptors of communities, they provide endpoints for understanding the effects of novel impacts, without *a priori* knowledge of species-specific responses. Furthermore, modelling pairwise interactions based on species-average body size and densities allow for the construction of potential food-web structure across spatial and temporal scales. This will aid in the ability to test foundational ecological theory, and provide evidence for the stability of communities.

References

Bartomeus, I., D. Gravel, J. M. Tylianakis, M. A. Aizen, I. A. Dickie, and M. Bernard-Verdier. 2016. A common framework for identifying linkage rules across different types of interactions. Functional Ecology **30**:1894–1903. https://doi.org/10.1111/1365-2435.12666.

Bernhardt, E. S., E. J. Rosi, and M. O. Gessner. 2017. Synthetic chemicals as agents of global change. Frontiers in Ecology and the Environment **15**:84–90. https://doi.org/10.1002/fee.1450.

Brose, U., T. Jonsson, E. L. Berlow, P. Warren, C. Banasek-Richter, L.-F. Bersier, J. L. Blanchard, T. Brey, S. R. Carpenter, M.-F. C. Blandenier, L. Cushing, H. A. Dawah, T. Dell, F. Edwards, S. Harper-Smith, U. Jacob, M. E. Ledger, N. D. Martinez, J. Memmott, K. Mintenbeck, J. K. Pinnegar, B. C. Rall, T. S. Rayner, D. C. Reuman, L. Ruess, W. Ulrich, R. J. Williams, G. Woodward, and J. E. Cohen. 2006. Consumer-resource body-size relationships in natural food webs. Ecology **87**:2411–2417. https://doi.org/10.1890/0012-9658(2006)87[2411:CBRINF]2.0.CO;2.

Chadderton, W. L. 1988. Faunal and chemical characteristics of some Stewart Island streams. New Zealand Natural Sciences **15**:43–50.

Costa-Pereira, R., M. S. Araújo, R. da S. Olivier, F. L. Souza, and V. H. W. Rudolf. 2018. Prey limitation drives variation in allometric scaling of predator-prey interactions. The American Naturalist **192**:E139–E149. https://doi.org/10.1086/698726.

DeNicola, D. M., and M. G. Stapleton. 2016. Using macroinvertebrates to assess ecological integrity of streams remediated for acid mine drainage. Restoration Ecology **24**:656–667. https://doi.org/10.1111/rec.12366.

Dossena, M., G. Yvon-Durocher, J. Grey, J. M. Montoya, D. M. Perkins, M. Trimmer, and G. Woodward. 2012. Warming alters community size structure and ecosystem functioning. Proceedings of the Royal Society B **279**:3011–3019. https://doi.org/10.1098/rspb.2012.0394.

Emmerson, M. C., and D. Raffaelli. 2004. Predator–prey body size, interaction strength and the stability of a real food web. Journal of Animal Ecology **73**:399–409.

Gravel, D., T. Poisot, C. Albouy, L. Velez, and D. Mouillot. 2013. Inferring food web structure from predator–prey body size relationships. Methods in Ecology and Evolution **4**:1083–1090. https://doi.org/10.1111/2041-210X.12103.

Gray, C., D. J. Baird, S. Baumgartner, U. Jacob, G. B. Jenkins, E. J. O'Gorman, X. Lu, A. Ma, M. J. O. Pocock, N. Schuwirth, M. Thompson, and G. Woodward. 2014. Ecological networks: the missing links in biomonitoring science. Journal of Applied Ecology **51**:1444–1449. https://doi.org/10.1111/1365-2664.12300.

Gray, C., D. Figueroa, L. Hudson, A. Ma, D. Perkins, and G. Woodward. 2015. Joining the dots: an automated method for constructing food webs from compendia of published interactions. Food Webs **5**:11–20. http://dx.doi.org/10.1016/j.fooweb.2015.09.001.

Hogsden, K. L., and J. S. Harding. 2012. Anthropogenic and natural sources of acidity and metals and their influence on the structure of stream food webs. Environmental Pollution **162**:466–474. https://doi.org/10.1016/j.envpol.2011.10.024.

