Cumulative Impacts of Multiple Stressors on Aquatic Communities: Novel Fish Predators in Warming Mountain Lakes

by

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Abstract

Novel anthropogenic perturbations (i.e. stressors) often mediate the effects of each other, generating “ecological surprises”. While the cumulative impact of certain stressors on individual organisms and populations is becoming better understood, little is known about how their net effects transfer to higher levels of biological organization. To address this knowledge gap, I investigated the influence of species co-tolerance (i.e. the correlation between species tolerances to two stressors) and meta-community dynamics on the net effects of two common stressors of planktonic food webs in naturally fishless lakes, namely introduced trout (*Oncorhynchus mykiss, Salvelinus fontinalis* and *Oncorhynchus clarkii*) and higher temperatures. To help formulate my hypotheses, I analyzed trends in observational data using 1) a survey of naturally fishless and stocked lakes positioned along an elevational (and thus climatic) gradient and, 2) monitoring records of an alpine lake when stocked trout were absent and present across a 24-year period of climatic variation. My main hypotheses were that 1) non-native trout synergistically increases the effect of higher temperatures on planktonic communities of montane lakes, 2) positively correlated species tolerances to non-native trout and warming, driven by selection for the same trait by both stressors (i.e. smaller body-size), can help explain the influence of exposure sequence on their cumulative effects on species composition and functional structure, and 3) regional zooplankton imported from a diverse array of lakes can functionally compensate for local alpine species suppressed by non-native trout and warming. I experimentally tested these hypotheses using controlled manipulations of fish and temperature on planktonic food webs from naturally fishless mountain lakes.

Empirical evidence from the spatial survey and outdoor mesocosm experiment following a two-factor, fully crossed design [(fish absent vs. present) × (ambient temperature vs. warmed)] with randomized blocking supported my first hypothesis by showing that the positive effect of higher temperatures on community biomass occurred only in the presence of non-native trout. The synergistic interaction between the stressors likely occurred because warming stimulated reproduction of smaller herbivores that were released from competition and predation in fish-stocked communities. Further, introduced trout stimulated primary production, likely by suppressing large efficient herbivores (*Daphnia* spp.), but also potentially by increasing nutrient recycling.

As hypothesized, both non-native trout and warming selected for small body-size and planktonic communities of naturally fishless montane lakes showed positive co-tolerance to the stressors. Using a novel approach integrating species traits and co-tolerances and a one-factor experiment consisting of three stressor treatments (Unstressed, Warming then fish, and Fish then warming) with randomized blocking, I discovered that positive co-tolerance to trout and warming helped explain their net effect on the functional structure of communities. Together, the stressors extirpated larger species occupying higher trophic levels, which lacked tolerance to either stressor, while stimulating smaller co-tolerant species, regardless of exposure sequence.

In the temporal survey and growth-chamber experiment following a three-factor, fully-crossed design [(size-selective predation absent vs. present) × (ambient temperature vs. warmed) × (local vs. local + regional species pools)] with randomized blocking, predation by non-native trout and higher temperatures each decreased community biomass in naturally fishless alpine lakes by suppressing large zooplankton. The experiment also revealed that declines of alpine species increased the establishment of regional zooplankton, including a diversity of montane

species under warmed conditions. Yet, contrary to my hypothesis, the introduction of regional species did not alleviate the negative effect of fish predation and warming on alpine communities, suggesting that upward dispersal of montane species is unlikely to provide functional compensation to stressed alpine communities.

My finding that non-native trout and warming had opposite effects on lower montane versus alpine lake communities highlights the challenging context dependency of their cumulative ecological impacts. Nevertheless, my integration of species co-tolerances with related key traits provides a promising tool to help predict the net effects of these and other stressors across ecological communities and habitats. A better understanding of the relationship between species tolerances to co-occurring stressors may also help prioritize management actions. For example, my discovery that non-native trout and higher temperatures similarly affect zooplankton communities suggests that fish removals may be best targeted in lakes that will experience less warming, such as those containing natural climate refugia (e.g. stable coldwater hypolimnia). Otherwise, climate warming may prevent planktonic food webs from recovering following the removal of introduced sportfish.

Preface

This thesis is an original work by Megan MacLennan. The use of rainbow trout fingerlings in experimental enclosures in Chapter 2 received research ethics approval from the University of Alberta Research Ethics Board, Project Name “Interactive effects of introduced trout and climate warming on planktonic food webs in alpine lakes of the Alberta Rockies”, No. 692, February 2, 2011.

Chapter 2 of this thesis has been published:

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I was responsible for conducting the survey and outdoor mesocosm experiment, data analysis, and manuscript composition. C. Dings-Avery assisted with zooplankton enumerations. R.D. Vinebrooke was the supervisory author and was involved with concept formation and study design, and contributed to manuscript edits.

Chapter 4 of this thesis has been accepted for publication in Hydrobiologia pending minor revisions. I was responsible for conducting the experiment, data analysis, and manuscript composition. R.D. Vinebrooke was the supervisory author and was involved in collection of biomonitoring data, concept formation and study design, and contributed to manuscript edits.

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CHAPTER 1: INTRODUCTION

"Global change" is defined by the cumulative effects of human-caused environmental changes around the world (Vitousek 1992, 1994). However, the net effects of these environmental changes (i.e. stressors; Jackson et al. 2015a) often cannot be predicted based solely on their individual effects (Crain et al. 2008, Darling and Cote 2008). Instead, stressors can mediate the effect of each other through unexpected interactions, resulting in "ecological surprises" (Paine et al. 1998, Christensen et al. 2006, Lindenmayer et al. 2010). Although interactions between stressors can be defined in several ways, they typically involve net effects that significantly differ from the sum of their individual effects (i.e. an additive expectation) (Folt et al. 1999). Antagonistic and synergistic interactions are generally denoted by net effects of stressors that are lesser or greater than additive, respectively (Figure 1-1). Such interactions are ubiquitous, occurring among many different stressors and affecting a diversity of organisms across various ecosystems (e.g., grasslands (Shaw et al. 2002), lakes (Schindler 1998a), coral reefs (Darling et al. 2010, Melbourne-Thomas et al. 2011), and kelp forests (Wernberg *et al.* 2010)). Consequently, potential interactions among stressors are a key source of uncertainty in forecasts of global change impacts (Sala et al. 2000a, Fisichelli et al. 2014).

Investigation of multiple stressors has been touted as a research priority because of their important management implications (Segner et al. 2014, Hering et al. 2015). Despite the surge in research interest, large knowledge gaps persist regarding the cumulative impacts of multiple stressors. In particular, we are unable to reliably explain why interactions among stressors are often context dependent, varying across both time and space (Blaustein and Kiesecker 2002). Further, our knowledge of multiple-stressor effects is disconnected across levels of biological organization (Sulmon et al. 2015) with effects at the community-level receiving little attention (Moe et al. 2013).

The primary objective of my research was to determine the individual and combined effects of non-native trout (*Oncorhynchus mykiss, Salvelinus fontinalis* and *Oncorhynchus clarkii*) and higher temperatures on planktonic communities of mountain lakes. Most importantly, I focused on potential mechanisms driving the effects of stressors on planktonic diversity and functional structure. To do so, I developed a conceptual framework integrating mechanisms that are expected to lead to stressor interactions across different levels of biological organization. The framework guided my research questions and formed the basis for generation of my hypotheses. Below I define "stressor" as it is used in the context of global change, present the conceptual framework for my research, describe approaches to study the effects of multiple stressors, discuss the rationale for my research, and outline the key objectives and hypotheses addressed in my thesis chapters.

Definition of a "stressor"

Ecological research on multiple stressors is relatively new, having emerged from an increasing need to better understand and predict the consequences of global change. The meaning of stressor within this anthropogenic context does not necessarily fit within traditional definitions from stress ecology. In that field, there is a general consensus that "stressor" refers to an external factor that causes "stress", which refers to an internal state (Van Straalen 2003). As such, a stressor is sometimes defined in relation to the physiological, hormonal or behavioural "stress" response that it elicits within an organism (Romero 2004). This usage is limiting in the context of multiple stressor effects where we are interested in effects on populations, communities and ecosystems, as well as individual organisms. Other classic definitions describe a “stressor” more generally as an external force that has an adverse effect on a biological system (Odum 1985, Grime 1989). This definition is also inadequate in the context of global environmental change because the same perturbation can have both positive and negative effects on different species, across different levels of biological organization, or even across different response variables (Crain et al. 2008, Piggott et al. 2015b).

The term “stressor” has also often been used synonymously with “disturbance” and the distinction between the two terms has been an ongoing debate (Kolasa and Pickett 1992, Rykiel 2006). Disturbance was originally viewed as a destructive force under the paradigm that ecological systems were stable and self-regulating in an equilibrium state. More modern perspectives have shifted to a “non-equilibrium” view of nature where disturbance is an integral component of ecosystems shaping species compositions (DeAngelis et al. 1985), while a stressor drives a biological system on a new trajectory outside of its natural dynamic equilibrium (Borics et al. 2013). Despite this, terms that conflate their meanings, such as “natural stressors” (e.g. Holmstrup et al. 2010), are pervasive.

Here, I define a stressor as a human-induced novel perturbation that displaces an environmental parameter beyond its natural range of variation. Instead of characterizing a stressor by the biological response it elicits, this definition is based on properties of the stressor itself. As a result, this definition captures how a stressor can exert either negative or positive influences on ecological responses, and also allows for a stressor to have no effect on some ecological properties. It also clearly differentiates between anthropogenic stressors and naturally-occurring disturbance regimes, while allowing changing frequencies or intensities of periodic disturbance events to be viewed as stressors. Further, this definition includes changes in the distribution or abundance of species caused by humans, allowing introduced invasive species to be considered a stressor, while excluding natural biotic interactions between native species, such as predation and competition.

A mechanistic framework of stressor interactions on communities

Improving our ability to predict when and how stressors interact will require a better understanding of the mechanisms driving their individual and combined effects (Darling et al. 2010, Jansen et al. 2011). Here, I present a conceptual framework linking mechanisms that generate interactive effects of stressors across different levels of biological organization, and spatial and temporal scales (Figure 1-2). I have grouped these potential drivers of interactive effects into four categories as depicted in Figure 1-2: (1) exogenous interactions (red arrow); (2) endogenous interactions (green arrows); (3) altered biotic interactions (blue arrows); and (4) dispersal within meta-populations and meta-communities (orange arrows). These mechanisms are not mutually exclusive and more than one may be operating to generate the observed net effect of stressors.

Exogenous interactions

An exogenous interaction is perhaps the simplest or most direct means by which stressors exert interactive effects (red arrow in Figure 1-2). Here, chemical or physical modification of one stressor by another takes place in the external environment prior to affecting species therein. For example, ultraviolet radiation can photochemically alter certain pollutants (i.e. pesticides and fertilizers) to synergistically increase their toxicity to amphibians (Blaustein et al. 2003), while lake acidification precipitates photoprotective dissolved organic matter out of the water column, thereby exposing aquatic biota to increased levels of damaging ultraviolet radiation (Hader et al. 2011). The processes underlying exogenous stressor interactions, such as altered chemical speciation and binding, have been well-studied in the field of ecotoxicology (Spurgeon et al. 2010).

Endogenous interactions

Endogenous interactions occur from within the biota when one stressor selects for functional traits that influence the tolerance of organisms to another stressor (Vinebrooke et al. 2004). Positively correlated tolerances to different stressors, referred to as positive co-tolerance, is hypothesized to lead to antagonistic interactions because exposure to one stressor selects for traits that, on average, increase resistance to the second stressor. In contrast, negative co-tolerance involves a trade-off in the ability of organisms to cope with different stressors, and is hypothesized to lead to synergistic interactions. Below I describe how correlated traits can influence the effects of multiple stressors on individuals, populations, and communities (green arrows in Figure 1-2).

Individual-level

The correlation between certain physiological, morphological, or behavioural traits can influence the ability of an individual to acclimate to different stressors (Todgham and Stillman 2013). Metabolic changes, diet shifts, altered habitat selection, and modified activity levels in response to one stressor can alter the tolerance of an individual to a subsequent stressor. For example, warming can reduce UV-induced DNA damage in certain zooplankton by enhancing photo-enzymatic repair (Williamson et al. 2002). Acclimation responses to one stressor can also increase or decrease an organism’s exposure to another stressor. For example, earlier sea ice break-up has caused polar bears to feed on more open-water seal species, which contain larger contaminant loads (McKinney et al. 2013). Recently, substantial progress has been made in integrating the effects of multiple stressors on individuals by applying the concept of “energy-limited tolerance” based on the premise that organisms need to allocate more energy to basal maintenance costs under stressful conditions (Sokolova 2013). Generally, it is expected that increased energy expenditure required to cope with one stressor will reduce an organism’s ability to deal with future stressors (Fischer et al. 2013, Queirós et al. 2015).

Population-level

At the population-level, adaptive selection of heritable traits can modify multiple regions of the genome to increase or decrease susceptibility to subsequent stressors (Sgro and Hoffmann 2004, Gonzalez and Bell 2013). Often maladaptive costs associated with stress tolerance, such as slower growth or decreased fertility, reduce average fitness and increase the susceptibility of a population to another stressor (Eranen et al. 2009). However, positive genetic correlation can also occur whereby certain genotypes are associated with increased tolerance to multiple stressors (Foo et al. 2012).

Further, some stressors can directly affect genetic diversity and influence the probability that a genotype that is tolerant to a subsequent stressor is present (Bach and Dahllof 2012). For example, isolation of populations through habitat fragmentation can erode genetic diversity, lowering the potential of a species to evolutionarily adapt to a future stressor (Dixo et al. 2009). Conversely, invasive species can increase genetic diversity and adaptive potential to future stressors by hybridizing with native species (Hoffmann and Sgro 2011). Evidence to date suggests that multiple stressors will increase extinction risk of populations by enhancing genetic erosion beyond those of single stressors (Leitao et al. 2013).

Community-level

At the community level, selection of functional traits in response to one stressor can drive compositional shifts toward species that are more tolerant or more sensitive to a subsequent stressor (Vinebrooke et al. 2004). For example, higher UV-radiation decreases the effects of cadmium toxicity on periphyton communities because sensitive diatoms are replaced by tolerant cyanobacteria (Navarro et al. 2008). Positive species co-tolerance is also the core mechanism underlying the “pollution-induced community tolerance” concept used in ecotoxicology, which posits that shifts toward tolerant species induced by a toxicant can increase the resistance of the community to a similar-acting toxicant (Blanck 2002). Theory on how species co-tolerance may influence community-level responses to multiple stressors is still in its early stages and has focused on biodiversity effects (Vinebrooke et al. 2004, Brown et al. 2013). Few studies have empirically tested for species co-tolerance and the potential use of species co-tolerance to predict the net effects of multiple stressors on communities remains unclear. Further, evidence of species co-tolerance has relied on expert-weighted indices to evaluate species sensitivities to each stressor, as a general approach for quantifying the sensitivity of species to different stressors is lacking (Graham et al. 2011).

Altered biotic interactions

Biotic interactions can also transfer the effect of a stressor across different levels of biological organization, thereby mediating the direct effect of another stressor (blue arrows in

Figure 1-2) (Walther 2010, Puccinelli 2012, Moe et al. 2013). Indeed, indirect effects of multiple stressors on species interactions can override their direct effects on species performance (Griffiths et al. 2015). Antagonistic interactions are the most common net effect of stressors at the community-level, while synergistic interactions are most common at the population-level, suggesting that interspecific interactions and functional compensation among species may mitigate the effects of multiple stressors (Crain et al. 2008, Piggott et al. 2015a). Functional redundancy among species allows competing species to take over a portion of the ecological process that would otherwise be lost when one or more of them is removed or reduced (Walker 1992, Naeem 1998). Thus, reductions in competition, followed by functional compensation by stress tolerant species may contribute to the prevalence of antagonistic interactions at the community level.

Stressor effects on biotic interactions also cause complex bottom-up and top-down effects that are often difficult to disentangle (Tylianakis et al. 2008). A recent meta-analysis found that nearly half of multiple stressors interactively affected trophic relationships and that net stressor effects on trophic relationships were highly context-dependent, often differing between sexes, stressor combinations, and habitats (Rosenblatt and Schmitz 2014). Generally, organisms from higher trophic levels seem to be more detrimentally affected by multiple stressors, having a greater propensity for synergistic declines in biodiversity and ecological function (Crain et al. 2008, Lakeman-Fraser and Ewers 2014). Stressor-induced changes in the abundance and species composition of their prey items may make coping with multiple stressors additionally challenging for higher trophic levels (Bundy et al. 2003, Lakeman-Fraser and Ewers 2014).

Most multiple stressors studies have focused on responses of individuals and species in isolation and therefore, fail to account for the potential modifying influence of biotic interactions. Yet altered species interactions are an important driver of responses to stressors and stressor effects on single species often cannot be extrapolated to more complex natural systems (Tylianakis et al. 2008). Therefore, reliable predictions of global change effects require consideration of the effects of multiple environmental drivers on multi-trophic food webs (Schweiger et al. 2010, Wernberg et al. 2012).

Dispersal within meta-populations and meta-communities

At the landscape scale, dispersal within meta-populations and meta-communities can influence the response of local and regional biota to multiple stressors (Leibold et al. 2004) (orange arrows in Figure 1-2). Dispersal can introduce novel tolerant genotypes and species to replace sensitive individuals, increase genetic variability and restructure communities (Bell and Gonzalez 2011, Urban et al. 2012). The Spatial Insurance Hypothesis predicts that regional biodiversity can insure local communities against changing environmental conditions because importation of functionally-redundant, yet tolerant species can replace sensitive local species to maintain ecological performance (Loreau et al. 2003). While there has been some evidence to support the Spatial Insurance Hypothesis under single stressor scenarios (Forrest and Arnott 2007, Steiner et al. 2011, Baho et al. 2012), the capacity of regional species to buffer local communities against multiple stressor effects is uncertain. However, experimental evidence suggests that regional species will be unable to fully compensate for functional losses in stressed local communities (Thompson and Shurin 2012).

Approaches for studying the effects of multiple stressors

Small-scale laboratory experiments have dominated multiple stressor research with very few studies conducted in the field (Crain et al. 2008). These small-scale experiments often involve highly artificial environments with little ecological complexity. In contrast, little is known about the effects of multiple stressors over realistic spatio-temporal scales in natural communities, where they are probably temporally variable and strongly influenced by biotic interactions and dispersal (Smith et al. 2009, Segner et al. 2014).

Multi-year monitoring of whole ecosystems is a valuable approach for studying multiple stressor effects across broad temporal scales, especially when the amount, intensity or overlap of stressors varies over time (Floury et al. 2013). Investigations spanning ecologically realistic temporal scales will enable us to detect time-lagged responses, including feedback effects of biotic changes on stressors and threshold effects of chronic exposure to multiple stressors (Luo et al. 2011). However, to date, most investigations using time-series observations have focused on only a narrow range of single-stressor effects (Woodward et al. 2013).

Alternatively, long-term stressor effects can be inferred based on spatial patterns across environmental gradients using space-for-time ‘natural experiments’. For example, elevational or latitudinal temperature gradients can be used to infer the effects of global warming. This approach is based on the assumption that observed relationships along environmental gradients are consistent with future ecological changes. However, even if the environmental trend is a good proxy for stressor changes over time, biological changes along the gradient may not represent future ecological responses if 1) stress-tolerant species are dispersal-limited, 2) the rate of environmental change exceeds that of acclimation or adaptation, or 3) the environmental gradient is confounded by other unmeasured ecological drivers. However, the robustness of inferences from space-for-time studies can be improved by selecting sites containing the same regional species pool within a region of similar geology where other abiotic variables are constant (Fukami and Wardle 2005). Additionally, ecological patterns along natural environmental gradients, which have been established over evolutionary time scales, may under- or over-estimate responses to relatively rapid changes associated with anthropogenic stressors (Petrin et al. 2008, Elmendorf et al. 2015). Consequently, space-for-time studies may be most appropriate for forecasting multi-decadal effects of stressors (Blois et al. 2013).

In contrast to observational studies that rely on correlative patterns, experiments are valuable for establishing causality between stressors and their biological effects. Large-scale field experiments can increase ecological realism relative to smaller-scale laboratory experiments while continuing to afford the control and replicability of stressor manipulation (Woodward et al. 2010). Additionally, designing multiple stressor experiments to test specific *a priori* mechanistic hypotheses increase their predictive value (Downes 2010).

Research rationale

Non-native fish (i.e. those that are introduced, deliberately or accidentally, outside of their natural geographic distribution) and climate warming threaten mountain lake communities worldwide (Gozlan et al. 2010). Starting in the early 20th century, about 20% of lakes in the Canadian Rocky Mountains were stocked with salmonids to create recreational fisheries (Schindler and Parker 2002). An estimated 84% of stocked lakes were naturally fishless (Schindler 2000). Salmonid introductions have particularly large effects on planktonic food webs of naturally fishless lakes that are evolutionarily naïve to fish predators (Eby et al. 2006). In fishless lakes, large *Daphnia* outcompete small planktonic herbivores because they capture small food particles more efficiently and can consume large food particles that are not available to smaller species (Brooks and Dodson, 1965). Size-selective predation by introduced trout strongly suppresses or extirpates large zooplankton species, including efficient *Daphnia* grazers, which often leads to increased algal biomass (Parker and Schindler 2006). Although many non-native fish populations in the national parks of the Canadian Rocky Mountains did not survive when stocking ceased in the 1970s, some species, particularly non-native rainbow trout (*Oncorhynchus mykiss*), eastern brook trout (*Salvelinus fontinalis*) and cutthroat trout (*Oncorhynchus clarkii*), reproduced and established self-sustaining populations (i.e. naturalized) (Schindler 2000, Schindler and Parker 2002, Messner et al. 2013).

In addition, mountain ranges are warming more rapidly than lower-elevation regions and some of the largest temperature increases are projected to occur in the Canadian Rocky Mountains (Nogués-Bravo et al. 2007, Pepin et al. 2015). By 2085, an average temperature increase of 4.1 - 6.7°C is predicted in the Canadian Rocky Mountains based on four different emission scenarios from the Intergovernmental Panel of Climate Change (IPCC) (Nogués-Bravo et al. 2007). An important consequence of warming in mountain regions is the upslope migration of montane species, leading to the creation of novel species assemblages (Parmesan 2006, Elsen and Tingley 2015). Warming is also promoting the upward expansion of fish populations, which have the potential to invade remaining naturally fishless mountain lakes (Hickling et al. 2006).

Planktonic communities of lower-elevation montane lakes (1000-1500 m a.s.l.) may be less detrimentally affected by non-native trout and warming than those of high-elevation alpine lakes above treeline (*c.* 2000 m a.s.l.). Zooplankton communities of naturally fishless montane lakes usually contain the dipteran larvae *Chaoborus* as a top predator, high abundances of *Daphnia* grazers, and a diversity of smaller species (Schindler 2000, Messner et al. 2013). In contrast, zooplankton communities of naturally fishless alpine lakes are less diverse and often dominated by an omnivorous top predator and a *Daphnia* alpine specialist (Anderson 1974, Bradford et al. 1998, Messner et al. 2013). Non-native fish tend to more negatively affect alpine zooplankton communities because they contain fewer small species to compensate for declines of large species relative to montane systems (Messner et al. 2013). Further, montane zooplankton communities may be better able to cope with increasing temperatures because they are adapted to warmer conditions, while alpine species tend to be cold-specialists (Krajick 2004).

Key objectives and hypotheses

My research addressed gaps in our knowledge of the effects of multiple stressors by empirically testing the net effect of non-native trout and higher temperatures on food webs of mountain lakes, and assessing the importance of species co-tolerance and meta-community dynamics for structuring ecological responses to these stressors. I used a combination of complementary approaches, including a space-for-time survey, a time-series analysis of multi-decadal biomonitoring data, and laboratory and field experiments to facilitate a more complete understanding of the ecological effects of these key stressors on mountain lake communities (Lindenmayer et al. 2010). Experiments enabled disentangling of stressor effects to provide insight into the mechanisms driving their individual and combined effects on food webs, while empirical surveys strengthened and generalized experiment findings by corroborating trends over realistic spatio-temporal scales (Woodward et al. 2010).

First, I conducted a survey of 22 naturally fishless and stocked lakes positioned along an elevational (and thus climatic) gradient and an experiment using outdoor mesocosms to develop and test the following two hypotheses relating to the net effect of non-native trout and higher temperatures on food webs of naturally fishless montane lakes:

Chapter 2

1. Predation by non-native trout and higher temperatures synergistically increase zooplankton biomass in naturally fishless montane lakes because fish release smaller species that are more responsive to warming from competition and predation.
2. Non-native trout stimulates phytoplankton biomass by suppressing large efficient *Daphnia* grazers and increasing nutrient availability.

I then developed a conceptual framework outlining how species traits and co-tolerance to stressors can mechanistically explain the effects of sequential stressors on the functional structure of communities, and applied this approach to the effects of non-native trout and warming on zooplankton communities of naturally fishless montane lakes. I used an outdoor mesocosm experiment to test the following hypotheses:

Chapter 3

1. Trait selection by two stressors determines the direction and degree of relatedness of species tolerances to each. In particular, I expected that warming and non-native trout select for the same key trait, namely reduced body size, resulting in positive co-tolerance to the stressors (Brooks and Dodson 1965, Moore et al. 1996). Consequently, zooplankton communities exposed to both stressors would consist primarily of small co-tolerant species.

2. The co-tolerance relationship determines the influence of stressor order on species composition and aggregate community properties. Specifically, I hypothesized that if zooplankton communities show positive co-tolerance to warming and non-native trout (i.e. stressors are highly redundant), then order of exposure to the stressors should not influence their net effect on zooplankton biomass. Conversely, the combined impact of the stressors should depend on their order of application if species tolerances to each are negatively correlated (i.e. low stressor redundancy).
3. The relationship between response and effect groups predicts the functional effect of stressors. I expected that body-size governs the tolerances of species to non-native trout and warming (i.e. is a response trait) while also influencing the ecological role of species (i.e. is also an effect trait). Thus, functional groups would have clustered tolerances to the stressors with species co-tolerant of non-native trout and warming being primarily small, inefficient grazers. In contrast, I expected that larger species would have low tolerances to the stressors and occupy higher trophic positions. Consequently, I hypothesized that the net impact of warming and non-native trout on naturally fishless lake communities would involve a decrease in large herbivores and a shift toward smaller herbivores, thereby decreasing grazing potential.

In my final data chapter, I compared the zooplankton community of an alpine lake when non-native trout were absent and present across a 24-year period of climatic variation, and conducted an experiment to test the net effect of non-native trout and warming on zooplankton communities of alpine lakes. I also investigated the influence of the regional species pool (including potential upward migration of montane species) on the responses of alpine communities to these stressors. Specifically I tested the following two hypotheses:

Chapter 4

1. Size-selective predation by non-native trout and higher temperature suppress large alpine zooplankton, while small species are resistant to both.
2. Species imported from a diverse array of lakes can, at least partially, compensate for resident alpine species suppressed by predation and warming.

In Chapter 5 conclusions from each of the three main data chapters are summarized and compared. Implications of my research findings and proposed future research directions are also discussed.

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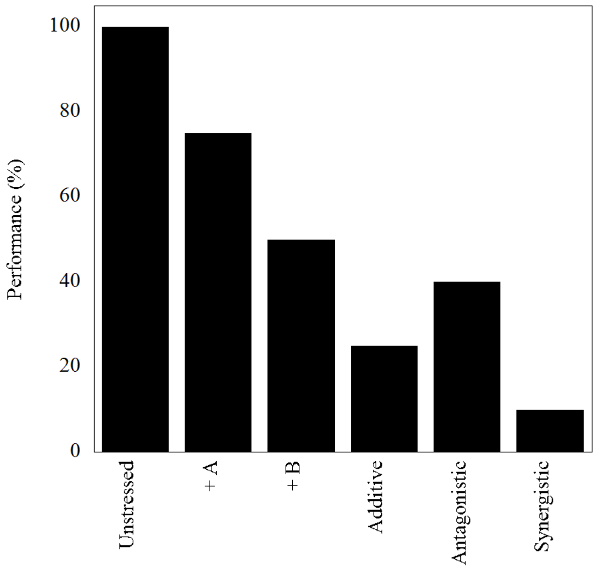

Figure 1-1 Hypothetical scenario where stressors A and B reduce performance by 25% and 50%, respectively. The additive null model predicts that performance should decline by 75% (i.e. 25% + 50%) in the presence of both stressors. An antagonistic interaction occurs when the net effect of stressors reduces performance significantly less than the additive expectation, while a synergistic interaction occurs when the net effect of stressors reduce performance significantly more than the additive expectation.

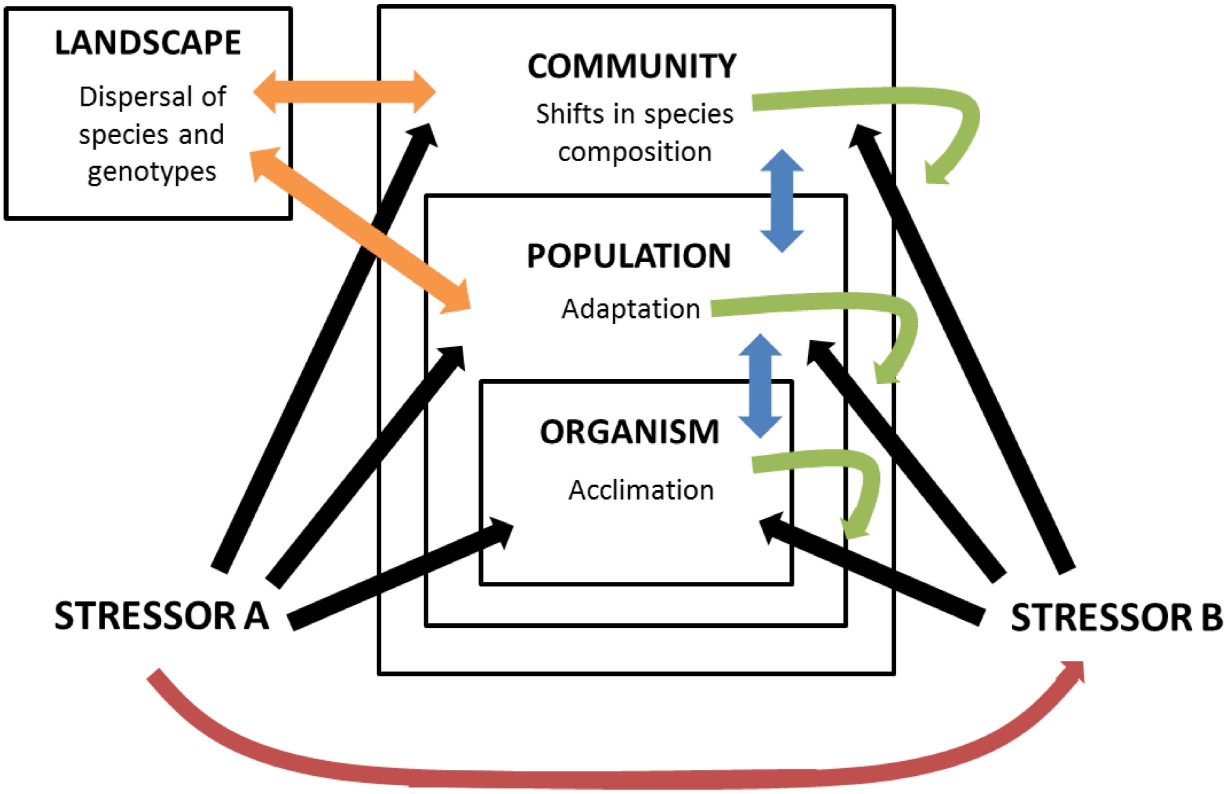

Figure 1-2 Conceptual framework of mechanisms that result in interactions that mediate the direct effects (black arrows) of stressors A and B on a metacommunity. Exogenous interactions between stressors (red arrow) occur in the external environment when one stressor (A) directly modifies the physical or chemical structure of the other (B), and therefore its impact on various levels of biological organization. Endogenous interactions (green arrows) occur from within the biota when a stressor (A) selects for functional traits that influence the tolerance of organisms to the other stressor (B). Here, functional traits are correlated as the result of acclimation at the organismal level, adaptation at the population-level, or species turnover at the community-level. Biotic interactions (blue arrows) can transfer the effect of a stressor across different levels of biological organization, thereby mediating the direct effect of the other stressor. Migration of species and genotypes (orange arrows) can also influence the response of local and regional biota to both stressors.

CHAPTER 2: NON-NATIVE TROUT INCREASE THE CLIMATIC SENSITIVITY OF ZOOPLANKTON COMMUNITIES IN NATURALLY FISHLESS LAKES[1]

Introduction

Stocking of mountain lakes around the world with salmonids for the purposes of angling has been a common practice for several decades (Schindler and Parker 2002, Eby et al. 2006, Miró and Ventura 2013). Prior to stocking, the majority of these lakes were naturally fishless (Bahls 1992). Despite the cessation of stocking in several protected areas and national parks, non-native trout populations have persisted in many lakes (Armstrong and Knapp 2004, Messner et al. 2013).

Non-native trout alter food webs and water quality in naturally fishless mountain lakes (Schindler and Parker 2002, Eby et al. 2006, Crawford and Muir 2008). Large *Daphnia* species are dominant in fishless lakes because their relatively high grazing efficiency allows them to out-compete smaller, less efficient grazers (Brooks and Dodson, 1965). When a novel fish predator is introduced, it typically suppresses large herbivorous zooplankton and macroinvertebrates that otherwise function as top predators in these lakes (Knapp et al. 2001, Schindler and Parker 2002). Several smaller invertebrates, not vulnerable to the introduced fish, then thrive as they experience less competition and predation (Paul et al. 1995, Eby et al. 2006, Tiberti et al. 2014). Consequently, a net shift toward smaller, less efficient grazers (Brooks and Dodson 1965) and enhanced nutrient recycling by the introduced fish can increase algal growth (Vanni and Layne 1997, Parker and Schindler 2006).

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Non-native trout and climate warming may synergistically affect planktonic communities in naturally fishless montane lakes (Rahel and Olden 2008, Jansson et al. 2010). For example, higher temperatures may exacerbate the effect of non-native trout by increasing their feeding rates (Jeppesen et al. 2010, Kratina et al. 2012) and nutrient recycling (Vanni 2002). Similarly, non-native trout may increase the effect of warming on planktonic communities by shifting communities toward small species that are more sensitive to temperature (Vinebrooke et al. 2004, Messner et al. 2013). An earlier survey of mountain lake communities found that increases in zooplankton biomass associated with higher temperatures were more pronounced in stocked lakes where small zooplankton were abundant, possibly because smaller-bodied zooplankton are more tolerant of higher temperature and warming disproportionately accelerates their development and reproduction relative to larger species (Messner et al. 2013, Moore et al. 1996). Therefore, current efforts to manage non-native trout and climate warming independently may be ineffective (Pyke et al. 2008, Alric et al. 2013).

To examine the robustness of earlier correlative findings, I analyzed data from another survey of 22 different mountain lakes located along an elevational (and therefore climatic) gradient. I also performed an outdoor mesocosm experiment to test the net effects of non-native rainbow trout (*Oncorhynchus mykiss*) and warming on planktonic food webs of naturally fishless montane lakes. My primary hypothesis was that predation by *O. mykiss* and higher temperatures would synergistically increase zooplankton biomass in naturally fishless montane lakes, because fish release smaller species that are more responsive to warming from competition and predation. I also hypothesized that *O. mykiss* would stimulate phytoplankton biomass by suppressing large efficient grazers and increasing nutrient availability.

Methods

Mountain lakes survey

From July 14-18 2009, zooplankton samples were collected from 22 lakes in Banff and Yoho National Parks, including 12 naturally fishless lakes and 10 lakes containing non-native trout (mainly eastern brook trout, *Salvelinus fontinalis,* and rainbow trout, but also cutthroat trout, *Oncorhynchus clarkii*) (Table 2-1). The lakes span an elevational gradient of *c*. 1450 m and range in mean temperature from 4.9 to 18.7^{0}C (Table 2-1). Quantitative zooplankton samples were collected at the maximum depth station using vertical hauls with a 30-cm diameter, 63-µm mesh-sized conical net from 1 m off the lake bottom to the surface. Zooplankton samples were preserved in the field in 70% ethanol. Water temperature profiles were measured at 1-m intervals from the surface of the lake to 1 m off the lake bottom using YSI Model 95 (YSI Incorporated, Yellow Springs, OH). The epilimnion was defined as the water from the surface to the depth where a greater than 1°C change per metre was observed and mean epilimnetic temperature was calculated for each lake.

Crustacean zooplankton were counted using a Leica MZ9s dissecting microscope at 32-times magnification. Specimens were identified to species when possible and genus otherwise (Ward and Whipple 1959). Samples were counted in their entirety. The lengths of the first 15 specimens of each developmental stage for each taxon were measured at 40-times magnification and used to derive biomass estimates using length-mass regressions (McCauley 1984, Culver et al. 1985). Zooplankton mean weighted length (herein referred to as mean length for simplicity) was calculated as the product of the mean length of a zooplankton taxon and its proportional relative abundance, summed across taxa. Zooplankton were classified as small herbivores, large daphniid grazers and omnivores/carnivores, based on functional characteristics such as body length, habitat, trophic group and feeding type (Barnett et al. 2007).

Analyses of covariance (ANCOVAs) were performed using PASW® Statistics 18 (SPSS Inc., Chicago, Illinois, USA) to test the direct and interactive effects of non-native trout and epilimnetic water temperature on zooplankton biomass and mean length. A significant interactive effect of introduced fish and the covariate temperature on total zooplankton biomass was found so separate regressions were performed to test the influence of temperature on zooplankton biomass for lakes with non-native trout absent and present. The lack of a significant interactive effect of non-native trout and epilimnetic water temperature on zooplankton mean length indicated that the assumption of homogeneity of slopes was valid. Therefore, I performed an ANCOVA without an interaction term to assess the independent effects of non-native trout and temperature on mean length.

Canonical correspondence analysis (CCA) using CANOCO 5.03 (ter Braak & Šmilauer, 2012) was then performed to examine the influence of non-native trout and epilimnetic temperature on zooplankton species composition relative to the other physical (i.e. lake surface area, maximum lake depth) and chemical (i.e. pH, total dissolved solids) variables measured. Although nutrient concentrations were not measured here, an earlier survey of 34 fishless and fish-stocked mountain lakes showed that total phosphorus and dissolved organic carbon concentrations were not significant predictors of zooplankton biomass in my study region (Messner et al. 2013). A unimodal species response model was used for ordination analyses because a preliminary analysis of the taxonomic data constructed a total primary gradient length of 4.1 standard deviation units (Šmilauer and Lepš 2014). Zooplankton taxa that were detected in fewer than three lakes were excluded from the analysis and biomasses were $\log_{10}$-transformed prior to analysis to down-weight the influence of dominant taxa. Forward selection using Monte Carlo permutation testing was performed to determine which environmental variables explained a significant amount of variance in the zooplankton compositional data after adjusting the threshold significance level to control the rate of Type 1 errors (i.e. the false discovery rate) (Benjamini and Hochberg 1995). Grizzly, Laryx, McConnell and Rock Isle lakes had missing values for environmental predictors and were treated as supplementary cases in this initial analysis. However, a subsequent ordination that included all lake cases was performed to isolate the influence of fish presence and epilimnetic water temperature, my variables of interest and the most significant explanatory predictors of species composition, while the other significant predictor, pH, was treated as a supplementary variable. Monte Carlo permutation testing was used to determine the significance of the first CCA axis and the overall ordination.

Mesocosm experiment

In July 2011, plankton was collected from five naturally fishless lakes in Banff National Park, Alberta, Canada - Copper (51°15'N: 155°55'W), Herbert (51°27'N: 116°13'W), Island (51°23'N: 116°6'W), Kingfisher (51°24'N: 116°10'W) and Lost (51°28'N: 116°16'W). Quantitative plankton samples were taken by vertically hauling nets through the water column from 1 m off the lake bottom. From July 13-14 2011, phytoplankton was collected using a 30-cm diameter, 10-µm mesh-sized conical net. The number of vertical hauls needed to equal the volume of the mesocosms was first multiplied by four and then performed at each lake to compensate for a measured net efficiency of 25%. Phytoplankton collections were passed through 63-µm sieve to remove zooplankton to prevent confounding my quantitative zooplankton collection. From July 18-19 2011, zooplankton was collected using a 30-cm diameter, 63-μm mesh-sized conical net assuming a net efficiency of 75% (McQueen and Yan 1993). Phytoplankton and zooplankton samples were transported in 20-L plastic carboys back to the Barrier Lake Field Research Station along the eastern front range of the Rocky Mountains, where they were distributed to mesocosms on the evening of their collection.

Intermediate bulk containers (W40” x L48” x H40”) made of translucent high-density polyethylene were used as experimental mesocosms. These units were entirely enclosed other than a 20-cm diameter opening at the top that was covered with 1-mm mesh. Snowmelt water was collected from a nearby pond, and then filtered through a 63-μm stainless sieve to prevent introduction of new zooplankton while filling each 1000-L capacity mesocosm. Each unit was aerated using a tube connected to an air pump to disturb the surface tension of the water and manually mixed twice daily. Plankton communities from each of the five lakes were distributed among four mesocosms for a total of 20 units. Natural densities of plankton were concentrated to a target of three zooplankters L^{-1} based on the mean density of zooplankton in the five lakes that was determined one week before collections.

The experiment followed a two-factor [(fish absent vs. present) × (ambient temperature vs. warmed)] randomized block (lake source of community) design with repeated measures. To establish the fish treatment, 10 mesocosms were randomly selected to remain fishless while the other 10 were stocked with a single rainbow trout (*O. mykiss*) fingerling (10 - 13 cm long) purchased from a local hatchery (Ackenberry Trout Farms, Camrose, Alberta). To simulate an allochthonous food subsidy and adhere to animal care protocol, each fish was fed ten 15-mg food pellets containing 1.2% phosphorus twice a week (Hi-Pro Feeds Inc., Okotoks, Alberta). This feeding regime accounted for only 5% of the recommended daily food intake for the rainbow trout fingerlings (Hinshaw 1990). Therefore, given that excretion of dissolved P by rainbow trout is negligible until the P requirement has been met (Sugiura et al. 2000, Dalsgaard and Pedersen 2011), I assumed high assimilation and retention of P by the fish (see Results).

The warming treatment consisted of 10 unheated mesocosms (mean 19.4ºC, ranging from 10.7ºC to 27.8ºC over the duration of the experiment) while the others were warmed by an average of 3.8°C (mean 23.2ºC, ranging from 10.9ºC to 28.6ºC) using four, 300-watt aquarium heaters (Hagen®, Montreal, Canada). A mean temperature increase of 3.8ºC is within the range of projected warming for 2055 in the study region (the high latitude mountains of North America) based on Atmosphere-Ocean General Circulation Models using four emission scenarios (A1FI, A2, B1 and B2) from the Intergovernmental Panel on Climate Change (Nogués-Bravo et al. 2007). Insulative covers (Heatsheets® Survival Blanket, AFMInc, California, USA) were also placed around the mesocosms to minimize diel fluctuations in water temperature and heat loss from warmed units. HOBO Pendant® data loggers (Massachusetts, USA) were deployed at a depth of 1 m in each mesocosm and recorded water temperatures at 1-hr intervals.

Mesocosms were thoroughly mixed with a paddle and then the plankton was sampled using a hose attached to a built-in spigot at the base of each mesocosm. Samples (40-L) of zooplankton were taken before treatments were applied (day 0) and at the mid-point of the experiment (day 21) with 80-L samples taken on the final day of the experiment (day 42). Zooplankton samples were concentrated using a 63-µm mesh-sized sieve and preserved in 70% ethanol. On the initial (day 0), middle (21) and final (42) days of the experiment, phytoplankton were concentrated by filtering 0.75 – 2.0 L samples on to GF/F glass-fibre filters (0.45-µm pore size), which were then frozen and kept in darkness. Filters were analyzed for chlorophyll *a* concentration as a proxy for algal biomass using standard HPLC procedures (Vinebrooke and Leavitt 1999). Unfiltered 500-mL water samples were also taken on the initial, middle and final days of the experiment and transported on ice to the Biogeochemical Analytical Facility at the University of Alberta, Edmonton (URL link: http://www.biology.ualberta.ca/basl) where they were chemically analyzed for total phosphorus, total nitrogen and dissolved organic carbon concentrations.

Crustacean zooplankton was counted using a Leica MZ9s dissecting microscope at 32-times magnification (a minimum of 300 individuals per sample). Samples containing more than 300 individuals were mixed so that the zooplankton was evenly distributed and a minimum of two sub-samples were counted. Subsamples were counted in their entirety and subsampling continued until the standard error of the total number of individuals per subsample was less than 10% of the mean number of individuals counted per subsample (Bade et al. 1998). To ensure detection of rare species, the remainder of the sample was examined for species that were not found in the completed subsamples or of which less than five individuals were found. The lengths of the first 15 specimens of each developmental stage for each taxon were measured at 40-times magnification and biomass was estimated using length-mass regressions (McCauley 1984, Culver et al. 1985). I used the ratio of zooplankton biomass to phytoplankton biomass (BZ:BP) as an indicator of trophic structure and potential grazing pressure by zooplankton. Measured chlorophyll *a* concentrations were converted into phytoplankton biomass using a conversion factor of 66 (Havens and Beaver 2013, Vijverberg et al. 2014).

Linear mixed models were performed using PASW® Statistics 18 (SPSS Inc., Chicago, Illinois, USA) to test for time-dependent direct and interactive effects of non-native trout and warming on total zooplankton biomass, *Chaoborus* spp. biomass, zooplankton mean length, chlorophyll *a* concentration, TP, TN and DOC (Zuur et al. 2009). Experimental treatments were treated as fixed variables and sampling occasion as a covariate. Random block (i.e. lake source) effects were tested by comparing models without and with a random intercept. The model that minimized the Akaike's Information Criterion corrected for small sample-size (AICc) based on restricted maximum likelihood estimation was used. Individual mesocosm was treated as a random factor and to account for potential temporal autocorrelation from repeated measures, several error covariance structures were tested, including autoregressive structures with homogeneous and heterogeneous variances. The covariance structure that minimized the AICc was used.

All variables were $\log_{10}$-transformed prior to analysis to test the multiplicative null expectation of net stressor effects. The multiplicative model tests the hypothesis that the effect of one stressor acts probabilistically on the effect of another to generate their net effect according to the equation:

$$P_{AB} = P_A + (1 - P_A)*P_B = P_A + P_B - P_A*P_B$$

where P_{AB} is the proportional effect of both stressors combined, P_A is the proportional effect of stressor A alone, and P_B is the proportional effect of stressor B alone (Folt et al. 1999, Dunne 2010). In contrast, the more commonly used additive model tests the hypothesis that the total effect of stressors is equal to the sum of their individual effects (Folt et al. 1999) according to the equation:

$$P_{AB} = P_A + P_B$$

However, the additive model was problematic in this study because it predicted net effects that exceeded 100% loss for some response variables that are necessarily non-negative, such as species density and mean species length (Sih et al. 1998). To avoid illogical predictions, I chose the multiplicative model, which never predicts a combined stressor effect larger than 100% loss. For example, if stressor A reduces the population size of a species by 60% and stressor B reduces it by 70%, the additive model predicts that the combined effect of the stressors is 130% loss; however, a negative population size is clearly impossible. In contrast, the multiplicative model predicts that the combined effect of the stressors will reduce the population size by 88%.

Partial redundancy analysis (RDA) was performed to examine the influence of non-native trout and warming on zooplankton species composition on the final day of the experiment, with the blocking variable, lake source, treated as a covariate. A linear species response model was used for ordination analyses because a preliminary analysis of the taxonomic data constructed a total primary gradient length of 2.6 standard deviation units (Šmilauer and Lepš 2014). Zooplankton taxa that were detected in fewer than three mesocosms on the final day of the experiment were excluded from the analysis and biomasses were $\log_{10}$-transformed prior to analysis to down-weight the influence of dominant taxa. Forward selection using Monte Carlo permutation testing was performed to determine whether treatment explained a significant amount of variance in zooplankton species composition. Monte Carlo permutations were also used to test the significance of the first CCA axis and the overall ordination.

Results

Mountain lakes survey

Total zooplankton biomass increased significantly with higher epilimnetic temperature only when non-native trout were present (ANCOVA Fish x Temperature $F_{1,18} = 33.16$, $P < 0.001$; Fish present: $r^2 = 0.79$, $P = 0.001$) (Figure 2-1a). In comparison, temperature had little effect on zooplankton biomass under fishless conditions (Fish absent: $r^2 = 0.002$, $P = 0.91$).

Zooplankton mean body size significantly decreased with fish (ANCOVA Fish $F_{1,19} = 11.92$, $P = 0.003$) and temperature (ANCOVA Temperature $F_{1,19} = 6.03$, $P = 0.02$) (Figure 2-1b).

The presence of non-native trout, and epilimnetic water temperature followed by pH, explained the greatest amount of variance in zooplankton species composition across lakes based on a forward selection criterion (Figure 2-2; Table 2-2). Fish status explained 18.8% and lake temperature 17.6% of species variance. Non-significant variables excluded from the final CCA were maximum lake depth, elevation, total dissolved solids, and lake surface area (Table 2-2). CCA axis 1 was significant ($F = 4.00$, $P = 0.002$), capturing 18.3% of total species variance and being best defined by the fish status of the lake. CCA axis 2 captured 16.3% of total species turnover and the overall ordination was also significant ($F = 4.80$, $P = 0.002$).

In general, smaller zooplankton species were positively associated with the presence of introduced trout (Figure 2-2). The zooplankton of stocked lakes consisted mainly of a variety of small herbivores (e.g. *Bosmina longirostris*, *Ceriodaphnia*, *Daphnia rosea*) and the herbivorous early-stages of copepods (i.e. cyclopoid and calanoid nauplii and copepodids). In contrast, the communities of fishless lakes contained mainly larger and often heavily pigmented *Daphnia* spp., *Hesperodiaptomus arcticus* and *Chydorus sphaericus*.

The secondary environmental gradient of lake temperature differentiated species characteristic of cold, alpine lakes from those in warmer, montane lakes. Cold-water communities consisted primarily of relatively large *Daphnia middendorffiana* and the omnivorous top predator *H. arcticus*. In contrast, warmer montane communities contained the top predator *Chaoborus* and a greater diversity of relatively smaller herbivores, such as *Alona* and *Daphnia catawba*.

Mesocosm experiment

The significant positive effect of introduced fish on total zooplankton biomass also increased the otherwise undetectable effect of warming by the end of the experiment (Table 2-3; Figure 2-3a). *Oncorhynchus mykiss* eliminated the large invertebrate predator, larvae of the dipteran *Chaoborus*, by day 21 (Figure 2-3b). Therefore, the synergistic positive response of planktonic communities to the combined effect of non-native trout and warming was attributable to the increase in crustacean zooplankton (see below). In particular, both *O. mykiss* and warming shifted communities toward small zooplankton by the final day of the experiment (Table 2-3; Figure 2-3c). The shift toward smaller zooplankton occurred most rapidly under combined exposure to non-native trout and warming, but the final body length of zooplankton was similar across single- and dual- stressor treatments (Figure 2-3c).

The presence of *O. mykiss*, but not warming, significantly decreased BZ:BP by disproportionately increasing chl *a*-inferred total phytoplankton biomass throughout most of the experiment (Table 2-3; Figure 2-3d,e). However, warming reversed the suppression of BZ:BP by non-native trout on day 42, owing to increased zooplankton biomass (*post hoc* ANOVA Fish x Temperature $F_{1,16} = 5.07$, $P = 0.04$) (Figure 2-3a,d). Otherwise, neither treatment significantly affected the other measured chemical variables (Table 2-3). In particular, lack of a significant difference in P concentration across stocked (4.11 ± 0.25 µg L^{-1}) and non-stocked mesocosms (7.32 ± 1.02 µg L^{-1}) supported my earlier assumption of high assimilation and retention of P by the fish.

The four treatment combinations explained a significant amount of variance in zooplankton species composition by the end of the experiment ($F = 8.20$, $P_{adj} = 0.008$) (Figure 2-4). RDA axes 1 and 2 accounted for 54.0% and 7.1% of the total variance in species data, respectively. RDA axis 1 was significant (*F* = 14.10, *P* = 0.002), as was the overall ordination (*F* = 6.6, *P* = 0.002).

Zooplankton communities exposed simultaneously to both stressors were further displaced from the controls in ordination space than were communities exposed to non-native trout or warming only (Figure 2-4). In particular, *O. mykiss* combined with warming shifted communities toward smaller herbivores (i.e. *Chydorus sphaericus* and *Acroperus harpae*). In comparison, the presence of *O. mykiss* alone favoured smaller omnivores (i.e. *Diacyclops thomasi* and *Cyclops vernalis*) and early herbivorous life stages of copepods. Warming alone had the least effect on species composition.

Discussion

Experimental findings supported the hypothesis generated by the space-for-time survey, namely that non-native trout increase climatic sensitivity in the zooplankton of naturally fishless montane lakes. Both lines of evidence agreed that the presence of introduced trout drove the positive effect of higher temperature on total zooplankton biomass, and also its effect on species composition. Otherwise, the direct effects of higher temperature on communities in fishless lakes were negligible. Size-selective predation by *O. mykiss* eliminated the top invertebrate predator and, as with warming, favoured smaller zooplankton. The shift toward a smaller body size was attributable to increased production of herbivorous early life stages of copepods, and a greater diversity of smaller herbivores. Introduction of *O. mykiss* also altered trophic structure primarily by stimulating the phytoplankton; however, warming reversed this effect by the end of the experiment by disproportionately increasing zooplankton biomass and potential grazing pressure.

The greater sensitivity of smaller-bodied species to warming can account for why the positive effect of higher temperatures on zooplankton biomass was only observed in the presence of non-native trout (Daufresne et al. 2009, Yvon-Durocher et al. 2011). Exclusion of *Chaoborus* by introduced visually-feeding fish would have released the smaller and less conspicuous zooplankton from predation (Hanazato and Yasuno 1989). Further, suppression of large *Daphnia* by the zooplanktivorous fish likely released smaller herbivores from competition (Brooks and Dodson 1965, Hart and Bychek 2011). Smaller-bodied zooplankton are not only more tolerant of higher temperatures than larger species, but also metabolically more sensitive to increased temperatures, as warming disproportionately accelerates their development and reproduction (Moore et al. 1996). For example, *Chydorus sphaericus* and *Acroperus harpae* thrived in the presence of both *O. mykiss* and warmer conditions, which reflects how their generation times decline logarithmically with increasing temperature (Bottrell 1975, Gillooly 2000, Gillooly et al. 2002). Small zooplankton also cycle more nutrients than large species and nutrient recycling by zooplankton and fish occurs more quickly at higher temperatures, which may help explain the increased algal biomass in fish-stocked (Moore et al. 1996).

The broad tolerance of *Daphnia* to thermal variation may explain the apparent insensitivity of the zooplankton of fishless systems to higher temperatures. Although my survey findings highlight the importance of temperature as a driver of zooplankton species turnover across a large spatial scale, moderate warming in the experiment had little effect on fishless communities dominated by *Daphnia*. Phenotypic plasticity may have enabled *Daphnia* to respond adaptively to higher temperatures, their maximum body size and mean generation time declining (Orcutt and Porter 1984, McKee and Ebert 1996). Indeed, shifts toward smaller adults and/or an increased proportion of juveniles could account for the significant decline in mean body length with warming (Daufresne et al. 2009). Similarly, large *Daphnia* occur in subtropical lakes, despite their high temperature, but only if fish are absent (Iglesias et al. 2011). These findings support the view that, at least in the near future, the direct effects of global warming on the communities of naturally fishless lakes will likely be small (McKee et al. 2002). However, the potential for non-native trout and other environmental drivers (e.g. Weidman et al. 2014) to synergistically increase the effects of warming on fishless mountain lakes suggests that indirect effects of global warming may be of much greater concern.

Suppression of large *Daphnia* grazers and a corresponding decrease in BZ:BP can explain the positive influence of non-native trout on primary production (Parker and Schindler 2006). Top-down control by fish can account for the decrease in the abundance of *Daphnia*, the reduced mean body-size of the zooplankton, and lower grazing pressure by zooplankton on phytoplankton (Jeppesen et al. 2007). Nutrient recycling by fish may also have contributed to increased primary production (Vanni and Layne 1997), but the absence of changes in TP and TN suggest that consumer-mediated fish effects were relatively more important.