Hogsden, K. L., and J. S. Harding. 2013. Leaf breakdown, detrital resources, and food webs in streams affected by mine drainage. Hydrobiologia **716**:59–73. https://doi.org/10.1007/s10750-013-1544-3.

Jourdan, J., M. Plath, J. D. Tonkin, M. Ceylan, A. C. Dumeier, G. Gellert, W. Graf, C. P. Hawkins, E. Kiel, A. W. Lorenz, C. D. Matthaei, P. F. M. Verdonschot, R. C. M. Verdonschot, and P. Haase. 2018. Reintroduction of freshwater macroinvertebrates: challenges and opportunities. Biological Reviews. https://doi.org/10.1111/brv.12458.

Lake, P. S. 2013. Resistance, resilience and restoration. Ecological Management and Restoration **14**:20–24. https://doi.org/10.1111/emr.12016.

Layer, K., J. O. Riede, A. G. Hildrew, and G. Woodward. 2010. Food web structure and stability in 20 streams across a wide pH gradient. Advances in Ecological Research **42**:265–299.

Martínez, A., A. Larrañaga, A. Miguélez, G. Yvon-Durocher, and J. Pozo. 2016. Land use change affects macroinvertebrate community size spectrum in streams: the case of Pinus radiata plantations. Freshwater Biology **61**:69–79. https://doi.org/10.1111/fwb.12680.

Morales-Castilla, I., M. G. Matias, D. Gravel, and M. B. Arau. 2015. Inferring biotic interactions from proxies. Trends in Ecology & Evolution **30**:347–356. https://doi.org/10.1016/j.tree.2015.03.014.

Nakazawa, T. 2017. Individual interaction data are required in community ecology: a conceptual review of the predator–prey mass ratio and more. Ecological Research **32**:5–12. https://doi.org/10.1007/s11284-016-1408-1.

Neutel, A., J. A. P. Heesterbeek, and P. C. de Ruiter. 2002. Stability in real food webs: weak links in long loops. Science **296**:1120–1124.

Norouzzadeh, M. S., A. Nguyen, M. Kosmala, A. Swanson, M. S. Palmer, C. Packer, and J. Clune. 2018. Automatically identifying, counting, and describing wild animals in camera-trap images with deep learning. Proceedings of the National Academy of Sciences **115**:E5716–E5725. https://doi.org/10.1073/pnas.1719367115.

Pereira, H. M., P. W. Leadley, V. Proença, R. Alkemade, J. P. W. Scharlemann, J. F. Fernandez-Manjarrés, M. B. Araújo, P. Balvanera, R. Biggs, W. W. L. Cheung, L. Chini, H. D. Cooper, E. L. Gilman, S. Guénette, G. C. Hurtt, H. P. Huntington, G. M. Mace, T. Oberdorff, C. Revenga, P. Rodrigues, R. J. Scholes, U. R. Sumaila, and M. Walpole. 2010. Scenarios for global biodiversity in the 21st century. Science **330**:1496–1501. https://doi.org/10.1126/science.1196624.

Perkins, D. M., I. Durance, F. K. Edwards, J. Grey, A. G. Hildrew, M. Jackson, J. I. Jones, R. B. Lauridsen, K. Layer-Dobra, M. S. A. Thompson, and G. Woodward. 2018. Bending the rules: exploitation of allochthonous resources by a top-predator modifies size-abundance scaling in stream food webs. Ecology Letters. https://doi.org/10.1111/ele.13147.

Petchey, O. L., and A. Belgrano. 2010. Body-size distributions and size-spectra: universal indicators of ecological status? Biology Letters **6**:434–437. https://doi.org/10.1098/rsbl.2010.0240.

Poisot, T., D. Gravel, S. Leroux, S. A. Wood, M. J. Fortin, B. Baiser, A. R. Cirtwill, M. B. Araújo, and D. B. Stouffer. 2016. Synthetic datasets and community tools for the rapid testing of ecological hypotheses. Ecography **39**:402–408. https://doi.org/10.1111/ecog.01941.

Poisot, T., D. B. Stouffer, and D. Gravel. 2015. Beyond species: why ecological interaction networks vary through space and time. Oikos **124**:243–251. https://doi.org/10.1111/oik.01719.