Higher temperature appeared to stimulate the feeding rate of fish, as shifts toward smaller zooplankton occurred most rapidly in the dual-stressed treatment (Jeppesen et al. 2010), suggesting that warming strengthened top-down effects of introduced fish, as observed elsewhere (Kratina et al. 2012, Alric et al. 2013). However, by the end of the experiment, the synergistic increase of small zooplankton under dual-stressed conditions reversed the suppression of BZ:BP by non-native trout. Despite this, chlorophyll *a* concentrations were unaffected, probably because of the absence of effective large-bodied *Daphnia* grazers and the proliferation of large, inedible algae that small zooplankton species were unable to consume (Hansson et al. 1998, Havens and Beaver 2013).

My discovery that non-native trout enhance the effects of higher temperatures on planktonic food webs of montane lakes adds to the growing evidence that interactions among stressors often produce unexpected net effects on sensitive mountain lake communities (Thompson et al. 2008, Weidman et al. 2014). Further, the subtle direct effect of high temperatures highlights the potential for forecasts of the effects of global warming to underestimate the actual outcome if the potential amplifying influences of other stressors are not considered. Therefore, my findings underscore the importance of managing non-native species in the context of other significant drivers of environmental change.

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Table 2-1 Environmental variables and stocked fish status of 22 mountain lakes surveyed during July 2009 in Banff and Yoho National Parks, Canada. Epilimnetic temperature was calculated as the mean water temperature of the epilimnion, based on measurements at 1-m intervals. To show the diversity of physical and chemical properties among lakes, maximum and minimum values for each variable are indicated in bold.

Lake	**Fish**	**Elevation (m a.s.l.)**	**Surface area (ha)**	**Maximum depth (m)**	**Epilimnetic temperature (°C)**	**TDS (μg L^{-1})**	**pH**
Bighorn	A	2347	2.2	9.2	8.5	86	8.2
Copper	A	1434	1.5	10.4	18.1	86	8.6
Emerald	P	**1302**	**116.0**	21.0	12.0	122	8.1
Grizzly	P	2314	N/A	6.8	13.2	100	8.4
Herbert	A	1585	5.7	13.3	15.7	162	8.2
Hungabee	A	2100	0.6	**4.0**	10.0	**10**	8.2
Island	A	1570	14.9	6.4	18.5	120	8.6
Johnson	P	1593	15.6	6.3	17.7	165	8.4
Kingfisher	A	1539	2.0	7.2	**21.0**	113	8.4
Laryx	P	2150	N/A	16.5	12.2	100	8.2
Little Herbert	P	1572	0.6	8.2	16.3	**194**	8.2
Lost	A	1692	**0.4**	5.5	16.3	163	8.2
Mary	A	2054	1.6	**4.0**	12.3	51	**8.8**
McConnell	A	2390	0.9	**28.0**	6.9	N/A	N/A
Moraine	P	1887	42.0	20.5	8.0	65	8.2
Opabin	A	2285	3.2	5.5	**4.9**	33	8.3
Pipit	A	2217	10.6	20.6	9.0	90	**8.0**
Rock Isle	P	**2755**	N/A	13.0	12.6	83	8.3
Snowflake	A	2320	7.1	12.8	9.6	102	**8.0**
Two Jack	P	1517	5.0	17.0	16.6	168	8.4
Vista	P	1567	6.6	6.5	14.1	130	8.4
Wapta	P	1656	24.0	8.5	7.8	74	8.3

Fish presence (P) or absence (A) of stocked non-native cutthroat trout, eastern brook trout and rainbow trout; *TDS* total dissolved solids

Table 2-2 Results of the CCA showing the proportion of total variance in zooplankton species composition among surveyed lakes explained by each environmental variable (λ), the *F*-statistics from a forward selection criteria, and the *P*-values based on 499 Monte Carlo simulations (both unadjusted and adjusted using the Bonferroni false discovery rate correction). *P*-values in bold indicate statistical significance.

Variable	**λ**	***F***	***P***	**P_{adj}**
Fish	0.19	3.70	**0.002**	**0.004**
Epilimnetic temperature	0.18	4.14	**0.002**	**0.003**
pH	0.09	2.52	**0.01**	**0.02**
Maximum depth	0.04	1.16	0.32	0.51
Elevation	0.04	1.08	0.41	0.65
Total dissolved solids	0.03	0.87	0.52	0.59
Area	0.02	0.61	0.78	0.78

Table 2-3 Results of the linear mixed models for the fixed effects of non-native trout (Fish), temperature (Temp) and time on zooplankton response variables, BZ:BP, chlorophyll *a* concentration, and water chemistry for the 42-day experiment. *P*-values in bold indicate statistical significance.

	Zooplankton biomass		Zooplankton length		BZ:BP		Chlorophyll *a* concentration	
Fixed Effect	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Fish	2.65	0.13	0.44	0.52	1.85	**0.03**	1.85	0.19
Temp	0.49	0.50	2.69	0.12	1.78	0.42	1.78	0.20
Time	7.78	**0.005**	152.33	**<0.001**	23.74	**0.04**	23.74	**<0.001**
Fish*Temp	0.74	0.41	0.09	0.77	0.08	0.61	0.08	0.78
Fish*Time	13.60	**0.002**	9.34	**0.005**	7.16	0.41	7.16	**0.01**
Temp*Time	0.17	0.68	11.21	**0.002**	1.33	0.93	1.33	0.26
Fish*Temp*Time	6.14	**0.02**	1.92	0.18	<0.001	0.15	<0.001	0.99

	Total phosphorus		Total nitrogen		Dissolved organic carbon	
Fixed Effect	*F*	*P*	*F*	*P*	*F*	*P*
Fish	0.45	0.83	0.72	0.48	5.45	**0.03**
Temp	1.19	0.28	1.03	0.33	2.86	0.11
Time	2.16	0.15	3.04	0.10	281.52	**<0.001**
Fish*Temp	0.28	0.60	0.11	0.92	0.23	0.63
Fish*Time	2.00	0.17	2.21	0.16	3.18	0.09
Temp*Time	0.54	0.47	1.26	0.28	3.59	0.07
Fish*Temp*Time	0.34	0.56	0.24	0.88	0.001	0.98

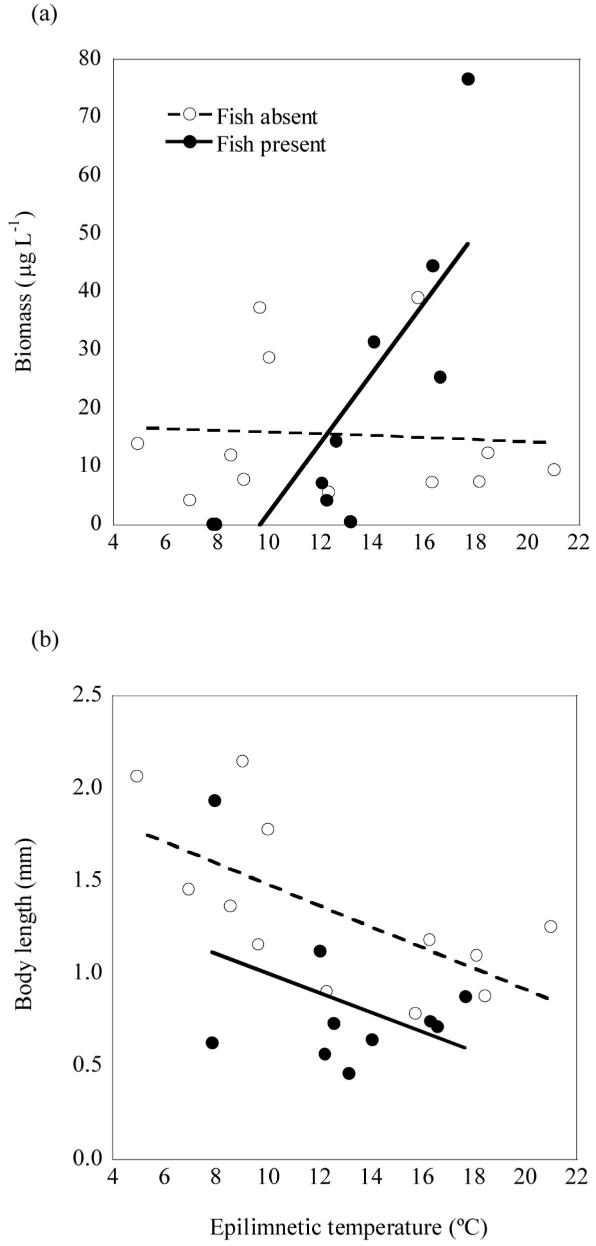

Figure 2-1 The effect of temperature on a) total zooplankton biomass and b) zooplankton mean body length across 12 naturally fishless lakes (open circles) and 10 lakes stocked with non-native trout (closed circles). The dashed and solid lines represent the best linear fits of significant relationships for fishless and stocked lakes, respectively.

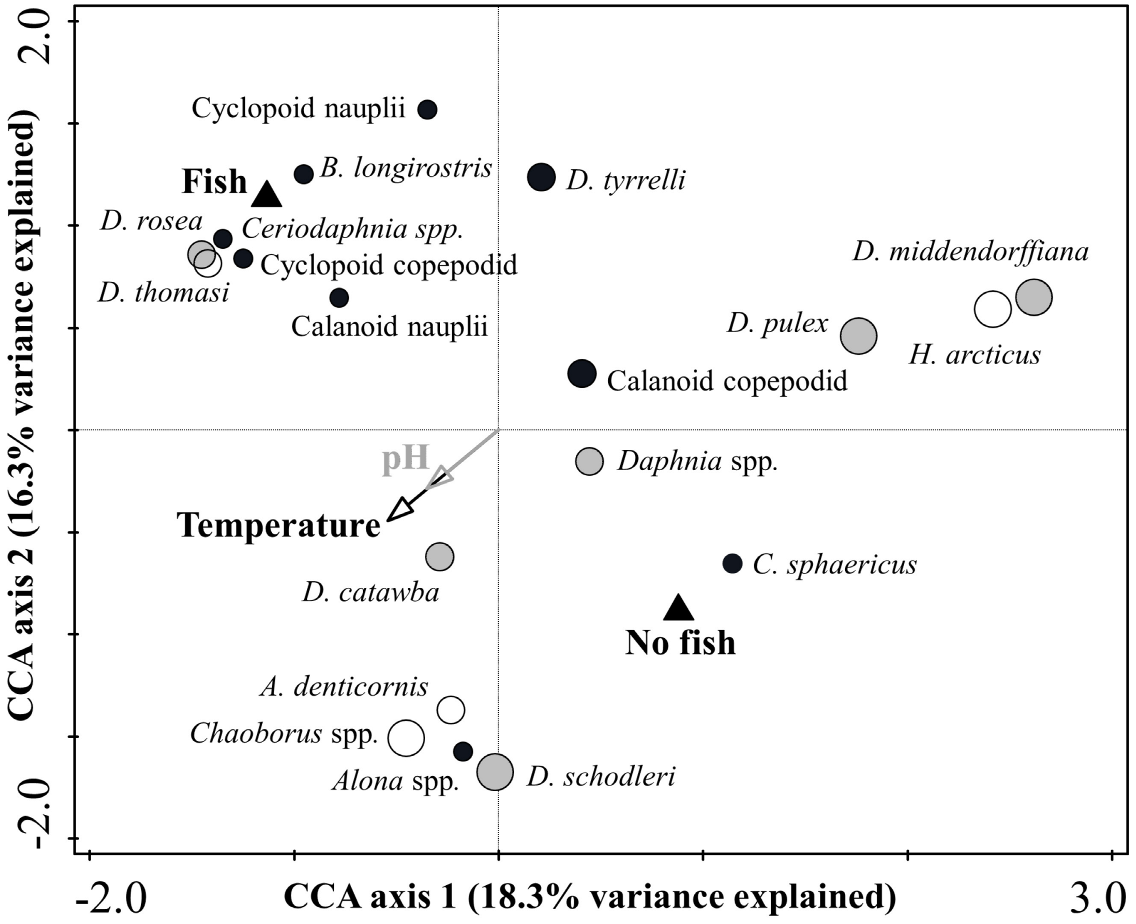

Figure 2-2 Biplot based on canonical correspondence analysis (CCA) using a forward selection of environmental variables that explained a significant amount of variance in zooplankton species composition across 22 mountain lakes surveyed in July 2009. To isolate the effects of my primary variables of interest, non-native trout (Fish) and epilimnetic temperature (Temperature), pH is plotted passively as a supplementary variable. Species are classified by functional group as small herbivores (closed circles), large *Daphnia* grazers (grey circles), and omnivores/carnivores (open circles). The mean body size of species is indicated by symbol size (small < 5 µg, medium 5 – 30 µg, large > 30 µg). See Appendix A-1 for full species names, mean body sizes of species, and mean biomasses in fishless and stocked lakes.

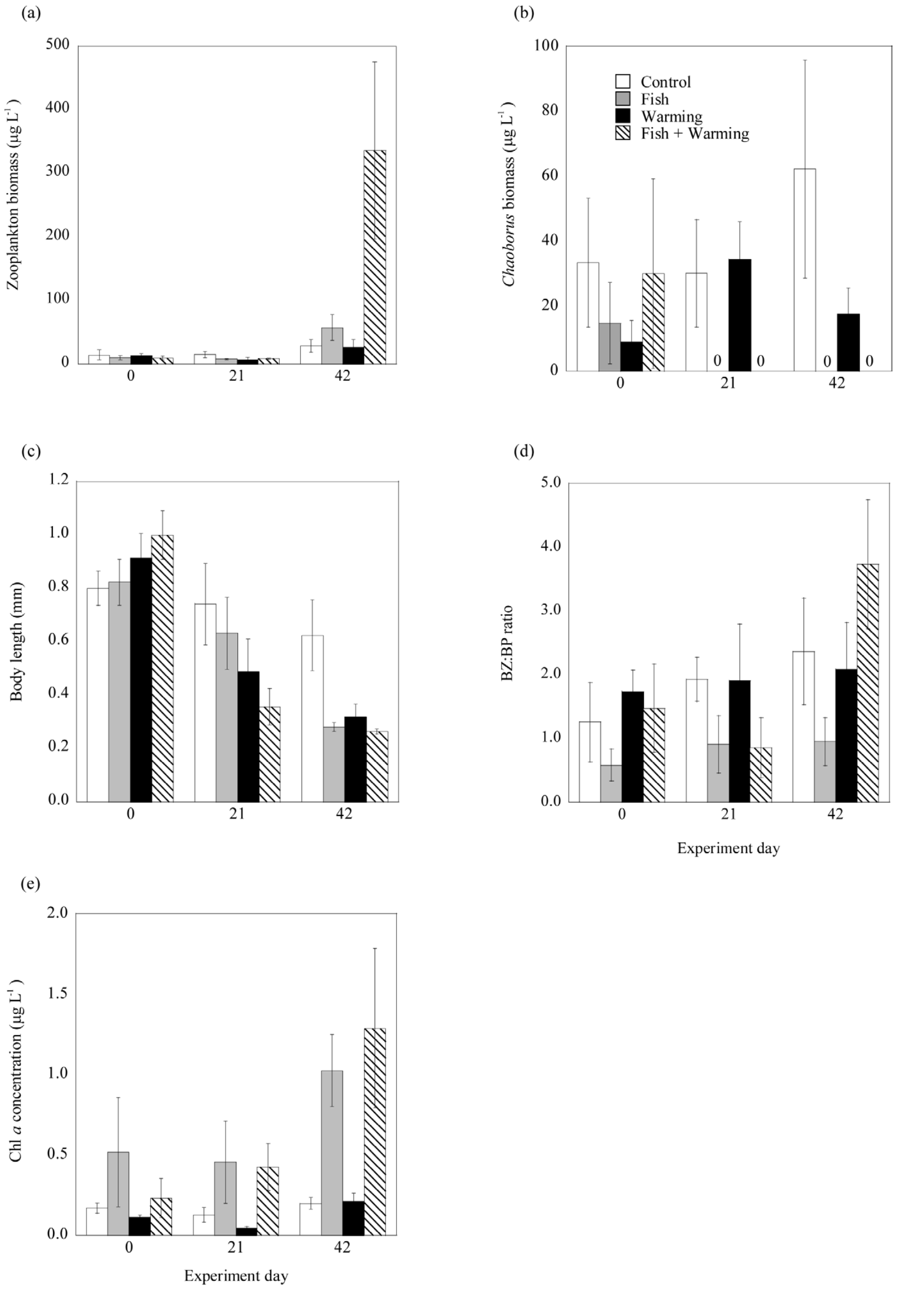

Figure 2-3 a) Total zooplankton biomass, b) *Chaoborus* spp. biomass, c) zooplankton mean body length, d) the ratio of zooplankton biomass to phytoplankton biomass (BZ:BP), and e) chlorophyll *a* concentration in unstressed control conditions and exposed to non-native trout and warming independently and in combination over the 42-day experiment. Vertical bars represent standard error.

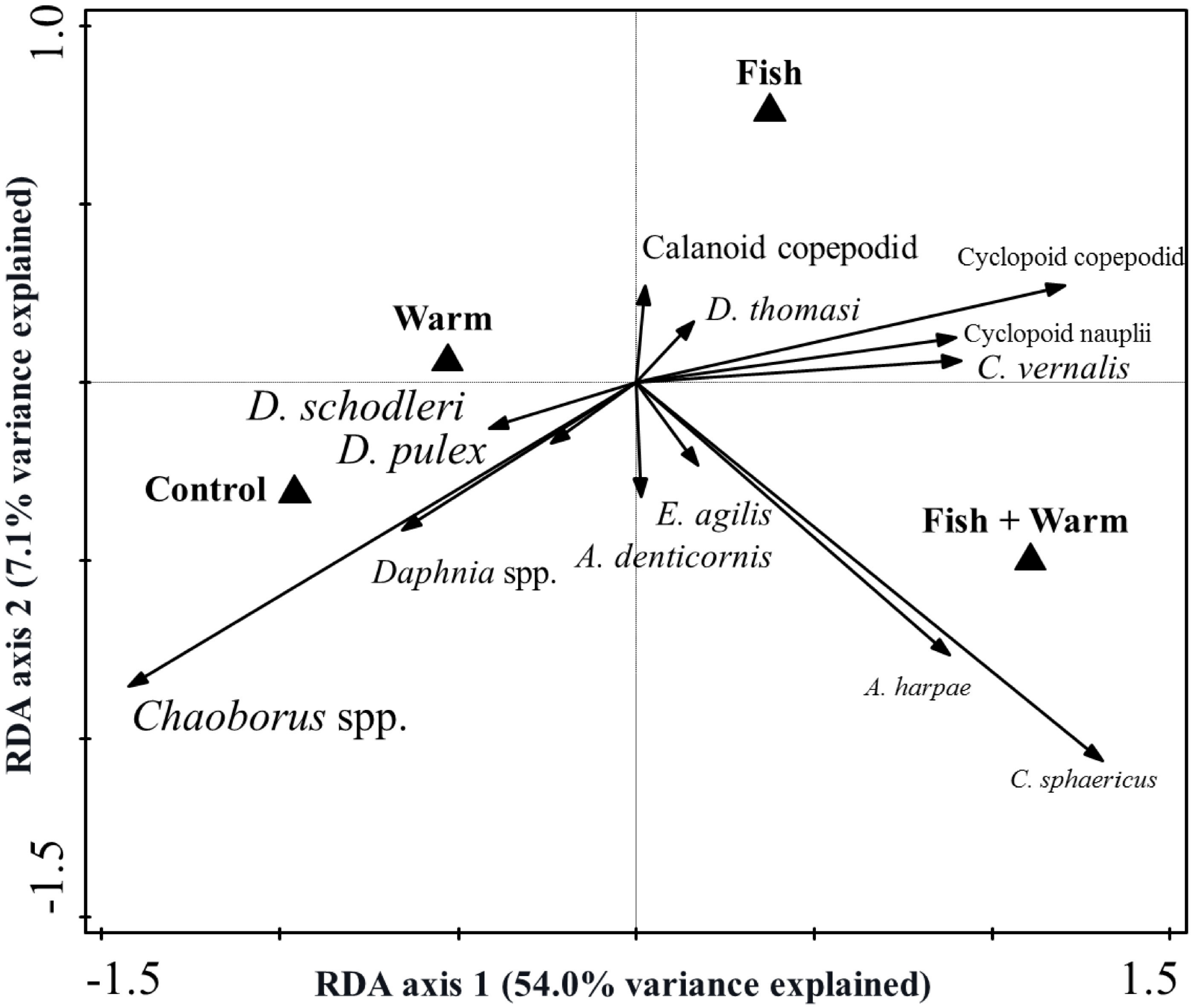

Figure 2-4 Partial redundancy analysis of zooplankton species and treatments on the final day of the mesocosm experiment (day 42). The blocking variable, lake source, was treated as a covariate. For clarity, only species that were strongly correlated along an axis are shown. The mean body size of species is indicated by font size (small < 5 µg, medium 5 – 30 µg, large > 30 µg). See Appendix A-2 for full species names, mean body sizes of species, and mean biomasses across treatments.

CHAPTER 3: LINKING SPECIES TRAITS AND CO-TOLERANCES TO PREDICT FUNCTIONAL EFFECTS OF SERIAL STRESSORS ON COMMUNITIES

Introduction

Our ability to predict the ecological effects of multiple stressors is hindered by uncertainty surrounding how and when they interact to mediate the effect of each other (Sala et al. 2000b, Fisichelli et al. 2014). Although how certain stressors interact to affect individual organisms and populations is becoming better understood, little is known about how their net effects transfer to higher levels of biological organization (Moe et al. 2013). Thus, a research framework is needed to predict cumulative impacts of stressors on aggregate properties of communities, such as species diversity and productivity (Duffy et al. 2009, Lindo et al. 2012). Despite this, many multiple stressor studies have been descriptive with findings that lack generality (Adams 2005, Munns Jr 2006, but see Halstead et al. 2014).

The species co-tolerance concept posits that population and community responses to two stressors may be predicted based on the correlation between species tolerances to each (Vinebrooke et al. 2004). Specifically, it is hypothesized that when tolerances of two stressors are unrelated, exposure to one stressor does not influence the effect of the other (Figure 3-1a). In other words, exposure to either stressor does not affect resistance to the other. Alternatively, positive correlation between tolerances (i.e. positive co-tolerance) expectedly increases resistance because of hard selection for a trait by the first stressor that confers tolerance of the other stressor (Figure 3-1b). Conversely, when tolerances are negatively correlated (i.e. negative co-tolerance) exposure to the one stressor is expected to decrease resistance to the other because of a trade-off between the traits selected for by the different stressors (Figure 3-1c).

Despite being well cited in the primary scientific literature since its introduction over a decade ago, the concept that species co-tolerance can drive the net effect of stressors on communities remains relatively untested (Brown et al. 2013). While there is some evidence of co-tolerance to stressors explaining species extinctions, the connection between co-tolerance and likely more common community-level responses to stressors, such as changes in species composition, is unclear (Graham et al. 2011, Tlili et al. 2011, Lucas et al. 2013). The limited integration of the co-tolerance concept in empirical studies may stem, in part, from the lack of a standard method for quantifying "tolerance" to a stressor (or conversely, "sensitivity"). Species tolerances to stressors are often not measured within natural communities, but instead inferred based on expert opinion or lethal limits determined in the laboratory (e.g. Graham et al. 2011, Darling et al. 2013, Boucek and Rehage 2014).

Stressor order may also influence the net effect of consecutive stressors. For example, the effect of multiple disturbances (i.e. short-term, discrete perturbations) on a community often depends on the order in which they occur (Bender et al. 1984, Fukami 2001, Floder and Hillebrand 2012). However, it is unknown whether exposure order also influences the net effect of chronic stressors (i.e. press perturbations) when they completely overlap and there is no recovery time between stressors. The often used cross-factorial experimental design where stressor treatments are applied simultaneously to assess their net effect is not suitable for investigating species co-tolerance relationships because the development of co-tolerance in a community depends on time-dependent processes (e.g. shifts toward tolerant species). Instead, exposing communities to stressors sequentially not only enables a more appropriate assessment of co-tolerance relationships, but also more realistically simulates exposure to stressors in natural environments (Giller et al. 2004, Fischer et al. 2013).

Here I integrate species co-tolerances with related key traits to hypothesize the responses of planktonic communities to the sequential effects of two widespread stressors of lake ecosystems, namely introduced fish and summer heating events (Rahel and Olden 2008). Previously, I showed that simultaneous exposure to these stressors synergistically increased total zooplankton biomass (Chapter 2; MacLennan et al. 2015). I now test whether species co-tolerance can explain the non-additive effect of warming and non-native trout on total zooplankton biomass and forecast functional responses to these stressors.

I created a conceptual framework (Figure 3-2) to help establish the rationale for the following hypotheses, which I then tested experimentally:

1) Trait selection by two stressors determines the direction and degree of correlation between species tolerances to each (Figure 3-1). In particular, I expected that warming and non-native trout select for the same key trait, namely reduced body size, resulting in positive co-tolerance to the stressors (Brooks and Dodson 1965, Moore et al. 1996). Consequently, zooplankton communities exposed to either or both stressors would consist primarily of small co-tolerant species.
2) The co-tolerance relationship determines the influence of stressor order on species composition and aggregate community properties (Figure 3-3). Specifically, I hypothesized that if zooplankton communities show positive co-tolerance to warming and non-native trout (i.e. stressors are highly redundant), then order of exposure to the stressors should not influence their net effect on zooplankton biomass. Conversely, the combined impact of the stressors should depend on their order of application if species tolerances to each are negatively correlated (i.e. low stressor redundancy).

3) The relationship between response and effect groups predicts the functional effect of stressors (Figure 3-4). I expected that body-size governs the tolerances of species to warming and non-native trout (i.e. is a response trait) while also influencing the ecological role of species (i.e. is also an effect trait). Thus, functional groups would have clustered tolerances to the stressors with species co-tolerant of warming and non-native trout being primarily small, inefficient grazers. In contrast, I expected that larger species would have low tolerances to the stressors and occupy higher trophic positions. Consequently, I hypothesized that the net impact of warming and non-native trout on naturally fishless lake communities would involve a decrease in larger efficient grazers and a shift toward smaller, less efficient herbivores, thereby increasing algal biomass.

Conceptual framework

The central tenets of the co-tolerance framework are that 1) trait selection drives patterns of co-tolerance, 2) co-tolerance relationships govern the effect of sequential stressors on a community, and 3) distribution of species tolerances within functional groups determines resistance of community functional structure (Figure 3-2). Here I define tolerance as the proportional change in the biomass of a species in the presence of a stressor relative to an unstressed control. However, other measures of performance (e.g. species abundance, reproductive rate) could be substituted for biomass to investigate different response variables of interest. Species tolerances are determined within a community context, thereby capturing both direct and indirect effects of stressors mediated by species interactions (Ives and Cardinale 2004). Using this metric, tolerance can be determined experimentally or by comparing natural communities in which a stressor is absent and present. Nevertheless, caution needs to be taken when establishing causality between stressors and biological variables based on observational data because unmeasured co-varying factors can confound stressor effects (Fukami and Wardle 2005) and the individual effects of co-varying stressors often cannot be disentangled (Niyogi et al. 2007).

1) Trait selection drives patterns of co-tolerance

Tolerance to a stressor is a function of species traits, including physiological, morphological and life history attributes that influence fitness (McGill et al. 2006). Identifying causal links between traits and tolerance to different stressors can inform *a priori* predictions of species co-tolerance relationships (Poff et al. 2010) (Figure 3-2, arrow 1). For example, selection for small-bodied organisms may drive positive co-tolerance in communities. Smaller-bodied organisms tend to have higher tolerances to a variety of stressors because of their greater capacity to acclimate and adapt to environmental change (Odum 1985, Vinebrooke et al. 2004, Woodward et al. 2005).

2) Co-tolerance relationships govern the effect of sequential stressors on a community

The correlation between species tolerances may forecast which species will “win” and “lose” when exposed to stressors sequentially (Figure 3-2, arrow 2). Species can be categorized into four response groups (i.e. groups of species that respond similarly to the stressors) corresponding to the four quadrats in a species co-tolerance bi-plot (Suding et al. 2008) (Figure 3-3). In general, exposure to both stressors is expected to suppress co-sensitive species (i.e. those that have low tolerance to both stressors) and shift communities toward co-tolerant species (i.e. those that have high tolerance to both stressors), regardless of stressor order (Figure 3-3c,e).

Therefore, if communities show positive co-tolerance to two stressors (i.e. stressors are highly redundant), then the order of the stressors should not influence their net effect on species composition and aggregate community properties.

However, if a community consists primarily of species showing a high degree of negative co-tolerance to two stressors, then their net effects should depend on the order of exposure to each. Consider the scenario in which a species is highly sensitive to the first stressor (stressor A) but tolerant of the second (stressor B) (Figure 3-3b, upper left quadrat). First, stressor A would directly suppress the species while having little, or even an indirect positive, effect on the more tolerant species through release from competition (Figure 3-3b). Continued exposure to stressor A would then negate any indirect positive effect of stressor B on that species as it is out-competed or preyed upon by the co-tolerant species (Figure 3-3c). Now consider the reverse scenario in which a species is tolerant of stressor A but sensitive to stressor B (Figure 3-3b, lower right quadrat). First, stressor A would not directly affect production by the species but may indirectly increase its production by suppressing some of its competitors and predators (Figure 3-3b). Further, stressor A may indirectly reduce the negative effect of stressor B on the species by suppressing some of its competitors and predators having a higher tolerance of stressor B (Figure 3-3c) (Ives and Cardinale 2004, Boucek and Rehage 2014). Therefore, if species show negative co-tolerance to stressors, then order of exposure to the stressors is expected to influence their net effect on species composition and aggregate community properties.

3) Distribution of species tolerances within functional groups determines resistance of community functional structure

The insurance hypothesis predicts that tolerant species can compensate for declines of functionally-redundant species that are intolerant of a stressor, thereby buffering communities against functional loss (Yachi and Loreau 1999). Hence, the functional effect of multiple stressors depends on the degree to which response groups are correlated with effects groups (i.e. groups of species that have similar ecological roles) (Figure 3-2, arrow 3). When tolerances of species to two stressors are clustered, response diversity (i.e. the diversity of responses to a stressor among species within a functional group) is low (Elmqvist et al. 2003) (Figure 3-4b). Here, the combined effect of stressors is more likely to decrease ecological functions than when tolerances of species within functional groups are randomly distributed (Figure 3-4a) (Vinebrooke et al. 2004). Without tolerant species to compensate for the reduced function of sensitive species, ecological functions are eroded. Conversely, when tolerances of species to two stressors are dispersed, response diversity is high (Figure 3-4c). Here, the likelihood that functional groups contain a species that is co-tolerant of the stressors is higher than when tolerances of species within functional groups are randomly distributed. As a result, there is greater potential for species compensation to buffer communities against stress-induced losses in ecological functions (Vinebrooke et al. 2004).

Positive and negative co-tolerance may also influence the likelihood that response and effect groups are correlated. For example, body-size often influences both the tolerances of species to stressors and their functional roles because it is closely linked to a suite of life-history traits, such as feeding strategy and trophic status (Woodward et al. 2005). Clustered tolerances to stressors combined with negative species co-tolerance is expected to represent a “worst case scenario” because trade-offs in tolerances will increase the number of species that are vulnerable to the stressors and entire functional groups may be lost (Vinebrooke et al. 2004).

Methods

Mesocosm experiment

In July 2011, fifteen 1000-L capacity mesocosms (W40” x L48” x H40”) made of translucent high-density polyethylene were established at the Barrier Lake Field Station along the eastern front range of the Rocky Mountains. The mesocosms were filled to capacity with filtered (63-µm mesh size) snowmelt water collected from a nearby pond. Each unit was wrapped in an insulative cover (Heatsheets® Survival Blanket, AFMInc, California, USA) except for a 20-cm diameter opening at the top that was covered with 1-mm mesh. Insulative covers minimized diel fluctuations in water temperature and heat loss from warmed treatments. Water in the mesocosms was aerated using a tube connected to an air pump to disturb surface tension and manually mixed with a paddle twice daily.

Plankton collected from five naturally fishless montane lakes (Copper (51°15’N: 155°55’W), Herbert (51°27’N: 116°13’W), Island (51°23’N: 116°6’W), Kingfisher (51°24’N: 116°10’W) and Lost (51°28’N: 116°16’W)) in Banff National Park, Alberta, Canada was used to inoculate mesocosms in a randomized block design. Quantitative plankton samples were taken from source lakes by vertically hauling a conical net from 1 m off the lake bottom through the entire water column, and then transported to the Barrier Lake Field Station in 20-L plastic carboys. On July 13-14 2011, phytoplankton was collected from the lakes using a 30-cm diameter, 10-µm mesh-sized conical net. After being passed through a 63-µm sieve to remove zooplankton, phytoplankton communities from each lake were randomly distributed in equal volumes to three mesocosms. On July 18-19 2011, zooplankton was collected from the lakes using a 30-cm diameter, 63-µm mesh-sized conical net. Natural densities of plankton were concentrated to a target of three zooplankters L^{-1} based on calculations of zooplankton density in each of the five lakes that were determined one week before collections. Zooplankton was evenly distributed among the three containers containing phytoplankton from their respective lake.

I performed a one-factor experiment consisting of three stressor treatments (unstressed, warming then fish, and fish then warming) using a randomized block design from July 23 to September 2 2011. The two sequential stressor scenarios were achieved by applying the first stressor on day 1 of the experiment and applying the second stressor at the mid-point of the experiment (after sampling on day 21). Mesocosms were warmed using four, 300-watt aquarium heaters (Hagen®, Montreal, Canada) to maintain an average difference of 3.6°C between heated and ambient tanks. HOBO Pendant® data loggers (Massachusetts, USA) were deployed at mid-depth in each mesocosm and recorded water temperatures at 1-hr intervals. During the first half of the experiment, unheated mesocosms averaged 19.6°C and ranged from 13.5°C to 24.0°C, while warmed mesocosms averaged 23.0°C and ranged from 14.3°C to 27.5°C. During the second half of the experiment, unheated mesocosms averaged 19.2°C and ranged from 10.8°C to 26.9°C, while warmed mesocosms averaged 23.1°C and ranged from 14.1°C to 29.3°C. The fish treatment was applied by stocking mesocosms with a single rainbow trout (*Oncorhynchus mykiss*) fingerling (10 - 13 cm long) purchased from a local hatchery (Ackenberry Trout Farms, Camrose, Alberta). Additional details on warming and fish treatments are provided in Chapter 2 (MacLennan et al. 2015).

Zooplankton was sampled from each mesocosm before the treatment was applied on day 0 and weekly thereafter until the final day of the experiment on day 42. Samples were collected using a hose attached to a built-in spigot at the base of each mesocosm while mixing the water with a paddle. Zooplankton was concentrated using a 63-μm mesh-sized sieve and preserved in

70% ethanol. On each sampling occasion, 40 L samples were taken, except on the final day of the experiment when 80 L samples were taken.

A minimum count of 300 zooplankters per sample was achieved using a Leica MZ9s dissecting microscope. A minimum of two subsamples were taken and counted in their entirety and subsampling continued until the standard error of the total number of individuals per subsample was less than 10% of the mean number of individuals counted per subsample (Bade et al. 1998). Individuals were identified to genus and species when possible using Ward and Whipple (1959). Lengths of the first 15 individuals of each developmental stage for each taxon were measured at 40× and biomass was estimated using length-mass regressions (McCauley 1984, Culver et al. 1985). Zooplankton taxa were classified into functional groups as defined by Barnett et al. (2007). *Chaoborus* was classified as the top predator and zooplankton taxa were grouped as omnivores, large herbivores, and small herbivores (Appendix B-1).

Species co-tolerances

Tolerance of each zooplankton taxon to introduced trout and warming was determined based on its response to each stressor in the absence of the other. Single stressor treatments were run concurrently with dual-stressor treatments in this study and are reported in Chapter 2. Tolerance to each of the stressors was calculated as:

$$\text{Tolerance} = (\bar{x}_{\text{stressor}} - \bar{x}_{\text{control}})/\bar{x}_{\text{control}}$$

where $\bar{x}_{\text{stressor}}$ is the mean biomass of the taxon in the presence of the stressor on the final day of the experiment and $\bar{x}_{\text{control}}$ is the mean biomass of the taxon in the unstressed control on the final day of the experiment. For both stressors, tolerance was determined for each taxon and averaged across lake blocks. Tolerances were bound by zero because negative biomass was not possible but positive responses to the stressors were not bound by an upper limit. As a result, the distribution of tolerances was inherently right-skewed. To normalize the data and rescale it around zero, I applied the following transformation to tolerances (X), in accordance with Kaiser et al. (2006):

$$Y = \ln(1 + [X/101])$$

As a result, tolerance represented a gradient of responses with negative values showing biomass loss and positive values indicating increased biomass in the presence of the stressor. A tolerance value of -4.6 showed loss of the taxon in the presence of the stressor, while a tolerance value of +4.6 indicated a 100-fold increase in biomass. I was unable to calculate tolerance for rare taxa that were not detected in at least one stressor treatment and the control within a lake block, and these taxa were excluded from the analysis of co-tolerance. One-tailed Pearson's correlations were used to test for positive co-tolerance of taxa to warming and non-native trout and for negative correlations between mean body length and tolerance to the stressors.

Community responses

Linear mixed models were used to test for time-dependent effects of stressor treatment on total zooplankton biomass, the biomass of functional groups (i.e. top predator, omnivores, large herbivores and small herbivores), and chlorophyll *a* concentration (Zuur et al. 2009). All response variables were $\log_{10}$-transformed prior to analysis to improve normality and homoscedasticity. Experimental treatment (Unstressed control, Warming then fish, Fish then warming) was treated as a fixed variable and sampling occasion as a covariate. Random block (i.e. lake source) effects were tested by comparing models including a random intercept against models without a random block effect. The model that minimized the Akaike's Information Criterion corrected for small sample-size (AICc) based on restricted maximum likelihood estimation was used (Appendix B-2). Individual mesocosm was treated as a random factor and to account for serial correlation from repeated measures, various error covariance structures were tested, including autoregressive structures with homogeneous and heterogeneous variances, to determine the optimal random structure. The covariance structure that minimized the AICc was used (Appendix B-2). Significant interactions between stressor treatment and time were further analyzed by performing separate univariate analyses of variances (ANOVAs) of stressor effects on response variables for each sampling date. I used Tukey's *post hoc* tests to examine significant differences between stressor treatments. Statistical analyses were performed using PASW® Statistics 18 (SPSS Inc., Chicago, Illinois, USA).

Results

1) Trait selection drives patterns of co-tolerance

Species tolerances of warming (r = -0.61, P = 0.01, n = 13) and non-native trout (r = -0.69, P = 0.004, n = 13) were both negatively correlated with body size (Figure 3-5). Further, species tolerances of the stressors were positively correlated (r = 0.48, P = 0.049, n = 13) as smaller species were most often co-tolerant, while larger species were intolerant of both stressors (Figure 3-6a). Only one species (*Diacyclops thomasi*) showed marked differences in its tolerance to warming versus non-native trout.

2) Co-tolerance relationships govern the net effects of sequential stressors on a community

Positive species co-tolerance within the community translated into exposure to either stressor causing a compositional shift toward taxa that were also tolerant of the subsequent stressor (Figure 3-6). Most taxa that were tolerant of the first stressor (i.e. having tolerances > 0) increased in biomass by day 21 (Figure 3-6b,d), and thereafter increased further with exposure to the second stressor (Figure 3-6c,e). In contrast, most taxa that were intolerant of the first stressor (i.e. having tolerances < 0) were suppressed by day 21 (Figure 3-6b,d), and subsequently either further suppressed or extirpated by the second stressor (Figure 3-6c,e).

The order of exposure to warming and non-native trout did not influence their cumulative effect on total community biomass (Table 3-1; Figure 3-7). In both sequences, joint exposure to the stressors suppressed or stimulated the same set of species (Figure 3-6c,e). However, the one species that responded dissimilarly to warming and non-native trout (*D. thomasi*) was influenced by order of exposure to the stressors. When exposed to warming first (the stressor to which it was sensitive), the stressors did not affect the biomass of *D. thomasi* (Figure 3-6c). However, when exposed to non-native trout first (the stressor to which it was tolerant), *D. thomasi* increased in biomass (Figure 3-6e). Although stressed communities had higher biomass than controls on the final day of the experiment, the effect of stressor treatment was only marginally significant (Table 3-1).

3) Distribution of species tolerances within functional groups determines resistance of community functional structure

The distribution of species co-tolerances within functional groups foreshadowed their responses to sequential exposure to warming and non-native trout. Taxa within functional groups tended to have clustered tolerances to both stressors, attesting to low response diversity (Figure 3-8). For example, large herbivores and the top predator *Chaoborus* all lacked tolerance to either stressor (Figure 3-8a,b,e,f), and as a result, they were extirpated upon exposure to both stressors, regardless of the order of exposure (Figure 3-9a,c). Omnivores were relatively more tolerant of non-native trout than warming (Figure 3-8c,d). Stressor treatment did not significantly affect omnivore biomass overall (Table 3-1). However, omnivore biomass was higher when exposed to Fish then warming relative to controls (Tukey's *post hoc* $P = 0.05$), but not Warming then fish (Tukey's *post hoc* $P= 0.53$) by the end of the experiment (Figure 3-9b). Small herbivores were co-tolerant (Figure 3-8g,h), increasing in total biomass regardless of order of exposure to the two stressors (Warming then fish: Tukey's *post hoc* $P = 0.007$; Fish then warming: Tukey's *post hoc* $P = 0.002$) (Table 3-1, Figure 3-9d). Both sequential applications of warming and non-native trout also significantly increased algal biomass as measured using chlorophyll *a* concentration (Warming then fish: Tukey's *post hoc* $P = 0.001$; Fish then warming: Tukey's *post hoc* $P < 0.001$) (Table 3-1; Figure 3-9e).

Discussion

Linking response traits, co-tolerance relationships, and effect groups offers a novel approach to predicting and testing effects of sequential stressors on the functional structure of communities. Using this framework, I found that species co-tolerance helped explain the net effects of warming and non-native trout on communities from naturally fishless lakes. As hypothesized, body-size was a key trait associated with tolerance of species to both warming and non-native trout, resulting in a community characterized by positively co-tolerant small species and highly sensitive larger species. Order of exposure to the stressors did not influence their net effect on total community biomass, supporting the hypothesis that the effect of highly redundant stressors should not depend on their order. Also, the distribution of species tolerances within functional groups predicted the effects of sequential exposures to warming and non-native trout on the functional structure of communities. The top predator *Chaoborus* and large herbivores were co-sensitive to warming and non-native trout and were always extirpated, stimulating smaller co-tolerant herbivores regardless of stressor order. In contrast, omnivores were more tolerant of non-native trout than warming, and their final biomass depended on stressor order. Below, I offer potential ecological explanations and for these key findings.

My finding that small body-size conferred tolerance to warming and non-native trout supported the hypothesis that smaller individuals are, in general, more stress-tolerant than larger organisms (Odum 1985). Small zooplankton are considered to be both better metabolically adapted to cope with higher temperatures (Brooks and Dodson 1965, Moore et al. 1996), and able to avoid detection by visually-feeding planktivorous fish (Brooks and Dodson 1965, Moore et al. 1996). Small body-size has also been associated with tolerance to a variety of other stressors, including pollution (Cattaneo et al. 1998), heavy metals (Vesela and Vijverberg 2007), hypoxia (Robb and Abrahams 2003), and ultraviolet radiation (Leech and Williamson 2000). The higher tolerance of small-bodied organisms to a multitude of stressors may be, in part, a consequence of their higher adaptive potential associated with larger population sizes and shorter generation times (Woodward et al. 2005).

My findings also supported the hypothesis that order of exposure does not influence the net effect of stressors on communities that exhibit positive species co-tolerance. Specifically, I discovered that stressor order did not influence the net effect of the highly-redundant stressors, warming and non-native trout, on zooplankton biomass. Therefore, the net effect of these stressors on communities of naturally fishless lakes will likely be similar between lakes containing historically stocked trout that experience a warming climate and lakes where warmer temperatures enable future invasions of trout.

Further, I found evidence supporting the hypothesis that order of exposure to two stressors influences the response of a species having highly dissimilar tolerances to each. Only one species (*Diacyclops thomasi*) had marked differences in its tolerances to warming and non-native trout, and its final biomass depended on order of exposure to the stressors. Therefore, it appears that the combined impact of sequential stressors on a community may depend on their order if species tolerances to each are negatively correlated.

My findings also suggest that positive co-tolerance can decrease the resistance of certain ecological functions if species tolerances within functional groups are clustered. Large-bodied species from higher trophic levels lacked tolerance to both warming and non-native trout, and sequential exposure to stressors extirpated the top predator and all large herbivores. Although an increase in small-bodied taxa maintained total zooplankton biomass, small-bodied taxa were unable to functionally compensate for large-bodied taxa because they performed different ecological roles. For example, higher algal biomass in stressed communities suggested that the loss of large efficient grazers was not offset by an increase in smaller less-efficient herbivores (Brooks and Dodson 1965); however, increased nutrient recycling by fish may also have contributed to this effect (Vanni and Layne 1997).

Although positive co-tolerance is hypothesized to increase the resistance of a community to two stressors (Vinebrooke et al. 2004), I discovered that positive co-tolerance of zooplankton communities to warming and non-native trout decreased resistance to their combined impact. Together, warming and non-native trout amplified shifts toward co-tolerant species, which likely drove their synergistic net effect on zooplankton biomass found in an earlier study (Chapter 2; MacLennan et al. 2015). Indeed, environmental drivers that have large impacts on species composition also tend to have large effects on aggregate community properties (Matulich and Martiny 2015). Therefore, it appears that although positive co-tolerance may mitigate negative effects of sequential stressors on communities (Vinebrooke et al. 2004, Brown et al. 2013), it can induce synergistic positive impacts if co-tolerant species are strongly favoured.

Caveats

Biotic interactions and length of exposure to a stressor can influence the tolerance of species. I determined tolerances of zooplankton taxa to warming and non-native trout based on their responses within natural communities over multiple generations, thereby capturing both potential direct effects of the stressors and indirect effects of altered species interactions (Gillooly 2000). Community responses to sequential stressors are likely to be less predictable using species tolerances determined in monocultures (Floder and Hillebrand 2012, Limberger et al. 2014). Further, exposing communities to single and dual stressor treatments for the same length of time controlled for the influence of time-dependent processes on tolerances of zooplankton taxa. Initial responses of species to stressors are primarily governed by direct physiological effects but tolerances of species may shift over time (Suttle et al. 2007, Smith et al. 2009), especially if evolutionary trade-offs affect the ability of species to cope with acute versus chronic stress (Magozzi and Calosi 2015). For example, increased tolerance to a stressor due to acclimation or adaptation would shift the position of a species upwards or to the right in the co-tolerance bi-plot (Vinebrooke et al. 2004). Conversely, if chronic exposure to a stressor degrades the resistance of a species over time, then its position in the co-tolerance bi-plot would shift down or to the left (Eggen et al. 2004).

Further, the capacity to predict the effects of sequential stressors on the functional structure of a community using species co-tolerances may be lessened by several factors. First, the assumption that the ecological role of a species is static is likely unrealistic, as community restructuring following exposure to a stressor can alter the trophic positions of tolerant species (Lindo et al. 2012). Also, highly uneven biomass contributions among species can cause the tolerance of a single high-performance species to drive the functional effect of sequential stressors (Floder and Hillebrand 2012). Additionally, responses to stressors other than changes in biomass, such as those due to behavioural shifts, could influence the resistance of ecological functions (McConkey and O'Farrill 2015).

Management implications

A greater ability to predict the effects of multiple stressors on communities will enable us to better evaluate potential management strategies. For example, my finding that zooplankton communities of naturally fishless lakes show positive co-tolerance to warming and non-native trout suggests that even if fish populations are removed, species composition may not fully recover because species that are intolerant of fish are also vulnerable to climate warming. If other biologically important species in lake (e.g. insects, plants) are similarly sensitive to warming, then fish removals may be best targeted in lakes that will experience less warming, such as those that contain natural climate refugia (e.g. stable coldwater hypolimnia). Conversely, negative co-tolerance in communities is hypothesized to lead to synergistic negative effects on communities and removal of a stressor is expected to facilitate ecological recovery (Brown et al. 2013).

Stressor removal could also be prioritized in communities in which traits associated with resistance and resilience (i.e. the ability to recover from a stressor) are negatively correlated. Here, the potential for management to accelerate recovery of communities is greatest because species that are most vulnerable to a stressor are also those that are able to recover most quickly following its removal (Díaz and Cabido 2001).

Conclusions

Linking species traits and co-tolerances provides a theoretical basis for generating testable hypotheses of the effects of sequential stressors on communities, using only knowledge of their individual effects. By identifying traits driving tolerances of species to stressors, my framework has the potential to improve our mechanistic understanding of the effects of multiple stressors on communities. Importantly, the framework can be broadly applied across ecological communities and as a result, may help reveal generalities among responses to multiple stressors across different organisms, habitats and stressor combinations.

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Table 3-1 Results of the linear mixed models for the fixed effects stressor treatment and time on total zooplankton biomass, the biomass of functional groups, and chlorophyll *a* concentration for the 42-day experiment. *P*-values in bold indicate statistical significance.

	Total biomass			Top predator			Omnivores		
Fixed effect	DF	*F*	*P*	DF	*F*	*P*	DF	*F*	*P*
Stressor treatment	2, 22.6	2.20	0.13	2, 12	10.40	**0.002**	2, 32.4	1.62	0.21
Time	1, 34.2	10.37	**0.003**	1, 12	7.38	**0.02**	1, 46.2	2.88	**0.002**
Stressor*Time	2, 34.2	2.54	0.09	2, 12	9.74	**0.003**	2, 46.2	2.34	0.11

	Large herbivores			Small herbivores			Chlorophyll *a*		
Fixed effect	DF	*F*	*P*	DF	*F*	*P*	DF	*F*	*P*
Stressor treatment	2, 8.0	2.89	0.11	2, 5.2	2.67	0.16	2, 3.7	26.19	**0.007**
Time	1, 7.2	10.09	**0.02**	1, 14.7	20.86	**<0.001**	1, 10.9	385.12	**<0.001**
Stressor*Time	2, 7.2	15.05	**0.003**	2, 14.7	5.30	**0.02**	2, 10.9	55.15	**<0.001**

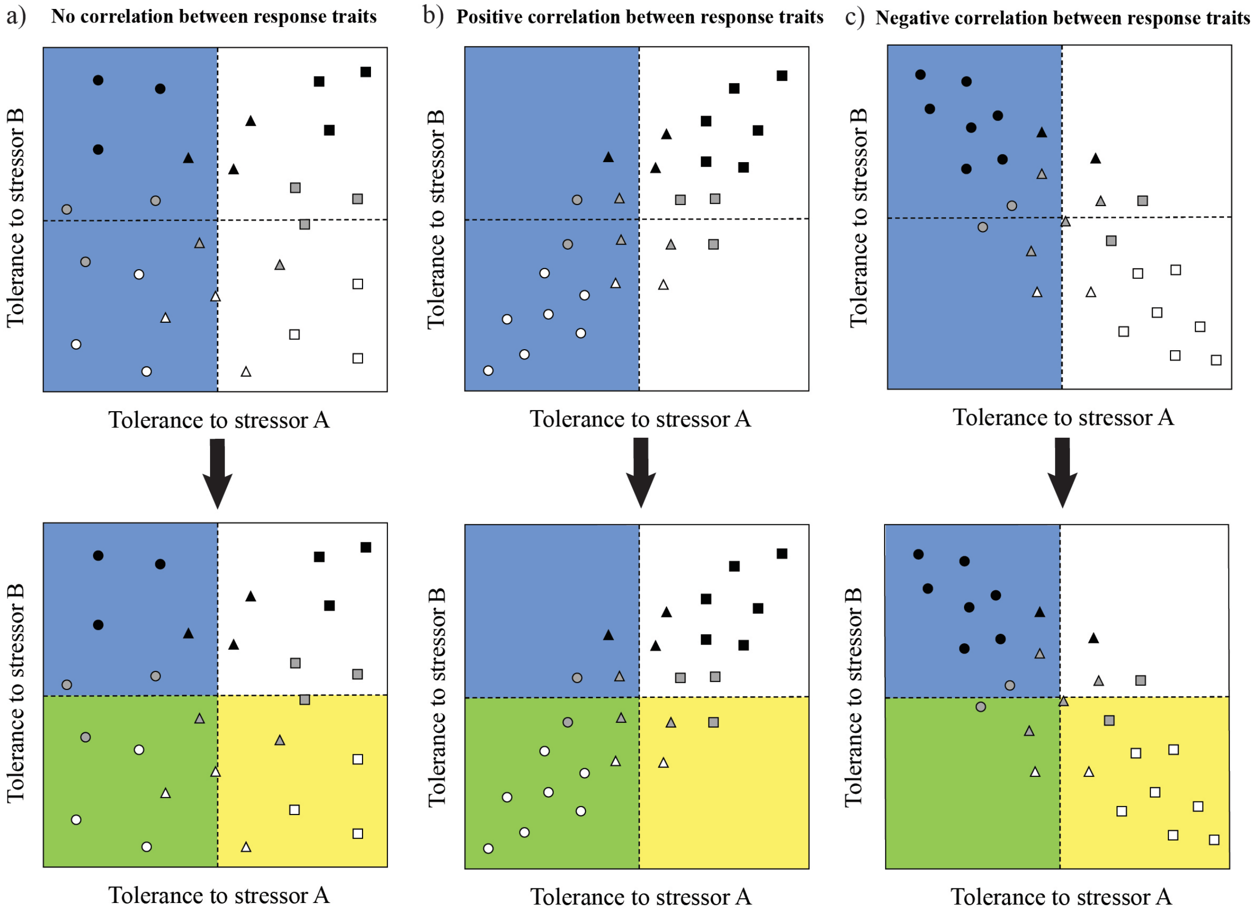
a)
No correlation between response traits
b)
Positive correlation between response traits
c)
Negative correlation between response traits
Tolerance to stressor B
Tolerance to stressor A

Figure 3-1 Three scenarios showing hypothesized relationships between response traits (i.e. traits that govern responses to environmental change) selected for by two stressors. Each point represents a species within a community. Symbols represent levels of a trait associated with tolerance to stressor A (square = high level, triangle = intermediate level, circle = low level). Symbol colours represent levels of a trait associated with tolerance to stressor B (black = high level, grey = intermediate level, white = low level). In the first scenario (a), response traits associated with tolerance to each stressor are uncorrelated. Here, selection by stressor A does not influence the distribution of the trait associated with tolerance to stressor B. Thus, exposure to stressor A does not influence the resistance of the community to stressor B. In the second scenario (b), response traits associated with tolerance to each stressor are positively correlated. Here, stressor A selects for species having levels of the trait that confers high tolerance to stressor B, thereby increasing the resistance of the community to stressor B. In the third scenario (c), response traits associated with tolerance to each stressor are negatively correlated. Here, stressor A selects for species having low levels of the trait that confers tolerance to stressor B, thereby decreasing the resistance of the community to stressor B.