Stock, M., T. Poisot, W. Waegeman, and B. D. Baets. 2017. Linear filtering reveals false negatives in species interaction data. Scientific Reports **7**:1–8. https://doi.org/10.1038/srep45908.

Thompson, R. M., E. D. Edwards, A. R. McIntosh, and C. R. Townsend. 2001. Allocation of effort in stream food-web studies: the best compromise? Marine and Freshwater Research **52**:339–345.

Thompson, R. M., and C. R. Townsend. 2004. Land-use influences on New Zealand stream communities: Effects on species composition, functional organisation, and food-web structure. New Zealand Journal of Marine and Freshwater Research **38**:595–608. https://doi.org/10.1080/00288330.2004.9517265.

Tylianakis, J. M., E. Laliberté, A. Nielsen, and J. Bascompte. 2010. Conservation of species interaction networks. Biological Conservation **143**:2270–2279. https://doi.org/10.1016/j.biocon.2009.12.004.

Tylianakis, J. M., and R. J. Morris. 2017. Ecological networks across environmental gradients. Annual Review of Ecology, Evolution, and Systematics **48**:25–48.

Willi, M., R. T. Pitman, A. W. Cardoso, C. Locke, A. Swanson, A. Boyer, M. Veldthuis, and L. Fortson. 2018. Identifying animal species in camera trap images using deep learning and citizen science. Methods in Ecology and Evolution. https://doi.org/10.1111/2041-210X.13099.

Acknowledgements

First, I would like to thank my advisor, Jon Harding. Thank you for letting me take some risks and explore what I was passionate about, even if you didn't always think it would work. The genetics idea didn't really pan out, but I think we were still able to do some cool work, and I hope it adds to the field of ecology.

Thanks to my committee for all the help throughout the last few years. Particular thanks for the feedback on my writing, and dealing with my jokes through email, without the benefit of non-verbal cues.

Thanks to all of my co-authors and colleagues, near and far. Collaboration is one of my highest aspirations as a scientist, and working with y'all has been a delight.

Thanks to my family back home for always being supportive, even when it means flying nearly to the antipodal point of this blue dust mote we all inhabit.

Thanks to the CO dream team. You weren't directly involved in this little project, but your WhatsApp messages were warmly received, and reminded me of how great our community is, and how lucky I am to be a part of it.

Last, but never least, thank you to my partner Carlyn Perovich. Thanks for joining me on this part of our adventure together. Thanks for being the best field technician, and bug picker a boy could ask for. Thanks for always being willing to talk to me about science, and particularly my hairbrained ideas and personal hypotheses. Thank you for your endless capacity to be my own personal editor, and doing your best to teach me proper grammar (some day I'll get it! (Maybe.)). Thank you for all of the nights spent under the open stars, the long backpacking trips to the middle of nowhere, always having me on belay, and always being ready to crack open a beer. You're without question the best.

I gratefully acknowledge a research grant from the New Zealand MBIE Endeavour Fund (Grant CLRE 1202), funding from the University of Canterbury Doctoral Scholarship, and travel funds

from the New Zealand Freshwater Science Society S.I.L. Trust Fund (1987), which made this work possible, and allowed me to share it with the scientific community.

Appendix: Site Locations

Table 1. Northing and Easting (New Zealand Transverse Mercator Projection) of all sites in this work. Stream X and Y were located on private property, and access was granted under the condition that their names and locations not be disclosed.

Site Name	Northing	Easting
Burke	534320	1510562
Cannel	5309056	1457153
One Horse Creek	5317165	1502552
Coorang	5317347	1502974
Lankey	5333406	1509167
Murray	5334950	1507735
Coal	5343749	1510569
Italian	5344326	1510157
Coalbrookedale	5376001	1500297
Burnett	5376034	1500272
Burnett Tributary	5376409	1500722
Sullivan West	5377541	1498061
Sullivan East	5377645	1498661
Kiwi	5381937	1497425
Portal	5389700	1506350
Hot	5389900	1506300
Packtrack	5389990	1506255
Granity	5390385	1505783
Miller	5390445	1505828
Mine	5390549	1507076
Charming	5395578	1512982
Warne	5395728	1512120
Chasm	5396388	1516324
Stream X	-	-
Stream Y	-	-