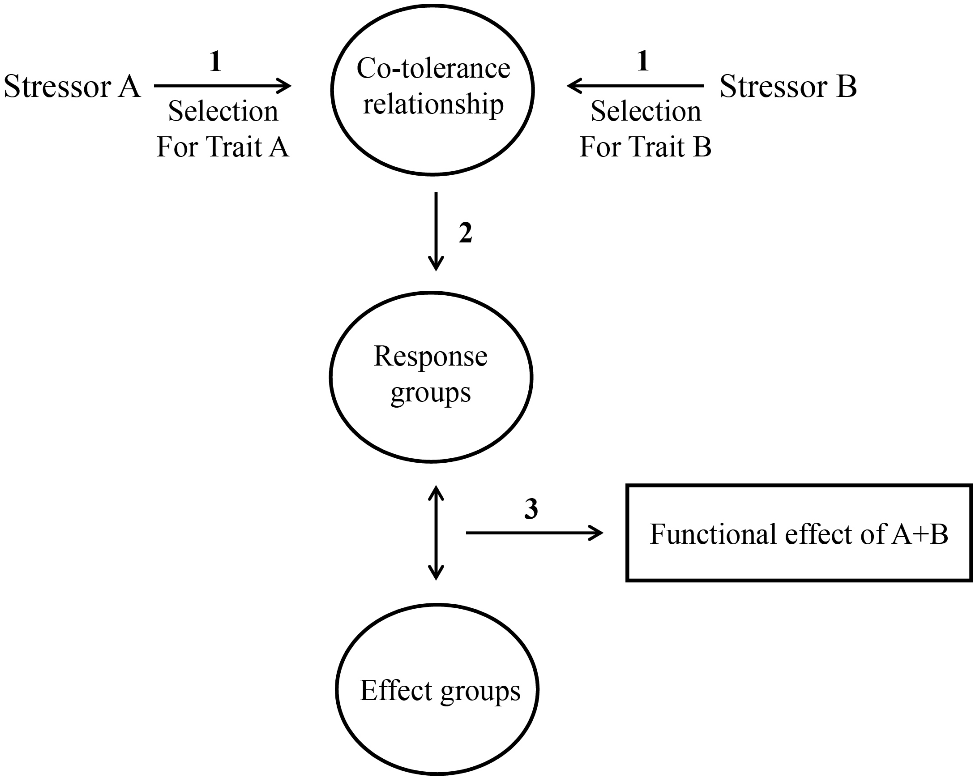

Figure 3-2 A flowchart for predicting the functional effect of stressors A and B on a community based on the correlation between species tolerances to each. Both stressors promote species-sorting by selecting for either the same or different key response traits, which ultimately influence their net functional effects on the community. 1) The relatedness of response traits associated with tolerance to each stressor drives the nature of the co-tolerance relationship (see Figure 3-1). 2) The co-tolerance relationship defines the distribution of species among response groups (i.e. species that respond similarly to the stressors) and determines how order of exposure influences the net effect of stressors on species composition and aggregate community properties (see Figure 3-3). 3) The relationship between response groups and effect groups (i.e. groups of species that affect similar ecological processes, such as herbivory or top predation) determines the cumulative functional effect of stressors A and B (see Figure 3-4).

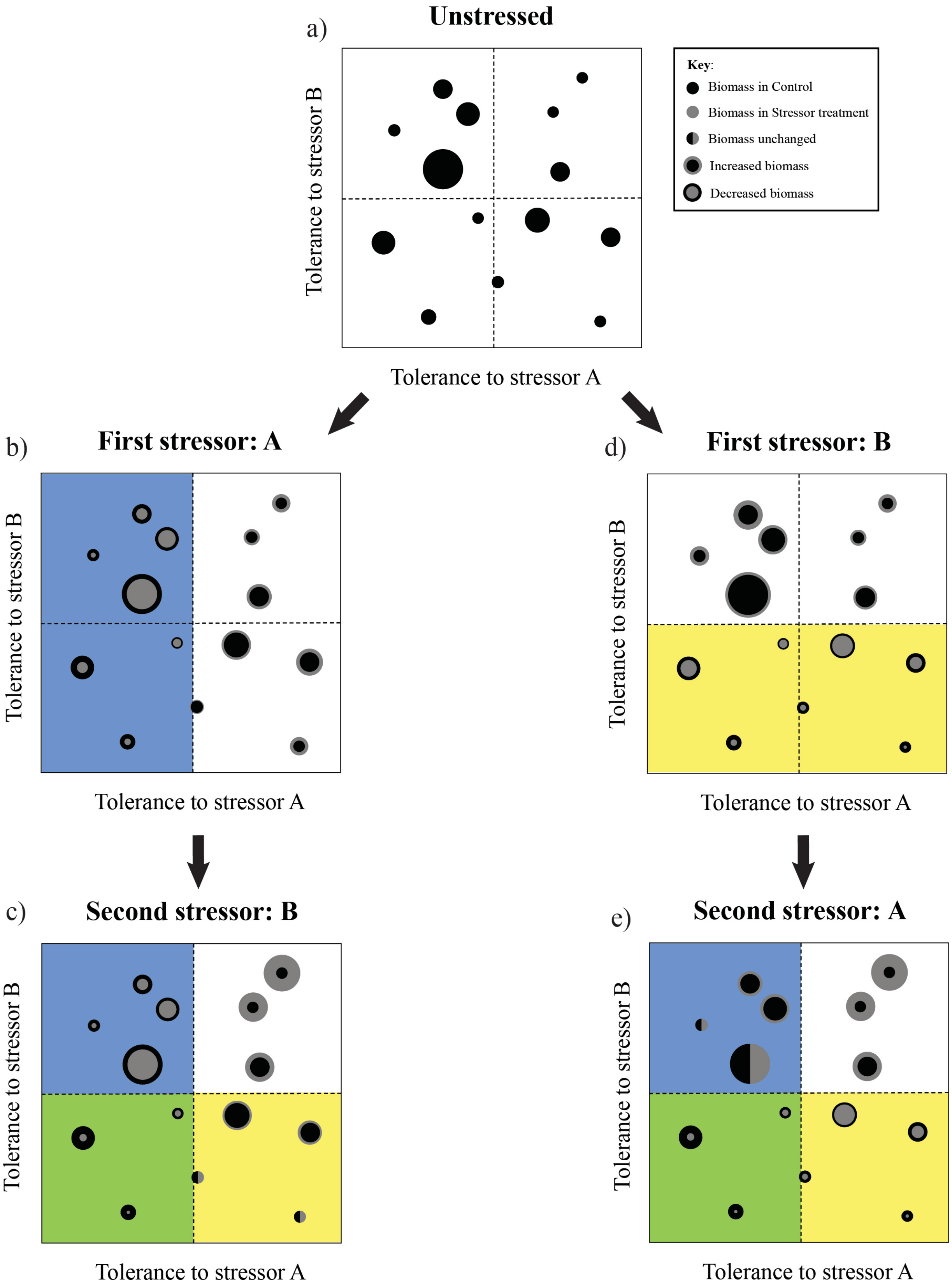
a)
Unstressed
Tolerance to stressor B
Tolerance to stressor A
Key:
● Biomass in Control
● Biomass in Stressor treatment
◐ Biomass unchanged
Increased biomass
Decreased biomass
b)
First stressor: A
Tolerance to stressor B
Tolerance to stressor A
c)
Second stressor: B
Tolerance to stressor B
Tolerance to stressor A
d)
First stressor: B
Tolerance to stressor B
Tolerance to stressor A
e)
Second stressor: A
Tolerance to stressor B
Tolerance to stressor A

Figure 3-3 Hypothetical responses of a community exposed to stressors A then B (left column) and B then A (right column). Circle size denotes relative functional performance of species. a) Here, the community consists of species having tolerances to stressors A and B that are not correlated, although other co-tolerance scenarios are possible (see Figure 3-1). Species are parsed into four response groups corresponding to the quadrats in the co-tolerance bi-plot (i.e. the co-tolerant group occupies the upper right quadrat, while the co-sensitive group is located in the lower left quadrat). b) Exposure to stressor A first suppresses species that lack tolerance to stressor A (left half of the bi-plot), while releasing species that have high tolerance to stressor A (right half of the bi-plot) from competition or predation. c) Next, exposure to stressor B further suppresses co-sensitive species, potentially promoting co-tolerant species. Species in the upper left quadrat continue to be suppressed by stressor A and also possibly the thriving co-tolerant species, thereby preventing a positive response to stressor B. Exposure to stressor B suppresses species in the lower right quadrat; however, their negative response to stressor B may be muted because stressor A has already reduced some of their potential competitors and predators having higher tolerances of stressor B. d) Alternatively, exposure to stressor B first suppresses species that lack tolerance to stressor B (lower half of the bi-plot) while stimulating species are tolerant of stressor B (upper half of the bi-plot). e) Subsequent exposure to stressor A suppresses co-sensitive species and increases co-tolerant species to a similar degree as the reverse stressor order. However, based on the same arguments as above, species in the upper left and lower right quadrats have opposite responses to the net effect of stressors relative to the reverse stressor order. Thus, stressor order is hypothesized to influence the net effect of stressors on species that have dissimilar responses to two stressors (i.e. upper left and lower right quadrats) but not species that respond similarly to two stressors (i.e. lower left and upper right quadrats).

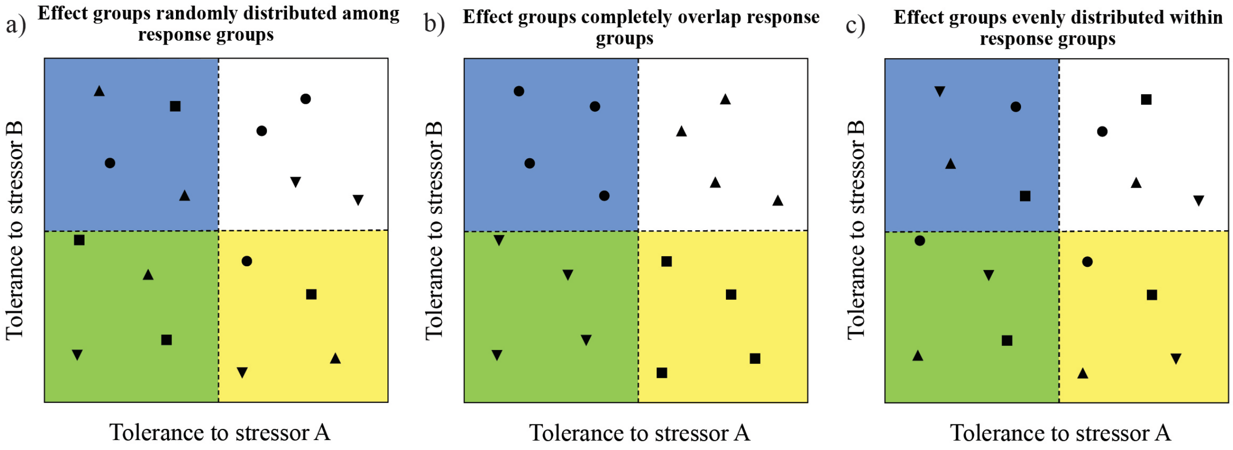

Figure 3-4 Hypothetical relationships between response and effect groups with species in each functional group represented by different symbols. Response groups are defined by the quadrats in the bi-plot as described in Figure 3-3. In the first scenario (a), effect groups are randomly distributed among response groups. In the second scenario (b), response and effect groups completely overlap such that all species within an effect group are similarly tolerant of stressors A and B (i.e. a clustered distribution). Here, low response diversity increases the likelihood that an entire functional group will be susceptible to both stressors relative to the random distribution. In the third scenario (c), effect groups are evenly distributed among response groups such that a species from each effect group is contained within each response group. Here, high response diversity decreases the likelihood that an entire functional group will be susceptible to the stressors relative to a random distribution. In each case, the number of functional groups and the number of species within each functional group are held constant.

a)

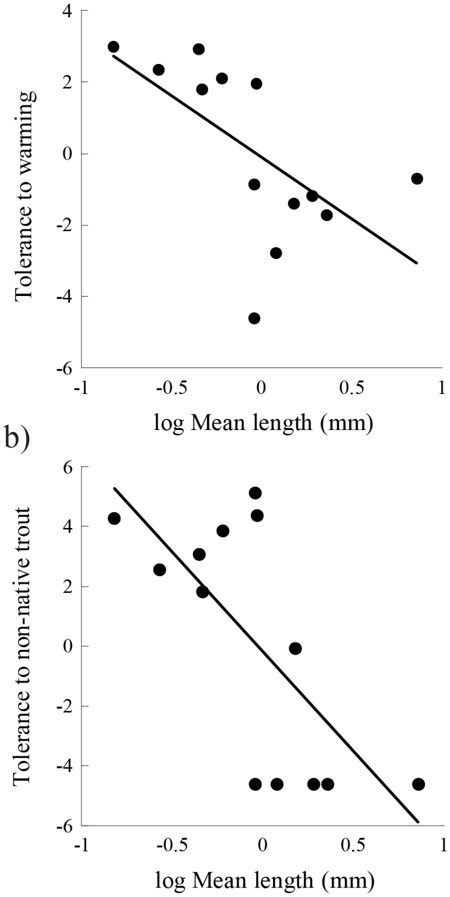

Figure 3-5 Relationship between $\log_{10}$-transformed mean body-length and a) tolerance to warming and b) tolerance to non-native trout. Solid lines represent the best linear fit of significant relationships.

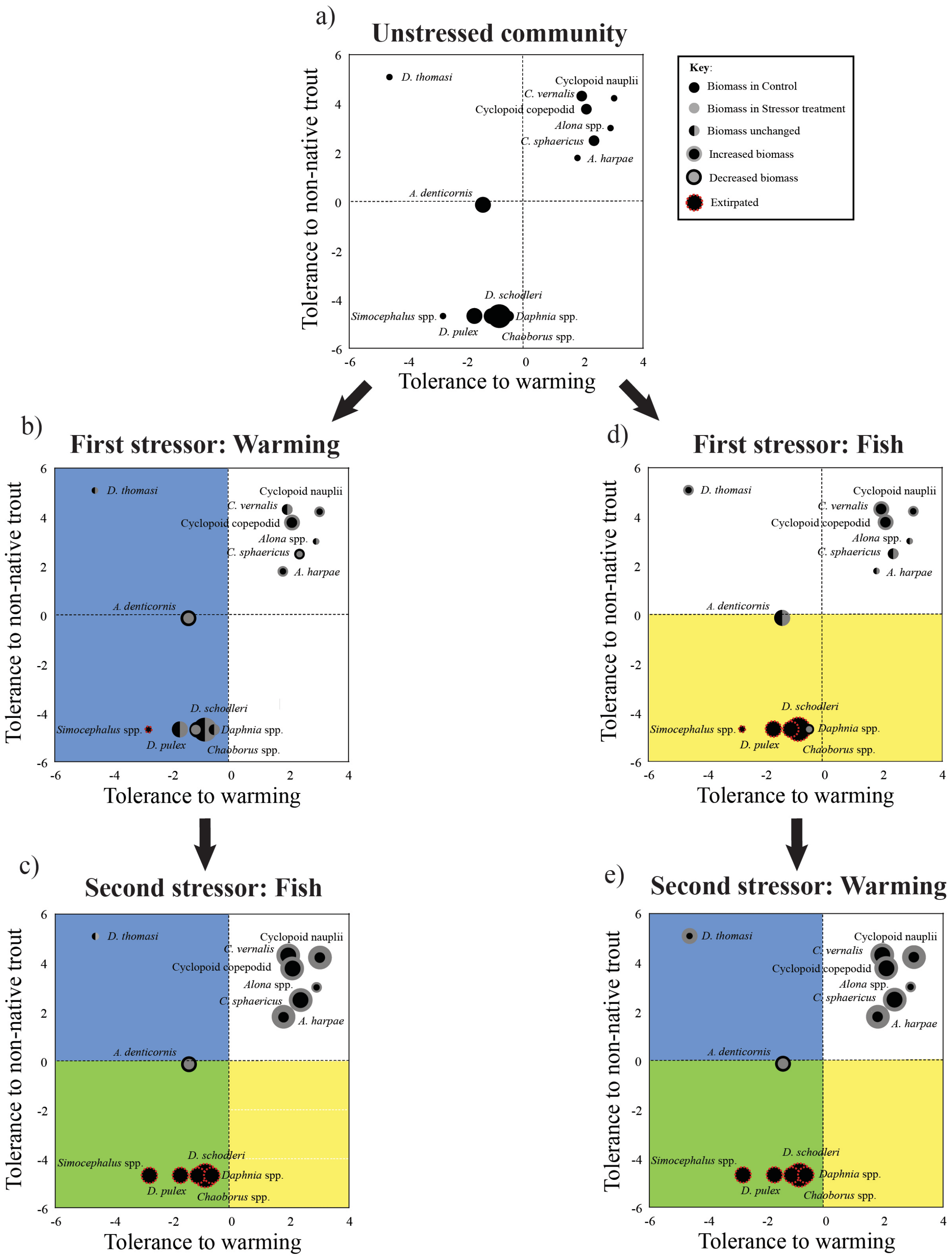
a)
Unstressed community
Key:
Biomass in Control
Biomass in Stressor treatment
Biomass unchanged
Increased biomass
Decreased biomass
Extirpated
b)
First stressor: Warming
c)
Second stressor: Fish
d)
First stressor: Fish
e)
Second stressor: Warming
D. thomasi
Cyclopoid nauplii
C. vernalis
Cyclopoid copepodid
Alona spp.
C. sphaericus
A. harpae
A. denticornis
D. schodleri
Simocephalus spp.
Daphnia spp.
D. pulex
Chaoborus spp.
Tolerance to non-native trout
Tolerance to warming
6
4
2
0
-2
-4
-6
-6
-4
-2
0
2
4

Figure 3-6 Species-sorting in response to Warming then fish (a-c) and Fish then warming (a,d,e) relative to their tolerances to each stressor. Zero tolerance values denote no net effect of the stressor on species biomass, while negative and positive values represent declines and increases in biomass, respectively. Tolerance values of -4.6 show species loss in the presence of the stressor, while tolerance values of +4.6 indicate a 100-fold increase in biomass. The size of the circles represents biomass with small circles = < 0.1 μg/L, medium circles = 0.1 - 1 μg/L, large circles = 1 – 10 μg/L, and extra-large circles = > 10 μg/L. See Appendix B-1 for full species names and exact biomass values.

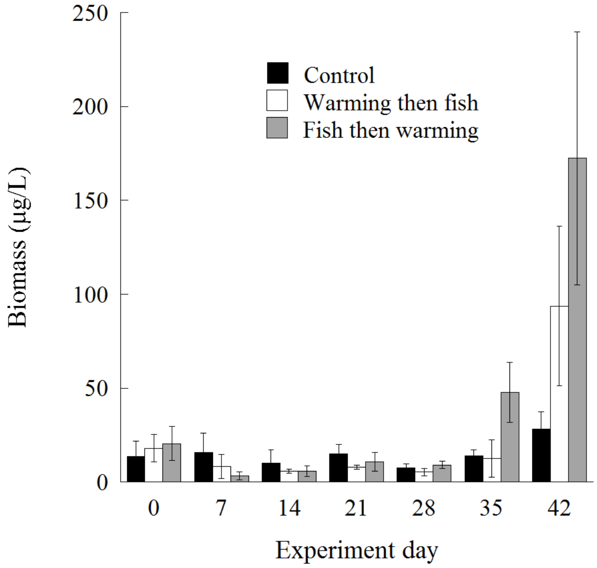

Figure 3-7 Total zooplankton biomass across stressor treatments over the 42-day experiment. Vertical bars represent standard error.

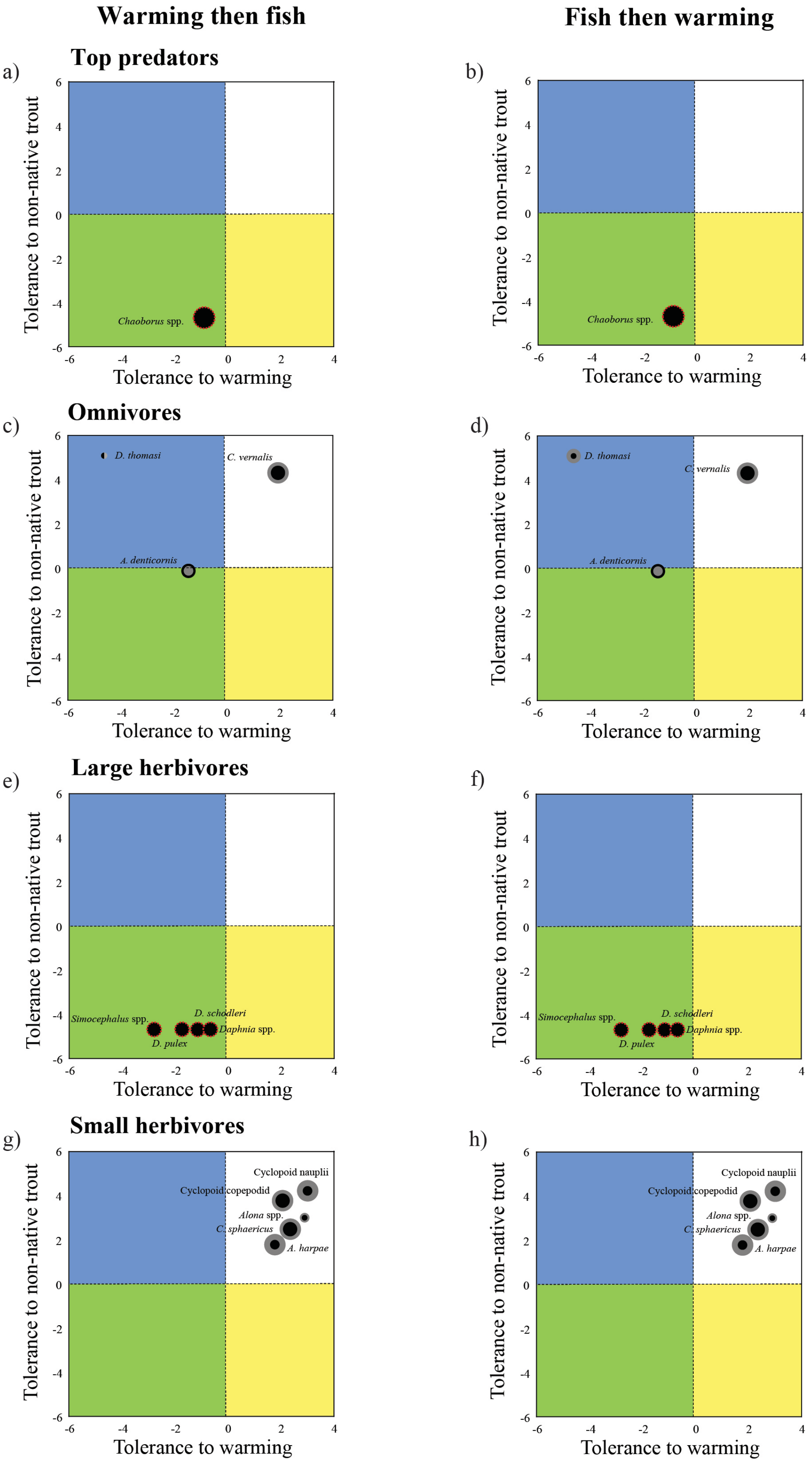Warming then fish
Fish then warming
Top predators
a)
b)
Omnivores
c)
d)
Large herbivores
e)
f)
Small herbivores
g)
h)
Tolerance to non-native trout
Tolerance to warming
6
4
2
0
-2
-4
-6
-6
-4
-2
0
2
4
Chaoborus spp.
D. thomasi
C. vernalis
A. denticornis
Simocephalus spp.
D. schodleri
Daphnia spp.
D. pulex
Cyclopoid nauplii
Cyclopoid copepodid
Alona spp.
C. sphaericus
A. harpae

Figure 3-8 Changes in biomass in response to Warming then fish (left column) and Fish then warming (right column) for the top predator *Chaoborus* (a,b), omnivores (c,d), large herbivores (e,f), and small herbivores (g,h). The taxa are positioned along gradients of tolerance to warming and non-native trout, as in Figure 3-6. The size of the circles denotes biomass with small circles = < 0.1 μg/L, medium circles = 0.1 - 1 μg/L, large circles = 1 - 10 μg/L, and extra-large circles = > 10 μg/L. See Appendix B-1 for full species names and exact biomass values.

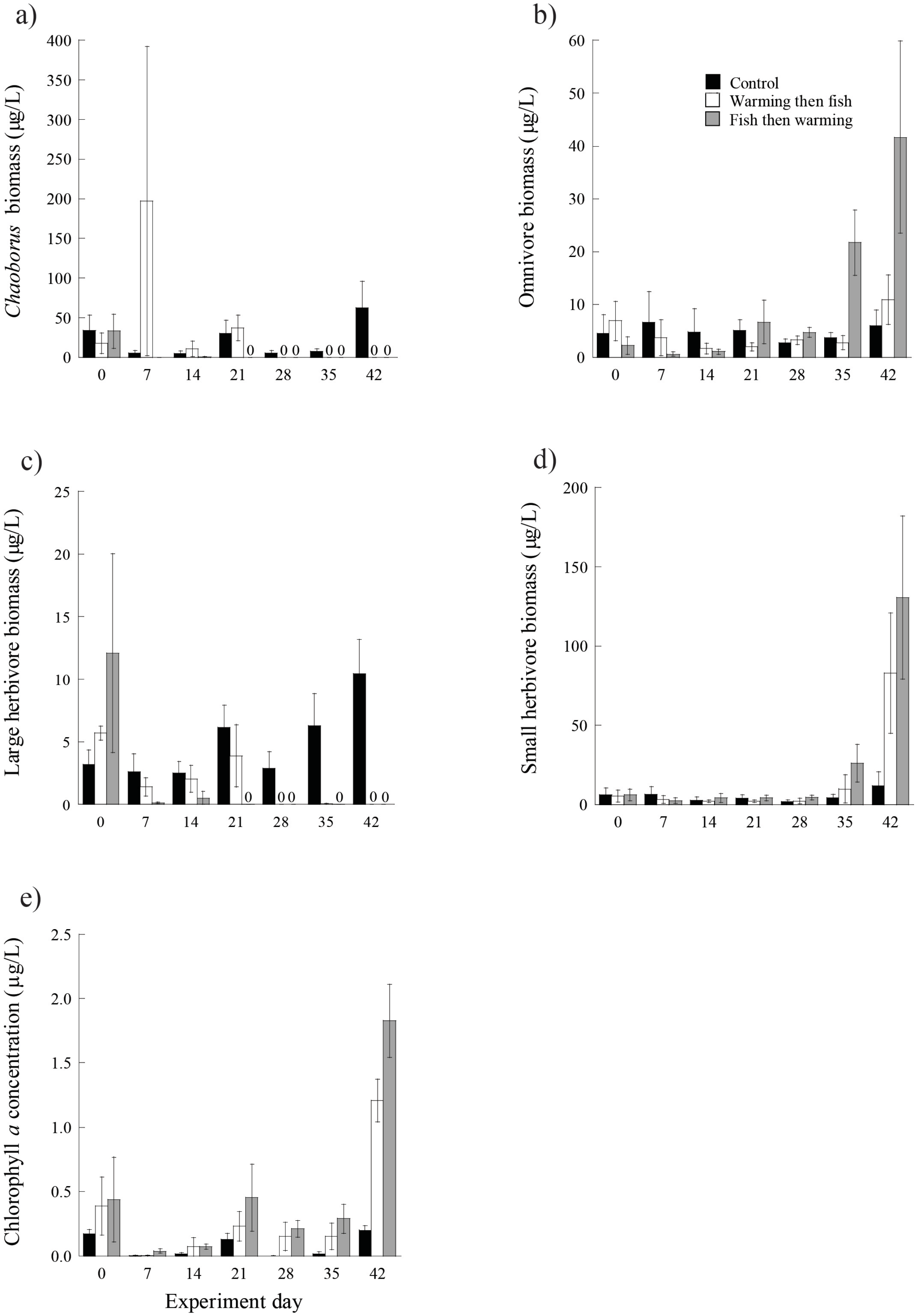
a)
Chaoborus biomass (µg/L)
0
50
100
150
200
250
300
350
400
0 0
0 0
0 0
0
b)
Omnivore biomass (µg/L)
0
10
20
30
40
50
60
Control
Warming then fish
Fish then warming
c)
Large herbivore biomass (µg/L)
0
5
10
15
20
25
0
0 0
0
0 0
d)
Small herbivore biomass (µg/L)
0
50
100
150
200
e)
Chlorophyll *a* concentration (µg/L)
0.0
0.5
1.0
1.5
2.0
2.5
0
7
14
21
28
35
42
Experiment day

Figure 3-9 a) Biomass of the top predator *Chaoborus*, b) biomass of omnivores, c) biomass of large herbivores, d) biomass of small herbivores, and e) chlorophyll *a* concentration across stressor treatments over the 42-day experiment. Vertical bars represent standard error.

CHAPTER 4: EFFECTS OF NON-NATIVE TROUT, HIGHER TEMPERATURES AND REGIONAL BIODIVERSITY ON ZOOPLANKTON COMMUNITIES OF ALPINE LAKES[2]

Introduction

Decades of stocking non-native sportfish to create recreational angling opportunities have biologically impoverished mountain lakes around the world (Pister 2001, Eby et al. 2006, Crawford and Muir 2008). In alpine regions above treeline (*c.* 2000 m a.s.l.), many lakes are naturally fishless, with native communities containing high abundances of conspicuously large and brightly coloured zooplankton (McNaught et al. 1999, Schindler and Parker 2002). Size-selective predation by salmonids introduced into these lakes has strongly suppressed and, at times, extirpated such prey species, substantially reducing zooplankton mean body-size and total biomass (Bradford et al. 1998, Brancelj 1999, Knapp et al. 2001, Schabetsberger et al. 2009, Messner et al. 2013). Although many stocking programs have ceased, non-native trout populations persist, especially in colder, alpine lakes (Messner et al. 2013).

Rapid and extreme warming occurring at high elevations is also expected to impair ecological function in alpine lakes, which are often considered sentinels of climate change (Sommaruga-Wograth et al. 1997, Skjelkvåle and Wright 1998, Parker et al. 2008). Alpine lake communities tend to be dominated by stenothermic endemic species that may be at risk of extinction if they are unable to evolve tolerance to rising temperatures (Krajick 2004). Experiments have shown that the ecological effects of higher temperatures on fishless alpine lake communities are similar to effects of fish predation with biomass losses occurring because large

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[2] This chapter has been accepted pending minor revisions at Hydrobiologia

zooplankton species are suppressed (Strecker et al. 2004, Holzapfel and Vinebrooke 2005). As a result, climate warming may cause further declines of large zooplankton and total biomass in stocked lakes.

Non-native trout and higher temperatures may also enable the colonization of novel species from the surrounding region into alpine lake communities. Evidence suggests that zooplankton species in low-elevation montane lakes (1000-1500 m a.s.l.) are currently excluded from alpine systems because they require warmer habitats (Anderson 1974) and interactions with local species prevent their establishment (Holzapfel and Vinebrooke 2005). Climate warming may remove these abiotic and biotic barriers and facilitate the upward spread of species by making alpine lakes thermally suitable and by suppressing local species (Nevalainen et al. 2014, Elsen and Tingley 2015). Likewise, non-native trout may alter local competitive and predatory interactions, thereby allowing new species, including those having eco-evolutionary experience with fish, to colonize alpine lakes (Gliwicz et al. 2010, Strecker and Arnott 2010). If fish presence and higher temperatures give regional species a competitive advantage over local specialists in alpine communities, interactions with regional species may contribute to further declines of alpine species (Gilman et al. 2010).

However, if regional species thrive in alpine lakes affected by non-native trout and warming, they could functionally compensate for declines of local species, thereby enabling aggregate community properties, such as total biomass, to be maintained. The Spatial Insurance Hypothesis predicts that in regions of high environmental heterogeneity, such as mountain landscapes, regional biodiversity can insure local communities against stress-induced losses of ecological function (Loreau et al. 2003, Leibold and Norberg 2004). The central tenet of this concept is that regional biodiversity increases the probability that a species tolerant to a given stressor is present by increasing both functional redundancy (i.e. the number of species performing similar ecological functions) and response diversity (i.e. the diversity of responses to an environmental change among functionally similar species) (Laliberte et al. 2010).

While evidence of spatial insurance in zooplankton communities has been mixed (Forrest and Arnott 2006, Thompson and Shurin 2012, Symons and Arnott 2013), the inherently low functional redundancy of alpine communities (Hauer et al. 1997) combined with high regional species diversity in mountain regions (Anderson 1974) suggests that potential dispersers may play an important role in structuring responses of alpine communities to non-native trout and warming. Importation of regional species may enhance the diversity and abundance of small zooplankton species that are more likely to avoid fish predation and be tolerant of higher temperatures. Small zooplankton species are rare in alpine lakes but low-elevation montane communities contain several small species that respond positively to both non-native trout and higher temperatures (Chapter 2; Messner et al. 2013, MacLennan et al. 2015). Additionally, large alpine species that are likely to be suppressed by non-native trout and higher temperatures (e.g. *Daphnia middendorffiana* and *Hesperodiaptomus arcticus*) may be replaced by functionally-similar regional species that co-exist with non-native fish in montane lakes (e.g. *Daphnia rosea* and *Diacyclops thomasi*) (Holzapfel and Vinebrooke 2005).

I analyzed long-term trends in a lake ecosystem and performed an experiment to investigate the effects of non-native trout and higher temperatures on the zooplankton communities of alpine lakes. I assessed the influence of temperature variation on zooplankton functional groups in a remote, naturally fishless alpine lake during years in which stocked trout were absent and present. I then performed an experiment to test whether a diverse pool of regional species provides a source of tolerant colonists that can functionally compensate for the effects of size-selective predation by non-native trout and warming on local alpine communities. I hypothesized that size-selective predation by non-native trout and warming would suppress large species (i.e. omnivores and *Daphnia* grazers), while smaller herbivores would be resistant to both, thereby decreasing the mean length of zooplankton in alpine communities. I also expected that predation and warming would facilitate the establishment of tolerant regional species and that regional species would buffer communities against biomass losses.

Methods

Comparative study of Pipit Lake

Pipit (51°61'N: 115°86'W, 2217 m.a.s.l) is a remote alpine cirque lake located in the Cascade Valley near the eastern boundary of Banff National Park, Alberta, Canada. This naturally fishless, non-glacial lake is an oligotrophic (7 μg L^{-1} total phosphorus), small (8.0 ha; 20.4 m maximum depth) headwater system that is readily accessible only via helicopter (Messner et al. 2013). Ice-out often does not occur in Pipit Lake until mid-July (as was the case in 2009), following which water temperatures can remain near freezing for several weeks with ice-cover returning by the end of September (McNaught et al. 1999, Parker et al. 2001). The zooplankton community of Pipit Lake is primarily comprised of two large crustacean species, *Hesperodiaptomus arcticus* and *Daphnia middendorffiana* (Appenidx C-1). Pipit Lake is representative of other naturally fishless alpine lakes in the region, which contain similar zooplankton communities, experience similar climates, and have a high degree of temporal synchrony in their water chemistries (Parker et al. 2008). Rainbow (*Oncorhynchus mykiss*) and cutthroat (*Oncorhynchus clarkii*) trout were stocked in high abundances in Pipit Lake from 1964 to 1966 but failed to reproduce with the last known fish being captured in 1977 (Parker and

Schindler 2006). These trout are highly size-selective visual predators that strongly suppress large (i.e. >1 mm) conspicuous zooplankton (Galbraith Jr 1967, Carlisle and Hawkins 1998). The zooplankton community composition of Pipit Lake recovered to its pre-stocking condition by 1990 (Anderson 1974, Parker and Schindler 2006).

The availability of unique multi-decadal data for Pipit Lake provided an excellent opportunity to test the independent and combined effects of non-native trout and long-term (i.e. interannual) temperature variation on an alpine lake community. I compared the influence of water temperature on the density of zooplankton in Pipit Lake during years when it contained stocked trout (1966, 1967, 1969, 1971, 1972) to years after it had recovered to its naturally fishless condition (1991-2009). Pipit Lake was sampled three times during July and August in most years (range 1-4). Surface water temperature was measured using a calibrated thermistor. Plankton throughout the water column from 1 m above the sediment to the water surface were integrated using vertical hauls of a 30-cm diameter, 63-µm mesh-sized conical net. Crustacean zooplankton was identified to species and enumerated using a dissecting microscope. Zooplankton was classified based on their size and trophic status into functional groups with *H. arcticus* and *Cyclops vernalis* grouped as omnivores, *D. middendorffiana* as large herbivores, and small cladocerans (< 1 mm) and early-life stages of copepods grouped as small herbivores (Barnett et al. 2007, MacLennan et al. 2012). I refer to these groups henceforth as functional groups. Mean surface water temperature and mean zooplankton density was determined for each year. There was no relationship between Julian date of sampling and zooplankton density ($r^2 = 0.01$, $P = 0.45$). Zooplankton lengths were not measured and therefore I was not able to analyze biomass responses or changes in body-size.

I tested for a significant interactive effect of non-native trout and the covariate temperature on total zooplankton density and the density of species within functional groups using analyses of covariance (ANCOVAs). The lack of a significant interaction in each of the above tests indicated that the assumption of homogeneity of slopes was valid. Therefore, I performed ANCOVAs without an interaction term to assess the independent effects of non-native trout and temperature on zooplankton total density and the density of omnivores, large herbivores, and small herbivores.

Experiment

On July 20, 2009, shortly after ice-out, plankton was collected from the remote fishless alpine lakes Bighorn, McConnell, Pipit, and Snowflake of Banff National Park, Alberta, Canada. All lakes contained similar zooplankton communities that were characteristic of naturally fishless alpine lakes, with > 96% of total biomass in each lake comprised of the large copepod *H. arcticus* and the cladoceran *D. middendorffiana* (Appendix C-1). Small herbivores, including *Bosmina longirostris*, *Diaptomus tyrrelli*, and immature cyclopoids were also present in low abundances (Appendix C-1). Zooplankton was quantitatively collected using vertical net hauls from 3 m off the lake bottom at maximum depth using a 30-cm diameter, 63-µm mesh-sized conical net, and a 30-cm diameter, 10-µm mesh-sized conical net was used to qualitatively collect edible phytoplankton as a food source. Phytoplankton collections were passed through a 63-µm sieve to remove zooplankton grazers. Plankton was transported in 20-L plastic carboys back to the University of Alberta in Edmonton, Canada within 24 hours of collection.

I performed a three-factor [(size-selective predation absent vs. present) × (ambient temperature vs. warmed) × (local vs. local + regional species pools)] experiment using a simple randomized block design (Figure 4-1). The blocking treatment consisted of communities collected from the four naturally fishless alpine lakes rather than a single site to maximize the generality of my findings. A single replicate from each treatment was assigned to each block for a total of 32 containers. White polyethylene buckets (28-cm diameter, 40-cm depth) filled with 22 L of Rocky Mountain spring water were used as containers. On July 21, 2009, zooplankton from each of the four lakes was distributed equally among eight containers. Inocula contained zooplankton at 20-times their natural density to ensure sufficient numbers of organisms for comparison between treatments. On the same day, the phytoplankton collections from each lake were distributed in equal-volume aliquots among the eight containers containing zooplankton from their respective lake to provide algal food. Growth chambers were set to a 12-h light/dark cycle. Local communities were given three days to adjust to growth chamber conditions at 10°C (the average temperature of source alpine lakes) before treatments were applied on July 24, 2009.

Size-selective fish predation was simulated by sieving the entire zooplankton community in the container three times using a 1-mm mesh-sized sieve following sampling on days 0, 12, and 21. Cottingham et al. (1997) also used a sieving approach to represent zooplanktivory and they showed that sieving simulated fish predation by shifting the zooplankton community composition from larger cladocerans to smaller cyclopoids, resulting in smaller zooplankton on average. A 1-mm mesh-sized sieve was chosen based on Brooks and Dodson (1965) showing that selective predation by fish extirpate zooplankton >1 mm. Zooplankton >1 mm that were removed through sieving were heat-killed and added back into their respective containers to control for nutrient differences and simulate nutrient recycling by fish (Shurin 2001, Vanni 2002, Forrest and Arnott 2006).

The temperature treatment was established by randomly assigning containers to either ambient control or warmed growth chambers, which were adjusted to operate at 10°C and 17°C. A temperature difference of 7°C was selected to represent the "worst case scenario" of projected warming in the study area (the high latitude mountain region of North America) for 2085 relative to 1961-1990 baseline temperatures based on atmospheric-ocean general circulation models using the IPCC A1FI global emissions scenario (Nogués-Bravo et al. 2007). The A1FI scenario describes a fossil-fuel intensive world of rapid economic growth where new and more efficient technology is rapidly introduced and the global population peaks mid-century (Nakicenovic et al. 2000). Although the bottoms of the source lakes were slightly cooler than the average temperature of 10ºC (i.e., 6-8ºC), the potential for cold-water refugia was limited in the source lakes because they were not stratified. Stratification in these lakes is infrequent and when it does occur, it is weak and intermittent (McNaught et al. 1999, Parker and Schindler 2006). Therefore a uniform temperature in the containers was a reasonable representation of lake conditions. Average photosynthetically active radiation was not significantly different between growth chambers (5.13 vs. 4.93 $\mu\text{mol}\bullet\text{s}^{-1}\bullet\text{m}^{-2}$, $t = 0.63$, $P = 0.53$, $df = 30$).

The regional species pool consisted of ambient densities of species assembled from 20 lakes in Banff National Park, representing a diverse set of lakes with and without fish present, ranging in mean epilimnetic temperatures from 5 to 21^{0}C. A heterogeneous array of montane, subalpine, and alpine lakes with and without stocked fish present was selected to maximize the potential ecological insurance effect of the regional species pool. Vertical hauls with a 30-cm diameter, 63-µm mesh-sized conical net were used to quantitatively collect regional zooplankton communities. Regional species collection occurred from July 14-18, 2009 and communities from each lake were held separately in cooled 20-L plastic carboys at low densities to minimize stress before transport to the University of Alberta on July 19. Regional communities were given seven days to adjust to growth chamber conditions before mixing into a regional species pool and distributing inocula to half of the containers on July 25 (experiment day 1).

Since natural rates of zooplankton dispersal are unknown, my goal was to characterize the potential for dispersal rather than simulate actual levels of dispersal (Bohonak and Jenkins 2003). The density of regional zooplankton that was distributed in each inoculum was equivalent to 10% of the density of local species, a concentration that has been used in similar investigations on the effect of regional species pool additions (e.g. Forrest and Arnott 2006). Five inocula were preserved in a 70% ethanol solution for enumeration. The regional species pool contained a high diversity of species including those found in local communities and 15 novel species not found in local communities (Appendix C-2).

The experiment was conducted for 31 days from July 24 to August 24, 2009. The experiment length reflected the short ice-free season of the study lakes. A 31-day duration allowed most cladoceran species to produce at least one cohort (Gillooly 2000). Copepod species in the local communities only produce a single cohort during the ice-free season, as is the case for most alpine lakes (Knapp et al. 2001, Catalan et al. 2006). Therefore, I was only able to assess mortality responses in copepods.

The placement of containers within each growth chamber was completely randomized weekly (i.e. not linked to blocks). Containers were mixed prior to sampling and depth-integrated zooplankton samples were collected using a clear acrylic tube. Two-litre samples without replacement were taken before the stressor treatments (size-selective predation and warming) were applied on days 0, 12 and 21, with the remaining 16 L collected on day 31. Zooplankton samples were concentrated using a 63-µm mesh-sized sieve and preserved in a 70% ethanol solution. The sampled water was not returned to the container to avoid the confounding sampling effect of altering density-dependent aspects of the communities. Crustacean zooplankters were enumerated using a Leica MZ9s dissecting microscope at 32-times magnification. Samples were counted in their entirety and individuals were identified to species when possible and genus otherwise using Ward and Whipple (1959) (Appendix C-2). Zooplankton were classified into functional groups (Appendix C-2; Barnett et al. 2007, MacLennan et al. 2012). The lengths of the first 15 individuals of each developmental stage for each taxon were measured and used to derive biomass estimates using length-weight regressions (McCauley 1984, Culver et al. 1985). Mean weighted length of the zooplankton community (herein referred to as mean length for simplicity) was calculated as the product of the mean length of a zooplankton taxa and its proportional relative abundance, summed across all taxa.

I tested the multiplicative null model, which predicts that the proportional effect of each stressor is independent of the presence of another stressor, by $\log_{10}$-transforming all response variables (e.g. biomass, length and species richness) prior to statistical analysis because the additive model was inappropriate for some response variables in the study (i.e. predicted net effects of predation and warming exceeding 100% loss) (Sih et al. 1998, Folt et al. 1999). Linear mixed models were performed using PASW® Statistics 18 (SPSS Inc., Chicago, Illinois, USA) to test for time-dependent direct and interactive effects of size-selective predation, warming, and regional species on total community biomass, mean zooplankton length, and species richness (Zuur et al. 2009). Experimental treatments were treated as fixed factors and sampling occasion as a covariate. Random block (i.e. lake source) effects were tested by comparing models without and with a random block effect included. The model that minimized the Akaike's Information Criterion corrected for small sample-size (AICc) based on restricted maximum likelihood

(REML) estimation was used (Appendix C-3). Individual container was treated as a random effect and to account for potential temporal autocorrelation from repeated measures, several error covariance structures were tested against an unstructured covariance matrix, including autoregressive structures with homogeneous and heterogeneous variances. The covariance structure that minimized the AICc was used (Appendix C-3). Stressor interactions were detected by a statistically significant interaction term. When the combined effect of stressors was significantly greater or lesser than the multiplicative prediction, the interactions were classified as synergistic and antagonistic, respectively (Folt et al. 1999).

General linear models were used to test the direct and interactive effects of size-selective predation, warming, and the regional species pool on the biomass of each functional group (i.e. omnivores, large herbivores, small herbivores) on the final day of experiment. In all general linear models, the blocking effect (i.e. lake source) was included when significant. Otherwise, it was removed from the model to increase statistical power.

The proportion of total biomass contributed by individuals from the regional pool was determined for each species by calculating the ratio of mean biomass in the regional inocula to mean biomass in the local + regional community on day 0. For example, the biomass contribution of zooplankton from the regional pool was 100% for species that were not found locally. This calculation was performed separately for each species within each lake community (i.e. lake block). I assumed that the proportional contribution of individuals from the regional pool for each species was constant throughout the experiment (e.g. if individuals from the regional pool comprised 25% of the total biomass of a given species in the lake community on day 0, I assumed this was also true on the final day of the experiment). I determined the total biomass comprised of regional zooplankton on the final day of the experiment as the sum of species biomasses weighted by their proportional contribution from the regional pool. I then calculated the percent of total biomass comprised of regional zooplankton on the final day of the experiment as the ratio of regional species biomass to total biomass in the local + regional communities, multiplied by 100. A linear model was used to test the direct and interactive effects of size-selective predation and warming on the percent of total biomass comprised of regional species on the final day of the experiment.

Partial redundancy analysis (RDA) was performed to examine the influence of size-selective predation and temperature on zooplankton species composition on the final day of the experiment. Lake source was treated as blocking variable defined as a covariate (i.e. the variation explained by lake source was removed before the variability explained by the treatments was quantified) (Šmilauer and Lepš 2014). A linear ordination method was selected because the community composition data had a gradient length of 2.9 standard deviation units (Šmilauer and Lepš 2014). Zooplankton taxa that were detected in fewer than four containers on the final day of the experiment were excluded from the analysis and biomasses were $\log_{10}$-transformed prior to analysis. Forward selection using Monte Carlo permutation testing was used to determine whether treatment explained a significant amount of variance in zooplankton species composition. Monte Carlo permutations were also used to test the significance of the first RDA axis and the overall ordination.

Results

Comparative study of Pipit Lake

Stocked trout and higher temperatures had non-interactive negative effects on total zooplankton density, although the influence of temperature was only marginally significant

(Fish: $F_{1,21}$ = 13.52, P = 0.001; Temperature: $F_{1,21}$ = 3.68, P = 0.07) (Figure 4-2a). Omnivores (i.e. *H. arcticus* and *C. vernalis*) were suppressed by both stressors, although again the influence of temperature was marginal (Fish: $F_{1,21}$ = 5.73, P = 0.03; Temperature: $F_{1,21}$ = 3.80, P = 0.07) (Figure 4-2b). Large herbivores (i.e. *D. middendorffiana*) were not detected in Pipit Lake when stocked trout were present except in the first year of monitoring in 1966. The density of large herbivores was not affected by temperature ($F_{1,21}$ = 0.22, P = 0.65) (Figure 4-2c). The density of small herbivores was not affected by non-native trout ($F_{1,21}$ = 4.02, P = 0.06) or temperature ($F_{1,21}$ = 0.17, P = 0.69) (Figure 4-2d).

Experiment

Size-selective predation and warming each negatively affected total zooplankton biomass (i.e. a non-interactive net effect) (Table 4-1, Figure 4-3a,b). While size-selective predation suppressed the biomass of omnivores and large herbivores, warming suppressed omnivores and small herbivores (Table 4-2, Figure 4-4). Together, size-selective predation and warming synergistically decreased zooplankton mean body-length (Table 4-1, Figure 4-3c,d). Zooplankton species richness was not affected by size-selective predation, warming, or their interaction (Table 4-1, Figure 4-3e,f).

Size-selective predation and warming each increased the proportion of regional zooplankton in communities (Predation: $F_{1,12}$ = 5.28, P = 0.04; Warming: $F_{1,12}$ = 11.70, P = 0.005) (Figure 4-5). However, regional zooplankton did not significantly alter treatment effects on total zooplankton biomass or mean body-length (Table 4-1, Figure 4-3b,d). The absence of significant effects of regional species on aggregate community properties was not attributable to a lack of novel species because amended communities had significantly higher species richness than unamended communities (Table 4-1, Figure 4-3f). Regional zooplankton addition did not alleviate the negative effects of size-selective predation and warming on the biomass of omnivores or the negative effect of warming on the biomass of small herbivores (Table 4-2, Figure 4-4). However, regional zooplankton enhanced the total biomass of large herbivores and appeared to increase their resistance to size-selective predation, although this effect was only marginally significant (Table 4-2, Figure 4-4).

The eight treatment combinations explained a significant amount of variance in species composition on the final day of the experiment ($F = 4.66$, $P = 0.01$) (Figure 4-6). RDA axes 1 and 2 accounted for 30.1% and 16.3% of the total variance in species data, respectively. RDA axis 1 was significant ($F = 9.00$, $P = 0.002$), as was the whole ordination ($F = 3.80$, $P = 0.002$).

Size-selective predation and warming had subtle direct effects on the composition of local zooplankton communities, with their primary effect being a decrease in the abundance of the large top predator, *H. arcticus* (Figure 4-6). *D. middendorffiana* was also suppressed by size-selective predation but was relatively more tolerant of warming. Dual-stressed communities were further displaced from controls in ordination space than communities exposed to either stressor individually. Exposure to both stressors shifted zooplankton communities toward smaller herbivorous species, namely *Chydorus sphaericus* and *Bosmina longirostris* (Figure 4-6). Regional species had the largest influence on the response of warmed-only communities, in which a diversity of large calanoid and *Daphnia* species native to montane lakes established. Otherwise, regional zooplankton did not strongly influence the response of zooplankton species to size-selective predation, alone or in combination with warming.

Discussion

The whole-lake and experimental findings agreed that size-selective predation by non-native trout and higher temperatures each decreased zooplankton biomass in alpine lake communities by disproportionately suppressing large species. Size-selective predation by non-native trout decreased omnivores and large herbivores, but did not affect small herbivores. Higher temperatures decreased omnivores and surprisingly, did not affect large herbivores, but suppressed small herbivores in the experiment. Size-selective predation and warming increased the biomass of regional zooplankton in alpine communities and warming promoted the establishment of a diversity of calanoid and *Daphnia* species from montane lakes. However, regional zooplankton did not alleviate the negative effects of these stressors on zooplankton total biomass. Below I consider potential explanations for key findings and discuss advantages and limitations of the whole-lake comparison and experimental approaches.

Together, my findings from the whole-lake comparison and experiment suggest that higher temperatures can suppress large zooplankton and total biomass in stocked alpine lakes if non-native trout alone have not already extirpated the dominant zooplankton species. In the experiment, exposure to both size-selective predation and warming led to the greatest decrease in zooplankton biomass. In contrast, the strong suppression of zooplankton by non-native trout in the whole-lake left little room for further negative effects of higher temperatures. Long-term exposure to high densities of stocked trout alone extirpated *H. arcticus* and *D. middendorffiana*, which together comprised > 99% of total zooplankton density in Pipit Lake. Therefore, in the whole lake, the net effect of non-native fish and higher temperatures would be most appropriately characterized by the comparative null model, in which the joint effect of stressors is equal to the effect of the single worst stressor (Folt et al. 1999). This type of interaction appears to be common when the effect of one stressor alone is particularly strong (Fausch et al. 2010). However, in some lakes the effect of non-native trout is weaker, enabling co-existence with large omnivores and *Daphnia* (e.g. Donald et al. 1994, Donald et al. 2001) and my experimental findings suggest that higher temperatures can suppress remaining large zooplankton in these systems. Additionally, the experiment revealed that even if non-native trout remove all large zooplankton, higher temperatures can suppress small herbivores that are tolerant of non-native trout.

While fish predation consistently reduced omnivorous and large herbivorous zooplankton, I discovered that body-size alone could not explain temperature effects. Contrary to my hypothesis, large herbivores were tolerant of warming in the experiment and there was no influence of temperature on the density of large herbivores in Pipit Lake, possibly due to the high capacity of *Daphnia* to adaptively respond to changes in temperature (Van Doorslaer et al. 2009, Geerts et al. 2015). Indeed, increases in *D. middendorffiana* densities in Pipit Lake concurrent with a general warming trend suggests that this species may ultimately benefit from higher temperatures (Fischer et al. 2011). Further, the biomass of small herbivores unexpectedly declined under warmed conditions in the experiment, likely because alpine specialists, such as *D. tyrrelli* and herbivorous early-life stages of *H. arcticus*, were intolerant of higher temperatures (Anderson 1974). Another potential explanation is that higher temperatures stimulated predation by *H. arcticus* on small herbivores (Moore et al. 1996), but this seems unlikely given that *H. arcticus* abundances decreased substantially with warming. Therefore, although I observed a general shift toward smaller zooplankton with warming consistent with earlier studies (e.g. Strecker et al. 2004, Holzapfel and Vinebrooke 2005, Daufresne et al. 2009), it appears that other organism traits may be necessary to explain the thermal tolerance of individual species.

Size-selective predation and warming increased the establishment of regional zooplankton in alpine communities concurrent with the suppression of local species, highlighting the potential importance of biotic interactions in governing the invasion resistance of alpine communities (Holzapfel and Vinebrooke 2005). However, I also found that warming promoted the establishment of a diversity of large calanoid and *Daphnia* species from montane lakes, suggesting that temperature is a key abiotic filter shaping alpine lake communities, and that climate warming may make alpine lakes more thermally suitable for montane species (Anderson 1974). Importantly, many of the montane species that thrived under warmed conditions were large (i.e. >1 mm) and therefore, were not tolerant of size-selective predation. Consequently, it appears that non-native trout may limit the potential establishment of montane species in alpine lake communities that might otherwise occur with warming.

Despite greater establishment of regional zooplankton in stressed alpine communities, imported species did not functionally compensate for losses of local species (i.e. did not offset losses in total biomass or losses of biomass within functional groups). The inability of montane species to offset biomass losses of alpine species was surprising as non-native trout and warming synergistically increased zooplankton biomass in montane lake communities (see Chapter 2; MacLennan et al. 2015). In particular, small herbivores, such as *C. sphaericus*, often increase in response to non-native trout and warming (Tiberti et al. 2014, MacLennan et al. 2015) but, despite being favoured under dual-stressed conditions, small herbivores remained rare in the experiment (i.e. comprised <1% of total zooplankton abundance). Competition with rotifers could account for the weak response of small herbivores to the combined influence of non-native trout and warming, as non-native trout and higher temperatures often increase the abundance of rotifers in alpine lakes (Strecker et al. 2004, Tiberti et al. 2014). However, the relatively low biomass of rotifers in the containers (1.4 μg L^{-1} on average) suggests that rotifers did not out-compete crustaceans in the experiment. A more likely explanation for the lack of functional compensation by regional species is that there was insufficient time for their populations to grow to offset losses of local species, especially given that the density of regional zooplankton in inocula was low (equivalent to 10% of the density of local species). The 31-day experiment represented the length of the ice-free season in our study lakes (McNaught et al. 1999, Parker et al. 2001) but allowed most species from the regional pool to reproduce only once or twice (Gillooly 2000). Therefore, it is possible that regional species might ultimately compensate for biomass losses in stressed alpine communities over longer time-scales with prolonged periods of colonization and reproduction over multiple generations.

Nevertheless, the long-term whole-lake investigation did not provide support for the hypothesis that regional species can offset the negative effects of non-native trout and warming on alpine lake communities, as there was no evidence of the establishment of novel species in Pipit Lake over the 24-year study period. Likewise, zooplankton biomass in a nearby alpine lake remained suppressed following the introduction of fish over three decades earlier, suggesting little potential for eventual functional compensation within communities of stressed alpine lakes in the Canadian Rocky Mountains (Parker and Schindler 2006). In contrast, higher abundances of small zooplankton have compensated for the loss of large species associated with fish introductions and higher temperatures in alpine lakes in the Alps mountain range, possibly, in part, due the upward migration of montane species (Nevalainen et al. 2014, Tiberti et al. 2014). Therefore, the potential for regional species to functionally insure alpine lake communities against the effects of non-native fish and higher temperatures may differ across geographical locations, supporting the assertion that biogeographical variation among mountain landscapes will lead to different regional responses of mountain lake communities to environmental change (Kernan et al. 2009).

The combination of biomonitoring and experimental approaches provided a balanced assessment of the net effect of non-native trout and higher temperatures on zooplankton communities of alpine lakes. The whole-lake time-series analysis enabled an investigation of long-term ecological responses to stressors in a natural environment over a realistic spatio-temporal scale involving multiple generations (Luo et al. 2011). Multi-decadal biomonitoring data are particularly important for assessing effects of gradual long-term climate warming but such data are rare for alpine lakes, as sampling in such remote locations can be difficult (Parker et al. 2008). Further, finding pristine reference conditions against which to gauge the effects of climate warming and introduced trout is challenging because stocking of mountain lakes dates back to the early 1900s and biomonitoring of alpine lakes has mostly occurred after non-native trout were introduced (Donald 1987, Schindler and Parker 2002). Consequently, the Pipit Lake data presented a valuable opportunity to study long-term trends associated with temperature variability in the absence and presence of non-native fish, but the uniqueness of the dataset limited the generality of findings. Nevertheless, the observation that non-native trout and higher temperatures disproportionately suppressed large alpine zooplankton in Pipit Lake is consistent with my experimental findings, as well as those from contemporary surveys (Donald et al. 2001, Knapp et al. 2001, Tiberti et al. 2014), paleolimnological studies (Luoto and Nevalainen 2013, Nevalainen et al. 2014) and an earlier mesocosm experiment (Holzapfel and Vinebrooke 2005).

The experiment complemented the whole-lake comparison by providing mechanistic insight afforded by controlled manipulations and greater generality across four alpine lake communities. However, container experiments inherently compromise ecological realism and therefore, conclusions are scale-dependent (Schindler 1998b, Holzapfel and Vinebrooke 2005, Stewart et al. 2013). For example, sieving was a reasonable simulation of the effect of non-native trout in naturally fishless alpine lake communities because vulnerability of zooplankton to fish predation is primarily determined by body-size in these simplistic food webs (Parker et al. 2001). However, I could not account for the potential influence of other factors, such as moving mode, pigmentation, and behavioural patterns (e.g. diel vertical migration) that can influence the susceptibility of zooplankton to fish predation. Likewise, the abrupt and intense nature of experimental warming by 7°C was more representative of an extreme summer heating event than more gradual warming associated with climate change (Jentsch et al. 2007). Further, I was only able to collect a regional species pool once due to the logistical constraints of reaching remote mountain lakes. Although simulating multiple dispersal events through the experiment would have more realistically represented natural passive species dispersal, Symons and Arnott (2013) found that zooplankton dispersers were most likely to colonize a stressed community if they arrived shortly after the stressor was applied. Therefore, to maximize potential colonization of regional species I added the regional species pool inocula on the day after applying the stressor treatments (i.e. day 1). Local zooplankton communities were also inoculated in the containers at higher than ambient densities, which are often very low (i.e. 3 animals L^{-1}), to ensure sufficient numbers of organisms for comparison across treatments (Paul et al. 1995, McNaught et al. 1999). However, higher densities likely resulted in an increase in density-dependent biotic interactions (e.g. competition, predation), as evidenced by a decline in total biomass in unstressed communities over the experiment, which could have reduced the potential establishment of regional species (Shurin 2000).

In conclusion, my discovery that warming can further impoverish zooplankton communities of alpine lakes containing non-native trout highlights the sensitivity of these communities to multiple stressors (Vinebrooke and Leavitt 2005). However, my findings also demonstrate the potential for alpine specialists, such as *H. arcticus*, to be useful bio-indicators of environmental change, as this species appears to be sensitive to even subtle variations in temperature at the whole-lake scale (Khamis et al. 2014). Additionally, this study reveals the capacity for non-native trout and higher temperatures to facilitate the colonization of regional zooplankton into alpine lakes of the Canadian Rocky Mountains. Regardless, it remains uncertain whether regional species will ultimately be able to offset biomass losses of local species in stressed alpine lakes and further research is needed to ascertain how the upward spread of montane species will influence alpine lake communities over ecologically-relevant time-scales.

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Table 4-1 Linear mixed model results for the fixed effects size-selective predation (Pred), temperature (Temp), regional species (Disp) and time on zooplankton total biomass, mean length, and species richness for the 31-day experiment. *P*-values in bold indicate statistical significance.

	Biomass			Length			Species richness		
Fixed Effect	DF	*F*	*P*	DF	*F*	*P*	DF	*F*	*P*
Pred	1, 46.5	0.33	0.57	1, 24	0.44	0.52	1, 113.7	0.25	0.62
Temp	1, 46.5	0.14	0.71	1, 24	1.27	0.27	1, 113.7	0.40	0.53
Disp	1, 46.5	4.52	**0.04**	1, 24	9.75	**0.005**	1, 113.7	50.57	**<0.001**
Time	1, 24.9	62.79	**<0.001**	1, 24	25.43	**<0.001**	1, 39.3	14.39	**0.001**
Pred*Temp	1, 46.5	0.32	0.57	1, 24	0.68	0.42	1, 113.7	0.07	0.79
Pred*Disp	1, 46.5	0.59	0.45	1, 24	0.09	0.77	1, 113.7	0.90	0.35
Pred*Time	1, 24.9	14.20	**0.001**	1, 24	22.28	**<0.001**	1, 39.3	0.59	0.45
Temp*Disp	1, 46.5	0.14	0.71	1, 24	0.03	0.86	1, 113.7	0.10	0.76
Temp*Time	1, 24.9	13.94	**0.001**	1, 24	28.18	**<0.001**	1, 39.3	0.01	0.92
Disp*Time	1, 24.9	0.46	0.50	1, 24	1.62	0.22	1, 39.3	0.07	0.79
Pred*Temp*Disp	1, 46.5	1.35	0.25	1, 24	1.85	0.19	1, 113.7	1.27	0.26
Pred*Temp*Time	1, 24.9	0.55	0.46	1, 24	5.72	**0.03**	1, 39.3	0.61	0.44
Pred*Disp*Time	1, 24.9	0.26	0.62	1, 24	0.003	0.96	1, 39.3	0.64	0.43
Temp*Disp*Time	1, 24.9	0.05	0.82	1, 24	1.00	0.33	1, 39.3	0.02	0.90
Pred*Temp*Disp*Time	1, 24.9	0.05	0.83	1, 24	0.51	0.48	1, 39.3	1.20	0.28

Table 4-2 General linear model results for the fixed effects size-selective predation (Pred), temperature (Temp), and regional zooplankton (Disp) on the biomass of functional groups on the final day of experiment (day 31). The random block treatment did not significantly affect omnivore biomass (*P* = 0.54) and was excluded from this model to increase statistical power. *P*-values in bold indicate statistical significance.

	Omnivores			Large herbivores			Small herbivores		
Fixed effect	DF	*F*	*P*	DF	*F*	*P*	DF	*F*	*P*
Pred	1, 24	13.90	**0.001**	1, 21	5.03	**0.04**	1, 21	0.004	0.95
Temp	1, 24	12.04	**0.002**	1, 21	2.79	0.11	1, 21	18.72	**<0.001**
Disp	1, 24	0.41	0.53	1, 21	6.98	**0.02**	1, 21	3.15	0.09
Pred*Temp	1, 24	0.06	0.82	1, 21	0.44	0.52	1, 21	0.29	0.60
Pred*Disp	1, 24	0.37	0.55	1, 21	3.61	0.07	1, 21	0.03	0.86
Temp*Disp	1, 24	0.80	0.38	1, 21	2.88	0.11	1, 21	1.52	0.23
Pred*Temp*Disp	1, 24	1.77	0.20	1, 21	0.38	0.55	1, 21	0.93	0.35

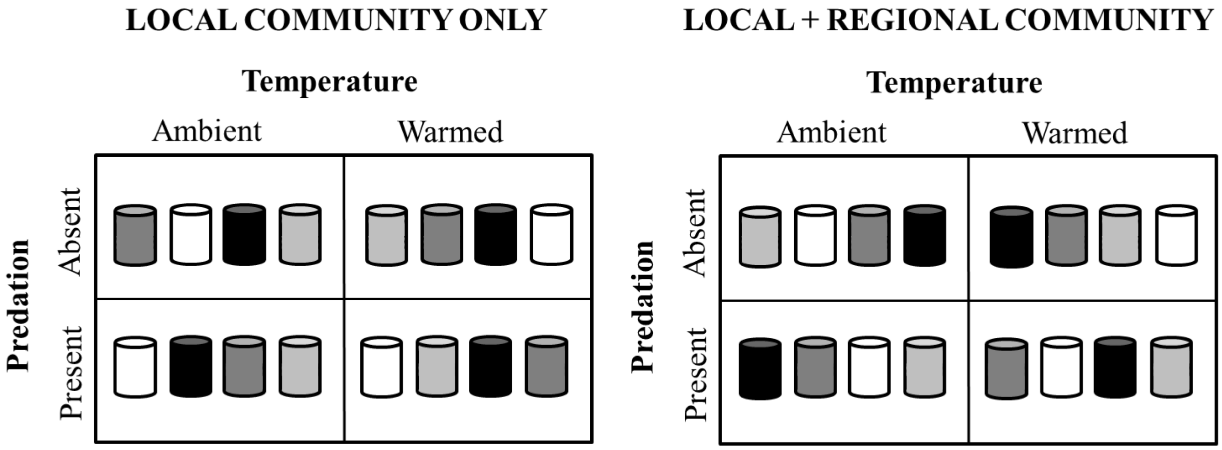

Figure 4-1 Schematic of the three-factor, fully-crossed experimental design [(size-selective predation absent vs. present) × (ambient temperature vs. warmed) × (local vs. local + regional species pools)] with randomized blocking. The blocking treatment consisted of communities collected from four naturally fishless alpine lakes, shown as different coloured containers.

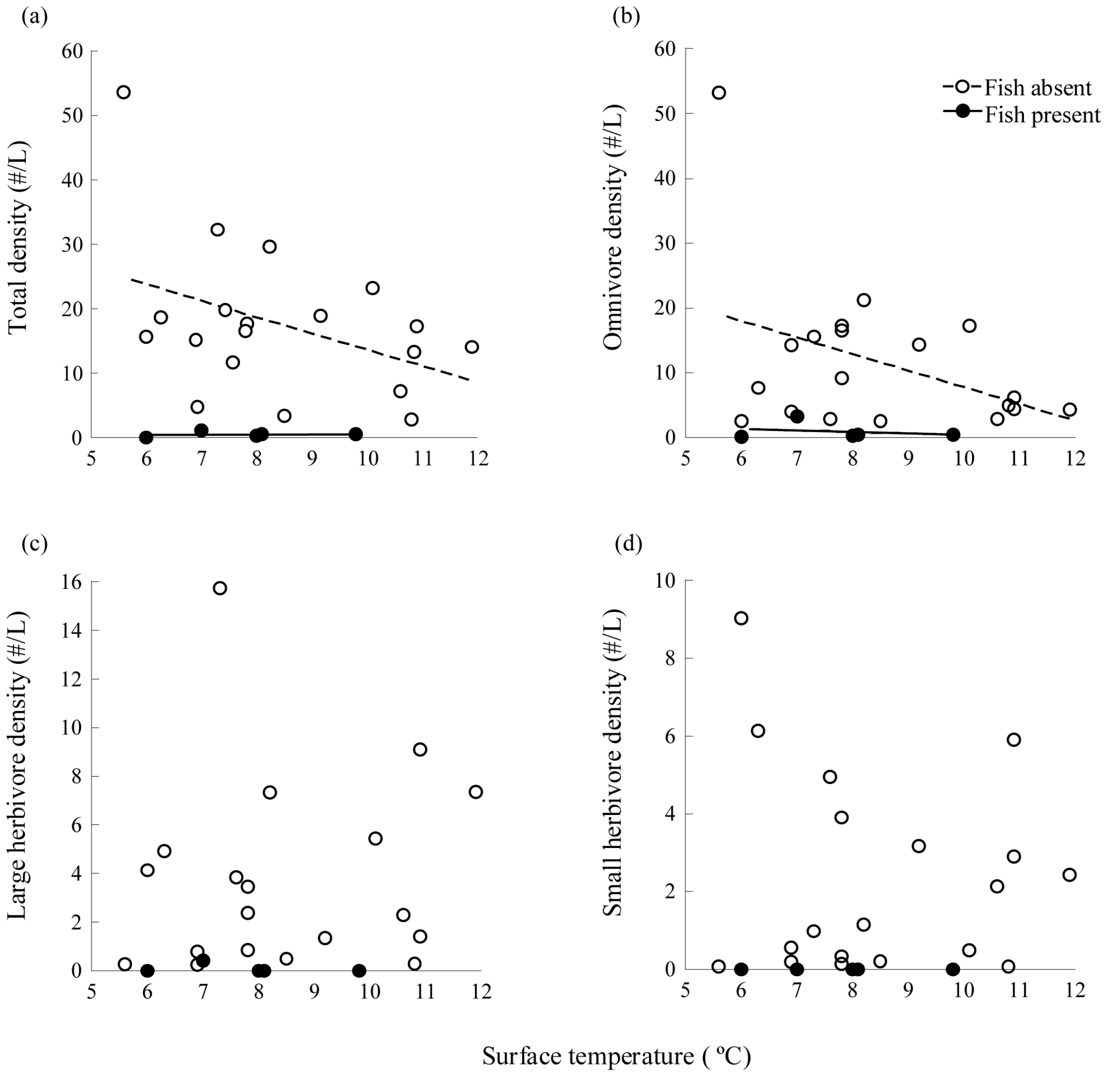

Figure 4-2 Relationships between surface water temperature in Pipit Lake and a) total zooplankton density, b) omnivore density, c) large herbivore density, and d) small herbivore density during years with stocked trout absent (open circles) and present (solid circles). Solid and broken lines represent lines of best fit for significant relationships.

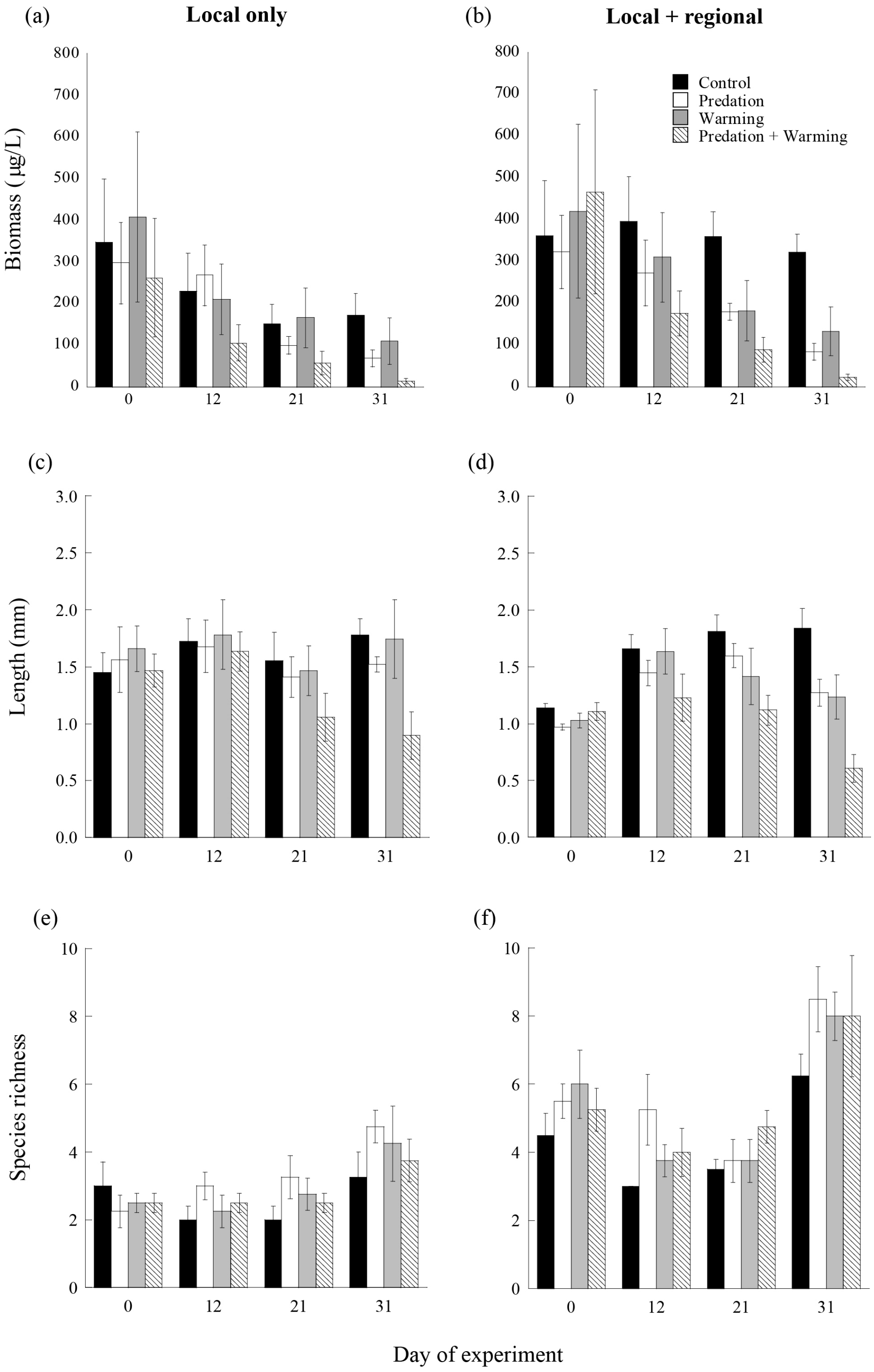
(a)
Local only
(b)
Local + regional
Biomass (µg/L)
0
100
200
300
400
500
600
700
800
■ Control
□ Predation
▒ Warming
▨ Predation + Warming
(c)
(d)
Length (mm)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
(e)
(f)
Species richness
0
2
4
6
8
10
0
12
21
31
Day of experiment

Figure 4-3 Total zooplankton biomass (a, b), mean body length (c, d) and species richness (e, f) over the 31-day experiment for communities with local species only (left column) and local + regional species pools (right column) in unstressed control conditions and exposed to size-selective predation and warming independently and in combination. Bars represent standard error.

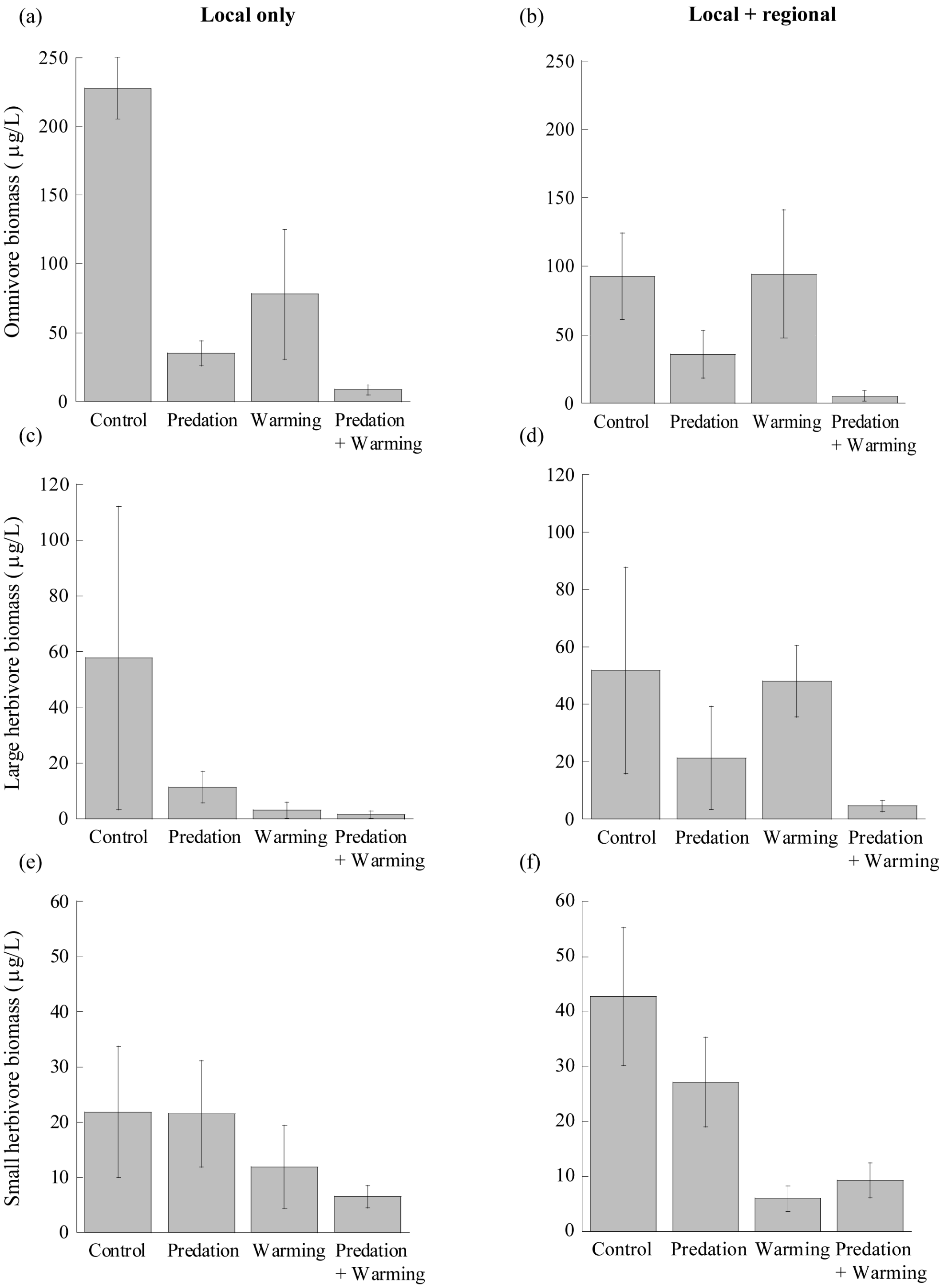
(a)
Local only
(b)
Local + regional
Omnivore biomass (µg/L)
250
200
150
100
50
0
Control
Predation
Warming
Predation
+ Warming
(c)
(d)
Large herbivore biomass (µg/L)
120
100
80
60
40
20
0
(e)
(f)
Small herbivore biomass (µg/L)
60
50
40
30
20
10
0

Figure 4-4 Omnivore biomass (a, b), large herbivore biomass (c, d) and small herbivore biomass (e, f) on the final day of the experiment (day 31) for communities with local species only (left column) and local + regional species pools (right column) in unstressed control conditions and exposed to size-selective predation and warming independently and in combination. Bars represent standard error.

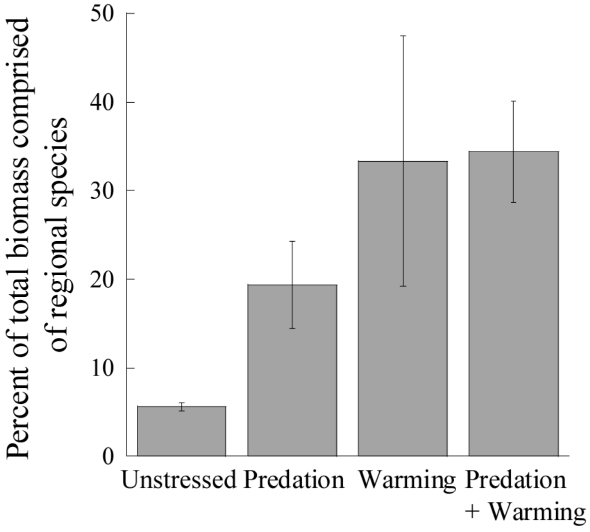

Figure 4-5 Percentage of total zooplankton biomass comprised of regional species for each stressor treatment on the final day of the experiment. Only communities containing local and regional species are included. Bars represent standard error.

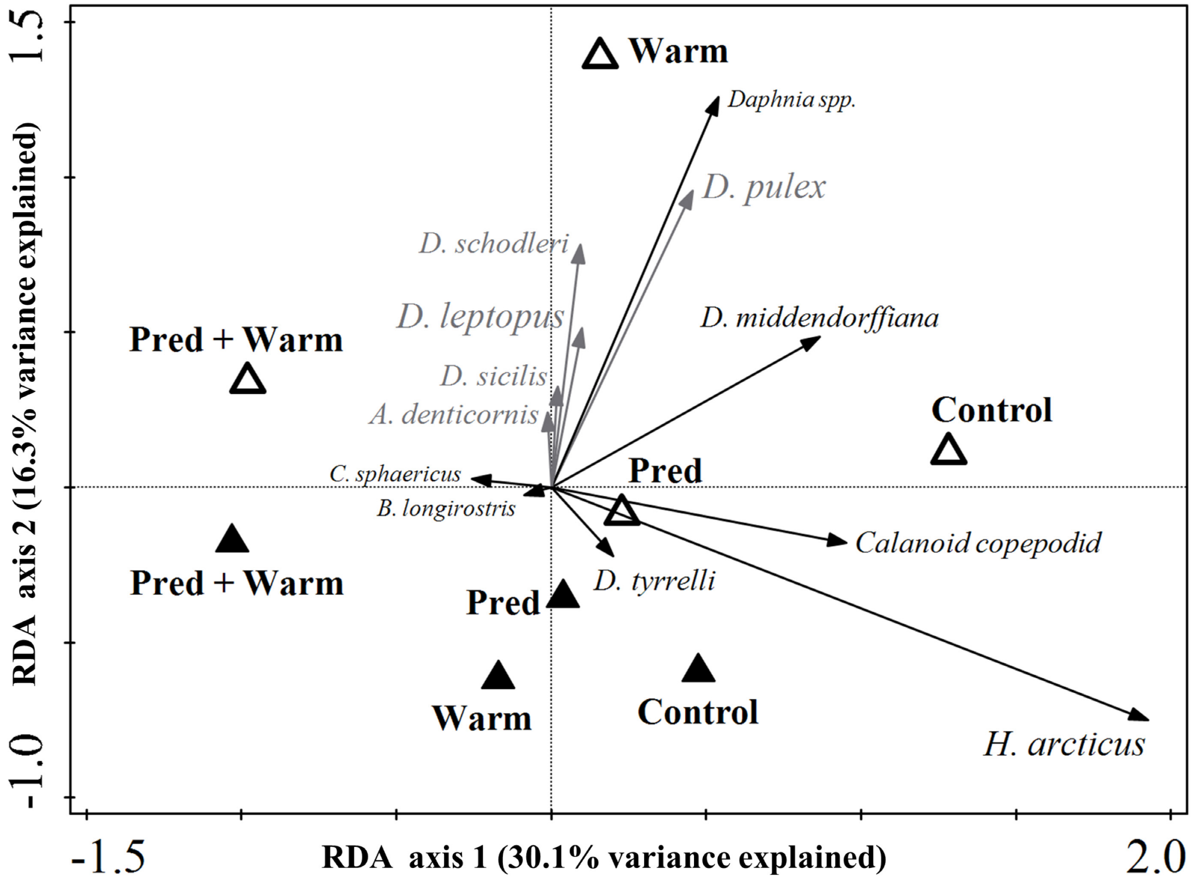

Figure 4-6 Partial redundancy analysis of zooplankton species and treatment combinations of size-selective predation (Pred) and warming (Warm) on the final day of the mesocosm experiment (day 31). Lake source was treated as a blocking variable defined as a covariate. Black triangles show communities containing local species only and white triangles show communities containing local + regional species pools. For clarity, only species that were strongly correlated along an axis are shown. The mean body length of species is indicated by font size (small < 1 mm, medium 1 - 2 mm, and large > 2 mm). Species present in both local and regional pools are shown in black and species exclusive to the regional pool (i.e. not found locally) are shown in grey. See Appendix C-1 for full species names, mean species lengths and mean biomasses across treatments.

CHAPTER 5: GENERAL CONCLUSIONS

My research showed that non-native trout and warming are, in combination, significant drivers of both zooplankton species composition and trophic structure of food webs of naturally fishless mountain lakes. Empirical evidence supported my hypothesis that fish release small zooplankton species that are more responsive to warming from competition and predation in montane lakes (Chapter 2, hypothesis 1). Both my space-for-time survey approach and outdoor mesocosm experiment generated findings showing that the positive influence of higher temperature on total zooplankton biomass occurred only in the presence of non-native trout, likely because warming stimulated reproduction of small herbivorous species that are favoured in lakes with stocked fish. Otherwise, the direct effects of higher temperatures on fishless communities were negligible. Experimental findings also supported my hypothesis that non-native trout stimulates phytoplankton biomass (Chapter 2, hypothesis 2), likely by suppressing large *Daphnia* grazers but also potentially by increasing nutrient cycling.

I then discovered that both non-native trout and warming select for small body-size, resulting in a community characterized by positively co-tolerant small species and highly sensitive larger species (Chapter 3, hypotheses 1). Experimental findings supported my hypothesis that order of exposure to non-native trout and warming would not influence their net effect on total community biomass (Chapter 3, hypothesis 2). I also found that taxa within functional groups had clustered tolerances to non-native trout and warming. The top predator *Chaoborus* and large herbivores were co-sensitive to warming and non-native trout and were always extirpated by sequential exposure to the stressors. Conversely, sequential exposure to the stressors always stimulated smaller co-tolerant herbivores (Chapter 3, hypothesis 3).

My findings from a growth-chamber experiment corroborated a pattern that I detected in the long-term data set for Pipit Lake, namely that non-native trout and higher temperatures both suppressed large alpine zooplankton species (Chapter 4, hypothesis 1). However, while small zooplankton were resistant to non-native trout, the experiment revealed that warming suppressed small herbivores as well as larger species, thereby contradicting my hypothesis that small species are resistant to both stressors (Chapter 4, hypothesis 1). The unexpected decline of small herbivores under warmed conditions appeared to be due to the suppression of coldwater specialist alpine species (Anderson 1974). Additionally, I did not find support for my hypothesis that regional species can functionally compensate for declines of alpine species suppressed by fish predation and warming (Chapter 4, hypothesis 2). Nevertheless, declines of local species in stressed alpine communities increased the establishment of imported zooplankton, including a diversity of montane species under warmed conditions. Therefore, it is possible that upward dispersal of montane species could eventually compensate for losses of alpine zooplankton over longer time-scales. However, there was no evidence of the establishment of novel species in Pipit Lake over the 24-year study period.

The individual and combined effects of non-native trout and warming differed among communities of naturally fishless lakes in montane and alpine regions. Although both stressors disproportionately suppressed large zooplankton species in montane and alpine lakes, non-native trout and warming had larger and more negative effects on zooplankton communities of alpine lakes. In montane lakes, a diversity of small species increased to offset suppression of large species by non-native trout and warming, maintaining total biomass under exposure to the individual stressors. In combination, non-native trout and warming strongly increased the abundance of small species, resulting in a synergistic positive net effect on total zooplankton biomass. In contrast, small species that were tolerant of non-native trout and warming were sparse in alpine lakes and unable to compensate for biomass losses of large species, resulting in a non-interactive negative net effect of the stressors on total zooplankton biomass. These findings highlight how the starting composition of a community can influence both the direction and magnitude of the individual and net effects of multiple stressors, even if the stressors operate via the same mechanism (e.g. selection of small species) (Floder and Hillebrand 2012).

Management implications

My findings add to the growing evidence that low diversity and lack of functional redundancy in food webs of alpine lakes increase their vulnerability to stressors relative to lower-elevation systems, thus highlighting them as a management priority (Parker et al. 2008, Williamson et al. 2009). However, my research also reveals that the impact of non-native trout on naturally fishless food webs in montane lakes may be amplified by continued climate warming. Although global stressors, such as climate warming, cannot be managed directly, the effectiveness of approaches to locally manage non-native trout depends on interactions with climate drivers (Brown et al. 2013).

Recently, non-native trout have been removed from mountain lakes to reverse their effects on food webs (Britton et al. 2011). The success of initiatives to restore native biodiversity by eradicating non-native trout has been varied (Donald et al. 2001, Parker et al. 2001, Knapp et al. 2007, Pope et al. 2009). Because introductions of non-native trout in mountain lakes have been widespread, fish removals should be prioritized in lakes where they will be most effective (Dunham et al. 2004). My finding that climate warming may exacerbate the effects of non-native trout on zooplankton communities of naturally fishless mountain lakes suggests that naturally fishless lakes may be strong candidates for fish removals. In contrast, non-native trout appear to have less severe ecological effects in lakes containing native fish, probably because organisms in these systems have adapted to fish predation (Nasmith et al. 2012, Hanisch et al. 2013). However, my finding that zooplankton species that are suppressed by non-native trout are also intolerant of higher temperatures suggests that the recovery of large zooplankton with fish removals may be partly offset by future negative effects of global warming. Consequently, fish removals may be best targeted in lakes that will experience less warming, such as those in natural climate refugia (Brown et al. 2014). Nonetheless, the responses of other biologically important species of fishless lake ecosystems, including insects and plants, to both stressors should be considered in a comprehensive assessment to inform management decisions.

Implications for multiple stressor research

The findings of my research underscore the merit of investigating the net effects of co-occurring stressors for predicting the consequences of global change. In particular, my finding of a synergistic interaction between non-native trout and warming on zooplankton biomass of montane lakes demonstrates that the ecological effects of stressors can be underestimated if they are not considered in the context of other significant drivers of environmental change. My findings also agree with earlier studies suggesting that the effects of multiple stressors are context-dependent by showing that the net effect of non-native trout and warming on mountain lake communities depended on habitat type. Nevertheless, the use of mechanistic trait-based approaches, such as the framework proposed in Chapter 3, has potential to reveal generalities among responses to stressors and therefore, may improve our predictive understanding of stressor effects.

Further, by providing evidence that species co-tolerance can structure the effects of multiple stressors, my research suggests that time-dependent responses to one stressor, such as adaptation and species turnover, can influence the resistance of a community to a subsequent stressor. Consequently, the often used experimental design in which stressors are applied simultaneously to assess their net effect should be modified to incorporate sequential stressor exposures for greater realism and applicability to natural systems (Giller et al. 2004, Fischer et al. 2013).

Future directions

To date, most studies have focused on classifying the nature of combined stressor effects on individuals and populations as non-interactive, synergistic or antagonistic. These descriptive studies have provided a good starting point for multiple stressor research; however, their findings cannot be easily extrapolated beyond the specific stressor combinations, organisms, and response variables tested because they do not explain how and why stressors interact (Adams 2005, Munns Jr 2006). Multiple stressor research will likely benefit from moving beyond discrete classifications of stressor interactions toward more complete descriptions of their net effects, which will likely depend on characteristics of the stressor and the affected biological community (Crain et al. 2008, Darling and Cote 2008, Segner et al. 2014). Below I outline potentially fruitful areas of research to improve our understanding of the effects of non-native trout and warming, as well as other stressors, on freshwater food webs.

Research priority 1: Assess the influence of dispersal on stressor effects

Few studies have investigated the potential for regional dispersers to functionally buffer sensitive local communities against the effects of multiple stressors. My study provided evidence that warming may increase the establishment of zooplankton species from montane lakes in alpine communities. However, the potential for upward shifts in the distribution of montane zooplankton species depends on their dispersal ability in mountain environments, which is still unknown and likely limited by topographic barriers (Holzapfel and Vinebrooke 2005). Large-scale studies are needed to investigate the net effects of stressors on zooplankton meta-communities of mountain lakes. In particular, evidence suggesting that diversity and stability of meta-communities will be eroded by a directional warming could have important implications for zooplankton communities in mountain regions where large environmental changes occur over short distances (Thompson et al. 2015).

Landscape-scale studies will also be necessary to quantify emigration responses to stressors by sensitive species that may be misinterpreted as mortality if only local censuses are performed (Kareiva and Wennergren 1995). For example, montane species that are intolerant of higher temperatures may be able to persist by dispersing to cooler lakes at higher elevations. Further, determining the dispersal ability of zooplankton species that were locally extirpated by non-native trout will help predict whether fish removals may lead to their re-establishment.

Research priority 2: Quantify effects across stressor intensities

There are a broad range of possible non-linear relationships between stressors and their effects (Mackey and Currie 2001). However, most multiple stressor studies employ an ANOVA-based factorial experimental design that implicitly assumes biological responses to stressors are linear over the range being tested (Dunne 2010). This approach is limited because it requires researchers to distil the broad range of stressor intensities affecting natural systems into a few, often arbitrary, categories of particular stressor magnitudes (e.g. “present vs. absent”, “before vs. after”, “control, low, high”). As a result, ANOVA-based factorial experiments only reveal the nature of net stressor effects at the specific stressor intensities selected, potentially leading to an overly simplistic view of interactions between stressors. Many studies classify the net effect of stressors as “synergistic”, “antagonistic”, or “additive/non-interactive” based on only two levels of stress (often an ambient control vs. stressed treatment). However, the direction and magnitude of interactions among stressors can vary non-linearly across gradients of stressor intensity (Vye et al. 2014, Sinclair and Arnott 2015). For example, the effects of multiple stressors can range from antagonistic to synergistic depending on the relative intensities of the individual stressors (Bansal et al. 2013).

A more predictive understanding of multiple stressor effects may be achieved using regression-based experiments, which allow researchers to describe how stressors affect ecological functions across a realistic range of intensities (Cottingham et al. 2005, Kreyling et al. 2014). Regression-based studies can be used to model the relationship between the magnitude of stressors and their net effects over a response surface, thereby allowing researchers to characterize non-linear effects of stressors and assess the changing strength and direction of stressor interactions along gradients of stressor intensity (Wagenhoff et al. 2012). Although including a greater number of stressor treatments in a regression-based design will compromise replication relative to ANOVA-based designs (assuming a given number of experimental units), regression-based approaches can reveal ecologically-important nonlinearities in responses to multiple stressors and provide insight into the mechanisms driving net stressor effects

(Wagenhoff et al. 2012). Alternatively, response surfaces to multiple stressors can be built using a survey-based approach by assessing stressor effects across sites that span gradients of stressor intensities (Lange et al. 2014).

Gradient studies investigating the effects of warming on lake ecosystems in particular, has been highlighted as a research need (Jeppesen et al. 2014). My space-for-time survey (Chapter 2) enabled a regression-based assessment of temperature effects across a climatic gradient; however my experiments included only two levels of temperature (ambient and warmed). Likewise, I assessed the effect of non-native trout by comparing fishless vs. stocked communities and therefore was not able to quantify how stocking density influenced the impact of non-native trout. Yet, the effects of invasive fish on zooplankton communities can be non-linear (Jackson et al. 2015b). Future studies could employ a regression-based design to investigate how different densities of non-native trout and magnitudes of warming jointly affect zooplankton communities. Ultimately, shifts toward regression-based approaches to assess the effects of stressors will facilitate predictions of their net effects at levels that were not explicitly tested, enable detection of threshold effects, and allow better parameterization of causal relationships, which can be more effectively incorporated into ecological models than the results of factorial, ANOVA-based designs (Cottingham et al. 2005, Kreyling et al. 2014).

Research priority 3: Incorporate projected increases in temperature variability and extreme heat events in experiments

Most experiments simulate climate change by applying constant temperature treatments, with the stressed condition having a set increase in temperature relative to controls (Helmuth et al. 2014). These “trend-focused” studies investigate the effects of mean shifts in temperature but are often associated with a lower degree of temperature variability than natural systems (Thompson et al. 2013). Yet, increased temperature variability, a higher frequency of extreme heat events, as well as more gradual directional changes is expected with global change (Jentsch et al. 2007). Importantly, increased variability in temperature will likely more detrimentally affect species than changes in the mean (Vasseur et al. 2014).

Few experiments have investigated the influence of climate warming on freshwater ecosystems relative to terrestrial and marine realms (Thompson et al. 2013). Warming experiments in freshwater ecosystems often use either a “fixed mean” approach or a “fixed increment” approach. “Fixed mean” experiments involve constant temperature treatments, usually comparing mean ambient conditions against projected increases in mean temperatures (Thompson et al. 2013). “Fixed increment” experiments also compare treatments differing in mean temperature, but natural variability is retained (Thompson et al. 2013). My growth chamber experiment (Chapter 4) employed static temperature treatments using a “fixed mean” approach, thereby limiting its realism. Despite its simplification, this experiment revealed potentially important interactions between non-native trout, warming and regional species on zooplankton communities of alpine lakes, suggesting that their effects merited further investigation. As a result, the net effect of these factors was recently tested using a larger-scale mesocosm experiment, which allowed for natural variation in temperature. In contrast, my outdoor mesocosm experiment (Chapter 2) employed a “fixed increment” approach, in which water temperatures tracked diel variations in temperature but temperature variability did not differ across treatments. Consequently, warmed communities experienced higher maximum temperatures during the day and higher minimum temperatures during the night relative to controls. Shifts in minimum and maximum temperatures can have large effects on the performance of ectotherms and therefore, may be particularly important to incorporate in climate change experiments on freshwater food webs (Hallman and Brooks 2015, Ma et al. 2015). Changes in temperature means and variances can also co-vary and may have non-additive effects on ectotherms (Vasseur et al. 2014). Further research is needed to evaluate the ecological effects of variance in temperature independent from, and in combination with, changes in mean temperature (Lawson et al. 2015).

Future research should also focus on the ecological consequences of extreme heat events (Thompson et al. 2013, Jeppesen et al. 2014). The effects of extreme heat events will likely be especially pronounced in small water bodies with low thermal mass, such as ponds and shallow lakes that do not stratify (including most of my study lakes) (Matthews 2010). The potential for heat events to restructure communities and prevent the re-establishment of locally extirpated species may also have implications for restoration efforts involving non-native trout removals in the face of climate change (Seifert et al. 2015).

Conclusion

A single-stressor perspective is no longer suitable for managing threatened ecosystems that are affected by multiple, often interacting, stressors. Thus, predicting the ecological consequences of multiple stressors is one of the greatest challenges facing ecologists. Despite the complex interplay between direct and indirect effects of stressors at the community-level, basic ecological theory can provide a useful foundation for predicting responses to multiple stressors (Halstead et al. 2014). Moving forward, a greater predictive understanding of the effects of multiple stressors on communities can be achieved by focusing on the relative importance of various mechanisms structuring their net effects (Helmuth et al. 2014).

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APPENDIX A: SUPPLEMENTARY MATERIAL FOR CHAPTER 2

Appendix A-1 List of species found in surveyed mountain lakes with mean body sizes, and mean biomasses across 12 fishless and 10 fish-stocked lakes.

Taxon	Mean body size (μg)	Mean biomass ($\mu g\ L^{-1}$) Fishless lakes	 Fish-stocked lakes
Acanthodiaptomus denticornis	10.91	0.71	0.09
Alona spp.	1.02	0.01	0.001
Bosmina longirostris	2.27	0.005	1.57
Calanoid copepodid	6.49	8.12	6.01
Calanoid nauplii	0.22	< 0.001	0.001
Ceriodaphnia spp.	1.32	< 0.001	0.79
Chaoborus spp.	640.0	14.16	0.87
Chydorus sphaericus	1.61	0.01	Not found
Cyclopoid copepodid	1.72	0.04	4.40
Cyclopoid nauplii	0.15	0.002	0.03
Daphnia catawba	10.99	0.14	0.06
Daphnia middendorffiana	43.39	0.85	Not found
Daphnia pulex	36.07	0.17	0.02
Daphnia rosea	7.95	Not found	1.87
Daphnia schodleri	32.24	1.42	Not found
Daphnia spp.	5.08	1.38	0.70
Diacyclops thomasi	5.61	0.01	2.78
Diaptomus tyrrelli	7.52	0.11	0.55
Hesperodiaptomus arcticus	53.03	1.40	0.02

Appendix A-2 List of species found in the mesocosm experiment with mean body sizes. When present, the mean biomass of each species on the final day of the experiment (day 42) is given for the unstressed control (Control), non-native trout-only (Fish), warming-only (Warming), and non-native trout + warming (Both) treatments.

Taxon	Mean body size (μg)	Mean biomass (μg L^{-1})			
		Control	Fish	Warming	Both
Acanthodiaptomus denticornis	9.59	1.31	0.33	0.45	1.81
Acroperus harpae	3.08	0.86	1.86	1.68	54.94
Alona affinis	1.81	Not found	0.05	0.02	0.003
Alona costata	2.80	0.007	0.26	0.36	0.26
Alona guttata	1.85	0.01	0.01	Not found	0.01
Alonella excisa	1.44	Not found	0.005	0.007	0.06
Calanoid copepodid	5.64	0.21	1.81	Not found	0.04
Chaoborus spp.	639.54	62.27	Not found	17.58	Not found
Chydorus sphaericus	1.03	9.04	11.78	5.35	173.25
Cyclopoid copepodid	1.48	1.11	16.76	2.58	52.52
Cyclopoid nauplii	0.14	0.43	6.74	1.44	14.54
Cyclops vernalis	6.36	3.39	15.43	2.21	33.13
Daphnia pulex	65.57	1.16	Not found	0.36	Not found
Daphnia schodleri	57.50	1.61	Not found	9.04	Not found
Daphnia spp.	6.33	2.70	Not found	1.11	0.002
Male *Daphnia* spp.	9.14	3.07	Not found	0.84	Not found
Diacyclops thomasi	5.19	0.009	1.54	Not found	0.64
Eucyclops agilis	5.16	0.02	Not found	0.02	1.76
Macrocyclops albidus	19.60	0.05	0.20	0.41	0.20
Scapholeberis kingi	1.92	0.03	0.15	0.02	Not found

APPENDIX B: SUPPLEMENTARY MATERIAL FOR CHAPTER 3

Appendix B-1 List of zooplankton taxa from naturally fishless lake communities for which tolerances to warming and non-native trout were determined. Species were classified by functional group (FG) as either a top predator (TP), omnivore (OM), large herbivore (LH) or small herbivore (SH). The mean biomass (μg/L) of each species on the final day of the experiment is given for unstressed controls (Control) and the two sequential stressor applications – Warming then Fish (W then F) and Fish then Warming (F then W).

		Day 21			**Day 42**		
Taxon	**FG**	**Control**	**W then F**	**F then W**	**Control**	**W then F**	**F then W**
Acanthodiaptomus denticornis	OM	3.34	0.78	1.81	1.31	0.91	0.64
Acroperus harpae	SH	< 0.01	0.28	0.01	0.86	10.46	10.43
Alona spp.	SH	0.03	0.03	0.05	0.02	0.96	0.97
Chaoborus spp.	TP	11.41	13.01	0	62.27	0	0
Chydorus sphaericus	SH	0.54	0.03	0.51	9.04	35.02	59.69
Cyclopoid copepodid	SH	0.79	1.17	1.24	1.11	21.4	46.1
Cyclopoid nauplii	SH	0.09	0.23	0.48	0.43	14.48	13.23
Cyclops vernalis	OM	0.99	0.96	4.23	3.39	10.76	39.36
Daphnia pulex	LH	2.02	2.22	0	1.56	0	0
Daphnia schodleri	LH	3.39	0.63	0	1.61	0	0
Daphnia spp.	LH	0.69	0.99	0.01	5.78	0	0
Diacyclops thomasi	OM	< 0.01	0.02	0.62	< 0.01	0.03	1.61
Simocephalus spp.	LH	0.06	0	0	1.52	0	0

Appendix B-2 AICc values based on restricted maximum likelihood estimation (REML) for linear mixed models testing the effect of stressor treatment (Unstressed, Warming then fish, and Fish then warming) and the covariate sampling occasion on total zooplankton biomass, the biomass of zooplankton within each functional group, and chlorophyll *a* concentration. Models were run with and without the random blocking effect (i.e. lake source) to determine whether its inclusion improved model fit. Then, to account for potential autocorrelation among repeated samples from the same containers, models were run with different covariance structures. The model with the lowest AICc value was used (in bold).

Model	**Total biomass**	**Top predator**	**Omnivores**	**Large herbivores**	**Small herbivores**	**Chlorophyll *a***
Including random block effect	**207.30**	183.39	135.02	**74.67**	**140.30**	**-217.18**
Excluding random block effect	210.96	**179.38**	**133.51**	75.10	156.36	-150.86
Covariance structures						
Autoregressive (1)	192.00	221.63	100.04	83.70	144.45	-144.64
Autoregressive (1) heterogeneous	**189.87**	196.26	**97.29**	71.46	146.34	-200.48
Autoregressive moving average (1,1)	193.51	220.19	102.11	84.53	151.25	-133.54
Compound symmetry	203.08	219.46	128.05	84.30	174.58	-115.22
Compound symmetry heterogeneous	200.18	197.22	123.51	70.77	141.83	-206.36
Diagonal	198.63	213.28	147.91	85.46	142.63	-202.64
First-order factor analytic	194.91	219.94	137.14	86.18	134.09	-119.33
First-order factor analytic heterogeneous	202.71	192.77	127.52	**65.85**	**123.10**	-204.17
Huynh-Feldt	212.67	191.73	132.47	82.69	170.47	-153.90
Scaled identity	200.96	226.77	145.90	87.81	172.61	-116.55
Unstructured	207.30	**179.38**	133.51	74.67	140.30	**-217.18**

APPENDIX C: SUPPLEMENTARY MATERIAL FOR CHAPTER 4

Appendix C-1 List of crustacean zooplankton species found in each of the four source lakes that were used as "blocks" in the mesocosm experiment. Samples were collected on July 20, 2009.

Lake	**Species**	**Mean length (mm)**	**Density (#/L)**	**Biomass (µg/L)**	**Proportion of total biomass**
Bighorn	*Daphnia middendorffiana*	2.22	0.13	6.29	0.52
	Daphnia middendorffiana (immature)	1.03	0.59	3.21	0.26
	Hesperodiaptomus arcticus	2.95	0.01	0.94	0.08
	Hesperodiaptomus arcticus copepodid	1.83	0.13	1.69	0.14
McConnell	*Bosmina longirostris*	0.31	0.01	0.01	< 0.01
	Cyclopoid nauplii	0.17	0.08	0.01	< 0.01
	Daphnia middendorffiana	1.47	0.01	0.09	0.02
	Hesperodiaptomus arcticus copepodid	1.91	0.30	4.14	0.97
Pipit	Cyclopoid copepodid	0.66	0.01	0.01	< 0.01
	Cyclopoid nauplii	0.16	0.01	< 0.01	< 0.01
	Daphnia middendorffiana (immature)	1.69	0.01	0.28	0.04
	Hesperodiaptomus arcticus	2.31	0.18	7.52	0.96
Snowflake	*Daphnia middendorffiana*	1.94	0.06	1.86	0.05
	Daphnia middendorffiana (immature)	1.03	0.18	0.94	0.03
	Diaptomus tyrrelli	1.26	0.22	1.28	0.03
	Hesperodiaptomus arcticus	2.36	0.16	7.42	0.20
	Hesperodiaptomus arcticus copepodid	1.11	4.71	25.88	0.69

Appendix C-2 List of juvenile and adult stages of zooplankton species that originated from the local species pool (L), the regional species pool (R) or both sources (L + R). Species are classified by functional group (FG) as omnivores (OM), large herbivores (LH), and small herbivores (SH). Species are listed by increasing mean length (± standard deviation when n>1) and when present, the mean biomass is given for each species before stressor application (Initial) and on the final date of the experiment in the unstressed control (Control) and exposed to size-selective predation (+P) and warming (+W) independently and in combination (+P+W).

Species	**FG**	**Mean length ± SD (mm)**	**Source**	**Mean biomass (μg/L)**				
				Initial	**Control**	**+P**	**+W**	**+P+W**
Alona spp	SH	0.18	R				0.03	
Calanoid nauplii	SH	0.18 ± <0.001	R				0.02	0.01
Alona rectangula	SH	0.22	R					0.04
Alona costata	SH	0.31	R				0.01	
Alona guttata	SH	0.31	L				0.01	
Chydorus spp	SH	0.33 ± 0.08	R					6.61
Chydorus sphaericus	SH	0.33 ± 0.07	L + R			0.51		2.43
Alona karau	SH	0.34	R				0.10	
Alona intermedia	SH	0.36 ± 0.02	R				0.33	0.12
Bosmina longirostris	SH	0.42 ± 0.08	L + R	3.08	0.31	1.15	0.34	0.59
Harpacticoid copepodid	SH	0.44 ± 0.03	L + R	0.23		0.02		
Ceriodaphnia spp	SH	0.58 ± 0.02	R	5.44				

Cyclopoid copepodid	SH	0.70 ± 0.17	L + R	15.29	0.77	0.64	1.13	0.94
Daphnia laevis	LH	0.83 ± 0.11	R				0.55	
Mesocyclops spp	OM	0.91 ± 0.05	R	2.39		0.86		0.27
Daphnia spp male	LH	0.94 ± 0.27	R		0.52	0.12	1.02	
Orthocyclops modestus	OM	0.96 ± 0.04	R	3.50		1.00		0.63
Daphnia spp	LH	0.98 ± 0.26	L + R	24.65	16.55	1.03	9.31	1.11
Macrocyclops distinctus	OM	1.02	R		< 0.01	< 0.01	< 0.01	< 0.01
Diacyclops thomasi	OM	1.04 ± 0.19	L + R	7.37	0.73	0.69	0.94	0.94
Eucyclops prionophorus	OM	1.15 ± 0.05	R				1.39	
Diaptomus sicilis	SH	1.16 ± 0.12	R	4.39	0.55	0.60	0.89	0.67
Daphnia rosea	LH	1.28 ± 0.78	R	5.35	0.90			
Cyclops vernalis	OM	1.29 ± 0.38	R			3.79		
Diaptomus tyrrelli	SH	1.30 ± 0.12	L + R	21.59	4.33	6.47	9.02	2.44
Daphnia catawba	LH	1.31 ± 0.20	R	5.51				
Calanoid copepodid	SH	1.33 ± 0.54	L + R	243.79	33.07	20.93	3.92	3.85
Daphnia similis/magna	LH	1.60	R			1.19		
Acanthocyclops robustus	OM	1.66 ± <0.001	R		1.89			
Acanthodiaptomus denticornis	OM	1.69 ± 0.17	R	8.48	0.77	1.36	1.43	1.10
Daphnia middendorffiana	LH	1.87 ± 0.51	L + R	89.55	56.34	16.68	14.95	3.92
Daphnia schodleri	LH	1.93 ± 0.51	R		2.65	0.90	10.90	2.75
Diaptomus sanguines	OM	1.95	R	14.22				
Diaptomus leptopus	OM	2.03 ± 0.20	R	13.34	5.85	5.41	4.42	4.70

Diaptomus clavipes	OM	2.15	L			2.26		
Daphnia pulex	LH	2.18 ± 0.45	R		4.37	3.67	7.53	1.30
Hesperodiaptomus arcticus	OM	2.42 ± 0.29	L + R	138.64	158.17	31.67	105.40	10.38
Diaptomus eiseni	OM	2.56 ± 0.24	L	36.16			40.92	

Appendix C-3 AICc values based on restricted maximum likelihood estimation (REML) for linear mixed models testing the fixed effects of size-selective predation, warming, and regional species, and the covariate sampling occasion, on the biomass, length and species richness of alpine zooplankton communities. Models were run with and without the random blocking effect (i.e. lake source) to determine whether its inclusion improved model fit. Then, to account for potential autocorrelation among repeated samples from the same containers, models were run with different covariance structures. The model with the lowest AICc value was used (in bold).

Model	**Biomass**	**Length**	**Species richness**
Including random block effect	**101.20**	-81.43	**16.66**
Excluding random block effect	127.74	**-83.89**	17.30
Covariance structures			
Autoregressive (1)	99.48	-70.77	18.75
Autoregressive (1) heterogeneous	100.77	-69.73	19.45
Autoregressive moving average (1,1)	99.93	-68.73	21.01
Compound symmetry	111.45	-65.50	18.86
Compound symmetry heterogeneous	110.08	-62.23	19.10
Diagonal	110.12	-60.88	17.38
First-order factor analytic	**94.13**	-51.83	21.56
First-order factor analytic heterogeneous	97.31	-72.19	**13.78**
Huynh-Feldt	105.75	-82.18	16.55
Scaled identity	112.27	-61.67	16.98
Toeplitz	101.31	-67.48	20.58
Toeplitz heterogeneous	102.61	-68.03	20.57
Unstructured	101.20	**-83.89**	16.66